JC06 Rec'd PCT/PTO 28 SEP 2005

Substitute for Form PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
033935-021	
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	10/551205

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US2004/009387	March 26, 2004	March 28, 2003

TITLE OF INVENTION

ORAL FORMULATIONS OF CLADRIBINE

APPLICANT(S) FOR DO/EO/US

Nicholas S. BODOR and Yogesh DANDIKER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission to items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (22) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 21 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 37 C.F.R. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information: Application Data Sheet.

Exhibit #:
Hopewell EX 1057
02/15/24
exhibitsticker.com

Hopewell EX1057 (Part 1 of 2)
Hopewell v. Merck
IPR2023-00480

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10755205	PCT/US2004/009387	033935-021

21. ☐ Applicant(s) requests that the published application include the following assignment information:
IVAX Corporation
4400 Biscayne Boulevard
Miami, Florida 33137 US

22. ☒ The following fees are submitted:

					CALCULATIONS PTO USE ONLY
Basic Filing Fee (1631)					$ 300.00
Surcharge of **$130.00 (1617)** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).					$ 130.00
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE	$
Total Claims	78	-20 =	58	x $50.00 (1615)	$ 2,900.00
Independent Claims	6	- 3 =	3	x $200.00 (1614)	$ 600.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ $360.00 (1616)	
Examination Fee				+ $200.00 (1633)	$ 200.00
Search Fee				+ $500.00 (1632)	$ 500.00
App. Size Fee (add $250.00 for each add'l 50 sheets exceeding 100 sheets)					
TOTAL OF ABOVE CALCULATIONS =					$ 4,630.00
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+	$ 0.00
SUBTOTAL =					$ 4,630.00
Processing fee of **$130.00 (1618)** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).					$ 0.00
TOTAL NATIONAL FEE =					$ 4,630.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (8021)** per property +					
TOTAL FEES ENCLOSED =					$ 4,630.00
					Amount to be refunded :
					charged :

a. ☒ A check in the amount of $ 4,630.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 02-4800 in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

d. ☐ Charge _____ to credit card. Form PTO-2038 is attached.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Burns, Doane, Swecker & Mathis, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Mary Katherine Baumeister
SIGNATURE

Mary Katherine Baumeister
NAME

26,254 — REGISTRATION NO.

September 28, 2005 — DATE

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SIGNATURE

Mary Katherine Baumeister
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September 28, 2005 — DATE

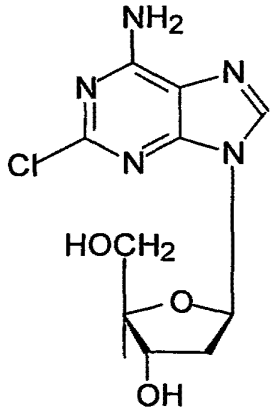
ORAL FORMULATIONS OF CLADRIBINE

FIELD OF THE INVENTION

The invention relates to a composition comprising a complex cladribine-cyclodextrin complex formulated into a solid oral dosage form and to a method for enhancing the oral bioavailability of cladribine.

BACKGROUND OF THE INVENTION

Cladribine, which is an acid-labile drug, has the chemical structure as set forth below:



It is also known as 2-chloro-2'-deoxyadenosine or 2-CdA. Cladribine exists as a white, nonhydroscopic, crystalline powder, consisting of individual crystals and of crystalline aggregates.

Cladribine is an antimetabolite which has use in the treatment of lymphoproliferative disorders. It has been used to treat experimental leukemias such as L1210 and clinically for hairy cell leukemia and chronic lymphocytic leukemia as well as Waldenstrom's macroglobulinaemia. It has

also been used as an immunosuppressive agent and as a modality for the treatment of a variety of autoimmune conditions including rheumatoid arthritis, inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis) and multiple sclerosis (see *e.g.*, J. Liliemark, *Clin. Parmacokinet*, 32(2): 120-131, 1997). It has also been investigated, either experimentally or clinically in, for example, lymphomas, Langerhan's cell histiocytosis, lupus erythematosus, chronic plaque psoriasis, Sezary syndrome, Bing-Neel syndrome, recurrent glioma, and solid tumors.

Oral delivery of drugs is often preferred to parenteral delivery for a variety of reasons, foremost patient compliance, or for cost or therapeutic considerations. Patient compliance is enhanced insofar as oral dosage forms alleviate repeated health care provider visits, or the discomfort of injections or prolonged infusion times associated with some active drugs. At a time of escalating health care costs, the reduced costs associated with oral administration versus parenteral administration costs gain importance. The cost of parenteral administration is much higher due to the requirement that a health care professional administer the cladribine in the health care provider setting, which also includes all attendant costs associated with such administration. Furthermore, in certain instances, therapeutic considerations such as the need for a slow release of cladribine over a prolonged period of time may be practically met only by oral or transmucosal delivery.

However, to date the oral delivery of cladribine has been plagued by low bioavailability (see, *e.g.*, J. Liliemark *et al., J. Clin. Oncol.*, 10(10): 1514-1518, 1992), and suboptimal interpatient variation (see, *e.g.*, J. Liliemark, *Clin. Pharmacokinet*, 32 (2): 120-131, 1997). See also, A. Tarasuik, *et al.* reporting poor absorption and pH dependent lability (*Arch. Immunol. et Therapiae Exper.*, 42: 13-15, 1994).

Cyclodextrins are cyclic oligosaccharides composed of cyclic α-(1→4) linked D-glucopyranose units. Cyclodextrins with six to eight units have

been named α-, β- and γ-cyclodextrin, respectively. The number of units determines the size of the cone-shaped cavity which characterizes cyclodextrins and into which drugs may be included to form stable complexes. A number of derivatives of α-, β- and γ-cyclodextrin are known in which one or more hydroxyl groups is/are replaced with ether groups or other radicals. These compounds are thus known complexing agents and have been previously used in the pharmaceutical field to form inclusion complexes with water-insoluble drugs and to thus solubilize them in aqueous media.

Recently, Schultz *et al.*, in U.S. Patent No. 6,194,395 B1, have described complexing and solubilizing cladribine with cyclodextrin. The Schultz *et al.* patent primarily addresses the problems inherent in previously described aqueous formulations of cladribine, particularly for subcutaneous and intramuscular injection. Schultz *et al.* have found that cladribine is not only significantly more soluble in aqueous media when formulated with cyclodextrin, but also is more stable against acid-catalyzed hydrolysis when combined with cyclodextrin. The latter finding is taught to be of particular benefit in the formulation of solid oral dosage forms, where the compound would normally undergo hydrolysis in the acid pH of the stomach contents. Schultz *et al.* do not appear to have described any actual work in connection with solid oral dosage forms. In fact, they describe only one method of preparing the solid dosage form, which is a melt extrusion process, in which the cladribine and cyclodextrin are mixed with other optional additives and then heated until melting occurs. Furthermore, the broad dosage ranges of 1 mg to 15 mg of cladribine and 100 mg to 500 mg of cyclodextrin listed in the patent suggest no criticality to the particular amount of cyclodextrin to be present with a given amount of cladribine in a solid oral dosage form. Indeed, these dosage ranges include many combinations which may be suitable as mixtures but not for complex formation. For example, a ratio of 1 mg of cladribine to 500 mg of cyclodextrin contains too much cyclodextrin, so

that the drug would not readily leave the complex and achieve its therapeutic function. On the other hand, 15 mg of cladribine and only 100 mg of cyclodextrin would not be enough to complex that amount of cladribine.

The Schultz *et al.* patent does suggest improving the stability of cladribine in oral dosage forms by combining/complexing it with cyclodextrin, but does not suggest improving the drug's oral bioavailability by such means; in fact, the patent does not describe or suggest a method for enhancing or maximizing the bioavailability of cladribine from a solid oral dosage form of cladribine and cyclodextrin, or a composition specially designed to do so.

Many workers have studied the solubility of specific drugs in water containing various concentrations of selected cyclodextrins in order to demonstrate that increasing concentrations of cyclodextrins increase the solubility of the drugs at selected temperatures and pH levels, as for example reported in the Schultz *et al.* patent. Phase solubility studies have also been performed by various workers in order to elucidate the nature of the complex formation, for example, whether the cyclodextrin and drug form a 1:1 complex or a 1:2 complex; see, for example, Harada *et al.* U.S. Patent No. 4,497,803, relating to inclusion complexes of lankacidin-group antibiotics with cyclodextrin, and Shinoda *et al.* U.S. Patent No. 4,478,995, relating to a complex of an acid addition salt of (2'-benzyloxycarbonyl)phenyl trans-4-guanidinomethylcyclohexanecarboxylate with a cyclodextrin.

While Schultz *et al.* teach that a cladribine-cyclodextrin complex improves the water solubility and acid stability of cladribine, the art does not suggest how to maximize or enhance the benefits of the complexation in terms of bioavailability and interpatient variation when the complex is to be administered in a solid oral dosage form.

SUMMARY OF THE INVENTION

It has now been found that amorphous cyclodextrins can be combined with cladribine to form a particularly advantageous product which can be incorporated into a solid oral dosage form. This product is a complex cladribine-cyclodextrin complex, and the solid oral dosage form containing it improves oral bioavailability and/or achieves lower interpatient and/or intrapatient variation of the drug.

The present invention provides a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, and a pharmaceutical composition comprising said complex, formulated into a solid oral dosage form. Thus, the cyclodextrin itself is amorphous, the inclusion complex with cladribine is amorphous (and is preferably saturated with cladribine) and the free cladribine which forms the non-inclusion complex is amorphous.

The invention also provides a method for increasing or enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof, a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form which maximizes the amount of cladribine in the inclusion and non-inclusion complexes.

The invention further provides for treatment of conditions responsive to administration of cladribine in mammals by administering thereto the composition of the invention. Use of cladribine in the preparation of the pharmaceutical compositions of the invention for administration to treat

cladribine-responsive conditions and for enhancing the oral bioavailability of cladribine is also provided.

Still further, the invention provides a process for the preparation of a complex cladribine-cyclodextrin complex which comprises the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature; and

(iii) lyophilizing the cooled solution to afford an amorphous product.

In yet a further aspect the invention provides a pharmaceutical composition obtainable by a process comprising the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature;

(iii) lyophilizing the cooled solution to afford an amorphous product; and

(iv) formulating the amorphous product into a solid oral dosage form.

BRIEF DESCRIPTION OF THE DRAWING

A more complete appreciation of the invention and its many attendant advantages will be readily understood by reference to the following detailed description and the accompanying drawing, wherein the sole Figure is a graphical representation of the results of a phase solubility study where various molar concentrations of hydroxypropyl-β-cyclodextrin (HPβCD) are plotted against various cladribine molar concentrations, with (●) representing

the data points obtained for complexation under conditions specified in EXAMPLE 2 below.

DETAILED DESCRIPTION OF THE INVENTION

Throughout the instant specification and claims, the following definitions and general statements are applicable.

The patents, published applications, and scientific literature referred to herein establish the knowledge of those with skill in the art and are hereby incorporated by reference in their entirety to the same extent as if each was specifically and individually indicated to be incorporated by reference. Any conflict between any reference cited herein and the specific teachings of this specification shall be resolved in favor of the latter. Likewise, any conflict between an art-understood definition of a word or phrase and a definition of the word or phrase as specifically taught in this specification shall be resolved in favor of the latter.

The term "inclusion complex" as used herein refers to a complex of cladribine with the selected cyclodextrin wherein the hydrophobic portion of the cladribine molecule (the nitrogen-containing ring system) is inserted into the hydrophobic cavity of the cyclodextrin molecule. This is often referred to simply as a cyclodextrin complex of the drug.

The term "non-inclusion complex" refers to a complex which is not an inclusion complex; rather than the hydrophobic portion of cladribine being inserted in the cyclodextrin cavity, the non-inclusion complex is formed primarily by hydrogen-bonding of the hydroxyls and amino group on "free" cladribine, (*i.e.* cladribine not in the inclusion complex) to the hydroxyls on the exterior of the cyclodextrin torus (*e.g.* in the case of hydroxypropyl-β-cyclodextrin, hydroxypropyl and hydroxyl groups on the glucose rings). This is a more loosely-held association than an inclusion complex.

As used herein, whether in a transitional phrase or in the body of a claim, the terms "comprise(s)" and "comprising" are to be interpreted as having an open-ended meaning. That is, the terms are to be interpreted synonymously with the phrases "having at least" or "including at least". When used in the context of a process, the term "comprising" means that the process includes at least the recited steps, but may include additional steps. When used in the context of a composition, the term "comprising" means that the composition includes at least the recited features or components, but may also include additional features or components.

The terms "consists essentially of" or "consisting essentially of" have a partially closed meaning, that is, they do not permit inclusion of steps or features or components which would substantially change the essential characteristics of a process or composition; for example, steps or features or components which would significantly interfere with the desired properties of the compositions described herein, *i.e.,* the process or composition is limited to the specified steps or materials and those which do not materially affect the basic and novel characteristics of the invention. The basic and novel features herein are the provision of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form, so as to provide improved bioavailability and/or lower interpatient and/or intrapatient variation following administration. Essential to the invention is the combination of the amorphous nature of the starting cyclodextrin, and the level of water solubility exhibited by cladribine (about 5 mg/ml at room temperature), and consequently its capability for hydrogen bonding, which can be taken advantage of under particular conditions described hereinafter, and which afford a special amorphous mixture uniquely well-suited for optimizing the oral bioavailability of cladribine.

The terms "consists of" and "consists" are closed terminology and allow only for the inclusion of the recited steps or features or components.

As used herein, the singular forms "a," "an" and "the" specifically also encompass the plural forms of the terms to which they refer, unless the content clearly dictates otherwise.

The term "about" is used herein to means approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" or "approximately" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

The term "amorphous" is used herein to refer to a noncrystalline solid. The cyclodextrins encompassed herein themselves are amorphous because they are each composed of a multitude of individual isomers, and their complexes with cladribine are also amorphous. Further, conditions for complexation can be selected (elevated temperature and prolonged complexation times, as described hereinafter) so that a supersaturated cladribine solution will be formed. When cooled, because of the amorphous nature of the complex and the cyclodextrin, some excess free cladribine does not precipitate but rather is trapped in amorphous form in intimate admixture with the (preferably saturated) amorphous cladribine-cyclodextrin inclusion complex. This excess cladribine forms a loosely-held association, or non-inclusion complex, with the cyclodextrin through hydrogen bonding. This, then, further increases the amount of cladribine in the product; this additional cladribine, because it is amorphous and also because it is in intimate admixture with the amorphous inclusion complex, is expected to be somewhat protected from degradation by stomach acid (although it may not be as protected as the cladribine which is in the form of the inclusion complex).

The term "saturated" when used in conjunction with a complex of cladribine in amorphous cyclodextrin means that the complex is saturated with cladribine, that is, the complex contains the maximum amount of cladribine which can be complexed (by means of both inclusion and non-inclusion complexes) with a given amount of cyclodextrin under the conditions of complexation used. A phase solubility study can be used to provide this information, as described in more detail hereinafter. (Conditions for the complexation are also described in more detail below.) Alternatively, a saturated complex may be arrived at empirically by simply adding cladribine to an aqueous solution of the selected cyclodextrin until no more cladribine goes into solution; ultimately, excess cladribine, if any, is removed (by filtration or centrifugation) and the solution lyophilized to provide the dry saturated complex.

The expression "substantially', as in "substantially free" means within 20% of the exact calculated amount, preferably within 10%, most preferably within 5%.

The term "interpatient variability" refers to variation among patients to which a drug is administered. The term "intrapatient variability" refers to variation experienced by a single patient when dosed at different times.

As used herein, the recitation of a numerical range for a variable is intended to convey that the invention may be practiced with the variable equal to any of the values within that range. Thus, for a variable which is inherently discrete, the variable can be equal to any integer value of the numerical range, including the end-points of the range. Similarly, for a variable which is inherently continuous, the variable can be equal to any real value of the numerical range, including the end-points of the range. As an example, a variable which is described as having values between 0 and 2, can be 0, 1 or 2 for variables which are inherently discrete, and can be 0.0,

0.1, 0.01, 0.001, or any other real value for variables which are inherently continuous.

In the specification and claims, the singular forms include plural referents unless the context clearly dictates otherwise. As used herein, unless specifically indicated otherwise, the word "or" is used in the "inclusive" sense of "and/or" and not the "exclusive" sense of "either/or."

Technical and scientific terms used herein have the meaning commonly understood by one of skill in the art to which the present invention pertains, unless otherwise defined. Reference is made herein to various methodologies and materials known to those of skill in the art. Standard reference works setting forth the general principles of pharmacology include Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 10th Ed., McGraw Hill Companies Inc., New York (2001).

Reference is made hereinafter in detail to specific embodiments of the invention. While the invention will be described in conjunction with these specific embodiments, it will be understood that it is not intended to limit the invention to such specific embodiments. On the contrary, it is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims. In the following description, numerous specific details are set forth in order to provided a thorough understanding of the present invention. The present invention may be practiced without some or all of these specific details. In other instances, well-known process operations have not been described in detail, in order not to unnecessarily obscure the present invention.

There is provided by the present invention compositions, as well as methods of making and of using pharmaceutical compositions, useful to achieve desirable pharmacokinetic properties. Such compositions stem from the discovery that solutions of cyclodextrin and cladribine in which cladribine is in a high thermodynamic state, when presented to the gastric mucosa

through which they are absorbed are associated with improved cladribine absorption, as reflected by higher bioavailability and/or lower interpatient variation.

It is postulated, without wishing to so limit the invention, that upon dissolution (*e.g.*, by contact with a fluid, such as a bodily fluid), dry compositions according to the invention form a locally saturated cladribine solution in which cladribine is in the state of highest thermodynamic activity (HTA), thus favoring absorption. Cladribine has a fairly low, although not insignificant, intrinsic aqueous solubility; it is in fact somewhat water soluble. The free cladribine formed from dissociation of the inclusion and non-inclusion complexes in a saturated aqueous solution seeks a more stable activity level by being absorbed through the gastric mucosa.

In view of the foregoing, it is apparent that to produce optimal pharmaceutical compositions, in a solid oral dosage form, these dosage forms should be formulated to release a localized saturated cladribine solution, upon contact of the solid dosage forms with body fluid at the mucosa, in which cladribine is in its HTA state. To provide such a localized saturated solution *in vivo*, it is important to first identify the optimal ratio of cladribine to amorphous cyclodextrin, which ratio is referred to herein as the HTA ratio, to be used in the solid dosage form.

The HTA ratio is empirically determined and is identified as the ratio of cladribine to amorphous cyclodextrin which corresponds to the maximum amount of cladribine that can be complexed with a given amount of the cyclodextrin. The HTA ratio may be determined using an empirical method such as a phase solubility study to determine the saturation concentration of cladribine that can be solubilized with different concentrations of amorphous cyclodextrin solutions. Hence, the method identifies the concentrations at which a saturated cladribine-cyclodextrin complex is formed. It is noted that the molar ratio represented by a point on the phase solubility graph shows

how many moles of amorphous cyclodextrin are the minimum needed to maintain the drug in the complex, under given conditions; this may then be converted to a weight ratio. For example, if a phase solubility diagram shows that 9 moles of a given cyclodextrin are needed to maintain the cladribine in a saturated complex, then multiplying the number of moles of cladribine by its molecular weight and multiplying the number of moles of the selected cyclodextrin by its molecular weight, one can arrive at the ratio of the products as an appropriate optimized weight ratio. A phase solubility study also provides information about the nature of the cladribine-cyclodextrin inclusion complex formed, for example whether the inclusion complex is a 1:1 complex (1 molecule of drug complexed with 1 molecule of cyclodextrin) or a 1:2 complex (1 molecule of drug complexed with 2 molecules of cyclodextrin).

In accordance with the present invention, one can start using either the selected amorphous cyclodextrin, such as hydroxypropyl-β-cyclodextrin (HPβCD) or hydroxypropyl-γ-cyclodextrin, or cladribine as the fixed variable to which an excess of the other is added to identify various solubility data points (indicating saturated cladribine-cyclodextrin complexes) and draw the resultant line. Typically, cladribine is added to an aqueous solution having a known concentration of amorphous cyclextrin under conditions empirically found to promote complex formation. Generally, the complexation is conducted with heating, for example at about 45 to about 60°C for a significant period of time, *e.g.*, at least 6-9 hours; it is believed that even better results can be obtained by heating at up to about 80°C for up to 24 hours. Excess precipitated cladribine is then removed and the cladribine concentration is subsequently measured. This concentration represents the amount of cladribine solubilized for a given amorphous cyclodextrin concentration. This process is repeated for a different known concentration of cyclodextrin until several data points are obtained. Each data point represents the concentration of the cladribine dissolved in a known

concentration of the selected amorphous cyclodextrin. The data points are then plotted to show the concentration of cladribine against the various cyclodextrin concentrations used. The graph is a phase solubility diagram which can be used to determine the amount of cladribine for any specific concentration of cyclodextrin used to form the solution under a given set of complexation conditions. It will be appreciated that the aqueous solubility of cladribine is about 5 mg/ml at room temperature and would be higher at elevated temperature. Consequently, the data points correspond to the amount of cladribine dissolved in aqueous HPβCD or other amorphous cyclodextrin under the selected conditions; when later lyophilized, the solution yields a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex. If equilibrium conditions are reached during the complexation, the amorphous cladribine-cyclodextrin complex will be saturated with cladribine.

One of skill in the art will appreciate that concentrations at which saturated complexes of cladribine with amorphous cyclodextrins are formed (and thus HTA ratios as well) may be identified by a variety of alternative methodologies. Accordingly, any method known in the field suitable to identify these concentrations is within the scope of the invention.

It has been discovered that desirable pharmacological properties (improved bioavailability and/or coefficient of variation as compared to traditional approaches) are associated with mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin.

Using intrinsically amorphous cyclodextrins, for example hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated cyclodextrins, and the like, with cladribine, which is a somewhat water soluble compound (capable of H-bonding through its free hydroxyl and

amino groups), the cladribine provides increased solubility in solutions of these cyclodextrins. Not only is there increased water solubility but also H-bonded association of the cladribine with the cyclodextrin, separately from the actual inclusion complexed material.

One of skill in the art will appreciate that the phase solubility diagram for each given starting concentration ratio represents the starting point of one's investigation on the basis of which variables (reactants' concentrations, temperature and time) may be altered to promote inclusion complex and non-inclusion complex associations favoring a higher or lower proportion of either type of association in the final product. Departure from the ratio of cladribine to cyclodextrin, the temperature and/or the dilution empirically found to promote equilibrium towards complex formation is then analyzed to promote the formation of mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin in various proportions according to the invention.

Thus, for example, by starting with more diluted cyclodextrin (*i.e.*, larger water volumes than that used for solubility plot analysis) logically will accommodate more cladribine in solution sequestering more of the same from complex formation. Upon evaporation, some of the solubilized cladribine will tend to associate with cyclodextrin in a non-inclusion complex fashion. By altering the initial dilution, one may shift equilibrium towards inclusion complex or non-inclusion complex formation. Similarly, by increasing complexation temperature, the water solubility of cladribine may be increased while decreasing the stability of inclusion complexes, thus promoting non-inclusion complexes. Thus, by altering complexation temperature, one may shift equilibrium towards inclusion complex or non-inclusion complex formation. Finally, complexation time may be altered to favor the formation of mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin according to the invention.

As exemplified hereinafter, it is possible to maximize the cladribine in solid amorphous mixtures, by forcing additional cladribine into solution (using more dilute solutions of cyclodextrin, higher temperatures and longer complexation times, as indicated above). When the solution is cooled off, the extensively amorphous nature of these cyclodextrins does not allow crystallization of an excess amount of cladribine beyond that which forms an inclusion complex with the cyclodextrin; and upon freeze-drying/lyophilization, one obtains an amorphous mixture of cladribine-cyclodextrin inclusion complex (which is amorphous) and amorphous free cladribine, loosely associated with uncomplexed cyclodextrin (and even with complexed cyclodextrin) by hydrogen-bonding, that is, the non-inclusion complex.

As shown in the EXAMPLES, this may be done by maximizing solubilization by elevating the temperature (for example, to about 50°to 80°C), and stirring for many hours (up to 24 hours) before freeze-drying. The weight/weight ratios obtained were about 1:14 and 1:11. The apparent optimum weight/weight ratio under these exemplified conditions is the higher of these, or about 1:14 of cladribine: cyclodextrin. If too much excess caldribine is added to the complexation medium, then crystallization of some of the cladribine takes place, which would in turn result in some crystalline cladribine in the product; this undesired excess cladribine is not in solution and is not H-bonded to the amorphous cyclodextrin and lowers the weight ratio. Therefore, it is desirable to carefully control the amount of excess cladribine beyond that which will form the inclusion complex to only the amount which will dissolve in the solution. The desired amorphous mixture of amorphous inclusion complex and amorphous free cladribine can be termed a "complex cladribine-cyclodextrin complex," which includes both inclusion and non-inclusion/H-bonded complexes. The inclusion complex is a complex of cladribine inserted into the hydrophobic cavity of the selected amorphous cyclodextrin, while the non-inclusion/H-bonded complex is

amorphous free cladribine loosely hydrogen-bonded to the cyclodextrin. It is estimated that about two-thirds (60 to 70%) of the cladribine will be in the non-inclusion complex, with the remaining one third (30 to 40%) being in the inclusion complex when the product is obtained as exemplified hereinbelow (17% HPβCD solution, 45 to 50°C complexation temperature for about 9 hours); by increasing the percentage of cyclodextrin used and/or manipulating the temperature, products can be readily obtained in which a much greater proportion of the amorphous mixture is in the form of the inclusion complex. In the case of a representative amorphous cyclodextrin, hydroxypropyl-β-cyclodextrin (HPβCD) a cladribine:cyclodextrin weight ratio of from about 1:10 to about 1:16 is appropriate for the exemplified conditions; the ratio is expected to be the same for hydroxypropyl-γ-cyclodextrin under those conditions. The material obtained is characterized by rapid dissolution of the cladribine in aqueous media.

Freeze-drying, also known as lyophilization, comprises three basic stages: first a freezing stage, then a primary drying stage and finally a secondary drying stage. EXAMPLE 2 below provides details of lyophilization as conducted on the batches described therein. This procedure can be further optimized by following the principles described by Xiaolin (Charlie) Tang and Michael J. Pikal in *Pharmaceutical Research*, Vol. 21, No. 2, February 2004, 191-200, incorporated by reference herein in its entirety and relied upon.

The above-described method requires amorphous cyclodextrins rather than originally crystalline cyclodextrins which have relatively low water solubilities, such as α-, β- or γ-cyclodextrin, 2,6-dimethyl-β-cyclodextrin and the like, because these cyclodextrins would allow crystallization of cladribine in excess of that forming an inclusion complex and therefore would not afford the desired amorphous mixture. The method also would not be useful if cladribine were highly hydrophobic/lipophilic, because in such a situation the drug would not have intrinsic aqueous solubility/H-bonding capability and

could not provide the unique mixture obtained herein. However, in point of fact, cladribine has an aqueous solubility of 5 mg/ml at room temperature, thus a significant amount of the drug will be simply soluble in the water phase especially at higher than room temperature; also, as in the case of HPβCD, for example, some of the cladribine will be associated by hydrogen-bonding to the 2-hydroxypropyl and free glucose-OH groups in the cyclodextrin via the two hydroxy functions found in the deoxyadenosine moiety of the cladribine.

The cyclodextrins within the scope of this invention are amorphous derivatives of the natural cyclodextrins α-, β- or γ-cyclodextrin wherein one or more of the hydroxy groups are substituted, for example, by alkyl, hydroxyalkyl, carboxyalkyl, alkylcarbonyl, carboxyalkoxyalkyl, alkylcarbonyloxyalkyl, alkoxycarbonylalkyl or hydroxy-(mono or polyalkoxy)alkyl groups; and wherein each alkyl or alkylene moiety preferably contains up to six carbons. Although commonly referred to as a single entity, an amorphous cyclodextrin is actually a mixture of many different entities, since the substituent groups can be located on various hydroxyls of the basic cyclodextrin structure. This in turn results in the amorphous nature of these cyclodextrins, which is indeed well-known. Moreover, these cyclodextrins can be obtained in varying degrees of substitution, for example from 1 to 14, preferably from 4 to 7; the degree of substitution is the approximate average number of substituent groups on the cyclodextrin molecule, for example, the approximate number of hydroxypropyl groups in the case of the hydroxpropyl-β-cyclodextrin molecule, and all such variations are within the ambit of this invention. Substituted amorphous cyclodextrins which can be used in the invention include polyethers, for example, as described in U.S. Patent No. 3,459,731. Further examples of substituted cyclodextrins include ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is replaced by C_{1-6}alkyl, hydroxy-C_{1-6}alkyl, carboxy-C_{1-6}alkyl or C_{1-6}alkyloxycarbonyl-

C_{1-6}alkyl groups or mixed ethers thereof. In particular, such substituted cyclodextrins are ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is replaced by C_{1-3}alkyl, hydroxy-C_{2-4}alkyl or carboxy-C_{1-2}alkyl or more particularly by methyl, ethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, carboxymethyl or carboxyethyl. The term "C_{1-6}alkyl" is meant to include straight and branched saturated hydrocarbon radicals, having from 1 to 6 carbon atoms such as methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, butyl, pentyl, hexyl and the like. Other cyclodextrins contemplated for use herein included glucosyl-β-cyclodextrin and maltosyl-β-cyclodextrin. Of particular utility in the present invention are randomly methylated β-cyclodextrin and polyethers such as hydroxypropyl-β-cyclodextrin, hydroxyethyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, and hydroxyethyl-γ-cyclodextrin, as well as sulfobutyl ethers, especially β-cyclodextrin sulfobutyl ether. In addition to simple cyclodextrins, branched cyclodextrins and cyclodextrin polymers may also be used. Other cyclodextrins are described, for example, in *Chemical and Pharmaceutical Bulletin* 28: 1552-1558 (1980); Yakugyo Jiho No. 6452 (28 March 1983); *Angew. Chem. Int. Ed. Engl.* 19: 344-362 (1980); U.S. Patent Nos. 3,459,731 and 4,535,152; European Patent Nos. EP 0 149 197A and EP 0 197 571A; PCT International Patent Publication No. WO90/12035; and UK Patent Publication GB 2,189,245.

References describing cyclodextrins for use in the compositions according to the present invention, and/or which provide a guide for the preparation, purification and analysis of cyclodextrins include the following: *Cyclodextrin Technology* by Jozsef Szejtli, Kluwer Academic Publishers (1988) in the chapter Cyclodextrins in Pharmaceuticals; *Cyclodextrin Chemistry* by M. L. Bender *et al.*, Springer-Verlag, Berlin (1978); *Advances in Carbohydrate Chemistry*, Vol. 12, Ed. By M. L. Wolfrom, Academic Press, New York in the chapter "The Schardinger Dextrins" by Dexter French, pp. 189-260; *Cyclodextrins and their Inclusion Complexes* by J. Szejtli,

Adakemiai Kiado, Budapest, Hungary (1982); I. Tabushi, *Acc. Chem. Research*, 1982, 15, pp. 66-72; W. Sanger, *Angewandte Chemie*, 92, p. 343-361 (1981); A. P. Croft *et al., Tetrahedron*, 39, pp. 1417-1474 (1983); Irie *et al. Pharmaceutical Research*, 5, pp. 713-716 (1988); Pitha *et al., Int. J. Pharm.* 29, 73 (1986); U.S. Patent Nos. 4,659,696 and 4,383,992; German Patent Nos. DE 3,118,218 and DE-3,317,064; and European Patent No. EP 0 094 157A. Patents describing hydroxyalkylated derivative of β- and γ-cyclodextrin include Pitha U.S. Patent Nos. 4,596,795 and 4,727,064, Müller U.S. Patent Nos. 4,764,604 and 4,870,060 and Müller *et al.* U.S. Patent No. 6,407,079.

Amorphous cyclodextrins of particular interest for complexation with cladribine include: hydroxyalkyl, *e.g.* hydroxyethyl or hydroxypropyl, derivatives of β- and γ-cyclodextrin; carboxyalkyl, *e.g.* carboxymethyl or carboxyethyl, derivatives of β- or γ-cyclodextrin; β-cyclodextrin sulfobutyl ether; and randomly methylated β-cyclodextrin. 2-Hydroxypropyl-β-cyclodextrin (HPβCD), 2-hydroxypropyl-γ-cyclodextrin (HPγCD), randomly methylated β-cyclodextrin, β-cyclodextrin sulfobutyl ether, carboxymethyl-β-cyclodextrin (CMβCD) and carboxymethyl-γ-cyclodextrin (CMγCD) are of special interest, especially hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin.

Compositions of an amorphous mixture of amorphous free cladribine and an amorphous, preferably saturated, cladribine-cyclodextrin inclusion complex for use in the present invention can be prepared under conditions favoring complex formation in a liquid environment as described and as exemplified herein. The resultant liquid preparations can be subsequently converted to a dry form suitable for administration as a solid oral or transmucosal dosage form.

One of skill will appreciate that a variety of approaches are available in the field to prepare compositions as described herein. One available

method exemplified herein includes the steps of mixing the cladribine in an aqueous solution of an amorphous cyclodextrin, separating un-dissolved cladribine (*e.g.*, by filtering or centrifugation), and lyophilizing or freeze-drying the saturated solution to form a solid amorphous mixture.

Pharmaceutical compositions according to the invention may optionally include one or more excipients or other pharmaceutically inert components. One of the advantages of the invention, however, is that cladribine drug forms as described herein can be prepared with the minimal amount of excipients necessary for shaping and producing the particular form, such as a tablet or patch. Excipients may be chosen from those that do not interfere with cladribine, with cyclodextrin or with complex formation.

Dosage forms are optionally formulated in a pharmaceutically acceptable vehicle with any of the well-known pharmaceutically acceptable carriers, diluents, binders, lubricants, disintegrants, scavengers, flavoring agents, coloring agents, and excipients (see *Handbook of Pharmaceutical Excipients*, Marcel Dekker Inc., New York and Basel (1998); Lachman *et al.* Eds., *The Theory and Practice of Industrial Pharmacy*, 3rd Ed., (1986); Lieberman *et al.*, Eds. *Pharmaceutical Dosage Forms,* Marcel Dekker Inc., New York and Basel (1989); and *The Handbook of Pharmaceutical Excipients*, 3rd Ed., American Pharmaceutical Association and Pharmaceutical Press, 2000); see also *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, Mack Publishing Co., Easton, PA (1990) and *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams & Wilkins, (1995)). A simple solid oral dosage form consists of the amorphous mixture of amorphous free cladribine and amorphous cladribine-cyclodextrin complex (preferably saturated) as described above, *i.e.* the complex cladribine-cyclodextrin complex, compressed with a small amount (*e.g.* about 1% by weight) of a suitable binder or lubricant such as magnesium stearate.

In certain instances, oral absorption may be further facilitated by the addition of various excipients and additives to increase solubility or to enhance penetration, such as by the modification of the microenvirionment.

The methods and pharmaceutical compositions described herein offer novel therapeutic modalities for the treatment of patients in need of treatment with cladribine. As shown herein, the invention addresses the problems of poor bioavailability traditionally associated with oral cladribine.

The compositions of the invention are particularly suitable as modalities for the treatment of any cladribine-responsive disease. Several disease states responsive to cladribine are well-documented in the literature (see *infra*). For any target disease state, an effective amount of the complex cladribine-cyclodextrin comples, *i.e.* the amorphous mixture of the optimized amorphous saturated cladribine-amorphous cyclodextrin complex with amorphous free cladribine as described above is used (*e.g.*, an amount affective for the treatment of multiple sclerosis, rheumatoid arthritis, or leukemia).

The term "therapeutically effective amount" or "effective amount" is used to denote treatments at dosages effective to achieve the therapeutic result sought. Therapeutically effective dosages described in the literature include those for hairy cell leukemia (0.09 mg/kg/day for 7 days), for multiple sclerosis (from about 0.04 to about 1.0 mg/kg/day (see U.S. Patent No. 5,506,214)); for other diseases, see also U.S. Patent Nos. 5,106,837 (autohemolytic anemia); 5,310,732 (inflammatory bowel disease); 5,401,724 (rheumatoid arthritis); 5,424,296 (malignant astrocytoma); 5,510,336 (histiocytosis); 5,401,724 (chronic myelogenous leukemia); and 6,239,118 (atherosclerosis).

Further, various dosage amounts and dosing regimens have been reported in the literature for use in the treatment of multiple sclerosis; see, for example: Romine et al., *Proceedings of the Association of American*

Physicians, Vol. 111, No. 1, 35-44 (1999); Selby et al., *The Canadian Journal of Neurological Sciences*, 25, 295-299 (1998); Tortorella et al., *Current Opinion in Investigational Drugs*, 2 (12), 1751-1756 (2001); Rice et al., *Neurology,* 54, 1145-1155 (2000); and Karlsson et al., *British Journal of Haematology*, 116, 538-548 (2002); all of which are incorporated by reference herein in their entireties and relied upon.

Moreover, the route of administration for which the therapeutically effective dosages are taught in the literature should be taken into consideration. While the instant compositions optimize the bioavailability of cladribine following oral administration, it will be appreciated that even optimal bioavailability from oral dosage forms is not expected to approach bioavailability obtain after intravenous administration, particularly at early time points. Thus, it is often appropriate to increase a dosage suggested for intravenous administration to arrive at a suitable dosage for incorporation into a solid oral dosage form. At the present time, it is envisioned that, for the treatment of multiple sclerosis, 10 mg of cladribine in the instant complex cladribine-cyclodextrin complex in the instant solid dosage form would be administered once per day for a period of five to seven days in the first month, repeated for another period of five to seven days in the second month, followed by ten months of no treatment. Alternatively the patient would be treated with 10 mg of cladribine in the instant complex cladribine-cyclodextrin complex in the instant dosage form once per day for a period of five to seven days per month for a total of six months, followed by eighteen months of no treatment. For further dosing information, see also U.S. Provisional Patent Application No. _____ [IVAX0021-P-USA/Attorney Docket No. 033935-011], and U.S. Provisional Patent Application No. _____ [IVAX0022-P-USA/Attorney Docket No. 033935-012], both entitled "Cladribine Regimen for Treating Multiple Sclerosis", both filed on March 25, 2004 and incorporated by reference herein in their entireties.

Furthermore, one of skill will appreciate that the therapeutically effective amount of cladribine administered herein may be lowered or increased by fine tuning and/or by administering cladribine according to the invention with another active ingredient. The invention therefore provides a method to tailor the administration/treatment to the particular exigencies specific to a given mammal. Therapeutically effective amounts may be easily determined, for example, empirically by starting at relatively low amounts and by step-wise increments with concurrent evaluation of beneficial effect.

As noted in the preceding paragraph, administration of cladribine in accord with this invention may be accompanied by administration of one or more additional active ingredients for treating the cladribine-responsive condition. The additional active ingredient will be administered by a route of administration and in dosing amounts and frequencies appropriate for each additional active ingredient and the condition being treated. For example, in the treatment of multiple sclerosis, other useful drugs include interferon beta (Rebif®, Betaseron®/Betaferon®, Avonex®), identical to the naturally occurring protein found in the human body; glatiramer acetate (Copaxone®), a random chain (polymer) of the amino acids glutamic acid, lysine, alanine and tyrosine; natalizumab (Antegren®), a monoclonal antibody; alemtuzumab (Campath-1H®), a humanized anti-CD52 monoclonal antibody; 4-aminopyridine (also known as 4-AP and Fampridine), a drug that blocks the potassium channels in neurons; and amantadine, an anti-viral agent which improves muscle control and reduces muscle stiffness and is used to alleviate the symptoms of fatigue in multiple sclerosis, a purpose for which pemoline (Cylert®) and L-Carnitine (a herbal product) may also be useful. In the treatment of hairy cell leukemia, additional active ingredients may include interferon alpha, pentostatin, fludarabine, rituximab (an anti-CD 20 monoclonal antibody) and the anti-CD22 recombinant immunotoxin BL 22; other additional active ingredients may be appropriate in other types of

leukemias. In the treatment of rheumatoid arthritis, there are many other active ingredients which may be selected. These include NSAIDS (non-steroidal anti-inflammatory drugs), which are of three types: salicylates such as aspirin, traditional NSAIDS such as ibuprofen and indomethacin, and COX-2 inhibitors such as celecoxib (Celebrex®), rofecoxib (Vioxx®), meloxicam (Mobic®), valdecoxib (Bextra®), lumiracoxib (Prexige®) and etoricoxib (Arcoxia®). Other drugs useful in treating rheumatoid arthritis which may be used in conjunction with the present invention include DMARDS, glucocorticoids, biological response modifiers and non-NSAID analgesics. DMARDS are disease-modifying anti-rheumatic drugs which include methotrexate, plaquenil, leflunomide (Arava®), sulfasalazine, gold, penicillamide, cyclosporine, methyl cyclophosamide and azathioprine. Glucocorticoids include dexamethasone, prednisolone, triamcinolone and many others. Biological response modifiers (which restore the disease-fighting ability of the immune system), include etanercept (Enrel®), a tumor-necrosis factor inhibitor, infliximab (Remicade®), which is also an anti-TNF drug, anakinra (Kineret®), a selective IL-1 blocker, and Humira®, a human monoclonal antibody which is another anti-TNF drug. The non-NSAID analgesics include acetaminophen as well as narcotic analgesics such as hydrocodone, oxycodone and propoxyphene. Generally speaking, those drugs which work by a mechanism different from that of cladribine are particularly useful for concomitant therapy with the cladribine composition described herein. Those drugs which are effective by the oral route of administration and which are compatible with the instant cladribine complexes in a single dosage form may be incorporated into the instant dosage forms; otherwise, they should of course be separately administered in amounts, frequencies and via administration routes suitable to them.

As used herein, "treating" means reducing, preventing, hindering the development of, controlling, alleviating and/or reversing the symptoms in the individual to which a compound of the invention has been administered, as

compared to the symptoms of an individual not being treated according to the invention. A practitioner will appreciate that the complexes, compositions, dosage forms and methods described herein are to be used in concomitance with continuous clinical evaluations by a skilled practitioner (physician or veterinarian) to determine subsequent therapy. Such evaluation will aid and inform in evaluating whether to increase, reduce or continue a particular treatment dose, and/or to alter the mode of administration.

The methods of the present invention are intended for use with any subject/patient that may experience the benefits of the methods of the invention. Thus, in accordance with the invention, the terms "subjects" as well as "patients" include humans as well as non-human subjects, particularly domesticated animals.

Any suitable materials and/or methods known to those of skill can be utilized in carrying out the present invention. However, preferred materials and methods are described. Materials, reagents and the like to which reference are made in the following description and examples are obtainable from commercial sources, unless otherwise noted.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein.

EXAMPLES

EXAMPLE 1

PHASE SOLUBILITY STUDY

A phase solubility study can be carried out as follows. Excess cladribine is added to cyclodextrin solutions of various concentrations of

hydroxypropyl-β-cyclodextrin (HPβCD) and allowed to complex as described in EXAMPLE 2 below. The excess, undissolved cladribine is removed by filtration. The amount of cladribine in the complexation solution is measured to obtain a data point. This process is repeated with different known concentrations of HPβCD until several data points are obtained. These data points are then plotted graphically, each data point representing the amount of cladribine that can be dissolved in water with a specific concentration of cyclodextrin. Points on the line generated by the data points represent ratios for the product. One of skill in the art will realize the same results will be generated if excess cyclodextrin is added to cladribine solutions of known concentration.

The molar concentrations of cladribine to cyclodextrin obtained are plotted and presented graphically. A representative phase solubility diagram is shown in the Figure. The plotted lines for cladribine-HPβCD represent cladribine solubilization for the conditions tested, that is, the ratio of the concentration of cladribine to the concentration of cyclodextrin. The area above each of the plotted lines represents conditions where excess insoluble cladribine is present. The area below each of the plotted lines represents the conditions where cyclodextrin is in excess.

The plot for cladribine-HPβCD shown in the Figure is approximately linear; this is indicative of a 1:1 complex, in which one molecule of the drug is complexed with one molecule of cyclodextrin. The Figure also shows that additional cyclodextrin is needed to maintain the cladribine in the complex. For example, about 0.14 mole of HPβCD is needed to maintain about 0.049 mole of cladribine dissolved under the selected conditions, which will ultimately provide the amorphous mixture of the amorphous, preferably saturated, cladribine-HPβCD inclusion complex and amorphous free cladribine (as a non-inclusion complex). Under the conditions of EXAMPLE 2 below, a significant portion of the cladribine in the product can be expected to be not in the inclusion complex but rather in amorphous form loosely held

in intimate admixture therewith by hydrogen bonding as a non-inclusion complex.

EXAMPLE 2

PREPARATION OF CLADRIBINE-CYCLODEXTRIN COMPLEX FOR HUMAN TRIALS

Cladribine is complexed with HPβCD by the following method.

In 825 mL of distilled water, 172.5 g of hydroxypropyl-β-cyclodextrin are dissolved (forming an approximately 17% solution), then cladribine is added and the mixture is stirred at about 45 to about 50°C for about nine hours. Stirring is continued for an additional 6 to 9 hours at room temperature. Any undissolved cladribine is removed by filtration and the solution is cooled to room temperature. To form the amorphous mixture of amorphous cladribine-cyclodextrin complex and amorphous free cladribine, the aqueous cladribine-cyclodextrin solution is dried by lyophilization prior to incorporation into solid oral tablets. The lyophilization procedure comprises a freezing stage of rapidly bringing the complexation solution to about -40°C to about -80°C (e.g., about -45°C) for approximately 2 to 4 hours (preferably about 3 to 4 hours), followed by a primary drying stage at about -25°C for approximately 80-90 hours, typically under low pressure, and a second drying stage at about 30°C for about 15-20 hours.

Product made by the foregoing general procedure can be analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based mobile phase, with UV detection at 264 nm) to find the weight ratio of cladribine to cyclodextrin in the final product. Final product preparations can be further characterized by methods known in the art, including, for example by inspecting appearance, ascertaining the overall impurity content by HPLC, ascertaining the water content using a Karl Fischer titrator, determining the dissolution profile by a standard method, for example using USP<711>Apparatus II equipment and UV detection at 264 nm, inspecting

the content uniformity and performing quantitative assay by HPLC analysis of the active ingredient.

Two batches of cladribine/cyclodextrin product, FD04 and FD05, were made by the foregoing general procedure as follows:

Purified water (825 mL) was pre-heated at 48°C (target range 45°C to 50°C) in a 1-liter glass vessel by immersion in a water bath. The heated water was stirred to achieve a controlled central vortex. 2-hydroxypropyl-β-cyclodextrin (172.50 g) was weighed and slowly added to the heated water over a period of 40 minutes. The resulting solution was stirred for a further 10 minutes to ensure complete dissolution of the cyclodextrin. Cladribine (12.00 g for FD04 and 18.75 g for FD05) was weighed and added to the stirred cyclodextrin solution, which turned cloudy before becoming clear. The resulting clear solution was maintained at 48°C and continually stirred for 9 hours. Stirring continued for a further 7 hours while the solution cooled to room temperature.

Use of a larger amount of cladribine in the preparation of FD05 was part of an attempt to optimize the procedure; however, it was found that the initial amount of cladribine in that case was too great and precipitation was observed at the end of the cooling step for batch FD05. The solution was filtered to remove the precipitate. Analysis of the resultant product revealed (assay value = 87.2%) that 16.35 g of cladribine had been incorporated into the cyclodextrin complex in the case of FD05. No filtration was required for batch FD04, indicating that the amounts used in the preparation of FD04 were more appropriate and that the FD05 procedure could be optimized by beginning with a smaller amount of cladribine (16.35 g rather than 18.75 g), thus avoiding the filtration step.

After cooling to room temperature and, in the case of FD05, filtering, the solutions were filled into 100 mL lyophilization vials (20 mL solutions per vial), the filled vials were partially stoppered and lyophilized. The

lyophilization included freezing at -45°C for about 200 minutes, a primary drying phase at -25°C under a pressure of 100 mTorr for about 5,200 minutes and a secondary drying phase at 30°C for about 1,080 minutes as set forth below:

TABLE I

Step	Process	Temperature	Pressure (mTorr)	Time (min)
1	Load	4°C		
2	Load Hold	4°C	n/a	120
3	Ramp	-45°C	n/a	120
4	Freezing	-45°C	n/a	200
5	Ramp	-25°C	100	120
6	Primary drying	-25°C	100	5200
7	Ramp	30°C	50	240
8	Secondary drying	30°C	50	1080
9	Finish	30°C	Vials closed under vacuum	

The FD04 and FD05 batches of cladribine/cyclodextrin product made by the foregoing procedure were analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based mobile phase with UV detection at 264 nm) and empirically found to have the following characteristics:

TABLE II

Lot No.	Cladribine: HPβCD w/w	Cladribine: HPβCD Weight Ratio
FD04	12.00g:172.50g	1:14.38
FD05	16.35g:172.50g	1:10.55

The products were analyzed by DSC thermograms and X-ray diffraction methods to determine any free crystalline cladribine in the lyophilized material. Importantly, the samples exhibited no transitions in the region of 210°C to 230°C, which is associated with the melting of crystalline cladribine. In both cases, no significant thermal activity was recorded in the range of 210°C to 230°C, suggesting that the complexes obtained at the end of the lyophilization do not have any significant amount of free crystalline cladribine, considering the sensitivity of the analytical method (up to 3% w/w). This conclusion was supported by the absence of peaks for crystalline cladribine from X-ray diffraction traces for both complexes FD04 and FD05.

The products are amorphous mixtures of amorphous cladribine-HPβCD inclusion complex and amorphous free cladribine hydrogen-bonded to the cyclodextrin as a non-inclusion complex. The cladribine:HPβCD weight ratios obtained were about 1:14 and 1:11.

Generally speaking, amorphous mixtures within the scope of the present invention have cladribine:HPβCD weight ratios of from about 1:10 to 1:16.

EXAMPLE 3

PREPARATION OF ORAL TABLETS

Tablets were manufactured using batches of amorphous mixtures FD04 and FD05 described in EXAMPLE 2 for use in a clinical study.

Batch N0120 was manufactured using cladribine-2-HPβCD complex mixture DF05 to a batch size of 3,000 tablets and batch N0126 was manufactured using cladribine-HPβCD complex mixture FD04 to a batch size of 800 tablets. The master formulations for the two batches are shown in TABLE III. Batch N0120 represented 3.0 g tablets and Batch N0126 represented 10 mg tablets for clinical study.

TABLE III

Constituent	Lot Number	mg/tablet 3.0 mg Batch N0120	mg/tablet 10.0 mg Batch N0126
Cladribine-HPβCD complex mix	FD05	30.60*	
Cladribine-HPβCD complex mix	FD04		153.75**
Sorbitol powder NF	1007403	68.4	44.25
Magnesium stearate NF	1006280	1.00	2.00
Total		100.00	200.00

*Equivalent to 3.0 mg cladribine per tablet.
**Equivalent to 10.0 mg cladribine per tablet.

The following table sets forth the method of manufacture of the Batch N0120 and N0126 tablets.

TABLE IV

1.	Pre-mix the magnesium stearate with an approximately equal quantity of sorbitol power.
2.	Pass the cladribine-HPβCD complex and the remainder of the sorbitol powder into a one-liter glass jar via a 40-mesh screen.
3.	Blend the contents for 10 minutes at 12 rpm.
4.	Pass the magnesium stearate/sorbitol powder pre-mix into the glass jar via the 40-mesh screen.
5.	Blend the final mixture for 5 minutes at 12 rpm.
6.	Compress into 3.0 mg and 10.0 mg tablets at a target compression weight of 100 mg and 200 mg, respectively.

Both the Batch N0120 3.0 mg tablets and the Batch N0126 10.0 mg tablets were round, with one side flat-beveled edged and the other side shallow convex. The Batch N0120 3.0 mg tablets had an average weight of 100 mg, a thickness of 2.7 mm, a friability of 0.2%, a hardness of 4 Kp and a disintegration time of 3 minutes. The Batch N0126 10.0 mg tablets had an average weight of 198 mg, a thickness of 4.2 mm, a friability of 1%, a hardness of 2.8 Kp and a disintegration time of 5 minutes 42 seconds.

The Batch N0120 3.0 mg and N0126 10.0 mg tablets were used in the clinical study summarized in EXAMPLE 5 below.

EXAMPLE 4

CLINICAL STUDY: RELATIVE BIOAVAILABILITY

The objective of this study was to assess the relative bioavailability of three oral cladribine formulations: (1) a cyclodextrin-based formulation according to the instant invention (Tablet 1: complex FD05, i.e. Batch No. N0120 tablets described above); (2) a mucoadhesive formulation (Tablet 2: containing 3.0 mg cladribine, 10 mg of Carbopol 71G NF, 22.2 mg of dicalcium phosphate, 64.3 mg of lactose and 0.5 mg of magnesium stearate, Batch No. N0121); and (3) a hard-gel capsule (Capsule containing 3.0 mg cladribine, 5.0 mg Carbopol 974P, 91.3 mg Avicel PH101, 100.0 mg Avicel PH102, 0.2 mg colloidal silicon dioxide and 0.5 mg magnesium stearate, Batch No. RD03030) in comparison with one fixed subcutaneous clardribine administration (reference formulation) in patients with MS (multiple sclerosis).

This study was a 2 center, open-label, randomized, 4-way crossover single dose study using twelve patients with MS. Patients received randomly three different fixed oral doses (3.0 mg) and a fixed subcutaneous dose of 3.0 mg. The four treatment days were separated by a drug-free interval of at

least 5 days. In each treatment period, blood samples were collected over a 24-hour period for evaluation of plasma cladribine.

The plasma concentration of cladribine was measured by a HPLC/MS/MS method. Using this method, the relationship between concentration versus peak area ratio was found to be linear within the range of 100 pg/ml to 50,000 pg/ml for cladribine. The limit of quantification was 100 pg/ml. Analysis of samples was carried out in 16 runs. No calibrator had to be excluded from fitting of the calibration curve and accuracy of each quality control sample met the GLP requirements.

576 clinical plasma samples were analyzed and concentration values of cladribine were determined. The results were compiled and are summarized in the tables below (Tables V and VI). In these tables, the following definitions are applicable: T_{max} is the time to reach maximum concentration in the plasma; $T_{½}$ is the half-life of cladribine in the plasma; C_{MAX} is the maximum concentration of cladribine in the plasma; AUC_{inf} is the area under the curve for the measured data from zero extrapolated to infinity; AUC_t is the area under the curve for the measured data (from zero to the last time point); Geom Mean is the geometric mean; CV is the coefficient of variation (relative standard deviation); LL is the lower limit; UL is the upper limit.

TABLE V
Summary Statistics for Pharmacokinetic Parameters for Cladribine Study
Obtained via Non-Compartmental Analyses. (n=12).

Pharmacokinetic Parameter	3.0 mg subcutaneous			3mg Tablet 1			3mg Tablet 2			3 mg Capsule		
	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)
T_{max}(hr)	N/A	.313 ±.113	36.2	N/A	.521 ±.167	32.1	N/A	1.25 ±.839	67.1	N/A	2.25 ±.622	27.7
$T_{½}$(hr)	N/A	6.69 ±2.01	30.1	N/A	7.55 ±2.50	33.1	N/A	6.73 ±2.82	41.9	N/A	6.27 ±2.31	36.9
C_{max}(pg/ml)	23186	N/A	40.1	6597	N/A	24.7	5041	N/A	52.6	3818	N/A	36.8
AUC_{inf} (hr·pg/ml)	57254	N/A	44.4	24936	N/A	28.8	21676	N/A	42.7	22604	N/A	39.5
AUC_t (hr·pg/ml)	54725	N/A	43.8	23182	N/A	28.0	20063	N/A	42.1	20951	N/A	42.0

**CV=SD/mean for T_{max} and $T_{½}$ and CV% geometric mean for C_{max}, AUC_{inf} and AUC_t.

TABLE VI

Ratios of Oral to Subcutaneous Pharmacokinetic Parameters and Corresponding Two-Sided 90% Confidence Intervals for Cladribine Study (n=12).

Pharmacokinetic Parameter	3 mg Tablet 1		3mg Tablet 2		3mg Capsule	
	Ratio*	LL, UL	Ratio*	LL, UL	Ratio*	LL, UL
AUC_{inf}	43.1	35.7, 52.1	38.4	31.8, 46.4	38.9	32.1, 47.0
AUC_t	41.9	34.6, 50.8	37.2	30.7, 45.0	37.6	31.0, 45.5

*Ratios (dose normalized) and Corresponding 95% LL obtained via inverse transformation of log-transformed data.

EXAMPLE 5

CLINICAL STUDY: DOSE RESPONSE AND ABSOLUTE BIOAVAILABILITY

The objective of this study was to assess the systemic availability of cladribine after oral administration in two different fixed oral doses, in comparison with one fixed intravenous administration (reference formulation) in patients with MS (multiple sclerosis), and to evaluate the safety and tolerability of cladribine in this population.

This study was a 3 center, open-label, randomized, 3-way crossover single dose study using twenty-six patients with MS. Patients received randomly two different fixed oral doses (3.0 mg and 10.0 mg) and a fixed intravenous dose of 3.0 mg (administered as a 1 hour infusion). The three treatment days were separated by a drug-free interval of at least 5 days. In

each treatment period blood samples were collected over a 24-hour period for evaluation of plasma cladribine.

The plasma concentrations of cladribine were measured by a HPLC/MS/MS method. Using this method the relationship between concentrations versus peak area ratios was found to be linear within the range of 100 pg/ml to 50,000 pg/ml for cladribine. The limit of quantification was 100 pg/ml. Analysis of samples was carried out in 16 runs. Except the first run (which had to be rejected because of equipment failure), all other runs could be accepted. No calibrator had to be excluded from fitting of the calibration curve and accuracy of each quality control sample met the GLP requirements.

858 clinical plasma samples were analyzed and concentration values of cladribine were determined. The results were compiled and are summarized in the tables below [TABLES VII through X]. In these tables, the following definitions are applicable: T_{max} is the time to reach maximum concentration in the plasma; $T_{1/2}$ is the half-life of cladribine in the plasma; C_{max} is the maximum concentration of cladribine in the plasma; AUC_{inf} is the area under the curve for the measured data from zero extrapolated to infinity; AUC_t is the area under the curve for the measured data (from zero to the last time point); Geom Mean is the geometric mean; CV is the coefficient of variation (relative standard deviation); LL is the lower limit; UL is the upper limit; σ^2 is the mean variance; σ_B^2 is the mean variance between subjects; σ_W^2 is the mean variance within subjects; CV_T is the total coefficient of variation; and CV_W is the coefficient of variation within subjects.

TABLE VII

Summary Statistics for Pharmacokinetic Parameters for Cladribine Study Obtained via Non-Compartmental Analysis (n=26)

Pharmaco-kinetic Parameter	3.0 mg IV infusion			Oral Administration					
				3.0 mg			10.0 mg		
	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)
T_{max}(hr)	N/A	.817 ±.397	48.6	N/A	.548 ±.300	54.8	N/A	.558 ±.204	36.5
$T_{½}$(hr)	N/A	6.50 ±1.27	19.5	N/A	5.85 ±1.18	20.2	N/A	5.60 ±0.75	13.3
C_{max}(pg/ml)	21425	N/A	27.6	5608	N/A	49.5	21242	N/A	50.5
AUC_{inf} (hr·pg/ml)	58528	N/A	24.0	20159	N/A	35.0	76690	N/A	30.3
AUC_t (hr·pg/ml)	56396	N/A	24.0	19166	N/A	36.9	74532	N/A	30.3

**CV=SD/mean for T_{max} and $T_{½}$ and CV% geometric mean for C_{max}, AUC_{inf} and AUC_t.

TABLE VIII

Ratios of Oral to I.V. Pharmacokinetic Parameters and Corresponding Lower Limit (LL) for the one-sided 95% Confidence Interval for Cladribine Study (n=26)

Pharmacokinetic Parameter	Oral Administration			
	3.0 mg		10.0 mg	
	Ratio*	LL	Ratio*	LL
AUC_{inf}	34.5	31.7	39.1	35.9
AUC_t	34.0	31.2	39.4	36.1

*Ratios (dose normalized) and Corresponding 95% LL obtained via inverse transformation of log-transformed data.

TABLE IX

Ratios and Corresponding two-sided 90% Confidence Intervals for Cladribine Study (n=26)

Pharmacokinetic Parameter	10.0 mg/3.0 mg		
	Ratio*	LL	UL
C_{max}	112.6	95.1	133.3
AUC_{inf}	113.3	104.2	123.3
AUC_t	115.8	106.1	126.5

*Ratios (dose normalized) and Corresponding 90% CI obtained via inverse transformation of log-transformed data.

TABLE X

Variance components for Cladribine Study (n=26)

Source of variation	C_{max}	AUC_{inf}	AUC_t
Between (σ_B^2)	.0380	.0487	.0492
With (σ_W^2)	.1315	.0330	.0357
TOTAL ($\sigma_B^2 + \sigma_W^2$)	.1695	.0816	.0849
CV_T(%)	43.0	29.2	29.8
CV_W(%)	37.5	18.3	19.1

Where PK parameters are dose-adjusted and $CV = \sqrt{\exp(\sigma^2) - 1}$

The foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents thereof may be resorted to, falling within the scope of the invention claimed.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

2. The pharmaceutical composition according to Claim 1, wherein the complex is saturated with cladribine.

3. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

4. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

5. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

6. The composition according to any one of Claims 1 to 3, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

7. The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

8. The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

9. The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

10. The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

11. The composition according to any one of Claims 1 to 10, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

12. The composition according to any one of Claims 1 to 11, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

13. A method for enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

14. The method according to Claim 13, wherein the complex is saturated with cladribine.

15. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

16. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

17. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

18. The method according to any one of Claims 13 to 15, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

19. The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

20. The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

21. The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

22. The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

23. The method according to any one of Claims 13 to 22, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin

corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

24. The method according to any one of Claims 13 to 23, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

25. A method for the treatment of symptoms of a cladribine-responsive condition in a subject suffering from said symptoms comprising orally administering to said subject a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

26. The method according to Claim 25, wherein the complex is saturated with cladribine.

27. The method according to Claim 25 or 26, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

28. The method according to Claim 27, wherein the cladribine-responsive condition is multiple sclerosis.

29. The method according to Claim 25, 26, 27 or 28, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin,

hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

30. The method according to any one of Claims 25 to 29, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

31. The method according to any one of Claims 25 to 30, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

32. The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

33. The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

34. The method according to Claim 25, 26, 27 or 28, wherein the amorphous cyclodextrin is hydropropyl-γ-cyclodextrin.

35. The method according to any one of Claims 25 to 34, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

36. Use of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, in the formulation of a solid oral dosage form, for administration in the treatment of symptoms of a cladribine-responsive condition.

37. Use according to Claim 36, wherein the complex is saturated with cladribine.

38. Use according to Claim 36 or 37, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

39. Use according to Claim 38, wherein the cladribine-responsive condition is multiple sclerosis.

40. Use according to Claim 36, 37, 38 or 39, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

41. Use according to any one of Claims 36 to 40, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

42. Use according to any one of Claims 36 to 41, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

43. Use according to Claim 42, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

44. Use according to Claim 42, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

45. Use according to any one of Claims 36 to 41, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

46. Use according to any one of Claims 36 to 45, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

47. Use of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, in the formulation of a solid oral dosage form, for enhancing the oral bioavailability of cladribine.

48. Use according to Claim 47, wherein the complex is saturated with cladribine.

49. Use according to Claim 47 or 48, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

50. Use according to any one of Claims 47 to 49, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

51. Use according to any one of Claims 47 to 50, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

52. Use according to Claim 51, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

53. Use according to Claim 51, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

54. Use according to any one of Claims 47 to 50, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

55. Use according to any one of Claims 47 to 54, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

56. A complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex.

57. The complex according to Claim 56, saturated with cladribine.

58. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

59. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

60. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

61. The complex according to any one of Claims 56 to 58, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

62. The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

63. The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

64. The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

65. The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

66. The complex according to any one of Claims 56 to 65, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

67. A process for the preparation of a complex cladribine-cyclodextrin complex which comprises the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature; and

(iii) lyophilizing the cooled solution to afford an amorphous product.

68. A process according to Claim 67, further comprising a filtration step following step (ii).

69. A process according to Claim 67 or 68, wherein step (i) is performed at a temperature of from about 45 to about 60°C.

70. A process according to any one of Claims 67 to 69, wherein step (i) is performed at a temperature of from about 45 to about 50°C.

71. A process according to Claim 69 or 70, wherein step (i) is performed with stirring.

72. A process according to Claim 71, wherein step (i) is performed for a period of from about 6 to about 9 hours.

73. A process according to any one of Claims 67 to 72, wherein step (ii) is performed for a period of from about 6 to about 9 hours.

74. A process according to any one of Claims 67 to 73, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80° C, and held at said temperature for a period of from about 2 to about 4 hours.

75. A process according to Claim 74, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

76. A process according to any one of Claims 67 to 75, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

77. A process according to any one of Claims 67 to 75, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

78. A process according to Claim 76 or 77, wherein 825 parts by volume of water are introduced in step (i).

79. A process according to any one of Claims 67 to 78, wherein the lyophilization step (iii) comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

80. A process according to Claim 79, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

81. A process according to Claim 79 or 80, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

82. A pharmaceutical composition obtainable by a process comprising the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature;

(iii) lyophilizing the cooled solution to afford an amorphous product; and

(iv) formulating the amorphous product into a solid oral dosage form.

83. A pharmaceutical composition according to Claim 82, wherein the process further comprises a filtration step following step (i) or (ii).

84. A pharmaceutical composition according to Claim 82 or 83, wherein step (i) of the process is performed at a temperature of from about 45 to about 60°C.

85. A pharmaceutical composition according to any one of Claims 82 to 84, wherein step (i) of the process is performed at a temperature of from about 45 to about 50°C.

86. A pharmaceutical composition according to Claim 84 or 85, wherein step (i) of the process is performed with stirring.

87. A pharmaceutical composition according to Claim 86, wherein step (i) of the process is performed for a period of from about 6 to about 9 hours.

88. A pharmaceutical composition according to any one of Claims 82 to 87, wherein step (ii) of the process is performed for a period of from about 6 to about 9 hours.

89. A pharmaceutical composition according to any one of Claims 82 to 88, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80°C, and held at said temperature for a period of from about 2 to about 4 hours.

90. A pharmaceutical composition according to Claim 89, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

91. A pharmaceutical composition according to any one of Claims 82 to 90, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

92. A pharmaceutical composition according to any one of Claims 82 to 90, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

93. A pharmaceutical composition according to Claim 91 or 92, wherein 825 parts by volume of water are introduced in step (i) of the process.

94. A pharmaceutical composition according to any one of Claims 82 to 93, wherein the lyophilization step (iii) of the process comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

95. A pharmaceutical composition according to Claim 94, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

96. A pharmaceutical composition according to Claim 94 or 95, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

97. A pharmaceutical composition according to any one of Claims 82 to 96, wherein the formulation step (iv) of the process comprises blending the complex with magnesium stearate and compressing into tablets.

98. A pharmaceutical composition according to Claim 97, wherein magnesium stearate is pre-mixed with sorbitol powder before blending with the complex.

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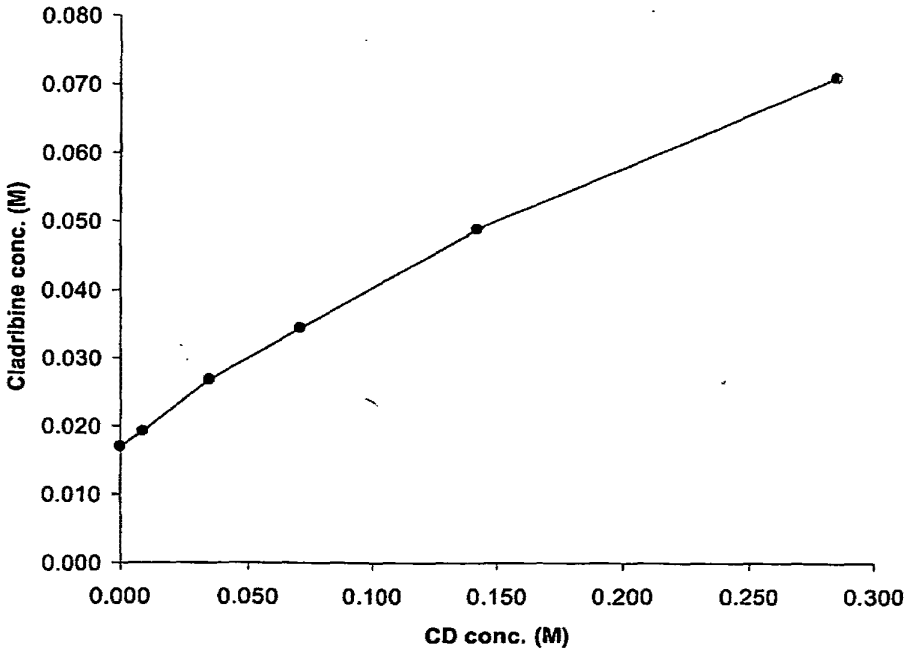
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[Continued on next page]

(54) Title: ORAL FORMULATIONS OF CLADRIBINE



(57) Abstract: ABSTRACT OF THE DISCLOSURE Provided are compositions of cladribine and cyclodextrin which are especially suited for the oral administration of cladribine.

