

CYCLODEXTRIN-BASED PHARMACEUTICS: PAST, PRESENT AND FUTURE

Mark E. Davis* and Marcus E. Brewster[‡]

Abstract | Cyclodextrins are cyclic oligomers of glucose that can form water-soluble inclusion complexes with small molecules and portions of large compounds. These biocompatible, cyclic oligosaccharides do not elicit immune responses and have low toxicities in animals and humans. Cyclodextrins are used in pharmaceutical applications for numerous purposes, including improving the bioavailability of drugs. Current cyclodextrin-based therapeutics are described and possible future applications discussed. Cyclodextrin-containing polymers are reviewed and their use in drug delivery presented. Of specific interest is the use of cyclodextrin-containing polymers to provide unique capabilities for the delivery of nucleic acids.

α -1,4- AND α -1,6- GLYCOSIDIC LINKAGES
The D-glucopyranoside unit contains six carbons and two of these units can be chemically linked from the 1-carbon of a unit to either the 4-carbon or the 6-carbon of the second unit.

Cyclodextrins (CDs) comprise a family of cyclic oligosaccharides, and several members of this family are used industrially in pharmaceutical and allied applications. CDs are manufactured from starch, one of the two glucose-containing polymers produced by photosynthesis (the other is cellulose). Starch consists of D-glucopyranoside building blocks that have both α -1,4- AND α -1,6-GLYCOSIDIC LINKAGES. The degradation of starch (which is primarily derived from corn, but also from potatoes and other sources) by the enzyme glucosyltransferase generates, by chain splitting and intramolecular rearrangement, primary products that are cyclic oligomers of α -1,4-D-glucopyranoside, or CDs. FIGURE 1 shows several schematic representations of β -CD. CDs derive their system of nomenclature from the number of glucose residues in their structure, such that the glucose hexamer is referred to as α -CD, the heptamer as β -CD and the octomer as γ -CD (FIG. 2). There are literally thousands of variations of CDs that have variable ring size and random or site-specific chemical functionalization. A comprehensive overview of all aspects of CDs is available¹.

The earliest reference to a substance that was later recognized as a CD was published in 1891 (REF. 2). By 1953, Freudenberg *et al.*³ had received the first patent on the use of CDs in drug formulations. This patent covered

most of the important concepts that are used even today, including the improvement of drug properties such as increased aqueous solubility and increased stability towards drug oxidation. Currently, CDs have found uses in many applications, such as in agrochemicals, pharmaceuticals, fragrances, foods and so on. This review will concentrate on the pharmaceutical uses of CDs.

The basis of CDs as pharmaceutical excipients

The three-dimensional structure of CDs endows them with properties that are useful for pharmaceutical applications. Because of the large number of hydroxyl groups on CDs, they are water-soluble. The water solubilities of α -, β - and γ -CD at ambient conditions are approximately 13%, 2% and 26% (weight by weight (w/w)), respectively (for β -CD this is approximately 18.8 g per l or 16.6 mM)¹. The lower solubility of β -CD compared with α -CD, even though the former contains a higher number of hydroxyl groups than the latter, is due to the formation of an internal hydrogen-bond network between the secondary hydroxyl groups. The disruption of hydrogen bonding via molecular manipulation gives rise to an increase in water solubility. For example, hydroxypropyl- β -CD (HP β CD) has an aqueous solubility of 60% (w/w) or more⁴. Although the entire CD molecule is water soluble, the

*Chemical Engineering,
California Institute of
Technology, Pasadena,
California 91125, USA.
[‡]Johnson & Johnson
Pharmaceutical Research
and Development,
Turnhoutseweg 30,
2340 Beerse, Belgium.
Correspondence to M.E.D.
e-mail:
mdavis@cheme.caltech.edu
doi:10.1038/nrd1576