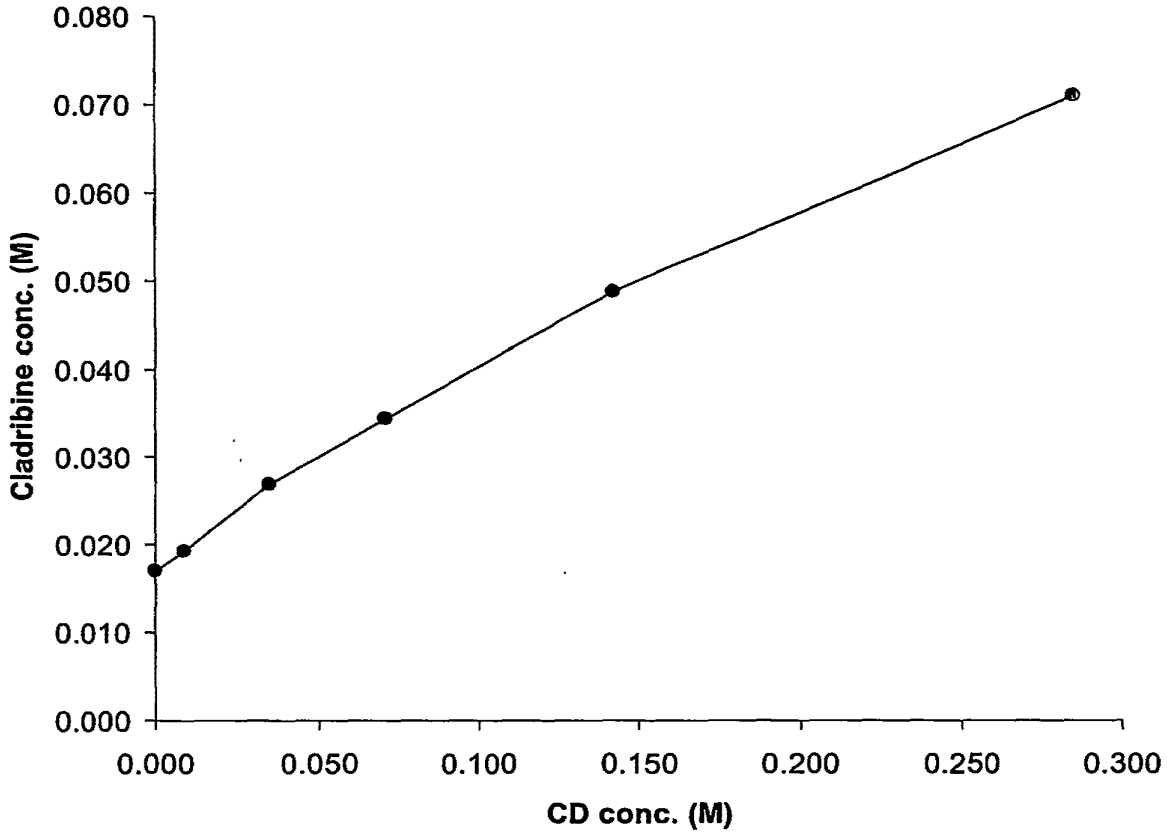
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1/1


0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
Cladribine conc. (M)
0.000
0.050
0.100
0.150
0.200
0.250
0.300
CD conc. (M)

10/551205

FILED UNDER 35 U.S.C. 371

PATENT NUMBER and ISSUE DATE

U.S. **UTILITY** Patent Application

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PATENT AND TRADEMARK OFFICE
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01 FC:1631	300.00	OP
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Nicholas S. BODOR et al.	Group Art Unit:
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Filed:	Confirmation No.:
For: ORAL FORMULATIONS OF CLADRIBINE	
National Phase of Intern'l Appln. No. PCT/US2004/009387 Intern'l Filing Date: March 26, 2004 Earliest Priority Date: March 28, 2003	

FIRST PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Prior to calculation of the claims fees and examination on the merits, please first amend the above-identified patent application as follows:

AMENDMENTS TO THE SPECIFICATION:

Page 1, line 3, please add the following new paragraph:

CROSS-REFERENCE TO EARLIER APPLICATIONS

This application is the U. S. national stage of International Application No. PCT/US2004/009387, filed March 26, 2004, which claims benefit under 35 U.S.C. §119(e) of United States Provisional Application No. 60/458,922, filed March 28, 2003; of United States Provisional Application No. 60/484,756, filed July 2, 2003; and of United States Provisional Application No. 60/541,247, filed February 4, 2004, all of said applications being hereby incorporated by reference herein in their entireties and relied upon.

Please cancel the abstract appearing on the cover of WO2004/087101 A2, the published version of PCT/FR2004/009387, and replace it with the following new Abstract:

ABSTRACT OF THE DISCLOSURE

Provided are compositions of cladribine and cyclodextrin which are especially suited for the oral administration of cladribine.

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (Original) A pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

2. (Original) The pharmaceutical composition according to Claim 1, wherein the complex is saturated with cladribine.

3. (Currently Amended) The composition according to Claim 1 ~~or 2~~, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

4. (Currently Amended) The composition according to Claim 1 ~~or 2~~, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

5. (Currently Amended) The composition according to Claim 1 ~~or 2~~, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

6. (Currently Amended) The composition according ~~to any one of Claims 1 to 3~~ Claim 1, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

7. (Original) The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

8. (Original) The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

9. (Original) The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

10. (Original) The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

11. (Currently Amended) The composition according to ~~any one of Claims 1 to 10~~ Claim 1, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

12. (Currently Amended) The composition according to ~~any one of Claims 1 to 11~~ Claim 1, wherein from about 30 to about 40 percent by weight of the

cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

13. (Original) A method for enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

14. (Original) The method according to Claim 13, wherein the complex is saturated with cladribine.

15. (Currently Amended) The method according to Claim 13 ~~or 14~~, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

16. (Currently Amended) The method according to Claim 13 ~~or 14~~, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

17. (Currently Amended) The method according to Claim 13 ~~or 14~~, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

18. (Currently Amended) The method according to ~~any one of Claims 13 to 15~~ Claim 13, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

19. (Original) The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

20. (Original) The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

21. (Original) The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

22. (Original) The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

23. (Currently Amended) The method according to ~~any one of Claims 13 to 22~~ Claim 13, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

24. (Currently Amended) The method according to ~~any one of Claims 13 to 23~~ Claim 13, wherein from about 30 to about 40 percent by weight of the

cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

25. (Original) A method for the treatment of symptoms of a cladribine-responsive condition in a subject suffering from said symptoms comprising orally administering to said subject a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

26. (Original) The method according to Claim 25, wherein the complex is saturated with cladribine.

27. (Currently Amended) The method according to Claim 25 ~~or 26~~, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

28. (Original) The method according to Claim 27, wherein the cladribine-responsive condition is multiple sclerosis.

29. (Currently Amended) The method according to Claim 25, ~~26, 27 or 28,~~ wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin,

hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

30. (Currently Amended) The method according to ~~any one of Claims 25 to 29~~ Claim 25, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

31. (Currently Amended) The method according to ~~any one of Claims 25 to 30~~ Claim 25, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

32. (Original) The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

33. (Original) The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

34. (Currently Amended) The method according to Claim 25, ~~26, 27 or 28,~~ wherein the amorphous cyclodextrin is hydropropyl-γ-cyclodextrin.

35. (Currently Amended) The method according to ~~any one of Claims 25 to 34~~ Claim 25, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

36.-55. (Cancelled)

56. (Original) A complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex.

57. (Original) The complex according to Claim 56, saturated with cladribine.

58. (Currently Amended) The complex according to Claim 56 ~~or 57~~, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

59. (Currently Amended) The complex according to Claim 56 ~~or 57~~, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

60. (Currently Amended) The complex according to Claim 56 ~~or 57~~, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

61. (Currently Amended) The complex according to ~~any one of Claims 56 to 58~~ Claim 56, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

62. (Original) The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

63. (Original) The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

64. (Original) The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

65. (Original) The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

66. (Currently Amended) The complex according to ~~any one of Claims 56 to 65~~ Claim 56, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

67. (Original) A process for the preparation of a complex cladribine-cyclodextrin complex which comprises the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature; and

(iii) lyophilizing the cooled solution to afford an amorphous product.

68. (Original) A process according to Claim 67, further comprising a filtration step following step (ii).

69. (Currently Amended) A process according to Claim 67 ~~or 68~~, wherein step (i) is performed at a temperature of from about 45 to about 60°C.

70. (Currently Amended) A process according to ~~any one of Claims 67 to 69~~ Claim 67, wherein step (i) is performed at a temperature of from about 45 to about 50°C.

71. (Currently Amended) A process according to Claim 69 ~~or 70~~, wherein step (i) is performed with stirring.

72. (Original) A process according to Claim 71, wherein step (i) is performed for a period of from about 6 to about 9 hours.

73. (Currently Amended) A process according to ~~any one of Claims 67 to 72~~ Claim 67, wherein step (ii) is performed for a period of from about 6 to about 9 hours.

74. (Currently Amended) A process according to ~~any one of Claims 67 to 73~~ Claim 67, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80° C, and held at said temperature for a period of from about 2 to about 4 hours.

75. (Original) A process according to Claim 74, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

76. (Currently Amended) A process according to ~~any one of Claims 67 to 75~~ Claim 67, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

77. (Currently Amended) A process according to ~~any one of Claims 67 to 75~~ Claim 67, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

78. (Currently Amended) A process according to Claim 76 ~~or 77~~, wherein 825 parts by volume of water are introduced in step (i).

79. (Currently Amended) A process according to ~~any one of Claims 67 to 78~~ Claim 67, wherein the lyophilization step (iii) comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

80. (Original) A process according to Claim 79, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

81. (Currently Amended) A process according to Claim 79 ~~or 80~~, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

82. (Original) A pharmaceutical composition obtainable by a process comprising the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature;

(iii) lyophilizing the cooled solution to afford an amorphous product; and

(iv) formulating the amorphous product into a solid oral dosage form.

83. (Original) A pharmaceutical composition according to Claim 82, wherein the process further comprises a filtration step following step (i) or (ii).

84. (Currently Amended) A pharmaceutical composition according to Claim 82 ~~or 83~~, wherein step (i) of the process is performed at a temperature of from about 45 to about 60°C.

85. (Currently Amended) A pharmaceutical composition according to ~~any one of Claims 82 to 84~~ Claim 82, wherein step (i) of the process is performed at a temperature of from about 45 to about 50°C.

86. (Currently Amended) A pharmaceutical composition according to Claim 84 ~~or 85~~, wherein step (i) of the process is performed with stirring.

87. (Original) A pharmaceutical composition according to Claim 86, wherein step (i) of the process is performed for a period of from about 6 to about 9 hours.

88. (Currently Amended) A pharmaceutical composition according to ~~any one of Claims 82 to 87~~ Claim 82, wherein step (ii) of the process is performed for a period of from about 6 to about 9 hours.

89. (Currently Amended) A pharmaceutical composition according to ~~any one of Claims 82 to 88~~ Claim 82, wherein step (iii) comprises an initial freezing

stage in which the solution is cooled to from about -40 to about -80°C, and held at said temperature for a period of from about 2 to about 4 hours.

90. (Original) A pharmaceutical composition according to Claim 89, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

91. (Currently Amended) A pharmaceutical composition according to ~~any one of Claims 82 to 90~~ Claim 82, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

92. (Currently Amended) A pharmaceutical composition according to ~~any one of Claims 82 to 90~~ Claim 82, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

93. (Currently Amended) A pharmaceutical composition according to Claim 91 ~~or 92~~, wherein 825 parts by volume of water are introduced in step (i) of the process.

94. (Currently Amended) A pharmaceutical composition according to ~~any one of Claims 82 to 93~~ Claim 82, wherein the lyophilization step (iii) of the process comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

95. (Original) A pharmaceutical composition according to Claim 94, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

96. (Currently Amended) A pharmaceutical composition according to Claim 94 ~~or 95~~, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

97. (Currently Amended) A pharmaceutical composition according to ~~any one of Claims 82 to 96~~ Claim 82, wherein the formulation step (iv) of the process comprises blending the complex with magnesium stearate and compressing into tablets.

98. (Original) A pharmaceutical composition according to Claim 97, wherein magnesium stearate is pre-mixed with sorbitol powder before blending with the complex.

REMARKS

The foregoing amendment to the specification adds an appropriate cross-reference to applicants' earlier U. S. provisional applications. The amendments to the claims delete multiple dependencies, many of which were improper according to U. S. practice, and also deletes European-style use claims. Method of treatment claims corresponding to the cancelled use claims are already in the application. Applicants reserve the right to submit additional claims drawn to combinations of features covered by the original multiple dependent claims but not specifically claimed in the claims as amended.

No new matter has been added.

Respectfully submitted,

BUCHANAN INGERSOLL PC

Date: September 28, 2005

By: Mary Katherine Baumeister
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10/551205
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APPLICATION DATA SHEET

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Number of Copies of CDs::	
Sequence Submission?::	
Computer Readable Form (CRF)?::	
Number of Copies of CRF::	
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Applicant Information

Applicant Authority Type:: Inventor

Primary Citizenship Country:: United States

Status:: Full Capacity

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Representative Customer Number:: 21839

Domestic Priority Information

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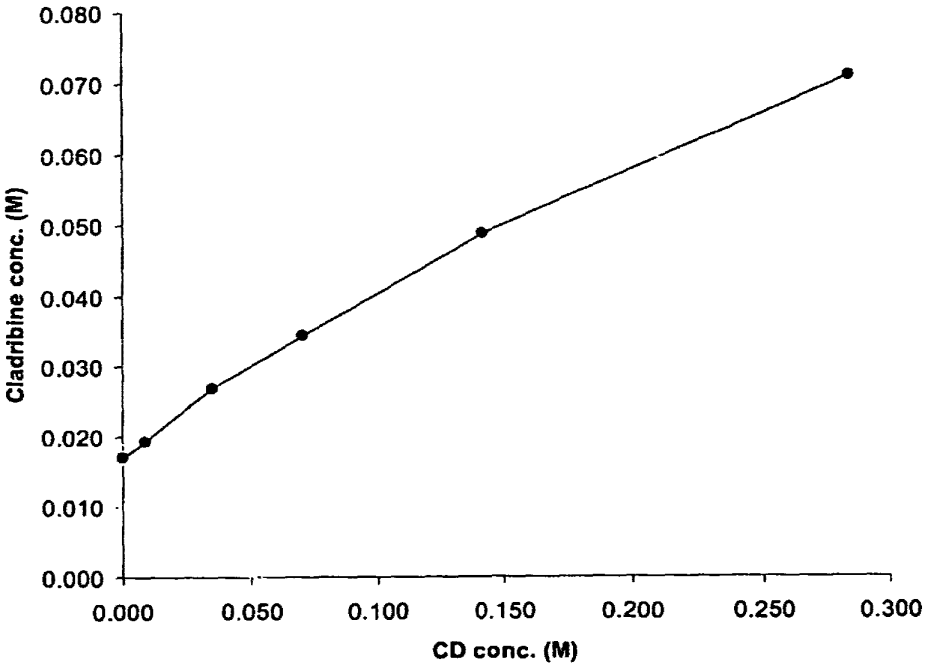
(74) Agents: **STEPNO, Norman, H.** et al.; BURNS, DOANE, SWECKER & MATHIS, LLP, PO BOX 1404, Alexandria, VA 22313-01404 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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[Continued on next page]

(54) Title: ORAL FORMULATIONS OF CLADRIBINE



(57) Abstract: ABSTRACT OF THE DISCLOSURE Provided are compositions of cladribine and cyclodextrin which are especially suited for the oral administration of cladribine.

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International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/20 A61K47/48 A61K31/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 194 395 B1 (NAEFF RAINER ET AL) 27 February 2001 (2001-02-27) cited in the application the whole document	1-98
A	US 4 727 064 A (PITHA JOSEF) 23 February 1988 (1988-02-23) the whole document	1-98
A	US 4 870 060 A (MUELLER BERND W W) 26 September 1989 (1989-09-26) the whole document	1-98

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
2 December 2004	10/12/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Toulacis, C

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/009387

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 13-35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/009387

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 6194395	B1	27-02-2001	NONE	
US 4727064	A	23-02-1988	US 4596795 A	24-06-1986
US 4870060	A	26-09-1989	AT 124055 T	15-07-1995
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			ZA 8601930 A	28-10-1987

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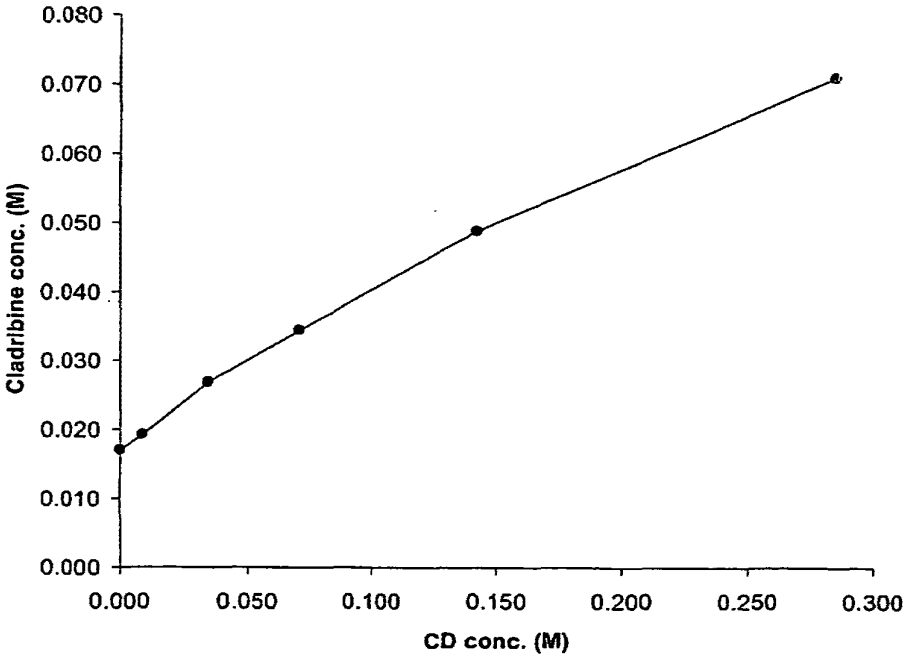
(74) Agents: STEPNO, Norman, H. et al.; BURNS, DOANE, SWECKER & MATHIS, LLP, PO BOX 1404, Alexandria, VA 22313-01404 (US).

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(57) Abstract: ABSTRACT OF THE DISCLOSURE Provided are compositions of cladribine and cyclodextrin which are especially suited for the oral administration of cladribine.

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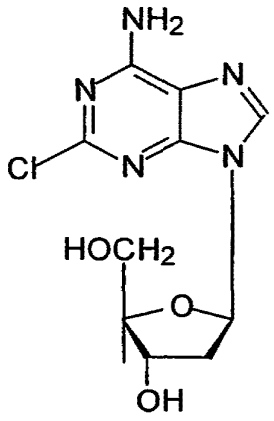
ORAL FORMULATIONS OF CLADRIBINE

FIELD OF THE INVENTION

The invention relates to a composition comprising a complex cladribine-cyclodextrin complex formulated into a solid oral dosage form and to a method for enhancing the oral bioavailability of cladribine.

BACKGROUND OF THE INVENTION

Cladribine, which is an acid-labile drug, has the chemical structure as set forth below:



It is also known as 2-chloro-2′-deoxyadenosine or 2-CdA. Cladribine exists as a white, nonhydroscopic, crystalline powder, consisting of individual crystals and of crystalline aggregates.

Cladribine is an antimetabolite which has use in the treatment of lymphoproliferative disorders. It has been used to treat experimental leukemias such as L1210 and clinically for hairy cell leukemia and chronic lymphocytic leukemia as well as Waldenstrom's macroglobulinaemia. It has

also been used as an immunosuppressive agent and as a modality for the treatment of a variety of autoimmune conditions including rheumatoid arthritis, inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis) and multiple sclerosis (see *e.g.*, J. Liliemark, *Clin. Parmacokinet*, 32(2): 120-131, 1997). It has also been investigated, either experimentally or clinically in, for example, lymphomas, Langerhan's cell histiocytosis, lupus erythematosus, chronic plaque psoriasis, Sezary syndrome, Bing-Neel syndrome, recurrent glioma, and solid tumors.

Oral delivery of drugs is often preferred to parenteral delivery for a variety of reasons, foremost patient compliance, or for cost or therapeutic considerations. Patient compliance is enhanced insofar as oral dosage forms alleviate repeated health care provider visits, or the discomfort of injections or prolonged infusion times associated with some active drugs. At a time of escalating health care costs, the reduced costs associated with oral administration versus parenteral administration costs gain importance. The cost of parenteral administration is much higher due to the requirement that a health care professional administer the cladribine in the health care provider setting, which also includes all attendant costs associated with such administration. Furthermore, in certain instances, therapeutic considerations such as the need for a slow release of cladribine over a prolonged period of time may be practically met only by oral or transmucosal delivery.

However, to date the oral delivery of cladribine has been plagued by low bioavailability (see, *e.g.*, J. Liliemark *et al., J. Clin. Oncol.*, 10(10): 1514-1518, 1992), and suboptimal interpatient variation (see, *e.g.*, J. Liliemark, *Clin. Pharmacokinet*, 32 (2): 120-131, 1997). See also, A. Tarasuik, *et al.* reporting poor absorption and pH dependent lability (*Arch. Immunol. et Therapiae Exper.*, 42: 13-15, 1994).

Cyclodextrins are cyclic oligosaccharides composed of cyclic α-(1→4) linked D-glucopyranose units. Cyclodextrins with six to eight units have

been named α-, β- and γ-cyclodextrin, respectively. The number of units determines the size of the cone-shaped cavity which characterizes cyclodextrins and into which drugs may be included to form stable complexes. A number of derivatives of α-, β- and γ-cyclodextrin are known in which one or more hydroxyl groups is/are replaced with ether groups or other radicals. These compounds are thus known complexing agents and have been previously used in the pharmaceutical field to form inclusion complexes with water-insoluble drugs and to thus solubilize them in aqueous media.

Recently, Schultz *et al.*, in U.S. Patent No. 6,194,395 B1, have described complexing and solubilizing cladribine with cyclodextrin. The Schultz *et al.* patent primarily addresses the problems inherent in previously described aqueous formulations of cladribine, particularly for subcutaneous and intramuscular injection. Schultz *et al.* have found that cladribine is not only significantly more soluble in aqueous media when formulated with cyclodextrin, but also is more stable against acid-catalyzed hydrolysis when combined with cyclodextrin. The latter finding is taught to be of particular benefit in the formulation of solid oral dosage forms, where the compound would normally undergo hydrolysis in the acid pH of the stomach contents. Schultz *et al.* do not appear to have described any actual work in connection with solid oral dosage forms. In fact, they describe only one method of preparing the solid dosage form, which is a melt extrusion process, in which the cladribine and cyclodextrin are mixed with other optional additives and then heated until melting occurs. Furthermore, the broad dosage ranges of 1 mg to 15 mg of cladribine and 100 mg to 500 mg of cyclodextrin listed in the patent suggest no criticality to the particular amount of cyclodextrin to be present with a given amount of cladribine in a solid oral dosage form. Indeed, these dosage ranges include many combinations which may be suitable as mixtures but not for complex formation. For example, a ratio of 1 mg of cladribine to 500 mg of cyclodextrin contains too much cyclodextrin, so

that the drug would not readily leave the complex and achieve its therapeutic function. On the other hand, 15 mg of cladribine and only 100 mg of cyclodextrin would not be enough to complex that amount of cladribine.

The Schultz *et al.* patent does suggest improving the stability of cladribine in oral dosage forms by combining/complexing it with cyclodextrin, but does not suggest improving the drug's oral bioavailability by such means; in fact, the patent does not describe or suggest a method for enhancing or maximizing the bioavailability of cladribine from a solid oral dosage form of cladribine and cyclodextrin, or a composition specially designed to do so.

Many workers have studied the solubility of specific drugs in water containing various concentrations of selected cyclodextrins in order to demonstrate that increasing concentrations of cyclodextrins increase the solubility of the drugs at selected temperatures and pH levels, as for example reported in the Schultz *et al.* patent. Phase solubility studies have also been performed by various workers in order to elucidate the nature of the complex formation, for example, whether the cyclodextrin and drug form a 1:1 complex or a 1:2 complex; see, for example, Harada *et al.* U.S. Patent No. 4,497,803, relating to inclusion complexes of lankacidin-group antibiotics with cyclodextrin, and Shinoda *et al.* U.S. Patent No. 4,478,995, relating to a complex of an acid addition salt of (2'-benzyloxycarbonyl)phenyl trans-4-guanidinomethylcyclohexanecarboxylate with a cyclodextrin.

While Schultz *et al.* teach that a cladribine-cyclodextrin complex improves the water solubility and acid stability of cladribine, the art does not suggest how to maximize or enhance the benefits of the complexation in terms of bioavailability and interpatient variation when the complex is to be administered in a solid oral dosage form.

SUMMARY OF THE INVENTION

It has now been found that amorphous cyclodextrins can be combined with cladribine to form a particularly advantageous product which can be incorporated into a solid oral dosage form. This product is a complex cladribine-cyclodextrin complex, and the solid oral dosage form containing it improves oral bioavailability and/or achieves lower interpatient and/or intrapatient variation of the drug.

The present invention provides a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, and a pharmaceutical composition comprising said complex, formulated into a solid oral dosage form. Thus, the cyclodextrin itself is amorphous, the inclusion complex with cladribine is amorphous (and is preferably saturated with cladribine) and the free cladribine which forms the non-inclusion complex is amorphous.

The invention also provides a method for increasing or enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof, a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form which maximizes the amount of cladribine in the inclusion and non-inclusion complexes.

The invention further provides for treatment of conditions responsive to administration of cladribine in mammals by administering thereto the composition of the invention. Use of cladribine in the preparation of the pharmaceutical compositions of the invention for administration to treat

cladribine-responsive conditions and for enhancing the oral bioavailability of cladribine is also provided.

Still further, the invention provides a process for the preparation of a complex cladribine-cyclodextrin complex which comprises the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature; and

(iii) lyophilizing the cooled solution to afford an amorphous product.

In yet a further aspect the invention provides a pharmaceutical composition obtainable by a process comprising the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature;

(iii) lyophilizing the cooled solution to afford an amorphous product; and

(iv) formulating the amorphous product into a solid oral dosage form.

BRIEF DESCRIPTION OF THE DRAWING

A more complete appreciation of the invention and its many attendant advantages will be readily understood by reference to the following detailed description and the accompanying drawing, wherein the sole Figure is a graphical representation of the results of a phase solubility study where various molar concentrations of hydroxypropyl-β-cyclodextrin (HPβCD) are plotted against various cladribine molar concentrations, with (●) representing

the data points obtained for complexation under conditions specified in EXAMPLE 2 below.

DETAILED DESCRIPTION OF THE INVENTION

Throughout the instant specification and claims, the following definitions and general statements are applicable.

The patents, published applications, and scientific literature referred to herein establish the knowledge of those with skill in the art and are hereby incorporated by reference in their entirety to the same extent as if each was specifically and individually indicated to be incorporated by reference. Any conflict between any reference cited herein and the specific teachings of this specification shall be resolved in favor of the latter. Likewise, any conflict between an art-understood definition of a word or phrase and a definition of the word or phrase as specifically taught in this specification shall be resolved in favor of the latter.

The term "inclusion complex" as used herein refers to a complex of cladribine with the selected cyclodextrin wherein the hydrophobic portion of the cladribine molecule (the nitrogen-containing ring system) is inserted into the hydrophobic cavity of the cyclodextrin molecule. This is often referred to simply as a cyclodextrin complex of the drug.

The term "non-inclusion complex" refers to a complex which is not an inclusion complex; rather than the hydrophobic portion of cladribine being inserted in the cyclodextrin cavity, the non-inclusion complex is formed primarily by hydrogen-bonding of the hydroxyls and amino group on "free" cladribine, (*i.e.* cladribine not in the inclusion complex) to the hydroxyls on the exterior of the cyclodextrin torus (*e.g.* in the case of hydroxypropyl-β-cyclodextrin, hydroxypropyl and hydroxyl groups on the glucose rings). This is a more loosely-held association than an inclusion complex.

As used herein, whether in a transitional phrase or in the body of a claim, the terms "comprise(s)" and "comprising" are to be interpreted as having an open-ended meaning. That is, the terms are to be interpreted synonymously with the phrases "having at least" or "including at least". When used in the context of a process, the term "comprising" means that the process includes at least the recited steps, but may include additional steps. When used in the context of a composition, the term "comprising" means that the composition includes at least the recited features or components, but may also include additional features or components.

The terms "consists essentially of" or "consisting essentially of" have a partially closed meaning, that is, they do not permit inclusion of steps or features or components which would substantially change the essential characteristics of a process or composition; for example, steps or features or components which would significantly interfere with the desired properties of the compositions described herein, *i.e.*, the process or composition is limited to the specified steps or materials and those which do not materially affect the basic and novel characteristics of the invention. The basic and novel features herein are the provision of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form, so as to provide improved bioavailability and/or lower interpatient and/or intrapatient variation following administration. Essential to the invention is the combination of the amorphous nature of the starting cyclodextrin, and the level of water solubility exhibited by cladribine (about 5 mg/ml at room temperature), and consequently its capability for hydrogen bonding, which can be taken advantage of under particular conditions described hereinafter, and which afford a special amorphous mixture uniquely well-suited for optimizing the oral bioavailability of cladribine.

The terms "consists of" and "consists" are closed terminology and allow only for the inclusion of the recited steps or features or components.

As used herein, the singular forms "a," "an" and "the" specifically also encompass the plural forms of the terms to which they refer, unless the content clearly dictates otherwise.

The term "about" is used herein to means approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" or "approximately" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

The term "amorphous" is used herein to refer to a noncrystalline solid. The cyclodextrins encompassed herein themselves are amorphous because they are each composed of a multitude of individual isomers, and their complexes with cladribine are also amorphous. Further, conditions for complexation can be selected (elevated temperature and prolonged complexation times, as described hereinafter) so that a supersaturated cladribine solution will be formed. When cooled, because of the amorphous nature of the complex and the cyclodextrin, some excess free cladribine does not precipitate but rather is trapped in amorphous form in intimate admixture with the (preferably saturated) amorphous cladribine-cyclodextrin inclusion complex. This excess cladribine forms a loosely-held association, or non-inclusion complex, with the cyclodextrin through hydrogen bonding. This, then, further increases the amount of cladribine in the product; this additional cladribine, because it is amorphous and also because it is in intimate admixture with the amorphous inclusion complex, is expected to be somewhat protected from degradation by stomach acid (although it may not be as protected as the cladribine which is in the form of the inclusion complex).

The term "saturated" when used in conjunction with a complex of cladribine in amorphous cyclodextrin means that the complex is saturated with cladribine, that is, the complex contains the maximum amount of cladribine which can be complexed (by means of both inclusion and non-inclusion complexes) with a given amount of cyclodextrin under the conditions of complexation used. A phase solubility study can be used to provide this information, as described in more detail hereinafter. (Conditions for the complexation are also described in more detail below.) Alternatively, a saturated complex may be arrived at empirically by simply adding cladribine to an aqueous solution of the selected cyclodextrin until no more cladribine goes into solution; ultimately, excess cladribine, if any, is removed (by filtration or centrifugation) and the solution lyophilized to provide the dry saturated complex.

The expression "substantially', as in "substantially free" means within 20% of the exact calculated amount, preferably within 10%, most preferably within 5%.

The term "interpatient variability" refers to variation among patients to which a drug is administered. The term "intrapatient variability" refers to variation experienced by a single patient when dosed at different times.

As used herein, the recitation of a numerical range for a variable is intended to convey that the invention may be practiced with the variable equal to any of the values within that range. Thus, for a variable which is inherently discrete, the variable can be equal to any integer value of the numerical range, including the end-points of the range. Similarly, for a variable which is inherently continuous, the variable can be equal to any real value of the numerical range, including the end-points of the range. As an example, a variable which is described as having values between 0 and 2, can be 0, 1 or 2 for variables which are inherently discrete, and can be 0.0,

0.1, 0.01, 0.001, or any other real value for variables which are inherently continuous.

In the specification and claims, the singular forms include plural referents unless the context clearly dictates otherwise. As used herein, unless specifically indicated otherwise, the word "or" is used in the "inclusive" sense of "and/or" and not the "exclusive" sense of "either/or."

Technical and scientific terms used herein have the meaning commonly understood by one of skill in the art to which the present invention pertains, unless otherwise defined. Reference is made herein to various methodologies and materials known to those of skill in the art. Standard reference works setting forth the general principles of pharmacology include Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 10th Ed., McGraw Hill Companies Inc., New York (2001).

Reference is made hereinafter in detail to specific embodiments of the invention. While the invention will be described in conjunction with these specific embodiments, it will be understood that it is not intended to limit the invention to such specific embodiments. On the contrary, it is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims. In the following description, numerous specific details are set forth in order to provided a thorough understanding of the present invention. The present invention may be practiced without some or all of these specific details. In other instances, well-known process operations have not been described in detail, in order not to unnecessarily obscure the present invention.

There is provided by the present invention compositions, as well as methods of making and of using pharmaceutical compositions, useful to achieve desirable pharmacokinetic properties. Such compositions stem from the discovery that solutions of cyclodextrin and cladribine in which cladribine is in a high thermodynamic state, when presented to the gastric mucosa

through which they are absorbed are associated with improved cladribine absorption, as reflected by higher bioavailability and/or lower interpatient variation.

It is postulated, without wishing to so limit the invention, that upon dissolution (*e.g.*, by contact with a fluid, such as a bodily fluid), dry compositions according to the invention form a locally saturated cladribine solution in which cladribine is in the state of highest thermodynamic activity (HTA), thus favoring absorption. Cladribine has a fairly low, although not insignificant, intrinsic aqueous solubility; it is in fact somewhat water soluble. The free cladribine formed from dissociation of the inclusion and non-inclusion complexes in a saturated aqueous solution seeks a more stable activity level by being absorbed through the gastric mucosa.

In view of the foregoing, it is apparent that to produce optimal pharmaceutical compositions, in a solid oral dosage form, these dosage forms should be formulated to release a localized saturated cladribine solution, upon contact of the solid dosage forms with body fluid at the mucosa, in which cladribine is in its HTA state. To provide such a localized saturated solution *in vivo*, it is important to first identify the optimal ratio of cladribine to amorphous cyclodextrin, which ratio is referred to herein as the HTA ratio, to be used in the solid dosage form.

The HTA ratio is empirically determined and is identified as the ratio of cladribine to amorphous cyclodextrin which corresponds to the maximum amount of cladribine that can be complexed with a given amount of the cyclodextrin. The HTA ratio may be determined using an empirical method such as a phase solubility study to determine the saturation concentration of cladribine that can be solubilized with different concentrations of amorphous cyclodextrin solutions. Hence, the method identifies the concentrations at which a saturated cladribine-cyclodextrin complex is formed. It is noted that the molar ratio represented by a point on the phase solubility graph shows

how many moles of amorphous cyclodextrin are the minimum needed to maintain the drug in the complex, under given conditions; this may then be converted to a weight ratio. For example, if a phase solubility diagram shows that 9 moles of a given cyclodextrin are needed to maintain the cladribine in a saturated complex, then multiplying the number of moles of cladribine by its molecular weight and multiplying the number of moles of the selected cyclodextrin by its molecular weight, one can arrive at the ratio of the products as an appropriate optimized weight ratio. A phase solubility study also provides information about the nature of the cladribine-cyclodextrin inclusion complex formed, for example whether the inclusion complex is a 1:1 complex (1 molecule of drug complexed with 1 molecule of cyclodextrin) or a 1:2 complex (1 molecule of drug complexed with 2 molecules of cyclodextrin).

In accordance with the present invention, one can start using either the selected amorphous cyclodextrin, such as hydroxypropyl-β-cyclodextrin (HPβCD) or hydroxypropyl-γ-cyclodextrin, or cladribine as the fixed variable to which an excess of the other is added to identify various solubility data points (indicating saturated cladribine-cyclodextrin complexes) and draw the resultant line. Typically, cladribine is added to an aqueous solution having a known concentration of amorphous cyclextrin under conditions empirically found to promote complex formation. Generally, the complexation is conducted with heating, for example at about 45 to about 60°C for a significant period of time, *e.g.*, at least 6-9 hours; it is believed that even better results can be obtained by heating at up to about 80°C for up to 24 hours. Excess precipitated cladribine is then removed and the cladribine concentration is subsequently measured. This concentration represents the amount of cladribine solubilized for a given amorphous cyclodextrin concentration. This process is repeated for a different known concentration of cyclodextrin until several data points are obtained. Each data point represents the concentration of the cladribine dissolved in a known

concentration of the selected amorphous cyclodextrin. The data points are then plotted to show the concentration of cladribine against the various cyclodextrin concentrations used. The graph is a phase solubility diagram which can be used to determine the amount of cladribine for any specific concentration of cyclodextrin used to form the solution under a given set of complexation conditions. It will be appreciated that the aqueous solubility of cladribine is about 5 mg/ml at room temperature and would be higher at elevated temperature. Consequently, the data points correspond to the amount of cladribine dissolved in aqueous HPβCD or other amorphous cyclodextrin under the selected conditions; when later lyophilized, the solution yields a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex. If equilibrium conditions are reached during the complexation, the amorphous cladribine-cyclodextrin complex will be saturated with cladribine.

One of skill in the art will appreciate that concentrations at which saturated complexes of cladribine with amorphous cyclodextrins are formed (and thus HTA ratios as well) may be identified by a variety of alternative methodologies. Accordingly, any method known in the field suitable to identify these concentrations is within the scope of the invention.

It has been discovered that desirable pharmacological properties (improved bioavailability and/or coefficient of variation as compared to traditional approaches) are associated with mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin.

Using intrinsically amorphous cyclodextrins, for example hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated cyclodextrins, and the like, with cladribine, which is a somewhat water soluble compound (capable of H-bonding through its free hydroxyl and

amino groups), the cladribine provides increased solubility in solutions of these cyclodextrins. Not only is there increased water solubility but also H-bonded association of the cladribine with the cyclodextrin, separately from the actual inclusion complexed material.

One of skill in the art will appreciate that the phase solubility diagram for each given starting concentration ratio represents the starting point of one's investigation on the basis of which variables (reactants' concentrations, temperature and time) may be altered to promote inclusion complex and non-inclusion complex associations favoring a higher or lower proportion of either type of association in the final product. Departure from the ratio of cladribine to cyclodextrin, the temperature and/or the dilution empirically found to promote equilibrium towards complex formation is then analyzed to promote the formation of mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin in various proportions according to the invention.

Thus, for example, by starting with more diluted cyclodextrin (*i.e.*, larger water volumes than that used for solubility plot analysis) logically will accommodate more cladribine in solution sequestering more of the same from complex formation. Upon evaporation, some of the solubilized cladribine will tend to associate with cyclodextrin in a non-inclusion complex fashion. By altering the initial dilution, one may shift equilibrium towards inclusion complex or non-inclusion complex formation. Similarly, by increasing complexation temperature, the water solubility of cladribine may be increased while decreasing the stability of inclusion complexes, thus promoting non-inclusion complexes. Thus, by altering complexation temperature, one may shift equilibrium towards inclusion complex or non-inclusion complex formation. Finally, complexation time may be altered to favor the formation of mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin according to the invention.

As exemplified hereinafter, it is possible to maximize the cladribine in solid amorphous mixtures, by forcing additional cladribine into solution (using more dilute solutions of cyclodextrin, higher temperatures and longer complexation times, as indicated above). When the solution is cooled off, the extensively amorphous nature of these cyclodextrins does not allow crystallization of an excess amount of cladribine beyond that which forms an inclusion complex with the cyclodextrin; and upon freeze-drying/lyophilization, one obtains an amorphous mixture of cladribine-cyclodextrin inclusion complex (which is amorphous) and amorphous free cladribine, loosely associated with uncomplexed cyclodextrin (and even with complexed cyclodextrin) by hydrogen-bonding, that is, the non-inclusion complex.

As shown in the EXAMPLES, this may be done by maximizing solubilization by elevating the temperature (for example, to about 50°to 80°C), and stirring for many hours (up to 24 hours) before freeze-drying. The weight/weight ratios obtained were about 1:14 and 1:11. The apparent optimum weight/weight ratio under these exemplified conditions is the higher of these, or about 1:14 of cladribine: cyclodextrin. If too much excess caldribine is added to the complexation medium, then crystallization of some of the cladribine takes place, which would in turn result in some crystalline cladribine in the product; this undesired excess cladribine is not in solution and is not H-bonded to the amorphous cyclodextrin and lowers the weight ratio. Therefore, it is desirable to carefully control the amount of excess cladribine beyond that which will form the inclusion complex to only the amount which will dissolve in the solution. The desired amorphous mixture of amorphous inclusion complex and amorphous free cladribine can be termed a "complex cladribine-cyclodextrin complex," which includes both inclusion and non-inclusion/H-bonded complexes. The inclusion complex is a complex of cladribine inserted into the hydrophobic cavity of the selected amorphous cyclodextrin, while the non-inclusion/H-bonded complex is

amorphous free cladribine loosely hydrogen-bonded to the cyclodextrin. It is estimated that about two-thirds (60 to 70%) of the cladribine will be in the non-inclusion complex, with the remaining one third (30 to 40%) being in the inclusion complex when the product is obtained as exemplified hereinbelow (17% HPβCD solution, 45 to 50°C complexation temperature for about 9 hours); by increasing the percentage of cyclodextrin used and/or manipulating the temperature, products can be readily obtained in which a much greater proportion of the amorphous mixture is in the form of the inclusion complex. In the case of a representative amorphous cyclodextrin, hydroxypropyl-β-cyclodextrin (HPβCD) a cladribine:cyclodextrin weight ratio of from about 1:10 to about 1:16 is appropriate for the exemplified conditions; the ratio is expected to be the same for hydroxypropyl-γ-cyclodextrin under those conditions. The material obtained is characterized by rapid dissolution of the cladribine in aqueous media.

Freeze-drying, also known as lyophilization, comprises three basic stages: first a freezing stage, then a primary drying stage and finally a secondary drying stage. EXAMPLE 2 below provides details of lyophilization as conducted on the batches described therein. This procedure can be further optimized by following the principles described by Xiaolin (Charlie) Tang and Michael J. Pikal in *Pharmaceutical Research*, Vol. 21, No. 2, February 2004, 191-200, incorporated by reference herein in its entirety and relied upon.

The above-described method requires amorphous cyclodextrins rather than originally crystalline cyclodextrins which have relatively low water solubilities, such as α-, β- or γ-cyclodextrin, 2,6-dimethyl-β-cyclodextrin and the like, because these cyclodextrins would allow crystallization of cladribine in excess of that forming an inclusion complex and therefore would not afford the desired amorphous mixture. The method also would not be useful if cladribine were highly hydrophobic/lipophilic, because in such a situation the drug would not have intrinsic aqueous solubility/H-bonding capability and

could not provide the unique mixture obtained herein. However, in point of fact, cladribine has an aqueous solubility of 5 mg/ml at room temperature, thus a significant amount of the drug will be simply soluble in the water phase especially at higher than room temperature; also, as in the case of HPβCD, for example, some of the cladribine will be associated by hydrogen-bonding to the 2-hydroxypropyl and free glucose-OH groups in the cyclodextrin via the two hydroxy functions found in the deoxyadenosine moiety of the cladribine.

The cyclodextrins within the scope of this invention are amorphous derivatives of the natural cyclodextrins α-, β- or γ-cyclodextrin wherein one or more of the hydroxy groups are substituted, for example, by alkyl, hydroxyalkyl, carboxyalkyl, alkylcarbonyl, carboxyalkoxyalkyl, alkylcarbonyloxyalkyl, alkoxycarbonylalkyl or hydroxy-(mono or polyalkoxy)alkyl groups; and wherein each alkyl or alkylene moiety preferably contains up to six carbons. Although commonly referred to as a single entity, an amorphous cyclodextrin is actually a mixture of many different entities, since the substituent groups can be located on various hydroxyls of the basic cyclodextrin structure. This in turn results in the amorphous nature of these cyclodextrins, which is indeed well-known. Moreover, these cyclodextrins can be obtained in varying degrees of substitution, for example from 1 to 14, preferably from 4 to 7; the degree of substitution is the approximate average number of substituent groups on the cyclodextrin molecule, for example, the approximate number of hydroxypropyl groups in the case of the hydroxpropyl-β-cyclodextrin molecule, and all such variations are within the ambit of this invention. Substituted amorphous cyclodextrins which can be used in the invention include polyethers, for example, as described in U.S. Patent No. 3,459,731. Further examples of substituted cyclodextrins include ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is replaced by C_{1-6}alkyl, hydroxy-C_{1-6}alkyl, carboxy-C_{1-6}alkyl or C_{1-6}alkyloxycarbonyl-

C_{1-6}alkyl groups or mixed ethers thereof. In particular, such substituted cyclodextrins are ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is replaced by C_{1-3}alkyl, hydroxy-C_{2-4}alkyl or carboxy-C_{1-2}alkyl or more particularly by methyl, ethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, carboxymethyl or carboxyethyl. The term "C_{1-6}alkyl" is meant to include straight and branched saturated hydrocarbon radicals, having from 1 to 6 carbon atoms such as methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, butyl, pentyl, hexyl and the like. Other cyclodextrins contemplated for use herein included glucosyl-β-cyclodextrin and maltosyl-β-cyclodextrin. Of particular utility in the present invention are randomly methylated β-cyclodextrin and polyethers such as hydroxypropyl-β-cyclodextrin, hydroxyethyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, and hydroxyethyl-γ-cyclodextrin, as well as sulfobutyl ethers, especially β-cyclodextrin sulfobutyl ether. In addition to simple cyclodextrins, branched cyclodextrins and cyclodextrin polymers may also be used. Other cyclodextrins are described, for example, in *Chemical and Pharmaceutical Bulletin* 28: 1552-1558 (1980); Yakugyo Jiho No. 6452 (28 March 1983); *Angew. Chem. Int. Ed. Engl.* 19: 344-362 (1980); U.S. Patent Nos. 3,459,731 and 4,535,152; European Patent Nos. EP 0 149 197A and EP 0 197 571A; PCT International Patent Publication No. WO90/12035; and UK Patent Publication GB 2,189,245.

References describing cyclodextrins for use in the compositions according to the present invention, and/or which provide a guide for the preparation, purification and analysis of cyclodextrins include the following: *Cyclodextrin Technology* by Jozsef Szejtli, Kluwer Academic Publishers (1988) in the chapter Cyclodextrins in Pharmaceuticals; *Cyclodextrin Chemistry* by M. L. Bender *et al.*, Springer-Verlag, Berlin (1978); *Advances in Carbohydrate Chemistry*, Vol. 12, Ed. By M. L. Wolfrom, Academic Press, New York in the chapter "The Schardinger Dextrins" by Dexter French, pp. 189-260; *Cyclodextrins and their Inclusion Complexes* by J. Szejtli,

Adakemiai Kiado, Budapest, Hungary (1982); I. Tabushi, *Acc. Chem. Research*, 1982, 15, pp. 66-72; W. Sanger, *Angewandte Chemie*, 92, p. 343-361 (1981); A. P. Croft *et al., Tetrahedron*, 39, pp. 1417-1474 (1983); Irie *et al. Pharmaceutical Research*, 5, pp. 713-716 (1988); Pitha *et al., Int. J. Pharm.* 29, 73 (1986); U.S. Patent Nos. 4,659,696 and 4,383,992; German Patent Nos. DE 3,118,218 and DE-3,317,064; and European Patent No. EP 0 094 157A. Patents describing hydroxyalkylated derivative of β- and γ-cyclodextrin include Pitha U.S. Patent Nos. 4,596,795 and 4,727,064, Müller U.S. Patent Nos. 4,764,604 and 4,870,060 and Müller *et al.* U.S. Patent No. 6,407,079.

Amorphous cyclodextrins of particular interest for complexation with cladribine include: hydroxyalkyl, *e.g.* hydroxyethyl or hydroxypropyl, derivatives of β- and γ-cyclodextrin; carboxyalkyl, *e.g.* carboxymethyl or carboxyethyl, derivatives of β- or γ-cyclodextrin; β-cyclodextrin sulfobutyl ether; and randomly methylated β-cyclodextrin. 2-Hydroxypropyl-β-cyclodextrin (HPβCD), 2-hydroxypropyl-γ-cyclodextrin (HPγCD), randomly methylated β-cyclodextrin, β-cyclodextrin sulfobutyl ether, carboxymethyl-β-cyclodextrin (CMβCD) and carboxymethyl-γ-cyclodextrin (CMγCD) are of special interest, especially hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin.

Compositions of an amorphous mixture of amorphous free cladribine and an amorphous, preferably saturated, cladribine-cyclodextrin inclusion complex for use in the present invention can be prepared under conditions favoring complex formation in a liquid environment as described and as exemplified herein. The resultant liquid preparations can be subsequently converted to a dry form suitable for administration as a solid oral or transmucosal dosage form.

One of skill will appreciate that a variety of approaches are available in the field to prepare compositions as described herein. One available

method exemplified herein includes the steps of mixing the cladribine in an aqueous solution of an amorphous cyclodextrin, separating un-dissolved cladribine (*e.g.*, by filtering or centrifugation), and lyophilizing or freeze-drying the saturated solution to form a solid amorphous mixture.

Pharmaceutical compositions according to the invention may optionally include one or more excipients or other pharmaceutically inert components. One of the advantages of the invention, however, is that cladribine drug forms as described herein can be prepared with the minimal amount of excipients necessary for shaping and producing the particular form, such as a tablet or patch. Excipients may be chosen from those that do not interfere with cladribine, with cyclodextrin or with complex formation.

Dosage forms are optionally formulated in a pharmaceutically acceptable vehicle with any of the well-known pharmaceutically acceptable carriers, diluents, binders, lubricants, disintegrants, scavengers, flavoring agents, coloring agents, and excipients (see *Handbook of Pharmaceutical Excipients*, Marcel Dekker Inc., New York and Basel (1998); Lachman *et al.* Eds., *The Theory and Practice of Industrial Pharmacy*, 3rd Ed., (1986); Lieberman *et al.*, Eds. *Pharmaceutical Dosage Forms,* Marcel Dekker Inc., New York and Basel (1989); and *The Handbook of Pharmaceutical Excipients*, 3rd Ed., American Pharmaceutical Association and Pharmaceutical Press, 2000); see also *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, Mack Publishing Co., Easton, PA (1990) and *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams & Wilkins, (1995)). A simple solid oral dosage form consists of the amorphous mixture of amorphous free cladribine and amorphous cladribine-cyclodextrin complex (preferably saturated) as described above, *i.e.* the complex cladribine-cyclodextrin complex, compressed with a small amount (*e.g.* about 1% by weight) of a suitable binder or lubricant such as magnesium stearate.

In certain instances, oral absorption may be further facilitated by the addition of various excipients and additives to increase solubility or to enhance penetration, such as by the modification of the microenvirionment.

The methods and pharmaceutical compositions described herein offer novel therapeutic modalities for the treatment of patients in need of treatment with cladribine. As shown herein, the invention addresses the problems of poor bioavailability traditionally associated with oral cladribine.

The compositions of the invention are particularly suitable as modalities for the treatment of any cladribine-responsive disease. Several disease states responsive to cladribine are well-documented in the literature (see *infra*). For any target disease state, an effective amount of the complex cladribine-cyclodextrin comples, *i.e.* the amorphous mixture of the optimized amorphous saturated cladribine-amorphous cyclodextrin complex with amorphous free cladribine as described above is used (*e.g.*, an amount affective for the treatment of multiple sclerosis, rheumatoid arthritis, or leukemia).

The term "therapeutically effective amount" or "effective amount" is used to denote treatments at dosages effective to achieve the therapeutic result sought. Therapeutically effective dosages described in the literature include those for hairy cell leukemia (0.09 mg/kg/day for 7 days), for multiple sclerosis (from about 0.04 to about 1.0 mg/kg/day (see U.S. Patent No. 5,506,214)); for other diseases, see also U.S. Patent Nos. 5,106,837 (autohemolytic anemia); 5,310,732 (inflammatory bowel disease); 5,401,724 (rheumatoid arthritis); 5,424,296 (malignant astrocytoma); 5,510,336 (histiocytosis); 5,401,724 (chronic myelogenous leukemia); and 6,239,118 (atherosclerosis).

Further, various dosage amounts and dosing regimens have been reported in the literature for use in the treatment of multiple sclerosis; see, for example: Romine et al., *Proceedings of the Association of American*

Physicians, Vol. 111, No. 1, 35-44 (1999); Selby et al., *The Canadian Journal of Neurological Sciences*, 25, 295-299 (1998); Tortorella et al., *Current Opinion in Investigational Drugs*, 2 (12), 1751-1756 (2001); Rice et al., *Neurology,* 54, 1145-1155 (2000); and Karlsson et al., *British Journal of Haematology*, 116, 538-548 (2002); all of which are incorporated by reference herein in their entireties and relied upon.

Moreover, the route of administration for which the therapeutically effective dosages are taught in the literature should be taken into consideration. While the instant compositions optimize the bioavailability of cladribine following oral administration, it will be appreciated that even optimal bioavailability from oral dosage forms is not expected to approach bioavailability obtain after intravenous administration, particularly at early time points. Thus, it is often appropriate to increase a dosage suggested for intravenous administration to arrive at a suitable dosage for incorporation into a solid oral dosage form. At the present time, it is envisioned that, for the treatment of multiple sclerosis, 10 mg of cladribine in the instant complex cladribine-cyclodextrin complex in the instant solid dosage form would be administered once per day for a period of five to seven days in the first month, repeated for another period of five to seven days in the second month, followed by ten months of no treatment. Alternatively the patient would be treated with 10 mg of cladribine in the instant complex cladribine-cyclodextrin complex in the instant dosage form once per day for a period of five to seven days per month for a total of six months, followed by eighteen months of no treatment. For further dosing information, see also U.S. Provisional Patent Application No. _____ [IVAX0021-P-USA/Attorney Docket No. 033935-011], and U.S. Provisional Patent Application No. _____ [IVAX0022-P-USA/Attorney Docket No. 033935-012], both entitled "Cladribine Regimen for Treating Multiple Sclerosis", both filed on March 25, 2004 and incorporated by reference herein in their entireties.

Furthermore, one of skill will appreciate that the therapeutically effective amount of cladribine administered herein may be lowered or increased by fine tuning and/or by administering cladribine according to the invention with another active ingredient. The invention therefore provides a method to tailor the administration/treatment to the particular exigencies specific to a given mammal. Therapeutically effective amounts may be easily determined, for example, empirically by starting at relatively low amounts and by step-wise increments with concurrent evaluation of beneficial effect.

As noted in the preceding paragraph, administration of cladribine in accord with this invention may be accompanied by administration of one or more additional active ingredients for treating the cladribine-responsive condition. The additional active ingredient will be administered by a route of administration and in dosing amounts and frequencies appropriate for each additional active ingredient and the condition being treated. For example, in the treatment of multiple sclerosis, other useful drugs include interferon beta (Rebif®, Betaseron®/Betaferon®, Avonex®), identical to the naturally occurring protein found in the human body; glatiramer acetate (Copaxone®), a random chain (polymer) of the amino acids glutamic acid, lysine, alanine and tyrosine; natalizumab (Antegren®), a monoclonal antibody; alemtuzumab (Campath-1H®), a humanized anti-CD52 monoclonal antibody; 4-aminopyridine (also known as 4-AP and Fampridine), a drug that blocks the potassium channels in neurons; and amantadine, an anti-viral agent which improves muscle control and reduces muscle stiffness and is used to alleviate the symptoms of fatigue in multiple sclerosis, a purpose for which pemoline (Cylert®) and L-Carnitine (a herbal product) may also be useful. In the treatment of hairy cell leukemia, additional active ingredients may include interferon alpha, pentostatin, fludarabine, rituximab (an anti-CD 20 monoclonal antibody) and the anti-CD22 recombinant immunotoxin BL 22; other additional active ingredients may be appropriate in other types of

leukemias. In the treatment of rheumatoid arthritis, there are many other active ingredients which may be selected. These include NSAIDS (non-steroidal anti-inflammatory drugs), which are of three types: salicylates such as aspirin, traditional NSAIDS such as ibuprofen and indomethacin, and COX-2 inhibitors such as celecoxib (Celebrex®), rofecoxib (Vioxx®), meloxicam (Mobic®), valdecoxib (Bextra®), lumiracoxib (Prexige®) and etoricoxib (Arcoxia®). Other drugs useful in treating rheumatoid arthritis which may be used in conjunction with the present invention include DMARDS, glucocorticoids, biological response modifiers and non-NSAID analgesics. DMARDS are disease-modifying anti-rheumatic drugs which include methotrexate, plaquenil, leflunomide (Arava®), sulfasalazine, gold, penicillamide, cyclosporine, methyl cyclophosamide and azathioprine. Glucocorticoids include dexamethasone, prednisolone, triamcinolone and many others. Biological response modifiers (which restore the disease-fighting ability of the immune system), include etanercept (Enrel®), a tumor-necrosis factor inhibitor, infliximab (Remicade®), which is also an anti-TNF drug, anakinra (Kineret®), a selective IL-1 blocker, and Humira®, a human monoclonal antibody which is another anti-TNF drug. The non-NSAID analgesics include acetaminophen as well as narcotic analgesics such as hydrocodone, oxycodone and propoxyphene. Generally speaking, those drugs which work by a mechanism different from that of cladribine are particularly useful for concomitant therapy with the cladribine composition described herein. Those drugs which are effective by the oral route of administration and which are compatible with the instant cladribine complexes in a single dosage form may be incorporated into the instant dosage forms; otherwise, they should of course be separately administered in amounts, frequencies and via administration routes suitable to them.

As used herein, "treating" means reducing, preventing, hindering the development of, controlling, alleviating and/or reversing the symptoms in the individual to which a compound of the invention has been administered, as

compared to the symptoms of an individual not being treated according to the invention. A practitioner will appreciate that the complexes, compositions, dosage forms and methods described herein are to be used in concomitance with continuous clinical evaluations by a skilled practitioner (physician or veterinarian) to determine subsequent therapy. Such evaluation will aid and inform in evaluating whether to increase, reduce or continue a particular treatment dose, and/or to alter the mode of administration.

The methods of the present invention are intended for use with any subject/patient that may experience the benefits of the methods of the invention. Thus, in accordance with the invention, the terms "subjects" as well as "patients" include humans as well as non-human subjects, particularly domesticated animals.

Any suitable materials and/or methods known to those of skill can be utilized in carrying out the present invention. However, preferred materials and methods are described. Materials, reagents and the like to which reference are made in the following description and examples are obtainable from commercial sources, unless otherwise noted.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein.

EXAMPLES

EXAMPLE 1

PHASE SOLUBILITY STUDY

A phase solubility study can be carried out as follows. Excess cladribine is added to cyclodextrin solutions of various concentrations of

hydroxypropyl-β-cyclodextrin (HPβCD) and allowed to complex as described in EXAMPLE 2 below. The excess, undissolved cladribine is removed by filtration. The amount of cladribine in the complexation solution is measured to obtain a data point. This process is repeated with different known concentrations of HPβCD until several data points are obtained. These data points are then plotted graphically, each data point representing the amount of cladribine that can be dissolved in water with a specific concentration of cyclodextrin. Points on the line generated by the data points represent ratios for the product. One of skill in the art will realize the same results will be generated if excess cyclodextrin is added to cladribine solutions of known concentration.

The molar concentrations of cladribine to cyclodextrin obtained are plotted and presented graphically. A representative phase solubility diagram is shown in the Figure. The plotted lines for cladribine-HPβCD represent cladribine solubilization for the conditions tested, that is, the ratio of the concentration of cladribine to the concentration of cyclodextrin. The area above each of the plotted lines represents conditions where excess insoluble cladribine is present. The area below each of the plotted lines represents the conditions where cyclodextrin is in excess.

The plot for cladribine-HPβCD shown in the Figure is approximately linear; this is indicative of a 1:1 complex, in which one molecule of the drug is complexed with one molecule of cyclodextrin. The Figure also shows that additional cyclodextrin is needed to maintain the cladribine in the complex. For example, about 0.14 mole of HPβCD is needed to maintain about 0.049 mole of cladribine dissolved under the selected conditions, which will ultimately provide the amorphous mixture of the amorphous, preferably saturated, cladribine-HPβCD inclusion complex and amorphous free cladribine (as a non-inclusion complex). Under the conditions of EXAMPLE 2 below, a significant portion of the cladribine in the product can be expected to be not in the inclusion complex but rather in amorphous form loosely held

in intimate admixture therewith by hydrogen bonding as a non-inclusion complex.

EXAMPLE 2

PREPARATION OF CLADRIBINE-CYCLODEXTRIN COMPLEX FOR HUMAN TRIALS

Cladribine is complexed with HPβCD by the following method.

In 825 mL of distilled water, 172.5 g of hydroxypropyl-β-cyclodextrin are dissolved (forming an approximately 17% solution), then cladribine is added and the mixture is stirred at about 45 to about 50°C for about nine hours. Stirring is continued for an additional 6 to 9 hours at room temperature. Any undissolved cladribine is removed by filtration and the solution is cooled to room temperature. To form the amorphous mixture of amorphous cladribine-cyclodextrin complex and amorphous free cladribine, the aqueous cladribine-cyclodextrin solution is dried by lyophilization prior to incorporation into solid oral tablets. The lyophilization procedure comprises a freezing stage of rapidly bringing the complexation solution to about -40°C to about -80°C (e.g., about -45°C) for approximately 2 to 4 hours (preferably about 3 to 4 hours), followed by a primary drying stage at about -25°C for approximately 80-90 hours, typically under low pressure, and a second drying stage at about 30°C for about 15-20 hours.

Product made by the foregoing general procedure can be analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based mobile phase, with UV detection at 264 nm) to find the weight ratio of cladribine to cyclodextrin in the final product. Final product preparations can be further characterized by methods known in the art, including, for example by inspecting appearance, ascertaining the overall impurity content by HPLC, ascertaining the water content using a Karl Fischer titrator, determining the dissolution profile by a standard method, for example using USP<711>Apparatus II equipment and UV detection at 264 nm, inspecting

the content uniformity and performing quantitative assay by HPLC analysis of the active ingredient.

Two batches of cladribine/cyclodextrin product, FD04 and FD05, were made by the foregoing general procedure as follows:

Purified water (825 mL) was pre-heated at 48°C (target range 45°C to 50°C) in a 1-liter glass vessel by immersion in a water bath. The heated water was stirred to achieve a controlled central vortex. 2-hydroxypropyl-β-cyclodextrin (172.50 g) was weighed and slowly added to the heated water over a period of 40 minutes. The resulting solution was stirred for a further 10 minutes to ensure complete dissolution of the cyclodextrin. Cladribine (12.00 g for FD04 and 18.75 g for FD05) was weighed and added to the stirred cyclodextrin solution, which turned cloudy before becoming clear. The resulting clear solution was maintained at 48°C and continually stirred for 9 hours. Stirring continued for a further 7 hours while the solution cooled to room temperature.

Use of a larger amount of cladribine in the preparation of FD05 was part of an attempt to optimize the procedure; however, it was found that the initial amount of cladribine in that case was too great and precipitation was observed at the end of the cooling step for batch FD05. The solution was filtered to remove the precipitate. Analysis of the resultant product revealed (assay value = 87.2%) that 16.35 g of cladribine had been incorporated into the cyclodextrin complex in the case of FD05. No filtration was required for batch FD04, indicating that the amounts used in the preparation of FD04 were more appropriate and that the FD05 procedure could be optimized by beginning with a smaller amount of cladribine (16.35 g rather than 18.75 g), thus avoiding the filtration step.

After cooling to room temperature and, in the case of FD05, filtering, the solutions were filled into 100 mL lyophilization vials (20 mL solutions per vial), the filled vials were partially stoppered and lyophilized. The

lyophilization included freezing at -45°C for about 200 minutes, a primary drying phase at -25°C under a pressure of 100 mTorr for about 5,200 minutes and a secondary drying phase at 30°C for about 1,080 minutes as set forth below:

TABLE I

Step	Process	Temperature	Pressure (mTorr)	Time (min)
1	Load	4°C		
2	Load Hold	4°C	n/a	120
3	Ramp	-45°C	n/a	120
4	Freezing	-45°C	n/a	200
5	Ramp	-25°C	100	120
6	Primary drying	-25°C	100	5200
7	Ramp	30°C	50	240
8	Secondary drying	30°C	50	1080
9	Finish	30°C	Vials closed under vacuum	

The FD04 and FD05 batches of cladribine/cyclodextrin product made by the foregoing procedure were analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based mobile phase with UV detection at 264 nm) and empirically found to have the following characteristics:

TABLE II

Lot No.	Cladribine: HPβCD w/w	Cladribine: HPβCD Weight Ratio
FD04	12.00g:172.50g	1:14.38
FD05	16.35g:172.50g	1:10.55

The products were analyzed by DSC thermograms and X-ray diffraction methods to determine any free crystalline cladribine in the lyophilized material. Importantly, the samples exhibited no transitions in the region of 210°C to 230°C, which is associated with the melting of crystalline cladribine. In both cases, no significant thermal activity was recorded in the range of 210°C to 230°C, suggesting that the complexes obtained at the end of the lyophilization do not have any significant amount of free crystalline cladribine, considering the sensitivity of the analytical method (up to 3% w/w). This conclusion was supported by the absence of peaks for crystalline cladribine from X-ray diffraction traces for both complexes FD04 and FD05.

The products are amorphous mixtures of amorphous cladribine-HPβCD inclusion complex and amorphous free cladribine hydrogen-bonded to the cyclodextrin as a non-inclusion complex. The cladribine:HPβCD weight ratios obtained were about 1:14 and 1:11.

Generally speaking, amorphous mixtures within the scope of the present invention have cladribine:HPβCD weight ratios of from about 1:10 to 1:16.

EXAMPLE 3

PREPARATION OF ORAL TABLETS

Tablets were manufactured using batches of amorphous mixtures FD04 and FD05 described in EXAMPLE 2 for use in a clinical study.

Batch N0120 was manufactured using cladribine-2-HPβCD complex mixture DF05 to a batch size of 3,000 tablets and batch N0126 was manufactured using cladribine-HPβCD complex mixture FD04 to a batch size of 800 tablets. The master formulations for the two batches are shown in TABLE III. Batch N0120 represented 3.0 g tablets and Batch N0126 represented 10 mg tablets for clinical study.

TABLE III

Constituent	Lot Number	mg/tablet 3.0 mg Batch N0120	mg/tablet 10.0 mg Batch N0126
Cladribine-HPβCD complex mix	FD05	30.60*	
Cladribine-HPβCD complex mix	FD04		153.75**
Sorbitol powder NF	1007403	68.4	44.25
Magnesium stearate NF	1006280	1.00	2.00
Total		100.00	200.00

*Equivalent to 3.0 mg cladribine per tablet.
**Equivalent to 10.0 mg cladribine per tablet.

The following table sets forth the method of manufacture of the Batch N0120 and N0126 tablets.

TABLE IV

1.	Pre-mix the magnesium stearate with an approximately equal quantity of sorbitol power.
2.	Pass the cladribine-HPβCD complex and the remainder of the sorbitol powder into a one-liter glass jar via a 40-mesh screen.
3.	Blend the contents for 10 minutes at 12 rpm.
4.	Pass the magnesium stearate/sorbitol powder pre-mix into the glass jar via the 40-mesh screen.
5.	Blend the final mixture for 5 minutes at 12 rpm.
6.	Compress into 3.0 mg and 10.0 mg tablets at a target compression weight of 100 mg and 200 mg, respectively.

Both the Batch N0120 3.0 mg tablets and the Batch N0126 10.0 mg tablets were round, with one side flat-beveled edged and the other side shallow convex. The Batch N0120 3.0 mg tablets had an average weight of 100 mg, a thickness of 2.7 mm, a friability of 0.2%, a hardness of 4 Kp and a disintegration time of 3 minutes. The Batch N0126 10.0 mg tablets had an average weight of 198 mg, a thickness of 4.2 mm, a friability of 1%, a hardness of 2.8 Kp and a disintegration time of 5 minutes 42 seconds.

The Batch N0120 3.0 mg and N0126 10.0 mg tablets were used in the clinical study summarized in EXAMPLE 5 below.

EXAMPLE 4

CLINICAL STUDY: RELATIVE BIOAVAILABILITY

The objective of this study was to assess the relative bioavailability of three oral cladribine formulations: (1) a cyclodextrin-based formulation according to the instant invention (Tablet 1: complex FD05, i.e. Batch No. N0120 tablets described above); (2) a mucoadhesive formulation (Tablet 2: containing 3.0 mg cladribine, 10 mg of Carbopol 71G NF, 22.2 mg of dicalcium phosphate, 64.3 mg of lactose and 0.5 mg of magnesium stearate, Batch No. N0121); and (3) a hard-gel capsule (Capsule containing 3.0 mg cladribine, 5.0 mg Carbopol 974P, 91.3 mg Avicel PH101, 100.0 mg Avicel PH102, 0.2 mg colloidal silicon dioxide and 0.5 mg magnesium stearate, Batch No. RD03030) in comparison with one fixed subcutaneous clardribine administration (reference formulation) in patients with MS (multiple sclerosis).

This study was a 2 center, open-label, randomized, 4-way crossover single dose study using twelve patients with MS. Patients received randomly three different fixed oral doses (3.0 mg) and a fixed subcutaneous dose of 3.0 mg. The four treatment days were separated by a drug-free interval of at

least 5 days. In each treatment period, blood samples were collected over a 24-hour period for evaluation of plasma cladribine.

The plasma concentration of cladribine was measured by a HPLC/MS/MS method. Using this method, the relationship between concentration versus peak area ratio was found to be linear within the range of 100 pg/ml to 50,000 pg/ml for cladribine. The limit of quantification was 100 pg/ml. Analysis of samples was carried out in 16 runs. No calibrator had to be excluded from fitting of the calibration curve and accuracy of each quality control sample met the GLP requirements.

576 clinical plasma samples were analyzed and concentration values of cladribine were determined. The results were compiled and are summarized in the tables below (Tables V and VI). In these tables, the following definitions are applicable: T_{max} is the time to reach maximum concentration in the plasma; $T_{½}$ is the half-life of cladribine in the plasma; C_{MAX} is the maximum concentration of cladribine in the plasma; AUC_{inf} is the area under the curve for the measured data from zero extrapolated to infinity; AUC_t is the area under the curve for the measured data (from zero to the last time point); Geom Mean is the geometric mean; CV is the coefficient of variation (relative standard deviation); LL is the lower limit; UL is the upper limit.

TABLE V
Summary Statistics for Pharmacokinetic Parameters for Cladribine Study
Obtained via Non-Compartmental Analyses. (n=12).

Pharmacokinetic Parameter	3.0 mg subcutaneous			3mg Tablet 1			3mg Tablet 2			3 mg Capsule		
	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)
T_{max}(hr)	N/A	.313 ±.113	36.2	N/A	.521 ±.167	32.1	N/A	1.25 ±.839	67.1	N/A	2.25 ±.622	27.7
$T_{½}$(hr)	N/A	6.69 ±2.01	30.1	N/A	7.55 ±2.50	33.1	N/A	6.73 ±2.82	41.9	N/A	6.27 ±2.31	36.9
C_{max}(pg/ml)	23186	N/A	40.1	6597	N/A	24.7	5041	N/A	52.6	3818	N/A	36.8
AUC_{inf} (hr·pg/ml)	57254	N/A	44.4	24936	N/A	28.8	21676	N/A	42.7	22604	N/A	39.5
AUC_t (hr·pg/ml)	54725	N/A	43.8	23182	N/A	28.0	20063	N/A	42.1	20951	N/A	42.0

**CV=SD/mean for T_{max} and $T_{½}$ and CV% geometric mean for C_{max}, AUC_{inf} and AUC_t.

TABLE VI

Ratios of Oral to Subcutaneous Pharmacokinetic Parameters and Corresponding Two-Sided 90% Confidence Intervals for Cladribine Study (n=12).

Pharmacokinetic Parameter	3 mg Tablet 1		3mg Tablet 2		3mg Capsule	
	Ratio*	LL, UL	Ratio*	LL, UL	Ratio*	LL, UL
AUC_{inf}	43.1	35.7, 52.1	38.4	31.8, 46.4	38.9	32.1, 47.0
AUC_t	41.9	34.6, 50.8	37.2	30.7, 45.0	37.6	31.0, 45.5

*Ratios (dose normalized) and Corresponding 95% LL obtained via inverse transformation of log-transformed data.

EXAMPLE 5

CLINICAL STUDY: DOSE RESPONSE AND ABSOLUTE BIOAVAILABILITY

The objective of this study was to assess the systemic availability of cladribine after oral administration in two different fixed oral doses, in comparison with one fixed intravenous administration (reference formulation) in patients with MS (multiple sclerosis), and to evaluate the safety and tolerability of cladribine in this population.

This study was a 3 center, open-label, randomized, 3-way crossover single dose study using twenty-six patients with MS. Patients received randomly two different fixed oral doses (3.0 mg and 10.0 mg) and a fixed intravenous dose of 3.0 mg (administered as a 1 hour infusion). The three treatment days were separated by a drug-free interval of at least 5 days. In

each treatment period blood samples were collected over a 24-hour period for evaluation of plasma cladribine.