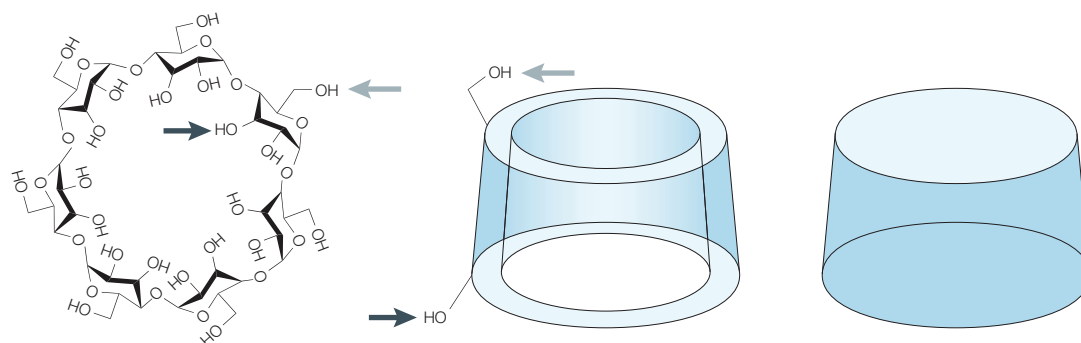


Figure 1 | Schematic representations of β -cyclodextrin. The open and closed arrows point to primary and secondary hydroxyl groups, respectively. The cyclodextrin (CD) architecture is a cup that is 0.79 ± 0.01 nm from top to bottom (primary OH face to secondary OH face), and is slightly larger on the face containing secondary hydroxyl groups. The cavity (0.47–0.53, 0.60–0.65 and 0.75–0.83 nm for α -, β - and γ -CD, respectively) and exterior diameters of the CDs (1.46 ± 0.04 , 1.54 ± 0.04 and 1.75 ± 0.04 nm for α -, β - and γ -CD, respectively, for the faces containing secondary hydroxyl groups) expand as the number of glucopyranoside units increase¹.

interior of the cup is relatively apolar and creates a **HYDROPHOBIC** micro-environment. CDs therefore have **HYDROPHILIC** cavity exteriors and hydrophobic cavity interiors. These properties are responsible for their aqueous solubility and ability to encapsulate hydrophobic moieties within their cavities, and the incorporation of 'guest' molecules in CD inclusion complexes in aqueous media has been the basis for most pharmaceutical applications. A dynamic equilibrium between free CDs, free drug molecules and their formed inclusion complexes is established if drug molecules are of

sufficient size and have appropriate properties for the formation of inclusion complexes. **FIGURE 3** schematically illustrates this dynamic equilibrium for 1:1 and 1:2 drug-CD complexes. The formation of inclusion complexes is possible with the entire drug molecule or only a portion of it. **FIGURE 3C** presents models of how α -, β - and γ -CD can form inclusion complexes with prostaglandin E_2 . Because of cavity size, α -CD complexes well with aliphatic chains and molecules such as polyethylene glycol (PEG), whereas β -CD is appropriate for aromatic rings, such as that in paclitaxel.

For CDs to be pharmaceutically useful, they must be biocompatible. CDs show resistance to degradation by human enzymes; CDs injected intravenously into humans are therefore essentially excreted intact via the kidney. However, bacterial and fungal enzymes (amylases) can degrade CDs. Ingested CDs can therefore be metabolized in the colon prior to excretion. The toxicities of CDs are dependent on their route of administration. For example, the dose that causes 50% death (LD_{50}) values of α -, β - and γ -CD administered intravenously into mice are approximately 1.0 g per kg⁵, 0.79 g per kg⁵ and more than 4.0 g per kg⁶, respectively. β -CD has an affinity for cholesterol and can extract it and other lipid membrane components from cells. At sufficiently high concentrations, β -CD can cause haemolysis of erythrocytes. Additionally, parenteral administration of β -CD is not possible because of its poor solubility (which leads to microcrystalline precipitation in the kidney), as well as the fact that it forms complexes with cholesterol that accumulate in the kidney and produce renal tubule damage. Functionalized β -CDs can mitigate these problems.

Chemically modified CDs result from etherification or the introduction of other functional groups at the 2-, 3- and 6-hydroxyl groups of the glucose residues. These changes improve solubility through two mechanisms: by breaking the 2-OH–3-OH hydrogen bonds, and by preventing crystallization due to creation of a statistically substituted material that is made up of many isomeric components and gives rise to an amorphous product.