The plasma concentrations of cladribine were measured by a HPLC/MS/MS method. Using this method the relationship between concentrations versus peak area ratios was found to be linear within the range of 100 pg/ml to 50,000 pg/ml for cladribine. The limit of quantification was 100 pg/ml. Analysis of samples was carried out in 16 runs. Except the first run (which had to be rejected because of equipment failure), all other runs could be accepted. No calibrator had to be excluded from fitting of the calibration curve and accuracy of each quality control sample met the GLP requirements.

858 clinical plasma samples were analyzed and concentration values of cladribine were determined. The results were compiled and are summarized in the tables below [TABLES VII through X]. In these tables, the following definitions are applicable: T_{max} is the time to reach maximum concentration in the plasma; $T_{1/2}$ is the half-life of cladribine in the plasma; C_{max} is the maximum concentration of cladribine in the plasma; AUC_{inf} is the area under the curve for the measured data from zero extrapolated to infinity; AUC_t is the area under the curve for the measured data (from zero to the last time point); Geom Mean is the geometric mean; CV is the coefficient of variation (relative standard deviation); LL is the lower limit; UL is the upper limit; σ^2 is the mean variance; σ_B^2 is the mean variance between subjects; σ_W^2 is the mean variance within subjects; CV_T is the total coefficient of variation; and CV_W is the coefficient of variation within subjects.

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TABLE VII

Summary Statistics for Pharmacokinetic Parameters for Cladribine Study Obtained via Non-Compartmental Analysis (n=26)

Pharmaco-kinetic Parameter	3.0 mg IV infusion			Oral Administration					
				3.0 mg			10.0 mg		
	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)	Geom Mean	Mean ± SD	CV** (%)
T_{max}(hr)	N/A	.817 ±.397	48.6	N/A	.548 ±.300	54.8	N/A	.558 ±.204	36.5
$T_{½}$(hr)	N/A	6.50 ±1.27	19.5	N/A	5.85 ±1.18	20.2	N/A	5.60 ±0.75	13.3
C_{max}(pg/ml)	21425	N/A	27.6	5608	N/A	49.5	21242	N/A	50.5
AUC_{inf} (hr·pg/ml)	58528	N/A	24.0	20159	N/A	35.0	76690	N/A	30.3
AUC_t (hr·pg/ml)	56396	N/A	24.0	19166	N/A	36.9	74532	N/A	30.3

**CV=SD/mean for T_{max} and $T_{½}$ and CV% geometric mean for C_{max}, AUC_{inf} and AUC_t.

TABLE VIII

Ratios of Oral to I.V. Pharmacokinetic Parameters and Corresponding Lower Limit (LL) for the one-sided 95% Confidence Interval for Cladribine Study (n=26)

Pharmacokinetic Parameter	Oral Administration			
	3.0 mg		10.0 mg	
	Ratio*	LL	Ratio*	LL
AUC_{inf}	34.5	31.7	39.1	35.9
AUC_t	34.0	31.2	39.4	36.1

*Ratios (dose normalized) and Corresponding 95% LL obtained via inverse transformation of log-transformed data.

TABLE IX

Ratios and Corresponding two-sided 90% Confidence Intervals for Cladribine Study (n=26)

Pharmacokinetic Parameter	10.0 mg/3.0 mg		
	Ratio*	LL	UL
C_{max}	112.6	95.1	133.3
AUC_{inf}	113.3	104.2	123.3
AUC_t	115.8	106.1	126.5

*Ratios (dose normalized) and Corresponding 90% CI obtained via inverse transformation of log-transformed data.

TABLE X

Variance components for Cladribine Study (n=26)

Source of variation	C_{max}	AUC_{inf}	AUC_t
Between (σ_B^2)	.0380	.0487	.0492
With (σ_W^2)	.1315	.0330	.0357
TOTAL ($\sigma_B^2 + \sigma_W^2$)	.1695	.0816	.0849
CV_T(%)	43.0	29.2	29.8
CV_W(%)	37.5	18.3	19.1

Where PK parameters are dose-adjusted and $CV = \sqrt{\exp(\sigma^2) - 1}$

The foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents thereof may be resorted to, falling within the scope of the invention claimed.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

2. The pharmaceutical composition according to Claim 1, wherein the complex is saturated with cladribine.

3. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

4. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

5. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

6. The composition according to any one of Claims 1 to 3, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

7. The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

8. The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

9. The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

10. The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

11. The composition according to any one of Claims 1 to 10, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

12. The composition according to any one of Claims 1 to 11, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

13. A method for enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

14. The method according to Claim 13, wherein the complex is saturated with cladribine.

15. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

16. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

17. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

18. The method according to any one of Claims 13 to 15, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

19. The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

20. The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

21. The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

22. The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

23. The method according to any one of Claims 13 to 22, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin

corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

24. The method according to any one of Claims 13 to 23, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

25. A method for the treatment of symptoms of a cladribine-responsive condition in a subject suffering from said symptoms comprising orally administering to said subject a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

26. The method according to Claim 25, wherein the complex is saturated with cladribine.

27. The method according to Claim 25 or 26, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

28. The method according to Claim 27, wherein the cladribine-responsive condition is multiple sclerosis.

29. The method according to Claim 25, 26, 27 or 28, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin,

hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

30. The method according to any one of Claims 25 to 29, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

31. The method according to any one of Claims 25 to 30, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

32. The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

33. The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

34. The method according to Claim 25, 26, 27 or 28, wherein the amorphous cyclodextrin is hydropropyl-γ-cyclodextrin.

35. The method according to any one of Claims 25 to 34, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

36. Use of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, in the formulation of a solid oral dosage form, for administration in the treatment of symptoms of a cladribine-responsive condition.

37. Use according to Claim 36, wherein the complex is saturated with cladribine.

38. Use according to Claim 36 or 37, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

39. Use according to Claim 38, wherein the cladribine-responsive condition is multiple sclerosis.

40. Use according to Claim 36, 37, 38 or 39, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

41. Use according to any one of Claims 36 to 40, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

42. Use according to any one of Claims 36 to 41, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

43. Use according to Claim 42, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

44. Use according to Claim 42, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

45. Use according to any one of Claims 36 to 41, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

46. Use according to any one of Claims 36 to 45, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

47. Use of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, in the formulation of a solid oral dosage form, for enhancing the oral bioavailability of cladribine.

48. Use according to Claim 47, wherein the complex is saturated with cladribine.

49. Use according to Claim 47 or 48, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

50. Use according to any one of Claims 47 to 49, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

51. Use according to any one of Claims 47 to 50, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

52. Use according to Claim 51, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

53. Use according to Claim 51, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

54. Use according to any one of Claims 47 to 50, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

55. Use according to any one of Claims 47 to 54, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

56. A complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex.

57. The complex according to Claim 56, saturated with cladribine.

58. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

59. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

60. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

61. The complex according to any one of Claims 56 to 58, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

62. The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

63. The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

64. The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

65. The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

66. The complex according to any one of Claims 56 to 65, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

67. A process for the preparation of a complex cladribine-cyclodextrin complex which comprises the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature; and

(iii) lyophilizing the cooled solution to afford an amorphous product.

68. A process according to Claim 67, further comprising a filtration step following step (ii).

69. A process according to Claim 67 or 68, wherein step (i) is performed at a temperature of from about 45 to about 60°C.

70. A process according to any one of Claims 67 to 69, wherein step (i) is performed at a temperature of from about 45 to about 50°C.

71. A process according to Claim 69 or 70, wherein step (i) is performed with stirring.

72. A process according to Claim 71, wherein step (i) is performed for a period of from about 6 to about 9 hours.

73. A process according to any one of Claims 67 to 72, wherein step (ii) is performed for a period of from about 6 to about 9 hours.

74. A process according to any one of Claims 67 to 73, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80° C, and held at said temperature for a period of from about 2 to about 4 hours.

75. A process according to Claim 74, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

76. A process according to any one of Claims 67 to 75, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

77. A process according to any one of Claims 67 to 75, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

78. A process according to Claim 76 or 77, wherein 825 parts by volume of water are introduced in step (i).

79. A process according to any one of Claims 67 to 78, wherein the lyophilization step (iii) comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

80. A process according to Claim 79, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

81. A process according to Claim 79 or 80, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

82. A pharmaceutical composition obtainable by a process comprising the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature;

(iii) lyophilizing the cooled solution to afford an amorphous product; and

(iv) formulating the amorphous product into a solid oral dosage form.

83. A pharmaceutical composition according to Claim 82, wherein the process further comprises a filtration step following step (i) or (ii).

84. A pharmaceutical composition according to Claim 82 or 83, wherein step (i) of the process is performed at a temperature of from about 45 to about 60°C.

85. A pharmaceutical composition according to any one of Claims 82 to 84, wherein step (i) of the process is performed at a temperature of from about 45 to about 50°C.

86. A pharmaceutical composition according to Claim 84 or 85, wherein step (i) of the process is performed with stirring.

87. A pharmaceutical composition according to Claim 86, wherein step (i) of the process is performed for a period of from about 6 to about 9 hours.

88. A pharmaceutical composition according to any one of Claims 82 to 87, wherein step (ii) of the process is performed for a period of from about 6 to about 9 hours.

89. A pharmaceutical composition according to any one of Claims 82 to 88, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80°C, and held at said temperature for a period of from about 2 to about 4 hours.

90. A pharmaceutical composition according to Claim 89, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

91. A pharmaceutical composition according to any one of Claims 82 to 90, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

92. A pharmaceutical composition according to any one of Claims 82 to 90, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

93. A pharmaceutical composition according to Claim 91 or 92, wherein 825 parts by volume of water are introduced in step (i) of the process.

94. A pharmaceutical composition according to any one of Claims 82 to 93, wherein the lyophilization step (iii) of the process comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

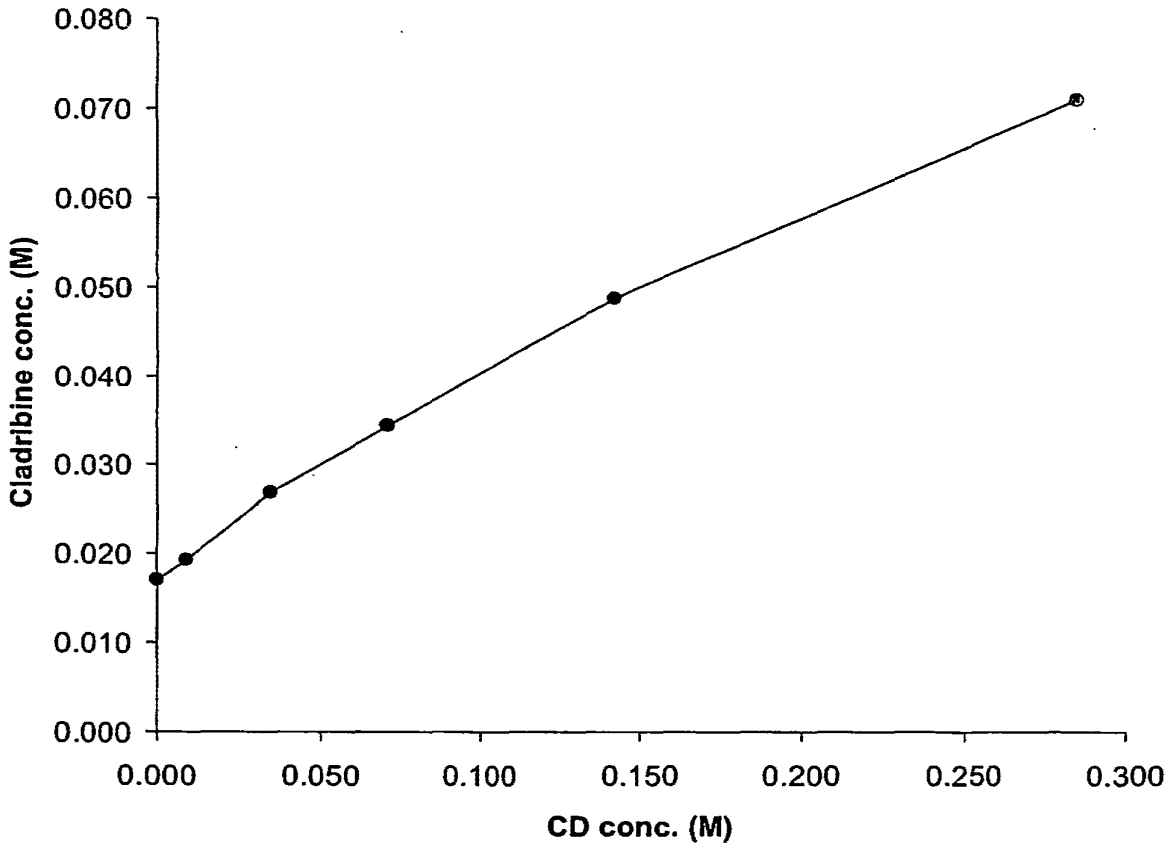
95. A pharmaceutical composition according to Claim 94, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

96. A pharmaceutical composition according to Claim 94 or 95, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

97. A pharmaceutical composition according to any one of Claims 82 to 96, wherein the formulation step (iv) of the process comprises blending the complex with magnesium stearate and compressing into tablets.

98. A pharmaceutical composition according to Claim 97, wherein magnesium stearate is pre-mixed with sorbitol powder before blending with the complex.

1/1


0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
Cladribine conc. (M)
0.000
0.050
0.100
0.150
0.200
0.250
0.300
CD conc. (M)

U.S NATIONAL STAGE WORKSHEET (DO/EO)

U.S. APPL. NO. 10/551205 INTERNATIONAL APPL. US04/09387

APPLICATION FILED BY: 20 MOS., ____ OR 30 MOS., ____ SCREENED BY

28 Sep 2006

Francine Young
PCT International Division

INTERNATIONAL APPLICATION PAPERS IN THE APPLICATION FILE:

- / International application
- ___ Article 19 amendments
- / Priority Document(s) No. 3
- ___ Request Form PCT/RO/101
- ___ PCT/IB/302
- ___ PCT/IB/304
- ___ PCT/IB/306
- ___ PCT/IB/308
- ___ PCT/IB/331
- ___ OTHER PCT/IB/____
- ___ PCT/IPEA/409 also 416
- ___ 409 annexes to IPER
- / PCT/ISA/210 (Search report) EP
- ___ Search report References
- ___ Other Papers filed

WIPO PUBLICATION
PUBLICATION NO. *WO* WO 2004/087101
PUBLICATION DATE 14 OCT 2004
PUBLICATION LANG., English
NOT PUBLISHED
_____U.S. only _____Requested

RECEIVED FROM THE APPLICANT: (other than checked above)

- / National application basic fee paid
- / Express Processing Requested
- ___ Translation of the International Application
- ___ Used the IB copy of the IA
- ___ Description
- ___ Claims____
- ___ Drawings /
- ___ Foreign Language in drawing
- ___ Article 19 Amendments
- ___ Amendment used in application
- ___ Article 34 Amendment
- ___ Amendment used in application
- ___ DNA
- ___ 1194 transaction done
- / Preliminary Amendment(s) filed 28 Sep 2005
- ____ second submission_____
- ____ Information Disclosure Statement_____
- ____ second submission_____
- ____ Assignment
- ____Forward to Assignment Branch
- ____Substitute Specification_____
- ____ Small Entity Statement _____
- type _____
- ____ Oath/Declaration (date submitted_____)
- ___ Not executed
- ____ Executed
- ____ Power of Attorney
- ____ Change of Address

Datasheet

28 SEP 2005

35 USC Receipt of Request (PTO – 1399 Transmittal Letter)

Date Acceptable oath/declaration received

102(e) Date

Date complete 35 USC 371 requirements met

DATE NOTICE COMPLETED

DO/EO 903	Notice of Acceptance	
DO/EO 905	Notice of Missing Requirements	13 Sep 2006
DO/EO 917	Notice of A defective oath or declaration	
DO/EO 916	Notice of defective response	
DO/EO 913	Notice of defective translation	
DO/EO 909	Notifiication of Abandonment	

PATENT APPLICATION FEE DETERMINATION RECORD

Effective December 8, 2004

Application or Docket Number: 10/551205

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)	SMALL ENTITY TYPE ☐		OR	OTHER THAN SMALL ENTITY	
			RATE	FEE		RATE	FEE
U.S. NATIONAL STAGE FEES							
BASIC FEE	SMALL ENT. = $ 150	LARGE ENT. = $ 300	BASIC FEE		OR	BASIC FEE	300
EXAMINATION FEE	Satisfies PCT Article 33(1)-(4) = $ 50 / $ 100	All other situations = $ 100 / $ 200	EXAM. FEE			EXAM. FEE	200
SEARCH FEE	All other situations (ie. No Search Rpt.) = $ 250 / $ 500	U.S. is ISA = $ 50 / $ 100 ALL other countries = $ 200 / $ 400	SEARCH FEE			SEARCH FEE	400
FEE FOR EXTRA SPEC. PGS.	minus 100 =	/ 50 =	X $ 125 =			X $ 250 =	
TOTAL CHARGEABLE CLAIMS	78 minus 20 =	* 58	X $ 25 =		OR	X $ 50 =	
INDEPENDENT CLAIMS	4 minus 3 =	* 3	X $ 100 =		OR	X $ 200 =	
MULTIPLE DEPENDENT CLAIM PRESENT ☐			+ $ 180 =		OR	+ $ 360 =	
			TOTAL		OR	TOTAL	900

* If the difference in column 1 is less than zero, enter "0" in column 2

CLAIMS AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDI-TIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDI-TIONAL FEE
AMENDMENT A	Total	*	Minus	**	=	X $ 25 =		OR	X $ 50 =	
	Independent	*	Minus	***	=	X $ 100 =		OR	X $ 200 =	
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM ☐					+ $ 180 =		OR	+ $ 360 =	
						TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE	ADDI-TIONAL FEE	OR	RATE	ADDI-TIONAL FEE
AMENDMENT B	Total	*	Minus	**	=	X $ 25 =		OR	X $ 50 =	
	Independent	*	Minus	***	=	X $ 100 =		OR	X $ 200 =	
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM ☐					+ $ 180 =		OR	+ $ 360 =	
						TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than '20', enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than '3', enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

(FOR USE WITH FORM PTO-875)

SERIAL NO. 10/551205

FILING DATE

APPLICANT(S)

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
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TOTAL IND.		↓	3	↓		↓
TOTAL DEP.		←	32	←		←
TOTAL CLAIMS			35			

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
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98				/		
99						
100						
TOTAL IND.		↓	3	↓		↓
TOTAL DEP.		←	40	←		←
TOTAL CLAIMS			43			

PTO - 1360 (REV. 11/04)

U.S. DEPARTMENT of COMMERCE
Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
10/551,205	Nicholas S. BODOR	033935-021

INTERNATIONAL APPLICATION NO.	
PCT/US04/09387	
I.A. FILING DATE	PRIORITY DATE
03/26/2004	03/28/2003

21839
BUCHANAN, INGERSOLL & ROONEY PC
POST OFFICE BOX 1404
ALEXANDRIA, VA 22313-1404

CONFIRMATION NO. 4092

371 FORMALITIES LETTER

OC000000020413402

Date Mailed: 09/14/2006

NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as a Designated / Elected Office (37 CFR 1.495).

- Copy of the International Application filed on 09/28/2005
- Copy of the International Search Report filed on 09/28/2005
- Preliminary Amendments filed on 09/28/2005
- Request for Immediate Examination filed on 09/28/2005
- U.S. Basic National Fees filed on 09/28/2005
- Priority Documents filed on 09/28/2005

The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.

ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTHS FROM THE DATE OF THIS NOTICE OR BY 32 MONTHS FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

*A copy of this notice **MUST** be returned with the response.*

FRANCINE YOUNG

Telephone: (703) 308-9140 EXT 215

PART 2 - OFFICE COPY

U.S. APPLICATION NUMBER NO.	INTERNATIONAL APPLICATION NO.	ATTY. DOCKET NO.
10/551,205	PCT/US04/09387	033935-021

FORM PCT/DO/EO/905 (371 Formalities Notice)

U.S NATIONAL STAGE WORKSHEET (DO/EO)

U.S. APPL. NO. 10/551205 INTERNATIONAL APPL. US04/09387

APPLICATION FILED BY: 20 MOS., ____ OR 30 MOS., ___ SCREENED BY

28 Sep 2006

Francine Young
PCT International Division

INTERNATIONAL APPLICATION PAPERS IN THE APPLICATION FILE:

- ✓ International application
- ___ Article 19 amendments
- ✓ Priority Document(s) No. 3
- ___ Request Form PCT/RO/101
- ___ PCT/IB/302
- ___ PCT/IB/304
- ___ PCT/IB/306
- ___ PCT/IB/308
- ___ PCT/IB/331
- ___ OTHER PCT/IB/_____
- ___ PCT/IPEA/409 also 416
- ___ 409 annexes to IPER
- ✓ PCT/ISA/210 (Search report) EP
- ___ Search report References
- ___ Other Papers filed

WIPO PUBLICATION
PUBLICATION NO. *WO* 04/087101
PUBLICATION DATE 14 OCT 2004
PUBLICATION LANG., English
NOT PUBLISHED
_____U.S. only _____Requested

RECEIVED FROM THE APPLICANT: (other than checked above)

- ✓ National application basic fee paid
- ✓ Express Processing Requested
- ___ Translation of the International Application
- ___ Used the IB copy of the IA
- ✓ Description
- ✓ Claims
- ___ Drawings /
- ___ Foreign Language in drawing
- ___ Article 19 Amendments
- ___ Amendment used in application
- ___ Article 34 Amendment
- ___ Amendment used in application
- ___ DNA
- ___ 1194 transaction done
- ___ Preliminary Amendment(s) filed 25 Sep 2005
- ___ second submission_____
- ✓ Information Disclosure Statement 11-14-06
- ___ second submission_____
- ✓ Assignment 09/29/06
- ___ Forward to Assignment Branch
- ___ Substitute Specification_____
- ___ Small Entity Statement _____
- type _____
- ✓ Oath/Declaration (date submitted 11-14-06)
- ___ Not executed
- ✓ Executed
- ___ Power of Attorney
- ___ Change of Address

Datasheet 9/28/05 11/14/06 EARLY Processing 9/28/05

28 SEP 2005

35 USC Receipt of Request (PTO – 1399 Transmittal Letter)

Date Acceptable oath/declaration received 14 NOV 2006

102(e) Date

Date complete 35 USC 371 requirements met

DATE NOTICE COMPLETED

DO/EO 903 Notice of Acceptance

DO/EO 905 Notice of Missing Requirements 13 Sep 2006

DO/EO 917 Notice of A defective oath or declaration

DO/EO 916 Notice of defective response

DO/EO 913 Notice of defective translation

DO/EO 909 Notifilcation of Abandonment

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

10/11/2005 ATRAN1 00000106 10551205

01 FC:1631	300.00	OP
02 FC:1633	200.00	OP
03 FC:1632	500.00	OP
04 FC:1617	130.00	OP
05 FC:1615	2900.00	OP
06 FC:1614	600.00	OP

04/23/2007 MPERSON 00000009 10551205

01 FC:1642 400.00 OP

03 FC:1632 -500.00 OP
Repln. Ref: 04/23/2007 MPERSON 0012184500
DA#:024800 Name/Number:10551205
FC: 9204 $100.00 CR

PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 489-257/60033

AP13 Rec'd PCT/PTO 14 NOV 2006

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NO. 0056192-000024
		U.S. APPLICATION NO. (If known) 10/551,205
INTERNATIONAL APPLICATION NO. PCT/US2004/009387	INTERNATIONAL FILING DATE 26 March 2004	PRIORITY DATE CLAIMED 28 March 2003

TITLE OF INVENTION
ORAL FORMULATIONS OF CLADRIBINE

APPLICANT(S) FOR DO/EO/US
Nicholas S. Bodor et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☐ This is a **FIRST** submission of items concerning a submission under 35 U.S.C. 371.
2. ☒ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a submission under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected (Article 31).
5. ☐ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98 and copies of 18 references and ISR.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A preliminary amendment.
14. ☐ An Application Data Sheet under 37 CFR 1.76.
15. ☐ A substitute specification.
16. ☐ A power of attorney and/or change of address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.2 and 37 CFR 1.821-1.825.
18. ☐ A second copy of the published International Application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the International Application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information: Copy of Notice of Missing Requirements enclosed. (Surcharge of $130 was submitted on September 28, 2005). Supplemental Application Data Sheet (showing new address for second inventor). Letter noting change in Attorney Docket No.

U.S. APPLICATION NO. (If known)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
10/551,205	PCT/US2004/009387	0056192-000024

The following fees have been submitted:		CALCULATIONS	PTO USE ONLY
21. ☐	Basic National fee (37 CFR 1.492(a)) (1631) $ 300	$ 0	
22. ☐	Examination fee (37 CFR 1.492(c)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) (1643) $ 0 All other situations (1633) $ 200	$ 0	
23. ☐	Search fee (37 CFR 1.492(b)) If the written opinion of the ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) (1640) $ 0 Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority (1641) $ 100 International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB (1642) $ 400 All other situations (1632) $ 500	$ 0	
	TOTAL OF 21, 22 AND 23 =	$ 0	
☐	Additional fee for specifications and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) or computer program listing in an electronic medium) (37 CFR 1.492(j)). The fee is $ 250 (1681) for each additional 50 sheets of paper or fraction thereof.		

Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof (round up to a whole number)	RATE		
0	-100 =	0	/50 =	0	x $ 250	$ 0	

☐ Surcharge of $ 130 (1617) for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).					$ 0	
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total claims	0	- 20 =	0	x $ 50 (1615)	$ 0	
Independent Claims	0	- 3 =	0	x $ 200 (1614)	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ $ 360 (1616)	$ 0	
TOTAL OF ABOVE CALCULATIONS =					$ 0	
☐ Applicant claims small entity status. See 37 CFR 1.27. Fees above are reduced by 1/2					0	
SUBTOTAL =					$ 0	
Processing fee of $ 130 (1618) for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)). +					0	
TOTAL NATIONAL FEE =					$ 0	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).$ 40 per property +						
TOTAL FEES ENCLOSED =					$	
				Amount to be refunded:	$	
				Amount to be charged:	$	

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 02-4800 in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

d. ☐ Charge $ 40.00 to credit card. Form PTO-2038 is attached.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

SEND ALL CORRESPONDENCE TO:

Customer No **21839**
P.O. Box 1404
Alexandria, VA 22313-1404
703 836 6620

Date: November 14, 2006

Mary Katherine Baumeister
SIGNATURE

Mary Katherine Baumeister — 26254
NAME — REGISTRATION NO.

D056192-000024

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
10/551,205	Nicholas S. BODOR	033935-021

INTERNATIONAL APPLICATION NO.	
PCT/US04/09387	
I.A. FILING DATE	PRIORITY DATE
03/26/2004	03/28/2003

21839
BUCHANAN, INGERSOLL & ROONEY PC
POST OFFICE BOX 1404
ALEXANDRIA, VA 22313-1404

CONFIRMATION NO. 4092

371 FORMALITIES LETTER

OC000000020413402

Date Mailed: 09/14/2006

NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as a Designated / Elected Office (37 CFR 1.495).

- Copy of the International Application filed on 09/28/2005
- Copy of the International Search Report filed on 09/28/2005
- Preliminary Amendments filed on 09/28/2005
- Request for Immediate Examination filed on 09/28/2005
- U.S. Basic National Fees filed on 09/28/2005
- Priority Documents filed on 09/28/2005

NHS/MKB
BUCHANAN INGERSOLL PC
SEP 18 2006
DOCKETED 9-18-06

The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.

ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTHS FROM THE DATE OF THIS NOTICE OR BY 32 MONTHS FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

*A copy of this notice **MUST** be returned with the response.*

Missing Parts 156 Due 11-14-06

SEP 13

FRANCINE YOUNG

Telephone: (703) 308-9140 EXT 215

PART 1 - ATTORNEY/APPLICANT COPY

U.S. APPLICATION NUMBER NO.	INTERNATIONAL APPLICATION NO.	ATTY. DOCKET NO.
10/551,205	PCT/US04/09387	033935-021

FORM PCT/DO/EO/905 (371 Formalities Notice)

Patent
Attorney's Docket No. 0056192-000024
(Formerly 033935-021)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	**MAIL STOP PCT**
Nicholas S. Bodor et al.	Group Art Unit:
Application No.: 10/551,205	Examiner:
Filed: PCT/US2004/009387 filed March 26, 2004	Confirmation No.: 4092
For: ORAL FORMULATIONS IN CLADRIBINE	

LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The subject application has been assigned a new Attorney Docket No. of 0056192-000024. Please mark your records accordingly and refer to the new number in all future communications related to this patent application.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date: November 14, 2006

By: Mary Katherine Baumeister
Mary Katherine Baumeister
Registration No. 26,254

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

COMBINED DECLARATION AND POWER OF ATTORNEY FOR UTILITY OR DESIGN PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ORAL FORMULATIONS OF CLADRIBINE

the specification of which **(check only one item below)**:

☐ is attached hereto.

☐ was filed as United States Patent application Number _____ on _____ and was amended on _____ (if applicable).

☒ was filed as PCT International application Number PCT/US2004/009387 on March 26, 2004 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §§ 119 (a)-(d), 172 or 365(a) of any foreign application(s) for patent or inventor's certificate or of any international (PCT) application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international (PCT) application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §§119(a)-(d), 172 or 365(a):				
COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (MM/DD/YYYY)	PRIORITY CLAIMED UNDER 35 U.S.C. §§119, 172 OR 365(a)	
			Yes	No

I hereby appoint the attorneys and agents associated with the following PTO Customer Number of Buchanan Ingersoll PC (including attorneys from Burns, Doane, Swecker & Mathis) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and transact all business in connection with international applications directed to said invention:

Customer Number **21839**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	Nicholas S. Bodor
Signature	Nicholas S. Bodor
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FULL NAME SECOND INVENTOR, IF ANY	Yogesh Dandiker
Signature	Y. Dandiker
Date	11/26/05
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Citizenship	United Kingdom
Mailing Address	57 FENN AVENUE
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FULL NAME OF THIRD INVENTOR, IF ANY	
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	

SUPPLEMENTAL APPLICATION DATA SHEET

Application Information

Application Number::	10/551,205
Filing Date::	
Application Type::	Regular
Subject Matter::	Utility
Suggested Classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	
Number of CD Disks::	
Number of Copies of CDs::	
Sequence Submission?::	
Computer Readable Form (CRF)?::	
Number of Copies of CRF::	
Title::	ORAL FORMULATIONS OF CLADRIBINE
Attorney Docket Number::	0056192-000024
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	1
Total Drawing Sheets::	1
Small Entity?::	No

Latin Name::

Variety Denomination Name::

Petition Included?:: No

Petition Type::

Licensed US Govt. Agency::

Contract or Grant Numbers::

Secrecy Order in Parent Appl.?:: No

Applicant Information

Applicant Authority Type:: Inventor

Primary Citizenship Country:: United States

Status:: Full Capacity

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Middle Name:: S.

Family Name:: BODOR

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Country of Mailing Address::	United States
Postal or Zip Code of Mailing Address::	33154
Applicant Authority Type::	Inventor
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Status::	Full Capacity
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Middle Name::	
Family Name::	DANDIKER
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Correspondence Information

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Phone Number:: (703) 836-6620

Fax Number: (703) 836-2021

Representative Information

Representative Customer Number:: 21839

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	National Stage of	PCT/US2004/009387	March 26, 2004
PCT/US2004/009387	Claims benefit under 35 U.S.C. §119(e) of	60/458,922	March 28, 2003
PCT/US2004/009387	Claims benefit under 35 U.S.C. §119(e) of	60/484,756	July 2, 2003
PCT/US2004/009387	Claims benefit under 35 U.S.C. §119(e) of	60/541,247	Feb 4, 2004

Foreign Priority Information

Country::	Application Number::	Filing Date::	Priority Claimed::

Assignee Information

Assignee Name::	ARES TRADING S.A.
Street of Mailing Address::	Zone Industrielle D L'Ouriettaz
City of Mailing Address::	Aubonne
State or Province of Mailing Address::	
Country of Mailing Address::	Switzerland
Postal or Zip Code of Mailing Address::	CH-1170

Attorney's Docket No. 0056192-000024

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	**MAIL STOP PCT**
Nicholas S. Bodor et al.	)	Group Art Unit:
Application No.: 10/551,205	)	Examiner:
Filed: PCT/US2004/009387 filed March 26, 2004	)	Confirmation No.: 4092
For: ORAL FORMULATIONS OF CLADRIBINE	)	

FIRST INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the duty of disclosure as set forth in 37 C.F.R. § 1.56, the accompanying information is being submitted in accordance with 37 C.F.R. §§ 1.97 and 1.98. Applicants request the Examiner's consideration of the documents listed on the accompanying Form PTO-1449. All of these documents are cited in the instant specifiation, including the documents cited in the International Search Report (copy enclosed) which was issued in connection with PCT/US2004/009387, filed March 26, 2004, of which this application is the national phase.

Pursuant to 37 C.F.R. § 1.98, a copy of each of the documents cited is enclosed. However, copies of any listed U.S. patents and U.S. patent application publications are not enclosed since it is no longer required according to the July 11, 2003 waiver of the requirement for copies of cited U.S. patents and U.S. patent application publications in national patent applications filed after June 30, 2003 and international applications entering the national stage under 35 U.S.C. § 371 after June 30, 2003.

This Statement, Form PTO-1449 and documents are being submitted within three (3) months of the filing or entry of the national stage of this application or before the first Office Action on the merits, whichever is later. Since these documents are being filed within the time period set forth in 37 C.F.R. § 1.97(b), no fee or statement is required.

The following remarks are offered with respect to the listed documents which are not in English:

DE 31 18 218 is in German. Applicants do not have an English translation. However, an English abstract, together with the citation of the document in the instant specification, are provided to serve as a brief statement of relevance. Applicants consider this a general state of the art reference.

DE 33 17 064 is in German. Applicants do not have an English translation but provides herewith an English abstract. The abstract and citation of the document in the specification serve as a brief statement of relevance. Applicants consider this a general state of the art reference.

EP 0 149 197 B1 is in German, although the claims are also present in English. Applicants enclose a full English translation of this document, which is cited in the instant specification and which applicants consider to be a general state of the art reference.

It is respectfully requested that an Examiner-initialed copy of the accompanying Form PTO-1449 be returned to the undersigned.

Respectfully submitted,

BUCHANAN INGERSOLL AND ROONEY PC

Date: November 14, 2006

By: Mary Katherine Baumeister
Mary Katherine Baumeister
Registration No. 26254

P.O. Box 1404
Alexandria, VA 22313-1404
703 836 6620

Substitute for form 1449/PTO & 1449B/PTO

FIRST INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 2

Complete if Known

Application Number	10/551,205
Filing Date	
First Named Inventor	Nicholas S. Bodor
Examiner Name	
Attorney Docket No.	0056192-000024

U.S. PATENT DOCUMENTS

Examiner Initials	Document Number	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Issue/Publication Date (MM-DD-YYYY)
	4,383,992		Lipari	05-17-1983
	6,239,118	B1	Schatz et al.	05-29-2001
	5,424,296		Saven et al.	06-13-1995
	5,510,336		Saven et al.	04-23-1996
	5,506,214		Beutler	04-09-1996
	4,659,696		Hirai et al.	04-21-1987
	3,459,731		Gramera et al.	08-05-1969
	4,478,995		Shinoda et al.	10-23-1984
	5,310,732		Carson et al.	05-10-1994
	5,401,724		Beutler	03-28-1995
	5,106,837		Carson et al.	04-21-1992
	4,497,803		Harada et al.	02-05-1985
	6,194,395	B1	Schultz et al.	02-27-2001
	6,407,079	B1	Müller et al.	06-18-2002
	4,870,060		Müller	09-26-1989
	4,727,064		Pitha	02-23-1988
	4,596,795		Pitha	06-24-1986
	4,764,604		Müller	08-16-1988
	4,535,152		Szejtli et al.	08-13-1985

FOREIGN PATENT DOCUMENTS

Examiner Initials	Document Number	Kind Code (if known)	Country	Date of Publication (MM-DD-YYYY)	STATUS						
					Translation	Partial Translation	Eng. Lang. Summary	Search Report	IPER	Abstract	Cited in Spec
	0 197 571	A2	EP	10-15-1986							X
	90/12035	A1	WO	10-18-1990							X
	31 18 218	A1	DE	04-22-1982						X	X
	33 17 064	A1	DE	11-15-1984						X	X
	2 189 245	A	GB	10-21-1987							X
	0 149 197	B1	EP	07-24-1985	X						X
	0 094 157	A1	EP	11-16-1983							X

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.
	Tarasiuk et al., "*Stability of 2-Chloro-2'-Deoxyadenosine at Various pH and Temperature*", Archivum Immunologiae et Therapiae Experimentalis, Vol. 42, pp. 13-15, 1994, published by Birkhauser Publishers Ltd., Basel, Switzerland
	Romine et al., "*A Double-Blind, Placebo-Controlled, Randomized Trial of Cladribine in Relapsing-Remitting Multiple Sclerosis*", Proceedings of the Association of American Physicians, Vol. 111, No. 1, pp. 35-44, 1999, published by Blackwell Publishing, Malden, MA

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with M.P.E.P. § 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

Substitute for form 1449/PTO & 1449B/PTO

FIRST INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	10/551,205
Filing Date	
First Named Inventor	Nicholas S. Bodor
Examiner Name	
Attorney Docket No.	0056192-000024

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.
	Tortorella et al., Current Opinion on Investigational Drugs, 2(12), pp. 1751-1756, 2001, published by PharmaPress Ltd., London, GB
	Selby et al., "*Safety and Tolerability of Subcutaneous Cladribine Therapy in Progressive Multiple Sclerosis*", Can. J. Neurol Sci., Vol. 25, pp. 295-299, 1998, published by Canadian Journal of Neurological Science, Calgary, Canada
	Rice et al., "*Cladribine and progressive MS Clinical and MRI outcomes of a multicenter controlled trial*", Neurology, Vol. 54, pp. 1145-1155, 2000, published by Lippincott Williams and Wilkins, Hagerstown, MD
	Liliemark et al., "*On the Bioavailability of Oral and Subcutaneous 2-Chloro-2'-Deoxyadenosine in Humans: Alternative Routes of Administration*", Journal of Clinical Oncology, Vol. 10, No. 10, pp. 1514-1518, 1992, published by American Society of Clinicial Oncology, Alexandria, VA
	Karlsson et al., "*Oral cladribine for B-cell chronic lymphocytic leukaemia: report of a phase II trial with a 3-d, 3-weekly schedule in untreated and pretreated patients, and a long-term follow-up of 126 previously untreated patients*", British Journal of Haematology, Vol. 116, pp. 538-548, 2002, published by Blackwell Science Ltd., Oxford, UK
	Liliemark, "*The Clinical Pharmacokinetics of Cladribine*" Clin. Pharmacokinet, Vol. 32 (2), pp. 120-131, 1997, published by Adis International Limited, Wolters Kluwer Health, Yardley, PA
	Nakai et al., "*Effects of Grinding on the Physical and Chemical Properties of Crystalline Medicinals with Microcrystalline Cellulose V: Comparison with Tri-O-methyl-β-cyclodextrin Ground Mixtures*", Chem. Pharm. Bulletin, Vol. 28(5), pp. 1552-1558, 1980, published by Pharmaceutical Society of Japan, Tokyo, Japan
	Saenger, "*Clyclodextrin Inclusion Compounds in Research and Industry*", Angew. Chem. Int. Ed. Engl., Vol. 19, pp. 344-362, 1980, published by Verlag Chemie, GmbH, Weinheim, Germany
	Tang et al., "*Design of Freeze-Drying Processes for Pharmaceuticals: Practical Advice*", Pharmaceutical Research, Vol. 21, No. 2, pp. 191-200, 2004, Springer, The Netherlands

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with M.P.E.P. § 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 197 571
A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 86200334.0

(22) Date of filing: 04.03.86

(51) Int. Cl.⁴: C 08 B 37/16
A 61 K 31/735

(30) Priority: 15.03.85 GB 8506792

(43) Date of publication of application:
15.10.86 Bulletin 86/42

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

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(72) Inventor: Müller, Bernd Willi Werner
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(54) Novel derivatives of gamma-cyclodextrin.

(57) Novel γ-cyclodextrin derivatives which are γ-cyclodextrin substituted with C_1-C_6 alkyl, hydroxy C_1-C_6 alkyl, carboxy C_1-C_6 alkyl or C_1-C_6 alkyloxycarbonylC_1-C_6 alkyl or mixed ethers thereof and their preparation. Compositions comprising such cyclodextrin derivatives and an active ingredient which is preferably a drug and the preparation of such compositions.

EP 0 197 571 A2

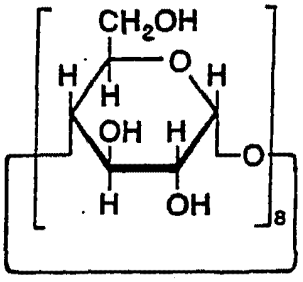
BEST AVAILABLE COPY

NOVEL DERIVATIVES OF γ-CYCLODEXTRIN.

Background of the invention

The present invention is concerned with new ethers of γ-cyclodextrin, their preparation and their use as complexants for chemicals and pharmaceuticals.

γ-cyclodextrin (γ-CD) is a cyclic oligosaccharide consisting of 8 glucose units which are joined together by α(1-4) linkages.



γ-CD is prepared by the enzymatic cleavage and religation of starch and a subsequent separation from the thus obtained cyclodextrin mixture containing i.a. α-cyclodextrin (containing 6 glucose units), ß-cyclodextrin (ß-CD) (7 glucose units) and γ-cyclodextrin (γ-CD).

Cyclodextrins are known in the art to possess the ability to form inclusion complexes and to have concomitant solubilizing properties. An exhaustive review which describes such complexes and their properties can be found in W. Sänger, Angewandte Chemie, 92, 343-361 (1981).

Derivatives of cyclodextrins are also known to possess the above-mentioned properties. Said derivatives have been reviewed in an article by A.P. Croft and R.A. Bartsch in Tetrahedron, 39, 1417-1474 (1983). More particularly, the German Offenlegungsschrift DE 3118218 discloses the 2,6-dimethyl derivatives of ß-CD, while in U.S. Patent No. 3,459,731 there are described hydroxyethyl, hydroxypropyl and hydroxypropyl/hydroxyethyl ethers of ß-CD. Furthermore, in U.S. Patent Appl. Ser. No. 6-603 839 there is described the use of specific derivatives of cyclodextrines to improve the systemic administration of sex hormones. Most of the cyclodextrin derivatives presently known in the art are derived from ß-CD, while the derivatives of α-CD and particularly of γ-CD remain relatively unknown.

The use of derivatives of ß-CD has the following advantages. ß-CD is only poorly water soluble and therefore it is disadvantageous to use it as a complexant and solubilizer. Derivatives of ß-CD on the other hand, due to their increased solubility, are more suitable complexants and solubilizers. In contrast herewith, α-CD and γ-CD having an excellent water solubility do not need such substitutions. Hence, it is obvious to use unsubstituted γ-CD (and α-CD) as complexant and solubilizer. Particularly for γ-CD, a number of such complexes with various useful compounds can be found in e.g. Int. J. Pharm. 10, 1-15 (1982) with steroid hormones, in Acta Pharm. Suec. 20, 11-20 (1983) with flurtripofen, in Chem. Pharm. Bull. 31, 286-291 (1983) with spirolacton and in Acta Pharm. Suec. 20, 287-294 (1983) with proscillaridin.

γ-CD does not form such inclusion complexes with any given compound. Often, such complexation is only established in the lower concentration range. At higher concentrations of γ-CD, the formed complex is precipitated.

It has now been found that an appropriately alkylated, hydroxyalkylated, carboxyalkylated or (alkyloxycarbonyl)alkylated form of γ-CD or a mixed ether thereof prevents the crystallization of such complexes. The advantages of γ-CD over its lower homologues, i.e. its larger cavity resulting in a superior propensity to form inclusion complexes, its favourable toxicological properties and the fact that it can be cleaved enzymatically by α-amylase (in contrast with ß-CD), can therefore fully be exploited.

γ-CD contains three free hydroxy functions per glucose unit which can completely or partially be derivatized. In view of this, the average degree of substitution (D.S.) is introduced, which is the average number of substituted hydroxy functions per glucose unit. Said D.S. can vary from its minimal value 0.125 up to its maximal value 3. In the latter case all 24 hydroxy groups are substituted, while in the former case only one is substituted. A minimal D.S. is especially preferred when γ-CD is used as solubilizer of pharmaceuticals for use in parenteral applications, while a higher D.S. is preferred when used in technical applications, such as, for example, for pesticides or enzymes. In the latter instance, the higher D.S. causes that also those hydroxy groups are functionalized which are located in the cavity of the γ-CD molecule. Consequently, the diameter of the cavity is decreased. By selecting the appropriate D.S. the size of the cavity can be adapted in order to obtain the optimum space required for a certain molecule to appropriately fit into the cavity of the cyclodextrin.

When introducing hydroxyalkyl substitutions on γ-CD, the hydroxy function of the thus obtained hydroxyalkyl ether group can further be hydroxyalkylated, generating multiple substitutions on one particular OH-group. In such cases the term average molar substitution (M.S.) is introduced. Said M.S. is defined as the average number of moles of the substituting agent per glucose unity. In view of this, it is evident that the M.S. can be greater than 3 and has, theoretically, no upper limit.

Description of the preferred embodiments.

The present invention is concerned with novel γ-CD derivatives, said novel γ-CD derivatives being γ-CD substituted with C_1-C_6 alkyl, hydroxy C_1-C_6 alkyl, carboxy C_1-C_6 alkyl or C_1-C_6 alkyloxycarbonylC_1-C_6 alkyl or mixed ethers thereof.

In the foregoing definitions the term "C_1-C_6-alkyl" is meant to include straight and branched saturated hydrocarbon radicals, having from 1 to 6 carbon atoms, such as, methyl, ethyl, 1-methylethyl,

1,1-dimethylethyl, propyl, 2-methylpropyl, butyl, pentyl, hexyl and the like.

Preferred compounds are those γ-CD derivatives being γ-CD substituted with C_1-C_3 alkyl, hydroxy C_2-C_4 alkyl, carboxy C_1-C_2 alkyl or (C_1-C_2 alkyloxycarbonyl)C_1-C_2 alkyl or mixed ethers thereof.

Particularly preferred new compounds are the methyl, ethyl, isopropyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, carboxymethyl and carboxyethyl substituted γ-cyclodextrins and further the (methyl)(hydroxyethyl), (methyl)(hydroxypropyl) and (methyl)(hydroxyethyl)(carboxymethyl) substituted γ-cyclodextrins having a D.S. or M.S. of from 0.125 to 3, more preferably of from 0.3 to 2.

The compounds of the present invention can generally be prepared by reacting the starting γ-CD with an appropriate O-alkylating agent or a mixture of such agents in a concentration being selected so that the desired D.S. is obtained. The said reaction is preferably conducted in a suitable solvent in the presence of an appropriate base. An appropriate O-alkylating agent is, for example, an alkyl, hydroxyalkyl, carboxyalkyl or (alkyloxycarbonyl)alkyl halide or sulfonate, e.g. methyl chloride, ethyl bromide, propyl methylsulfonate, ethyl chloroacetate, α-chloroacetic acid; or an oxirane, e.g. oxirane, methyloxirane. Suitable solvents are, for example, water; an alcohol or polyalcohol, e.g. methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 1,2-ethanediol, 1,2-propanediol and the like; a ketone, e.g. 2-propanone, 2-butanone, 4-methyl-2-pentanone, and the like; an ether or polyether, e.g. ethoxyethane, 2-(2-propyloxy)propane, tetrahydrofuran, 1,2-dimethoxyethane and the like; and C_1-C_4-alkyloxy-C_2-C_3-alkanol and mixtures of such solvents. An appropriate base is, for example, an alkali or earth alkaline metal hydroxide, e.g. sodium hydroxide, potassium hydroxide; or an alkali or earth alkaline metal hydride or amide, e.g. sodium hydride, calcium hydride, sodium amide and the like bases.

Preferably the said O-alkylation reaction is conducted with 0.1 to 3 parts by weight of water per part by weight γ-CD in case there is no organic solvent used, and with 1 to 40 parts by weight organic solvent per part by weight γ-CD in case no water is used.

In a particularly preferred way of preparing the compounds of the present invention, the reaction mixture containing the starting γ-CD, the solvent, base and O-alkylating agent is heated in an autoclave at a temperature comprised between 30° and 200°C. Depending on the reactivity of the O-alkylating agent, the reaction mixture is allowed to react at this temperature for 15 minutes up to 24 hours. Subsequently, the mixture is acidified and the reaction product is isolated and purified by standard separation and purification procedures such as, for example, column chromatography, ultra filtration, centrifugation, and dried.

The compounds of the present invention can also be converted into each other. For example, the (alkyloxycarbonyl)alkyl substituted γ-cyclodextrines may conveniently converted to the corresponding carboxyalkyl substituted γ-cyclodextrines following art-known saponification procedures, e.g. by treating the starting compounds with an aqueous acidic or basic solution.

The compounds of the present invention are useful due to their ability to form inclusion complexes having a stabilizing effect on the complexed compounds, and due to their concomitant solubilizing activity. Compounds exhibiting a significantly increased water solubility and improved stability after having been transferred to inclusion complexes with the above-mentioned γ-CD derivatives, are those having the required shape and size, i.e. which fit into the cavity. The size of the cavity can be adapted by selecting the appropriate γ-CD derivatives having a suitable D.S. Examples of such compounds are, for example, non-steroid anti-rheumatic agents, steroids, cardiac glycosides and derivatives of benzodiazepine, benzimidazole, piperidine, piperazine,

imidazole, triazole, pyridazine, 1,2,4-triazinedione or 2,3,5,6-tetrahydro-imidazo[2,1-b]thiazoles, or amides, hydratropic acid derivatives or trialkylamines, whereby the derivatives of benzodiazepine, benzimidazole, piperidine, piperazine, imidazole, triazole, pyridazine, 1,2,4-triazinedione or 2,3,5,6- tetrahydro-imidazo[2,1-b]thiazole, or amides, hydratropic acid derivatives or trialkylamines are preferred.

Useful benzimidazole derivatives are thiabendazole, fuberidazole, ciclobendazole, oxibendazole, parbendazole, cambendazole, mebendazole, fenbendazole, flubendazole, albendazole, oxfendazole, nocodazole and astemizole.

Suitable piperidine derivatives are diphenoxylate, phenoperidine, haloperidol, haloperidol decanoate, bromperidol decanoate, bromperidol, moperone, trifluperidol, pipamperone, piritramide, fentanyl, benperidol, droperidol, benzitramide, benzetimide, domperidone, sufentanil, carfentanil, alfentanil, dexetimide, milenperone, difenoxin, fluspirilene, penfluridol, pimozide,, lorcainide, loperamide, astemizole, ketanserine, levocabastine, cisapride, altanserin, ritanserin, 3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,7-dimethyl-4H-pyrido[1,2-a]pyrimidin-4-one, 3-[2-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]-ethyl]-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one and 3-[2-[4-[[3-(2-furanylmethyl)-3H-imidazo[4,5-b]pyridin-2-yl]amino]-1-piperidinyl]ethyl]-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one. Suitable piperazine derivatives include azaperone, fluanisone, lidoflazine, flunarizine, mianserine, oxatomide, mioflazine, clocinizine and cinnarizine.

Examples of suitable imidazole derivatives are metronidazole, ornidazole, ipronidazole, tinidazole, isoconazole, nimorazole, miconazole, burimamide, metiamide, metomidate, enilconazole or imazalil, etomidate, econazole, clotrimazole, carnidazole, cimetidine, doconazole, sulconazole, parconazole, orconazole, butoconazole, triadiminole, tioconazole, valconazole, fluotrimazole, ketoconazole, oxiconazole, lombazole, bifonazole, oxmetidine, fenticonazole, tubulazole and (Z)-1-[2-chloro-2-(2,4-dichlorophenyl)ethenyl]-1H-imidazole.

As suitable triazole derivatives there may be mentioned virazole, azaconazole, etaconazole, propiconazole, penconazole, itraconazole and terconazole.

Useful pyridazine derivative are, for example, 3-chloro-6-[3,6-dihydro-4-(3-methylphenyl)-1(2H)-pyridinyl]pyridazine, 3-methoxy-6-[4-(3-methylphenyl)-1-piperazinyl]pyridazine and the compounds of Publ. Eur. Pat. Appl. No. 0,156,433.

Useful 1,2,4-triazinediones are, for example, 2-chloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)benzeneacetonitrile, 2,6-dichloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)benzeneacetonitrile and the compounds of Publ. Eur. Pat. Appl. No. 0,170,316.

Useful trialkylamines are, for example, diisopromine, prozapine.

Useful 2,3,5,6-tetrahydro-imidazo[2,1-b]thiazoles comprise, for example, tetramisole or levamisole.

Useful amides are, for example, closantel, ambucetamide, isopropamide, buzepide metiodide, dextromoramide.

A useful hydratropic acid derivative is, for example, suprofen.

Particularly valuable pharmaceutical compositions are obtained when converting etomidate, ketoconazole, tubulazole, itraconazole, levocabastine or flunarizine into a water-soluble form using the complex forming agents of the invention. Such compositions are therefore a special subject of the present invention.

The invention is further directed to a method of preparing compositions of sparingly water-soluble or water-instable compounds which method is characterized by dissolving the γ-cyclodextrin ether in water and adding thereto the selected compound as well as optionally drying the solution of the formed inclusion compound using methods known per se. Formation of the solution may preferably take place at temperatures between 15 and 35°C.

The drug is suitably added batchwise. The water may further comprise physiologically compatible compounds such as sodium chloride, potassium nitrate, glucose, mannitol, sorbitol, xylitol or buffers such as phosphate, acetate or citrate buffer.

Using γ-cyclodextrin ethers in accordance with the invention it is possible to prepare commonly known application forms of drugs for oral, parenteral, topical, rectal or vaginal application, e.g. infusion and injection solutions, drop solutions (e.g. eye drops or nasal drops), sprays, tablets, powders, capsules, aerosols, sirups, jellies, ointments, medical baths, rectalia and vaginalia.

The aqueous solutions may further comprise suitable physiologically compatible preserving agents such as, for example, quaternary ammonium soaps, chlorbutanol, phenoxetol, bromopol, and the like, and also anti-oxidantia, such as, for example, ascorbic acid.

For the preparation of solid formulations the solutions of the inclusion compounds are dried using conventional methods; thus the water may be evaporated in a rotation evaporator or by lyophilisation. The residue is pulverized and, optionally after addition of further inert ingredients, converted into uncoated or coated tablets, suppositories, capsules, creams or ointments.

Examples

The following examples are meant to illustrate and not to limit the present invention in all its aspects. Unless stated otherwise, all parts therein are by weight.

A. Preparation examples.

Example 1

1 Part of γ-CD and a solution of 1.5 parts of sodium hydroxide in 1.5 parts of water were mixed in an autoclave. Then there were added 3 parts of methyl chloride and 0.5 parts of methyloxirane. The mixture was heated for 1 hour at 65°C and subsequently for 2 hours at 100°C. After cooling, the remaining methyloxirane was expelled and the reaction mixture was neutralized with hydrochloric acid. The volatile components were evaporated and the remainder was filtered. The filtrate was liberated from sodium chloride over an ion exchanger and subsequently freeze-dried, yielding the (methyl)(hydroxypropyl) derivative of γ-CD. Following the same procedures and using the appropriate starting materials the (ethyl)(hydroxyethyl) derivative of γ-CD was also prepared.

Example 2

In an autoclave there were mixed 2.5 parts of 1,2-dimethoxyethane, 1 part of γ-CD and a solution of 1 part of sodium hydroxide in 1.2 parts of water. To this mixture, there were added 2 parts of oxirane and the whole was heated to 110°C for 5 hours. After cooling, the remaining oxirane was expelled and the reaction mixture was neutralized with hydrochloric acid. The volatile components were evaporated and the remainder was filtered. The filtrate was subsequently liberated from sodium chloride over an ion exchanger and subsequently freeze-dried, yielding the hydroxyethyl derivative of γ-CD with a M.S. of 0.77.

Following the same procedures and using the appropriate starting materials there was also prepared the 2-hydroxypropyl derivative of γ-CD with a M.S. of 0.66.

Example 3

1 Part of γ-CD, 3 parts of 1,2-dimethoxyethane and 1.5 parts of sodium hydroxide in 1.5 parts of water were mixed in an autoclave. Subsequently, there were added 4 parts of chloromethane and the whole was heated at 120°C for 4 hours. After cooling the reaction mixture was neutralized with hydrochloric acid and the volatile components evaporated. The remainder was filtered and the filtrate was liberated fom sodium chloride over an ion exhanger and subsequently freeze-dried, yielding the methyl derivative of γ-CD with a D.S. of 1.49.

Following the same procedures and using the appropriate starting materials there were also prepared the methyl derivative of γ-CD with a D.S. of 0.13; the carboxymethyl derivative of γ-CD with a D.S. of 0.86; and the (ethoxycarbonyl)methyl derivative of γ-CD; the ethyl derivative of γ-CD; the butyl derivative of γ-CD; the isobutyl derivative of γ-CD; the isopropyl derivative of γ-CD; the carboxyethyl derivative of γ-CD; the 3-hydroxypropyl derivative of γ-CD; and the 4-hydroxybutyl derivative of γ-CD.

B. Examples illustrating the properties of the γ-CD derivatives

Example 4

Starting from a 5% stock solution of a particular γ-CD derivative in a phosphate buffer of pH 7.4, a dilution series was obtained with concentrations varying of from 0% to 5% with 0.5% steps. 3 ml of these solutions were pipetted into a closed container containing an appropriate amount of progesteron. After 5 days shaking at 25°C, the thus obtained mixture was filtered over a membrane filter (pore diameter: 0.22 µm), and the content of progesteron was determined with high pressure liquid chromatography (using a column of 25 cm length; 5 mm internal diameter; packed with 5 µm ODS-hypersil (RP-18); eluent: acetonitrile/water; U.V. detection). The results of these concentration measurements for a number of the γ-CD derivatives of the present invention and for unsubstituted γ-CD gathered in the following table.

concentration of γ-CD derivative in % (weight by volume)	content of progesteron in µg/ml					
	unsubsti-tuted γ-CD	methyl substi-tuted D.S.=0.13	methyl substi-tuted D.S.=1.49	carboxy-methyl subst. M.S.=0.86	hydroxy ethyl subst. M.S.=0.77	hydroxy propyl subst. M.S.=0.66
0	5.9	5.9	5.9	5.9	5.9	5.9
0.5	425	488	379	102	234	302
1	343	972	748	209	452	582
1.5	275	1458	1144	313	673	872
2	203	1902	1470	417	860	1165
2.5	163	2149	1888	517	1055	1431
3	93	2258	2260	610	1291	1704
3.5	60	2392	2686	79	1472	1987
4	54	2592	3050	796	1722	2287
4.5	46	2627	3411	891	1817	2595
5	45	2602	3876	979	2065	2865

Table: Content of progesteron in solutions containing various concentrations of γ-CD derivative and γ-CD.

Example 5.

Following the procedures described in example 4 the content of 3-chloro-6-[3,6-dihydro-4-(3-methylphenyl)-1(2H)-pyridinyl]pyridazine was determined in solutions containing various concentrations of γ-CD derivatives. Said pyridazine compound is described in Published Europ. Pat. Appl. No. 0,156,433 as a useful anti-viral agent.

concentration of γ-CD derivative in % (weight by volume)	content of 3-chloro-6-[3,6-dihydro-4-(3-methylphenyl)-1(2H)-pyridinyl]pyridazine in µg/ml		
	unsubstituted γ-CD	methyl-substituted D.S.=1.49	hydroxypropyl substituted M.S.=0.66
0	0.4	0.4	0.4
1	2.0	2.0	1.5
2.5	0.8	8.0	4.5
3.5	-	12.6	7.0
5	0.8	20.0	10.0

C. Composition examples.

Example 6

In 100 ml water 7 g hydroxyethyl-γ-CD (M.S. = 0.77) and 0.5 g medroxyprogesterone acetate were dissolved. The water was evaporated. 75 mg of the residue was powdered and mixed with 366 mg $CaHPO_4.2H_2O$, 60 mg corn starch, 120 mg cellulose powder (microcrystalline), 4.2 mg highly dispersed silica (Aerosil® 200) and 4.8 mg magnesium stearate and pressed to a tablet.

Example 7

5 g hydroxyethyl γ-cyclodextrin (M.S. = 0.77) and 0.5 g lidocaine were dissolved in 100 ml of a physiological sodium chloride solution at 30°C and filtered through a membrane filter (0.45 microns). The solution was filled into ampules and sterilized.

CLAIMS

1. A γ-cyclodextrin ether or mixed ether wherein the ether substituents are C_1-C_6 alkyl, hydroxy C_1-C_6 alkyl, carboxy C_1-C_6 alkyl or (C_1-C_6 alkyloxycarbonyl)C_1-C_6 alkyl.

2. A γ-cyclodextrin ether or mixed ether according to claim 1 wherein the ether substituents are C_1-C_3 alkyl, hydroxy C_2-C_4 alkyl or carboxy C_1-C_2 alkyl.

3. A γ-cyclodextrin derivative according to claim 1, wherein the substituents are methyl, ethyl, isopropyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, carboxymethyl and carboxyethyl.

4. A γ-cyclodextrin derivative according to any of claims 1-3 wherein the degree of substitution is in the range of 0.125 to 3 and the M.S. is in the range of 0.125 to 10.

5. A γ-cyclodextrin derivative according to any of claims 1-3, wherein the degree of substitution is in the range of 0.3 to 2 and the M.S. is in the range of 0.3 to 3.

6. A process for preparing a compound as defined in any of the claims 1-5, characterized by reacting γ-cyclodextrin with an O-alkylating agent.

7. A process according to claim 6, wherein the process is conducted in a reaction-inert solvent in the presence of a base and at a temperature in the range from 30° to 200°.

8. A composition comprising an active ingredient and a γ-cyclodextrin derivative as claimed in any of claims 1-5.

9. A composition according to claim 8, wherein the active ingedient is a drug.

10. A composition according to claim 9, wherein the drug is a non-steroid anti-rheumatic agent, a steroid, a cardiac glycoside or a derivative of benzodiazepine, benzimidazole, piperidine, piperazine, imidazole, triazole, pyridazine, 1,2,4-triazinedione, 2,3,5,6-tetrahydroimidazo[2,1-b]thiazole or hydratropic acid, or an amide or trialkylamine derivative.

11. A composition according to claim 8 wherein the drug is etomidate.

12. A composition according to claim 8 wherein the drug is ketoconazole.

13. A composition according to claim 8 wherein the drug is itraconazole.

14. A composition according to claim 8 wherein the drug is flunarizine.

15. A method of preparing a composition according to anyone of the claims 8-14, characterized in that the γ-cyclodextrin ether is dissolved in water and that the active ingredient is added whereafter the thus obtained solution is optionally dried.

16. A method according to claim 15, wherein the residue after removal of the solvent is pulverized and, optionally after addition of further ingredients, converted into a solid form for administration.

17. A method according to claims 15 or 16, wherein further physiologically acceptable substances are added to the water.

18. A method according to claim 17, wherein sodium chloride, glucose, mannitol, sorbitol, xylitol or a phosphate or citrate buffer are added to the water.

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(54) **Novel derivatives of gamma-cyclodextrin.**

(57) Novel γ-cyclodextrin derivatives which are γ-cyclodextrin substituted with C_1–C_6 alkyl, hydroxy C_1–C_6 alkyl, carboxy C_1–C_6 alkyl or C_1–C_6 alkyloxycarbonyl C_1–C_6 alkyl or mixed ethers thereof and their preparation. Compositions comprising such cyclodextrin derivatives and an active ingredient which is preferably a drug and the preparation of such compositions.

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EUROPEAN SEARCH REPORT

Application number
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	WO-A-8 200 251 (U.S. DEPARTMENT OF COMMERCE) * Page 2, lines 22-23; page 7, lines 12-22; claim 3 *	1-5,8-10	C 08 B 37/16 A 61 K 31/735
X	PROCEEDINGS OF THE FIRST INTERNATIONAL SYMPOSIUM ON CYCLODEXTRINS, 30th September - 2nd October 1981, Budapest, HU, pages 275-287, edited by J. SZEJTLI, D. Reidel Publishing CO., Dordrecht, NL; A. LIPTAK et al.: "The chemistry of cyclodextrin derivatives" * Page 278, paragraph 3; page 279, paragraphs 2,3 *	1-7	
A	CHEMICAL ABSTRACTS, vol. 98, no. 2, 10th January 1983, page 225, abstract no. 8163z, Columbus, Ohio, US; & JP-A-57 106 698 (KYOWA HAKKO KOGYO CO., Ltd.) 02-07-1982 * Abstract *	15,16	TECHNICAL FIELDS SEARCHED (Int. Cl.4) C 08 B A 61 K

-/-

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15-07-1987	SOMERVILLE F.M.

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EUROPEAN SEARCH REPORT

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
P,X	JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 74, no. 9, September 1985, pages 987-990, American Pharmaceutical Association, Washington, US; J. PITHA et al.: "Amorphous water-soluble derivatives of cyclodextrins: nontoxic dissolution enhancing excipients" * Table 1; page 989, paragraph 2; page 990, paragraph 5 *	1-5,8-10	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15-07-1987	SOMERVILLE F.M.

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(54) Title: REGIOSELECTIVE SUBSTITUTIONS IN CYCLODEXTRINS

(57) Abstract

A process for preparing regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins wherein the substitution is either directed toward hydroxyls 2 or 2,3 of the glucose units with little substitution on hydroxyl 6 or toward hydroxyls 6 and with little substitution on the secondary hydroxyls. The regiospecificity is obtained through the proper control of basicity of the reaction mixtures which are comprised of epoxide and cyclodextrins and a suitable solvent.

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REGIOSELECTIVE SUBSTITUTIONS IN CYCLODEXTRINS

The development of procedures which would yield mixtures of cyclodextrin derivatives in which substitution at either the wide or narrow side of the toroid would be predominant was desired. Such a specific pattern of substitution has not been thought to be realizable by simple means, i.e., using cheap reagents without fractionation of the product. Nevertheless, that has been accomplished and here we disclose that by proper selection of preparative conditions mixtures of cyclodextrin derivatives with a specific pattern of substitution can be obtained. That discovery was made possible through a detailed analysis of cyclodextrin mixtures. That analysis, in conjunction with a fortuitous choice of reaction conditions, is the basis of the present invention. It should be noted that reagents and reaction conditions similar to those previously used by us and others have been employed. The novelty is the finding that there exist regions of reaction conditions which previously were not used and in which mixtures of cyclodextrin derivatives with unique substitution patterns are obtained; furthermore, these patterns are only slightly affected by the overall degree of substitution. That finding may be of importance since on its basis mixtures of cyclodextrin derivatives can be taylored for uses where recognition of a specific guest compound by a host is desired.

The usefulness of those derivatives of polysaccharides which assume random coil conformation depends primarily on their average degree of substitution and is only slightly affected by the differences in substitution patterns. Polysaccharide derivatives with an ordered conformation and derivatives of cyclic oligosaccharides (e.g. α-, β- or γ-cyclodextrins), which are *de facto* ordered by the presence of a cycle, present a different problem; there the substitution pattern may prefoundly affect their usefulness. The shape of cyclodextrins is a toroid : on the narrower side of the toroids are located all primary hydroxyls (-CH_2-OH) and on the wider sides are the secondary hydroxyls. Thus, substitution on secondary hydroxyls puts the substituents close to the wider entry of the cavity of the toroid, whereas substitutions on the primary hydroxyls close to the narrower entry. The principal use of cyclodextrins is in inclusion complexation : a guest lipophilic compound is accepted into the toroidal cavity of the host compound, i.e., of the cyclodextrin. This process is bound to be affected by specific changes at the entry sites of the host molecule. That was well demonstrated using chemically pure cyclodextrin derivatives. These compounds were prepared by multi-step synthesis

requiring multiple extensive purifications and thus are available only in small quantities and at a great price. In many applications the chemical purity (individuality) of cyclodextrin derivatives is not required or may even be of a detriment. Using mixtures of cyclodextrins is often preferred since these usually do not crystallize and thus have much higher solubilities and are also better suited as coatings.

Cyclodextrins, such as α-, β- or γ-cyclodextrins, similarly to other carbohydrates, react with epoxides yielding mixtures of oligosubstituted hydroxyalkylcyclodextrins. The latter compounds were first disclosed U.S. Patent 3,459,731. These cyclodextrins were found eminently useful for pharmaceutical purposes and this use was disclosed in U.S. Patent 4,596,795, U.S. 4,727,064, U.S. Patent 4,870,060, U.S. Patent 4,764,604, Eur. Patent No. 149,197, Int. J. Pharm. 26, 77, 1985, J. Pharm. Res. 309, 1985 and J. Pharm. Sci. 75, 571, 1986. Hydroxyalkylcyclodextrins were also prepared by reaction of cyclodextrins with ethylene or propylene carbonate catalyzed by potassium carbonate; R.B. Friedman, Modified Cyclodextrins, abstract B6 of the 4th International Symposium on Cyclodextrins, April 1988, Munich, West Germany. Furthermore, preparation of mixed alkyl and hydroxyalkylcyclodextrins was the subject of two patent applications, namely Eur. Patent Appl. EP 146,841 and EP 147,685. The multicomponent mixtures of hydroxyalkylcyclodextrins could be characterized using mass spectrometry, as far as number of substituent per cyclodextrin is concerned. Each of the peaks in such a spectrum corresponds to certain degree of substitution, but since there is a great number of possible isomeric compounds at any degree of substitution, the mixtures are only partially characterized by direct mass spectrometry. An advance in characterization was obtained by hydrolysis of hydroxypropylcyclodextrin mixtures and evaluation of the hydroxypropylglucose mixtures thus obtained by mass spectrometry (Pharmaceut. Res. 5,713-717, 1988). These results show that the substituents in hydroxypropylcyclodextrins are not evenly distributed between the glucose residues. A large number of hydroxyalkylcyclodextrins has been prepared and characterized in this manner and the average degree of substitution was found to depend primarily on the ratio of reagents used. These quite diverse reaction conditions yielded mixtures with a rather similar distribution of degree of substitution (Int. J. Pharm. 29 : 73-82, 1986; Pharmaceut. Res. 5 : 713-717, 1988). Consequently, the reaction conditions (i.e., strength of alkali added) were chosen primarily on the basis of convenience of manipulation of the mixtures. In different protocols (Int. J. Pharm. 29 : 73-82, 1986; Pharmaceut. Res. 5 : 713-717, 1988) the concentration of sodium hydroxide solution, which is used as a solvent for the other component, ranged between 5-17% w/w preferably about 11% w/w. At concentrations lower than these the reaction proceeds sluggishly; at higher

concentrations the solubility of β-cyclodextrin decreases and also the removal of sodium hydroxide after the reaction becomes tedious. Thus, in production of hydroxyalkyl-cyclodextrins the practical range of the concentrations of sodium hydroxide solution used as a solvent were 5-17% and there was no incentive to venture outside of this range.