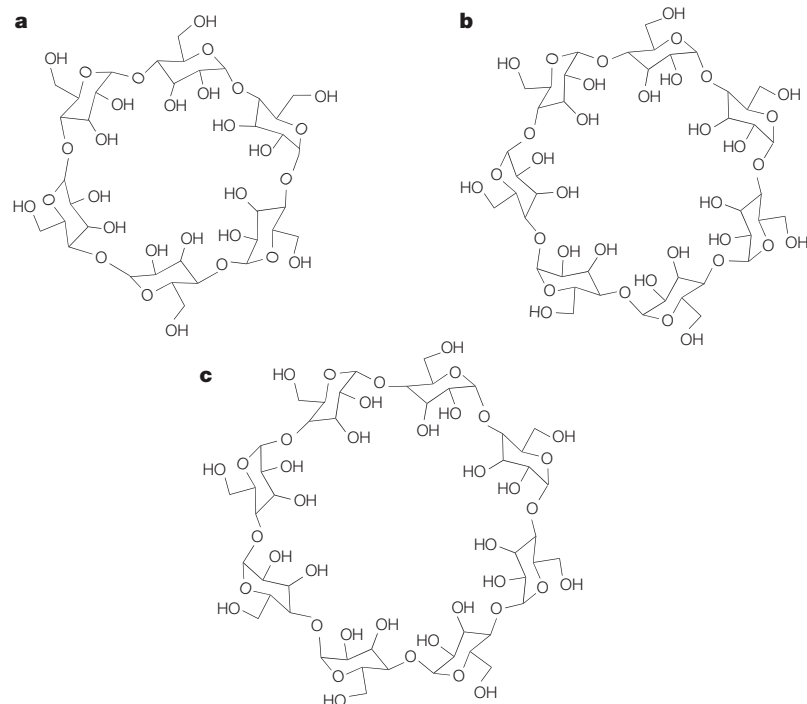


Figure 2 | Schematic representations of cyclodextrins. α -CD (a), β -CD (b) and γ -CD (c) contain 6, 7 and 8 glucopyranoside units, respectively. The molecular masses of α -, β - and γ -CD are 972, 1,135 and 1,297 Da, respectively.

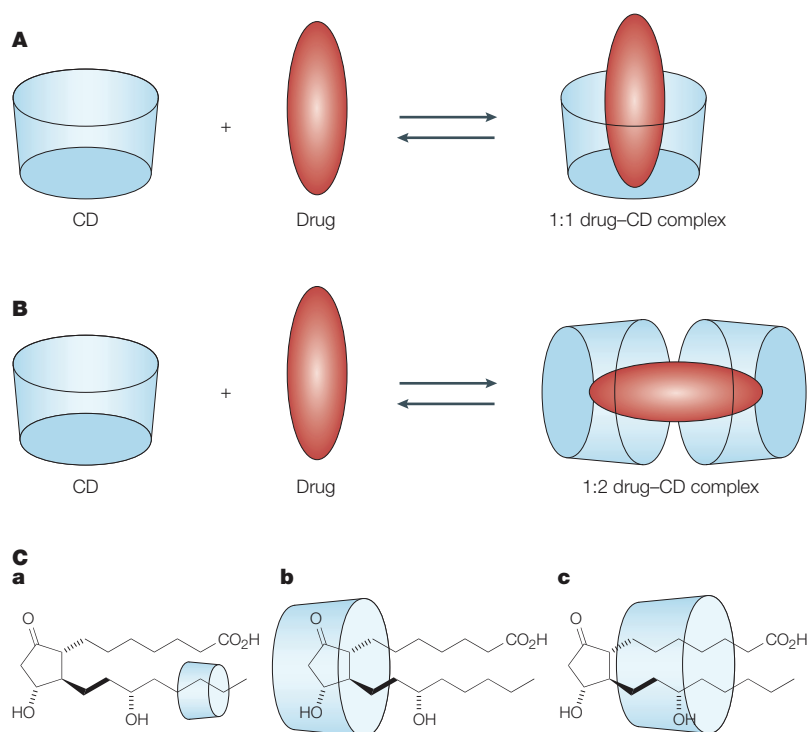


Figure 3 | **Schematic illustration of the association of free cyclodextrin (CD) and drug to form drug-CD complexes.** **A** | 1:1 drug-CD complex. **B** | 1:2 drug-CD complex. **C** | Proposed models of inclusion complexes between prostaglandin E_2 and (a) α -CD, (b) β -CD and (c) γ -CD. Adapted from REF. 1.