The aim of the present invention is to provide a process enabling the attainment of a desired pattern of substitution by hydroxyalkyl groups onto α-, β- or γ-cyclodextrins through the control of basicity of the reaction mixtures which are comprised of epoxide and cyclodextrins and a suitable solvent. It was found that through the proper control of basicity the substitution may be directed either toward the wide or the narrow opening of the cavity of cyclodextrins i.e. (1) toward hydroxyls 2 or 2,3 of glucose residues with little substitution on hydroxyl 6, or (2) toward hydroxyl 6 and with little substitution on the secondary hydroxyls 2 and 3. In aqueous media the basicity of the reaction mixtures required for said regiospecificity may be obtained by a decrease or an increase of the previously used concentration range (5-17%) of sodium hydroxide solution, which is used as a reaction solvent for other components of the reaction mixtures. These concentrations represent typically less than 2.5% or more than 10.5% of sodium hydroxide content in the fully assembled reaction mixtures. In non-aqueous media the desired basicity may preferably be obtained using sodium methylsulfinylmethanide as a base and dimethyl sulfoxide as a solvent. It is however understood that other organic solvents or bases may be applied. The above method may also be applied for the preparation of mixtures of hydroxyalkylcyclodextrins which vary in their average degree of substitution, but in which the pattern of substitution is not changed.

A further aspect of the invention is to provide regiospecific hydroxyalkylated α-, β- or γ-cyclodextrins wherein the substitution is mainly on the hydroxyls 2 or 2,3 of the glucose residues with little substitution on hydroxyl 6, or wherein the substitution is mainly on the hydroxyl 6 with little substitution on the secondary hydroxyls 2 and 3, and fully or partly alkylated derivatives of these regiospecific hydroxyalkylated α-, β- or γ-cyclodextrins. Particular hydroxyalkylcyclodextrins substituted mainly on the wide side of the cavity have a relative distribution of the substitution on the 2 hydroxyl groups versus the 6 hydroxyl groups which varies from about 2:1 to about 20:1, preferably from about 5:1 to about 20:1, or from about 10:1 to about 15:1. Particular hydroxyalkylcyclodextrins substituted mainly on the narrow side of the cavity have a relative distribution of the substitution on the 6 hydroxyl groups versus the 2 hydroxyl groups from about 1.5:1 to 20:1, preferably from about 2.5:1 to 20:1, or from about 3:1 to about 15:1.

Still a further aspect of the invention is to provide mixtures comprising the above regiospecific hydroxyalkylated α-, β-, or γ-cyclodextrins.

In the foregoing definitions the term "hydroxyalkyl " defines bivalent straight or branch chained hydrocarbon radicals containing form 1 to 6 carbon atoms such as hydroxyethyl, hydroxypropyl or hydroxyisobutyl groups.

Since a hydroxy moiety of the cyclodextrin can be substituted by a hydroxyalkyl unit which itself can be substituted with yet another hydroxyalkyl unit, the average molar substitution (M.S.) is used as a measure of the average number of alkylated hydroxy functions per mole of glucose unit. Particular cyclodextrins according to the present invention have a M.S. which is in the range of 0.125 to 10, in particular of 0.3 to 3, or from 0.3 to 1.5. The average substitution degree (D.S.) expresses the average number of substituted hydroxyls per glucose unit. Particular cyclodextrins according to the present invention have a D.S. which is in the range of 0.125 to 3, in particular of 0.2 to 2, or from 0.2 to 1.5.

Hydroxyalkylated α-, β- or γ-cyclodextrins according to the present invention are prepared by an alkali catalyzed reaction of epoxides with cyclodextrins in a suitable solvent preferably at a temperature between 0 to 100°C, or between 0 to 70°C. A suitable solvent for carrying out the process of the invention is an aqueous alkali metal hydroxide solution. As the alkali metal hydroxide used may be mentioned lithium hydroxide, barium hydroxide, sodium hydroxide and potassium hydroxide. Of these, sodium hydroxide is preferable. The concentration of the sodium hydroxide solution which is used as a reaction solvent for other components of the reaction mixtures is either lower than 5% (w/w), preferably lower than 4% (w/w), or higher than 17%(w/w), preferably higher than 18%(w/w). In some instances, equinormal lithium, potassium or barium hydroxide solutions may also be applied. These concentrations represent typically less than 2.5% or more than 10.5% of alkali metal hydroxide content in the fully assembled reaction mixtures. The molar ratio of alkali metal hydroxide versus cyclodextrin should preferably be in the range of 0.5 to 3.5, more in particular less than 2.5, or should be in the range of 10 to 80, more in particular more than 13.8. The epoxide concentration in the final mixture may vary from about 1% to about 30%, more in particular from about 2% to about 20%. Particular samples of hydroxypropylated β-cyclodextrin were prepared by reacting β-cyclodextrin with propylene oxide in aqueous sodium hydroxide (Examples 1-7). The reaction conditions used in these preparations are summarized in Table I.

Table I.

Summary of preparative Conditions of Hydroxypropyl-β-cyclodextrins							
	Examples						
	1	2	3	4	5	6	7
sodium hydroxide solution used as a solvent (%w/w)	16.9%	17.5%	5.7%	1.5%	4.8%	17%	30%
Final reaction mixture (%w/w)							
sodium hydroxide	10.4%	10.5%	2.9%	1.1%	2.7%	10.3%	23.4%
cyclodextrin (anhydrous)	29.6%	21.3%	28.6%	15.1%	23.0%	21.3%	11.6%
propylene oxide	4.0%	15.4%	14.4%	10.9%	16.6%	15.4%	8.4%
Final reaction mixture (molar ratio)							
sodium hydroxide / cyclodextrin	10.0	13.9	2.9	2.1	3.4	13.8	57.2
propylene oxide / cyclodextrin	2.6	14.1	9.8	14.3	14.3	14.3	14.1

A suitable solvent for carrying out the present invention may also dimethyl sulfoxide, N,N-dimethylformamide, dioxane or mixtures thereof with water in the presence of a base. It is however understood that other organic solvents or bases may be applied. In the preparation described in Example 8 anhydrous conditions were used with sodium methylsulfinylmethanide in dimethylsulfoxide as catalyst and solvent, respectively. Pure regiospecific hydroxypropylated cyclodextrin may be isolated from the mixtures by removal of the unreacted starting material by art known procedures such as, extraction with organic solvents, adsorption chromatograpy, selective crystallization and combinations of these techniques.

In order to determine the distribution of substituents between the different positions in the α-D-glucopyranosyl residues of β-cyclodextrin each product was permethylated (Example 9), hydrolysed, and the resulting glucose ethers reduced, acetylated, and analyzed as alditol acetates, by gas liquid chromatography (Example 10).

There are several points to be clarified before the results are evaluated. Etherification with an epoxide such as propylene oxide is a complicated reaction. When racemic propylene oxide is used, diastereomeric ethers are formed, which are only partially separated by the analytical method used. In order to fully address this complication three examples (Examples 1-3) were prepared using racemic propylene oxide, whereas in Examples 4-8 (S)-propylene oxide was used, which is bound to yield a simpler pattern. Another complication is that the oxiran ring in propylene oxide can be opened either by attack on 0-1, which is the predominating reaction and gives a 2-hydroxypropyl ether, or on 0-2, giving a 2-(1-hydroxypropyl)ether. Two derivatives of the latter type were observed in the present study. The third type of complication is due to the introduction of additional hydroxyls by the substituent. Fortunately, the secondary hydroxyl of the 2-hydroxypropyl group should not be very reactive, and alkylation in this position should consequently not be very important. Nevertheless, small amounts of such derivatives were observed. The results of the analyses are summarized in Table II.

Conventional abbreviations were used, e.g., S_2 denotes mono-substitution on 0-2, S_{226} denotes bi-substitution on 0-2 (by $-CH_2-C(CH_3)H-O-CH_2-CH(OCH_3)-CH_3$ group) and mono-substitution on 0-6; glucose-derived numbering was used for alditols. In some analyses under methylation, especially in the 3-position, was observed. The products, however, were identified from their mass spectra, and the molar percentages added to those of the corresponding fully methylated components. Two 2-(1-methoxypropyl) ethers were observed with this group in the 2- and the 6-position of a glucosyl residue, respectively. The yields of these ethers were 2-4% of the corresponding 1-(2-methoxypropyl)ethers, and reflects the relative reactivities at the primary and the secondary position of propylene oxide, respectively.

Table II

Composition of Alditol Acetates in Mole % obtained from various 2-hydroxypropyl-β-cyclodextrin Preparations								
	Examples							
Substitution pattern by 2-methoxypropyl groups	1	2	3	4	5	6	7	8
S_0	77.8	43.9	39.3	74.4	40.2	42.9	53.2	65.5
S_0 non-methylated on 0-3	-	-	-	-	2.8	2.2	-	-
Total non-substituted	77.8	43.9	39.3	74.4	43.0	45.1	53.2	65.5
S_2	5.2	10.9	30.3	14.6	23.0	8.4	3.1	2.3
S_2 non-methylated on 0-3	-	-	-	-	0.6	0.2	-	-
S_2 2-(1-methoxypropyl)-	-	-	-	-	0.6	-	-	-
S_3	2.7	5.2	5.4	4.8	6.1	3.0	1.4	0.9
S_3 non-methylated on 0.6	-	-	-	-	0.5	-	-	-
S_6	12.5	23.6	3.8	2.6	7.0	26.4	33.0	23.3
S_6 non-methylated on 0-3	-	-	-	-	0.5	1.5	-	-
S_6 2-(1-methoxypropyl)-	-	-	-	-	-	0.6	-	-
Total non-substituted	20.4	39.7	39.5	22.0	38.3	40.1	37.5	36.5
S_{23}	0.6	3.9	14.3	2.2	8.9	2.8	0.7	-
S_{26}	0.9	7.5	3.7	0.9	5.2	6.4	1.9	1.8
S_{26} non-methylated on 0.3	-	-	-	-	0.7	-	-	-
S_{36}	0.3	2.3	1.4	0.5	1.6	2.2	0.9	-
S_{66}	-	-	-	-	-	0.3	4.7	6.0
Total disubstituted	1.8	13.7	19.4	3.6	16.4	11.7	8.2	7.8
S_{226}	-	-	-	-	-	0.2	0.2	-
S_{236}	0.1	2.7	1.7	-	2.4	2.3	0.7	-
S_{266}	-	-	-	-	-	0.5	0.4	-
S_{666}	-	-	-	-	-	-	0.2	-
Total trisubstituted	0.1	2.7	1.7	0	2.4	3.0	1.5	0

The relative reactivities at the three different positions in the α-D-glucopyranosyl groups may be determined from the molar percentages of the ethers. Sperlin equations (H.M. Sperlin in E. Ott, H.M. Sperlin and M.W. Grafflin (Eds.) Cellulose and Cellulose Derivatives, Part II, Interscience, New York, 1954, pp. 673-712) were used to determine the relative reactivities, k_2, k_3 and k_6, from the distribution of the substituents. The results in Table II can thus be reduced to those three parameters (Table III). The value for k_2 and k_3 there concern the relative reactivities when the other hydroxyl is not alkylated. Further calculations indicate that these reactivities are considerably enhanced when the other hydroxyl becomes alkylated, in particular the substitution on 0-3 increases the reactivity of 0-2 hydroxyls.

Table III

Relative Reactivities at the 2,3- and 6-Positions and Average Degree of Substitution Values for the Different 2-Hydroxypropyl Ethers of β-cyclodextrin					
				Average Degree of Substitution	
Example	propylene oxide	%NaOH[a]	$k_2:k_3:k_6$	From mole % of ethers	From m.s.
1	(RS)	16.9	1 : 0.43 : 2.1	1.7	2.5
2	(RS)	17.5	1 : 0.40 : 1.6	5.3	6.8
3	(RS)	5.7	1 : 0.15 : 0.12	5.8	6.6
4	(S)	1.5	1 : 0.36 : 0.08	2.0	3.4
5	(S)	4.8	1 : 0.27 : 0.32	5.5	6.0
6	(S)	17.0	1 : 0.28 : 2.2	5.2	5.8
7	(S)	30.0	1 : 0.41 : 7.6	4.0	5.2
8	(S)	b	1 : 0.17 : 8.3	3.0	-

a Concentration of aqueous sodium hydroxide solution (w/w) used as solvent for the other reaction components.

b Sodium methylsulfinylmethide in dimethyl sulfoxide

From the results given in Table III it is evident that the relative reactivities at 0-2 and 0-3 are rather independent of the alkali concentration during the etherification. The relative reactivity of 0-6 versus 0-2, however, varies from approximately 1:5 at low alkali concentration to 7:1 at high alkali concentration. For the reaction promoted by sodium methylsulfinylmethanide in dimethyl sulfoxide, the alkylation in the 6-position is even more favored. These drastic changes in the reactivity of 0-6 are the basis for the

regiospecificity observed at extremely low or high alkali concentrations, a phenomenon which is the subject of the present invention.

The thus prepared regiospecific hydroxyalkylated cyclodextrins may also be derivatized with an alkylating agent to obtain fully or partly substituted mixed ethers. The alkylation reaction may be carried out with appropriate alkylating agents such as alkylsulfates or alkylhalogenides in a base, liquid reaction medium containing an alkali metal hydroxide, water and, optionally, at least one organic solvent such as, for example, dimethoxy-ethane or isopropanol. In this regard, it is important to point out that if a regiospecific substitution is followed by a non-specific one even the latter acquires a measure of regiospecificity.

The following examples are intended to illustrate and not to limit the scope of the present invention in all its aspects.

Example 1

Preparation of hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (200 g of hydrate corresponding to 173.2 g anhydrous and 0.153 moles) was dissolved with stirring in warm (60°C) solution of sodium hydroxide (61.2 g or 0.53 moles in 300 ml of distilled water, i.e., 16.9% w/w). The solution was placed into round flask, cooled to ice bath temperature and after attachment of reflux condenser containing dry ice-acetone mixture, propylene oxide (25 ml, 23.2 g, 0.40 moles) was added dropwise with constant stirring. Stirring was continued for 3 hours at ice bath temperature and overnight at room temperature. Then the mixture was neutralized with concentrated hydrochloric acid and evaporated in vacuo to a consistency of thick syrup, which was added to 1 l of ethanol (190 proof). After several hours of stirring the insoluble sodium chloride was filtered off, washed with ethanol (190 proof, 200 ml). The ethanolic solutions were evaporated in vacuo, residue dissolved in distilled water (300 ml) and dialyzed for 5 hours at 0°C against several charges of distilled water. The retained fraction was freeze-dried and the resulting powder stirred with acetone (1.5 l) for one day. The acetone was decanted and residue stirred with an additional acetone (1 l) again for one day and the precipitate of hydroxypropyl-β-cyclodextrin filtered off and dried for 2 hours in vacuo. Acetone solutions upon evaporation yielded oily residue (3g) principally oligopropyleneglycols. The dried powder of hydroxypropyl-β-cyclo-dextrin was dissolved in distilled water (300 ml) and the solution freeze-dried to yield a white powder (98g).

Example 2

Preparation of hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (200 g hydrate, i.e., 173 g anhydrous, 0.153 moles) was, as above, dissolved in a solution of sodium hydroxide (85 g, 2.12 moles in 400 ml distilled water, i.e., 17.5% w/w) and in the same manner as above treated with propylene oxide (150 ml, 125 g, 2.152 moles). Using processing analogous to that above a fraction of oligopropylene glycols amounted to 38 g while altogether 193 g of hydroxypropyl-β-cyclodextrin was obtained.

Example 3

Preparation of hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (500 g hydrate, i.e., 432 g anhydrous, 0.382 moles) was, as above, dissolved in a solution of sodium hydroxide (45 g, 1.1 moles in 750 ml distilled water, i.e., 5.7% w/w) and under the same conditions as above treated with propylene oxide (260 ml, 217 g, 3.73 moles). The reaction mixture was left for five hours in an ice bath and kept at room temperature for two days. After processing similar to that described above and including extraction of oligopropylene glycols with acetone a white powder of hydroxypropyl-β-cyclodextrin (490 g) was obtained.

Example 4

Preparation of (S)-hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (13.3 g of hydrate, i.e., 11.5 g anhydrous, 0.010 moles) was dissolved in a solution of sodium hydroxide (0.822 g, 0.0206 mol in 54 ml distilled water, i.e., 1.5%) by stirring at 60°C. The increased amount of alkaline solution used was necessitated by the low solubility of β-cyclodextrin at very low (present case) or very high (30%) concentration of sodium hydroxide. The solution was cooled in an ice bath and in the same manner as above (S)-propylene oxide (10 ml, 8.29 g, 0.143 moles), a commercial preparation obtained from Aldrich Chemical Co., was added. Reaction mixture was kept overnight at 0-5°C and thereafter for 4 hours at room temperature. Then the mixture was neutralized with sulfuric acid (10%) to pH 7.5 and evaporated to dryness. Since the product is not well soluble either in ethanol or in water the residue, after evaporation, was suspended in distilled water (100 ml) and dialyzed against distilled water for 5 hours at room temperature. The retained suspension was evaporated to dryness, yielding a white powdery product (14.23 g).

Example 5

Preparation of (S)-hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (13.3 g of hydrate, i.e., 11.5 g anhydrous, 0.010 moles) was dissolved in a process as described above in a solution of sodium hydroxide (1.35 g, 0.034 moles in 27 ml distilled water, i.e., 4.8%) and treated in the manner described above with (S)-propylene oxide (10 ml, 8.29 g, 0.143 moles). The reaction mixture was kept overnight at 0-5°C and thereafter for 3 hours at room temperature. After neutralization with diluted sulfuric acid (10%) the solution was evaporated in vacuo nearly to dryness and residue stirred with ethanol (100 ml, 190 proof) for 30 minutes. After filtering off the insoluble sodium sulfate the ethanolic extracts were evaporated to dryness, dissolved in distilled water (35 ml), and dialyzed against distilled water for 3 hours at 0°C. Evaporation of the retained materials yielded a white powder of (S)-hydroxypropyl-β-cyclodextrin (17.3 g).

Example 6

Preparation of (S)-hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (13.3 g hydrate, i.e., 11.5 g anhydrous, 0.010 moles) was dissolved as above in the solution of sodium hydroxide (5.53 g, 0.13 moles in 27 ml distilled water, i.e., 17.0%) and treated in the manner described above with (S)-propylene oxide (10 ml, 8.29 g, 0.143 moles). The same isolation procedure as above yielded a white powder of (S)-hydroxypropyl-β-cyclodextrin (17.9 g).

Example 7

Preparation of (S)-hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (8.02 g hydrate, 6.93 g anhydrous, 6.1 moles) was added to a solution of sodium hydroxide (13.955 g, 0.349 moles in water 32.6 ml, i.e., 30%) and dissolved by stirring and heating to 70°C to a clear yellowish solution. Then the mixture was cooled in an ice bath and to the solution which remained homogeneous was added, while stirring, (S)-propylene oxide (5 g, 0.086 moles). After neutralization, evaporation, ethanol extraction, and dialysis all performed as above, a white powdery product (9.22 g) was obtained.

Example 8

One pot preparation of Permethyl (S)-hydroxypropyl-β-cyclodextrin

Sodium hydride (5.51 g of 80% dispersion in mineral oil, i.e., 0.31 moles) was added to anhydrous dimethyl sulfoxide (65 ml) and left to react at 60°C with stirring under argon for 1 hour. Then anhydrous β-cyclodextrin (10 g, 0.0088 moles) dissolved

in anhydrous dimethyl sulfoxide (65 ml) was added, stirred for 3 hours at room temperature and to this solution then slowly added a solution of (S)-propylene oxide (2.05 g, 0.035 moles) in dimethyl sulfoxide (10 ml). The reaction mixture was stirred for 15 hours at room temperature. Thereafter, methyl iodide (26 ml) was added dropwise (ice bath cooling) and the mixture stirred for one day at room temperature. After decomposition with water (100 ml) the product was extracted with trichloromethane (2 x 150 ml). Trichloromethane extracts were washed with water (100 ml), saturated sodium chloride, and evaporated. The residue was partitioned between water (25 ml) and diethyl ether (2 x 100 ml). Ethereal extracts were washed with water (20 ml), dried with anhydrous sodium sulfate, filtered through aluminum oxide (8 g), and evaporated to yield a product in the form of a pale yellow syrup (10.2 g).

Example 9

Permethylation of (S)-hydroxypropyl-β-cyclodextrins

All the procedures used were similar to the following : sodium hydride (2.1 g, as above, i.e., 0.07 moles) was added to anhydrous dimethyl sulfoxide (20 ml) under argon and the mixture heated for 1 hour to about 60°C. Thereafter, well dried (3 hours, 110°C) hydroxypropyl-β-cyclodextrin (4 g) dissolved in dimethyl sulfoxide (15 ml) was added and left to react, under argon and while stirring at room temperature, for an additional 3 hours. Then the reaction mixture was cooled in an ice bath and methyl iodide (10 ml, 0.161 moles) added dropwise. After another hour at ice bath temperature the mixture was left stirring overnight. Then water (24 ml) was added while cooling and the product extracted twice by trichloromethane (total 90 ml). The trichloromethane extract was washed with water (20 ml) and evaporated. The residue was treated with water (25 ml) and three times extracted with ether (total 75 ml), ether extracts washed with water, and evaporated. The residue was dissolved in ether (100 ml), stirred for 30 minutes with neutral alumina, filtered, and evaporated yielding 3.7 g of permethylated product.

Example 10

Analysis of Permethyl Derivatives of hydroxypropyl-β-cyclodextrins

The permethylated product (3 mg) was dissolved in M aqueous trifluoroacetic acid (0.5 ml), kept in a screw-cap tube at 100°C overnight and concentrated by flushing with air. The residue and sodium borohydride (10 mg) were dissolved in M aqueous ammonia (0.5 ml) and kept at room temperature for 1 hour. The solution was acidified with 50% acetic acid (2 drops) and concentrated. Boric acid was removed by codistillation first with acetic acid-methanol (1:9, 5 ml) and then with methanol (25 ml).

The residue was treated with acetic anhydride and pyridine (2:1, 0.5 ml) at 100°C for 30 minutes, concentrated, and partitioned between trichloromethane and water (2:1, 6 ml). The trichloromethane phase was concentrated and the residue analysed by g.l.c. and g.l.c.-m.s.

G.l.c. was performed on a Hewlett Packard 5830 A instrument fitted with a flame ionization detector, with hydrogen as the carrier gas. G.l.c.-m.s. was performed on a Hewlett Packard 5790-5970 system with helium as the carrier gas. A Hewlett Packard Ultra 2 (cross-linked 5% phenyl methyl silicone) fused silica, capillary column (25 m, 0.20 mm i.d.) was used. Temperature program : 8 minutes at 185°C, → 250°C at 5° per minute, 250°C for 10 minutes.

Claims

1. A process for preparing regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins wherein the substitution is directed either toward the narrow or toward the wider opening of the cavity of the cyclodextrins in a reaction mixture comprising epoxide, cyclodextrin and a solvent characterized by controlling the basicity of the reaction mixture.

2. A process according to claim 1 wherein the reaction mixture is comprised of propylene oxide, β-cyclodextrin and a solvent.

3. A process according to claim 1 or 2 wherein the solvent is an alkali metal hydroxide solution.

4. A process according to any of claims 1 to 3 wherein the solvent is a sodium hydroxide solution having a concentration lower than 5% (w/w) or higher than 17%(w/w).

5. A process according to any of claims 1 to 3 wherein the solvent is a sodium hydroxide solution having a concentration lower than 4% (w/w) or higher than 18%(w/w).

6. A process according to claim 3 wherein the molar ratio of alkali metal hydroxide/cyclodextrin is in the range of 0.5 to 3.5 or in the range of 10 to 80.

7. A process according to claim 3 wherein the alkali metal hydroxide concentration in the fully assembled reaction mixture is less than 2.5% or more than 10.5%

8. A process according to claim 1 or 2 wherein the solvent is dimethyl sulfoxide and the desired basicity is obtained by using sodium methylsulfinylmethanide as a base.

9. A process according to any of claims 1-8 for the preparation of mixtures of α-, β- or γ-hydroxyalkylcyclodextrins which vary in their average degree of substitution but in which the pattern of substitution is not changed.

10. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins wherein the hydroxyalkyl substitution is directed either toward the narrow or the wider opening of the cavity of the cyclodextrins.

11. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 10 wherein the substitution is mainly on the hydroxyls 2 or 2,3 of the glucose residues with little substitution on hydroxyl 6, or wherein the substitution is mainly on the hydroxyl 6 with little substitution on the secondary hydroxyls 2 and 3.

12. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 11 wherein the relative distribution of the substitution on the 2 hydroxyl groups versus the 6 hydroxyl groups varies from 2:1 to 20:1.

13. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 11 wherein the relative distribution of the substitution on the 2 hydroxyl groups versus the 6 hydroxyl groups varies from 10:1 to 20:1.

14. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 11 wherein the relative distribution of the substitution on the 6 hydroxyl groups versus the 2 hydroxyl groups varies from 1.5:1 to 20:1.

15. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 14 wherein the relative distribution of the substitution on the 6 hydroxyl groups versus the 2 hydroxyl groups varies from 2.5:1 to 20:1.

16. A process for preparing fully or partly alkylated derivatives of regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins defined in any of claims 10-15 characterized by reacting the latter with an alkylating agent in a basic, liquid reaction medium.

17. Fully or partly alkylated derivatives of the regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins defined in any of claims 10-15.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 90/00524

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 08 B 37/16

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 08 B

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	DE, A, 2260785 (MORISHITA PHARMACEUTICAL) 20 June 1974 see pages 5,6; claims	1-17
A	J. Carbohydrate Chemistry, volume 7, no. 2, 1988, Marcel Dekker, Inc., Ken'ichi Takeo et al.: "Derivatives of alpha-cyclodextrin and the synthesis of 6-O-α-D-glucopyranosyl-α-cyclodextrin", pages 293-308 see abstract	1-17

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
4th July 1990	26.07.90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	F.W. HECK

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9000524 SA 35772

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/07/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A- 2260785	20-06-74	None	

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

Water-soluble inclusion complexes of biologically active organic compounds which are insoluble or have only limited solubility in water, and their aqueous solutions, as well as their preparation and pharmaceutical products containing these compounds

Publication number: DE3118218
Publication date: 1982-04-22
Inventor: SZEJTLI JOZSEF DIPL ING CHEM D (HU); BOLLA GEB PUSZTAI (HU); STADLER GEB SZABO (HU)
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Classification:
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CH661517 (A5)
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Abstract not available for DE3118218
Abstract of corresponding document: **FR2484252**

The invention relates to water-soluble inclusion complexes of biologically active organic compounds which are insoluble or have only limited solubility in water, particularly of fat-soluble vitamins, steroid hormones, prostanoids, local anaesthetics and the like, to their aqueous solutions and to their preparation, wherein the biologically active compounds are converted into an aqueous solution in the form of inclusion complexes which have been formed with dimethyl- beta -cyclodextrins having an average degree of substitution of 14, in particular with heptakis(2,6-di-O-methyl)- beta -cyclodextrin, by dissolving the organic compounds which are insoluble or have only limited solubility in water in an aqueous solution of 1 to 8 mol (calculated per 1 mol of the compound to be dissolved) of dimethyl- beta -cyclodextrin. The aqueous solutions, obtained according to the invention, of the biologically active organic compounds can be processed to give pharmaceutical products which can be administered orally or parenterally.

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DEUTSCHES PATENTAMT

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DE 31 18218 A1

(54) **Wasserlösliche Einschluß-Komplexe von in Wasser nicht oder nur begrenzt löslichen biologisch aktiven organischen Verbindungen und deren wässrige Lösungen sowie deren Herstellung und diese Verbindungen enthaltende Arzneimittelpräparate**

Die Erfindung betrifft wasserlösliche Einschluß-Komplexe von in Wasser unlöslichen oder nur begrenzt löslichen, biologisch aktiven organischen Verbindungen, besonders von fettlöslichen Vitaminen, Steroidhormonen, Prostanoiden, lokalanästhetischen Mitteln u.ä., deren wäßrige Lösungen und deren Herstellung, wobei die biologisch aktiven Verbindungen in Form von Einschluß-Komplexen, die mit Dimethyl-β-cyclodextrinen mit einem durchschnittlichen Substitutionsgrad von 14, insbesondere mit Heptakis-(2,6-di-O-methyl)-β-cyclodextrin, gebildet worden sind, in wäßrige Lösung gebracht werden, in dem man die in Wasser unlöslichen bzw. nur begrenzt löslichen organischen Verbindungen in einer wäßrigen Lösung von 1 bis 8 Mol (pro 1 Mol der zu lösenden Verbindung berechnet) Dimethyl-β-cyclodextrin löst. Die gemäß Erfindung erhaltenen wäßrigen Lösungen der biologisch aktiven organischen Verbindungen können zu oral oder parenteral verabreichbaren Arzneimittelpräparaten verarbeitet werden. (31 18 218)

DE 31 18218 A1

BUNDESDRUCKEREI BERLIN 03. 82 230 016/676 17/80

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3118218

Y/ho **FRANKFURT (MAIN),** 5.Mai 1981

Chinoin Gyógyszer és Vegyészeti Termékek Gyára RT.,
H 1045 Budapest, Tó utca 1-5, Ungarn

Patentansprüche

1) Wasserlösliche Einschluß-Komplexe von in Wasser unlöslichen oder nur begrenzt löslichen, biologisch aktiven organischen Verbindungen mit Dimethyl-β-cyclodextrinen, deren durchschnittliche Substitutionszahl 14 beträgt, sowie deren wässrige Lösungen.

2) Einschluß-Komplexe nach Anspruch 1, dadurch gekennzeichnet, daß sie als Dimethyl-β-cyclodextrin mit einer durchschnittlichen Substitutionszahl 14 Heptakis-(2,6-di-O-methyl)-β-cyclodextrin enthalten.

3) Einschluß-Komplexe nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß sie in Wasser unlösliche oder nur begrenzt lösliche Vitamine, Steroidhormone, Prostanoide oder Lokalanästhetica als biologisch aktive organische Verbindungen enthalten.

4) Einschluß-Komplexe nach Anspruch 1, 2 oder 3, dadurch gekennzeichnet, daß sie als in Wasser unlösliche oder nur begrenzt lösliche Vitamine die fettlöslichen Vitamine A, B, D, E oder K enthalten.

5) Einschluß-Komplexe nach Anspruch 1, 2 oder 3, dadurch gekennzeichnet, daß sie als in Wasser unlösliche oder nur begrenzt lösliche Steroidhormone

(3β,4β)-3-[(O-2,6-Di-desoxy-β-D-ribo-hexopyranosyl-⟨1→4⟩-2,6-didesoxy-β-D-ribo-hexapyranosyl-⟨1→4⟩-2,6-didesoxy-β-D-ribo-hexapyranosyl)-oxy]-14-hydroxy-card-20(22)-enolid,
(3β,5β,12β)-3-[(O-β-D-Glukopyranosyl-⟨1→4⟩-O-3-O-acetyl-2,6-didesoxy-β-D-ribo-hexopyranosyl-⟨1→4⟩-O-2,6-didesoxy-β-D-ribo-hexopyranosyl-⟨1→4⟩-2,6-didesoxy-β-D-ribo-hexopyranosyl)-oxy]-12,14-dihydro-card-20(22)-enolid,
(17β)-17-Hydroxy-östr-4-en-3-on,
11,17,21-Trihydroxy-pregn-4-en-3,20-dion,
Methylsecodin,
Androst-4-en-3,17-dion,
(17β)-Östra-1,3,5-(10)-trien-3,17-diol-3-benzoat,
17,21-Dihydroxy-pregn-4-en-3,20-dion-17-acetat,
17,21-Dihydroxy-pregn-4-en-3,20-dion,
17β-Hydroxy-17-methyl-androst-4-en-3-on,
Pregn-4-en-3,20-dion,
3β,17α,21-Triacetoxy-pregn-5-en-20-on oder
3β,17α,21-Trihydroxy-pregn-5-en-20-on-21-acetat
enthalten.

6) Einschluß-Komplexe nach Anspruch 1, 2 oder 3, dadurch gekennzeichnet, daß sie als in Wasser unslösliche oder nur begrenzt lösliche Prostanoide PGI_2-äthylester enthalten.

7) Einschluß-Komplexe nach Anspruch 1, 2 oder 3, dadurch gekennzeichnet, daß sie als in Wasser unlösliche oder nur begrenzt lösliche Lokalanästhetica
2-(Diäthylamino)-N-(2,6-dimethylphenyl)-acetamid oder
1-Butyl-N-(2,6-dimethyl-phenyl)-2-piperidin-carboxamid
enthalten.

8) Einschluß-Komplexe nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß sie als in Wasser unslösliche oder nur begrenzt lösliche biologisch aktive organische Verbindung 1-(p-Chlorbenzoyl)-2-methyl-5-methoxy-indol-3-yl-essigsäure enthalten.

9) Verfahren zur Herstellung von wasserlöslichen Einschluß-Komplexen von in Wasser unlöslichen oder nur begrenzt löslichen, biologisch aktiven organischen Verbindungen in Form ihrer wässrigen Lösungen, dadurch gekennzeichet, daß man 1 Mol der zu lösenden Verbindung in der wässrigen Lösung von 1 bis 8 Mol eines Dimethyl-β-cyclodextrins mit einem auf die Methylgruppen bezogenen durchschnittlichen Substitutionsgrad 14 löst.

10) Verfahren nach Anspruch 9, dadurch gekennzeichnet, daß man Heptakis-(2,6-di-O-methyl)-β-cyclodextrin als Dimethyl-β-cyclodextrin mit Substitutionsgrad 14 verwendet.

11) Verfahren nach Anspruch 9 oder 10, dadurch gekennzeichnet, daß man als in Wasser unlösliche oder nur begrenzt lösliche biologische aktive Verbindungen Vitamine, Steroidhormone, Prostanoide oder Lokalanästhetica verwendet.

12) Verfahren nach Anspruch 9, 10 oder 11, dadurch gekennzeichnet, daß man als in Wasser unlösliche oder nur begrenzt lösliche Vitamine die fettlöslichen Vitamine A, B, D, E oder K verwendet.

13) Verfahren nach Anspruch 9, 10 oder 11, dadurch gekennzeichnet, daß man als in Wasser unlösliche oder nur begrenzt lösliche Steroidhormone

(3β,4β)-3-[(O-2,6-Di-desoxy-β-D-ribo-hexopyranosyl-⟨1→4⟩-2,6-didesoxy-β-D-ribo-hexapyranosyl-⟨1→4⟩-2,6-didesoxy-β-D-ribo-hexapyranosyl)-oxy]-14-hydroxy-card-20(22)-enolid,
(3β,5β,12β)-3-[(O-β-D-Glukopyranosyl-⟨1→4⟩-O-3-O-acetyl-2,6-didesoxy-β-D-ribo-hexopyranosyl-⟨1→4⟩-O-2,6-didesoxy-β-D-ribo-hexopyranosyl-⟨1→4⟩-2,6-didesoxy-β-D-ribo-hexopyranosyl)-oxy]-12,14-dihydro-card-20(22)-enolid,
(17β)-17-Hydroxy-östr-4-en-3-on,
11,17,21-Trihydroxy-pregn-4-en-3,20-dion,
Methylsecodin,
Androst-4-en-3,17-dion,
(17β)-Östra-1,3,5-(10)-trien-3,17-diol-3-benzoat,

17,21-Dihydroxy-pregn-4-en-3,20-dion-17-acetat,
17,21-Dihydroxy-pregn-4-en-3,20-dion,
17β-Hydroxy-17-methyl-androst-4-en-3-on,
Pregn-4-en-3,20-dion,
3β,17α,21-Triacetoxy-pregn-5-en-20-on oder
3β,17α,21-Trihydroxy-pregn-5-en-20-on-21-acetat
verwendet.

14) Verfahren nach Anspruch 9, 10 oder 11, dadurch gekennzeichnet, daß man als in Wasser unlösliche oder nur begrenzt lösliche Prostanoide PGI_2-äthylester verwendet.

15) Verfahren nach Anspruch 9, 10 oder 11, dadurch gekennzeichnet, daß man als in Wasser unlösliche oder nur begrenzt lösliche Lokalanästhetica
2-(Diäthylamino)-N-(2,6-dimethylphenyl)-acetamid oder
1-Butyl-N-(2,6-dimethyl-phenyl)-2-piperidin-carboxamid
verwendet.

16) Verfahren nach Anspruch 9 oder 10, dadurch gekennzeichnet, daß man als in Wasser unlösliche oder nur begrenzt lösliche biologisch aktive Verbindung 1-(p-Chlorbenzoyl)-2-methyl-5-methoxy-indol-3-yl-essigsäure verwendet.

17) Oral oder parenteral verabreichbare Arzneimittelpräparate, dadurch gekennzeichnet, daß sie als Wirkstoff einen Einschluß-Komplex der Ansprüche 1 bis 8 enthalten.

Wasserlösliche Einschluß-Komplexe von in Wasser nicht oder nur begrenzt löslichen biologisch aktiven organischen Verbindungen und deren wässrige Lösungen sowie deren Herstellung und diese Verbindungen enthaltende Arzneimittelpräparate.

Die Erfindung betrifft ein Verfahren zur Herstellung von in Wasser unlöslichen oder nur in begrenztem Mass löslichen, biologisch aktiven organischen Verbindungen und gegebenenfalls zur Beeinflussung der Wirkungsdauer von solchen Lösungen.

Ein grosser Teil von biologisch aktiven organischen Verbindungen ist in Wasser nicht oder nur in begrenztem Mass löslich. Durch diesen Umstand wird auch die Verwendung von solchen organischen Verbindungen in Injektionspräparaten unmöglich gemacht. In solchen Fällen, wenn die zu verwendenden biologisch aktiven organischen Verbindungen saure oder basische Gruppen, z.B. Carboxyl-, Sulfonsäure-, primäre, sekundäre oder tertiäre Aminogruppen besitzen, bietet die Möglichkeit von Salzbildungen eine mehr oder minder annehmbare Lösung dieses Problems. Oft treten aber dabei Schwierigkeiten infolge der nicht entsprechend hohen Azidität bzw. Basizität der zur Salzbildung eingesetzten Gruppen oder wegen der ungenügenden Stabilität des in ionische Form gebrachten Moleküls auf. Im ersten Falle zeigt die wässrige Lösung eine von der erwünschten Neutralität abweichende Reaktion, während im letzteren Falle die Lagerbeständigkeit der Lösung stark herabgesetzt wird. Oft wird durch die Salzbildung auch

die Transportgeschwindigkeit des Wirkstoffes in den Geweben nachteilig beeinflusst. Dieser Umstand führt z.B. im Falle von lokalanästhetischen Mitteln zum Ergebnis, dass die gewünschte Wirkung nur durch eine Überdosierung des Wirkstoffes erreicht werden kann, wodurch aber auch die Nebenwirkungen des Wirkstoffes in erhöhtem Mass auftreten können oder die unerwünschte Abwanderung des Wirkstoffes muß mit Hilfe von zusätzlichen Wirkstoffen, etwa von Vasokonstriktoren gehemmt werden, wodurch aber wieder neuere Nebenwirkungen auftreten könne. In vielen Fällen kommen aber solche Methoden überhaupt nicht in Frage, da viele biologisch aktive organische Verbindungen, wie z.B. die meisten Steroide keine zur Salzbildung geeigneten Gruppen besitzen. Bei solchen Verbindungen versucht man die Wasserlöslichkeit des Wirkstoffes dadurch zu erreichen, dass man die Verbindung mit zweibasischen organischen Carbonsäuren verestert und dann die freie Carboxylgruppe auf bekannte Weise in ein Salz überführt. Diese Methode ist aber nur begrenzt anwendbar und kann gegebenenfalls auch den Wirkstoff nachteilig beeinflussen. Wirkstoffe, bei welchen die obigen Methoden nicht anwendbar sind, wie z.B. die fettlöslichen Vitamine, können in der Form von öligen Lösungen parenteral verabreicht werden.

Es ist aber allgemein bekannt, dass durch ölige Injektionen schädliche Gewebeveränderungen an der Verabreichungsstelle verursacht werden können.

Auf Grund der obigen Ausführungen kann man mit Recht feststellen, dass zur Herstellung von wässrigen Lösungen von in Wasser unlöslichen oder nur begrenzt löslichen, biologisch aktiven organischen Verbindungen bisher keine allgemein anwendbare, befriedigende Methode bekannt ist.

Auf dem Gebiet der Therapie besteht seit langer Zeit die Bestrebung, fettlösliche Wirkstoffe in der Form von den Wirkstoff in wässriger Lösung enthaltenden Präparaten verabreichen zu können. Der Wirkstoff wird nämlich aus wässrigen Lösungen besser resorbiert, und es können auf diese Weise auch die durch den öligen Träger verursachten Nebenwirkungen vermieden werden.

Es wurde nun in überraschender Weise gefunden, dass therapeutisch vorteilhaft verwendbare, stabile wässrige Lösungen von in Wasser unlöslichen oder nur in begrenztem Mass löslichen, biologisch aktiven organischen Verbindungen hergestellt werden können, wenn man die erwähnten in Wasser unlöslichen bzw. schwer löslichen organischen Verbindungen in

wässrigen Lösungen von 1 bis 8 Mol (auf 1 Mol der zu lösenden Verbindung berechnet) Dimethyl-ß-cyclodextrin mit einem durchschnittlichen Substitutionsgrad 14 löst.

Es ist allgemein bekannt, dass wenn man in einer wässrigen Cyclodextrinlösung eine weitere Substanz löst, deren Moleküle mindestens teilweise apolar sind und der Durchmesser dieser Moleküle oder einer ihrer Seitenketten nicht grösser als der Durchmesser der Hohlräume der Cyclodextrin-Moleküle ist, so zeigen diese schlecht hydratisierbaren apolaren Molekülteile die Bestrebung, sich in die ebenfalls apolaren Cyclodextrin-Hohlräure einzufügen.

Es ist eine charakteristische Eigenschaft der auf diese Weise entstandenen Einschluss-Komplexe, dass sie in Wasser wesentlich schlechter löslich sind, als das freie Cyclodextrin selbst [Chem. Berichte, 90, 2561-2573 (1957)]. Da aber auch das ß--Cyclodextrin selbst bei Raumtemperatur eine Wasserlöslichkeit von nur 1,8 g/100 ml zeigt, scheiden sich die auf obige Weise gebildeten Einschluss-Komplexe aus den wässrigen Lösungen von ß-Cyclodextrin meistens in kristalliner Form aus. Somit können derartige, mit ß-Cyclodextrin gebildete Einschluss-Komplexe wegen ihrer schlechten Wasserlöslichkeit in

Injektionspräparaten nicht verwendet werden. Dabei verursachen zwar die Cyclodextrine bei oraler Anwendung keine toxischen Erscheinungen, doch können bei ihrer intraperitonealen, intravenösen, intramuscularen oder subcutanen Verabreichung in gewissen Fällen Nieren-Schädigungen auftreten [Amer. J. Pathol., 83, 367 (1976)].

Die vorliegende Erfindung basiert auf der Erkenntnis, dass die partiell methylierten ß-Cyclodextrine beinahe um zwei Grössenordnungen grössere Wasserlöslichkeit zeigen, als das unsubstituierte ß-Cyclodextrin, wobei diese partiell methylierten Derivate des ß-Cyclodextrins ebenfalls zur Bildung von kristallinen Einschluss-Komplexen fähig sind [vgl. Carbohydrate Research, 76, 59 (1979)], und zwar nach ähnlichen Prinzipien, wie dies bei dem unsubstituierten ß-Cyclodextrin erfolgt [Advances in Catalysis, 23, 209 (1973)].

Unter "partiell methylierten ß-Cyclodextrinen" sind solche methylierte Derivate des ß-Cyclodextrins zu verstehen, in welchen jedes Cyclodextrin-Molekül durch 1 bis 20 Methylgruppen substituiert ist. In der Reihe dieser partiell methylierten Derivate sind besonders das je Cyclodextrin-Molekül durchschnittlich 7 Methylgruppen enthaltende Monomethylderivat

und das durchschnittlich 14 Methylgruppen enthaltende Dimethylderivat hervorzuheben. Im allgemeinen können diese partiell methylierten Derivate des ß--Cyclodextrins jeweils durch den auf die Methylgruppen bezogenen Substitutionsgrad gekennzeichnet werden.

Es wurden schon zahlreiche Verfahren zur Herstellung von methylierten Derivaten der Cyclodextrine beschrieben. Zur vollständigen Methylierung des α-Cyclodextrins wurde die Muskat'sche Methylierungsmethode (in flüssigem Ammoniak, in Gegenwart von Kaliummetall) angewendet, wodurch in einem einzigen Schritt kristallines Hexakis-(2,3,6-tri-O-methyl)--α-cyclodextrin erhalten wurde [Berichte, 69, 2041 (1936)]. Im Falle von ß-Cyclodextrin konnte nach dem-selben Verfahren nur nach 18-facher Wiederholung das vollständig methylierte, 21 Methylgruppen enthaltende Derivat erhalten werden. Nach einer speziellen Form des Kuhn'schen Methylierungsverfahrens (mit Methyljodid in Dimethylformamid, in Gegenwart von Bariumoxyd) konnten sowohl das α- als auch das ß-Cyclodextrin vollständig methyliert werden [Tetrahedron, 24, 803 (1968)]. In dem-selben Referat wurde auch eine andere Variante des Kuhn'schen Methylierungsverfahrens (mit Dimethylsulfat im 1:1 Gemisch

von Dimethylformamid und Dimethylsulfoxyd, in Gegenwart von Bariumoxyd und Bariumhydroxyd) beschrieben; nach dieser Methode wurden kristallines Hexakis-(2,6-di-O-methyl)-α-cyclodextrin und Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin hergestellt.

Zur Herstellung von den Monomethylderivaten der Cyclodextrine und zwar von Heptakis-(3-O-methyl)-ß-cyclodextrin und Heptakis-(2-O-methyl)-ß-cyclodextrin wurden verschiedene mehrstufige komplette Synthesen beschrieben [Bioorg. Chem., 5, 121 (1976); Stärke, 28,226 (1976); Stärke, 26, 111 (1974)], deren gemeinsamer charakteristischer Zug darin liegt, dass die nicht zu methylierenden Kohlenstoffatome durch geeignete Substitutionen geschützt wurden, und/oder die Methylierungsreaktion in organischen Lösungsmitteln, in Gegenwart von Bariumsalzen, selektiv durchgeführt wurde. Nach der Methylierung konnten dann die gewünschten Monomethylderivate erst nach der Freisetzung der während der Methylierung geschützten Kohlenstoffatomen gewonnen werden.

Die oben beschriebenen Verfahren dienten dem Zweck, die Substituierbarkeit und die Möglichkeiten der selektiven partiellen Substituierung von Cyclodextrinen als speziellen (cyclischen) Oligosacchariden zu untersuchen. Die Durchführung einer selektiven

Substitution bietet meistens (und auch im vorliegenden Fall) wesentlich schwierigere Probleme, als die Herstellung von persubstituierten Produkten.

Bei der Herstellung sämtlicher aus der Literatur bekannten partiell methylierten bzw. permethylierten Cyclodextrinderivate wurde die Methylierung in organischen Lösungsmitteln ausgeführt, wobei in sämtlichen Fällen, wo es beabsichtigt war, die Substitution der Hydroxylgruppe in der 3-Stellung zu vermeiden, wurden zur Gewährleistung der Selektivität der Substitution Bariumsalze in organischen Lösungsmitteln eingesetzt.

Die gezielte Herstellung von Trimethylderivaten oder von verschiedenen Monomethylderivaten ist zur Zeit nur von theoretischem Interesse, da bezüglich ihrer komplexbildenden Eigenschaften bisher nur das Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin untersucht wurde [Carbohydrate Research, 76, 59 (1979)].

Zu den im erfindungsgemässen Verfahren zum Lösen der biologisch aktiven organischen Verbindungen verwendbaren Dimethyl-ß-cyclodextrinen mit einem durchschnittlichen Substitutionsgrad 14 gehören sämtliche solche methylierte ß-Cyclodextrine, die an den einzelnen Cyclodextrinringen durchschnittlich

14 Methylgruppen tragen. Dieses Produkt kann auch eine homogene, aus lauter gleichen Molekülen bestehende Substanz sein, welche durch die Fraktionierung des in der Methylierungsreaktion gewonnenen Produktgemisches erhalten werden kann, kann aber auch ein aus in verschiedenen Graden methylierten Cyclodextrinringen bestehendes Mischprodukt sein; es ist nur erforderlich, dass die durchschnittliche Substitutionszahl der Cyclodextrinringe 14 betragen soll. Die Methylgruppen können in gleichmässiger Verteilung stehen, so dass je zwei Methylgruppen an jeder Glukose-Einheit vorhanden sind, sie können aber auch ungleichmässig verteilt sein, so dass die Cyclodextrinringe aus unsubstituierten und aus mit 1, 2 oder 3 Methylgruppen substituierten Glukose-Einheiten aufgebaut sind, wobei aber der durchschnittliche Methylierungsgrad der einzelnen, aus 7 Glukose-Einheiten aufgebauten Cyclodextrinringe 14 beträgt.
In dieser Beschreibung bezieht sich die Bezeichnung "Dimethyl-cyclodextrin" (ohne nähere Angabe der chemischen Struktur) auf Dimethyl-ß-cyclodextrine mit einem durchschnittlichen Substitutionsgrad 14 und mit beliebiger, meistens ungleichmässiger Verteilung der Methylgruppen. Solche Dimethyl-ß-cyclodextrine können durch die direkte Methylierung von ß-Cyclo-

dextrin hergestellt werden.

Nach dem erfindungsgemässen Verfahren können die verschiedensten in Wasser unlöslichen oder nur begrenzt löslichen, biologisch aktiven organischen Verbindungen in wässrige Lösungen gebracht werden. So können wässrige Lösungen von verschiedenen fettlöslichen Vitaminen, z.B. von A-, D-, E- und K-Vitaminen, von verschiedenen Steroiden, z. B. von Hydrocortison oder 1,2-Dehydrocortison, von als Basen wasserunlöslichen lokalanästhetischen Wirkstoffen, z. B. von 2-(Dimethylamino-methyl)-1-äthyl-cyclohexanon-benzoat oder 2-(Diäthylamino)-N-(2,6-dimethylphenyl)-acetamid, von wasserunlöslichen Prostanoiden, z. B. von $PGF_{2\alpha}$ oder von Prostacyclin, von verschiedenen anderen Pharmakonen, wie z. B. von Indomethacin [1-(p-Chlorbenzoyl)-2-methyl-5-methoxy-indol-3-yl-essigsäure] oder von Acetylsalicylsäure hergestellt werden. Der Kreis der Wirkstoffe, welche nach dem erfindungsgemässen Verfahren in wässrige Lösungen gebracht werden können, ist in chemischer Hinsicht nur insofern beschränkt, dass die zu diesem Zweck geeigneten organischen Verbindungen einen solchen apolaren Molekülteil besitzen müssen, dessen Ausmass den Einbau in den Hohlraum des Cyclodextrinringes ermöglicht.

Die in Wasser unlöslichen oder nur begrenzt löslichen, biologisch aktiven organischen Verbindungen werden nach dem erfindungsgemässen Verfahren zweckmässig derart in wässrige Lösungen gebracht, dass man zuerst das Dimethyl-cyclodextrin in Wasser von geeigneter Qualität oder z. B. in physiologischer Kochsalz- oder Glukoselösung löst, die Lösung gewünschtenfalls (z. B. wenn der zu lösende organische Wirkstoff empfindlich gegen Oxydation ist) von Sauerstoff befreit und dann in dieser Lösung den zu lösenden Wirkstoff unter Rühren auflöst.
Das Auflösen dieser Stoffe kann bei Raumtemperatur oder bei mässig erhöhter Temperatur, z. B. bei 35-50°C erfolgen. Höhere Temperaturen sind im allgemeinen nicht vorteilhaft, weil unter solchen Umständen die Löslichkeit nicht mehr befriedigend ist. Die Löslichkeit des Dimethyl-cyclodextrins und seiner Komplexe nimmt bei der Steigerung der Temperatur ab; diese Verminderung der Löslichkeit ist aber reversibel, so dass der aus der erwärmten Lösung sich ausscheidende kristalline Niederschlag bei dem Abkühlen der Lösung wieder gelöst wird. Diese Erscheinung kann bei der Wärmesterilisierung der Präparate oft beobachtet werden, hat aber infolge der erwähnten Reversibilität keine schädlichen Wirkungen.

Die nach dem erfindungsgemässen Verfahren hergestellten wässrigen Lösungen der biologisch aktiven organischen Verbindungen können nach an sich bekannten Methoden zu enteral, parenteral oder lokal Anwendbaren Arzneimittelpräparaten formuliert werden. Zur oralen Verabreichung können z.B. Löffel-Arzneimittel oder Tropfen, zur parenteralen Verabreichung Infusions- und Injektionslösungen, zur lokalen Anwendung Umschläge, Abwaschflüssigkeiten und Heilpackungen zubereitet werden. Bei der Herstellung von solchen Arzneimittelpräparaten können die üblichen Füllstoffe, Verdünnungsmittel, Stabilisatoren, gegebenenfalls auch geschmack- und geruchverbessernden Zusätze, sowie übliche Mittel zur Einstellung des pH-Wertes oder des osmotischen Druckes der Lösungen verwendet werden.

Es wurde ferner überraschender Weise gefunden, dass durch die Herstellung der erfindungsgemässen wässrigen Lösungen in gewissen Fällen auch die Wirkungsdauer der gelösten Wirkstoffe verlängert wird. Das ist besonders bei lokalanästhetischen Mitteln von erheblicher Bedeutung, da infolge der auf diese Weise verlängerten Wirkungsdauer des Wirkstoffes die Anwendung des viele Nebenwirkungen hervorrufenden Adrenalins vermieden werden kann.

Die näheren Einzelheiten der Erfindung werden durch die nachstehenden Beispiele veranschaulicht; es ist aber zu bemerken, dass die Erfindung in keiner Weise auf den Inhalt dieser Beispiele beschränkt ist.

Beispiel 1:

10 g Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin werden in 100 ml Wasser bei 22 °C gelöst und dann wird bei der-selben Temperatur, unter Rühren, die zu lösende, in Wasser nicht oder nur beschränkt lösliche, biologisch aktive organische Verbindung allmählich, in kleinen Portionen zugegeben. Die allmähliche Zugabe der zu lösenden organischen Verbindung wird so lange fortgesetzt, bis die jeweils zugesetzten Portionen immer gelöst werden; nach der Zugabe der letzten, sich nicht mehr vollständig auflösenden Portion wird das Rühren des Gemisches noch 2 Stunden fortgesetzt, dann wird die Lösung filtriert und die Menge der gelösten biologisch aktiven Verbindung wird spektrophotometrisch bestimmt. Zur Kontrolle wird dieser Lösungsversuch unter den selben Bedingungen, aber mit reinem Wasser, ohne Zugabe von Cyclodextrin wiederholt. Die in den beiden Fällen gemessenen Löslichkeitswerte (in g/100 ml), sowie die für die Erhöhung der Löslichkeit charakteristischen Quotienten

S_2/S_1 für die verschiedenen biologisch aktiven Verbindungen sind in der nachstehenden Tabelle I zusammengefasst.

Tabelle I

Verbindung	Löslichkeit in Wasser g/100 ml S_1	Löslichkeit in Dimethyl-CD-Lösung g/100 ml S_2	S_2/S_1
Indomethacin	0,0078	0,159	20,4
Digoxin	0,0272	2,22	81,6
Lanatosid C	0,018	0,908	50,4
Nortestosteron	0,031	1,47	47,4
Hydrocortison	0,036	2,3	56,4
Methylsecodion	0,0057	0,45	79,0
Androst-4-en--3,17-dion	0,0082	1,3	158,5
Östron	0,003	0,475	158,33
Reichstein-S--17-acetat	0,0111	1,9	171,17
Methyltestosteron	0,0071	1,37	193
Reichstein-S	0,006	1,7	283
Progesteron	0,0016	1,30	812,5
Prolec	0,001	1,025	1025
Monac	0,0008	0,91	1137,5
16α-Methyl-Reichstein-S	0,0011	1,37	1245,5

Prolac: 3ß,17α,21-Triacetoxy-5-pregnen-20-on

Monac: 3ß,17α,21-Trihydroxy-5-pregnen-20-on-21-acetat

Beispiel 2:

In 10 destilliertem Wasser, dessen Temperatur mit einem Thermostat bei 40 °C gehalten wird, werden in Stickstoffatmosphäre, vom Licht geschützt, 20 mg Dimethyl-cyclodextrin unter ständigem Rühren gelöst. Es wird in einigen Minuten eine klare Lösung erhalten. Dann wird 1,0 mg kristallines Vitamin-D_3 langsam, in zwei Portionen zugegeben; in 3,5 bis 4 Stunden wird das Vitamin vollständig aufgelöst.

Separat wird eine äthanolische Vitamin-D_3-Lösung von gleicher Konzentration hergestellt und beide Lösungen werden mit einem Lichtstrahl von 400 bis 600 nm Wellenlänge und 2900 Lux Lichtstärke 34 Tage lang bestrahlt. Während dieser Lichtbehandlung wird der Vitamin-D_3-Gehalt von beiden Lösungen in gewissen Zeitabständen spektrophotometrisch bestimmt. Die erhaltenen Ergebnisse sind in der nachstehenden Tabelle II zusammengefasst:

Tabelle II

Zeit (Tage)	Vitamin-D_3-Gehalt, in 96%igem äthanol	% der Anfangskonzentration in 0,2%iger Dimethylcyclodextrinlösung
0	100 %	100 %
2	77,2 %	97,0 %

6	65,3 %	85,3 %
9	45,8 %	83,5 %
11	33,2 %	80,2 %
15	10,0 %	75,0 %
20	0,0 %	48,8 %
34	0,0 %	48,3 %

Aus den Daten der obigen Tabelle ist es ersichtlich, dass die Lichtbeständigkeit von Vitamin-D_3 durch die Komplexbildung mit Dimethyl-cyclodextrin erheblich erhöht wird.

Beispiel 3:

In 10 ml destilliertem Wasser werden unter den im Beispiel 2 angegebenen Bedingungen 2,0 g Dimethyl-cyclodextrin gelöst, dann werden in dieser Lösung in fünf Portionen insgesamt 100 mg kristallines Vitamin-D_3 gelöst, und zwar so, dass jede weitere Portion des Vitamins nur nach der vollständigen Auflösung der vorherigen Portion zugegeben wird.
Es wird auf diese Weise eine klare Lösung erhalten.
Die Lösung wird in diffusem Licht 6 Monate gelagert;
Eine spektrophotometrische Bestimmung des Vitamin-D_3-Gehalts zeigt, dass nach der sechsmonatigen Lagerung noch immer 85 % des ursprünglichen Vitamin-D_3-Gehalts in der Lösung anwesend ist.

Eine auf obige Weise hergestellte Vitamin-D_3-

Lösung wird im Vakuum, bei 40 °C zur Trockne eingedampft. Es wird ein filmartiger Rückstand erhalten; dieser Rückstand wird gepulvert und auf diese Weise wird ein stabiles pulverförmiges Vitamin-D_3--Präparat erhalten, welches in 5-500 ml Wasser zu einer klaren Lösung gelöst werden kann.

Beispiel 4:

2,0 g Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin gelöst und in dieser Lösung werden auf die im Beispiel 3 angegebenen Weise 100 mg kristallines Vitamin-D_3 aufgelöst. Diese Lösung wird dann auf 60 °C erwärmt und der in kristalliner Form ausgeschiedene Einschluss-Komplex von Vitamin-D_3 mit Dimethyl-cyclodextrin wird bei dieser Temperatur abfiltriert und warm getrocknet.

Mit einem Röntgen-Difraktometer wurden die Diagramme des erhaltenen kristallinen Produkts, sowie auch jene des Heptakis-(2,6-di-O-methyl)-ß-cyclodextrins aufgenommen; die erhaltenen charakteristischen Reflexionsbande sind in der nachstehenden Tabelle III angegeben, wo zum Vergleich auch die entsprechenden Reflexionsbande von Vitamin-D_3 angegeben sind.

Tabelle III

Charakteristische Reflexionsbande

(2 θ^o Winkelwerte)

Heptakis-(2,6-di-O--methyl-ß-cyclodextrin	Einschluss-Komplex	Vitamin-D_3
8,4	8,7	5,1
10,0	9,4	5,3
10,2	10,1	6,7
12,3	10,3	8,8
13,5	12,4	13,9
17,1	16,9	15,7
18,4	19,1	15,9
19,3	19,6	16,4
20,7	20,2	18,3
29,8	20,4 und 21,4	22,0

Beispiel 5:

253,2 mg (0,194 mMol) Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin werden in 1,8 ml Wasser gelöst und dann wird bei 30 °C, unter Rühren, die Lösung von 28 mg (0,076 mMol) PGI_2-äthylester in 2 ml Diäthyläther zugesetzt. Die Lösung wurd auf Raumtemperatur abkühlen gelassen und dann auf übliche Weise lyophilisiert. Es werden 254 mg amorphes weisses Pulver erhalten, welches in Wasser fünfmal besser löslich ist, als der mit ß-Cyclodextrin hergestellte Einschluss-Komplex von PGI_2-äthylester. Dieses Pulver wird in

Ampullen abgefüllt und die zugeschmolzenen Ampullen werden an einem kühlen Ort gelagert. Nach zwei Monaten wurde der Wirkstoffgehalt des Präparats bestimmt und es wurde gefunden, dass der Wirkstoffverlust nach 2 Monaten weniger als 5 % war. Die Bestimmung des Wirkstoffgehalts des Komplexes erfolgt auf die folgende Weise: das Präparat wird in einer Tris--Pufferlösung gelöst, die Lösung wird mit Natriumchlorid gesättigt und anschliessend mit Diäthyläther extrahiert. Der sich im Extrakt befindliche PGI_2--äthylexter wird silyliert und die Menge des Silylderivats geschromatographisch gemessen. Der PGI_2--äthylestergehalt des Komplexes beträgt 10,0 %.

Die Aggregation von Thrombocyten hemmende Konzentration des Komplexes ist im Born'schen Test 300 ng/ml. Diese Aktivität wird nach der Auflösung des Komplexes 2 Stunden lang unverändert beibehalten, woraus folgt, dass der PGI_2-äthylester durch die Komplexbildung in erheblichem Mass stabilisert wird. Es ist auch ein bedeutsamer Vorteil dieses Produkts, dass die bei der parenteralen Anwendung festgestellte nierenschädigende Wirkung von ß-Cyclodextrin bei dem hier zur Komplexbildung verwendeten Heptakis--/2,6-di-O-methyl/-ß-cyclodextrin nicht vorhanden ist.

Beispiel 6:

0,3 g Dimethyl-cyclodextrin werden in 2 ml

physiologischer Kochsalzlösung gelöst, die Temperatur der Lösung wird mittels eines Thermostats auf 35 °C eingestellt 5,2 mg in Portionen zugesetztes Vitamin-K_3 werden darin gelöst. Die auf diese Weise erhaltene Lösung wird durch Filtrieren sterilisiert und in Ampullen abgefüllt. Dieses Produkt kann als Injektionspräparat verwendet werden.

Beispiel 7:

0,05 g Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin werden in 1 ml destilliertem Wasser gelöst und dann wird die Lösung in Stickstoffatmosphäre, vom Licht geschützt mit 1,72 mg Retinolacetat (Vitamin-A-acetat), 25 µg Vitamin D_3 und 4,0 mg dl-α--Tokopherolacetat (Vitamin-E-acetat) versetzt. Es wird eine klare Lösung erhalten, welche als oral verabreichbare Tropfen verwendet werden kann.

Aus der obigen Lösung kann durch die Zugabe von 2 mg Aneurin-chlorid-hydrochlorid (Vitamin-B_1-salz), 0,8 mg Riboflavin-5'-phosphorsäureester-natriumsalz (Vitamin-B_2-salz), 30 mg Nikotinamid (Vitamin-B_3), 4 mg Pyridoxin-hydrochlorid (Vitamin-B_6-salz), 100 mg Vitamin-C und 10 mg Pantheol (reduzierte, alkoholische Form von Vitamin B_5) ein oral verabreichbares Polyvitaminpräparat hergestellt werden. Die Tagesdose dieses Präparats ist 3-mal 5 bis 10 Tropfen.

Beispiel 6:

2 ml einer 10 Gew.%-igen wässrigen Lösung von Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin werden in einem Stickstoffstrom, vom Licht geschützt, von Sauerstoff befreit und dann werden unter Rühren, in mehreren Portionen insgesamt 34,4 mg Retinolacetat darin gelöst. Das Auflösen des Retinolacetats nimmt bei Raumtemperatur etwa 3 Stunden in Anspruch. Die erhaltene Lösung wird dann in Stickstoffatmosphäre mit Wärme sterilisiert; der bei dem Aufwärmen sich ausscheidende Einschlusskomplex löst sich wieder bei dem Abkühlen. Die auf diese Weise erhaltene Lösung kann als Injektionspräparat oder als orale Tropfen verwendet werden; die Tagesdose beträgt 15 bis 30 Tropfen.

Beispiel 9:

In 2 ml einer 10 Gew.%-igen wässrigen Lösung von Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin in der im Beispiel 8 beschriebenen Weise, bei 30 °C 3,44 g Retinolacetat und anschliessend 5,0 mg dl-α--Tokopherolacetat unter Rühren gelöst. Die erhaltene Lösung wird im Vakuum, bei 35 °C zur Trockne eingedampft, der filmartige Rückstand wird gepulvert und in Ampullen abgefüllt. Das auf diese Weise erhaltene trockene Präparat kann in 10 ml von beliebigen

üblichen Infusionslösungen schnell und klar gelöst werden. Das Produkt kann für Infusionen verwendet werden.

Beispiel 10:

15 g Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin werden in 100 destilliertem Wasser bei 25 °C gelöst und die Lösung wird mit 1,5 g gepulverten Lidocain-Base [2-(Diäthylamino)-N-(2,6-dimethyl-phenyl)-acetamid] versetzt. Es wird eine klare, stabile Lösung erhalten, welche unbeschränkt ohne Veränderung gelagert werden kann. Der gelöste Wirkstoff scheidet sich auch beim Verdünnen mit Wasser nicht aus.

Beispiel 11:

15 g Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin werden in 95 ml physiologischer Kochsalzlösung gelöst. Die Temperatur der Lösung wird mittels eines Thermostats auf 30 °C eingestellt und es wird unter Rühren 1,0 g Lidocain-Base zugegeben. Nach dem Auflösen des Lidocains wird das Volumen der Lösung mit physiologischer Kochsalzlösung auf 100 ml ergänzt (Präparat A).

Separat wird aus Lidocain-hydrochlorid mit physiologischer Kochsalzlösung eine in 100 ml 1,0 g Lidocain-Base in der Form von Hydrochloridsalz enthaltende Lösung hergestellt (Präparat B).

Aus beiden Präparaten A und B werden dann durch Verdünnung mit physiologischer Kochsalzlösung 0,25, 0,50 und 0,75 Gew.%-ige Testlösungen hergestellt.

Mit den auf obige Weise hergestellten Testlösungen wurden die folgenden Untersuchungen durchgeführt:

In das eine Auge von Hasen wurde die aus dem Präparat A und in das andere Auge des selben Tieres die aus dem Präparat B hergestellte Testlösung eingetropft. Beide Augen wurden dann mit einer Wildschweinborste gereizt und das Auftreten der Cornealreflexe wurde in Abhängigkeit von der Zeit registriert. Die bei 10 Tieren gemessenen Reflexzahlen wurden in Abhängigkeit von der Zeit in zweifacher logarithmischer Zusammenhang graphisch dargestellt; aus den sich auf diese Weise ergebenden geraden Linien wurden die zum Auslösen von 50 %-igen Reflexen gehörenden Zeitwerte als charakteristisch betrachtet. Diese "$t_{eff\ 50}$"-Werte wurden in der nachstehenden Tabelle IV zusammengefasst.

Tabelle IV

Konzentration %	Präparat B $t_{eff\ 50}$-Werte Min.(') Sek. ('')	Präparat A	Aenderung von $t_{eff\ 50}$ %

./.

0,25	4'54''	6'36''	+ 34,69
0,50	11'45''	18'40''	+ 58,85
0,75	12'10''	24'40''	+102,73

Aus den Daten der obigen Tabelle ist es klar ersichtlich, dass die nach dem erfindungsgemässen Verfahren hergestellten Lösungen bei dem selben Wirkstoffgehalt eine wesentlich längere Wirkungsdauer zeigen.

Beispiel 12:

Die nach Beispiel 11 hergestellten Präparate A und B wurden auch an Meerscheinchen mit 350 bis 400 g Einzelgewicht in intracutanem Haut-Test geprüft. Einen Tag vor den Versuchen wurden die Tiere am Rücken depiliert. Am Rücken der einzelnen Tiere wurden rechts und links von der Wirbelsäule, vorne und hinten je 0,1 ml der nach Beispiel 11 hergestellten Verdünnungen der Präparate A bzw. B intracutan injiziert und dann wurde die Haut der Tiere durch standarde Nadelstiche gereizt. Die normale Schmerzreaktion der Tiere (Kreischen) wurde durch ausserhalb der sich an den Injektionstellen subcutan ausbildenden Lidocain-Depots gemachte Stiche kontrolliert. Es wurden die Zeiten registriert, bei welchen die Tiere auf die in den Bereichen der Injektionen gemachten Stiche keine Schmerzreaktion zeigten.

Die Ergebnisse wurden ebenfalls in zweifach logarithmischem Maßstab graphisch dargestellt und es wurden die zur 50 %-iger Reflexion gehörenden Zeitwerte ermittelt. Diese sind in der nachstehenden Tabelle V zusammengefasst.

Tabelle V

Konzentration %	Präparat B $t_{eff\ 50}$-Werte Min. (')	Präparat A $t_{eff\ 50}$-Werte Sek.('')	Aenderung von $t_{eff\ 50}$ %
0,50	19'55''	31'00''	+55,64
0,75	28'10''	46'45''	+65,97
1,00	38'00''	60'00''	+57,89

Die Daten der obigen Tabelle zeigen klar di Verlängerung der Wirkungsdauer bei den erfindungsgemässen Präparaten.

Beispiel 13:

Die nach Beispiel 11 hergestellten Präparate A und B wurden auch in Fällen von Leitungs-Anästhesie an Ratten geprüft. Den Tieren wurden in 1 cm Entfernung von der Schwanzwurzel, neben den rechts- bzw. linksseiteigen Nervenstämmen je 0,15 ml Lösung injiziert. Die Tiere wurden mit elektrischem Strom (Spannung: 90 V, Frequenz: 100 Hz) gereizt; sie haben den auftretenden

Schmerz durch ein energisches Wegreissen des Schwanzes und durch Kreischen angezeigt. Die gemessenen Anästhesie-Zeitdauer sind in der nachstehenden Tabelle VI zusammengefasst.

Tabelle VI

Konzentration %	Präparat B	Präparat A	Verlängerung der Anästhesie %
	Zeitdauer der Anästhesie Minuten		
0,50	76	146	+ 92,10
0,75	117	215	+ 83,76
1,00	222	464	+109,0

Die Daten der obigen Tabelle zeigen, dass die nach dem erfindungsgemässen Verfahren hergestellten Lösungen bei den selben Wirkstoffdosen um 90 bis 100 % längere Wirkung haben.

Beispiel 14:

9 g Dimethyl-cyclodextrin werden in 60 ml destilliertem Wasser gelöst und es werden 0,33 g Bupivacain-Base [1-Butyl-N-(2,6-dimethyl-phenyl)-2-piperidin-carboxamid] zugesetzt. Es wird eine stabile, klare Lösung erhalten, aus welcher Injektionslösungen hergestellt werden können.

Beispiel 15:

Es wird auf die im Beispiel 14 beschriebene

Weise eine 150 mg/ml Dimethyl-cyclodextrin und 4,5 mg/ml Bupivacain-Base enthaltene Injektionslösung hergestellt und sterilisiert (Präparat C).

Freiwilligen Versuchspersonen wurden am Unterarm 0,2 ml der zu untersuchenden Lösung subcutan injiziert, wobei die Versuchspersonen in vier Gruppen eingeteilt wurden und eine Gruppe mit dem Präparat C, die zweite Gruppe mit einer handelsüblichen Bupivacain-hydrochlorid Injektionslösung, die dritte Gruppe mit einer 2 %-igen Lidocain-hydrochlorid Injektionslösung und die vierte Gruppe mit steriler physiologischer Kochsalzlösung behandelt wurde. Während 270 Minuten nach der Verabreichung der Injektion wurde in jeden 30 Minuten die Schmerzempfindung durch Nadelstiche untersucht. Während dieser Zeit wurde bei den mit dem Präparat C behandelten Personen in 66 % der Fälle keine Schmerzempfindung festgestellt; diese Zahl war bei den mit physiologischer Kochsalzlösung behandelten Personen 20 %, bei den mit Bupivacain-hydrochlorid bzw. Lidocain-hydrochlorid behandelten Personen gleichermassen 38 %.

Beispiel 16:

In 2 ml einer 10 Gew.%-igen Lösung von Hepta-kis-(2,6-di-O-methyl)-ß-cyclodextrin werden 30 mg Hydrocortison gelöst. Die Lösung wird sterilisiert und kann als Injektionspräparat verwendet werden.

Beispiel 17:

100 mg Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin werden in 1 ml destilliertem Wasser gelöst, dann werden zu der erhaltenen Lösung bei 30 °C, langsam, portionsweise 25 mg Prednisolon zugesetzt und darin aufgelöst. Die auf diese Weise erhaltene wässrige Lösung des Einschluss-Komplexes wird dann in inerter Gasatmosphäre durch Erwärmen sterilisiert. Die erhaltene stabile sterile Lösung kann als Injektionspräparat verwendet werden.

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Process for the preparation of cyclooctaamylose

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Abstract of **DE3317064**

The invention relates to a process for the preparation of cyclooctaamylose by enzymatic cleavage of starch and subsequent fractionation of the cleavage products, in which cyclodextrin glycosyltransferase is added to an aqueous preparation of starch, and bromobenzene is added to the starch preparation at least after the amount of initially formed cyclodextrin no longer increases.

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(54) Verfahren zur Herstellung von Cyclooctaamylose

Die Erfindung betrifft ein Verfahren zur Herstellung von Cyclooctaamylose durch enzymatische Spaltung von Stärke und anschließende Auftrennung der Spaltprodukte, wobei einer wäßrigen Zubereitung von Stärke Cyclodextrin-glykosyltransferase zugegeben wird und zumindest nachdem die Menge an primär gebildetem Cyclodextrin nicht mehr zunimmt, die Stärkezubereitung mit Brombenzol versetzt wird.

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Patentansprüche

1. Verfahren zur Herstellung von Cyclooctaamylose durch enzymatische Spaltung von Stärke und anschließender Auftrennung der Spaltprodukte, dadurch gekennzeichnet, daß

   a) einer wäßrigen Zubereitung von Stärke Cyclodextringlykosyltransferase zugegeben wird und

   b) zumindest nachdem die Menge an primär gebildetem Cyclodextrin nicht mehr zunimmt, die Zubereitung gemäß a) mit Brombenzol versetzt wird.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die wäßrige Zubereitung von Stärke vor der Zugabe von Cyclodextringlykosyltransferase mit Alkalisalzen kurzkettiger Alkansäuren versetzt wird.

3. Verfahren nach den Ansprüchen 1 und 2, dadurch gekennzeichnet, daß bei der Auftrennung der Spaltprodukte Cyclooctaamylose aus pyridinischer Suspension durch Zugabe von Alkohol gewonnen wird.

C o n s o r t i u m
für elektrochemische Industrie
GmbH

München, den 3.5.1983
PAT/Dr.Ra/we

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Verfahren zur Herstellung von Cyclooctaamylose

Die Erfindung betrifft ein Verfahren zur Herstellung von Cyclooctaamylose, im weiteren auch als γ-Cyclodextrin bezeichnet, durch enzymatische Spaltung von Stärke und anschließende Auftrennung der Spaltprodukte.

γ-Cyclodextrin ist relativ gut wasserlöslich, besitzt einen hydrophoben Torus von ca. 10 Angström Durchmesser, in den Gastmoleküle eingeschlossen werden können. Aufgrund dieser Eigenschaften ist γ-Cyclodextrin ein an sich begehrter Einsatzstoff u.a. auf dem Arzneimittelsektor, auf den Gebieten des Pflanzenschutzes, der Kosmetik oder in der Nahrungsmittelindustrie.

Gemäß EP-OS 45 464 ist es Stand der Technik, Cyclodextrine aus Stärkehydrolysaten durch Fällung mit z.B. Brombenzol abzutrennen. Aus der genannten Veröffentlichung ist es ferner bekannt, γ-Cyclodextrin aus Stärke durch enzymatische Spaltung zu gewinnen und aus dem Reaktionsprodukt mit Hilfe chromatographischer Methoden zu isolieren.

Generell ist festzustellen, daß die bekannten Herstellungsmethoden für γ-Cyclodextrin so aufwendig und damit so kostenintensiv sind, daß γ-Cyclodextrin bisher nicht die an sich durch seine Eigenschaften begründete breite industrielle Nutzung finden konnte.

Aufgabe der Erfindung war es, ein Verfahren zur Herstellung von Cyclooctaamylose zu entwickeln, das verbesserte Ausbeuten liefert.

Gegenstand der Erfindung ist ein Verfahren zur Herstellung von Cyclooctaamylose durch enzymatische Spaltung von Stärke und anschließender Auftrennung der Spaltprodukte, das dadurch gekennzeichnet ist, daß

a) einer wäßrigen Zubereitung von Stärke Cyclodextringlykosyltransferase zugegeben wird und

b) zumindest nachdem die Menge an primär gebildetem Cyclodextrin nicht mehr zunimmt, die Zubereitung gemäß a) mit Brombenzol versetzt wird.

Grundsätzlich kann erfindungsgemäß jede Art von Stärke, einschließlich nativer Stärke oder Stärke-Partialhydrolysate, eingesetzt werden. Beispiele sind Kartoffelstärke, Maisstärke, Maniokstärke u.a.

Als wäßrige Zubereitungen von Stärke können alle wäßrigen Zubereitungen eingesetzt werden, die auch bereits bisher zur enzymatischen Spaltung von Stärke verwendet werden konnten. Es sind dies insbesondere 5 bis 30 Gew.-%-ige wäßrige Lösungen von gelifizierter Stärke. Sie werden im einfachsten Fall durch Kochen entsprechender Mengen von Stärke in Wasser gewonnen.

Die genannten Zubereitungen enthalten zur Enzymstabilisierung zumeist kleinere Mengen an Calciumchlorid, insbesondere ca. 5 mMol Calciumchlorid/l.

Vorzugsweise enthalten die erfindungsgemäß einer enzymatischen Spaltungsreaktion zu unterwerfenden Stärkezubereitungen weiterhin Alkalisalze kurzkettiger Alkansäuren. Die Mengen betragen zweckmäßigerweise 5 bis 300 mMol/l Stärkezubereitung. Beispiele für die genannten Alkalisalze sind Natriumformiat, Kaliumformiat, Natriumacetat, Kaliumacetat, Natriumpropionat, Kaliumpropionat u.a., insbesondere Natriumacetat. Durch diese Maßnahme wird die Bildung von Cyclooctaamylose begünstigt.

Den genannten Stärkezubereitungen wird nun das an sich bekannte Enzym Cyclodextringlykosyltransferase zugegeben. Als Quelle für dieses Enzym dienen Mikroorganismen, wie Bacillus macerans, Bacillus megaterium, Bacillus circulans, Bacillus stearo-thermophylus, Micrococcus spp., alkalophiler Bacillus (z.B. Bacillus Nr. 38-2 oder 17-1), Klebsiella pneumoniae, insbesondere Klebsiella pneumoniae.

Das Enzym wird vorzugsweise in solchen Mengen zugegeben, daß das Gewichtsverhältnis von Enzym : Stärke 1 : 2000 bis 1 : 10 000, insbesondere 1 : 4000 bis 1 : 5000 beträgt.

Die Spaltungsreaktion wird bei Temperaturen von 30 bis 55 °C, insbesondere 40 bis 45 °C unter Rühren durchgeführt. Der pH-Wert der Stärkezubereitung beträgt 4,5 bis 7,9.

Vorzugsweise wird ein Teil der Enzymmenge, ca. 10 bis 40 Gew.-%, insbesondere 25 bis 35 Gew.-%, der Gesamtmenge des eingesetzten Enzyms bereits bei einer Temperatur des Reaktionsgemisches von 60 bis 70 °C zugegeben. Danach wird das Reaktionsgemisch unter Rühren auf eine Temperatur von 30 bis 55 °C abgekühlt und schließlich die restliche Menge an Enzym zudosiert.

Der Fortschritt der enzymatischen Spaltungsreaktion kann beispielsweise durch Probenentnahme und chromatographische Analyse überwacht werden. Als Maßstab hierfür dient die Menge der sich primär bildenden Cyclodextrine. Abhängig von der Abstammung der Cyclodextringlykosyltransferase ist dies in der überwiegenden Menge α- oder β-Cyclodextrin. Beispielsweise bildet die Cyclodextringlykosyltransferase von Bacillus macerans und von Klebsiella pneumoniae in der überwiegenden Menge α-Cyclodextrin, während die Cyclodextringlykosyltransferase des alkalophilen Bacillus primär überwiegend β-Cyclodextrin bildet.

Die enzymatische Spaltungsreaktion führt schließlich zu einem Zustand, in dem die Menge dieser primär gebildeten Cyclodextrine im Reaktionsgemisch nicht mehr zunimmt. Zumindest nachdem dieses Stadium erreicht ist, wird erfindungsgemäß Brombenzol zugegeben. Typische Inkubationszeiten bis zum Erreichen dieses Stadiums sind 5 bis 9 Stunden. Zweckmäßigerweise erfolgt die Brombenzolzugabe erst, wenn mehr als 50 Gew.-%, insbesondere mehr als 70 Gew.-%, an sich primär bildendem Cyclodextrin im Reaktionsgemisch vorliegt.

Durch Zugabe von Brombenzol fallen ß-Cyclodextrin und γ-Cyclodextrin als schwerlösliche Clathrate aus. Die eingesetzte Menge an Brombenzol ist, bezogen auf die Menge im Reaktionsgemisch anwesender und sich weiterbildender Cyclodextrine mindestens äquimolar. Vorzugsweise wird jedoch, bezogen auf die genannte Menge anfallender Cyclodextrine ein 1,5- bis 10-facher, insbesondere ein 2- bis 3-facher molarer Überschuß an Brombenzol zugesetzt.

Danach wird das Reaktionsgemisch bei 30 bis 50 °C, insbesondere bei 40 bis 45 °C, weitergerührt bis keine Zunahme an Zielprodukt (Cyclooctaamylose) mehr festgestellt wird. Die Reaktionskontrolle kann beispielsweise durch Probenentnahme und chromatographische Analyse erfolgen. Typische Gesamtinkubationszeiten bis zum Endpunkt der Reaktion sind 24 bis 48 Stunden.

Zur Aufarbeitung des Reaktionsgemisches werden zunächst die Feststoffe durch z.B. Dekantieren, Filtrieren, Zentrifugieren und dergleichen abgetrennt. Das Zielprodukt liegt als Feststoff (Brombenzol-Clathrat) im Gemisch mit Brombenzol-Clathrat des ß-Cyclodextrins und ebenfalls als Nebenprodukten gebildeten lang- und kurzkettigen, nicht cyclischen Spaltprodukten vor.

Zur Auftrennung des Gemisches werden nun die Cyclodextrine durch Zerstören der entsprechenden Brombenzol-Clathrate wieder in Lösung gebracht. Eine geeignete Methode hierfür ist die Behandlung des genannten Feststoffgemisches mit heißem oder kochendem Wasser oder Wasserdampf, wobei nach Art einer Wasserdampfdestillation Brombenzol aus dem Gemisch entfernt wird. Aus der schließlich brombenzolfreien, wäßrigen Lösung können die langkettigen, nicht cyclischen Spaltprodukte durch Fällen mit Alkoholen, wie beispielsweise Methanol, abgetrennt werden.

Die Hauptmenge des ß-Cyclodextrins wird nun vom Zielprodukt durch Kristallisation abgetrennt. Hierzu wird die wäßrige Lösung zweckmäßigerweise eingeengt und die Hauptmenge des entstandenen ß-Cyclodextrins bei einer Temperatur von 2 bis 10 °C, insbesondere ca. 4 °C, auskristallisiert. Es verbleibt als Überstand eine wäßrige Lösung von γ-Cyclodextrin, dem Zielprodukt, mit kleineren Mengen an ß-Cyclodextrin und an kurzkettigen, nicht cyclischen Spaltprodukten. Die Entfernung der kurzkettigen, nicht cyclischen Spaltprodukte ist oftmals nicht erforderlich, da diese Produkte zumeist nur in geringen Mengen vorliegen. Falls eine Abtrennung erwünscht ist, werden in der gleichen Verfahrensweise wie bereits beschrieben die Brombenzol-Clathrate des ß- und γ-Cyclodextrins gebildet und isoliert. Die kurzkettigen, nicht cyclischen Spaltprodukte verbleiben dabei in Lösung. Danach werden durch Austreiben des Brombenzols aus dem Gemisch die Cyclodextrine wieder in Lösung gebracht.

Beim Eindampfen der erhaltenen Lösung bis zur Trockne oder durch Sprühtrocknung und dergleichen wird als Zielprodukt Roh-Cyclooctaamylose mit einem Reinheitsgehalt von > 90 % erhalten.

Falls ein reineres Produkt erwünscht wird, wird die Roh-Cyclooctaamylose mit Pyridin bei einer Temperatur von 20 bis 60 °C, insbesondere ca. 50 °C, behandelt. Durch Zugabe von Alkohol, z.B. Methanol, zur pyridinischen Suspension fällt Cyclooctaamylose als leicht sedimentierbarer Niederschlag an. Als weiterer Reinigungsschritt bietet sich das Umfällen der Cyclooctaamylose aus wäßriger Lösung mit z.B. Ethanol an.

Das erfindungsgemäße Verfahren gestattet es, ausgehend von Stärke, Cyclooctaamylose in stark verbesserten Ausbeuten sowie in hoher Reinheit herzustellen. Das Verfahrensprodukt findet Verwendung u.a. als Bestandteil von Pflanzenschutzmitteln, Medikamenten, Kosmetika oder Lebensmitteln.

Die Erfindung wird nun anhand von Beispielen näher erläutert:

Beispiel 1

150 g Kartoffelstärke wurden in 1 l Wasser, das noch 200 mMol Natriumacetat und 5 mMol Calciumchlorid enthielt, suspendiert und durch Erhitzen auf 120 °C innerhalb von 30 Minuten gelifiziert. Der pH-Wert betrug 6,9. Nach Abkühlen der Mischung auf 70 °C wurden unter Rühren und weiterer Abkühlung 10 mg Cyclodextrin-glykosyltransferase von Klebsiella pneumoniae, gelöst in 20 ml eines 20 millimolaren Triethanolamin-hydrochlorid-puffers (pH 7,2; 5 mMol Calciumchlorid), zugesetzt. Unter weiterem Rühren wurde auf 40 °C abgekühlt und danach noch 20 mg Cyclodextrin-glykosyltransferase von Klebsiella pneumoniae in der gleichen Zubereitung wie oben beschrieben, zugegeben.

Nach einer Inkubationszeit von 7 Stunden bei 40 °C wurde durch chromatographische Analyse keine Zunahme des primär gebildeten α-Cyclodextrins mehr festgestellt.

Das Reaktionsgemisch wurde nun mit 26 g Brombenzol versetzt. Nach einer weiteren Inkubationszeit von 15 Stunden bei 40 °C unter Rühren waren 61,4 Gew.-% der Stärke zu Cyclodextrinen abgebaut. Das Verhältnis von α- / β- / γ-Cyclodextrin betrug 1 : 4,1 : 2,23.

Die schwerlöslichen Bestandteile der Reaktionsmischung wurden nun durch Zentrifugieren abgetrennt, in 1 l Wasser suspendiert und zum Sieden erhitzt, wobei Brombenzol als Aceotrop mit Wasser abdestillierte.

Die erhaltene leicht trübe Lösung wurde auf Raumtemperatur abgekühlt und mit 1,2 l Methanol versetzt. Nach Abtrennen des gebildeten Niederschlags wurde das Methanol abdestilliert und die wäßrige Lösung soweit eingeengt, daß die Konzentration an γ-Cyclodextrin 20 Gew.-% betrug. Das erhaltene Konzentrat wurde anschließend 12 Stunden bei 4 °C aufbewahrt.

Es wurden 48 g farblose Kristalle von β-Cyclodextrin erhalten, die von der Lösung abgetrennt und einmal mit 200 ml eiskaltem Wasser gewaschen wurden.

Die vereinigten Überstände wurden mit 7,2 g Brombenzol versetzt und 6 Stunden bei Raumtemperatur gerührt. Der entstandene farblose Niederschlag wurde anschließend abzentrifugiert, in 150 ml Wasser aufgenommen und durch Kochen unter aceotropen Abdestillieren des Brombenzols in Lösung gebracht.

Die erhaltene wäßrige Lösung wurde schließlich lyophilisiert. Es wurden 30 g eines Gemisches aus 2,4 g β-Cyclodextrin und 27,6 g γ-Cyclodextrin erhalten.

Die Ausbeute an γ-Cyclodextrin betrug demnach 98,4 %, bezogen auf den γ-Cyclodextrin-Gehalt der Konversionsmischung.

Zur weiteren Reinigung wurde das Gemisch nach 2-stündigem Trocknen bei 105 °C in 100 ml Pyridin suspendiert und 2 Stunden bei 50 °C gerührt. Anschließend wurde mit 200 ml Methanol versetzt. Das erhaltene farblose Präzipitat wurde in 150 ml Wasser suspendiert und durch Erhitzen in Lösung gebracht. Das γ-Cyclodextrin wurde mit 450 ml Ethanol gefällt und nach erneutem Umfällen mit Ethanol im Vakuum getrocknet.

Es wurden 27,2 g γ-Cyclodextrin mit einem Reinheitsgehalt von 98,2 % erhalten. Die Ausbeute, bezogen auf den γ-Cyclodextrin-Gehalt der Konversionsmischung betrug 95,2 %.

Eine Wiederholung des beschriebenen Reinigungsverfahrens führte zu 25,6 g γ-Cyclodextrin vom Reinheitsgehalt 99,8 %.

Die Ausbeute an Reinprodukt betrug, bezogen auf eingesetzte Stärke, 17 %.

Beispiel 2

200 g Maniok-Stärke wurden in 1 l Wasser mit einem zusätzlichen Gehalt von 200 mMol Natriumacetat und 5 mMol Calciumchlorid suspendiert und durch 30-minütiges Erhitzen auf 120 °C gelifiziert. Der pH-Wert der Reaktionsmischung betrug 6,9. Nach Abkühlen auf 70 °C wurden unter Rühren und weiterer Abkühlung 13 mg Cyclodextrin-Glykosyltransferase von Klebsiella pneumoniae in der gleichen Zubereitung, wie im Beispiel 1 beschrieben, zugesetzt. Unter weiterem Rühren wurde auf 43 °C abgekühlt und danach noch 27 mg Cyclodextrin-glykosyltransferase von Klebsiella pneumoniae in der gleichen Zubereitung wie oben beschrieben zugegeben.

Nach einer Inkubationszeit von 8 Stunden bei 43 °C wurde, durch chromatographische Analyse ermittelt, keine weitere Zunahme an initial gebildetem α-Cyclodextrin ermittelt.

Danach wurden dem Reaktionsgemisch 32,6 g Brombenzol zugegeben. Nach einer weiteren Inkubationszeit von 40 Stunden bei 43 °C waren 59 Gew.-% der Stärke zu Cyclodextrinen abgebaut. Das Verhältnis von α- / β- / γ-Cyclodextrin betrug 1 : 10,6 : 3,92.

Es wurden die unlöslichen Bestandteile der Konversionsmischung durch Zentrifugation abgetrennt und in 1 l Wasser aufgenommen. Die Suspension wurde zum Sieden erhitzt, wobei Brombenzol als Aceotrop mit Wasser abdestillierte.

Die erhaltene leicht trübe Lösung wurde nun soweit eingeengt, daß die Konzentration an γ-Cyclodextrin 20 Gew.-% betrug. Das erhaltene Konzentrat wurde 12 Stunden bei 4 °C aufbewahrt.

Es wurden 74 g Roh-β-Cyclodextrin erhalten, die von der Lösung abgetrennt und einmal mit eiskaltem Wasser gewaschen wurden.

Die vereinigten Überstände wurden wie gemäß Beispiel 1 beschrieben aufgearbeitet.

Es wurden erhalten:

29,2 g Roh-γ-Cyclodextrin, entsprechend einer Ausbeute von 98,6 %, bezogen auf den γ-Cyclodextrin-Gehalt der Konversionsmischung

27 g γ-Cyclodextrin vom Reinheitsgehalt 99,7 %, entsprechend einer Ausbeute, bezogen auf eingesetzte Stärke von 13,5 %.

Beispiel 3

Es wurde die Arbeitsweise gemäß Beispiel 2 wiederholt, mit der Abänderung, daß anstatt in Gegenwart von 200 mMol Natriumacetat in Gegenwart von 200 mMol Natriumformiat gelifiziert wurde.

Nach einer Gesamt-Inkubationszeit von 48 Stunden waren 57 Gew.-% der Stärke zu Cyclodextrinen abgebaut. Der Anteil an γ-Cyclodextrin betrug 14 Gew.-%.

Beispiel 4

Die Arbeitsweise gemäß Beispiel 2 wurde wiederholt, mit der Abänderung, daß anstatt in Gegenwart von 200 mMol Natriumacetat in Gegenwart von 200 mMol Kaliumpropionat gelifiziert wurde.

Nach einer Gesamt-Inkubationszeit von 48 Stunden waren 56,7 Gew.-% der Stärke zu Cyclodextrinen abgebaut. Der Anteil an γ-Cyclodextrin betrug 13,8 %.

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(54) Producing modified cyclodextrins

(57) A process for modifying and increasing the water solubility of cyclodextrins in a moderate controlled progressive reaction comprises forming ether derivatives of cyclodextrins by reaction with an alkylene carbonate, such as ethylene carbonate, under anhydrous reaction conditions and in an alkaline environment at a temperature of at least 100°C.

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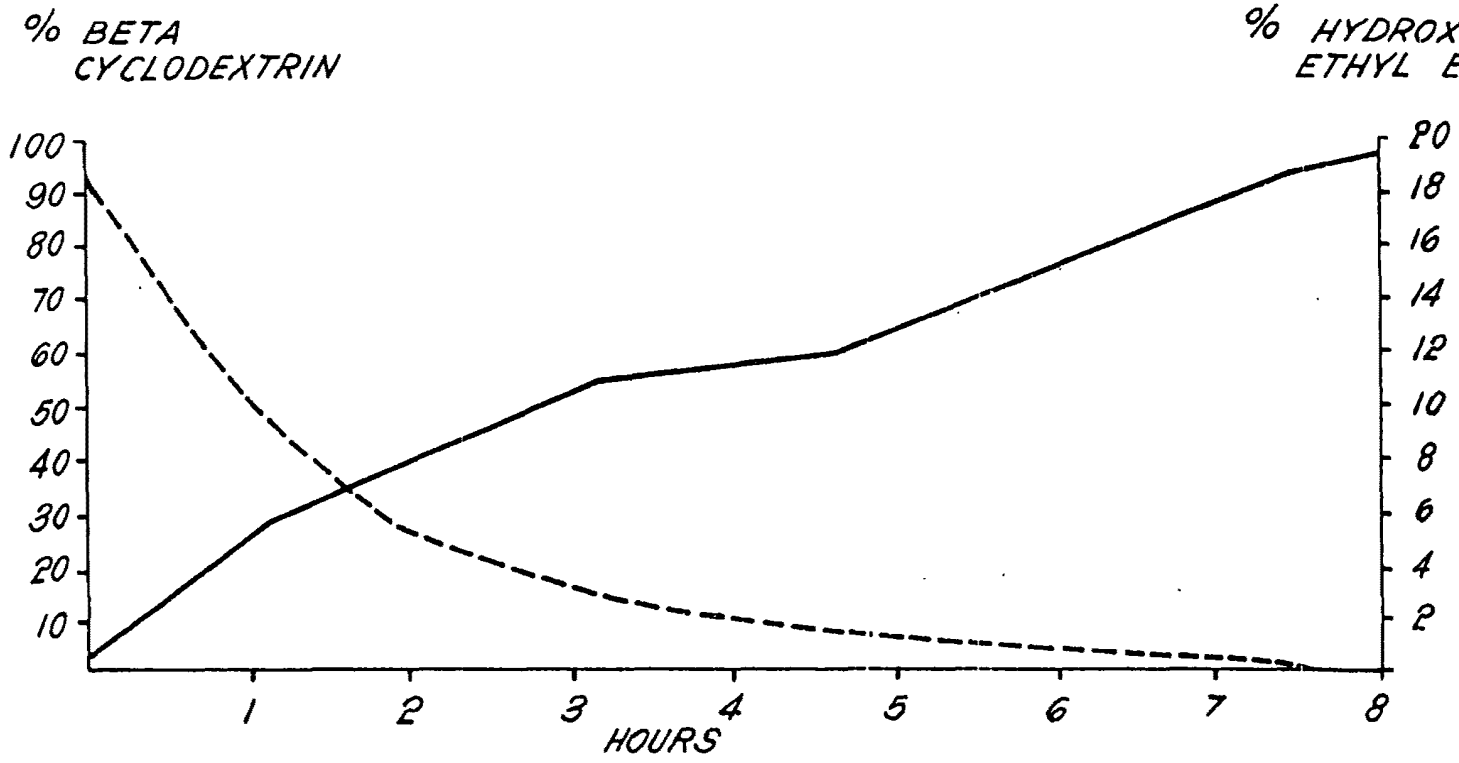


Fig. 1

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SPECIFICATION

Process for producing modified cyclodextrins

The present invention is directed to a process for modifying and increasing the water solubility of cyclodextrins in a moderate controlled progressive reaction and in particular to producing derivatives of cyclodextrins in a substantially anhydrous reaction with an alkylene carbonate such as ethylene carbonate in an alkaline environment.

Cyclodextrins also called "Schardinger dextrins" are known to be cyclic oligosaccharides composed of glucose residues bonded together by alpha 1,4 bonds. The six membered ring structure is called alph-cyclodextrin, the seven membered ring is beta-cyclodextrin and the eight membered ring is gamma-cyclodextrin. The cyclodextrins have different chemical and physical properties from the linear oligosaccharides derived from starch in that they are non-reducing dextrins and the ring structure is widely used as a host for the inclusion of various compounds usually organic compounds for the food, pharmaceutical and chemical fields.

As is also well-known, cyclodextrins are produced from starch of any selected plant variety such as corn, potato, waxy maize and the like which may be modified or unmodified starch derived from cereal or tuber origin and the amylose or amylopectin fractions thereof. The selected starch is aqueous slurry at selected concentration up to about 35% by weight solids is usually liqufied as by gelatinization or treatment with a liquefying enzyme such as bacterial alpha-amylase enzyme and then subject to treatment with a transglycosylase (CGT) enzyme to form the cyclodextrins.

The amount of the individual alpha, beta and gamma-cyclodextrins produced by treating the starch with the CGT enzyme will vary depending on the selected starch, selected CGT enzyme and processing conditions. The parameters to select for the CGT enzyme conversion for the desired result in the amount of each individual cyclodextrin to be produced is conventional and well-described in the literature.

Conventionally, the DE of the liquefied starch is maintained below about 20 DE, the starch solids concentration is below about 35% by weight, the pH for conversion may be about 4.5 to 8.5 at a selected temperature from ambient and up to about 75°C for a selected period of time typically from about 10 hours up to seven days and more. The amount of CGT enzyme used for conversion is conventional and well-known in the art.

Precipitation and separation of the individual cyclodextrins described in the prior art include solvent systems (D. French et al. J. Am. Chem. Soc. 71, 353 (1949)), inclusion compounds such as trichloroethylene (U.S. Patent 3,425,910) as well as non-solvent systems utilizing selected ion exchange resins and chromatographic gel filtration (u.S. Patents 4,418,144 and 4,303,787). The individual cyclodextrins and mixtures thereof are readily available in the market.

Beta-cyclodextrin is most widely used to form inclusion complexes particularly in the pharmaceutical field. The six membered ring alpha-cyclodextrin has a cavity size of about 5 to 6 Å which is too small to take up most of the commonly used pharmeceuticals, whereas the beta-cyclodextrin cavity measures about 7 to 8 Å which accommodates most pharmaceuticals as does the gamma-cyclodextrin cavity of about 9 to 10 Å. Additionally, by making beta-cyclodextrin more soluble, it is highly economically attractive. The beta-cyclodextrin tends to form stable complexes with many pharmaceuticals but it is frequently very difficult to use because of its low water solubility. Only about 1.8 grams of beta-cyclodextrin will dissolve in 100 mls. of water as compared to the about 14 grams of alpha-cyclodextrin and about 23 grams of gamma-cyclodextrin that dissolve in 100 mls. of water at room temperature.

It has now been discovered that water solubility of the cyclodextrins may be drastically increased by modification with alkylene carbonates and preferably ethylene carbonate to form hydroxyethyl ethers on the ring structure. In a preferred embodiment of the present invention, the water solubility of the beta-cyclodextrin was increased up to about 60 grams in 100 mls. of water without necessarily interfering with its capacity to form inclusion complexes. In fact, water solubility has been measured at greater than 60 grams in 100 mls. of water at room temperature. Another advantage of the present invention is that the modification to hydroxythyl ethers is carried out in a moderate progressive reaction under readily controlled conditions and it may be stopped to obtain the desired degree of modification in a predicatable manner.

In accordance with the present invention, the modification with the alkylene carbonate is carried out in a substantially anhydrous system in an alkaline environment which is of advantage in that the reaction is carried out with the reagent as reaction medium. The alkylene oxides have been used heretofore for modifying starch and the reducing sugars of starch hydrlysates and long chain oligosaccharides but the known processes involve either an aqueous system wherein water interferes with etherification of the sugars is a non-aqueous system wherein a complicated multistage procedure is used which makes it virtually impossible to control the reaction for predictable results. This is especially true of the highly aggressive alkylene oxide reagents such as ethylene and propylene oxides which require special precautions to avoid the danger of

explosion and the serious hazard to health because of toxicity. The alkylene carbonates of the present invention are non-toxic as are the resulting cyclodextrin hydroxyethyl ethers and there is no need for any special precautions or controls during etherification since the reaction is a moderate one that is readily controlled in predictable manner to the desired degree of etherification.

In accordance with the present invention, it is only necessary to mix the selected cyclodextrin and alkylene carbonate in a basic environment and heat the mixture to initiate the moderate etherification reaction that progressively proceeds in the formation of the cyclodextrin hydroxyether. The reaction mixture is then held at the elevated temperature for a period of time to allow the reaction to progressively proceed. Preferably, the dry, selected cyclodextrin is mixed with a dry basic catalyst and a liquefied alkylene carbonate is added to form a homogeneous slurry prior to the addition of heat. The amount of water present in the substantially anhydrous reaction mixture is maintained below about 20% by weight of the ingredients and preferably below 15% by weight.

The substantially anhydrous reaction is carried out in an alkaline environment. In order to form the basic environment a basic catalyst is used. The basic catalyst is present in the reaction vessel in an amount sufficient to initiate the reaction. Initiation of the reaction is apparent by visually observing bubbles coming off of the reaction medium. It is preferred that the amount of basic catalyst present for the reaction of the present invention is about 2% to about 10% by weight based on the weight of the cyclodextrin present. Best results are achieved when reaction is carried out in the presence of a basic catalyst such as potassium carbonate in an amount of about 4 to 6% by weight based on the weight of cyclodextrin. Other catalysts that may be used to advantage include sodium hydroxide and triethylamine.

The amount of alkylene carbonate used for reaction may, of course, be varied but for best results an excess of the selected alkylene carbonate is used for the maximum degree of modification. Reaction is stopped by cooling the mix to ambient temperature when the desired degree of modification has been achieved. For best results in control of reaction and predictability, ethylene carbonate is used in an amount of from about 12 moles to about 40 moles for each mole of cyclodextrin in the reaction mixture. Any alkylene carbonate can be used. Preferably, reaction is carried out with just one selected alkylene carbonate but, if desired, a mixture of alkylene carbonates may be used in the reaction mixture. Suitable alkylene carbonate for the present invention include propylene carbonate, ethylene carbonate, butylene carbonate and glyceryl carbonate. The amount of aklylene carbonate used is about 12 moles to about 40 mols per mole cyclodextrin. Greater amounts of alkylene carbonate can be used without seriously effecting the reaction.

The preferred cyclodextrin is beta-cyclodextrin for use in the pharmaceutical and food fields. Preferably, reaction is carried out with just one selected cyclodextrin but, if desired, a mixture of cyclodextrins may be used in the reaction mixture.

The temperature of reaction may also be varied from about 100°C up to about 200°C and preferably it is between about 100 to 150°C.

Further details and advantages of the present invention are most readily understood by reference to preferred embodiments herein chosen for illustration and to the drawing in which:-

Figure 1 which illustrates the controlled progressive modification of beta-cyclodextrin in accordance with the invention.

EXAMPLE 1

In one preferred embodiment of the invention, 100 grams of beta-cyclodextrin were mixed with 6 grams of potassium carbonate. The dry powder mix was placed in a glass conventional three-necked flask fitted with a condenser. 250 grams of liquefied ethylene carbonate were added and mixed with the dry powder to form a homogeneous slurry. In this example the ethylene carbonate was liquefied by melting the solid ethylene carbonate. The cyclodextrin contained a small amount of about 10% of moisture present in the substantially anhydrous reaction mixture. The temperature was raised to 125°C and held at 125°C for 7–1/2 hours whereupon all of the ingredients were in solution according to visual observation.

The reaction mass was allowed to cool to room temperature and about 1 liter of acetone was added and mixed vigorously with the solution to precipitate the hydroxyethyl ether non-toxic beta-cyclodextrin. The precipitate was removed from the reaction liquor by vacuum filtration and then redissolved in a minimal amount of methanol, reprecipitated by acetone and then filtered and dried. Dissolving the precipitate in methanol is effective for removing the acetone by distillation if this should be desired and the methonol solution may be treated with an ion exchange resin in conventional manner to remove residual catalyst salts and conventional carbon bleaching may also be used in conventional manner for decolorization. After a final precipitation with acetone, the non-toxic hydroxyethyl ether beta-cyclodextrin was a white powder which exhibited the high water solubility of 60 grams dissolved in 100 mls. of water.

The moderate progression of the controlled reaction is shown in Fig. 1. As there shown the

modification of the beta-cyclodextrin quite unexpectedly progressed in a linear manner and at the end of 7–1/2 hours there was no discernible unreacted beta-cyclodextrin left in the reaction liquor. The % of hydroxyethyl ether was at a maximum of about 19% at that point which is equal to a degree of substitution of about 0.7. The moderate progression in linear manner as a correlation of time and temperature and concentrations in the example provides excellent control whereby the reaction may be stopped at any desired point to obtain a desired degree of substitution in a predictable manner for the application use at hand where the control of water solubility of the inclusion complex is desirable.

EXAMPLE II

In this second embodiment of the invention, gamma-cyclodextrin is substituted for the beta-cyclodextrin in Example I using the same proportions of ingredients and reaction condition of Example I. The modification of the gamma-cyclodextrin to hydroxyethyl ether proceeds in a comparable linear predictable manner.

It will be understood that progression of the moderate reaction is a function of time and temperature for any given concentration of ingredients and that the progression of the modification may vary albeit the reaction will still progress in a controlled linear manner.

EXAMPLE III

Example I is repeated by substituting alpha–cyclodextrin for the beta–cyclodextrin using the same procedure and proportion of ingredients for controlled linear conversion to alpha hydroxyethyl ether cylodextrin.

Acetone is preferred for recovery of the hydroxyethyl ether cyclodextrins since its boiling point is below that of the alkylene carbonates which may be readily recovered from the acetone by conventional distillation and recycled for the modification reaction. The alkylene carbonates are soluble in a wide range of organic solvents which provides flexibility in selection of a solvent system in which the cyclodextrin derivatives are insoluble. For example, N-propanol, ethyl acetate, toluene and chloroform may be substituted for acetone if desired.

EXAMPLE IV

Example I is repeated by substituting propylene carbonate for the ethylene carbonate and heating to, and carrying out the reaction at 150°C; otherwise, the same procedure and proportion of ingredients for controlling linear conversion to beta-hydroxypropyl ether cyclodextrin. It is noted that propylene carbonate is a liquid and therefore did not need to be melted prior to addition.

The cyclodextrins of the present invention may be recovered after reaction in any convenient manner. Conventional freeze-drying may be employed, for example, precipitating the hydroxy either cyclodextrin with a suitable precipitant such as acetone. Thereafter the precipitate is dissolved in water, preferably deionized and decolorized in conventional manner and then freeze dried.

CLAIMS

1. The process of modifying cyclodextrins to produce hydroxy ethers which comprises the steps of forming a substantially anhydrous mixture of one or more alpha, beta and gamma-cyclodextrins and an alkylene carbonate in a basic environment and heating the resulting mixture to a temperature of at least about 100°C to modify the one or more cyclodextrins and form an ether derivative or derivatives thereof.

2. A process as claimed in Claim 1, wherein beta-cyclodextrin is reacted with the alkylene carbonate.

3. A process as claimed in Claim 2, wherein the alkylene carbonate is ethylene carbonate which reacts with the beta-cyclodextrin to form a hydroxyethyl ether modified beta-cyclodextrin.

4. A process as claimed in Claim 2, wherein the alkylene carbonate is propylene carbonate which reacts with the beta-cyclodextrin to form a hydroxyethyl ether modified beta-cyclodextrin.

5. A process as claimed in Claim 3, wherein the reaction is continued until the water solubility of the hydroxyethyl ether formed is greater than about 1.8 grams of the beta-cyclodextrin hydroxy ether in 100 mls of water and up to about 60 grams in 100 mls of water.

6. A process as claimed in any one of the preceding Claims, wherein from about 12 moles to about 40 moles of alkylene carbonate is present in the reaction mixture for each mole of cyclodextrin.

7. A process as claimed in any one of the preceding Claims, wherein a basic catalyst is added to produce a basic environment.

8. A process as claimed in Claim 7, wherein said basic catalyst is potassium carbonate.

9. A process as claimed in any one of the preceding Claims, wherein the amount of water present in the substantially anhydrous reaction mixture is not more than about 20% by weight of the dry solids therein.

10. A process of modifying beta-cyclodextrin with an alkylene carbonate and a basic catalyst to form a substantially anhydrous reaction mixture, heating the anhydrous reaction mixture to a temperature of at least about 100°C to modify the beta-cyclodextrin by forming an ether derivative of increased water solubility and recovering the modified beta-cyclodextrin ether derivative.

11. A process as claimed in Claim 10, wherein the alkylene carbonate is ethylene carbonate and is present in the reaction mixture in an amount of about 12 moles to about 40 moles for each mole of beta-cyclodextrin.

12. A process as claimed in Claim 10, wherein the alkylene carbonate is propylene carbonate and is present in the reaction mixture in an amount of about 12 moles to about 40 moles for each mole of beta-cyclodextrin.

13. A process as claimed in any one of Claims 10 to 12, wherein the basic catalyst is potassium carbonate.

14. A process as claimed in any one of Claims 10 to 13, wherein the modified beta-cyclodextrin is recovered by adding acetone to precipitate the beta-cyclodextrin product from the reaction mixture.

15. A process as claimed in Claim 14, wherein the modified beta-cyclodextrin is removed from the acetone and the ethylene carbonate is recovered from the acetone for recycling.

16. A process as claimed in Claim 14 or Claim 15, wherein the recovered beta-cyclodextrin product is redissolved in methanol and reprecipitated from the methanol solution by adding acetone thereto.

17. A process as claimed in any one of Claims 10 to 16, wherein the reaction mixture is heated to a temperature of about 125°C to provide a moderate progressive reaction that proceeds in linear manner with time.

18. A process as claimed in any one of Claims 10 to 17, wherein the substantially anhydrous reaction mixture contains not more than about 20% of water by weight of the dry solids therein.

19. A process for modifying cyclodextrins to produce hydroxy ethers, substantially as hereinbefore described with reference to any of the foregoing examples.

20. Ether derivatives of cyclodextrins prepared by modifying cyclodextrins by a process as claimed in any one of Claims 1 to 19.

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LIBERGRAF, STOCKHOLM 1990

Pharmaceutical compositions containing drugs which are instable or sparingly soluble in water and methods for their preparation

Description

The invention relates to pharmaceutical compositions containing drugs which are instable or only sparingly soluble in water, and methods for their preparation. The compositions are characterized by increased water solubility and improved stability.

A large number of drugs is only poorly or sparingly soluble in water so that suitable application forms like drop solutions or injection solutions are being prepared using other polar additives like propylene glycol etc. If the drug molecule has basic or acidic groups there exists the further possibility of increasing the water solubility by salt formation. As a rule this results in decreased efficacy or impaired chemical stability. Due to the shifted distribution equilibrium the drug may penetrate the lipophilic membrane only slowly corresponding to the concentration of the non-dissociated fraction while the ionic fraction may be subject to a rapid hydrolytic decomposition.

Additional "water-like" solvents like low molecular polyethylene glycols or 1,2-propylene glycol are therefore used in the preparation of aqueous solutions of sparingly water-soluble drugs which glycols, however, cannot be considered pharmacologically inert, or the drug is solubilized using surfactants so that the drug molecules are occluded in micelles. This solubilization has numerous disadvantages: The surfactant molecules used have frequently a strongly haemolytic effect and the drug needs to pass out of the micelle by diffusion after the application. This results in a retard effect (compare B.W. Müller, Gelbe Reihe, Vol. X, pages 132ff (1983)).

Accordingly it may be stated that there exists no satisfactory and generally applicable method of solubilization.

For solid drugs it is also important to render the sparingly water-soluble drug water-soluble since a good solubility increases the bioavailability of the drug. It has been described that inclusion compounds, e.g. with urea or complexes of polyvinyl pyrrolidone may improve the solubility of a compound but in aqueous solution they are not stable. Such inclusion compounds are therefore at best suitable for solid application forms of drugs.

This is different when using α-, β-, and γ-cyclodextrin which can bind a drug in its ring also in aqueous solution (W. Sänger, Angewandte Chemie 92, 343 (1980)). However, it is disadvantageous that the β-cyclodextrin itself is only poorly water-soluble (1.8 g/100 mL) so that the therapeutically necessary drug concentrations are not achieved.

If a derivative is formed of the cyclodextrin its solubility and therefore the amount of dissolved drug may be considerably increased. Thus, German Offenlegungsschrift 31 18 218 discloses a solubilization method using methylated β-cyclodextrin as monomethyl derivative with 7 methyl groups and especially as dimethyl derivative with 14 methyl groups. With the 2,6-di-0-methyl derivative it is for instance possible to increase the water solubility of indomethacin 20.4-fold and that of digitoxin 81.6-fold.

In WO-A-820 051 inclusion complexes of retinoids with cyclodextrins and cyclodextrin derivatives are described. The rationale of the publication (or: the publication is based on the problem...) is the reduction of toxic side effects of retinoids by modification of the molecular structure and/or by complexation with cyclodextrins. The solubility of these substances (retinoids) in lipids shall be lowered and the tendency of accumulation in adipose tissue shall be reduced or avoided. In the publication, α, β and γ cyclodextrins and their derivatives are described to be equally suitable for complexation whereas mainly methoxy-derivatives of α, β and γ cyclodextrins are mentioned (listed) as examples.

However, for therapeutical use the methyl derivatives of β-cyclodextrin show serious draw backs. Due to their increased lipophilicity they have a haemolytic effect and they further cause irritations of the mucosa and eyes. Their acute intravenous toxicity is still higher than the already considerable toxicity of the unsubstituted β-cyclodextrin. It is a further serious disadvantage for the practical application that the solubility of the dimethyl β-cyclodextrin and its complexes suffers a steep decrease at higher temperatures so that crystalline dextrin precipitates upon heating. This phenomenon makes it very difficult to sterilize the solutions at the usual temperatures of 100 to 121°C.

Quite surprisingly it has now been found that certain other β-cyclodextrin derivatives can form inclusion compounds which also considerably increase the water-solubility of sparingly water-soluble and instable drugs without showing the disadvantages described above.

Subject of the invention are therefore novel pharmaceutical compositions comprising inclusion compounds of only sparingly water-soluble or in water instable drugs with a partially etherified β-cyclodextrin in which the residues are hydroxyethyl, hydroxypropyl or dihydroxypropyl groups and part of the residues may optionally be methyl or ethyl groups. The β-cyclodextrin ether have a water-solubility of more than 1.8 g in 100 mL water, except for inclusion complexes of retinoids with β-cyclodextrin ethers if the residues are hydroxyethyl, hydroxypropyl or dihydroxypropyl groups. The use of partially methylated β-cyclodextrin ethers with 7 to 14 methyl groups in the β-cyclodextrin molecule, as they are known from German Offenlegungsschrift 31 18 218 do not come under the present invention. Furthermore, inclusion complexes of retinoids with β-cyclodextrin ethers are excluded, as far as methyl-ethyl- and 2-hydroxyethyl substituents are concerned (WO-A-820 051).

β-cyclodextrin is a compound with ring structure consisting of 7 anhydro glucose units; it is also referred to as cycloheptaamylose. Each of the 7 glucose rings contains in 2-, 3-, and 6-position three hydroxy groups which may be etherified. In the partially etherified β-cyclodextrin derivatives used according to the invention only part of these hydroxy groups is etherified with hydroxyalkyl groups and optionally further with methyl or ethyl groups. When etherifying with 5-hydroxy alkyl groups which can be carried out by reaction with the corresponding alkylene oxides, the degree of substitution is stated as molar substitution (MS), viz. in mole alkylene oxide per anhydroglucose unit, compare US patent specification 34 59 731, column 4. In the hydroxyalkyl ethers of β-cyclodextrin used in accordance with the invention the molar substitution is between 0.05 and 10, preferably between 0.2 and 2. Particularly preferred is a molar substitution of about 0.25 to about 1.

The etherification with alkyl groups may be stated directly as degree of substitution (DS) per glucose unit which - as stated above - is 3 for complete substitution. Partially etherified β-cyclodextrins are used within the invention which comprise besides hydroxyalkyl groups also alkyl groups, especially methyl or ethyl groups, up to a degree of substitution of 0.05 to 2.0, preferably 0.2 to 1.5. Most preferably the degree of substitution with alkyl groups is between about 0.5 and about 1.2.

The molar ratio of drug to β-cyclodextrin ether is preferably about 1:6 to 4:1, especially about 1:2 to 1:1. As a rule it is preferred to use the complex forming agent in a molar excess.

Useful complex forming agents are especially the hydroxyethyl, hydroxypropyl and dihydroxypropyl ether, their corresponding mixed ethers, and further mixed ethers with methyl or ethyl groups, such as methyl-hydroxyethyl, methyl-hydroxypropyl, ethyl-hydroxyethyl and ethyl-hydroxypropyl ether of β-cyclodextrin.

The preparation of the hydroxyalkyl ethers of β-cyclodextrin may be carried out using the method of US patent specification 34 59 731. Suitable preparation methods for β-cyclodextrin ethers may further be found in J. Szejtli et al., Stärke 32, 165 (1980) and A.P. Croft and R.A. Bartsch, Tetrahedron 39, 1417 (1983). Mixed ethers of β-cyclodextrin can be prepared by reacting β-cyclodextrin in a basic liquid reeaction medium comprising an akali metal hydroxide, water and optionally at least one organic solvent (e.g. dimethoxyethane or isopropanol) with at least two different hydroxyalkylating and optionally alkylating etherifying agents (e.g. ethylene oxide, propylene oxide, methyl or ethyl chloride).

Drugs exhibiting a significantly increased water-solubility and improved stability, respectively, after having been transferred into inclusion compounds with the above-mentioned β-cyclodextrin ethers are those having the required shape and size, i.e. which fit into the cavity of the β-cyclodextrin ring system. This includes for instance non-steroid anti-rheumatic agents, steroids, cardiac glycosides and derivatives of benzodiazepine, benzimidazole, piperidine, piperazine, imidazole or triazole.

Useful benzimidazole derivatives are thiabendazole, fuberidazole, oxibendazole, parbendazole, cambendazole, mebendazole, fenbendazole, flubendazole, albendazole, oxfendazole, nocodazole and astemisole. Suitable piperadine derivatives are fluspirilene, pimozide, penfluridole, loperamide, astemizole, ketanserine, levocabastine, cisapride, altanserine, and ritanserine. Suitable piperazine derivatives include lidoflazine, flunarizine, mianserine, oxatomide, mioflazine and cinnarizine. Examples of suitable imidazole derivatives are metronidazole, ornidazole, ipronidazole, tinidazole, isoconazole, nimorazole, burimamide, metiamide, metomidate, enilconazole, etomidate, econazole, clotrimazole, carnidazole, cimetidine, docodazole, sulconazole, parconazole, orconazole, butoconazole, triadiminole, tioconazole, valconazole, fluotrimazole, ketoconazole, oxiconazole, lombazole, bifonazole,

oxmetidine, fenticonazole and tubulazole. As suitable triazole derivatives there may be mentioned virazole, itraconazole and terconazole.

Particularly valuable pharmaceutical compositions are obtained when converting etomidate, ketoconazole, tubulazole, itraconazole, levocabastine or flunarizine into a water-soluble form using the complex forming agents of the invention. Such compositions are therefore a special subject of the present invention.

The invention is further directed to a method of preparing pharmaceutical compositions of sparingly water-soluble or water-instable drugs which is characterized by dissolving the β-cyclodextrin ether in water and adding thereto the selected drug as well as optionally drying the solution of the formed inclusion compound using methods known per se. Formation of the solution may take place at temperatures between 15 and 35°C.

The drug is suitably added stepwise. The water may further comprise physiologically compatible compounds such as sodium chloride, potassium nitrate, glucose, mannitole, sorbitol, xylitol or buffers such as phosphate, acetate or citrate buffer.

Using β-cyclodextrin ethers in accordance with the invention it is possible to prepare application forms of drugs for oral, parenteral or topical application, e.g. infusion and injection solutions, drop solutions (e.g. eye drops or nasal drops), sprays, aerosols, syrups, and medical baths.

The aqueous solutions may further comprise suitable physiologically compatible preserving agents such as quaternary ammonium soaps or chlorbutanol.

For the preparation of solid formulations the solutions of the inclusion compounds are dried using conventional methods; thus the water may be

evaporated in a rotation evaporator or by lyophilization. The residue is pulverized and, optionally after addition of further inert ingredients, converted into uncoated or coated tablets, suppositories, capsules, creams or ointments.

The following examples serve to illustrate the invention which, however, is not restricted to the examples.

The phosphate buffer solution mentioned in the examples had a pH of 6.6 and the following composition:

KH_2PO_4	68.05 g
NaOH	7.12 g
Aqua demin. ad.	5000.0 g

All percentages are percent by weight.

Example 1

Starting from a 7% stock solution of hydroxyethyl β-cyclodextrin (MS 0.43) in phosphate buffer solution a dilution series was prepared so that the complex forming agent concentration was increased in steps of 1%. 3 mL of these solutions were pipetted into 5 mL snap-top-glasses containing the drug to be tested. After shaking for 24 hours at 25°C the solution was filtered through a membrane filter (0.22 microns) and the dissolved drug content was determined spectrophotometrically. Figures 1, 3 and 4 show the increase of the drug concentration in solution in relation to the concentration of the complex forming agent for indomethacin (figure 1), piroxicam (figure 3) and diazepam (figure 4). The maximum drug concentration is limited by the saturation solubility of the cyclodextrin derivative in the buffer which in the case of hydroxyethyl-β-cyclodextrin (MS 0.43) is reached at 7.2 g/100 mL.

When comparing for instance the results obtained with indomethacin to those given in German Offenlegungsschrift 31 18 218 for 2,6-di-0-methyl-β-cyclodextrin (figure 2) it will be observed that the hydroxyethyl derivative has a significantly higher complex formation constant (compare the different slopes in figures 1 and 2).

Example 2

A. The saturation solubility at 25°C of different drugs was determined using a 10% hydroxypropyl-β-cyclodextrin solution (MS 0.35) in phosphate buffer solution under the same conditions as in example 1. The saturation solubilities S_1 in phosphate buffer solution and S_2 in phosphate buffer solution and 10% added hydroxypropyl-β-cyclodextrin are given in table 1.

Table 1

Drugs	**S_1 (mg/mL)**	**S_2 (mg/mL)**	**Ratio S_1:S_2**
Indomethacin	0.19	5.72	1: 30.1
Digitoxin	0.002	1.685	1: 842.5
Progesterone	0.0071	7.69	1:1083.0
Dexamethasone	0.083	14.28	1: 172.0
Hydrocortisone	0.36	21.58	1: 59.9
Diazepam	0.032	0.94	1: 29.4

B. The solubility of drugs in a 4% aqueous solution of hydroxypropyl-methyl-β-cyclodextrin (DS 0.96; MS 0.43) was determined in a similar manner. The results obtained are summarized in the following table 2 in which the ratio R of the saturation solubility in water or at the stated pH, respectively, with and without addition of β-cyclodextrin derivative is stated for each drug. The solutions

prepared according the invention were further found to be significantly more stable when compared with aqueous solutions.

Table 2

Drug		R
Itraconazole	at pH 5	96
	at pH 2.5	75
Flunarizine		18
Levocabastine	at pH 9.5	81
	at pH 7.4	8
Ketoconazole		85
Flubendazole		30
Tubulazole		43
Cisapride		3
Loperamide		62
Etomidate		8.5
Cinnarizine	at pH 5	28
	at pH 3	12

Example 3

In 10 mL phosphate buffer solution 0.7 g hydroxyethyl-β-cyclodextrin (MS 0.43) were dissolved together with 0.04 g indomethacin at 25°C until a clear solution was formed. This solution was filtered through a membrane filter (0.22 microns) and filled under laminar flow into a pre-sterilized injection bottle which was stored at 21°C (B). In a parallel test a saturated indomethacin solution in a phosphate buffer solution (0.21 mg/mL) was stored under the same conditions (A). The drug concentrations determined by high pressure liquid chromatography are given in table 3. The great improved stability of the composition according to the invention is apparent.

Table 3

Storing Time in weeks	Indomethacin Content (%) A	B
0	100.1	99.7
2	91.2	99.9
4	79.1	98.1
6	69.8	98.6
8	64.8	98.4

Example 4 (Injectable formulation)

0.35 g hydroxypropyl-β-cyclodextrin (MS 0.35) were dissolved in 5 mL of physiological sodium chloride solution and warmed to about 35°C whereafter 3 mg diazepam were added. After storing for a short time a clear solution was obtained which was filled into an ampule after filtration through a membrane filter (0.45 microns).

Example 5 (Tablet)

In 100 mL water 7 g hydroxyethyl-β-cyclodextrin (MS 0.43) and 0.5 g medroxyprogesterone acetate were dissolved. The water was then evaporated in a rotation evaporator. The residue (75 mg) was powdered and after addition of 366 mg calcium hydrogen phosphate.$2H_20$, 60 mg corn starch, 120 mg cellulose powder (microcrystalline), 4.2 mg highly dispersed silica (AEROSIL^R 200) and 4.8 mg magnesium stearate tablets with a weight of 630.0 mg and comprising 5 mg drug per unit dose were made. The dissolution rate of the medroxyprogesterone acetate from this formulation is 21 times higher when compared to a tablet comprising the same inert ingredients without addition of the β-cyclodextrin ether.

Example 6

5 g hydroxyethyl-β-cyclodextrin (MS 0.43) and 14 mg vitamin A-acetate were dissolved under stirring in 100 mL water or sugar solution (5% aqueous solution) within 2.5 hours under a nitrogen atmosphere. After filtration through a membrane filter (0.45 microns) the solution was filled into ampules and sterilized or filled into dropper bottles with addition of 0.4% chlor butanol as preserving agent.

Example 7

5 or 7.5 g hydroxyethyl-β-cyclodextrin (MS 0.43) and 0.5 or 0.75 g Lidocaine were dissolved in 100 mL of physiological sodium chloride solution at 30°C (B). Injection solutions, eye droplets and solutions for topical use were prepared therefrom as described in example 6. When comparing the anaethesic effect of these solutions in animal tests with an aqueous lidocaine HCl solution (A) one observes an extension of the duration of the effect by 300%. Test: rats, injection of 0.1 mL into the tail root in the vicinity of the right or left nerve filaments and electrical irritation. The test results are summarized in table 4.

Table 4

Drug Concentration (%)	Duration of effect (min) A	B	Extension %
0.5	56	163	291
0.75	118	390	330

Example 8

6 mg dexamethasone and 100 mg hydroxyethyl-β-cyclodextrin (MS 0.43) were dissolved in 5 mL water, sterilized by filtration through a membrane

filter (0.22 microns) and packed into an aerosol container allowing to dispense 0.1 mL per dose.

Example 9

The acute intravenous toxicity of some β-cyclodextrins was tested on rats with the following results. It was surprisingly found that the toxicity of the derivatives used according to the invention is lower by an entire order of magnitude.

Table 5

	LD_{50} in rats (i.v.) in mg/Kg bodyweight
β-cyclodextrin	453
dimethyl-β-cyclodextrin (DS 2.0)	200-207
hydroxypropyl-methyl-β-cyclodextrin (DS 0.96; MS 0.43)	> 2000*

* a higher dose has not been tested. In mice the value was > 4000 mg/Kg.

The haemolytic effect of the methylether according to German Offenlegungsschrift 31 18 218 was compared to that of an ether used according to the invention. To this end 100 µl of a physiological sodium chloride solution with a cyclodextrin content of 10%, 800 µl of a buffer (400 mg MOPS, 36 mg Na_2HPO_4 . 2 H_2O, 1.6 g NaCl in 200 mL H_2O) and 100 µl of a suspension of human red blood cells (three times washed with sodium chloride solution) were mixed for 30 minutes at 37°C. Thereafter the mixture was centrifuged and the optical density was determined at 540 nm.

Controls:

a) 100 µl sodium chloride solution + buffer → 0% haemolysis

b) 900 µl water → 100% haemolysis.

The results obtained are summarized in the following table 6 in which the concentrations are stated at which 50% and 100% haemolysis occurred.

Table 6

Substance	C_{50}%	C_{100}%
Dimethyl-β-CD (DS 2.0)	0.33%	0.5%
Methyl-β-CD (DS 1.79)	0.53	0.8
Hydroxypropyl-methyl-β-CD (DS 0.96; MS 0.43%)	1.5	4

The results show that the haemolytic effect of the hydroxypropylmethyl ether is about 5 to 8 times weaker than that of the dimethyl ether according to the prior art. Animal tests have further shown that the hydroxyalkyl ethers do not cause irritation of the mucosa and eyes in contrast to the methyl ethers.

Claims

1. A pharmaceutical preparation containing inclusion complexes of medicinal substances which are sparingly water-soluble or are instable in water, with a partially etherified β-cyclodextrin, the ether substituents of which are hydroxyethyl, hydroxypropyl or dihydroxypropyl groups, wherein one portion of the ether substituents may optionally be methyl or ethyl groups and the β-cyclodextrin ether has a water-solubility of more than 1.8 g in 100 mL of water,

wherein inclusion complexes of retinoids with β-cyclodextrin ethers are excluded insofar as their ether substituents are methyl, ethyl and 2-hydroxy-ethyl groups.

2. A preparation according to Claim 1, characterized in that it contains a partially etherified β-cyclodextrin which has a molar degree of substitution for the hydroxyalkyl substituents of from 0.05 to 10 and a degree of substitution for the alkyl substituents of from 0.05 to 2.0.

3. A preparation according to either Claim 1 or Claim 2, characterized in that it contains the medicinal substance and the β-cyclodextrin ether in a molar ratio of from 1:6 to 4:1.

4. A preparation according to any one of Claims 1 to 3, characterized in that as medicinal substance it contains a non-steroid antirheumatic agent, a steroid, a cardiac glycoside or derivatives of benzodiazepin, benzimidazole, piperidine, piperazine, imidazole or triazole.

5. A preparation according to any one of Claims 1 to 4, characterized in that as medicinal substance it contains etomidate.

6. A preparation according to any one of Claims 1 to 4, characterized in that as medicinal substance it contains ketoconazole.

7. A preparation according to any one of Claims 1 to 4, characterized in that as medicinal substance it contains itraconazole.

8. A preparation according to any one of Claims 1 to 4, characterized in that as medicinal substance it contains levocabastine.

9. A preparation according to any one of Claims 1 to 4, characterized in that as medicinal substance it contains flunarizine.

10. A preparation according to any one of Claims 1 to 4, characterized in that as medicinal substance it contains tubulazole.

11. A process for the preparation of a pharmaceutical preparation according to any one of Claims 1 to 10, characterized in that the β-cyclodextrin ether is dissolved in water and the corresponding medicinal substance is added and, optionally, the resultant solution of the inclusion complex is dried in accordance with per se known methods.

12. A process according to Claim 11, characterized in that the residue obtained after removal of the solvent is pulverized and, optionally after the addition of further adjuvants, is converted into a solid administration form.

13. A process according to Claim 11 or Claim 12, characterized in that further physiologically compatible substances are added to the water.

14. A process according to Claim 13, characterized in that common salt, glucose, mannitol, sorbitol, xylitol or a phosphate or citrate buffer is added to the water.

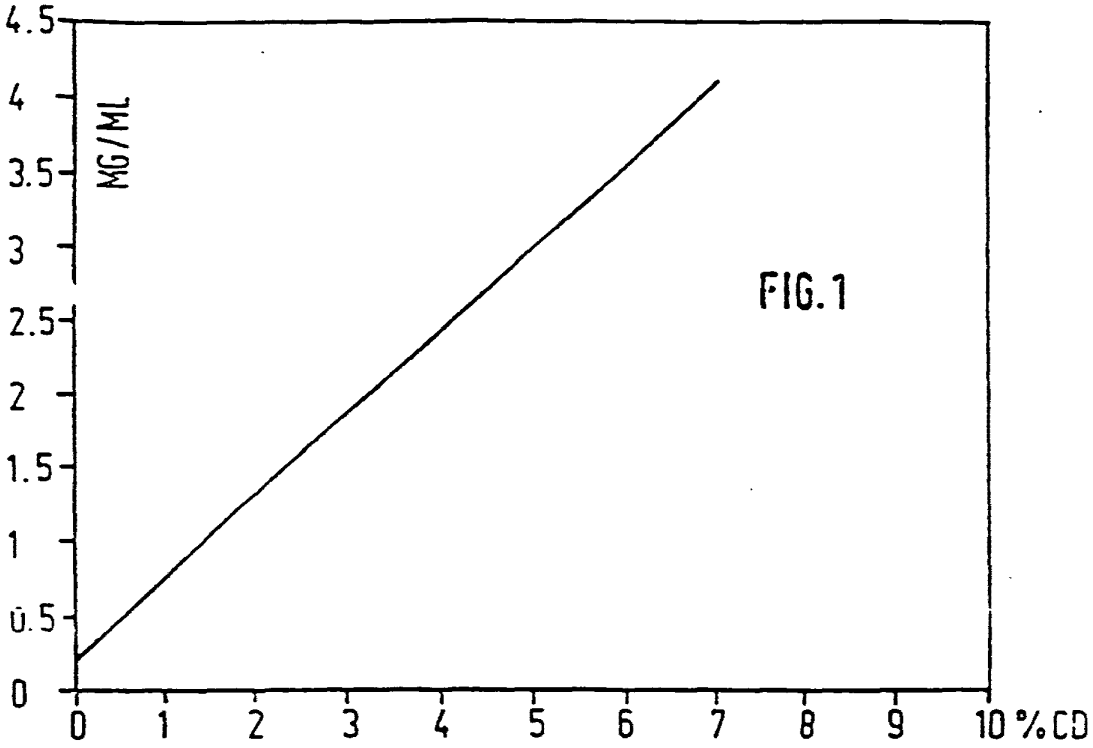

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
MG/ML
0 1 2 3 4 5 6 7 8 9 10 % CD

FIG.1

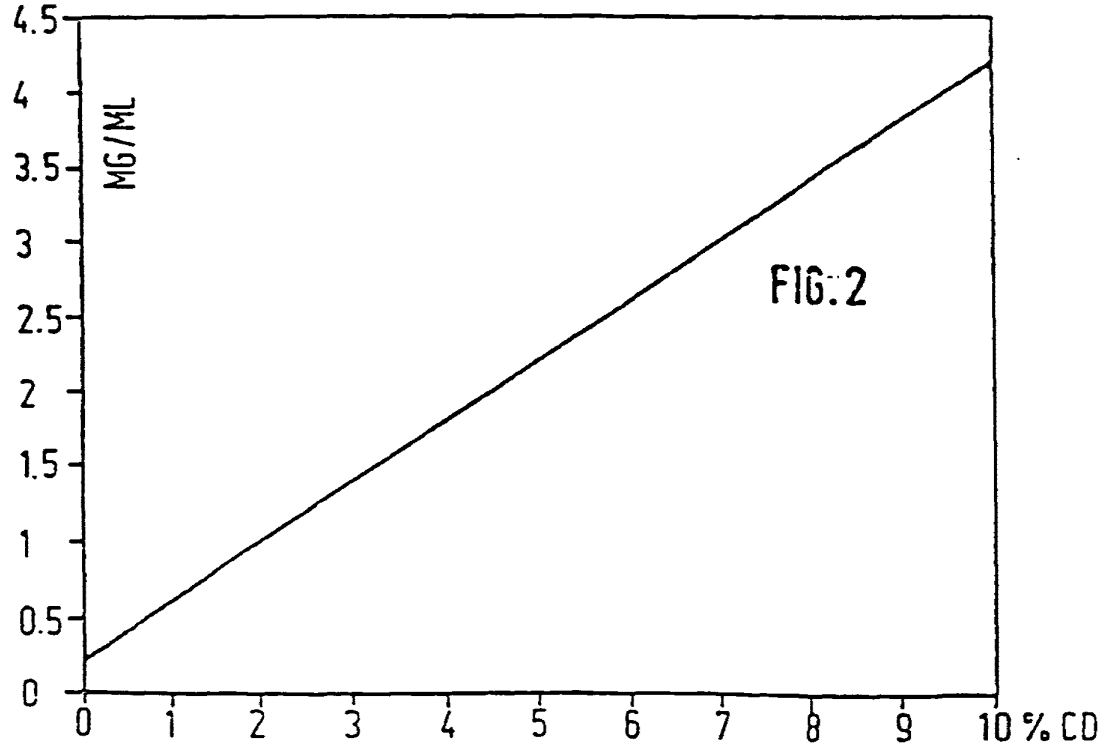

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
MG/ML
0 1 2 3 4 5 6 7 8 9 10 % CD

FIG.2

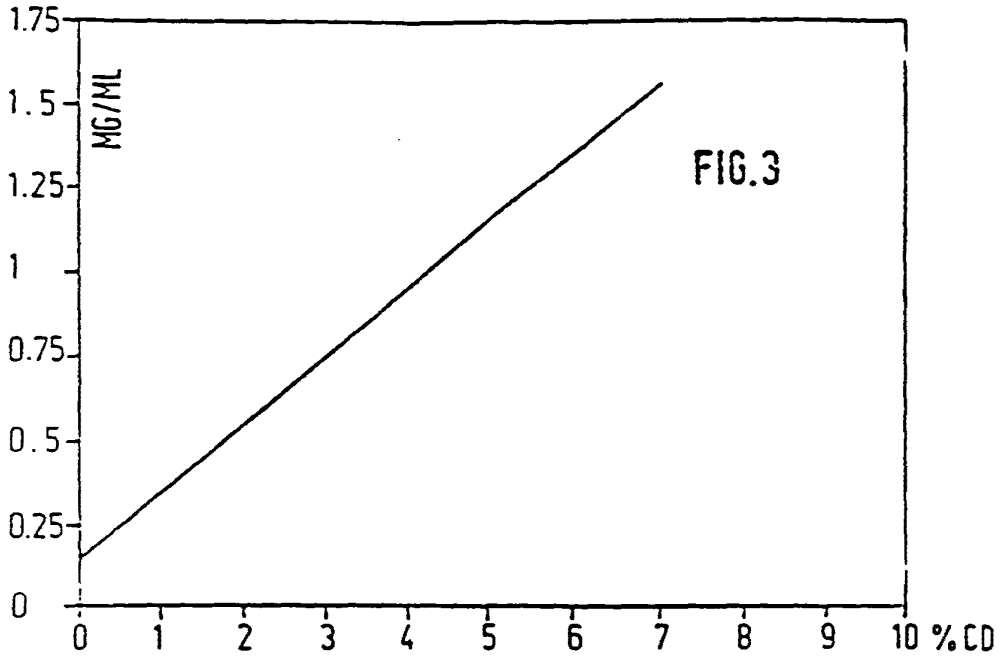

1.75
1.5
1.25
1
0.75
0.5
0.25
0
MG/ML
0 1 2 3 4 5 6 7 8 9 10 %CD

FIG.3

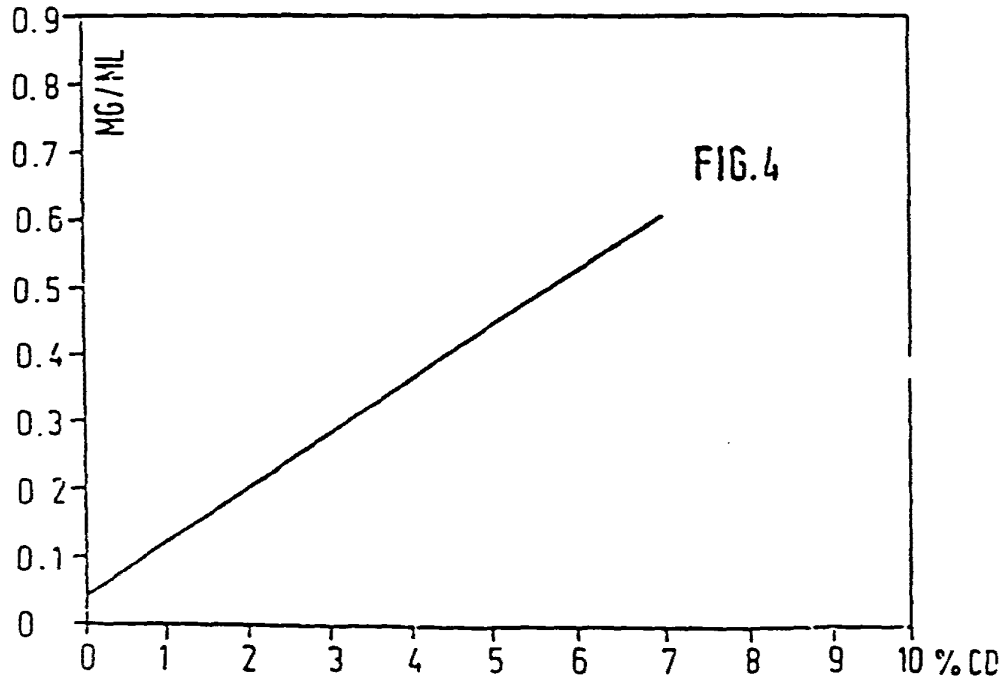

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0 2
0.1
0
MG/ML
0 1 2 3 4 5 6 7 8 9 10 %CD

FIG.4

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(54) **Pharmazeutische Präparate von in Wasser schwerlöslichen oder instabilen Arzneistoffen und Verfahren zu ihrer Herstellung.**

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LIBERGRAF, STOCKHOLM 1990

Beschreibung

Die Erfindung betrifft pharmazeutische Präparate von in Wasser schwer löslichen oder instabilen Arzneistoffen sowie Verfahren zur deren Herstellung. Die Präparate zeichnen sich durch eine erhöhte Wasserlöslichkeit und verbesserte Stabilität aus.