The complexity of these mixtures can be appreciated by considering β -CD. For this compound, there are 21 hydroxyl functional groups and therefore $2^{21} - 1$ possible combinations for substitutions (that is, more than 2 million). If an optically active centre is introduced, as in the case of 2-hydroxypropylation, the number of geometrical and optical isomers is truly astronomical, given that the β -CD nucleus contains 28 chiral centres. It is conceivable that the pharmaceutical performance of these isomeric mixtures can change with the extent and degree of substitution, and so these factors have to be assessed and specified in the excipient. In practice, this is done by analogy with other chemically modified pharmaceutical starches and celluloses, such as hydroxypropyl cellulose and hydroxypropylmethyl cellulose. Both the European Pharmacopoeial monograph and the proposed United States Pharmacopoeial monograph on HP β CD, for example, specify that the material should have a molar substitution (expressed as the number of hydroxypropyl groups per anhydroglucose unit) between 0.4 and 1.5; this means 2.8–10.5 hydroxypropyl functional groups per cyclodextrin molecule. They also specify that less than 1.5% unmodified β -CD should be present. The molar substitution can be determined using nuclear magnetic resonance and infra-red methods. Two functionalized CDs, hydroxypropyl β -CD (HP β CD) and sulphobutyl ether β -CD (SBE β CD), are available in FDA-approved products for human use (see below). In addition, CDs do not produce an immune

response in mammals. Because of these highly desirable properties, CDs have found numerous pharmaceutical applications; reviews on the use of CDs in drug delivery are available^{7–13}.

Pharmaceutical applications of CDs

Current pharmaceutical research has a number of drivers, including the nature of the drugs being developed, the need for generating orally bioavailable dosage forms and the preparation of solubilized parenteral formulations. Drug discovery has evolved over the years to the point that high-throughput screening techniques have become routine. These approaches put significant evolutionary pressure on emerging drug candidates, and this has led to a systematic increase in molecular mass, lipophilicity and a decrease in water solubility for lead compounds over time^{14,15}. This, in turn, has had a significant impact on what is required from drug delivery formulators, in that the number of formulation options has had to be increased to address the larger diversity of challenges presented.

For a drug to be orally available, the compound must dissolve and be absorbed through the gastrointestinal tract in such a way that it generates adequate drug levels at the pharmacologically active site to ensure that the desired action is obtained in a reproducible manner. Retrospective studies show that >40% of drug failures in development can be traced to poor biopharmaceutical properties, specifically poor dissolution or poor permeability¹⁶. In recognition of the importance of these factors, the FDA and other drug regulatory organizations have defined a Biopharmaceutical Classification System in which drugs are divided into four types on the basis of their solubility and permeability characteristics (FIG. 4)^{17–19}. High-throughput drug discovery methodologies are increasingly selecting difficult Type II compounds, and CDs can be an important enabling technology for these compounds in particular^{20,21}. By increasing the apparent water solubility of a drug candidate, formulations can be generated such that a Type II material behaves like a Type I compound, with a resulting increase in oral bioavailability^{20,22} (BOX 1).

The reasons for the inclusion of CDs in a particular formulation can vary widely (BOX 2)²³, and are specific to the circumstance — that is, the specific physicochemical issues that have to be overcome and the administration route^{24–26}.

Initial applications of α - and β -CDs: prostaglandin and nonsteroidal anti-inflammatory agents. CDs first came to the fore in marketed products as drug delivery technologies that enabled the development of various prostaglandins^{27,28}. One of first of these compounds, PGE₂, a substance with potent oxytocin-like effects, was of interest as a possible agent for the induction of labour in childbirth^{29,30}. As with other members of the E-type prostoglandins, these compounds are highly unstable, and this feature complicated their formulation and development. β -CD complexes of PGE₂ resulted in a significant increase in their solid-state stability, and a product designed along these lines

WETTABILITY

The wettability of a liquid is defined as the contact angle between a droplet of the liquid in thermal equilibrium on a horizontal surface.