Eine große Zahl von Arzneistoffen ist in Wasser nur sehr schlecht oder wenig löslich, so daß entsprechende Arzneiformen wie Tropfen oder Injektionslösungen unter Verwendung anderer polarer Hilfstoffe wie Propylenglykol usw. hergestellt werden. Falls das Arzneistoffmolekül basische oder saure Gruppen trägt, besteht ferner die Möglichkeit, durch Salzbildung die Wasserlöslichkeit zu erhöhen. In der Regel geht dies mit einer Verminderung der Wirkung oder mit einem Verlust der chemischen Stabilität einher. Aufgrund des veränderten Verteilungsgleichgewichtes vermag der Arzneistoff nämlich nur noch langsam entsprechend der Konzentration seines nichtdissoziierten Anteils in die lipophile Membran einzudringen, während der ionogene Anteil einer schnellen hydrolytischen Zersetzung unterliegen kann.

Bei der Herstellung von wässrigen Lösungen schwer wasserlöslicher Wirkstoffe verwendet man daher entweder zusätzliche "wasserähnliche" Lösungsmittel wie niedermolekulare Polyethylenglykole oder 1,2-Propylenglykol, die jedoch nicht als pharmakologisch inert zu bezeichnen sind, oder man solubilisiert den Wirkstoff unter Verwendung von Tensiden durch Einschluß der Arzneistoffmoleküle in Mizellen. Diese Solubilisation hat aber zahlreiche Nachteile: die verwendeten Tensidmoleküle wirken häufig stark hämolytisch und der Arzneistoff muß nach Applikation durch Diffusion die Mizelle verlassen. Dies führt zu einem Retardeffekt (vgl. B. W. Müller, Gelbe Reihe, Band X, S. 132 ff (1983)).

Man kann also sagen, daß es keine befriedigende und allgemein anwendbare Methode zur Lösungsvermittlung gibt.

Auch für feste Arzneistoffe ist es wichtig, daß der schwer wasserlösliche Wirkstoff in eine wasserlösliche Form gebracht wird, da eine gute Löslichkeit die Verfügbarkeit des Wirkstoffes erhöht. Es wurde schon beschrieben, daß Einschlußverbindungen z. B. mit Harnstoff oder Komplexe mit Polyvinylpyrrolidon die Löslichkeit eines Stoffes verbessern können, jedoch sind sie in wässriger Lösung nicht mehr beständig. Solche Einschlußverbindungen sind daher allenfalls für feste Arzneiformen geeignet.

Anders ist dies beim Einsatz von (α-, β- und γ-Cyclodextrin, das einen Arzneistoff auch in wässriger Lösung in seinem Ring komplex binden kann (W. Sänger, Angew. Chemie 92, 343 (1980)). Nachteilig ist jedoch, daß das β-Cyclodextrin selbst nur schlecht wasserlöslich ist (1,8 g pro 100 ml), so daß die therapeutisch erforderlichen Arzneistoffkonzentrationen nicht erreicht werden.

Derivatisiert man den Hilfsstoff Cyclodextrin, so kann seine Löslichkeit und damit auch die Menge des in Lösung gebrachten Arzneistoffes erheblich erhöht werden. So wird in der DE-A-3 118 218 die Lösungsvermittlung mit methyliertem β-Cyclodextrin als Monomethylderivat mit 7 Methylgruppen und insbesondere als Dimethylderivat mit 14 Methylgruppen beschrieben. Mit dem 2,6-Di-O-methylderivat kann z. B. die Löslichkeit von Indometacin in Wasser um das 20,4-fache, diejenige von Digitoxin um das 81,6-fache gesteigert werden.

In der WO-A-820 051 werden Einschlußverbindungen von Retinoiden mit Cyclodextrinen und Cyclodextrinderivaten beschrieben. Das der Veröffentlichung zugrundeliegende Problem ist die Senkung der toxischen Nebenwirkungen von Retinoiden durch Modifikation der Molekularstruktur und/oder Komplexierung mit Cyclodextrinen. Die Fettlöslichkeit der Substanzen soll verringert und damit deren Neigung zur Konzentration im Fettgewebe gesenkt oder vermieden werden. In der Veröffentlichung werden α-, β- und γ-Cyclodextrine sowie deren Derivate für die Komplexierung als gleichermaßen geeignet bezeichnet, während als Einzelbeispiele für verwendbare Derivate in erster Linie Methoxyderivate von α-, β- und γ-Cyclodextrinen genannt werden.

Für die therapeutische Anwendung weisen die Methylderivate des β-Cyclodextrins jedoch schwerwiegende Nachteile auf. Aufgrund ihrer erhöhten Lipophilität wirken sie hämolytisch und führen auch zu Reizungen der Schleimhäute und Augen. Ihre akute intravenöse Toxizität ist noch höher als die bereits beträchtliche Toxizität des unsubstituierten β-Cyclodextrins. Für die praktische Anwendung besteht ein weiterer schwerwiegender Nachteil darin, daß die Löslichkeit des Dimethyl-β-cyclodextrins und seiner Komplexe bei höheren Temperaturen stark abnimmt, so daß bei einem Erhitzen das Dextrin auskristallisiert. Durch diese Erscheinung wird die Sterilisation der Lösungen bei den üblichen Temperaturen von 100 bis 121°C stark erschwert.

Es wurde nun überraschenderweise gefunden, daß bestimmte andere derivatisierte β-Cyclodextrine unter Bildung von Einschlußverbindungen die Wasserlöslichkeit von schwer wasserlöslichen und die Stabilität von in Wasser instabilen Arzneistoffen ebenfalls erheblich erhöhen, die geschilderten Nachteile aber nicht aufweisen.

Gegenstand der Erfindung sind demgemäß pharmazeutische Präparate mit einem Gehalt an Einschlußverbindungen schwer wasserlöslicher oder in Wasser instabiler Arzneistoffe mit einem partiell veretherten β-Cyclodextrin, deren Ethersubstituenten Hydroxyethyl-, Hydroxypropyl- oder Dihydroxypropylgruppen sind, wobei ein Teil der Ethersubstituenten gegebenenfalls Methyl- oder Ethylgruppen sein können und der β-Cyclodextrinether eine Wasserlöslichkeit von über 1,8 g in 100 ml Wasser aufweist, wobei Einschlußverbindungen von Retinoiden mit β-Cyclodextrinethern ausgenommen sind, soweit deren Ethersubstituenten Methyl-, Ethyl- und 2-Hydroxyethylgruppen sind.

Die Verwendung von partiell methylierten β-Cyclodextrinethern mit 7 bis 14 Methylgruppen im β-Cyclodextrinmolekül, wie sie aus der DE-A-3 118 218 bekannt sind, fällt nicht unter die Erfindung. Ferner sind erfindungsgemäß Einschlußverbindungen von Retinoiden mit β-Cyclodextrinethern ausgenommen, soweit deren Ethersubstituenten Methyl-, Ethyl- und 2-Hydroxyethylgruppen sind (vgl. WO-A-820 051).

β-Cyclodextrin ist eine ringförmig aufgebaute Verbindung aus sieben Anhydroglucoseeinheiten; sie wird auch als Cyclohepta-amylose bezeichnet. Jeder der sieben Glucoseringe enthält jeweils drei Hydroxygruppen in 2-, 3- und 6-Stellung, welche verethert werden können. Bei den partiell veretherten Cyclodextrinderivaten, welche erfindungsgemäß eingesetzt werden, ist nur ein Teil dieser Hydroxygruppen mit den angegebenen Hydroxyalkylresten sowie gegebenenfalls ferner mit Methyl- oder Ethylresten verethert. Bei der Veretherung mit- 5 Hydroxyalkylresten, welche durch Umsetzung mit den ent-

sprechenden Alkylenoxiden erfolgen kann, wird der Substitutionsgrad als molarer Substitutionsgrad (MS), nämlich in Mol Alkylenoxid je Anhydroglucoseeinheit angegeben, vgl. US-A-3 459 731, Spalte 4. Bei den erfindungsgemäß eingesetzten Hydroxyalkylethern des β-Cyclodextrins beträgt der molare Substitutionsgrad zwischen 0,05 und 10, vorzugsweise zwischen 0,2 und 2.

Besonders bevorzugt ist ein molarer Substitutionsgrad von etwa 0,25 bis etwa 1.

Für die Veretherung mit Alkylresten läßt sich unmittelbar der Substitutionsgrad (DS) je Glucoseeinheit angeben, welcher – wie bereits oben erwähnt – bei vollständiger Substitution 3 beträgt. Im Rahmen der Erfindung werden partiell veretherte β-Cyclodextrine eingesetzt, welche neben den Hydroxyalkylresten Alkylreste, nämlich Methyl- oder Ethylreste bis zu einem Substitutionsgrad von 0,05 bis 2,0, vorzugsweise 0,2 bis 1,5 enthalten. Besonders bevorzugt beträgt der Substitutionsgrad für die Alkylreste etwa 0,5 bis etwa 1,2.

Das Molverhältnis von Arzneistoff zu β-Cyclodextrinether beträgt vorzugsweise etwa 1 : 6 bis 4 : 1, insbesondere etwa 1 : 2 bis 1 : 1. In der Regel ist es bevorzugt, den Komplexbildner in einem molaren Überschuß einzusetzen.

Als Komplexbildner kommen die Hydroxyethyl-, Hydroxypropyl- und Dihydroxypropylether sowie deren entsprechende gemischte Ether und ferner Mischether mit Methyl- oder Ethylgruppen, wie z. B. Methyl-hydroxyethyl-, Methyl-hydroxypropyl-, Ethyl-hydroxyethyl- und Ethyl-hydroxypropylether des β-Cyclodextrins in Betracht.

Die Herstellung der Hydroxyalkylether des β-Cyclodextrins kann nach dem Verfahren der US-A-3 459 731 erfolgen. Geeignete Herstellungsverfahren für β-Cyclodextrinether finden sich ferner bei J. Szejtli et al., Stärke 32, 165 (1980) und A. P. Croft und R.A. Bartsch, Tetrahedron 39, 1417 (1983). Mischether des β-Cyclodextrins lassen sich herstellen, indem β-Cyclodextrin in einem ein Alkalimetallhydroxid, Wasser und ggf. mindestens ein organisches Lösungsmittel (z. B. Dimethoxyethan oder Isopropanol) enthaltenden basischen flüssigen Reaktionsmedium mit mindestens zwei unterschiedlichen hydroxyalkylierenden und ggf. alkylierenden Veretherungsmitteln (z. B. Ethylenoxid, Propylenoxid, Methyl- oder Ethylchlorid) umgesetzt wird.

Arzneistoffe, die in Form von Einschlußverbindungen mit den genannten β-Cyclodextrinethern eine erheblich gesteigerte Wasserlöslichkeit bzw. eine höhere Stabilität zeigen, sind solche, die die entsprechende Paßform haben, d.h. sie müssen in den Hohlraum des β-Cyclodextrin-Ringsystems passen. Hierzu gehören z. B. nichtsteroide Antirheumatika, Steroide, Herzglykoside und Derivate des Benzodiazepins, Benzimidazols, Piperidins, Piperazins, Imidazols oder Triazols.

Infrage kommende Benzimidazolsderivate sind Thiabendazol, Fuberidazol, Oxibendazol, Parbendazol, Cambendazol, Mebendazol, Fenbendazol, Flubendazol, Albendazol, Oxfendazol, Nocodazol und Astemisol. Als Piperidinderivate kommen Fluspirilen, Pimozid, Penfluridol, Loperamid, Astemizol, Ketanserin, Levocabastin, Cisaprid, Altanserin, und Ritanserin in Betracht. Geeignete Piperazinderivate sind Lidoflazin, Flunarizin, Mianserin, Oxatomid, Mioflazin und Cinnarizin. Als Imidazolderivate sind Metronidazol, Ornidazol, Ipronidazol, Tinidazol, Isoconazol, Nimorazol, Burimamid, Metiamid, Metomidat, Enilconazol, Etomidat, Econazol, Clotrimazol, Carnidazol, Cimetidin, Docodazol, Sulconazol, Parconazol, Orconazol, Butoconazol, Triadiminol, Tioconazol, Valconazol, Fluotrimazol, Ketoconazol, Oxiconazol, Lombazol, Bifonazol, Oxmetidin, Fenticonazol und Tubulazol zu nennen. Zu den infrage kommenden Triazolderivaten gehören Virazol, Itraconazol und Terconazol.

Besonders wertvolle pharmazeutische Präparate werden erhalten, wenn man mit Hilfe der erfindungsgemäß verwendeten Komplexbildner Etomidat, Ketoconazol, Tubulazol, Itraconazol, Levocabastin oder Flunarizin in wasserlösliche Form überführt. Diese Präparate sind deshalb ein besonderer Gegenstand der vorliegenden Erfindung.

Die Erfindung betrifft ferner ein Verfahren zur Herstellung pharmazeutischer Zubereitungen schwer wasserlöslicher oder in Wasser instabiler Arzneistoffe, welches dadurch gekennzeichnet ist, daß man den β-Cyclodextrinether in Wasser löst und den entsprechenden Arzneistoff zufügt sowie gegebenenfalls die erhaltene Lösung der Einschlußverbindung nach an sich bekannten Methoden trocknet. Die Bildung der Lösung kann bei Temperaturen zwischen 15 und 35°C erfolgen.

Der Arzneistoff wird dabei zweckmäßigerweise portionsweise zugesetzt. Das Wasser kann beispielsweise noch physiologisch verträgliche Substanzen wie Kochsalz, Kaliumnitrat, Glukose, Mannitol, Sorbitol, Xylitol oder Puffer wie Phosphat-, Acetat- oder Citratpuffer enthalten.

Es lassen sich mit den erfindungsgemäß verwendeten β-Cyclodextrinderivaten Arzneiformen sowohl für die orale, die parenterale als auch lokale Anwendung herstellen z. B. Infusions- und Injektionslösungen, Tropfen (z. B. Augentropfen, Nasentropfen), Sprays, Aerosole, Sirups und Bäder.

Die wässrigen Lösungen können noch geeignete, physiologisch verträgliche Konservierungsmittel enthalten, z. B. quartäre Ammoniumseifen, Chlorbutanol.

Für die Herstellung fester Zubereitungen werden die Lösungen der Einschlußverbindungen nach üblichen Methoden getrocknet; z. B. kann das Wasser am Rotationsverdampfer abgezogen oder durch Gefriertrocknung entfernt werden. Der Rückstand wird pulverisiert und gegebenenfalls nach Zusatz von weiteren Hilfsstoffen in Tabletten, Dragees Suppositorien, Kapseln oder Cremes bzw. Salben überführt.

Die nachfolgenden Beispiele erläutern die Erfindung, die jedoch keinesfalls auf die hier angeführten Beispiele beschränkt ist.

Die in den Beispielen genannte Phosphatpuffer-Lösung hatte einen pH-Wert von 6.6 und folgende Zusammensetzung:

KH_2PO_4	68,05 g
NaOH	7,12 g
Aqua demin. ad	5000,0 g

Alle Prozentangaben sind Gewichtsprozent.

Beispiel 1

Ausgehend von einer 7 %-igen Stammlösung von Hydroxyethyl-β-cyclodextrin (MS 0.43) in Phosphatpufferlösung wurde eine Verdünnungsreihe so hergestellt, daß die Komplexbildnerkonzentration in 1 % Schritten abgestuft vorlag. 3 ml von diesen Lösungen wurden in 5 ml-Schnappdeckelgläser pipettiert, die den entsprechenden Arzneistoff enthielten. Nach 24-stündigem Schütteln bei 25°C wurde die Lösung über ein Membranfilter (0.22 µm) abfiltriert und der Gehalt an gelöstem Arzneistoff spektralphotometrisch bestimmt. Die Figuren 1, 3 und 4 zeigen die Zunahme der Arzneistoffkonzentration in Lösung in Abhängigkeit von dem Komplexbildnerzusatz für Indometacin (Fig. 1), Piroxicam (Fig. 3) und Diazepam (Fig. 4). Die maximal mögliche Arzneistoffkonzentration wird durch die Sättigungslöslichkeit des Cyclodextrinderivates im Puffer begrenzt, die im Fall des Hydroxyethyl-β-cyclodextrins (MS 0.43) bei 7,2 g/100 ml liegt.

Vergleicht man z. B. die Resultate von Indometacin mit denjenigen, die für 2,6-Di-O-methyl-β-cyclodextrin in der DE-A-3 118 218 angegeben sind (Fig. 2), so zeigt das Hydroxyethyl-Derivat eine deutlich höhere Komplexbildungskonstante (vergl. die unterschiedlichen Steigungen in Fig. 1 und 2).

Beispiel 2

A. Unter Verwendung einer 10 %-igen Hydroxypropyl-β-cyclodextrinlösung (MS 0.35) in Phosphatpufferlösung wurde unter denselben Bedingungen wie in Beispiel 1 die Sättigungslöslichkeit bei 25°C für verschiedene Arzneistoffe bestimmt. In Tabelle 1 sind die Sättigungslöslichkeit S_1 in Phosphatpufferlösung und S_2 in Phosphatpufferlösung unter Zusatz von 10 % Hydroxypropyl-β-cyclodextrin angegeben.

Tabelle 1

Arzneistoff	S_1 (mg/ml)	S_2 (mg/ml)	Verh. S_1 zu S_2
Indometacin	0,19	5,72	1: 30,1
Digitoxin	0,002	1,685	1: 842,5
Progesteron	0,0071	7,69	1: 1083,0
Dexamethason	0,083	14,28	1: 172,0
Hydrocortison	0,36	21,58	1: 59,9
Diazepam	0,032	0,94	1: 29,4

B. In ähnlicher Weise wurde die Löslichkeit von Arzneistoffen in einer 4 %-igen wässrigen Lösung von Hydroxypropyl-methyl-β-cyclodextrin (DS = 0,96; MS = 0,43) untersucht. Die erhaltenen Ergebnisse sind in der nachfolgenden Tabelle 2 zusammengefaßt, wobei für jeden Arzneistoff das Verhältnis R von Sättigungslöslichkeit in Wasser bzw. beim angegebenen pH-Wert mit und ohne Zusatz des β-Cyclodextrinderivats angegeben ist. In allen Fällen erwiesen sich die erfindungsgemäß hergestellten Lösungen darüber hinaus im Vergleich zu wässrigen Lösungen als wesentlich stabiler.

Tabelle 2

Arzneistoff		R
Itraconazol	bei pH 5	96
	bei pH 2,5	75
Flunarizin		18
Levocabastin	bei pH 9,5	81
	bei pH 7,4	8
Ketoconazol		85
Flubendazol		30
Tubulazol		43
Cisaprid		3
Loperamid		62
Etomidat		8,5
Cinnarizin	bei pH 5	28
	bei pH 3	12

Beispiel 3

In 10 ml Phosphatpufferlösung wurden bei 25°C 0,7 g Hydroxyethyl-β-cyclodextrin (MS 0.43) und 0,04 g Indometacin bis zur klaren Lösung eingearbeitet. Diese wurde durch ein Membranfilter (0,22 µm) filtriert und unter Laminarflow in eine vorsterilisierte Injektionsflasche abgefüllt, die bei 21°C eingelagert wurde (B). Parallel dazu wurde eine gesättigte Indometacinlösung in Phosphatpufferlösung (0,21 mg/ml) unter gleichen Bedingungen aufbewahrt (A). Die hochdruckflüssigkeitschromatographisch ermittelten Wirkstoff-Konzentrationen sind in Tabelle 3 wiedergegeben. Die wesentlich verbesserte Stabilität des erfindungsgemäßen Präparates ist offensichtlich.

Tabelle 3

Lagerzeit in Wochen	Gehalt an Indometacin (%) A	B
0	100,1	99,7
2	91,2	99,9
4	79,1	98,1
6	69,8	98,6
8	64,8	98,4

Beispiel 4 (Injektionspräparat)

Man löste 0,35 g Hydroxypropyl-β-cyclodextrin (MS 0.35) in 5 ml physiologischer Kochsalzlösung, temperierte auf ca. 35°C und gab 3 mg Diazepam hinzu. Nach kurzem Rühren erhielt man eine klare Lösung, die nach Filtration durch ein 0,45 µm Membranfilter in eine Ampulle abgefüllt wurde.

Beispiel 5 (Tablette)

In 100 ml Wasser wurden 7 g Hydroxyethyl-β-cyclodextrin (MS 0.43) sowie 0,5 g Medroxyprogesteronacetat gelöst. Anschließend wurde das Wasser auf einem Rotationsverdampfer abgezogen. Der Rückstand (75 mg) wurde pulverisiert und mit 366 mg Calcium-hydrogenphosphat. $2H_2O$, 60 mg Maisstärke, 120 mg Cellulosepulver(mikrokristallin), 4,2 mg hochdisperse Kieselsäure (Aerosil® 200) und 4,8 mg Magnesiumstearat zu Tabletten mit einem Endgewicht von 630,0 mg und pro Einzeldosis 5 mg Wirkstoff verarbeitet. Die Lösungsgeschwindigkeit des Medroxyprogesteronacetats aus dieser Zubereitung ist etwa 21 mal höher als bei Einsatz der gleichen Tablettenhilfsstoffe ohne Zusatz des β-Cyclodextrinethers.

Beispiel 6

In 100 ml Wasser oder Zuckerlösung (5 %-ige wässrige Lösung) wurden 5 g Hydroxyethyl-β-cyclodextrin (MS 0.43) und 14 mg Vitamin A-acetat durch Rühren über ca. 2,5 h unter Stickstoffatmosphäre gelöst. Nach Filtration über ein 0,45 µm Membranfilter wurde die Lösung entweder in Ampullen abgefüllt und sterilisiert oder unter Verwendung von 0,4 % Chlorbutanol als Konservierungsmittel in Tropfflaschen abgefüllt.

Beispiel 7

In 100 ml physiologischer Kochsalzlösung wurden 5 g bzw. 7,5 g Hydroxyethyl-β-cyclodextrin (MS 0.43) und 0,5 bzw. 0,75 g Lidocain bei 30°C gelöst (B). Analog Beispiel 6 wurden daraus Injektionslösungen, Augentropfen und Lösungen für die cutane Anwendung hergestellt. Vergleicht man die anaesthesierende Wirkung dieser Lösungen am Tiermodell gegenüber einer wässrigen Lidocain-HCl-Lösung (A), so ist eine Wirkungsverlängerung von 300 % festzustellen. Versuch: Ratte, Injektion von 0,1 ml in die Schwanzwurzel in die Nähe der rechten oder linken Nervenstränge und elektrische Reizung. Die Versuchsergebnisse sind in Tabelle 4 zusammengefaßt.

Tabelle 4

Arzneistoffkonz. (%)	Wirkungsdauer (min) A	B	Verlängerung (%)
0,5	56	163	291
0,75	118	390	330

Beispiel 8

Es wurden 6 mg Dexamethason und 100 mg Hydroxyethyl-β-cyclodextrin (MS 0.43) in 5 ml Wasser gelöst, über ein 0,22 µm Membranfilter steril filtriert und in eine Dosier-Aerosoldose verpackt, die 0,1 ml pro Dosis freizusetzen erlaubt.

Beispiel 9

Die akute intravenöse Toxizität von einigen β-Cyclodextrinen wurde an Ratten untersucht, wobei die nachfolgenden Ergebnisse erhalten wurden. Dabei zeigte sich, daß die Toxizität der erfindungsgemäß eingesetzten Derivate überraschend eine ganze Größenordnung niedriger ist.

Tabelle 5

LD_{50} bei Ratten (i.v.) in mg/kg Körpergewicht

β-Cyclodextrin	453
Dimethyl-β-cyclodextrin (DS = 2,0)	200 – 207
Hydroxypropyl-methyl-β-cyclodextrin (DS = 0,96; MS = 0,43)	> 2000*

* eine höhere Dosis wurde nicht getestet. Bei der Maus lag der Wert > 4000 mg/kg.

In einer weiteren Versuchsreihe wurde die hämolytische Wirkung der Methylether gemäß DE-A-3 118 218 mit derjenigen eines erfindungsgemäß verwendeten Ethers verglichen. Dazu wurden 100 µl einer physiologischen Kochsalzlösung mit einem Cyclodextringehalt von 10 %, 800 µl eines Puffers (400 mg MOPS, 36 mg $Na_2HPO_4 \cdot 2\ H_2O$, 1,6 g NaCl in 200 ml H_2O) und 100 µl einer Suspension von roten Blutkörperchen (menschlichen Ursprungs, dreimal mit Kochsalzlösung gewaschen), 30 Min. lang bei 37°C gemischt. Anschließend wurde zentrifugiert und die Extinktion bei 540 nm bestimmt.

Kontrollen:
a) 100 µl Kochsalzlösung + Puffer → 0 % Hämolyse
b) 900 µl Wasser → 100 % Hämolyse

Die gefundenen Ergebnisse sind in der nachfolgenden Tabelle 6 zusammengefaßt, wobei die Konzentrationen angegeben sind, bei denen eine 50 %-ige bzw. eine 100 % Hämolyse stattfand.

Tabelle 6

Substanz	C_{50} %	C_{100} %
Dimethyl-β-CD (DS = 2.0)	0,33 %	0,5 %
Methyl-β-CD (DS = 1,79)	0,53 %	0,8 %
Hydroxypropyl-methyl-β-CD (DS = 0,96 MS = 0,43 %)	1,5 %	4 %

Die Ergebnisse zeigen, daß die hämolytische Wirkung des Hydroxypropyl-methylethers etwa 5 bis 8-mal geringer ist als die des Dimethylethers gemäß Stand der Technik.

Tierversuche haben ferner ergeben, daß die Hydroxyalkylether im Gegensatz zu den Methylethern keine Irritation der Schleimhäute und Augen hervorrufen.

Patentansprüche

1. Pharmazeutisches Präparat mit einem Gehalt an Einschlußverbindungen schwer wasserlöslicher oder in Wasser instabiler Arzneistoffe mit einem partiell veretherten β-Cyclodextrin, dessen Ethersubstituenten Hydroxyethyl-, Hydroxypropyl- oder Dihydroxypropylgruppen sind, wobei ein Teil der Ethersubstituenten gegebenenfalls Methyl- oder Ethylgruppen sein können und der β-Cyclodextrinether eine Wasserlöslichkeit von über 1,8 g in 100 ml Wasser aufweist, wobei Ein-

schlußverbindungen von Retinoiden mit β-Cyclodextrinethern ausgenommen sind, soweit deren Ethersubstituenten Methyl-, Ethyl- und 2-Hydroxyethylgruppen sind.

2. Präparat nach Anspruch 1, *dadurch gekennzeichnet*, daß es ein partiell verethertes β-Cyclodextrin enthält, welches einen molaren Substitutionsgrad für die Hydroxyalkylreste von 0,05 bis 10 und einen Substitutionsgrad für die Alkylreste von 0,05 bis 2,0 aufweist.

3. Präparat gemäß einem der Ansprüche 1 oder 2, *dadurch gekennzeichnet*, daß es Arzneistoff und β-Cyclodextrinether im Molverhältnis von 1 : 6 bis 4 : 1 enthält.

4. Präparat gemäß einem der Ansprüche 1 bis 3, *dadurch gekennzeichnet*, daß es als Arzneistoff ein nicht steroides Antirheumatikum, ein Steroid, ein Herzglycosid oder Derivate des Benzodiazepins, Benzimidazols, Piperidins, Piperazins, Imidazols oder Triazols enthält.

5. Präparat nach einem der Ansprüche 1 bis 4, *dadurch gekennzeichnet*, daß es als Arzneistoff Etomidat enthält.

6. Präparat nach einem der Ansprüche 1 bis 4, *dadurch gekennzeichnet*, daß es als Arzneistoff Ketoconazol enthält.

7. Präparat nach einem der Ansprüche 1 bis 4, *dadurch gekennzeichnet*, daß es als Arzneistoff Itraconazol enthält.

8. Präparat nach einem der Ansprüche 1 bis 4, *dadurch gekennzeichnet*, daß es als Arzneistoff Levocabastin enthält.

9. Präparat nach einem der Ansprüche 1 bis 4, *dadurch gekennzeichnet*, daß es als Arzneistoff Flunarizin enthält.

10. Präparat nach einem der Ansprüche 1 bis 4, *dadurch gekennzeichnet*, daß es als Arzneistoff Tubulazol enthält.

11. Verfahren zur Herstellung eines pharmazeutischen Präparates gemäß einem der Ansprüche 1 bis 10, *dadurch gekennzeichnet*, daß man den β-Cyclodextrinether in Wasser löst und den entsprechenden Arzneistoff zufügt sowie ggf. die erhaltene Lösung der Einschlußverbindung nach an sich bekannten Methoden trocknet.

12. Verfahren nach Anspruch 11, *dadurch gekennzeichnet*, daß man den nach Entfernen des Lösungsmittels erhaltenen Rückstand pulverisiert und, gegebenenfalls nach Zusatz weiterer Hilfsstoffe, in eine feste Applikationsform überführt.

13. Verfahren nach einem der Ansprüche 11 oder 12, *dadurch gekennzeichnet*, daß man dem Wasser noch weitere physiologisch verträgliche Stoffe zusetzt.

14. Verfahren nach Anspruch 13, *dadurch gekennzeichnet*, daß man dem Wasser Kochsalz, Glucose, Mannitol, Sorbitol, Xylitol oder einen Phosphat- oder Citratpuffer zusetzt.

Claims

1. A pharmaceutical preparation containing inclusion complexes of medicinal substances which are sparingly water-soluble or are instable in water, with a partially etherified β-cyclodextrin, the ether substituents of which are hydroxyethyl, hydroxypropyl or dihydroxypropyl groups, wherein one portion of the ether substituents may optionally be methyl or ethyl groups and the β-cyclodextrin ether has a water-solubility of more than 1.8 g in 100 ml of water, wherein inclusion complexes of retinoids with β-cyclodextrin ethers are excluded insofar as their ether substituents are methyl, ethyl and 2-hydroxyethyl groups.

2. A preparation according to Claim 1, characterised in that it contains a partially etherified β-cyclodextrin which has a molar degree of substitution for the hydroxyalkyl substituents of from 0.05 to 10 and a degree of substitution for the alkyl substituents of from 0.05 to 2.0.

3. A preparation according to either Claim 1 or Claim 2, characterised in that it contains the medicinal substance and the β-cyclodextrin ether in a molar ratio of from 1 : 6 to 4 : 1.

4. A preparation according to any one of Claims 1 to 3, characterised in that as medicinal substance it contains a non-steroid antirheumatic agent, a steroid, a cardiac glycoside or derivatives of benzodiazepin, benzimidazole, piperidine, piperazine, imidazole or triazole.

5. A preparation according to any one of Claims 1 to 4, characterised in that as medicinal substance it contains etomidate.

6. A preparation according to any one of Claims 1 to 4, characterised in that as medicinal substance it contains ketoconazole.

7. A preparation according to any one of Claims 1 to 4, characterised in that as medicinal substance it contains itraconazole.

8. A preparation according to any one of Claims 1 to 4, characterised in that as medicinal substance it contains levocabastine.

9. A preparation according to any one of Claims 1 to 4, characterised in that as medicinal substance it contains flunarizine.

10. A preparation according to any one of Claims 1 to 4, characterised in that as medicinal substance it contains tubulazole.

11. A process for the preparation of a pharmaceutical preparation according to any one of Claims 1 to 10, characterised in that the β-cyclodextrin ether is dissolved in water and the corresponding medicinal substance is added and, optionally, the resultant solution of the inclusion complex is dried in accordance with per se known methods.

12. A process according to Claim 11, characterised in that the residue obtained after removal of the solvent is pulverised and, optionally after the addition of further adjuvants, is converted into a solid administration form.

13. A process according to Claim 11 or Claim 12, characterised in that further physiologically compatible substances are added to the water.

14. A process according to Claim 13, characterised in that common salt, glucose, mannitol, sorbitol, xylitol or a phosphate or citrate buffer is added to the water.

Revendications

1. Composition pharmaceutique contenant des composés d'inclusion de médicaments peu solubles dans l'eau ou instables dans l'eau et d'une bêta-cyclodextrine partiellement éthérifiée dont les radicaux d'éthers sont des groupes hydroxyéthyle, hydroxypropyle ou dihydroxypropyle, une partie des radicaux d'éthers pouvant consister le cas échéant en groupes méthyle ou éthyle et l'éther de bêta-cyclodextrine ayant une solubilité dans l'eau supérieure à 1,8 g pour 100 ml d'eau, sous réserve que les composés d'inclusion des rétinoïdes et des éthers de bêta-cyclodextrine sont exclus lorsque leurs radicaux d'éthers sont des groupes méthyle, éthyle et 2-hydroxyéthyle.

2. Composition selon la revendication 1, caractérisée en ce qu'elle contient une bêta-cyclodextrine partiellement éthérifiée présentant un degré de substitution molaire de 0,05 à 10 pour les groupes hydroxyalkyle et un degré de substitution de 0,05 à 2,0 pour les groupes alkyle.

3. Composition selon l'une des revendications 1 ou 2, caractérisée en ce qu'elle contient le médicament et l'éther de bêta-cyclodextrine dans un rapport molaire de 1 : 6 à 4 : 1.

4. Composition selon l'une des revendications 1 à 3, caractérisée en ce qu'elle contient en tant que médicament un médicament antirhumatismal non stéroïdique, un stéroïde, un cardioglucoside ou des dérivés de la benzodiazépine, du benzimidazole, de la pipéridine, de la pipérazine, de l'imidazole ou du triazole.

5. Composition selon l'une des revendications 1 à 4, caractérisée en ce qu'elle contient en tant que médicament l'Etomidat.

6. Composition selon l'une des revendications 1 à 4, caractérisée en ce qu'elle contient en tant que médicament le Ketoconazol.

7. Composition selon l'une des revendications 1 à 4, caractérisée en ce qu'elle contient en tant que médicament l'Itraconazol.

8. Composition selon l'une des revendications 1 à 4, caractérisée en ce qu'elle contient en tant que médicament le Levocabastin.

9. Composition selon l'une des revendications 1 à 4, caractérisée en ce qu'elle contient en tant que médicament le Flunarizin.

10. Composition selon l'une des revendications 1 à 4, caractérisée en ce qu'elle contient en tant que médicament le Tubulazol.

11. Procédé de préparation d'une composition pharmaceutique selon l'une des revendications 1 à 10, caractérisé en ce

que l'on dissout l'éther de bêta-cyclodextrine dans l'eau et on ajoute le médicament voulu, puis, le cas échéant, on sèche la solution du composé d'inclusion ainsi obtenue par des procédés connus en soi.

12. Procédé selon la revendication 11, caractérisé en ce que l'on broie le résidu obtenu après élimination du solvant et, le cas échéant, après addition d'autres produits auxiliaires, on met sous une forme d'administration solide.

13. Procédé selon l'une des revendications 11 ou 12, caractérisé en ce que l'on ajoute encore à l'eau d'autres substances acceptables pour l'usage pharmaceutique.

14. Procédé selon la revendication 13, caractérisé en ce que l'on ajoute à l'eau du chlorure de sodium, du glucose, du mannitol, du sorbitol, du xylitol ou un tampon au phosphate ou au citrate.

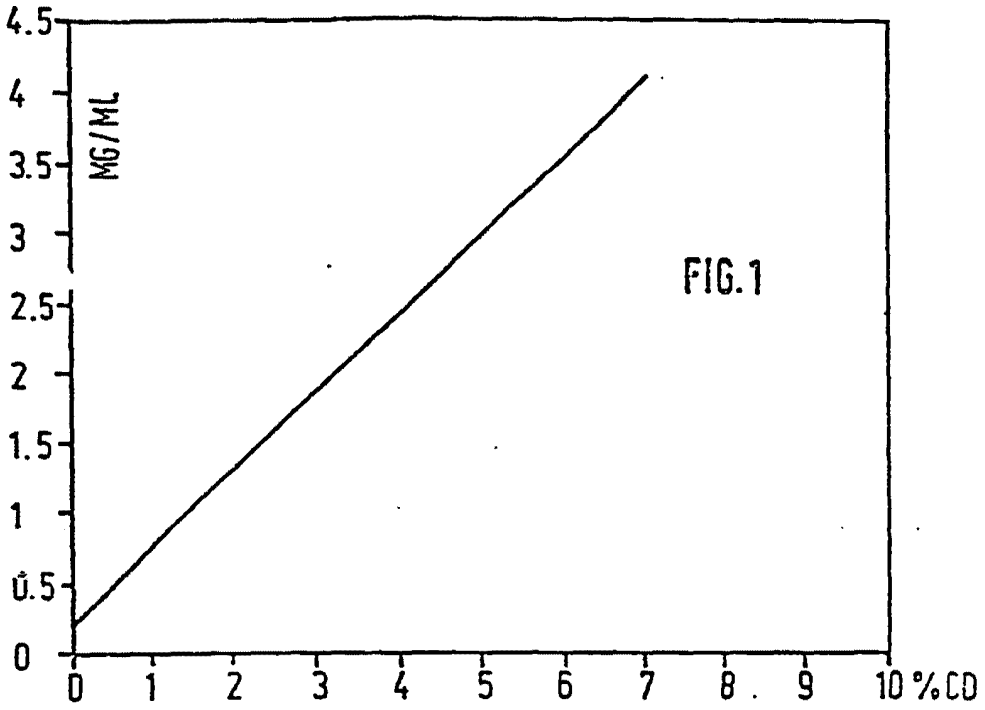

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
MG/ML
0 1 2 3 4 5 6 7 8 9 10 %CD

FIG.1

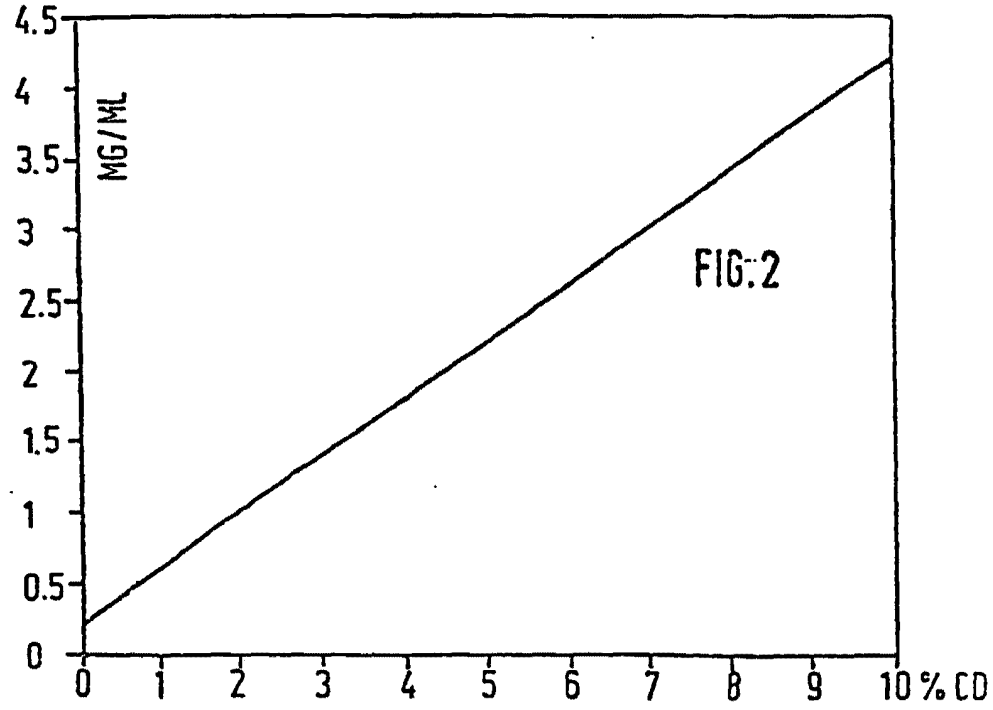

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
MG/ML
0 1 2 3 4 5 6 7 8 9 10 %CD

FIG.2

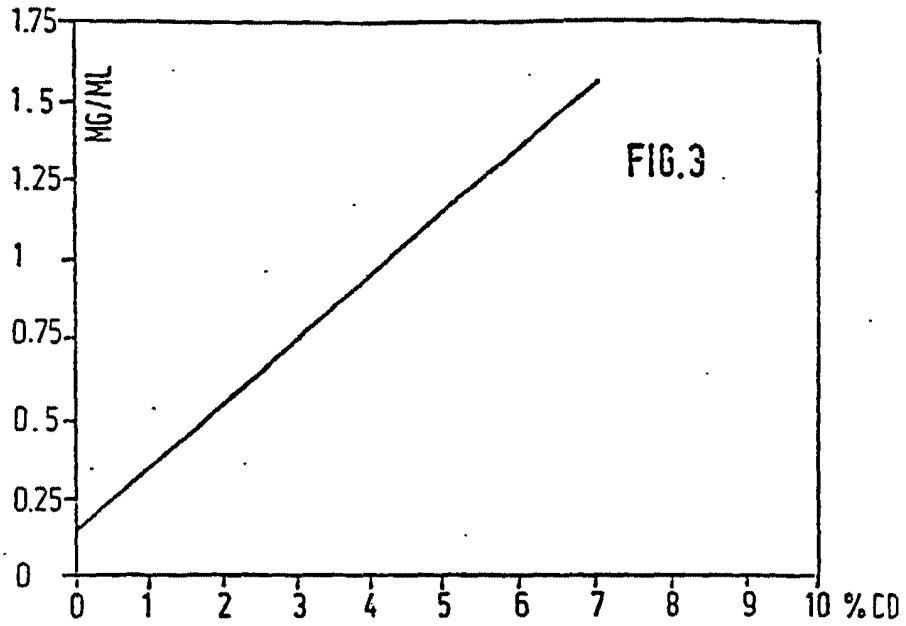

1.75
1.5
1.25
1
0.75
0.5
0.25
0
MG/ML
0 1 2 3 4 5 6 7 8 9 10 %CO

FIG.3

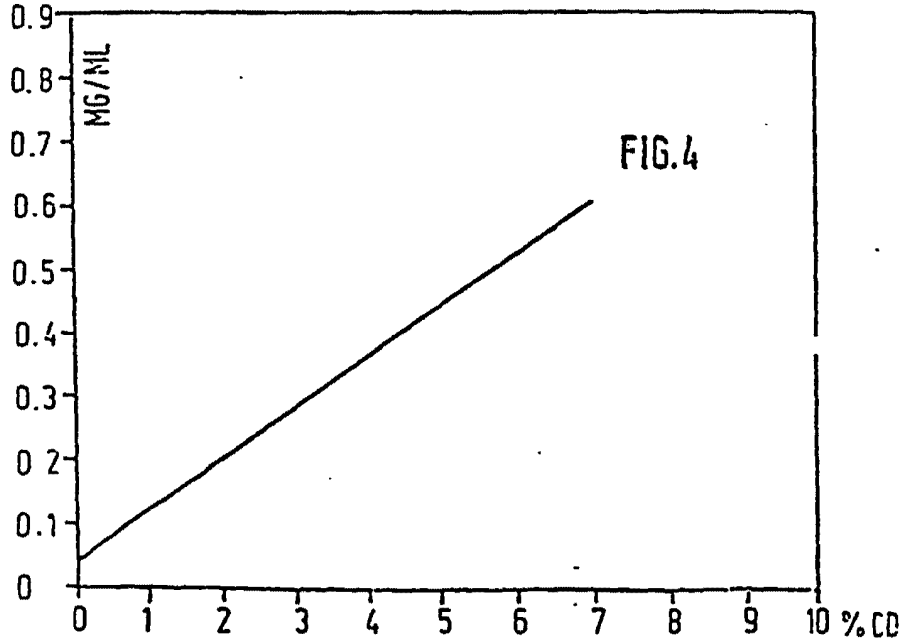

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0 2
0.1
0
MG/ML
0 1 2 3 4 5 6 7 8 9 10 %CO

FIG.4

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(54) **Pharmaceutical composition and its use.**

(57) A pharmaceutical composition containing a hydrophilic drug which is poorly absorbable through the gastrointestinal tract and cyclodextrin brings an increased absorbability of the drug into the mammalian body when administered non-oral or non-injection.

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PHARMACEUTICAL COMPOSITION AND ITS USE

The present invention relates to a pharmaceutical composition and its use.

It is generally known that any highly hydrophilic drug having a small oil/water partition coefficient is hardly absorbed through the gastrointestinal tract and consequently the bioavailability of such drug is small. Therefore, in order to achieve a sufficient clinical effect, such a hydrophilic drug is administered in the form of an injection. However, the administration of a drug by way of injection requires an expert hand and causes a pain in the recipient and, for these reasons, it is desired to develop a dosage form other than the injection but capable of being applied in an easy and simple manner and affording a high level of bioavailability.

Under these circumstances, the present inventors conducted an intensive study to develop a preparation form for non-oral and non-injection administration that would be conductive to an improved bioavailability, hence an improved pharmacological effect, of a hydrophilic or water-soluble drug which is poorly absorbable through the gastrointestinal tract. As a result, the present inventors found that when such a drug is used by non-oral and non-injection administration in combination with cyclodextrin, the absorption of the drug is markedly increased. This finding and subsequent research have resulted in the development of the present invention.

The present invention realtes to (1) a pharmaceutical composition which contains a hydrophilic drug, which is poorly absorbable through the gastrointestinal tract, and cyclodextrin and (2) a method of administering a pharmaceutical composition, which contains a hydrophilic drug, which is poorly absorbable through the gastrointestinal tract, and cyclodextrin, from the nasal cavity, the vagina or rectum.

In the present pharmaceutical composition, a hydrophilic drug which is poorly absorbable through gastrointestinal tracts is contained as the drug.

The drug which is poorly absorbable through the gastrointestinal tract to be used according to this invention is a drug, the bioavailability of which is preferably not more than about 70 percent, more preferably not more than about 50 percent, and most preferably not more than about 20 percent, for example in experimental animals (rat, dog, rabbit etc.), preferably in human.

In another aspect, the hydrophilic drug to be used according to this invention is a drug having a small oil/water partition coefficient and more particularly an n-octanol/water partition coefficient of not more than about 10, preferably not more than about 1, more preferably not more than about 0.1.

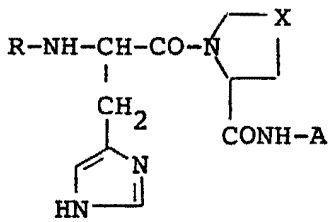
The oil/water partition coefficient can be determined by the method described in Robert E. Notari "Biopharmaceutics and Pharmacokinetics", Marcel Dekker Inc., 1975, New York, U.S.A. Thus, equal amount of n-octanol and a buffer solution (pH 5.5) are placed in a test tube to give a 1:1 mixture. The buffer solution is exemplified by Sörensen buffer [Ergebniss der Physiology 12, 393 (1912)], Clark-Lubs buffer [Journal or Bacteriology 2, (1), 109, 191 (1971)], Macllvaine buffer [Journal Biological Chemistry 49, 183 (1921)], Michaelis buffer [Die Wasser-Stoffionenkonzentration, p. 186 (1914)], Kolthoff buffer [Biochemische Zeitschrift 179, 410 (1926)] and so on.

An adequate amount of the drug to be tested is added to the mixture, and the test tube is stoppered, immersed in a constant-temperature bath (25°C) and shaken vigorously. When it appears that the drug has been dissolved in between both the liquid layers and an equilibrium has been reached, the mixture is allowed to stand or is centrifuged, and aliquots of the upper and lower liquid layers are pipetted separately and analyzed for the concentration of the drug in each layer. The ratio of the concentration of the drug in the n-octanol layer to the concentration of the drug in the aqueous layer is the oil/water partition coefficient.

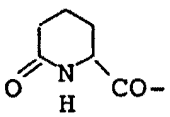
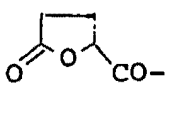
As the drug employed in the present invention, there may be mentioned, for example, physiologically active polypeptides, polysaccharides, aminoglycoside antibiotics, beta-lactam antibiotics, and nucleic acid drugs.

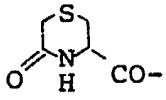
The polypeptides are exemplified by peptides which comprises two or more than two of amino acid residues. The polypeptides preferably have a molecular weight of about 200 to 60000.

As the physiologically active polypeptide, there may be mentioned, for example, L-pyroglutamyl-L-histidyl-L-prolinamide (thyrotropin releasing hormone; hereinafter referred to briefly as TRH) or its salts, especially its tartrate [U.S. Patent No. 3,957,247], and polypeptide represented by the formula (I)

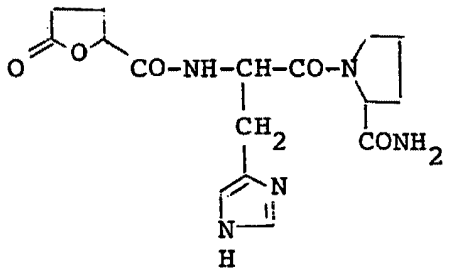


(I)

wherein A stands for hydrogen, alkyl, aralkyl, alkoxyalkyl, hydroxyalkyl or alkoxy, R stands for  , 

or  , X stands for $-CH_2-$, $-CH_2CH_2-$ or -S-, R and each of the other constituent amino acid residues may have L- or D-configuration or be racemic] and salts thereof [U.S. Patent No. 4,100,152].

Among the compound represented by the formula (I), the compound shown below is referred to briefly as "DN-1417".



(γ-butyrolactone-γ-carbonyl-L-histydyl-L-prolinamide)

Furthermore, as the polypeptide, there may be mentioned luteinizing hormone-releasing hormone (hereinafter referred to briefly as "LH-RH") or peptides which have the LH-RH activity and have the formula (II).

(Pyr)Glu-R_1-Trp-Ser-R_2-R_3-R_4-Arg-Pro-R_5 (II)

[wherein R_1 stands for His, Tyr, Trp or p-NH_2-Phe; R_2 stands for Tyr or Phe; R_3 stands for Gly or a D-amino acid residue: R_4 stands for Leu, Ile or Nle; R_5 stands for Gly-NH-R_6 (R_6 stands for H or a lower alkyl group which may optionally have a hydroxyl group) or NH-R_6 (R_6 stands for as defined above)] [U.S. Patent No. 3,853,837, U.S. Patent No. 4,008,209, U.S. Patent No. 3,972,859. British Patent No. 1,423,083, Proceedings of the National Academy of Science of the United States of America, vol. 78, pp. 6509-6512 (1981)].

As examples of the D-amino acid residue R_3 there may be mentioned the residues of alpha-D-amino acids containing up to 9 carbon atoms (e.g. D-Leu, Ile, Nle, Val, Nval, Abu, Phe, Phg, Ser, Thr, Met, Ala, Trp, α-Aibu, etc.),

which may have suitable protective groups (e.g. t-butyl, t-butoxy, t-butoxycarbonyl, etc.). Of course, salts of peptide (II) with acids as well as metal complex compounds of peptide (II) may also be employed just as peptide (II). All abbreviations, wherever they are used in this specification to denote amino acids, peptides, protective groups, etc., are those according to IUPAC-IUB Commission on Biochemical Nomenclature or those commonly employed in the particular field of art. Where any of the amino acids named herein is subject to optical isomerism, all references to such amino acid mean the L-form unless otherwise indicated.

In the present specification, the polypeptide (II) in which R_1=His, R_2=Tyr, R_3=D-Leu, R_4=Leu, R_5=$NHCH_2$-CH_3 is referred to briefly as TAP-144.

Examples of said polypeptide include insulin, somatostatin, somatostatin derivatives (U.S. Patent No. 4,093,573, U.S. Patent No. 4,100,117, U.S. Patent No. 4,253,998), growth hormone, prolactin, adrenocorticotrophic hormone (ACTH), melanocyte stimulating hormone (MSH), thyrotropin releasing hormone (TRH), its salts or its derivatives [U.S. Patent No. 3,957,247, U.S. Patent No. 4,100,152.], thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), vasopressin, vasopressin derivatives {desmopressin [Folia Endocrinologica Japonica, 54, 5, pp. 676-691, 1978)]},oxytocin, carcitonin, parathyroid hormone, glucagon, gastrin, secretin, pancreozymin, cholecystokinin, angiotensin, human placental lactogen, human chorionic gonadotropin (HCG), enkephalin, enkephalin derivatives [U.S. Patent 4,277,394; European Patent Application Publication No. 31567], endorphin, interferon (α, β, γ), urokinase, kallikrein, thymopoietin, thymosin, motilin, dynorphin, bombesin, neurotensin, caerulein, bradykinin, substance P, kyotorophin and nerve growth factor, peptide type antibiotics such as polymyxin B, colistin,

gramicidin, bacitracin, peptide type anti-tumor agents such as bleomycin, neocarzinostatin.

The polysaccharide drugs mentioned above include, among others, heparin and such antitumor agents as lentinan, zymosan and PS-K (krestin).

The aminoglycoside antibiotics mentioned above include, among others, gentamycin, streptomycin, kanamycin, dibekacin, paromomycin, kanendomycin, lipidomycin, tobramycin, amikacin, fradiomycin and sisomicin.

The beta-lactam antibiotics mentioned above include, among others, penicillins such as sulbenicillin, mecillinam, carbenicillin, piperacillin and ticarcillin, thienamycin, and cephalosporins such as cefotiam, cefsulodine, cefmenoxime, cefmetazole, cefazolin, cefotaxime, cefoperazone, ceftizoxime and moxalactam.

The nucleic acid drugs mentioned above include, among others, citicoline and such antitumor agents as cytarabine and 5-FU (5-fluorouracil).

Examples of the cyclodextrin used according to this invention include various cyclodextrins obtainable by hydrolysis of starch with acid or amylase and various cyclodextrin derivatives.

Such cyclodextrins include α(degree of polymerization 6), β(degree of polymerization 7) and γ(degree of polymerization 8) cyclodextrins [Farumashia vol. 16, No. 1 (1980), 33 Yakugaku Zasshi vol. 101 (10), 857-873 (1981), and Japanese Patent Application Publication Sho 53 (1978)-31223]. As examples of said cyclodextrin derivatives include tri-O-methylcyclodextrin [Chemical & Pharmaceutical Bulletin 28, 1552-1558 (1980)], triaminocyclodextrin [Angewandte Chemie: International Edition in English 19, 344-362 (1980)] and so forth. In the practice of this invention, α-cyclodextrin is particularly preferable.

The pharmaceutical composition of the present invention is formed into a nasal, vaginal or rectal preparation.

In the nasal preparation, the physiologically active

polypeptide is contained as the drug.

The nasal preparation according to the present invention can be produced by the per se conventional processes. For example, small amounts of a pH adjusting agent, preservative, thickening agent (natural gums, cellulose derivatives, acrylic acid polymers, vinyl polymers, etc.) or/and excipient is/are incorporated.

The nasal preparation of the present invention may take a solid, liquid or semi-solid form. In the case of a solid form, the above components may be simply blended or be freeze-dried to provide a powdery composition, the preferred particle size in either case being about 20 to 250 microns. In the case of a liquid preparation, it is preferably an aqueous solution, an aqueous suspension or an oil suspension. The semi-solid preparation is preferably an aqueous or oleaginous gel or ointment.

As to the proportion of each component in the nasal preparation, the polypeptide content of the final preparation is about 0.005 to 50 w/v % and preferably about 0.01 to 30 w/v % and the proportion of cyclodextrin is about 2 to 99.995 w/v % and preferably about 5 to 99.99 w/v %. In the case of a liquid or semi-solid preparation, the amount of the polypeptide in the preparation is about 0.001 to 50 w/v % and preferably about 0.05 to 40 w/v %, while the amount of cyclodextrin is about 0.5 to 50 w/v % and preferably about 1 to 30 w/v %.

The solid preparation can be produced by the per se known procedure. For example, a mixer is charged with cyclodextrin or, if required, a mixture of cyclodextrin and an excipient and, then, the polypeptide dissolved in a small amount of water is gradually added and mixed in. Thereafter, the mixture is dried in vacuo at a suitable temperature and the dried composition is pulverized to give a solid preparation. Alternatively, the polypeptide and cyclodextrin, plus an excipient if required, are dissolved well in water and freeze-dried or spray-

dried to give a dehydrated composition which is then pulverized into a solid preparation.

The excipient is exemplified by glucose, mannitol, inositol, sucrose, lactose, fructose, starch, corn strach, microcrystalline cellulose, hydroxypropyl-cellulose, hydroxypropylmethyl-cellulose, polyvinyl pyrrolidone, etc.

The liquid preparation can be produced by the per se known procedure. For example, an aqueous preparation for nasal administration can be produced by dissolving, suspending or emulsifying the polypeptide and cyclodextrin in water, a buffer solution or an aqueous medium. The oil suspension for nasal use can be produced by suspending or emulsifying the polypeptide and cyclodextrin in an oleaginous base. The buffer solution is exemplified as those mentioned above.

The above-mentioned oleaginous basis is exemplified by various oils and fats such as sesame oil, olive oil, corn oil, soybean oil, cotton-seed oil, peanut oil, lanoline, vaseline, paraffin, coparaffinate, silicone oil, glycerin fatty acid having 6 to 30 carbon atoms or its glycerin ester or its alcoholic ester, or a mixture thereof.

As to the semi-solid preparation, an aqueous or oleaginous gel or ointment can be produced by the per se conventional procedure. For example, such an aqueous gel for nasal administration can be produced in the following manner. First, an aqueous solution or suspension of cyclodextrin is prepared and, if required, a pH adjusting agent, a preservative or/and the like are added. The solution is divided into halves and an aqueous gel base is dissolved or dispersed in one of the halves and heated or cooled to give a stable gel. In the other half is dissolved the polypeptide. Then, the two halves are combined and evenly mixed to give an aqueous gel preparation.

Adjustment of the pH of preparation can be effected by adding an acid, a base, a buffer solution or the like in the course of production of the preparations. As examples of the acid, there may be mentioned inorganic acids (e.g. hydrochloric acid, boric acid, phosphoric acid, carbonic acid, bicarbonic acid, etc.), amino acids and organic acids (e.g. monocarboxylic acids, oxycarboxylic acids, polycarboxylic acids). The base is exemplified by sodium hydroxide, potassium hydroxide, sodium hydrogen carbonate, sodium carbonate, etc. The buffer solution is exemplified by those mentioned above.

Examples of the aqueous gel basis include natural gums (e.g. gum tragacanth, gum acasia, gum karaya, Irish moss, gum guaiac, gum xanthane, locust bean gum, etc.), cellulose derivatives (e.g. methylcellulose, carboxymethylcellulose, etc.), acrylic acid polymers (e.g. polyacrylic acid, polymethacrylic acid, etc.), vinyl polymers (e.g. polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl methyl ether, carboxypolymethylene, etc.), synthetic polysaccharides (e.g. polysucrose, polyglucose, polylactose, etc.), starch, dextrin, pectin, sodium alginate, etc. These bases may be used in the form of appropriate mixtures of two or more species.

The oleaginous ointment for nasal administration can be produced by dispersing the polypeptide and cyclodextrin evenly in a hot melt of an oleaginous base and cooling the same under stirring. The oleaginous base may be one of those mentioned hereinbefore.

Preservatives may be incorporated in nasal preparations. Examples of such preservatives include phenolic compounds such as phenol, cresol, etc.; alcohols such as chlorobutanol, phenylethyl alcohol, propylene glycol, etc.; invert soaps such as benzalkonium chloride, benzethonium chloride, etc.; benzoic acid, sorbic acid, dehydroacetic acid and sulfurous acid and salts thereof; acids and their salts such as sodium hydrogen sulfite.

The nasal preparation of this invention, if it is a solid preparation, can be administered by the following exemplary procedure. Thus, a capsule containing the powdery preparation is set in an exclusive dust applicator equipped with needles to pierce the capsule at the top and bottom thereof and an air balloon is used to drive the powdery contents into the nasal cavity.

In the case of a liquid preparation, it is put in a nasal douche, an atomizer or a spray-mist applicator suited for nasal application of liquids and dripped or sprayed into the nasal cavity.

The semi-solid preparation can be administered, for example by filling a tube with the preparation and sending the preparation directly into the nasal cavity through an applicator attached to the mouth of the tube or by administering the indicated dose of the preparation by means of a nasal insertion device.

While the dosage of the polypeptide varies with its kind, the disease to be managed and the animals to be treated (e.g. warm-blooded mammalian animals such as rat, rabbit, hourse, cattle, human). The proper amount of the solid preparation per dose is about 5 mg to 100 mg, that of the liquid preparation is about 0.05 ml to 0.5 ml, and that of the semi-solid preparation is about 50 mg to 500 mg. The nasal preparation may be administered once to four times per day.

By the administration of the nasal preparation of this invention, it brings the follwing advantageous features.

1) The physiologically active polypeptide which is poorly absorbed through the gastrointestinal tract can be administered by a route other than injection to achieve an improved bioavailability.

2) The physiologically active polypeptide can be administered without an accompanying pain.

3) Self-medication at home is possible. This is especially beneficial when repeated administration is necessary.

4) Cyclodextrin as an absorption promoting component

is tasteless, odorless, low toxic and not irritating to the mucous membrane, thus permitting production of pharmaceutical preparations which can be safely used in repeated dose regimens.

In the vaginal preparation of the present invention, the physiologically active polypeptide mentioned above is contained as the drug.

The pharmaceutical preparation for vaginal administration according to this invention can be produced by per se conventional processes.

The pharmaceutical preparation for vaginal administration according to this invention may have, among others, the form of a vaginal suppository which retains its solid form at room temperature and melts at the body temperature, or the form of an ointment or liquid contained in a tube, for instance. In the former case, the preparation of solid form is vaginally administered, and the preparation is melted at the body temperature. In the latter case, the tube containing the ointment or liquid is vaginally administered, and the content is pushed out and the tube is taken out.

The preparation may also be made available in the form of a tablet which, after administration, is dissolved or disintegrated in the vaginal fluid. In this case, the preparation can be easily administered into vagina when an adequate device, preferably an inserter, is used.

The vaginal suppository or ointment can be produced by the per se known processes, for instance by dissolving or dispersing a physiologically active polypeptide and cyclodextrin in a preliminarily molten oleaginous or aqueous base, attaining homogeneous dispersion by adequate warming and stirring, and molding the mixture.

In the preparation for vaginal administration, any of the known suppository bases or ointment bases can be employed. As the water-soluble bases, there may be

mentioned polyethylene glycols (e.g. those having the mean molecular weight of 200, 300, 400, 1000, 4000, 6000), propylene glycol, grycerol. These bases may be used either alone or as a mixture. As examples of said oleaginous bases there may be mentioned such oils and fats as sesame oil, olive oil, corn oil, soybean oil, cotton seed oil, peanut oil, cacao butter, castor oil, wool fat, squalene, etc., the corresponding modified materials as modified by such procedures as hydrogenation, fatty acid interchange, acetylation, fractional extraction, etc.; mineral oils such as vaseline, paraffin, silicone oil, etc.; glycerin esters of fatty acids of 6 to 30 carbon atoms, particularly higher fatty acid esters such as glycerin palmitate, glycerin laurate, glycerin stearate, glycerin myristate, etc.; esters of fatty acids of 6 to 30 carbon atoms with alcohols of 2 to 8 carbon atoms, particularly waxes such as isopropyl myristate, butyl stearate, diisopropyl adipate, diethyl sebacate, etc.; and higher fatty acids of 6 to 30 carbon atoms, particularly stearic acid, oleic acid, etc. Such oleaginous bases may be used either alone or as a mixture.

To the aqueous pharmaceutical preparation for vaginal administration in accordance with the present invention, there may be added, if necessary, an isotonizing agent (e.g. sodium chloride, potassium chloride, sorbitol), a wetting agent (e.g. glycerol, propylene glycol), a preservative (e.g. benzyl alcohol), a pH-adjusting agent (e.g. hydrochloric acid, acetic acid, citric acid, phosphoric acid, sodium hydroxide, potassium hydroxide, ammonia, a salt of any of these), a thickening agent (e.g. methylcellulose, carboxymethylcellulose), a stabilizer (e.g. sodium ethylenediaminetetraacetate, human serum albumin, citric acid), a dispersing agent [e.g. lecithin, Tween (polyoxyethylenesorbitan fatty acid ester, Kao-Atlas Co., Ltd. Japan), Span (higher fatty acid sorbitan ester, Kao-Atlas Co.)], and so on.

The preparation of the present invention for vaginal administration may be gel suppository prepared by a per se conventional manner from an aqueous solution or suspension containing the present polypeptide by adding a water-soluble gel-forming bases. As examples of the water-soluble gel bases, there may be mentioned naturally occurring gums (e.g. gum tragacanth, gum acacia, karaya gum, Irish moss, gum guaiac, gum xanthane, locust-bean gum, etc.), cellulose derivatives (e.g. methyl-cellulose, carboxymethyl-cellulose, etc.), acrylic acid polymers (e.g. polyacrylic acid, polymethacrylic acid, etc.), vinyl polymers (e.g. polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl methyl ether, carboxypolymethylene, etc.), synthetic polysaccharides (e.g. polysucrose, polyglucose, polylactose, etc.), starch, dextrin, pectin, sodium alginate and so forth. These bases may be employed either singly or, if necessary, as a mixture of two or more different bases and copolymers of the polymer mentioned above are also employed.

The aqueous solution of the preparation for vaginal administration is also vaginally administered as supported on a solid matrix, for instance. The solid matrix may be one of the known matrixes such as porous materials made of high molecular compounds (e.g. silicon rubber, polyurethane, etc.), biological polymers (e.g. collagen, gelatin, etc.), cellulosic materials (e.g. cotton, paper, etc.) and so forth.

The preparation of solution may be made to foam or aerosol by a per se conventional manner.

To prepare vaginal tablets, the active component is compressed into appropriate dosage units generally by a procedure analogous to the known procedure, using diluent such as lactose, sucrose, starch, etc., disintegrating agents such as starch, sodium hydrogen carbonate, etc.; binders such as starch, gelatin, carboxymethyl-cellulose, polyvinylpyrrolidone, hydroxypropyl-cellulose, etc.;

lubricants such as talc, magnesium stearate, polyethylene glycol (6000), stearic acid, etc. When the required dosage is very small, an increased product uniformity may be obtained by preparing a mixed solution of the polypeptide with a diluent such as lactose, starch or mannitol beforehand then drying the mixed solution by way of freeze-drying or spray-drying to make a diluted powder and molding this diluted powder into tablets. In view of the relative scarcity of vaginal fluids as compared with gastrointestinal fluids disintegration and dissolution are important considerations.

To assist in disintegration and dissolution, there may be prepared effervescent tablets with combination of alkali metal carbonates (e.g. sodium carbonate) or bicarbonates with citric acid or tartaric acid.

Tablets thus prepared can be inserted into vagina as they are.

The aqueous solution or aqueous suspension containing cyclodextrin and a physiologically active polypeptide combinedly dissolved or dispersed therein may also serve as the pharmaceutical preparation for vaginal administration according to this invention.

The content, in the pharmaceutical preparation, of the polypeptide depends on the kind of the polypeptide, the pharmacological effect desired, the number of administrations, the interval between administrations, the severity of the disease, and other factors. However, it may be at any level which is sufficient for the development of the desired pharmacological effect. Thus, an adequate content can be selected, for instance, within the range of about 0.000025% to 90% by weight, preferably within the range of 0.0001% to 50% by weight, based on the composition according to this invention.

The addition level or concentration of cyclodextrin in a solid preparation is generally about 1 to 90% by weight, preferably about 2 to 50% by weight. In the case

of a liquid or semi-solid preparation, the level is generally about 0.5 to 50% by weight, preferably about 1 to 30% by weight. In each case, it is especially preferable that the concentration is about 2 to 20% by weight.

The single dose of the pharmaceutical preparation for vaginal administration may vary depending on the form of the preparation, the kind of the principal drug (i.e. physiologically active polypeptide), the animal to be treated (e.g. warm-blooded mammalian animals such as rat, rabbit, horse, cattle, human) and the purpose of administration. The only requirement is that the principal drug should be administered in an effective dose. Thus, an adequate single dose can be selected, for instance within the range of about 1 mg to 10 g, preferably about 20 mg to 2 g. The number of administrations per day may also vary in the manner mentioned above but can be adequately selected from among once to three times.

By the administration of the pharmaceutical preparation for vaginal administration according to this invention, it brings following characteristic features:

(1) Those physiologically active polypeptides that are hardly absorbed through the gastrointestinal tract can be administered by a route other than injection and at the same time a high level of bioavailability can be attained. Accordingly, the desired pharmacological effect can be obtained with a small dose of said polypeptide.

(2) Physiologically active polypeptides can be administered expediently without any accompanying pain.

(3) In cases where frequent repeated administration is necessary, for instance in cases where the treatment of congenital metabolic disorder is contemplated or a contraceptive or anti-tumor action is expected, the pharmaceutical preparation according to this invention can be easily administered to the patient by self-medication, thus it enables therapy at home.

(4) Since a sustained drug level in the blood is obtainable as compared with injections,the release of the polypeptide from the preparation can be easily controlled and if desired, a further sustained pharmacological effect can be obtained.

(5) Even if the vaginal mucous membrane is the site of action, an efficient pharmacological effect can be expected.

(6) Cyclodextrin used as an absorption-promoting component is low toxic and little irritating to the mucous membrane, thus it permits the production of pharmaceutical preparations which can be safely used in repeated administrations.

In the present rectal preparation, the hydrophilic drug which is poorly absorbable through the gastrointestinal tract is contained as the drug.

The rectal preparation according to this invention can be produced by per se known processes. For example, the drug and cyclodextrin employed according to this invention are added to an oleaginous or aqueous base and the mixture is warmed to an adequate temperature (about 40° to 60°C) for dissolution or dispersion, then poured into a mold and cooled (about 10° to 25°C).

The above-mentioned oleaginous base is, for example, a higher fatty acid glyceride [e.g. cacao butter, which is of the natural origin, Witepsols (Dynamite Nobel, Federal Republic of Germany) (which is a semisynthetic base)], a medial fatty acid glyceride [e.g. Miglyols (Dynamite Nobel)] or a vegetable oil (e.g. sesame oil, soybean oil, corn oil, cottonseed oil, olive oil).

The aqueous base mentioned above include, among others, polyethylene glycols, polypropylene glycols and glycerin as well as hydrogel bases such as natural gum (e.g. gum tragacanth, gum arabic, karaya gum, Irish moth, gum guaiac, xanthan gum, locust bean gum), cellulose derivatives (e.g. methylcellulose, carboxymethylcellulose), acryl polymers (e.g. polyacrylic acid, polymethacrylic

acid), vinyl polymers (e.g. polyvinylpyrrolidone, polyvinyl alcohol, polyvinyl methyl ether, carboxypolymethylene), synthetic polysaccharides (e.g. polysucrose, polyglucose, polylactose), starch, dextrin, pectin and sodium alginate.

These bases may be used either alone or in the form of a mixture of two or more of them.

In producing the pharmaceutical preparation for rectal administration according to this invention, small amounts of preservatives, pH adjusting agents, thickening agents and/or excipients may be incorporated.

The preservatives include, for example, alcohols such as chlorobutanol, quaternary ammonium salts such as benzalkonium chloride, benzethonium chloride and cetrimide, sorbic acid and chlorhexidines.

The pH adjusting agents include inorganic acids such as hydrochloric acid, boric acid, phosphoric acid, carbonic acid and bicarbonic acid, organic acids inclusive of mono- and polycarboxylic acids, and amino acids as well as bases such as sodium hydroxide, potassium hydroxide, sodium hydrogen carbonate and sodium carbonate.

As the buffer solution, there may be mentioned those mentioned above.

The thickening agents include, for example, natural gums such as xanthan gum and locust bean gum, cellulose derivatives such as methylcellulose and carboxymethyl-cellulose, acrylic acid polymers such as polyacrylic acid, and vinyl polymers such as polyvinylpyrrolidone and polyvinyl alcohol.

In this manner, the pharmaceutical preparation for rectal administration is produced in the form of solid preparation (e.g. oleaginous suppository, water-soluble suppository), a semi-solid preparation (e.g. ointment suppository, gel or jelly suppository), suspension (e.g. rectal capsule or enema containing an oleaginous or water-soluble vehicle and the drug), or liquid preparation (e.g. rectal capsule or enema containing an oleaginous

or water-soluble vehicle and the drug), for instance.

The effective single dose of the drug used in accordance with this invention may vary depending on the kind of the drug and the conditions of the animal to be treated. A peptide drug is used, for example in a dose of about 25 µg to 250 mg, a polysaccharide drug in a dose of about 500 mg to 2,000 mg, for instance, an aminoglycoside or beta-lactam antibiotic in a dose of, for example, about 50 to 1,000 mg, and a nucleic acid drug in a dose of about 20 to 1,000 mg, for instance.

The addition level of cyclodextrin in the preparation is generally 1 to 50 w/w percent, preferably about 2 to 20 w/w percent, most preferably about 2 to 10 w/w percent.

These preparations can be administered rectally by direct insertion into the anus of the animals to be treated (e.g. warm-blooded mammalian animals such as rat, rabbit, hourse, cattle, human). Semi-solid, foamy or liquid preparations can also be administered by using an inserter. The rectal preparation may be administered once to three times per day.

By the administration of the present rectal preparation, it brings the following advantageous features:

1) Since the rate of absorption of the drug into the body is improved, a smaller dose of the drug can exert its effect efficiently.

2) The preparation can be expediently administered without a substantial accompanying pain.

3) Self-medication at home is possible. This is beneficial when repeated administration is necessary.

4) The blood level of the drug can be sustained through sustained release thereof from the preparation and therefore the drug efficacy can be maintained longer as compared with injections.

5) Cyclodextrin used as an absorption promoting component is low toxic and little irritating to the mucous membrane. Thus it permits production of pharmaceutical preparations which can be safely used in repeated dose regimens.

6) In contrast with nasal administration, the present

preparation can be practiced with those drugs that are to be administered in large doses or have unpleasant taste. The present preparation can also be practiced with those drugs that are unstable in aqueous bases, by using oleaginous bases.

The following Experimental Examples and Examples are further illustrative of this invention.

Experimental Example 1

Male SD strain rats (body weights 250 g, approx.) fasted for 16 hours are used in groups of at least 3 animals. Each animal is anesthetized with pentobarbital and operated for nasal medication in accordance with the method described in International Journal of Pharmaceutics 7, 317 (1981). Then, 0.1 ml/kg of insulin solution is directly administered into the nasal cavity with a micropipette via the nostril. Blood samples are taken from the tail vein at timed intervals and the plasma glucose levels are determined.

The insulin solution used is a mixed solution of 10 U or 20 U of porcine insulin (about 0.2 mg or 0.8 mg) and 0 mg to 10 mg [which correspond to 0 to 10%] of α-, β- or γ-cyclodextrin in 0.1 ml of an isotonic buffer solution (pH 7.4). In the case of β-cyclodextrin whose saturation solubility is about 1.8%, it is administered as suspensions when its concentrations are over the above solubility limit.

As control, insulin is intravenously administered and plasma glucose levels are determined in the manner as above.

The results are shown in Table 1. As shown in Table 1, the addition of α-, β- or γ-cyclodextrin causes a remarkable depression of plasma glucose level as compared with the control, indicating that insulin is efficiently absorbed through the nasal mucous membrane.

Table 1: Change in plasma glucose level after nasal administration of insulin to rats

	Route	Dose of insulin U/Kg	Kind and concentration of cyclodextrin	Change (%) in plasma glucose level			
				Before administration	1 hr.	2 hr.	4 hr.
Control	Intravenous	5	-	100	29.6	31.6	41.7
	Intranasal	10	-	100	93.7	99.3	103.0
	Intranasal	20	-	100	92.4	90.6	99.9
This invention	Intranasal	10	α, 3%	100	73.5	59.7	62.9
	Intranasal	10	α, 5%	100	59.4	46.8	54.4
	Intranasal	10	α, 10%	100	33.8	24.8	40.3
	Intranasal	20	α, 5%	100	64.3	38.4	47.9
	Intranasal	10	β, 10%	100	62.5	48.6	49.7
	Intranasal	10	γ, 10%	100	80.0	81.0	74.2

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Experimental Example 2

The 2 mg/kg equivalent of ^{14}C-DN-1417 and 5 mg (5% equivalent) of α-cyclodextrin are dissolved in 0.1 ml of physiological saline and 0.1 ml of the solution is administered into the nasal cavity of each rat with a micropipette in the manner as Experimental Example 1. Blood samples are taken from the tail vein at timed intervals and the total radioactivity in the plasma is measured to ascertain the blood concentration. As controls, the same dose is given subcutaneously or ^{14}C-DN-1417 alone without addition of α-cyclodextrin, is administered similarly.

The results are shown in Table 2. It is apparent that the nasal administration of the preparation according to this invention causes marked increases in the absorption of the peptide and that as compared with subcutaneous administration, the bioavailability of the peptide is increased about 5-fold from about 10% to about 50%.

Table 2: Concentration of DN-1417 in plasma after nasal administration of DN-1417 (2 mg/kg) to rats

	Route	Content of cyclodextrin	Concentration in plasma μg/ml		
			1 hr.	2 hr.	4 hr.
Control	Subcutaneous	-	2.3	1.6	0.76
	Intranasal	-	0.22	0.21	0.23
This invention	Intranasal	α, 5%	1.3	1.0	0.51

Experimental Example 3

In 0.1 ml of physiological saline are dissolved 100 μg of TAP-144 and 5 mg of α-cyclodextrin, and in the manner as Experimental Example 1, the 0.1 ml/kg equivalent of the solution is administered into the nasal cavity

(the dose of TAP-144: 100 µg/kg). Blood samples are taken from the tail vein at timed intervals and the serum concentration of TAP-144 is determined by radioimmunoassay. Control animals are either subcutaneously injected at the same dose or given nasally a similar preparation which does not contain α-cyclodextrin.

The results are shown in Table 3. It is apparent that the nasal administration of the composition of this invention results in a marked increase in the absorption of the peptide and that as compared with subcutaneous administration, the bioavailability of the drug is increased about 3.5 times from about 20% to about 70%.

Table 3: Concentration of TAP-144 in serum after nasal administration of TAP-144 (100 µg/kg) to rats

	Route	Cyclo-dextrin	Concentration in serum ng/ml			
			0.5 hr.	1 hr.	2 hr.	4 hr.
Control	Subcutaneous	-	44.0	40.2	24.0	6.7
	Intranasal	-	3.2	3.9	3.5	3.9
This invention	Intranasal	α, 5%	32.1	30.3	16.4	6.8

Experimental Example 4

SD-strain mature female rats (weighing about 270 g, 14 to 18 weeks old, in groups of 4 to 5 individuals) at diestrus as selected by the vaginal smear test covering a period of at least one week are anesthetized with pentobarbital and phenobarbital at 8-11 a.m. and a solution prepared by dissolving 500 µg of TAP-144 and 20 mg (2%), 50 mg (5%) or 100 mg (10%) of α-cyclodextrin in physiological saline in an amount to make 1 ml is administered into the vagina with about 12 mg of cotton soaked therewith (at the dose level of 0.2 ml/kg (corresponding 100 µg/kg of TAP-144). Blood samples are taken from the tail vein at

timed intervals and assayed for the serum TAP-144 level by radioimmunoassay [refer to Endocrinologia Japonica, vol. 27, pages 593-605 (1980)]. In control runs, the same dose of TAP-144 is intravenously administered or a similar preparation produced without the addition of α-cyclodextrin is vaginally administered, and the serum level is determined.

The results shown in Table 4 indicate that the vaginal absorption of TAP-144 administered as the α-cyclodextrin-containing preparation is about 6 times promoted as compared with the control vaginal preparation, and that a high serum level is maintained longer than it is the case with the control intravenous administration run.

Table 4

	Route	Concentration of α-cyclodextrin	Concentration of TAP-144 in serum (ng/ml)						
			Time after administration						
			5 min.	10 min.	30 min.	1 hr.	2 hr.	4 hr.	6 hr.
Control	Intravenous	-	212	173	74.2	39.3	6.76	1.83	<0.13
	Intravaginal	0%	-	-	4.75	4.98	5.37	7.98	5.94
This invention	Intravaginal	2%	-	-	28.1	33.8	39.2	32.1	23.5
	Intravaginal	5%	-	-	32.1	44.3	40.2	24.1	10.5
	Intravaginal	10%	-	-	45.3	75.3	71.1	32.7	12.5

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Experimental Example 5

Following the procedure of Experimental Example 4, a suspension of 5% β-cyclodextrin (β-CD) in physiological saline (containing 100 μg/kg/0.2 ml of TAP-144), a 5% methylcellulose jelly preparation containing 5% α-cyclodextrin (α-CD) (containing 100 μg/kg/400 mg of TAP-144) and an oleaginous suppository (base: Witepsol, containing 100 μg/kg/200 mg of TAP-144) are vaginally administered to rats (in groups of 4 to 5 individuals) under anesthetization, and the serum TAP-144 level determination is performed.

The results shown in Table 5 indicate that inclusion of α-cyclodextrin produces an evident absorption-promoting effect.

Table 5

	Preparation	Kind and concentration of cyclodextrin	Concentration of TAP-144 in serum (ng/ml) Time after administration (hour) 0.5	1	2	4	6
Control	Physiological saline	0%	4.75	4.98	5.37	7.98	5.94
This invention	5% methylcellulose jelly	α-CD, 5%	20.1	23.9	21.0	16.1	10.3
	Oil suppository	α-CD, 5%	14.5	19.7	20.3	29.9	20.2
	Physiological saline	β-CD, 5%	18.4	15.0	20.2	20.6	16.3

As is evident from the foregoing, administration, in the form of the pharmaceutical preparation according to this invention makes it possible for a highly active LH-RH

derivative such as TAP-144 to be absorbed into the body in an efficient manner. Therefore, it is expected that the pharmaceutical preparation according to this invention can be used in expedient drug administration for the purpose of treating breast cancer, which inevitably requires prolonged administration [Lancet, vol. 1, pages 1213-1216 (1982)], or of contraception through shortening of the luteal phase [Science, vol. 215, pages 170-172 (1982)], for instance.

Experimental Example 6

Following the procedure of Experimental Example 4, a solution of porcine insulin and 5% α-cyclodextrin (α-CD) in physiological saline (containing 20 units of porcine insulin/kg/0.2 ml) is vaginally administered to rats. Blood samples are taken from the tail vein at timed intervals and assayed for the plasma glucose level. The plasma glucose level just before administration of insulin is expressed as 100%. In control studies, an α-cyclodextrin-free physiological saline solution of insulin is administered either subcutaneously (5 units/kg) or intravaginally (20 units/kg) and the plasma glucose level is determined in the same manner.

The results shown in Table 6 indicate that when insulin is administered intravaginally as the α-cyclodextrin-containing pharmaceutical preparation for vaginal administration according to this invention, a marked decrease in the plasma glucose level is obtained as compared with the control vaginal administration group, the reduction in the area under the plasma glucose level-time curve within 6 hours after administration being 339.9 ± 11.2 (mean ± standard error) % × hour for the invention group and 158.2 ± 29.6 (mean ± standard error) % × hour for the control group; in this case the pharmacological effect is almost doubled. When compared with the control subcutaneous administration group, a prolonged hypoglycemic effect is obtained in the invention group.

Table 6

	Route	Dose of insulin (Unit/kg)	Plasma glucose level just before the administration (%)	Plasma glucose level (%) Time after the administration (hour)					
				0.5	1	2	3	4	6
Control	Subcutaneous	5	100	67.0	54.7	42.2	35.1	37.5	74.1
	Intravaginal (physiological saline)	20	100	80.7	75.8	77.2	74.2	66.9	67.8
This invention	Intravaginal (5% α-CD)	20	100	66.5	52.7	40.0	33.7	31.4	41.5

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Experimental Example 7

Male SD strain rats (body weights, approx. 300 g) fasted in advance are used in groups of 3 individuals. Under pentobarbital anesthetization, each animal is rectally given 0.1 ml of a ^{14}C-DN-1417 preparation [solution or suspension of 0.6 mg of ^{14}C-DN-1417 and 5 mg of α- or β-cyclodextrin in 0.1 ml of an isotonic hydrochloric acid-potassium chloride buffer (pH 3.0)] by means of a micropipet. Blood samples are taken from the tail vein at timed intervals and the plasma level is determined from the total radioactivity in the blood. In the control group, ^{14}C-DN-1417 is administered in the same manner but without the addition of cyclodextrin. The results, which are shown in Table 7, indicate that the plasma level is markedly increased as compared with the control group. It is thus evident that ^{14}C-DN-1417 is absorbed efficiently through the rectum.

Table 7

Level of addition of cyclodextrin	Plasma level (μg/ml) 0.08	0.25	0.5hr.	1hr.	2hr.	4hr.	6hr.	AUC* (0-6 hr.)
Control -	0.21	0.25	0.25	0.30	0.22	0.21	0.23	1.40
This invention								
5% (w/v) α-cyclodextrin	0.17	1.15	1.36	1.06	0.68	0.66	0.71	4.61
5% (w/v) β-cyclodextrin	-	0.62	0.68	0.54	0.33	0.23	0.28	2.06

*(Note) AUC: Area under the plasma level-time curve (μg·hr/ml)

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Experimental Example 8

DN-1417 with or without 5 mg of α-cyclodextrin is dissolved in 0.1 ml of hydrochloric acid-potassium chloride buffer, pH 3.0. The solution is administered into the rectum of each male SD strain rat (weighing about 250 g, each group containing of 3 individuals) with a micropipet and 60 minutes later, pentobarbital (40 mg/kg) is intraperitoneally injected. The percent reduction in the sleeping time, which is the time interval between the loss of righting reflex and the reaquisition thereof, is determined. Percent reduction in sleeping time

$$= \left(1 - \frac{\text{Sleeping time after administration of DN-1417}}{\text{Sleeping time after administration of vehicle}}\right) \times 100$$

The results, which are shown in Table 8, indicate that the addition of 5% α-cyclodextrin almost doubles the pharmacological activity of DN-1417 in positive correlation with the increased absorption.

Table 8

	Cyclodextrin addition level	Dose of DN-1417 (mg/kg)	% Reduction in sleeping time
Control	-	5	7.8
	-	10	14.8
This invention	5% (w/v) α-cyclodextrin	5	15.2
	5% (w/v) α-cyclodextrin	10	26.4

Experimental Example 9

A Witepsol W-35-based suppository (weight: about 45 mg) containing DN-1417 in an amount corresponding to 2 mg/kg and 5 w/w percent of α- or β-cyclodextrin is administered into the rectum of each male SD strain rat weighing about 90 g (4 weeks of age, each group consisting of 10 individuals), and the discharge rate is examined with

a suppository without cyclodextrin used as the control. The results, which are shown in Table 9, indicate that both the α- and β-cyclodextrin-containing suppositories does not differ in the discharge rate from the control suppositories. This means that the cyclodextrin-added suppositories are hardly irritatable to the rectal mucous membrane.

Table 9

	Base	Cyclodextrin addition level	Rate of discharge** after 15 min.	30 min.	45 min.
Control	Witepsol W-35	—	3/10	3/10	3/10
This invention	Witepsol W-35	5% (w/w) α-cyclodextrin	2/10	2/10	3/10
	Witepsol W-35	5% (w/w) β-cyclodextrin	1/10	3/10	3/10

** The number of animals which discharged the suppository/ the number of animals tested

Experimental Example 10

Porcine insulin (in an amount corresponding to 50 IU/kg) and 5 mg of α-, β- or γ-cyclodextrin are dissolved in 0.1 ml of physiological saline, the solution is administered into the rat rectum in the manner in Experimental Example 7, and plasma glucose levels are determined at timed intervals. In the control group, porcine insulin is administered in the manner but without the addition of cyclodextrin.

The results, which are shown in Table 10, indicate that the addition of α-, β- or γ-cyclodextrin results in a greater hypoglycemic effect than in the control group. It is thus evident that insulin is absorbed more efficiency through the rectum

Table 10

	Cyclodextrin addition level	Change (%) in plasma glucose level				
		Before administration	0.5 hr.	1 hr.	1.5 hr.	2 hr.
Control	-	100	102.2	86.0	95.2	121.9
This invention	5% (w/v) α-cyclodextrin	100	92.9	39.1	39.6	69.6
	5% (w/v) β-cyclodextrin	100	94.3	70.1	76.3	90.1
	5% (w/v) γ-cyclodextrin	100	94.8	79.0	81.3	93.0

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Experimental Example 11

A Witepsol W-35-based suppository (weight: about 45 mg) containing porcine insulin in an amount corresponding to 50 IU/kg and 5 w/w percent of α- or β-cyclodextrin is prepared in the conventional manner and administered to each male SD strain rat (body weight: about 300 g; 3 animals per group) after fasting at the site about 1.5 cm from the anus. Thereafter, blood samples are taken from the tail vein and assayed for the blood sugar level. The results, which are shown in Table 11, indicate that the addition of α- or β-cyclodextrin results in an increased absorption of insulin.

Table 11

	Cyclodextrin addition level	Change (%) in plasma glucose level					
		Before administration	0.5 hr.	1 hr.	1.5 hr.	2 hr.	3 hr.
Control	-	100	98.1	94.1	97.4	98.1	102.5
This invention	5% (w/w) α-cyclodextrin	100	93.1	61.1	63.5	86.1	93.4
	5% (w/w) β-cyclodextrin	100	87.1	81.5	80.1	92.5	100.4

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Experimental Example 12

Heparin sodium 600 U (corresponding to 3.7 mg) and 5 mg of α-cyclodextrin are dissolved in 0.1 ml of physiological saline and the solution is rectally administered to rats in the manner in Experimental Example 7. Blood samples are taken from the tail vein at timed intervals. A 0.27 ml portion of each blood sample is placed in a polyethylene microtube containing 0.03 ml of 3.8 w/v percent of sodium citrate, the tube contents are stirred well and then centrifuged, and the plasma portion is subjected to prothrombin time (blood coagulation time) determination using a thromboplastin reagent (Simplastin, Ono Pharmaceutical Co., Japan). In the control group, the same procedure is followed with α-cyclodextrin-free heparin sodium. The results, which are shown in Table 12, indicate that the addition of α-cyclodextrin results in a prolonged blood coagulation time as compared with the control group. An increased absorption of heparin is thus shown.

Table 12

	Cyclodextrin addition level	Blood coagulation time (in seconds)					
		Before administration	0.5 hr.	0.75 hr.	1 hr.	1.5 hr.	2 hr.
Control	-	13.6	14.3	13.7	14.7	15.3	14.7
This invention	5% (w/v) α-cyclodextrin	14.3	17.2	18.4	19.7	18.9	19.4

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Experimental Example 13

An amount (corresponding to 50 mg/kg) of 5-FU and 5 mg of α-cyclodextrin are finely milled in a mortar and added to 0.1 ml of physiological saline. The mixture is subjected to ultrasonic treatment (27 KHz, 5 minutes). The thus-obtained suspension is rectally administered to rats in the manner in Experimental Example 7. Blood samples are taken from the tail vein at timed intervals and 5-FU plasma levels are determined by bioassay using Micrococcus luteus ATCC-10240 as the test organism. In the control group, the procedure is followed with α-cyclodextrin-free 5-FU. The results, which are shown in Table 13, indicate that the addition of α-cyclodextrin results in an increased absorption of 5-FU as compared with the control group.

Table 13

	Cyclodextrin addition level	Plasma level (μg/ml) 0.5 hr.	1 hr.	2 hr.	4 hr.	AUC (0-4 hr.)
Control	-	0.97	0.43	0.13	0.06	1.1
This invention	5% (w/v) α-cyclodextrin	1.37	1.72	1.05	0.49	4.0

Experimental Example 14

An amount (corresponding to 12 mg/kg) of gentamycin sulfate and 50 mg of α-cyclodextrin are added to 1 ml of physiological saline and the mixture is administered into the rectum of a male rabbit weighing about 2.5 kg in the manner in Experimental Example 7. Blood sampling is performed from the auricular vein at timed intervals and the plasma gentamycin level is determined by bioassay using Bacillus subtilis PCI 219 as the test organism. In the control study, the same procedure is followed with α-cyclodextrin-free gentamycin sulfate. The results are

shown in Table 14. It is indicated that the addition of α-cyclodextrin results in an increased plasma level and in an increased absorption.

Table 14

	Cyclodextrin addition level	Plasma level (μg/ml) 0.5 hr.	1 hr.	2 hr.	4 hr.
Control	-	0.2	0.5	0.4	0.2
This invention	5% (w/v) α-cyclodextrin	0.7	2.5	1.7	1.2

Experimental Example 15

A Witepsol W-35-based suppository (total weight: 150 mg) containing an amount (corresponding to 50 mg/kg) of sodium cefazolin and 10 w/w percent of α-cyclodextrin is prepared by a conventional method and administered to each male SD strain rat (body weight: about 400 g; 3 animals per group) after fasting at the site inner about 1.5 cm from the anus. Thereafter, blood sampling from the tail vein is performed at timed intervals and the plasma cefazolin level is determined by bioassay using Bacillus subtilis PCI-219 as the test organism. As the control study, the same experiment is conducted with α-cyclodextrin-free sodium cefazolin. The results are shown in Table 15, from which it can be seen that the addition of α-cyclodextrin results in an increased plasma level, hence in an increased absorption, as compared with the control group.

Table 15

	Cyclo-dextrin addition level	Plasma level (μg/ml) 0.5 hr.	1 hr.	2 hr.	3 hr.	4 hr.	AUC (0-4 hr.)
Con-trol	-	1.76	2.21	3.00	3.49	2.54	10.32
This inven-tion	10% (w/w) α-cyclo-dextrin	3.84	5.32	7.37	6.26	5.32	22.20

Example 1

In 8 ml of an isotonic phosphate buffer (pH 7.4) is dissolved 5000 U (about 200 mg) of porcine insulin. Then, 500 mg of α-cyclodextrin and 20 mg of chlorobutanol are added and thoroughly dissolved. The mixed solution is diluted with physiological saline to make 10 ml. This solution is put in a nasal spray applicator and administered at the dose level of 0.1 ml per dose.

Example 2

In 40 ml of purified water are dissolved 200 mg of DN-1417, 200 mg of mannitol and 200 mg of β-cyclodextrin and the solution is freeze-dried. The dry product is then pulverized to give a powder about 20 to 250 microns in diameter. A 30 mg portion of the powder is filled into No. 4 (U. S. Pharmacopoeia XX) hard gelatin capsules. To administer the drug, this capsule is set on an exclusive dust applicator equipped with needles for piercing holes inthe capsule and a rubber balloon for sending air into the capsule both ends of the capsule are pierced, a rubber balloon is compressed to dispense the powdery contents into the nasal cavity via the top hole.

Example 3

In 16 ml of an isotonic buffer solution (pH 7.4) containing 0.12% of methylparaben and 0.01% of propyl-paraben are dissolved 1 g of α-cyclodextrin and 2 g of

TAP-144, followed by addition of 200 mg of methylcellulose (Metolose 90SH 4000, Shin-Etsu Chemical Co., Ltd., Japan). The mixture is stirred well to give a homogeneous viscous liquid. This liquid is diluted with the buffer solution to make a total of 20 g. This liquid is put in a nasal spray applicator and administered into the nasal cavity.

Example 4

In 16 ml of an isotonic buffer solution (pH 7.4) containing 0.03% of p-chloro-m-xylenol is dissolved 1 g of α-cyclodextrin and 2 g of TAP-144, followed by addition of 200 mg of methylcellulose (Metolose 90SH 4000, Shin-Etsu Chemical Co., Ltd., Japan). The mixture is stirred well to give a homogeneous viscous liquid. This liquid is diluted with the buffer solution to make a total of 20 g. This liquid is put in a nasal spray applicator and administered into the nasal cavity.

Example 5

500 mg of natural LH-RH [the peptide of general formula (II) wherein R_1=His, R_2=Tyr, R_3=Gly, R_4=Leu, R_5=Gly-NH_2] and 1 g of α-cyclodextrin are taken in a mortar, in which they are mixed with a hot melt of 1 g lanolin. Then, Miglyol 812 [Dynamite Nobel] is gradually added under stirring to make 10 g of an oil suspension. This suspension is put in an applicator, equipped with a dropping pipet and directly administered into the nasal cavity at the dose level of 0.1 g/dose.

Example 6

In 1 ml of physiological saline are dissolved 50 mg of α-cyclodextrin and 100000 U of α-interferon (human leukocyte interferon). This solution is put in a nasal applicator with a dropping pipet and administered into the nasal cavity at the level of 0.1 ml dose.

Example 7

In 10 ml of physiological saline are dissolved 2 mg of desmopressin and 1 g of γ-cyclodextrin followed by addition of 100 mg of methyl cellulose to give a viscous liquid. A 0.2 ml portion of this liquid is taken in an applicator and administered directly into the nasal cavity.

Example 8

In physiological saline are dissolved 1 g of enkepharin and 3 g of α-cyclodextrin. This solution is put in a spray applicator and administered at the level of 0.2 ml per dose into the nasal cavity.

Example 9

To 90 ml of warm water (about 60° to 80°C), there is added 10 g of methylcellulose (Metolose 90SH 4000, Shin-Etsu Chemical Co.) and dispersed therein by adequate stirring. Then, 200 mg of TAP-144 and 10 g of α-cyclodextrin are together dissolved therein, 100 ml of cooled (about 4° to 10°C) aqueous solution is added, the mixture is stirred well at room temperature until a homogeneous gel is obtained. The total quantity is adjusted to 200 g by addition of distilled water. The gel is defoamed by centrifugation and distributed into tubes, which are then sealed. A vaginal dosage form containing a single dose of 1 mg of TAP-144 is prepared by placing 1 g of this gel in an applicator.

Example 10

Oxytocin (20,000 units) and 10 g of α-cyclodextrin are dissolved in an aqueous solution preliminarily prepared by dissolving 5.0 ml of acetic acid and 2.15 g of sodium acetate trihydrate in one liter of water and having a pH of 3.5 to 4.5, to make 200 ml of a solution. A pharmaceutical preparation for vaginal administration which contains 10 units of oxytocin per 0.1 ml (single dose) is prepared by filling a nozzle device-equipped applicator with the above solution.

Example 11

In 200 ml of water, there are dissolved and dispersed 20 g of lactose and 20,000 units (about 800 mg) of porcine insulin, followed by lyophilization. Thereafter, the lyophilizate is ground and stirred well. To a 10.4 g portion of the lyophilizate, there is added a new 61.35 g portion of lactose, and the mixture is stirred well. Further, 10 g of α-cyclodextrin and 10 g of corn starch are added. After adequate mixing, 20 ml of a preliminarily prepared 10% hydroxypropylcellulose (HPC-L) in ethanol solution is added, the mixture is kneaded and granulated by sieving, and the granules are dried at room temperature under reduced pressure for 16 hours. To the granules, there are added 5 g of corn starch and 1.25 g of magnesium stearate. After adequate mixing, each 1 g portion of the mixture is compressed into a tablet. In this way, tablets for vaginal administration each containing 100 units of insulin are produced.

Example 12

To a mixture of 125 mg of an LH-RH analog which is a polypeptide having the formula (Pyr)Glu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-$NHCH_2$-CH_3 [Biochemical and Biophysical Research Communications, vol. 60, No. 1, pages 406-413 (1974)] and 5 g of α-cyclodextrin, there is added 5 g of lanolin preliminarily melted by warming. After sufficient milling and mixing, 89.9 g of an oleaginous base (Witepsol) melted in advance at 50°C was added portionwise with stirring. After adequate homogenization, a plastic container for making a vaginal suppository is filled with 0.8 g of the mixture and the whole is cooled to give a pharmaceutical preparation form for vaginal administration which contains 1 mg of the LH-RH analog per container.

Example 13

α-Cyclodextrin (1 g) and 50,000,000 units of α-interferon (human leukocyte-derived interferon) are dissolved in a 0.2% aqueous carboxymethylcellulose solution to make 10 ml. A pharmaceutical preparation for vaginal administration containing 1,000,000 units of α-interferon per 0.2 ml thereof, which is a single dose, is produced by filling a nozzle device-equipped spray with the solution.

Example 14

α-Cyclodextrin (0.5 g), thyroid hormone-releasing hormone (TRH) tartrate (141.4 mg; 100 mg as TRH) and glycerin (180 ml) are dissolved in distilled water to make 10 ml. A paper tampon (ϕ 10 mm × 25 mm) fixed on a plastic inserter is soaked with 1 ml of the solution to give a pharmaceutical preparation for vaginal administration containing 10 mg of TRH.

Example 15

α-Cyclodextrin (1 g), 50,000,000 units of γ-interferon and 400 mg of human serum albumin are dissolved in 10 ml of distilled water. Glass bottles are each filled with 2 ml of the solution and the contents are lyophilized. Immediately before use, the lyophilizate is dissolved in 2 ml of a diluent of distilled water and the bottle is mounted on the adapter of a nozzle device-equipped spray to give a pharmaceutical preparation for vaginal administration containing 1,000,000 units of γ-interferon per 0.2 ml thereof (single dose).

Example 16

Witepsol W-35 (9.316 g, Dynamite Nobel), a base, is weighed, placed in a mortar and melted by warming at 40-45°C, and 500 mg of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. Then, 183.6 mg of DN-1417 citrate (corresponding to 120 mg of DN-1417) is added. The resultant mixture is stirred well and poured into a 1 g suppository mold and cooled gradually to give ten 1 g rectal suppositories.

Example 17

In a mortar, there is placed 9.316 g of a mixed base composed of 75 w/w percent of polyethylene glycol (PEG) 1000 and 25 w/w percent of PEG 4000. The base is melted with warming at 50-60°C. α- or β-cyclodextrin and DN-1417 citrate are added thereto and the mixture is treated in the manner in Example 16 to give ten 1 g rectal suppositories.

Example 18

To 50 ml of an aqueous solution containing 0.12% of methyl paraben and 0.01% (w/w) of propyl paraben preliminarily dissolved therein by heating to 80-90°C (hereinafter, such solution is referred to as solution A), there is added 5 g of methylcellulose (Metolose 90SH 4000, Shin-Etsu Chemical Co.), and the mixture is stirred to prepare a dispersion. Thereto is added 38 ml of solution A containing 1.414 g of TRH tartrate (corresponding to 1 g of TRH) and 5 g of α-cyclodextrin dissolved therein. The resultant mixture is cooled to 4 to 10°C and stirred well to give a homogeneous gel. After adjusting the total amount to 100 g, 1 g portion each of the gel is poured into applicators for rectal administration. There are thus produced gel suppositories for rectal administration.

Example 19

To 50 ml of an aqueous solution containing 0.03% of p-chloro-m-xylenol (hereinafter, such solution is referred to as solution A), there is added 5 g of methylcellulose (Metolose 90SH 4000, Shin-Etsu Chemical Co.), and the mixture is stirred to prepare a dispersion. Thereto is added 38 ml of solution A containing 1.414 g of TRH tartrate (corresponding to 1 g of TRH) and 5 g of α-cyclodextrin dissolved therein. The resultant mixture is cooled to 4 to 10°C and stirred well to give a homogeneous gel. After adjusting the total amount to 100 g, 1 g portion each of the gel is poured into applicators for rectal administration. There are thus produced gel suppositories for rectal administration.

Example 20

Witepsol W-35 (a base, 9.388 g) is weighed, placed in a mortar and melted by warming at 40-45°C, and 500 mg of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. Then, 112.4 mg of TAP-144 acetate (corresponding to 100 mg of TAP-144) is added. The resultant mixture is stirred well, poured into a 1 g suppository mold and cooled gradually to give ten 1 g rectal suppositories.

Example 21

Porcine insulin (500 U, about 20 mg) is dissolved in 8 ml of an isotonic phosphate buffer (pH 7.4), and further 500 mg of α-, β- or γ-cyclodextrin and 20 mg of chlorobutanol are added. The mixture is stirred well and made 10 ml by addition of physiological saline, and 1 ml portions of the resulting solution are distributed into inserter for rectal administration to give liquid dosage units for rectal administration.

Example 22

Witepsol W-35 (a base, 9.25 g) is weighed, placed in a mortar and melted by warming at 40 to 45°C, and 500 mg of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. Then, 250 mg of enkephalin is added, and the resultant mixture is stirred well, poured into a 1 g suppository mold and cooled gradually to give ten 1 g rectal suppositories.

Example 23

In a mortar, 3 g of lanolin is melted with warming, 616 mg (100,000 U) of sodium heparin and 1 g of α-cyclodextrin are added thereto, the mixture is mixed well for

homogenization, and Miglyol 812 (Dynamite Nobel) is added gradually with stirring to make the whole weight 10 g. No. 0 (U.S. Pharmacopoeia XX) hard capsules are filled with 500 mg each portions of the resultant oleaginous suspension to give 20 rectal capsules.

Example 24

Witepsol W-35 (a base, 15.5 g) is weighed, placed in a mortar and melted by warming at 40 to 45°C, and 2 g of α- or β-cyclodextrin is added thereto. The mixture is warmed and stirred. Then, 2.5 g of citicoline is added. The resultant mixture is stirred well, poured into a 2 g suppository mold and cooled gradually to give ten 2 g rectal suppositories.

Example 25

In a mortar, 3 g of lanolin is melted with warming, 2 g of finely pulverized crystalline 5-FU and 1 g of α-cyclodextrin are added, the mixture is mixed well for homogenization, and Miglyol 812 (Dynamite Nobel) is added gradually with stirring to make the whole weight 10 g. No. 0 hard capsules are filled with 500 mg portions of the resultant oleaginous suspension to give 20 rectal capsules each containing 100 mg of 5-FU.

Example 26

Witepsol W-35 (a base, 7 g) is weighed, placed in a mortar and melted by warming at 40 to 45°C, and 1.0 g of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. Then, 12 g of kanamycin sulfate [corresponding to 10 g (potency) of kanamycin] is added. The resultant mixture is stirred well, poured into a 2 g suppository mold and cooled gradually to give ten rectal suppositories.

Example 27

Witepsol W-35 (a base, 7.885 g) is weighed, placed in a mortar and melted by warming at 40 to 45°C, and 1.000 g of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. Then, 11.115 g of sodium

sulbenicillin [corresponding to 10 g (potency) of sulbenicillin] is added. The resultant mixture is stirred well, poured into a 2 g suppository mold and cooled gradually to give ten 2 g rectal suppositories.

Example 28

Witepsol H-15 (61.5g) is melted by warming, and 10 g of α-cyclodextrin and 28.5 g of finely pulverized cefotiam hydrochloride [corresponding to 25 g (potency) of cefotiam] are added thereto. After homogenization, the mixture is poured into a 2 g suppository mold and cooled gradually to give fifty 2 g rectal suppositories.

What we claim is:

1. A pharmaceutical composition which comprises containing a hydrophilic drug which is poorly absorbable through the gastrointestinal tract and cyclodextrin.

2. A pharmaceutical composition as claimed in Claim 1, wherein the hydrophilic drug is physiologically active polypeptides.

3. A pharmaceutical composition as claimed in Claim 1, wherein the hydrophilic drug is one selected from the group consisting of polysaccharides, aminoglycoside antibiotics, beta-lactam antibiotics and nucleic acid drugs.

4. A pharmaceutical composition as claimed in Claim 2, wherein the composition is formed into a preparation for nasal administration.

5. A pharmaceutical composition as claimed in Claim 2, wherein the composition is formed into a preparation for vaginal administration.

6. A pharmaceutical composition as claimed in Claim 3, wherein the composition is formed into a preparation for rectal administration.

7. A pharmaceutical composition as claimed in Claim 1, wherein the cyclodextrin is α-cyclodextrin.

8. A method for administering a pharmaceutical composition, which comprises administering a pharmaceutical composition containing a physiologically active polypeptide and cyclodextrin through nasal cavity.

9. A method for administering a pharmaceutical composition, which comprises administering a pharmaceutical composition containing a physiologically active polypeptide and cyclodextrin through vagina.

10. A method for administering a pharmaceutical composition, which comprises administering a pharmaceutical composition containing a hydrophilic drug, which is poorly in absorbable through the gastrointestinal tract, selected from the group consisting of polysaccharides, aminoglycoside antibiotics, beta-lactam antibiotics and nucleic acid drug and cyclodextrin through rectum.

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which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application number

EP 83 30 2118

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
X	BE - A - 513 723 (KNOLL AG CHEMISCHE FABRIKEN) * page 2, lines 1-7; page 3, lines 17-23 * --	1-7	A 61 K 9/18 A 61 K 31/715 C 08 B 37/16
E	EP - A - 0 056 995 (THE WELLCOME FOUNDATION LIMITED) * claims 1-11 * --	1-7	
E	GB - A - 2 090 738 (CHINOIN GYOGYSZER ES VEGYESZETI TERMEKEK GYARA RT) * page 9, lines 14-31; claims 1-11 * ----	1-7	

TECHNICAL FIELDS SEARCHED (Int. Cl. ³)

A 61 K
C 08 B

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely:

Claims searched incompletely:

Claims not searched: 8-10

Reason for the limitation of the search:

Method for treatment of the human or animal body by surgery or therapy (see article 52 (4) of the European Patent Convention).

Place of search	Date of completion of the search	Examiner
The Hague	05-08-1983	BRINKMANN

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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EP-A-0 070 368
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EP-A-0 082 921
EP-A-0 091 782
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The file contains technical information submitted after the application was filed and not included in this specification

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(56) References cited:
WO-A-82/04052
BE-A- 513 723
GB-A-2 090 738

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Courier Press, Leamington Spa, England.

EP 0 094 157 B1

Description

The present invention relates to a pharmaceutical composition and its use.

It is generally known that any highly hydrophilic drug having a small oil/water partition coefficient is scarcely absorbed through the gastrointestinal tract and consequently the bioavailability of such drug is small. Therefore, in order to achieve a sufficient clinical effect, such hydrophilic drugs are administered by injection. However, the administration of a drug by way of injection requires an expert hand and causes pain to the recipient and, for these reasons, it is desired to develop a dosage form other than an injectable and that is capable of being applied in an easy and simple manner and affording a high level of bioavailability.

Under these circumstances, the present inventors conducted an intensive study to develop a preparation form for non-oral and non-injection administration that would provided improved bioavailability, hence an improved pharmacological effect, of a hydrophilic or water-soluble drug which is poorly absorbable through the gastrointestinal tract. As a result, the present inventors found that when such a drug is used by non-oral and non-injection administration in combination with cyclodextrin, the absorption of the drug is markedly increased. Pharmaceutical compositions comprising a hydrophilic drug and cyclodextrin in the form of inclusion complexes or compounds are disclosed, for example, in BE—A—513 723, EP—A—0 018 773, WO—A—82 100 251, EP—A—0 056 995, EP—A—0 070 368 and WO—A—82 104 052. A composition in a form suitable for rectal administration is disclosed in EP—A—0 018 773. The state of the art (Article 54(3) and Article 56) also comprises the earlier European Patent Applications EP—A—0 091 782, EP—A—0 078 599 and EP—A—0 082 921. The pharmaceutical compositions of the present invention, however, are not in the form of inclusion complexes or compounds.

The present invention provides a pharmaceutical composition in a form suitable for administration through mucous membranes, characterised by (i) a hydrophilic drug which is poorly absorbable through the gastrointestinal tract, the bioavailability of the drug being not more than 70% and the n-octanol/water partition coefficient of the drug being not more than 10; and (ii) cyclodextrin, tri-O-methylcyclodextrin or triaminocyclodextrin. In a preferred embodiment, the composition is formed into a form suitable for nasal, vaginal or rectal administration, the preferred cyclodextrin is α-cyclodextrin. In one embodiment, the hydrophilic drug is a physiologically active polypeptide. In another embodiment, the hydrophilic drug is selected from polysaccharides, aminoglycoside antibiotics, beta-lactam antibiotics and nucleic acid drugs.

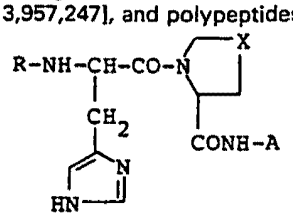
As stated above the present invention relates to (1) a pharmaceutical composition which contains a hydrophilic drug, which is poorly absorbable through the gastrointestinal tracts, and (2) cyclodextrin, tri-O-methylcyclodextrin or triaminocyclodextrin. The hydrophilic drug which is poorly absorbable through the gastrointestinal tracts is a drug, the bioavailability of which is not more than 70%, preferably not more than 50%, more preferably not more than 20% [for example in experimental animals (rat, dog or rabbit), preferably in humans] and with an n-octanal/water partition coefficient of not more than 10, preferably not more than 1, more preferably not more than 0.1.

The oil/water partition coefficient can be determined by the method described in Robert E. Notari "Biopharmaceutics and Pharmacokinetics", Marcel Dekker Inc., 1975, New York, U.S.A. Thus, equal volumes of n-octanol and a buffer solution (pH 5.5) are placed in a test tube to give a 1:1 mixture. The buffer solution is exemplified by Sorensen buffer [Ergebniss der Physiology *12*, 393 (1912)], Clark-Lubs buffer [Journal of Bacteriology *2*, (1), 109, 191 (1971)], McIlvaine buffer [Journal Biological Chemistry *49*, 183 (1921)] and Michaelis buffer [Die Wasser-Stoffionenkonzentration, p. 186 (1914)], Kolthoff buffer [Biochemische Zeitschrift *179*, 410 (1926)]. A suitable amount of the drug to be tested is added to the mixture, and the test tube is stoppered, immersed in a constant-temperature bath (25°C) and shaken vigorously. When it appears that the drug has been dissolved in both of the liquid layers and an equilibrium has been reached, the mixture is allowed to stand or is centrifuged, and aliquots of the upper and lower liquid layers are pipetted separately and analyzed for the concentration of the drug in each layer. The ratio of the concentration of the drug in the n-octanol layer to the concentration of the drug in the aqueous layer is the oil/water partition coefficient.

As the drug employed in the present invention, there may be mentioned, for example, physiologically active polypeptides, polysaccharides, aminoglycoside antibiotics, beta-lactam antibiotics, and nucleic acid drugs.

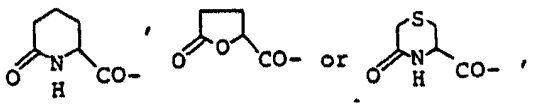
The polypeptides are exemplified by peptides which comprises two or more than two amino acid residues. The polypeptides preferably have a molecular weight of about 200 to 60000.

As the physiologically active polypeptide, there may be mentioned, for example, L-pyroglutamyl-L-histidyl-L-prolinamide (thyrotropin releasing hormone; hereinafter referred to briefly as TRH) or its salts, especially its tartrate [U.S. Patent No. 3,957,247], and polypeptides represented by the formula (I)



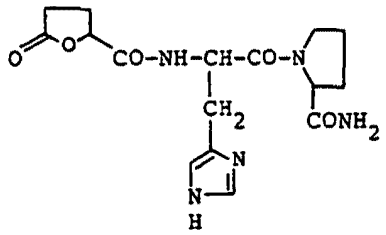
(I)

wherein A stands for hydrogen, alkyl, aralkyl, alkoxyalkyl, hydroxyalkyl or alkoxy, R stands for



X stands for —CH_2—, —CH_2CH_2— or —S—, R and each of the other constituent amino acid residues may have L- or D-configuration or be racemic] and salts thereof [U.S. Patent No. 4,100,152].

Among the compound represented by the formula (I), the compound shown below is referred to briefly as "DN—1417".



(γ-butyrolactone-γ-carbonyl-L-histydyl-L-prolinamide).

Furthermore, as the polypeptide, there may be mentioned luteinizing hormone-releasing hormone (hereinafter referred to briefly as "LH—RH") or peptides which have the LH—RH activity and have the formula (II).

$$(Pyr)Glu—R_1—Trp—Ser—R_2—R_3—R_4—Arg—Pro—R_5 \quad (II)$$

[wherein R_1 stands for His, Tyr, Trp or P—NH_2—Phe; R_2 stands for Tyr or Phe; R_3 stands for Gly or a D-amino acid residue: R_4 stands for Leu, Ile or Nle; R_5 stands for Gly—NH—R_6 (R_6 stands for H or a lower alkyl group which may optionally have a hydroxyl group) or NH—R_6 (R_6 is as defined above)] [U.S. Patent No. 3,853,837, U.S. Patent No. 4,008,209, U.S. Patent No. 3,972,859. British Patent No. 1,423,083, Proceedings of the National Academy of Science of the United States of America, vol. 78, pp. 6509—6512 (1981)].

As examples of the D-amino acid residue R_3 there may be mentioned the residues of *alpha*-D-amino acids containing up to 9 carbon atoms (e.g. D—Leu, Ile, Nle, Val, Nval, Abu, Phe, Phg, Ser, Thr, Met, Ala, Trp, α-Aibu, etc.), which may have suitable protective groups (e.g. t-butyl, t-butoxy, t-butoxycarbonyl). Of course, salts of peptide (II) with acids as well as metal complex compounds of peptide (II) may also be employed just as peptide (II). All abbreviations, wherever they are used in this specification to denote amino acids, peptides, protective groups, etc., are those according to IUPAC—IUB Commission on Biochemical Nomenclature or those commonly employed in the particular art. Where any of the amino acids named herein is subject to optical isomerism, all references to such amino acid mean the L-form unless otherwise indicated.

In the present specification, the polypeptide (II) in which R_1 = His, R_2 = Tyr, R_3 = D—Leu, R_4 = Leu, R_5 = $NHCH_2$—CH_3 is referred to briefly as TAP—144.

Examples of said polypeptide include insulin, somatostatin, somatostatin derivatives (U.S. Patent No. 4,093,573, U.S. Patent No. 4,100,117, U.S. Patent No. 4,253,998), growth hormone, prolactin, adrenocorticotrophic hormone (ACTH), melanocyte stimulating hormone (MSH), thyrotropin releasing hormone (TRH), its salts or its derivatives [U.S. Patent No. 3,957,247, U.S. Patent No. 4,100,152.], thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), vasopressin, vasopressin derivatives {desmopressin [Folia Endocrinologica Japonica, *54*, 5, pp. 676—691, 1978)]}, oxytocin, carcitonin, parathyroid hormone, glucagon, gastrin, secretin, pancreozymin, cholecystokinin, angiotensin, human placental lactogen, human chorionic gonadotropin (HCG), enkephalin, enkephalin derivatives [U.S. Patent 4,277,394; European Patent Application Publication No. 31567], endorphin, interferon (α, β, γ), urokinase, kallikrein, thymopoietin, thymosin, motilin, dynorphin, bombesin, neurotensin, caerulein, bradykinin, substance P, kyotorophin and nerve growth factor, peptide type antibiotics such as polymyxin B, colistin, gramicidin, bacitracin, peptide type anti-tumour agents such as bleomycin, neocarzinostatin.

The polysaccharide drugs mentioned above include, among others, heparin and such antitumour agents as lentinan, zymosan and PS—K (krestin).

The aminoglycoside antibiotics mentioned above include, among others, gentamycin, streptomycin, kanamycin, dibekacin, paromomycin, kanendomycin, lipidomycin, tobramycin, amikacin, fradiomycin and sisomicin.

The beta-lactam antibiotics mentioned above include, among others, penicillins such as sulbenicillin, mecillinam, carbenicillin, piperacillin and ticarcillin, thienamycin, and cephalosporins such as cefotiam, cefsulodine, cefmenoxime, cefmetazole, cefazolin, cefotaxime, cefoperazone, ceftizoxime and moxalactam.

The nucleic acid drugs, compounds with a base (which constitutes a nucleic acid) in the molecule, mentioned above include, among others, citicoline and such antitumour agents as cytarabine and 5—FU (5-fluorouracil).

Examples of the cyclodextrin used according to this invention include various cyclodextrins obtainable by hydrolysis of starch with acid or amylase and various cyclodextrin derivatives.

Such cyclodextrins include α (degree of polymerization 6), β (degree of polymerization 7) and γ (degree of polymerization 8) cyclodextrins [Farumashia vol. 16, No. 1 (1980), 33 Yakugaku Zasshi vol. 101 (10), 857—873 (1981), and Japanese Patent Application Publication Sho 53 (1978) — 31223], tri-O-methylcyclodextrin [Chemical & Pharmaceutical Bulletin *28*, 1552—1558 (1980) and triaminocyclodextrin [Angewandte Chemie: International Edition in English *19*, 344—362 (1980)]. In the practice of this invention, α-cyclodextrin is particularly preferable.

The pharmaceutical composition of the present invention is formed into a preparation suiable for administration through mucous membranes, in particular into a nasal, vaginal or rectal preparation.

The nasal preparation according to the present invention can be produced by the *per se* conventional processes. For example, small amounts of a pH adjusting agent, preservative, thickening agent (natural gums, cellulose derivatives, acrylic acid polymers, vinyl polymers) or/and excipient is/are incorporated.

The nasal preparation of the present invention may take a solid, liquid or semi-solid form. In the case of a solid form, the above components may be simply blended or be freeze-dried to provide a powdery composition, the preferred particle size in either case being about 20 to 250 μm. In the case of a liquid preparation, it is preferably an aqueous solution, an aqueous suspension or an oil suspension. The semi-solid preparation is preferably an aqueous or oleaginous gel or ointment.

As to the proportion of each component in the solid nasal preparation, the polypeptide content of the final preparation is 0.005 to 50 w/v % and preferably 0.01 to 30 w/v % and the proportion of cyclodextrin is 2 to 99.995 w/v % and preferably 5 to 99.99 w/v %. In the case of a liquid or semi-solid preparation, the amount of the polypeptide in the preparation is 0.001 to 50 w/v % and preferably 0.05 to 40 w/v %, while the amount of cyclodextrin is 0.5 to 50 w/v % and preferably 1 to 30 w/v %.

The solid preparation can be produced by the *per se* known procedure. For example, a mixer is charged with cyclodextrin or, if required, a mixture of cyclodextrin and an excipient and, then, the polypeptide dissolved in a small amount of water is gradually added and mixed in. Therafter, the mixture is dried in vacuo at a suitable temperature and the dried composition is pulverized to give a solid preparation. Alternatively, the polypeptide and cyclodextrin, plus an excipient if required, are dissolved in water and freeze-dried or spray-dried to give a dehydrated composition which is then pulverized into a solid preparation.

The excipient is exemplified by glucose, mannitol, inositol, sucrose, lactose, fructose, starch, corn starch, microcrystalline cellulose, hydroxypropyl-cellulose, hydroxypropylmethyl-cellulose, polyvinyl pyrrolidone.

The liquid preparation can be produced by the *per se* known procedure. For example, an aqueous preparation for nasal administration can be produced by dissolving, suspending or emulsifying the polypeptide and cyclodextrin in water, a buffer solution or an aqueous medium. The oil suspension for nasal use can be produced by suspending or emulsifying the polypeptide and cyclodextrin in an oleaginous base. The buffer solution is exemplified as those mentioned above.

The above-mentioned oleaginous basis is exemplified by various oils and fats such as sesame oil, olive oil, corn oil, soybean oil, cotton-seed oil, peanut oil, lanoline, Vaseline®, paraffin, coparaffinate, silicone oil, a fatty acid having 6 to 30 carbon atoms or an ester thereof, for example a glycerin ester, or a mixture thereof.

As to the semi-solid preparation, an aqueous or oleaginous gel or ointment can be produced by the *per se* conventional procedure. For example, such an aqueous gel for nasal administration can be produced in the following manner. First, an aqueous solution or suspension of cyclodextrin is prepared and, if required, a pH adjusting agent and/or a preservative is added. The solution is divided into halves and an aqueous gel base is dissolved or dispersed in one of the halves and heated or cooled to give a stable gel. In the other half is dissolved the polypeptide. Then, the two halves are combined and evenly mixed to give an aqueous gel preparation.

Adjustment of the pH of preparation can be effected by adding an acid, a base or a buffer solution in the course of production of the preparations. As examples of acids, there may be mentioned inorganic acids (e.g. hydrochloric acid, boric acid, phosphoric acid, carbonic acid, bicarbonate), amino acids and organic acids (e.g. monocarboxylic acids, hydroxycarboxylic acids, polycarboxylic acids). The bases are exemplified by sodium hydroxide, potassium hydroxide, sodium hydrogen carbonate, sodium carbonate. The buffer solution is exemplified by those mentioned above.

Examples of the aqueous gel basis include natural gums (e.g. gum tragacanth, gum acasia, gum karaya, Irish moss, gum guaiac, gum xanthane, locust bean gum), cellulose derivatives (e.g. methylcellulose, carboxymethylcellulose), acrylic acid polymers (e.g. polyacrylic acid, polymethacrylic acid), vinyl polymers (e.g. polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl methyl ether, carboxypolymethylene), synthetic polysaccharides (e.g. polysucrose, polyglucose, polylactose), starch, dextrin, pectin, sodium alginate. These bases may be used in the form of appropriate mixtures or two or more species.

The oleaginous ointment for nasal administration can be produced by dispersing the polypeptide and cyclodextrin evenly in a hot melt of an oleaginous base and cooling the same under stirring. The oleaginous base may be one of those mentioned hereinbefore.

Preservatives may be incorporated in nasal preparations. Examples of such preservatives include phenolic compounds such as phenol, cresol; alcohols such as chlorobutanol, phenylethyl alcohol, propylene glycol; invert soaps such as benzalkonium chloride, benzethonium chloride; benzoic acid, sorbic acid, dehydroacetic acid and sulfurous acid and their salts such as sodium hydrogen sulfite.

The nasal preparation of this invention, if it is a solid preparation, can be administered by the following exemplary procedure. Thus, a capsule containing the powdery preparation is placed in an exclusive dust applicator equipped with needles to pierce the capsule at the top and bottom thereof and an air balloon is used to drive the powdery contents into the nasal cavity.

In the case of a liquid preparation, it is put in a nasal douche, an atomizer or a spray-mist applicator suited for nasal application of liquids and dripped or sprayed into the nasal cavity.

The semi-solid preparation can be administered, for example by filling a tube with the preparation and applying the preparation directly into the nasal cavity through an applicator attached to the mouth of the tube or by administering the indicated dose of the preparation by means of a nasal insertion device.

While the dosage of the polypeptide varies with its kind, the disease to be managed and the animals to be treated (e.g. warm-blooded mammalian animals such as rat, rabbit, horse, cattle, human). A suitable amount of the solid preparation per dose is 5 mg to 100 mg, that of the liquid preparation is 0.05 ml to 0.5 ml, and that of the semi-solid preparation is 50 mg to 500 mg. The nasal preparation may be administered once to four times per day.

By the administration of the nasal preparation of this invention, it brings the following advantageous features.

1) The physiologically active polypeptide which is poorly absorbed through the gastrointestinal tract can be administered by a route other than injection to achieve an improved bioavailability.

2) The physiologically active polypeptide can be administered without accompanying pain.

3) Self-medication at home is possible. This is especially beneficial when repeated administration is necessary.

4) Cyclodextrin as an absorption promoting component is tasteless, odorless, of low toxicity and non-irritating to the mucous membrane, thus permitting production of pharmaceutical preparations which can be safely used in repeated dose regimens.

The pharmaceutical preparation for vaginal administration according to this invention can be produced by *per se* conventional processes.

The pharmaceutical preparation for vaginal administration according to this invention may have, among others, the form of a vaginal suppository which retains its solid form at room temperature and melts at the body temperature, or the form of an ointment or liquid contained in a tube, for instance. In the former case, the preparation of solid form is vaginally administered, and the preparation is melted at the body temperature. In the latter case, the tube containing the ointment or liquid is vaginally administered, and the content is pushed out and the tube is taken out.'

The preparation may also be made available in the form of a tablet which, after administration, is dissolved or disintegrated in the vaginal fluid. In this case, the preparation can be easily administered into vagina when an adequate device, preferably an inserter, is used.

The vaginal suppository or ointment can be produced by the *per se* known processes, for instance by dissolving or dispersing a physiologically active polypeptide and cyclodextrin in a preliminarily molten oleaginous or aqueous base, attaining homogeneous dispersion by adequate warming and stirring, and molding the mixture.

In the preparation for vaginal administration, any of the known suppository bases or ointment bases can be employed. As the water-soluble bases, there may be mentioned polyethylene glycols (e.g. those having the mean molecular weight of 200, 300, 400, 1000, 4000, 6000), propylene glycol, glycerol. These bases may be used either alone or as a mixture. As examples of said oleaginous bases there may be mentioned such oils and fats as sesame oil, olive oil, corn oil, soybean oil, cotton seed oil, peanut oil, cacao butter, castor oil, wool fat, squalene, the corresponding modified materials as modified by such procedures as hydrogenation, fatty acid interchange, acetylation, fractional extraction, etc.; mineral oils such as Vaseline®, paraffin, silicone oil; esters of fatty acids of 6 to 30 carbon atoms with glycerin, particularly higher fatty acid esters such as glycerin palmitate, glycerin laurate, glycerin stearate, glycerin myristate; esters of fatty acids of 6 to 30 carbon atoms with alcohols of 2 to 8 carbon atoms, particularly waxes such as isopropyl myristate, butyl stearate, diisopropyl adipate, diethyl sebacate; and higher fatty acids of 6 to 30 carbon atoms, particularly stearic acid, oleic acid. Such oleaginous bases may be used either alone or as a mixture.

To the aqueous pharmaceutical preparation for vaginal administration in accordance with the present invention, there may be added, if necessary, an isotonizing agent (e.g. sodium chloride, potassium chloride, sorbitol), a wetting agent (e.g. glycerol, propylene glycol), a preservative (e.g. benzyl alcohol), a pH-adjusting agent (e.g. hydrochloric acid, acetic acid, citric acid, phosphoric acid, sodium hydroxide, potassium hydroxide, ammonia, a salt of any of these acids), a thickening agent (e.g. methylcellulose, carboxymethylcellulose), a stabilizer (e.g. sodium ethylenediaminetetraacetate, human serum albumin,

citric acid), a dispersing agent [e.g. lecithin, Tween® (polyoxyethylenesorbitan fatty acid ester, Kao-Atlas Co., Ltd. Japan) and Span® (higher fatty acid sorbitan ester, Kao-Atlas Co.)].

The preparation of the present invention for vaginal administration may be a gel suppository prepared by a *per se* conventional manner from an aqueous solution or suspension containing the hydrophilic drug by adding a water-soluble gel-forming bases. As examples of the water-soluble gel bases, there may be mentioned naturally occurring gums (e.g. gum tragacanth, gum acacia, karaya gum, Irish moss, gum guaiac, gum xanthane, locust-bean gum), cellulose derivatives (e.g. methyl-cellulose, carboxymethyl-cellulose), acrylic acid polymers (e.g. polyacrylic acid, polymethacrylic acid), vinyl polymers (e.g. polvyinyl pyrrolidone, polyvinyl alcohol, polvyinyl methyl ether, carboxypolymethylene), synthetic polysaccharides (e.g. polysucrose, polyglucose, polylactose), starch, dextrin, pectin and sodium alginate. These bases may be employed either singly or, if necessary, as a mixture of two or more different bases and copolymers of the polymer mentioned above are also employed.

The aqueous solution for vaginal administration is also vaginally administered as supported on a solid matrix, for instance. The solid matrix may be one of the known matrixes such as porous materials made from high molecular compounds (e.g. silicon rubber, polyurethane), biological polymers (e.g. collagen, gelatin) and cellulosic materials (e.g. cotton, paper).

The prepared solution may be made into a foam or an aerosol preparation in a *per se* conventional manner.

To prepare vaginal tablets, the active component is compressed into appropriate dosage units generally by a procedure analogous to the known procedure, using diluent such as lactose, sucrose, starch, disintegrating agents such as starch, sodium hydrogen carbonate; binders such as starch, gelatin, carboxymethyl-cellulose, polyvinylpyrrolidone, hydroxypropyl-cellulose; lubricants such as talc, magnesium stearate, polyethylene glycol (6000), stearic acid. When the required dosage is very small, an increased product uniformity may be obtained by preparing a mixed solution of the hydrophilic drug with a diluent such as lactose, starch or mannitol beforehand then drying the mixed solution by way of freeze-drying or spray-drying to make a diluted powder and molding this diluted powder into tablets. In view of the relative scarcity of vaginal fluids as compared with gastrointestinal fluids disintegration and dissolution are important considerations.

To assist in disintegration and dissolution, there may be prepared effervescent tablets with combination of alkali metal carbonates (e.g. sodium carbonate) or bicarbonates with citric acid or tartaric acid.

Tablets thus prepared can be inserted directly into vagina.

The aqueous solution or aqueous suspension containing cyclodextrin and a physiologically active hydrophilic drug dissolved or dispersed therein may also serve as the pharmaceutical preparation for vaginal administration according to this invention.

The content, in the pharmaceutical preparation, of the hydrophilic drug depends on the kind of the drug, the pharmacological effect desired, the number of administrations, the interval between administrations, the severity of the disease, and other factors. However, it may be at any level which is sufficient for the development of the desired pharmacological effect. Thus, an adequate content can be selected, for instance, within the range of 0.000025% to 90% by weight, preferably within the range of 0.0001% to 50% by weight, based on the composition according to this invention.

The addition level or concentration of cyclodextrin in a solid preparation is generally 1 to 90% by weight, preferably 2 to 50% by weight. In the case of a liquid or semi-solid preparation, the level is generally 0.5 to 50% by weight, preferably 1 to 30% by weight. In each case, it is especially preferable that the concentration is 2 to 20% by weight.

The single dose of the pharmaceutical preparation for vaginal administration may vary depending on the form of the preparation, the kind of the principal drug (e.g. physiologically active polypeptide), the animal to be treated (e.g. warm-blooded mammalian animals such as rat, rabbit, horse, cattle, human) and the purpose of administration. The only requirement is that the principal drug should be administered in an effective dose. Thus, an adequate single dose can be selected, for instance within the range of 1 mg to 10 g, preferably 20 mg to 2 g. The number of administrations per day may also vary in the manner mentioned above but can be adequately selected from among once to three times.

By the administration of the pharmaceutical preparation for vaginal administration according to this invention, it brings following characteristic features:

(1) Those physiologically active hydrophilic drugs that are scarcely absorbed through the gastrointestinal tract can be administered by a route other than injection and at the same time a high level of bioavailability can be attained. Accordingly, the desired pharmacological effect can be obtained with a small dose of said drug.

(2) Physiologically active hydrophilic drug can be administered expediently without any accompanying pain.

(3) In cases where frequent repeated administration is necessary, for instance in cases where the treatment of congenital metabolic disorder is contemplated or a contraceptive or anti-tumour action is expected, the pharmaceutical preparation according to this invention can be easily administered by the patient, thus it enables therapy at home.

(4) Since a sustained drug level in the blood is obtainable as compared with injections, the release of the

hydrophilic drug from the preparation can be easily controlled and if desired, a further sustained pharmacological effect can be obtained.

(5) Even if the vaginal mucous membrane is the site of action, an efficient pharmacological effect can be expected.

(6) Cyclodextrin used as an absorption-promoting component has a low toxicity and is not very irritating to the mucous membrane, thus it permits the production of pharmaceutical preparations which can be safely used in repeated administrations.

The rectal preparation according to this invention can be produced by *per se* known processes. For example, the drug and cyclodextrin employed according to this invention are added to an oleaginous or aqueous base and the mixture is warmed to a suitable temperature (about 40° to 60°C) for dissolution or dispersion, then poured into a mold and cooled (about 10° to 25°C).

The above-mentioned oleaginous base is, for example, a higher fatty acid glyceride [e.g. cacao butter, which is of the natural origin, Witepsols® (Dynamite Nobel, Federal Republic of Germany) (which is a semisynthetic base)], a medium chain fatty acid glyceride [e.g. Miglyols® (Dynamite Nobel)] or a vegetable oil (e.g. sesame oil, soybean oil, corn oil, cottonseed oil, olive oil).

The aqueous base mentioned above include, among others, polyethylene glycols, polypropylene glycols and glycerin as well as hydrogel bases such as natural gum (e.g. gum tragacanth, gum arabic karaya gum, Irish moth, gum guaiac, xanthum gum, locust bean gum), cellulose derivatives (e.g. methylcellulose, carboxymethylcellulose), acryl polymers (e.g. polyacrylic acid, polymethacrylic acid), vinyl polymers (e.g. polyvinylpyrrolidone, polyvinyl alcohol, polyvinyl methyl ether, carboxypolymethylene), synthetic polysaccharides (e.g. polysucrose, polyglucose, polylactose), starch, dextrin, pectin and sodium alginate.

These bases may be used either alone or in the form of a mixture of two or more of them.

In producing the pharmaceutical preparation for rectal administration according to this invention, small amounts of preservatives, pH adjusting agents, thickening agents and/or excipients may be incorporated.

The preservatives include, for example, alcohols such as chlorobutanol, quaternary ammonium salts such as benzalkonium chloride, benzethonium chloride and cetrimide, sorbic acid and chlorhexidines.

The pH adjusting agents include inorganic acids such as hydrochloric acid, boric acid, phosphoric acid, carbonic acid and bicarbonate, organic acids inclusive of mono- and polycarboxylic acids, and amino acids as well as bases such as sodium hydroxide, potassium hydroxide, sodium hydrogen carbonate and sodium carbonate.

As the buffer solution, there may be mentioned those mentioned above.

The thickening agents include, for example, natural gums such as xanthan gum and locust bean gum, cellulose derivatives such as methylcellulose and carboxymethylcellulose, acrylic acid polymers such as polyacrylic acid, and vinyl polymers such as polyvinylpyrrolidone and polyvinyl alcohol.

In this manner, the pharmaceutical preparation for rectal administration is produced in the form of solid preparation (e.g. oleaginous suppository, water-soluble suppository), a semi-solid preparation (e.g. ointment suppository, gel or jelly suppository), suspension (e.g. rectal capsule or enema containing an oleaginous or water-soluble vehicle and the drug), or liquid preparation (e.g. rectal capsule or enema containing an oleaginous or water-soluble vehicle and the drug), for instance.

The effective single dose of the drug used in accordance with this invention may vary depending on the kind of the drug and the conditions of the animal to be treated. A peptide drug is used, for example in a dose of 25 µg to 250 mg, a polysaccharide drug in a dose of 500 mg to 2,000 mg, for instance, an aminoglycoside or beta-lactam antibiotic in a dose of, for example, 50 to 1,000 mg, and a nucleic acid drug in a dose of 20 to 1,000 mg, for instance.

The addition level of cyclodextrin in the preparation is generally 1 to 50 w/w percent, preferably 2 to 20 w/w percent, most preferably 2 to 10 w/w percent.

These preparations can be administered rectally by direct insertion into the anus of the animals to be treated (e.g. warm-blooded mammalian animals such as rat, rabbit, horse, cattle, human). Semi-solid, foamy or liquid preparations can also be administered by using an inserter. The rectal preparation may be administered once to three times per day.

By the administration of the present rectal preparation, it brings the following advantageous features:

1) Since the rate of absorption of the drug into the body is improved, a smaller dose of the drug can exert its effect efficiently.

2) The preparation can be expediently administered without any substantial accompanying pain.

3) Self-medication at home is possible. This is beneficial when repeated administration is necessary.

4) The blood level of the drug can be sustained through sustained release thereof from the preparation and therefore the drug efficiency can be maintained longer as compared with injections.

5) Cyclodextrin used as an absorption promoting component has a low toxicity and is not very irritating to the mucous membrane. Thus it permits production of pharmaceutical preparations which can be safely used in repeated dose regimens.

6) In contrast with nasal administration, the present preparation can be applied to those drugs that are to be administered in large doses or have unpleasant taste. The present preparation can also be applied to those drugs that are unstable in aqueous bases, by using oleaginous bases.

The following Experimental Examples and Examples are futher illustrative of this invention.

Experimental Example 1

Male SD strain rats (body weights 250 g, approx.) fasted for 16 hours are used in groups of at least 3 animals. Each animal is anesthetized with pentobarbital and operated for nasal medication in accordance with the method described in International Journal of Pharmaceutics 7, 317 (1981). Then, 0.1 ml/kg of insulin solution is directly administered into the nasal cavity with a micropipette via the nostril. Blood samples are taken from the tail vein at timed intervals and the plasma glucose levels are determined.

The insulin solution used is a mixed solution of 10 U or 20 U of porcine insulin (about 0.2 mg or 0.8 mg) and 0 mg to 10 mg [which correspond to 0 to 10%] of α-, β- or γ-cyclodextrin in 0.1 ml of an isotonic buffer solution (pH 7.4). In the case of β-cyclodextrin whose saturation solubility is about 1.8%, it is administered as suspensions when its concentrations are over the above solubility limit.

As control, insulin is intravenously administered and plasma glucose levels are determined in the manner as above.

The results are shown in Table 1. As shown in Table 1, the addition of α-, β- or γ-cyclodextrin causes a remarkable depression of plasma glucose level as compared with the control, indicating that insulin is efficiently absorbed through the nasal mucous membrane.

TABLE 1

Change in plasma glucose level after nasal administration of insulin to rats

	Route	Dose insulin U/Kg	Kind and concentration of cyclodextrin	Change (%) in plasma glucose level			
				Before administration	1 hr.	2 hr.	4 hr.
Control	Intravenous	5	—	100	29.6	31.6	41.7
	Intranasal	10	—	100	93.7	99.3	103.0
	Intranasal	20	—	100	92.4	90.6	99.9
This invention	Intranasal	10	α, 3%	100	73.5	59.7	62.9
	Intranasal	10	α, 5%	100	59.4	46.8	54.4
	Intranasal	10	α, 10%	100	33.8	24.8	40.3
	Intranasal	20	α, 5%	100	64.3	38.4	47.9
	Intranasal	10	β, 10%	100	62.5	48.6	49.7
	Intranasal	10	γ, 10%	100	80.0	81.0	74.2

Experimental Example 2

The 2 mg/kg equivalent of ^{14}C—DN—1417 and 5 mg (5% equivalent) of α-cyclodextrin are dissolved in 0.1 ml of physiological saline and 0.1 ml of the solution is administered into the nasal cavity of each rat with a micropipette in the manner as Experimental Example 1. Blood samples are taken from the tail vein at timed intervals and the total radioactivity in the plasma is measured to ascertain the blood concentration. As controls, the same dose is given subcutaneously or ^{14}C—DN—1417 alone without addition of α-cyclodextrin, is administered similarly.

The results are shown in Table 2. It is apparent that the nasal administration of the preparation according to this invention causes marked increases in the absorption of the peptide and that as compared with the control nasal administration, the bioavailability of the peptide is increased about 5-fold from about 10% to about 50%.