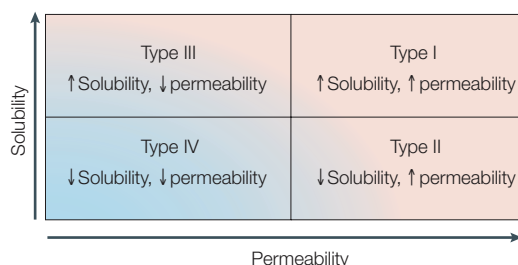


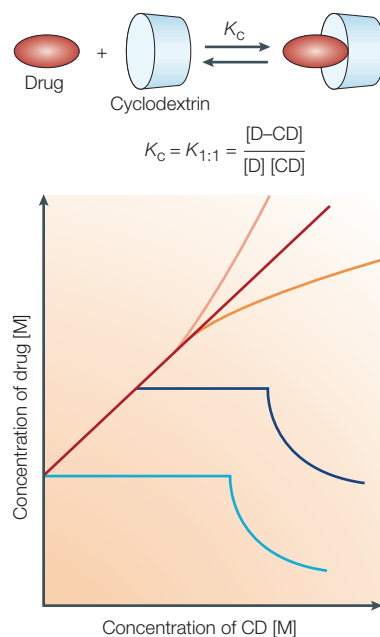
Figure 4 | Biopharmaceutical Classification System (BCS) characterization of drugs based on solubility and permeability measures. Drug solubility is defined by the dose/solubility ratio, with a soluble drug defined as one in which the highest dose intended for human use will dissolve in 250 ml of water (the so-called FDA glass of water). Permeability can be defined by various *in vivo* or *in vitro* assays, but a permeable drug is one associated with $\geq 90\%$ oral bioavailability or $\geq 90\%$ absorption as assessed by urinary excretion data.

(dinoprostone betadex; Prostarmon E; Ono) was approved for the Japanese market in 1976 (TABLE 1). Prostarmon E is highly effective and represented a significant medical advance, especially for the induction of labour in oxytocin-insensitive individuals, but also for its tendency to produce less bleeding after delivery.

The second prostaglandin marketed as a CD complex was PGE₁ (alprostadil alphadex; Prostavasin/Edex/Caverject/Prostandin; Schwarz Pharma). PGE₁ relaxes smooth muscle and increases blood flow, and was initially developed as a therapeutic to treat peripheral circulatory disorders. Given its limited metabolic stability, initial therapies with PGE₁ required intra-arterial administration to obtain useful clinical results.

Box 1 | Solubilization with cyclodextrins

Cyclodextrins (CDs) can enhance apparent water solubility by forming dynamic, non-covalent, water-soluble inclusion complexes as depicted in the figure. This interaction is an equilibrium governed by an equilibrium constant, K_c . The nature of the complex, as well as the numerical value of the equilibrium constant, can be derived from measuring a particular property of the complex as a function of drug and CD concentrations. In phase-solubility analysis, the increased solubility is assessed as a function of CD concentration. As illustrated, a number of solubility profiles are possible, each giving insight into the type of complex formed, as well as its stoichiometry. An A-type profile (red line) represents the formation of soluble CD complexes, whereas B-type systems (blue line) indicate the formation of complexes of limited solubility.



On the basis of this administration route, the stabilizing effect of α -CD on PGE₁ and the suitability of this CD for parenteral use (unlike β -CD), formulations of PGE₁/ α -CD complexes were developed. In 1979, alprostadil alphadex (Prostavasin) was approved for the treatment of peripheral vascular complications, including **Buerger's disease**^{29,30}. The compound also showed activity against chronic arterial occlusions and arteriosclerosis.

Another vascular malady that can be treated with alprostadil alphadex is male erectile dysfunction, for which the complex is given by intracavernous injection^{31–34}. The PGE₁- α -CD complex was found to be effective in subjects who were not responsive to sildenafil citrate (Viagra; Pfizer), an oral inhibitor of **phosphodiesterase-5**³⁵. In a study of 67 patients that failed sildenafil citrate therapy at 50 and 100 mg, 85–90% reported improvements in erectile function when PGE₁- α -CD was self-administered after 'at-home' treatment. Caverject Impluse is approved for use in the United States on the basis of these medical needs.

The complexity of the administration route prompted the development of more convenient dosing options, including intravenous dosage forms. These drivers resulted in a modified formulation (Prostandin), which was approved in 1982 in Japan and subsequently in a number of other countries, including Germany^{29,30}.

A third example of a prostaglandin marketed as a CD complex is limaprost alphadex (Opalmon/ Prorenal; Ono)^{29,30,36,37}. This prostaglandin analogue was developed for the treatment of vascular disease and was shown to have improved antiplatelet aggregation and vasodilation activity relative to PGE₁. Importantly, the compound was orally available and showed a good separation between its therapeutic action and unwanted side effects (mainly oxytocin-like effects). The limoprost- α -CD complex was found to be safe and effective in the treatment of Buerger's disease and was approved in 1988.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a mainstay for the treatment of pain³⁸. The major drawbacks of these otherwise useful compounds include upper gastrointestinal irritation and bleeding³⁹. Piroxicam (Feldene; Pfizer) is an illustrative example. The drug is useful in the treatment of **osteoarthritis** and **rheumatoid arthritis**, as well as gout, acute musculoskeletal disorders and dysmenorrhea^{40–43}. It has a relatively long pharmacokinetic half-life, meaning that it can be taken once a day, in contrast to many other NSAIDs. The parent drug is also poorly water soluble (~ 30 μ g per ml), poorly **WETTABLE** (that is, with a contact angle of 70°) and highly crystalline (with a melting point of 202 °C and a ΔH_{melt} of 106 J per g)^{44–47}. This imparts to the compound poor dissolution properties, as well as dissolution-limited oral pharmacokinetics.