TABLE 2
Concentration of DN—1417 in plasma after nasal administration of DN—1417 (2 mg/kg) to rats

	Route	Content of cyclodextrin	Concentration in plasma μg/ml 1 hr.	2 hr.	4 hr.
Control	Subcutaneous	—	2.3	1.6	0.76
	Intranasal	—	0.22	0.21	0.23
This invention	Intranasal	α, 5%	1.3	1.0	0.51

Experimental Example 3

In 0.1 ml of physiological saline are dissolved 100 μg of TAP—144 and 5 mg of α-cyclodextrin, and in the manner as Experimental Example 1, the 0.1 ml/kg equivalent of the solution is administered into the nasal cavity (the dose of TAP—144: 100 μg/kg). Blood samples are taken from the tail vein at timed intervals and the serum concentration of TAP—144 is determined by radioimmunoassay. Control animals are either subcutaneously injected at the same dose or given nasally a similar preparation which does not contain α-cyclodextrin.

The results are shown in Table 3. It is apparent that the nasal administration of the composition of this invention results in a marked increase in the absorption of the peptide and that as compared with with control nasal administration, the bioavailability of the drug is increased about 3.5 times from about 20% to about 70%.

TABLE 3
Concentration of TAP—144 in serum after nasal administration of TAP—144 (100 μg/kg) to rats

	Route	Cyclodextrin	Concentration in serum ng/ml 0.5 hr.	1 hr.	2 hr.	4 hr.
Control	Subcutaneous	—	44.0	40.2	24.0	6.7
	Intranasal	—	3.2	3.9	3.5	3.9
This invention	Intranasal	α, 5%	32.1	30.3	16.4	6.8

Experimental Example 4

SD-strain mature female rats (weighing about 270 g, 14 to 18 weeks old, in groups of 4 to 5 individuals) at diestrus as selected by the vaginal smear test covering a period of at least one week are anesthetized with pentobarbital and phenobarbital at 8—11 a.m. and a solution prepared by dissolving 500 μg of TAP—144 and 20 mg (2%), 50 mg (5%) or 100 mg (10%) of α-cyclodextrin in physiological saline in an amount to make 1 ml is administered into the vagina with about 12 mg of cotton soaked therewith (at the dose level of 0.2 ml/kg (corresponding 100 μg/kg of TAP—144). Blood samples are taken from the tail vein at timed intervals and assayed for the serum TAP—144 level by radioimmunoassay [refer to Endocrinologia Japonica, vol. 27, pages 593—605 (1980)]. In control runs, the same dose of TAP—144 is intravenously administered or a similar preparation produced without the addition of α-cyclodextrin is vaginally administered, and the serum level is determined.

The results shown in Table 4 indicate that the vaginal absorption of TAP—144 administered as the α-cyclodextrin-containing preparation is about 6 times greater as compared with the control vaginal preparation, and that a high serum level is maintained longer than is the case with the control intravenous administration run.

TABLE 4

	Route	Concentration of α-cyclodextrin	Concentration of TAP—144 in serum (ng/ml) Time after administration 5 min.	10 min.	30 min.	1 hr.	2 hr.	4 hr.	6 hr.
Control	Intravenous	—	212	173	74.2	39.3	6.76	1.83	<0.13
	Intravaginal	0%	—	—	4.75	4.98	5.37	7.98	5.94
This invention	Intravaginal	2%	—	—	28.1	33.8	39.2	32.1	23.5
	Intravaginal	5%	—	—	32.1	44.3	40.2	24.1	10.5
	Intravaginal	10%	—	—	45.3	75.3	71.1	32.7	12.5

Experimental Example 5

Following the procedure of Experimental Example 4, a suspension of 5% β-cyclodextrin (β-CD) in physiological saline (containing 100 μg/kg/0.2 ml of TAP—144), a 5% methylcellulose jelly preparation containing 5% α-cyclodextrin (α-CD) (containing 100 μg/kg/400 mg of TAP—144) and an oleaginous suppository (base: Witepsol®, containing 100 μg/kg/200 mg of TAP—144) are vaginally administered to rats (in groups of 4 to 5 individuals) under anesthetization, and the serum TAP—144 level determination is performed.

The results shown in Table 5 indicate that inclusion of α-cyclodextrin produces an evident absorption-promoting effect.

TABLE 5

	Preparation	Kind and concentration of cyclodextrin	Concentration of TAP-144 in serum (ng/ml) Time after administration (hour) 0.5	1	2	4	6
Control	Physiological saline	0%	4.75	4.98	5.37	7.98	5.94
This invention	5% methyl cellulose jelly	α-CD, 5%	20.1	23.9	21.0	16.1	10.3
	Oil suppository	α-CD, 5%	14.5	19.7	20.3	29.9	20.2
	Physiological saline	β-CD, 5%	18.4	15.0	20.2	20.6	16.3

As is evident from the foregoing, administration, in the form of the pharmaceutical preparation according to this invention makes it possible for a highly active LH—RH derivative such as TAP—144 to be absorbed into the body in an efficient manner. Therefore, it is expected that the pharmaceutical preparation according to this invention can be used in drug administration for the purpose of treating breast cancer, which inevitably requires prolonged administration [Lancet, vol. 1, pages 1213—1216 (1982)], or of contraception through shortening of the luteal phase [Science, vol. 215, pages 170—172 (1982)], for instance.

Experimental Example 6

Following the procedure of Experimental Example 4, a solution of porcine insulin and 5% α-cyclodextrin (α-CD) in physiological saline (containing 20 units of porcine insulin/kg/0.2 ml) is vaginally administered to rats. Blood samples are taken from the tail vein at timed intervals and assayed for the plasma glucose level. The plasma glucose level just before administration of insulin is expressed as 100%. In control studies, an α-cyclodextrin-free physiological saline solution of insulin is administered either subcutaneously (5 units/kg) or intravaginally (20 units/kg) and the plasma glucose level is determined in the same manner.

The results shown in Table 6 indicate that when insulin is administered intravaginally as the α-cyclodextrin-containing pharmaceutical preparation for vaginal administration according to this invention, a marked decrease in the plasma glucose level is obtained as compared with the control vaginal administration group, the reduction in the area under the plasma glucose level-time curve within 6 hours after administration being 339.9±11.2 (mean±standard error) % × hour for the invention group and 158.2±29.6 (mean± standard error) % × hour for the control group; in this case the pharmacological effect is almost doubled. When compared with the control subcutaneous administration group, a prolonged hypoglycemic effect is obtained in the invention group.

TABLE 6

	Route	Dose of insulin (Unit/kg)	Plasma glucose level just before the administration (%)	Plasma glucose level (%) Time after the administration (hour)					
				0.5	1	2	3	4	6
Control	Subcutaneous	5	100	67.0	54.7	42.2	35.1	37.5	74.1
	Intravaginal (physiological saline)	20	100	80.7	75.8	77.2	74.2	66.9	67.8
This invention	Intravaginal (5% α-CD)	20	100	66.5	52.7	40.0	33.7	31.4	41.5

Experimental Example 7

Male SD strain rats (body weights, approx. 300 g) fasted in advance are used in groups of 3 individuals. Under pentobarbital anesthetization, each animal is rectally given 0.1 ml of a ^{14}C—DN—1417 preparation [solution or suspension of 0.6 mg of ^{14}C—DN—1417 and 5 mg of α- or β-cyclodextrin in 0.1 ml of an isotonic hydrochloric acid-potassium chloride buffer (pH 3.0)] by means of a micropipet. Blood samples are taken from the tail vein at timed intervals and the plasma level is determined from the total radioactivity in the blood. In the control group, ^{14}C—DN—1417 is administered in the same manner but without the addition of cyclodextrin. The results, which are shown in Table 7, indicate that the plasma level is markedly increased as compared with the control group. It is thus evident that ^{14}C—DN—1417 is absorbed efficiently through the rectum.

TABLE 7

Level of addition of cyclodextrin	Plasma level (μg/ml) 0.08	0.25	0.5hr.	1hr.	2hr.	4hr.	6hr.	AUC* (0—6 hr.)
Control —	0.21	0.25	0.25	0.30	0.22	0.21	0.23	1.40
This invention								
5% (w/v) α-cyclodextrin	0.17	1.15	1.36	1.06	0.68	0.66	0.71	4.61
5% (w/v) β-cyclodextrin	—	0.62	0.68	0.54	0.33	0.23	0.28	2.06

* (Note) AUC: Area under the plasma level-time curve (μg·hr/ml)

Experimental Example 8

DN—1417 with or without 5 mg of α-cyclodextrin is dissolved in 0.1 ml of hydrochloric acid-potassium chloride buffer, pH 3.0. The solution is administered into the rectum of each male SD strain rat (weighing about 250 g, each group containing of 3 individuals) with a micropipet and 60 minutes later, pentobarbital (40 mg/kg) is intraperitoneally injected. The percent reduction in the sleeping time, which is the time interval between the loss of righting reflex and the reaquisition thereof, is determined. Percent reduction in sleeping time

$$= \left(1 - \frac{\text{Sleeping time after administration of DN—1417}}{\text{Sleeping time after administration of vehicle}}\right) \times 100$$

The results, which are shown in Table 8, indicate that the addition of 5% α-cyclodextrin almost doubles the pharmacological activity of DN—1417 in positive correlation with the increased absorption.

TABLE 8

	Cyclodextrin addition level	Dose of DN—1417 (mg/kg)	% Reduction in sleeping time
Control	—	5	7.8
	—	10	14.8
This invention	5% (w/v) α-cyclodextrin	5	15.2
	5% (w/v) α-cyclodextrin	10	26.4

Experimental Example 9

A Witepsol® W—35-based suppository (weight: about 45 mg) containing DN—1417 in an amount corresponding to 2 mg/kg and 5 w/w percent of α- or β-cyclodextrin is administered into the rectum of each male SD strain rat weighing about 90 g (4 weeks of age, each group consisting of 10 individuals), and the discharge rate is examined with a suppository without cyclodextrin used as the control. The results, which are shown in Table 9, indicate that both the α- and β-cyclodextrin-containing suppositories does not differ in the discharge rate from the control suppositories. This means that the cyclodextrin-added suppositories are hardly irritatable to the rectal mucous membrane.

TABLE 9

	Base	Cyclodextrin addition level	Rate of discharge** after 15 min.	30 min.	45 min.
Control	Witepsol W—35	—	3/10	3/10	3/10
This invention	Witepsol W—35	5% (w/w) α-cyclodextrin	2/10	2/10	3/10
	Witepsol W—35	5% (w/w) β-cyclodextrin	1/10	3/10	3/10

** The number of animals which discharged the suppository/the number of animals tested

Experimental Example 10

Porcine insulin (in an amount corresponding to 50 IU/kg) and 5 mg of α-, β- or γ-cyclodextrin are dissolved in 0.1 ml of physiological saline, the solution is administered into the rat rectum in the manner in Experimental Example 7, and plasma glucose levels are determined at timed intervals. In the control group, porcine insulin is administered in the manner but without the addition of cyclodextrin.

The results, which are shown in Table 10, indicate that the addition of α, β or γ-cyclodextrin results in a greater hypoglycemic effect than in the control group. It is thus evident that insulin is absorbed more efficiency through the rectum.

TABLE 10

	Cyclodextrin addition level	Change (%) in plasma glucose level				
		Before administration	0.5 hr.	1 hr.	1.5 hr.	2 hr.
Control	—	100	102.2	86.0	95.2	121.9
This invention	5% (w/v) α-cyclodextrin	100	92.9	39.1	39.6	69.6
	5% (w/v) β-cyclodextrin	100	94.3	70.1	76.3	90.1
	5% (w/v) γ-cyclodextrin	100	94.8	79.0	81.3	93.0

Experimental Example 11

A Witepsol® W—35-based suppository (weight: about 45 mg) containing porcine insulin in an amount corresponding to 50 IU/kg and 5 w/w percent of α- or β-cyclodextrin is prepared in the coventional manner and administered to each male SD strain rat (body weight: about 300 g; 3 animals per group) after fasting at a site about 1.5 cm from the anus. Thereafter blood samples are taken from the tail vein and assayed for the blood sugar level. The results, which are shown in Table 11, indicate that the addition of α- or β-cyclodextrin results in an increased absorption of insulin.

TABLE 11

	Cyclodextrin addition level	Before administration	Change (%) in plasma glucose level 0.5 hr.	1 hr.	1.5 hr.	2 hr.	3 hr.
Control	—	100	98.1	94.1	97.4	98.1	102.5
This invention	5% (w/w) α-cyclodextrin	100	93.1	61.1	63.5	86.1	93.4
	5% (w/w) β-cyclodextrin	100	87.1	81.5	80.1	92.5	100.4

Experimental Example 12

Heparin sodium 600 U (corresponding to 3.7 mg) and 5 mg of α-cyclodextrin are dissolved in 0.1 ml of physiological saline and the solution is rectally administered to rats in the manner in Experimental Example 7. Blood samples are taken from the tail vein at timed intervals. A 0.27 ml portion of each blood sample is placed in a polyethylene microtube containing 0.03 ml of 3.8 w/v percent of sodium citrate, the tube contents are stirred well and then centrifuged, and the plasma portion is subjected to prothrombin time (blood coagulation time) determination using a thromboplastin reagent (Simplastin, Ono Pharmaceutical Co., Japan). In the control group, the same procedure is followed with α-cyclodextrin-free heparin sodium. The results, which are shown in Table 12, indicate that the addition of α-cyclodextrin results in a prolonged blood coagulation time as compared with the control group. An increased absorption of heparin is thus shown.

TABLE 12

	Cyclodextrin addition level	Before administration	Blood coagulation time (in seconds) 0.5 hr.	0.75 hr.	1 hr.	1.5 hr.	2 hr.
Control	—	13.6	14.3	13.7	14.7	15.3	14.7
This invention	5% (w/v) α-cyclodextrin	14.3	17.2	18.4	19.7	18.9	19.4

Experimental Example 13

An amount (corresponding to 50 mg/kg) of 5-FU and 5 mg of α-cyclodextrin are finely milled in a mortar and added to 0.1 ml of physiological saline. The mixture is subjected to ultrasonic treatment (27 KHz, 5 minutes). The thus-obtained suspension is rectally administered to rats in the manner in Experimental Example 7. Blood samples are taken from the tail vein at timed intervals and 5-FU plasma levels are determined by bioassay using *Micrococcus luteus* ATCC-10240 as the test organism. In the control group, the procedure is followed with α-cyclodextrin-free 5-FU. The results, which are shown in Table 13, indicate that the addition of α-cyclodextrin results in an increased absorption of 5-FU as compared with the control group.

TABLE 13

	Cyclodextrin addition level	Plasma level (μg/ml) 0.5 hr.	1 hr.	2 hr.	4 hr.	AUC (0—4 hr.)
Control	—	0.97	0.43	0.13	0.06	1.1
This invention	5% (w/v) α-cyclodextrin	1.37	1.72	1.05	0.49	4.0

Experimental Example 14

An amount (corresponding to 12 mg/kg) of gentamycin sulfate and 50 mg of α-cyclodextrin are added to 1 ml of physiological saline and the mixture is administered into the rectum of a male rabbit weighing about 2.5 kg in the manner in Experimental Example 7. Blood sampling is performed from the auricular vein at timed intervals and the plasma gentamycin level is determined by bioassay using *Bacillus subtilis* PCI 219 as the test organism. In the control study, the same procedure is followed with α-cylcodextrin-free gentamycin sulfate. The results are shown in Table 14. It is indicated that the addition of α-cyclodextrin results in an increased plasma level and in an increased absorption.

TABLE 14

	Cyclodextrin addition level	Plasma level (μg/ml) 0.5 hr.	1 hr.	2 hr.	4 hr.
Control	—	0.2	0.5	0.4	0.2
This invention	5% (w/v) α-cyclodextrin	0.7	2.5	1.7	1.2

Experimental Example 15

A Witepsol® W-35-based suppository (total weight: 150 mg) containing an amount (corresponding to 50 mg/kg) of sodium cefazolin and 10 w/w percent of α-cyclodextrin is prepared by a conventional method and administered to each male SD strain rat (body weight: about 400 g; 3 animals per group) after fasting at a site about 1.5 cm from the anus. Thereafter, blood sampling from the tail vein is performed at timed intervals and the plasma cefazolin level is determined by bioassay using *Bacillus subtilis* PCI-219 as the test organism. As the control study, the same experiment is conducted with α-cyclodextrin-free sodium cefazolin. The results are shown in Table 15, from which it can be seen that the addition of α-cyclodextrin results in an increased plasma level, hence in an increased absorption, as compared with the control group.

TABLE 15

	Cyclodextrin addition level	Plasma level (μg/ml) 0.5 hr.	1 hr.	2 hr.	3 hr.	4 hr.	AUC (0—4 hr.)
Control	—	1.76	2.21	3.00	3.49	2.54	10.32
This invention	10% (w/w) α-cyclodextrin	3.84	5.32	7.37	6.26	5.32	22.20

Example 1

In 8 ml of an isotonic phosphate buffer (pH 7.4) is dissolved 5000 U (about 200 mg) of porcine insulin. Then, 500 mg of α-cyclodextrin and 20 mg of chlorobutanol are added and thoroughly dissolved. The mixed solution is diluted with physiological saline to make 10 ml. This solution is put in a nasal spray applicator and administered at the dose level of 0.1 ml per dose.

Example 2

In 40 ml of purified water are dissolved 200 mg of DN-1417, 200 mg of mannitol and 200 mg of β-cyclodextrin and the solution is freeze-dried. The dry product is then pulverized to give a powder about 20 to 250 μm in diameter. A 30 mg portion of the powder is filled into No. 4 (U.S. Pharmacopoeia XX) hard gelatin capsules. To administer the drug, this capsule is set on an exclusive dust applicator equipped with needles for piercing holes in the capsule and a rubber balloon for sending air into the capsule. Both ends of the capsule are pierced and the rubber balloon is compressed to dispense the powdery contents into the nasal cavity via the top hole.

Example 3

In 16 ml of an isotonic buffer solution (pH 7.4) containing 0.12% of methylparaben and 0.01% of propylparaben are dissolved 1 g of α-cyclodextrin and 2 g of TAP-144, followed by addition of 200 mg of methyl-cellulose (Metolose 90SH, 4000, Shin-Etsu Chemical Co., Ltd., Japan). The mixture is stirred well to give a homogeneous viscous liquid. This liquid is diluted with the buffer solution to make a total of 20 g. This liquid is put in a nasal spray applicator and administered into the nasal cavity.

Example 4

In 16 ml of an isotonic buffer solution (pH 7.4) containing 0.03% of p-chloro-m-xylenol is dissolved 1 g of α-cyclodextrin and 2 g of TAP-144, followed by addition of 200 mg of methylcellulose (Metolose 90SH 400, Shin-Etsu Chemical Co., Ltd., Japan). The mixture is stirred well to give a homogeneous viscous liquid. This liquid is diluted with the buffer solution to make a total of 20 g. This liquid is put in a nasal spray applicator and administered into the nasal cavity.

Example 5

500 mg of natural LH—RH [the peptide of general formula (II) wherein R_1 = His, R_2 = Tyr, R_3 = Gly, R_4 = Leu, R_5 = Gly—NH_2] and 1 g of α-cyclodextrin are taken in a mortar, in which they are mixed with a hot melt of 1 g lanolin. Then, Miglyol®812 [Dynamite Nobel] is gradually added under stirring to make 10 g of an oil suspension. This suspension is put in an applicator, equipped with a dropping pipet and directly administered into the nasal cavity at the dose level of 0.1 g/dose.

Example 6

In 1 ml of physiological saline are dissolved 50 mg of α-cyclodextrin and 100000 U of α-interferon (human leukocyte interferon). This solution is put in a nasal applicator with a dropping pipet and administered into the nasal cavity at the level of 0.1 ml dose.

Example 7

In 10 ml of physiological saline are dissolved 2 mg of desmopressin and 1 g of γ-cyclodextrin followed by addition of 100 mg of methylcellulose to give a viscous liquid. A 0.2 ml portion of this liquid is taken in an applicator and administered directly into the nasal cavity.

Example 8

In physiological saline are dissolved 1 g of enkepharin and 3 g of α-cyclodextrin. This solution is put in a spray applicator and administered at the level of 0.2 ml per dose into the nasal cavity.

Example 9

To 90 ml of warm water (about 60° to 80°C), there is added 10 g of methylcellulose (Metolose 90SH 4000, Shin-Etsu Chemical Co.) and dispersed therein by adequate stirring. Then, 200 mg of TAP-144 and 10 g of α-cyclodextrin are together dissolved therein, 100 ml of cooled (about 4° to 10°C) aqueous solution is

added, the mixture is stirred well at room temperature until a homogeneous gel is obtained. The total quantity is adjusted to 200 g by addition of distilled water. The gel is defoamed by centrifugation and distributed into tubes, which are then sealed. A vaginal dosage form containing a single dose of 1 mg of TAP-144 is prepared by placing 1 g of this gel in an applicator.

Example 10

Oxytocin (20,000 units) and 10 g of α-cyclodextrin are dissolved in an aqueous solution preliminarily prepared by dissolving 5.0 ml of acetic acid and 2.15 g of sodium acetate trihydrate in one liter of water and having a pH of 3.5 to 4.5, to make 200 ml of a solution. A pharmaceutical preparation for vaginal administration which contains 10 units of oxytocin per 0.1 ml (single dose) is prepared by filling a nozzle device-equipped applicator with the above solution.

Example 11

In 200 ml of water, there are dissolved and dispersed 20 g of lactose and 20,000 units (about 800 mg) of porcine insulin, followed by lyophilization. Thereafter, the lyophilizate is ground and stirred well. To a 10.4 g portion of the lyophilizate, there is added a new 61.35 g portion of lactose, and the mixture is stirred well. Further, 10 g of α-cyclodextrin and 10 g of corn starch are added. After adequate mixing, 20 ml of a preliminarily prepared 10% hydroxypropylcellulose (HPC-L) in ethanol solution is added, the mixture is kneaded and granulated by sieving, and the granules are dried at room temperature under reduced pressure for 16 hours. To the granules, there are added 5 g of corn starch and 1.25 g of magnesium stearate. After adequate mixing, each 1 g portion of the mixture is compressed into a tablet. In this way, tablets for vaginal administration each containing 100 units of insulin are produced.

Example 12

To a mixture of 125 mg of an LH—RH analog which is a polypeptide having the formula (Pyr)Glu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-$NHCH_2$—CH_3 [Biochemical and Biophysical Research Communications, vol. 60, No. 1, pages 406—413 (1974)] and 5 g of α-cyclodextrin, there is added 5 g of lanolin preliminarily melted by warming. After sufficient milling and mixing, 89.9 g of an oleaginous base (Witepsol) melted in advance at 50°C was added portionwise with stirring. After adequate homogenization, a plastic container for making a vaginal suppository is filled with 0.8 g of the mixture and the whole is cooled to give a pharmaceutical preparation form for vaginal administration which contains 1 mg of the LH—RH analog per container.

Example 13

α-Cyclodextrin (1 g) and 50,000,000 units of α-interferon (human leukocyte-derived interferon) are dissolved in a 0.2% aqueous carboxymethylcellulose solution to make 10 ml. A pharmaceutical preparation for vaginal administration containing 1,000,000 units of α-interferon per 0.2 ml thereof, which is a single dose, is produced by filling a nozzle device-equipped spray with the solution.

Example 14

α-Cyclodextrin (0.5 g), thyroid hormone-releasing hormone (TRH) tartrate (141.4 mg; 100 mg as TRH) and glycerin (180 ml) are dissolved in distilled water to make 10 ml. A paper tampon (φ 10 mm × 25 mm) fixed on a plastic inserter is soaked with 1 ml of the solution to give a pharmaceutical preparation for vaginal administration containing 10 mg of TRH.

Example 15

α-Cyclodextrin (1 g), 50,000,000 units of γ-interferon and 400 g of human serum albumin are dissolved in 10 ml of distilled water. Glass bottles are each filled with 2 ml of the solution and the contents are lyophilized. Immediately before use, the lyophilizate is dissolved in 2 ml of a diluent of distilled water and the bottle is mounted on the adapter of a nozzle device-equipped spray to give a pharmaceutical preparation for vaginal administration containing 1,000,000 units of γ-interferon per 0.2 ml thereof (single dose).

Example 16

Witepsol® W-35 (9.316 g, Dynamite Nobel), a base, is weighed, placed in a mortar and melted by warming at 40—45°C, and 500 mg of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. then, 183.6 mg of DN-1417 citrate (corresponding to 120 mg of DN-1417) is added. The resultant mixture is stirred well and poured into a 1 g suppository mold and cooled gradually to give ten 1 g rectal suppositories.

Example 17

In a mortar, there is placed 9.316 g of a mixed base composed of 75 w/w percent of polyethylene glycol (PEG) 1000 and 25 w/w percent of PEG 4000. The base is melted with warming at 50—60°C. α- or β-cyclodextrin and DN-1417 citrate are added thereto and the mixture is treated in the manner in Example 16 to give ten 1 g rectal suppositories.

Example 18

To 50 ml of an aqueous solution containing 0.12% of methyl paraben and 0.01% (w/w) of propyl paraben preliminarily dissolved therein by heating to 80—90°C (hereinafter, such solution is referred to as solution A), there is added 5 g of methylcellulose (Metolose 90SH 4000, Shin-Etsu Chemical Co.), and the mixture is stirred to prepare a dispersion. Thereto is added 38 ml of solution A containing 1.414 g of TRH tartrate (corresponding to 1 g of TRH) and 5 g of α-cyclodextrin dissolved therein. The resultant mixture is cooled to 4 to 10°C and stirred well to give a homogeneous gel. After adjusting the total amount to 100 g, 1 g portion each of the gel is poured into applicators for rectal administration. There are thus produced gel suppositories for rectal administration.

Example 19

To 50 ml of an aqueous solution containing 0.03% of p-chloro-m-xylenol (hereinafter, such solution is referred to as solution A), there is added 5 g of methylcellulose (Metolose 90SH 4000, Shin-Etsu Chemical Co.), and the mixture is stirred to prepare a dispersion. Thereto is added 38 ml of solution A containing 1.414 g of TRH tartrate (corresponding to 1 g of TRH) and 5 g of α-cyclodextrin dissolved therein. The resultant mixture is cooled to 4 to 10°C and stirred well to give a homogeneous gel. After adjusting the total amount to 100 g, 1 g portion each of the gel is poured into applicators for rectal administration. There are thus produced gel suppositories for rectal administration.

Example 20

Witepsol® W-35 (a base, 9.388 g) is weighed, placed in a mortar and melted by warming at 40—45°C, and 500 mg of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. Then, 112.4 mg of TAP-144 acetate (corresponding to 100 mg of TAP-144) is added. The resultant mixture is stirred well, poured into a 1 g suppository mold and cooled gradually to give ten 1 g rectal suppositories.

Example 21

Porcine insulin (500 U, about 20 mg) is dissolved in 8 ml of an isotonic phosphate buffer (pH 7.4), and further 500 mg of α-, β- or γ-cyclodextrin and 20 mg of chlorobutanol are added. The mixture is stirred well and made 10 ml by addition of physiological saline, and 1 ml portions of the resulting solution are distributed into inserter for rectal administration to give liquid dosage units for rectal administration.

Example 22

Witepsol® W-35 (a base, 9.25 g) is weighed, placed in a mortar and melted by warming at 40 to 45°C, and 500 mg of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. Then, 250 mg of enkephalin is added, and the resultant mixture is stirred well, poured into a 1 g suppository mold and cooled gradually to give ten 1 g rectal suppositories.

Example 23

In a mortar, 3 g of lanolin is melted with warming, 616 mg (100,000 U) of sodium heparin and 1 g of α-cyclodextrin are added thereto, the mixture is mixed well for homogenization, and Myglyol®812 (Dynamite Nobel) is added gradually with stirring to make the whole weight 10 g. No. 0 (U.S. Pharmacopoeia XX) hard capsules are filled with 500 mg each portions of the resultant oleaginous suspension to give 20 rectal capsules.

Example 24

Witepsol® W-35 (a base, 15.5 g) is weighed, placed in a mortar and melted by warming at 40 to 45°C, and 2 g of α- or β-cyclodextrin is added thereto. The mixture is warmed and stirred. Then, 2.5 g of citicoline is added. The resultant mixture is stirred well, poured into a 2 g suppository mold and cooled gradually to give ten 2 g rectal suppositories.

Example 25

In a mortar, 3 g of lanolin is melted with warming, 2 g of finely pulverized crystalline 5-FU and 1 g of α-cyclodextrin are added, the mixture is mixed well for homogenization, and Miglyol®812 (Dynamite Nobel) is added gradually with stirring to make the whole weight 10 g. No. 0 hard capsules are filled with 500 mg portions of the resultant oleaginous suspension to give 20 rectal capsules each containing 100 mg of 5-FU.

Example 26

Witepsol®W-35 (a base, 7 g) is weighed, placed in a mortar and melted by warming at 40 to 45°C, and 1.0 g of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. Then, 12 g of kanamycin sulfate [corresponding to 10 g (potency) of kanamycin] is added. The resultant mixture is stirred well, poured into a 2 g suppository mold and cooled gradually to give ten rectal suppositories.

Example 27

Witepsol®W-35 (a base, 7.885 g) is weighed, placed in a mortar and melted by warming at 40 to 45°C, and 1.000 g of α- or β-cyclodextrin is added thereto. The mixture is stirred with warming. then, 11.115 g of

sodium sulbenicillin [corresponding to 10 g (potency) of sulbenicillin] is added. The resultant mixture is stirred well, poured into a 2 g suppository mold and cooled gradually to give ten 2 g rectal suppositories.

Example 28

Witepsol® H-15 (61.5 g) is melted by warming, and 10 g of α-cyclodextrin and 28.5 g of finely pulverized cefotiam hydrochloride [corresponding to 25 g (potency) of cefotiam] are added thereto. After homogenization, the mixture is poured into a 2 g suppository mold and cooled gradually to give fifty 2 g rectal suppositories.

Claims

1. A pharmaceutical composition in a form suitable for administration through mucous membranes, characterised by (i) a hydrophilic drug which is poorly absorbable through the gastrointestinal tract, the bioavailability of the drug being not more than 70% and the n-octanol/water partition coefficient of the drug being not more than 10; and (ii) cyclodextrin, tri-O-methylcyclodextrin or triaminocyclodextrin.

2. A pharmaceutical composition as claimed in Claim 1, wherein the composition is formed into a preparation suitable for nasal, vaginal or rectal administration.

3. A pharmaceutical composition as claimed in Claim 1, wherein the cyclodextrin is α-cyclodextrin.

4. A pharmaceutical composition as claimed in Claim 1, wherein the hydrophilic drug is a physiologically active polypeptide.

5. A pharmaceutical composition as claimed in Claim 1, wherein the hydrophilic drug is selected from polysaccharides, aminoglycoside antibiotics, beta-lactam antibiotics and nucleic acid drugs.

6. A pharmaceutical composition as claimed in Claim 4, wherein the composition is formed into a preparation suitable for nasal administration.

7. A pharmaceutical composition as claimed in Claim 4, wherein the composition is formed into a preparation suitable for vaginal administration.

8. A pharmaceutical composition as claimed in claim 4, wherein the composition is formed into a preparation suitable for rectal administration.

9. A pharmaceutical composition as claimed in Claim 5, wherein the composition is formed into a preparation suitable for nasal administration.

10. A pharmaceutical composition as claimed in Claim 5, wherein the composition is formed into a preparation suitable for vaginal administration.

11. A pharmaceutical composition as claimed in Claim 5, wherein the composition is formed into a preparation suitable for rectal administration.

Patentansprüche

1. Pharmazeutische Zusammensetzung in einer für die Verabreichung durch die Schleimhäute geeigneten Form, gekennzeichnet durch (i) ein hydrophiles Medikament, das durch den Magen-Darm-Trakt schlecht absorbiert wird, wobei die Bioverfügbarkeit des Medikaments nicht mehr als 70% beträgt und der n-Octanol/Wasser-Verteilungskoeffizient des Medikaments nicht größer als 10 ist, und (ii) Cyclodextrin, Tri-O-methylcyclodextrin oder Triaminocyclodextrin.

2. Pharmazeutische Zusammensetzung nach Anspruch 1, dadurch gekennzeichnet, daß die Zusammensetzung zu einem für die nasale, vaginale oder rektale Verabreichung geeigneten Präparat formuliert wird.

3. Pharmazeutische Zusammensetzung nach Anspruch 1, dadurch gekennzeichnet, daß das Cyclodextrin α-Cyclodextrin ist.

4. Pharmazeutische Zusammensetzung nach Anspruch 1, dadurch gekennzeichnet, daß das hydrophile Medikament ein physiologisch aktives Polypeptid ist.

5. Pharmazeutische Zusammensetzung nach Anspruch 1, dadurch gekennzeichnet, daß das hydrophile Medikament aus Polysacchariden, Aminoglycosid-Antibiotika, Beta-Lactam-Antibiotika und Nucleinsäure-Medikamenten ausgewählt ist.

6. Pharmazeutische Zusammensetzung nach Anspruch 4, dadurch gekennzeichnet, daß die Zusammensetzung zu einem für die nasale Verabreichung geeigneten Präparat formuliert wird.

7. Pharmazeutische Zusammensetzung nach Anspruch 4, dadurch gekennzeichnet, daß die Zusammensetzung zu einem für die vaginale Verabreichung geeigneten Präparat formuliert wird.

8. Pharmazeutische Zusammensetzung nach Anspruch 4, dadurch gekennzeichnet, daß die Zusammensetzung zu einem für die rektale Verabreichung geeigneten Präparat formuliert wird.

9. Pharmazeutische Zusammensetzung nach Anspruch 5, dadurch gekennzeichnet, daß die Zusammensetzung zu einem für die nasale Verabreichung geeigneten Präparat formuliert wird.

10. Pharmazeutische Zusammensetzung nach Anspruch 5, dadurch gekennzeichnet, daß die Zusammensetzung zu einem für die vaginale Verabreichung geeigneten Präparat formuliert wird.

11. Pharmazeutische Zusammensetzung nach Anspruch 5, dadurch gekennzeichnet, daß die Zusammensetzung zu einem für die rektale Verabreichung geeigneten Präparat formuliert wird.

Revendications

1. Composition pharmaceutique sous une forme adaptée à l'administration à travers les muqueuses, caractérisée par (i) un médicament hydrophile qui est faiblement absorbable par le tractus gastro-intestinal, la biodisponibilité du médicament n'étant pas supérieure à 70% et le coefficient de répartition du médicament dans le n-octanol/eau n'étant pas supérieur à 10; et (ii) la cyclodextrine, la tri-O-méthylcyclodextrine ou la triaminocyclodextrine.

2. Composition pharmaceutique selon la revendication 1, dans laquelle la composition est présentée sous une forme adaptée à l'administration par voie nasale, vaginale ou rectale.

3. Composition pharmaceutique selon la revendication 1, dans laquelle la cyclodextrine est l'α-cyclodextrine.

4. Composition pharmaceutique selon la revendication 1, dans laquelle le médicament hydrophile est un polypeptide doué d'activité physiologique.

5. Composition pharmaceutique selon la revendication 1, dans laquelle le médicament hydrophile est choisi parmi les polysaccharides, les antibiotiques aminoglycosides, les antibiotiques béta-lactamiques et les médicaments acides nucléiques.

6. Composition pharmaceutique selon la revendication 4, dans laquelle la composition est présentée sous une forme adaptée à l'administration par voie nasale.

7. Composition pharmaceutique selon la revendication 4, dans laquelle la composition est présentée sous une forme adaptée à l'administration par voie vaginale.

8. Composition pharmaceutique selon la revendication 4, dans laquelle la composition est présentée sous une forme adaptée à l'administration par voie rectale.

9. Composition pharmaceutique selon la revendication 5, dans laquelle la composition est présentée sous une forme adaptée à l'administration par voie nasale.

10. Composition pharmaceutique selon la revendication 5, dans laquelle la composition est présentée sous une forme adaptée à l'administration par voie vaginale.

11. Composition pharmaceutique selon la revendication 5, dans laquelle la composition est présentée sous une forme adaptée à l'administration par voie rectale.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
10/551,205	11/14/2006	1614	4530	033935-021	78	6

CONFIRMATION NO. 4092

21839
BUCHANAN, INGERSOLL & ROONEY PC
POST OFFICE BOX 1404
ALEXANDRIA, VA22313-1404

FILING RECEIPT

Date Mailed: 04/24/2007

Receipt is acknowledged of this regular Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please mail to the Commissioner for Patents P.O. Box 1450 Alexandria Va 22313-1450. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Nicholas S. Bodor, Bal Harbour, FL;
Yogesh Dandiker, Toronto, CANADA;

Assignment For Published Patent Application

ARES TRADING S.A., Aubonne, SWITZERLAND

Power of Attorney: The patent practitioners associated with Customer Number 21839

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/US04/09387 03/26/2004
which claims benefit of 60/458,922 03/28/2003
and claims benefit of 60/484,756 07/02/2003
and claims benefit of 60/541,247 02/04/2004

Foreign Applications

If Required, Foreign Filing License Granted: 04/21/2007

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US10/551,205**

Projected Publication Date: 08/02/2007

Non-Publication Request: No

Early Publication Request: No

Title

Oral formulations of cladribine

Preliminary Class

514

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Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
10/551,205	Nicholas S. Bodor	033935-021

INTERNATIONAL APPLICATION NO.	
PCT/US04/09387	
I.A. FILING DATE	PRIORITY DATE
03/26/2004	03/28/2003

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CONFIRMATION NO. 4092

371 ACCEPTANCE LETTER

OC000000023497427

Date Mailed: 04/24/2007

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as a Designated / Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above and the relevant dates are:

11/14/2006	11/14/2006
DATE OF RECEIPT OF 35 U.S.C. 371(c)(1), (c)(2) and (c)(4) REQUIREMENTS	DATE OF COMPLETION OF ALL 35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE " FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1), (c)(2) and (c)(4) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

The following items have been received:

- Copy of the International Application filed on 09/28/2005
- Copy of the International Search Report filed on 09/28/2005
- Preliminary Amendments filed on 09/28/2005
- Information Disclosure Statements filed on 11/14/2006
- Oath or Declaration filed on 11/14/2006
- Request for Immediate Examination filed on 09/28/2005
- U.S. Basic National Fees filed on 09/28/2005
- Assignment filed on 09/29/2006
- Priority Documents filed on 09/28/2005

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

DIANE L SMITH
Telephone: (703) 308-9290 EXT 121

PART 3 - OFFICE COPY

FORM PCT/DO/EO/903 (371 Acceptance Notice)

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Nicholas S. Bodor et al.	Group Art Unit: 1614
Application No.: 10/551,205	Examiner:
Filing Date: November 14, 2006	Confirmation No.: 4092
Title: ORAL FORMULATIONS OF CLADRIBINE	

SECOND
INFORMATION DISCLOSURE STATEMENT
TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Enclosed is a Second Information Disclosure Statement (IDS) and accompanying form PTO-1449 for the above-identified patent application.

☒ No additional fee for submission of an IDS is required.

☐ The fee of $ 180 as set forth in 37 C.F.R. § 1.17(p) is also enclosed.

☐ A statement under 37 C.F.R. § 1.97(e) is also enclosed.

☐ A statement under 37 C.F.R. § 1.97(e), and the fee of $ 180 as set forth in 37 C.F.R. § 1.17(p) are also enclosed.

☐ Charge _____ to Deposit Account No. 02-4800 for the fee due.

☐ A check in the amount of _____ is enclosed for the fee due.

☐ Charge _____ to credit card for the fee due. Form PTO-2038 is attached.

☒ The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in duplicate.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date August 10, 2007

By: Mary Katherine Baumeister
Mary Katherine Baumeister
Registration No. 26254

P.O. Box 1404
Alexandria, VA 22313-1404
703 836 6620

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AUG 10 2007
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Nicholas S. Bodor et al.	Group Art Unit: 1614
Application No.: 10/551,205	Examiner:
Filed: November 14, 2006	Confirmation No.: 4092
For: ORAL FORMULATIONS OF CLADRIBINE	

SECOND INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the duty of disclosure as set forth in 37 C.F.R. § 1.56, the accompanying information is being submitted in accordance with 37 C.F.R. §§ 1.97 and 1.98.

Applicants request the Examiner's consideration of the enclosed publication cited during prosecution of the corresponding Chinese application in an Official Action dated May 11, 2007. The publication is listed on the accompanying Form PTO-1449. The reference is in Chinese; therefore, the cited section (page 105, lines 25-29) is accompanied by an English translation thereof. This reference was cited as relevant only to some of the process and product-by-process claims.

This statement, Form PTO-1449 and enclosed citation and translation are believed to be filed prior to an action on the merits. In addition, the undersigned hereby states under 37 C.F.R. § 1.97(e) that each item of information contained in this information disclosure statement was first cited in any communication from a patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement. Therefore, no fee is required to obtain consideration under 37 C.F.R. §1.97(b) or (c), whichever is applicable.

It is respectfully requested that the Examiner returned an initialed copy of applicants' enclosed Form PTO-1449 with the next official communication or with the first Action on the merits.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date: August 10, 2007

By: Mary Katherine Baumeister
Mary Katherine Baumeister
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Substitute for form 1449/PTO & 1449B/PTO

SECOND INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 1

Complete if Known

Application Number	10/551,205
Filing Date	November 14, 2006
First Named Inventor	Nicholas S. Bodor et al.
Examiner Name	
Attorney Docket No.	0056192-000024

OIPE IAP4 AUG 10 2007 PATENT & TRADEMARK OFFICE

U.S. PATENT DOCUMENTS

Examiner Initials	Document Number	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Issue/Publication Date (MM-DD-YYYY)

FOREIGN PATENT DOCUMENTS

Examiner Initials	Document Number	Kind Code (if known)	Country	Date of Publication (MM-DD-YYYY)	STATUS						
					Translation	Partial Translation	Eng. Lang. Summary	Search Report	IPER	Abstract	Cited in Spec

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.
	Gao, Shen, *New Dosage Form and New Technology of Modern Drugs*, first edition, January 2002, Chapter 6, Section 3 (III) Procedures, page 105, lines 25-29 (published by People's Military Medical Publisher), and English translation thereof

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with M.P.E.P. § 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/551,205	11/14/2006	Nicholas S. Bodor	0056192-000024

CONFIRMATION NO. 4092

21839
BUCHANAN, INGERSOLL & ROONEY PC
POST OFFICE BOX 1404
ALEXANDRIA, VA22313-1404

Title: Oral formulations of cladribine

Publication No. US-2007-0197468-A1
Publication Date: 08/23/2007

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Pre-Grant Publication Division, 703-605-4283

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
10/551,205	11/14/2006	

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)	SMALL ENTITY ☐ RATE ($)	SMALL ENTITY ☐ FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
FOR	NUMBER FILED	NUMBER EXTRA					
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	09/28/2007	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 78	Minus	** 78	= 0	X $ =		OR	X $50=	0
	Independent (37 CFR 1.16(h))	* 6	Minus	***6	= 0	X $ =		OR	X $200=	0
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
DONNA D. SMALLS-LOGAN

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

OIPE IAP97
NOV 08 2007
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	**MAIL STOP AMENDMENT**
Nicholas S. Bodor et al.	Group Art Unit: 1614
Application No.: 10/551,205	Examiner:
Filing Date: November 14, 2006	Confirmation No.: 4092
Title: ORAL FORMULATIONS OF CLADRIBINE	

THIRD INFORMATION DISCLOSURE STATEMENT TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Enclosed is a Third Information Disclosure Statement (IDS) and accompanying form PTO-1449 for the above-identified patent application.

☒ No additional fee for submission of an IDS is required.

☐ The fee of $ 180 as set forth in 37 C.F.R. § 1.17(p) is also enclosed.

☐ A statement under 37 C.F.R. § 1.97(e) is also enclosed.

☐ A statement under 37 C.F.R. § 1.97(e), and the fee of $ 180 as set forth in 37 C.F.R. § 1.17(p) are also enclosed.

☐ Charge _____ to Deposit Account No. 02-4800 for the fee due.

☐ A check in the amount of _____ is enclosed for the fee due.

☐ Charge _____ to credit card for the fee due. Form PTO-2038 is attached.

☒ The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in duplicate.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date November 8, 2007

By: Mary Katherine Baumeister
Mary Katherine Baumeister
Registration No. 26254

P.O. Box 1404
Alexandria, VA 22313-1404
703 836 6620

OIPE IAP97
NOV 08 2007
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	**MAIL STOP AMENDMENT**
Nicholas S. Bodor et al.	Group Art Unit: 1614
Application No.: 10/551,205	Examiner:
Filed: November 14, 2006	Confirmation No.: 4092
For: ORAL FORMULATIONS OF CLADRIBINE	

THIRD INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the duty of disclosure as set forth in 37 C.F.R. § 1.56, the accompanying information is being submitted in accordance with 37 C.F.R. §§ 1.97 and 1.98. Applicants request the Examiner's consideration of the documents listed on the accompanying Form PTO-1449.

Pursuant to 37 C.F.R. § 1.98, a copy of each of the documents cited is enclosed.

The documents are being submitted within three (3) months of the filing or entry of the national stage of this application or before the first Office Action on the merits, whichever is later. Since these documents are being filed within the time period set forth in 37 C.F.R. § 1.97(b), no fee or statement is required.

It is respectfully requested that an Examiner-initialed copy of the accompanying Form PTO-1449 be returned to the undersigned.

The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in duplicate.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date: November 8, 2007

By: Mary Katherine Baumeister
Mary Katherine Baumeister
Registration No. 26254

P.O. Box 1404
Alexandria, VA 22313-1404
703 836 6620

Substitute for form 1449/PTO & 1449B/PTO

THIRD
INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(use as many sheets as necessary)

Sheet 1 of 2

Complete if Known

Application Number	10/551,205
Filing Date	November 14, 2006
First Named Inventor	Nicholas S. Bodor
Examiner Name	
Attorney Docket No.	0056192-000024

OIPE IAP97 NOV 08 2007 PATENT & TRADEMARK OFFICE

U.S. PATENT DOCUMENTS

Examiner Initials	Document Number-Kind Code	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines Where Relevant Passages or Figures Appear
	US-			
	US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials	Foreign Patent Document: Country Code[1], Number, Kind Code	Publication Date (MM-DD-YYYY)	Name of Patentee or Applicant of Cited Document	STATUS: Translation	Partial Translation	Eng. Lang. Summary	Search Report	IPER	Abstract	Cited in Spec. / Pg. No(s).	

[1]Enter Office that issued the document, by the two-letter code.

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.
	Albertioni et al., "On the bioavailability of 2-chloro-2'-deoxyadenosine (CdA)", Eur J Clin Pharmacol., Vol. 44, pp. 579-582, 1993, Springer-Verlag, Germany
	Ahn et al., "Chiral Recognition in Gas-Phase Cyclodextrin: Amino Acid Complexes-Is the Three Point Interaction Still Valid in the Gas Phase?", J Am Soc Mass Spectrom, Vol. 12, pp. 278-287, 2001, Elsevier Science, Inc., US
	Bakthiar et al., "A study of the complexation between dimethyl-β-cyclodextrin and steroid hormones using electrospray ionization mass spectrometry", Rapid Communications in Mass Spectrometry, Vol. 11, pp. 1478-1481, 1997, John Wiley And Sons Ltd, England
	Beutler et al., "The treatment of chronic progressive multiple sclerosis with cladribine", Proc. Natl. Acad. Sci. USA, Medical Sciences, Vol. 93, pp. 1716-1720, 1996, National Academy of Sciences, US
	Cheng et al., "Measurement of chiral complexes of cyclodextrin and amino acids by electrospray ionization time-of-flight mass spectrometry", J. Mass Spectrom, Vol. 36, pp. 834-836, 2001, John Wiley & Sons, Ltd., England
	Choi et al., "FT-Raman and FT-IR Spectra of the Non-steroidal Anti-inflammatory Drug Ketoprofen Included in Cyclodextrins", Analytical Sciences, Vol. 17 Supplement, pp. i785-i788, 2001, The Japan Society for Analytical Chemistry, Japan
	Giordano et al., "Thermal analysis of cyclodextrins and their inclusion compounds", Thermochimica Acta 380, pp. 123-151, 2001, Elsevier Science B.V., The Netherlands
	Hwang et al., "Water Suppression That Works. Excitation Sculpting Using Arbitrary Waveforms and Pulsed Field Gradients", Journal of Magnetic Resonance, Series A, Vol. 112, pp. 275-279, 1995, Academic Press, Inc., US
	Lamcharfi et al., "Electrospray Ionization Mass Spectrometry in Supramolecular Chemistry: Characterization of Non-covalent Cyclodextrin Complexes", Journal of Mass Spectrometry, Vol. 31, pp. 982-986, 1996, John Wiley & Sons, Ltd., England
	Loftsson et al., "Pharmaceutical Applications of Cyclodextrin. 1. Drug Solubilization and Stabilization", Journal of Pharmaceutical Sciences, Vol. 85, No. 10, pp. 1017-1025, 1996, American Pharmaceutical Association and the American Chemical Society, US
	Meier et al., "The Influence of β- and γ-Cyclodextrin Cavity Size on the Association Constant with Decanoate and Octanoate Anions", Journal of Inclusion Phenomena and Macrocyclic Chemistry, Vol. 40, pp. 291-295, 2001, Kluwer Academic Publishers, The Netherlands
	Mura et al., "Interactions of ketoprofen and ibuprofen with β-cyclodextrins in solution and in the solid state", International Journal of Pharmaceutics, Vol. 166, pp. 189-203, 1998, Elsevier Science B.V., The Netherlands

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with M.P.E.P. § 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

Substitute for form 1449/PTO & 1449B/PTO

THIRD
INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(use as many sheets as necessary)

Sheet 2 of 2

Complete if Known

Application Number	10/551,205
Filing Date	November 14, 2006
First Named Inventor	Nicholas S. Bodor
Examiner Name	
Attorney Docket No.	0056192-000024

U.S. PTO
IAP91
NOV 08 2007
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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.
	Nolan et al., "Preparation of Vesicles and Nanoparticles of Amphiphilic Cyclodextrins Containing Labile Disulfide Bonds", Langmuir, Vol. 19, pp. 4469-4472, 2003, American Chemical Society, US
	Ramanathan et al., "Electrospray Ionization Mass Spectrometric Study of Encapsulation of Amino Acids by Cyclodextrins", J. Am Soc Mass Spectrom, Vol. 6, pp. 866-871, 1995, American Society for Mass Spectrometry, US
	Redenti et al., "Raman and Solid State ^{13}C-NMR Investigation of the Structure of the 1 : 1 Amorphous Piroxicam : β-Cyclodextrin Inclusion Compound", Biospectroscopy, Vol. 5, pp. 243-251, 1999, John Wiley & Sons, Inc., US
	Sipe et al., "Cladribine in treatment of chronic progressive multiple sclerosis", The Lancet, Vol. 344, pp. 9-13, 1994, Lancet Publishing Group, England
	Szejtli, "Introduction and General Overview of Cyclodextrin Chemistry", Chem. Rev., Vol. 98, pp. 1743-1753, 1998, American Chemical Society, US
	Uekama et al., "Cyclodextrin Drug Carrier Systems", Chem. Rev., Vol. 98, pp. 2045-2076, 1998, American Chemical Society, US
	Uekama et al., "Peracylated β-Cyclodextrins as Novel Sustained-release Carriers for a Water-soluble Drug, Molsidomine", J. Pharm. Pharmacol., Vol. 46, pp. 714-717, 1994, Pharmaceutical Press, England
	Taddei et al., "Influence of Environment on Piroxicam Polymorphism: Vibrational Spectroscopic Study", Biopolymers (Biospectroscopy), Vol. 62, pp. 68-78, 2001, John Wiley & Sons, Inc., US

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with M.P.E.P. § 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/551,205	11/14/2006	Nicholas S. Bodor	0056192-000024	4092

21839 7590 12/06/2007

BUCHANAN, INGERSOLL & ROONEY PC
POST OFFICE BOX 1404
ALEXANDRIA, VA 22313-1404

EXAMINER	
LAU, JONATHAN S	

ART UNIT	PAPER NUMBER
4173	

NOTIFICATION DATE	DELIVERY MODE
12/06/2007	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ADIPFDD@bipc.com
debra.hawkins@bipc.com

Office Action Summary	Application No.	Applicant(s)	
	10/551,205	BODOR ET AL.	
	Examiner	Art Unit	
	Jonathan S. Lau	4173	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *1* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-35 and 56-98* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-35 and 56-98* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Restriction Requirement

Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claim(s) 1-12, 56-66 and 82-98, drawn to a cladribine-cyclodextrin complex and pharmaceutical compositions thereof.

Group II, claim(s) 13-35, drawn to a method of enhancing oral bioavailability of cladribine or treating symptoms of a cladribine-responsive condition comprising administering a pharmaceutical composition comprising a cladribine-cyclodextrin complex.

Group III, claim(s) 67-81, drawn to a process of making a cladribine-cyclodextrin complex.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The common feature of the inventions of Groups I-III is a cladribine-cyclodextrin complex. However, a cladribine-cyclodextrin complex is a known product. See Schultz et al. (US Patent 6,194,395, provided by Applicant in IDS filed 14 Nov 2006) column 1, lines 4-10 and column 2, lines 10-14. The formation of an inclusion complex with cyclodextrin is known in the art to be a reversible process governed by an equilibrium; therefore a composition of cladribine and cyclodextrin inherently comprises both the inclusion complex of cladribine and cyclodextrin and the non-inclusion complex of cladribine associated with cyclodextrin. Therefore a cladribine-cyclodextrin complex is not the special technical feature of a single general inventive concept. The special technical feature of the invention of Group I is the specific chemical structure of the cyclodextrin in the cladribine-cyclodextrin complex. The special technical feature of the invention of Group II is the specific method of enhancing bioavailability or treating symptoms of a cladribine-responsive condition by administering a specifc cladribine-cyclodextrin complex. The special technical feature of the invention of Group III is the specific steps of the process of making a specifc cladribine-cyclodextrin complex.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or invention to be examined even though the requirement be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

The election of an invention or species may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse.

Should applicant traverse on the ground that the inventions or species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions or species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C.103(a) of the other invention.

The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and the product claims are subsequently found allowable, withdrawn process claims that depend from or otherwise require all the limitations of the allowable product claim will be considered for rejoinder. All claims directed to a nonelected process invention must require all the limitations of an allowable product claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product are found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product claim will not be rejoined. See MPEP § 821.04(b). Additionally, in order to retain the right to rejoinder in accordance with the above policy, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder**. Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jonathan S. Lau whose telephone number is 571-270-3531. The examiner can normally be reached on Monday - Thursday, 9 am - 4 pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisors, Ardin Marschel can be reached on 571-272-0718 or Cecilia Tsang can be reached on 571-272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

JSL

/Ardin Marschel/
Supervisory Patent Examiner, Art Unit 1614

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	10551205	BODOR ET AL.
	Examiner	Art Unit
	Jonathan S Lau	4173

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/26/2007								
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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	10551205	BODOR ET AL.
	Examiner	Art Unit
	Jonathan S Lau	4173

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
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☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/26/2007								
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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	10551205	BODOR ET AL.
	Examiner	Art Unit
	Jonathan S Lau	4173

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	11/26/2007								
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JAN 04 2008
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	**MAIL STOP AMENDMENT**
Nicholas S. Bodor et al.	)	Group Art Unit: 1614
Application No.: 10/551,205	)	Examiner: Jonathan S. LAU
Filed: November 14, 2006	)	Confirmation No.: 4092
For: ORAL FORMULATIONS OF CLADRIBINE	)	

FOURTH INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the duty of disclosure as set forth in 37 C.F.R. § 1.56, the accompanying information is being submitted in accordance with 37 C.F.R. §§ 1.97 and 1.98. Applicants request the Examiner's consideration of the document listed on the accompanying Form PTO-1449.

Pursuant to 37 C.F.R. § 1.98, a copy of the document listed is enclosed.

The document is being submitted within three (3) months of the filing or entry of the national stage of this application or before the first Office Action on the merits, whichever is later. Since this document is being filed within the time period set forth in 37 C.F.R. § 1.97(b), no fee or statement is required.

It is respectfully requested that an Examiner-initialed copy of the accompanying Form PTO-1449 be returned to the undersigned.

The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in duplicate.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date: January 4, 2008

By: Mary Katherine Baumeister
Mary Katherine Baumeister
Registration No. 26254

P.O. Box 1404
Alexandria, VA 22313-1404
703 836 6620

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re Patent Application of	**MAIL STOP AMENDMENT**
Nicholas S. Bodor et al.	Group Art Unit: 4173
Application No.: 10/551,205	Examiner: JONATHAN S LAU
Filed: November 14, 2006	Confirmation No.: 4092
For: ORAL FORMULATIONS OF CLADRIBINE	

REPLY AND AMENDMENT IN RESPONSE TO RESTRICTION REQUIREMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Office Action dated December 6, 2007, please first amend the above-identified patent application as follows:

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (Original) A pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

2. (Original) The pharmaceutical composition according to Claim 1, wherein the complex is saturated with cladribine.

3. (Previously Presented) The composition according to Claim 1, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

4. (Previously Presented) The composition according to Claim 1, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

5. (Previously Presented) The composition according to Claim 1, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

6. (Currently Amended) The composition according to Claim 1, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

7. (Original) The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

8. (Original) The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

9. (Original) The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

10. (Original) The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

11. (Previously Presented) The composition according to Claim 1, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

12. (Previously Presented) The composition according to Claim 1, wherein from about 30 to about 40 percent by weight of the cladribine is in the

inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

13. (Original) A method for enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

14. (Original) The method according to Claim 13, wherein the complex is saturated with cladribine.

15. (Previously Presented) The method according to Claim 13, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

16. (Previously Presented) The method according to Claim 13, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

17. (Previously Presented) The method according to Claim 13, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

18. (Previously Presented) The method according to Claim 13, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

19. (Original) The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

20. (Original) The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

21. (Original) The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

22. (Original) The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

23. (Previously Presented) The method according to Claim 13, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

24. (Previously Presented) The method according to Claim 13, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion

complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

25. (Original) A method for the treatment of symptoms of a cladribine-responsive condition in a subject suffering from said symptoms comprising orally administering to said subject a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

26. (Original) The method according to Claim 25, wherein the complex is saturated with cladribine.

27. (Previously Presented) The method according to Claim 25, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

28. (Original) The method according to Claim 27, wherein the cladribine-responsive condition is multiple sclerosis.

29. (Previously Presented) The method according to Claim 25, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin,

hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

30. (Previously Presented) The method according to Claim 25, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

31. (Previously Presented) The method according to Claim 25, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

32. (Original) The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

33. (Original) The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

34. (Previously Presented) The method according to Claim 25, wherein the amorphous cyclodextrin is hydropropyl-γ-cyclodextrin.

35. (Previously Presented) The method according to Claim 25, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

36.-55. (Cancelled)

56. (Original) A complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex.

57. (Original) The complex according to Claim 56, saturated with cladribine.

58. (Previously Presented) The complex according to Claim 56, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, randomly methylated β-cyclodextrin, carboxymethyl-β-cyclodextrin or sulfobutyl-β-cyclodextrin.

59. (Previously Presented) The complex according to Claim 56, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

60. (Previously Presented) The complex according to Claim 56, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

61. (Previously Presented) The complex according to Claim 56, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

62. (Original) The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-β-cyclodextrin.

63. (Original) The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:14.

64. (Original) The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:11.

65. (Original) The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl-γ-cyclodextrin.

66. (Previously Presented) The complex according to Claim 56, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

67. (Currently Amended) A process for the preparation of a complex cladribine-cyclodextrin complex as claimed in Claim 56, which comprises the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature; and

(iii) lyophilizing the cooled solution to afford an amorphous product.

68. (Original) A process according to Claim 67, further comprising a filtration step following step (ii).

69. (Previously Presented) A process according to Claim 67, wherein step (i) is performed at a temperature of from about 45 to about 60°C.

70. (Previously Presented) A process according to Claim 67, wherein step (i) is performed at a temperature of from about 45 to about 50°C.

71. (Previously Presented) A process according to Claim 69, wherein step (i) is performed with stirring.

72. (Original) A process according to Claim 71, wherein step (i) is performed for a period of from about 6 to about 9 hours.

73. (Previously Presented) A process according to Claim 67, wherein step (ii) is performed for a period of from about 6 to about 9 hours.

74. (Previously Presented) A process according to Claim 67, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from

about -40 to about -80° C, and held at said temperature for a period of from about 2 to about 4 hours.

75. (Original) A process according to Claim 74, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

76. (Previously Presented) A process according to Claim 67, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

77. (Previously Presented) A process according to Claim 67, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl-β-cyclodextrin are introduced in step (i).

78. (Previously Presented) A process according to Claim 76, wherein 825 parts by volume of water are introduced in step (i).

79. (Previously Presented) A process according to Claim 67, wherein the lyophilization step (iii) comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

80. (Original) A process according to Claim 79, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

81. (Previously Presented) A process according to Claim 79, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

82. (Original) A pharmaceutical composition obtainable by a process comprising the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature;

(iii) lyophilizing the cooled solution to afford an amorphous product; and

(iv) formulating the amorphous product into a solid oral dosage form.

83. (Original) A pharmaceutical composition according to Claim 82, wherein the process further comprises a filtration step following step (i) or (ii).

84. (Previously Presented) A pharmaceutical composition according to Claim 82, wherein step (i) of the process is performed at a temperature of from about 45 to about 60°C.

85. (Previously Presented) A pharmaceutical composition according to Claim 82, wherein step (i) of the process is performed at a temperature of from about 45 to about 50°C.

86. (Previously Presented) A pharmaceutical composition according to Claim 84, wherein step (i) of the process is performed with stirring.

87. (Original) A pharmaceutical composition according to Claim 86, wherein step (i) of the process is performed for a period of from about 6 to about 9 hours.

88. (Previously Presented) A pharmaceutical composition according to Claim 82, wherein step (ii) of the process is performed for a period of from about 6 to about 9 hours.

89. (Previously Presented) A pharmaceutical composition according to Claim 82, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80°C, and held at said temperature for a period of from about 2 to about 4 hours.

90. (Original) A pharmaceutical composition according to Claim 89, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

91. (Previously Presented) A pharmaceutical composition according to Claim 82, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

92. (Previously Presented) A pharmaceutical composition according to Claim 82, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

93. (Previously Presented) A pharmaceutical composition according to Claim 91, wherein 825 parts by volume of water are introduced in step (i) of the process.

94. (Previously Presented) A pharmaceutical composition according to Claim 82, wherein the lyophilization step (iii) of the process comprises:

(a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;

(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

95. (Original) A pharmaceutical composition according to Claim 94, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

96. (Previously Presented) A pharmaceutical composition according to Claim 94, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

97. (Previously Presented) A pharmaceutical composition according to Claim 82, wherein the formulation step (iv) of the process comprises blending the complex with magnesium stearate and compressing into tablets.

98. (Original) A pharmaceutical composition according to Claim 97, wherein magnesium stearate is pre-mixed with sorbitol powder before blending with the complex.

REMARKS

Entry of the foregoing, reconsideration of the restriction requirement and examination of all of the claims on the merits are respectfully requested in light of the following remarks:

STATUS OF CLAIMS

Claims 1-35 and 56-98 remain in this application. Claims 36-55 were previously cancelled.

Claim 6 has been amended hereinabove to correct an obvious clerical error.

Claim 67 has been amended above to specify that the claimed process is for the preparation of a complex cladribine-cyclodextrin complex as claimed in Claim 56. This limits the process of Group III, Claim 67 (and its dependent claims) to the preparation of a complex of Group I, Claim 56.

RESTRICTION REQUIREMENT

The Examiner has required election of a single invention, which he considers is one of Group I, Claims 1-12, 56-66 and 82-98, drawn to the cladribine-cyclodextrin complex and pharmaceutical compositions thereof; Group II, Claims 13-35, drawn to methods of enhancing the oral bioavailability of cladribine or treating symptoms of a cladribine-responsive condition; and Group III, Claims 67-81, drawn to a process of making. Applicants hereby elect, with traverse, Group I, Claims 1-12, 56-66 and 82-98.

The requirement is traversed because the claims are indeed so linked as to form a single general inventive concept under PCT RUle 13.1. The claims in Groups I-III all have the same special technical features and those are, in fact, the features of the claimed complex. This is not simply any cladribine-cyclodextrin complex, as the Examiner seems to think, however, but rather, a very specific complex which is a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex (Claim 56). The claimed composition comprising this complex is a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amprphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form (Claim 1).

The Examiner has ignored the amorphous nature of the entities which constitute the claimed complex cladribine-cyclodextrin complex and the solid oral dosage forms containing it, which amorphous entities maximize or enhance the benefits of complexation in terms of bioavailability and interpatient variation when administered in solid oral dosage form. Thus, the Examiner has ignored the novel and inventive features of the claimed complex in assessing the claimed subject matter.

Schultz et al. U.S. Patent No. 6,194,395, does not disclose a solid oral dosage form of a complex as claimed in present Claim 1, or the instantly claimed complex itself, either explicitly or inherently. The Examiner refers to the formation of

an inclusion complex with cyclodextrin to be a reversible process governed by an equilibrium and therefore inherently comprises both an inclusion complex and a non-inclusion complex. The process which the Examiner refers to can occur only in solution. Applicants' claims are limited to a complex cladribine-cyclodextrin complex which is an amorphous admixture of (a) an amorphous inclusion complex and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, and to solid oral dosage forms containing these. The word "amorphous" is defined on page 9 of the specification as referring to a noncrystalline solid. Schultz et al. do not describe any actual work done with solid oral dosage forms. In fact, they describe only one general method of preparing a solid dosage form, which is a melt extrusion process, in which the cladribine and cyclodextrin are mixed with other optional additives and then heated until melting occurs. The Schultz et al. patent does not describe or suggest a method for enhancing or maximizing the bioavailability of cladribine from a solid oral dosage form or a complex or composition specially designed to do so. The reference is moreover silent about amorphous forms; and applicants' amorphous inclusion complex and other amorphous materials are in no way inherent in what Schultz et al. describe.

Schultz et al. U.S.P. 6,194,395 was correctly classified as a general state-of-the-art "A" reference in the International Search Report, a copy of which was provided with applicants' First Information Disclosure Statement of November 14, 2006. Applicants are submitting with the accompanying Fourth Information Disclosure Statement a copy of the International Preliminary Report on Patentability issued in connection with the international phase of this application. In particular, we draw the Examiner's attention to the Written Opinion of the International Searching

Authority (ISA) issued by the European Patent Office in its capacity as ISA, in particular the remarks regarding novelty, inventive step and industrial applicability. The present Examiner's position, which is contrary to a reasonable interpretation of the art as pointed out above, is also contrary to the opinion of the International Searching Authority.

For the reasons set forth above, it is respectfully submitted that the restriction requirement is without merit as the Examiner's basis for it is not sound. Withdrawal of the restriction requirement and examination of all of the claims on the merits are respectfully requested.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date: January 4, 2008

By: Mary Katherine Baumeister
Mary Katherine Baumeister
Registration No. 26254

P.O. Box 1404
Alexandria, VA 22313-1404
703 836 6620

Substitute for form 1449/PTO & 1449B/PTO

FOURTH INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

OIPE JAP76 JAN 04 2008 PATENT & TRADEMARK OFFICE

Sheet 1 of 1

Complete if Known	
Application Number	10/551,205
Filing Date	November 14, 2006
First Named Inventor	Nicholas S. Bodor
Examiner Name	
Attorney Docket No.	0056192-000024

U.S. PATENT DOCUMENTS

Examiner Initials	Document Number-Kind Code	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines Where Relevant Passages or Figures Appear
	US-			
	US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials	Foreign Patent Document: Country Code[1], Number, Kind Code	Publication Date (MM-DD-YYYY)	Name of Patentee or Applicant of Cited Document	STATUS: Translation	Partial Translation	Eng. Lang. Summary	Search Report	IPER	Abstract	Cited in Spec. / Pg. No(s).	

[1]Enter Office that issued the document, by the two-letter code.

OTHER DOCUMENTS

Examiner Initials	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.
	PCT International Preliminary Report on Patentability and Written Opinion for International Application No. PCT/US2004/009387, International filing date March 26, 2004.

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with M.P.E.P. § 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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PATENT & TRADEMARK OFFICE

Patent
Attorney Docket No. 0056192-000024

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

1Fee

In re Patent Application of	)	**MAIL STOP AMENDMENT**
Nicholas Bodor et al.	)	Group Art Unit: 1614
Application No.: 10/551,205	)	Examiner: JONATHAN S LAU
Filing Date: November 14, 2006	)	Confirmation No.: 4092
Title: ORAL FORMULATIONS OF CLADRIBINE	)	

AMENDMENT/REPLY TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Enclosed is a reply for the above-identified patent application.

☐ A Petition for Extension of Time is enclosed.

☐ _____ Terminal Disclaimer(s) and the ☐ $ 65 ☐ $ 130 fee per Disclaimer due under 37 C.F.R. § 1.20(d) are enclosed.

☒ Also enclosed is/are: FOURTH IDS and FORM 1449

☐ Small entity status is hereby claimed.

☐ Applicant(s) requests continued examination under 37 C.F.R. § 1.114 and enclose the ☐ $ 405 ☐ $ 810 fee due under 37 C.F.R. § 1.17(e).

☐ Applicant(s) requests that any previously unentered after final amendments not be entered. Continued examination is requested based on the enclosed documents identified above.

☐ Applicant(s) previously submitted _____ on _____ for which continued examination is requested.

☐ Applicant(s) requests suspension of action by the Office until at least _____, which does not exceed three months from the filing of this RCE, in accordance with 37 C.F.R. § 1.103(c). The required fee under 37 C.F.R. § 1.17(i) is enclosed.

☐ A Request for Entry and Consideration of Submission under 37 C.F.R. § 1.129(a) (1809/2809) is also enclosed.

☒ No additional claim fee is required.

☐ An additional claim fee is required, and is calculated as shown below:

AMENDED CLAIMS					
	No. of Claims	**Highest No. of Claims Previously Paid For**	**Extra Claims**	**Rate**	**Additional Fee**
Total Claims	78	78	0	x $ 50 (1202)	$ 0
Independent Claims	5	6	0	x $ 210 (1201)	0
☐ If Amendment adds multiple dependent claims, add $ 370 (1203)					$ 0
Total Claim Amendment Fee					**$ 0**
☐ Small Entity Status claimed - subtract 50% of Total Claim Amendment Fee					0
TOTAL ADDITIONAL CLAIM FEE DUE FOR THIS AMENDMENT					**$ 0**

☐ Charge _____ to Deposit Account No. 02-4800 for the fee due.

☐ A check in the amount of _____ is enclosed for the fee due.

☐ Charge_____ to credit card for the fee due. Form PTO-2038 is attached.

☒ The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.20(d) and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in duplicate.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date January 6, 2008

By: Mary Katherine Baumeister
Mary Katherine Baumeister
Registration No. 26254

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
10/551,205	11/14/2006	

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)	SMALL ENTITY ☐ RATE ($)	SMALL ENTITY ☐ FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
FOR	NUMBER FILED	NUMBER EXTRA					
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	01/04/2008	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 78	Minus	** 78	= 0	X $ =		OR	X $50=	0
	Independent (37 CFR 1.16(h))	* 5	Minus	***6	= 0	X $ =		OR	X $210=	0
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/VIKKI SHORT/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.