CDs were applied to this compound in an effort to improve several properties, including safety and drug dissolution rate. These improved characteristics reduced gastrointestinal irritation, and allowed more rapid drug absorption and a more rapid onset of the analgesic effect. Complexation studies indicated that a molar ratio of 2.5 per 1 was optimal for the drug-CD combination

and, given the low piroxicam dose (20 mg), this did not add excessive bulk to the formulation⁴⁸. The apparent solubility of piroxicam in the β -CD formulation was increased fivefold relative to the uncomplexed drug substance. This enhanced solubility was associated with an increased dissolution rate and higher plasma levels at early time in humans, which, in turn, directly correlated with an increased absorption rate^{48,49}. There was no change in terminal half-life or total area under the curve when piroxicam or the piroxicam- β -CD complex were compared. With regard to efficacy, piroxicam- β -CD has been demonstrated to have a faster onset of action in a number of clinical trials^{48,50,51}.

Although differences in the tolerability of piroxicam and its β -CD complex require the analysis of epidemiological data, acute studies might provide some useful insight. Several studies that have assessed the endoscopic appearance of the stomach of volunteers taking either piroxicam or the piroxicam- β -CD complex have revealed significantly better outcomes in the case of administration of the complexed drug^{48,52,53}. Similarly, radio-adhesive substrates demonstrated fewer gastric lesions with the piroxicam- β -CD complex relative to piroxicam^{48,54}. Several branded piroxicam- β -CD products are available, including Brexin (Chiesi) and Cicladol (Chiesi).

Applications of randomly methylated β -CD. Randomly methylated β -CD (RM β CD) (FIG. 5) provides good biocompatibility and useful complexing efficiencies, and is beginning to be used in marketed products throughout the world. An eye drop preparation of the antibiotic chloramphenicol has been developed by Oftalder and marketed as Clorocil in Portugal⁵⁵. The recently introduced nasal product Aerodil, which contains RM β CD, is a complex between the indicated CD and β -oestradiol^{56–60}. A number of potential advantages are apparent for such an oestradiol delivery system. Although the safety of hormone-replacement therapy has been the subject of recent debate^{61,62}, its efficacy in reducing menopausal symptomatology is well established. Traditional dosing strategies include oral administration of conjugated equine oestrogens, oestradiol esters or micronized oestradiol, as well as the use of transdermal patches to deliver this sex hormone^{63–65}. Although both routes have a number of limitations, both provide relatively constant blood levels of drug, in contrast to endogenous oestrogen release, which tends to be more pulsatile.

The RM β CD-based nasal product avoids a number of issues related to oral or transdermal administration⁵⁶. Nasal administration results in direct systemic uptake, and so the first-pass effect is reduced or eliminated. In addition, the nasal route is convenient, non-invasive and provides for consistency of drug absorption. Drug uptake is rapid, and peak plasma concentrations are achieved within 10–30 min of drug dosing. Drug levels also dissipate rapidly and return close to baseline within 2 hours⁵⁶. This pattern is more akin to physiological oestrogen secretion than that associated with oral or transdermal approaches. Another feature of this route is that, unlike oral dosing of oestrogens that generate a

Box 2 | Pharmaceutical applications of CDs

Cyclodextrins (CDs) can be used to achieve the following:

- Enhance solubility
- Enhance bioavailability
- Enhance stability
- Convert liquids and oils to free-flowing powders
- Reduce evaporation and stabilize flavours
- Reduce odours and tastes
- Reduce haemolysis
- Prevent admixture incompatibilities

high oestrone/oestradiol ratio, nasal delivery gives rise to a more physiological ratio of the two hormones^{56–60}.

A number of clinical trials have confirmed these product design principles. Studd *et al.* studied 420 postmenopausal women and found that the nasal product based on oestradiol and RM β CD was effective in ameliorating menopausal symptoms as early as four weeks after initiation of therapy in a dose-dependent manner, and continued to improve the post-menopausal symptoms even after 12 weeks⁵⁶. The minimum effective oestradiol dose was 200–400 μ g per day. These doses were similar in efficacy to 2 mg of oestrogen administered orally, although the reduced systemic exposure results in a potentially improved safety profile. Inter- and intrasubject variability was less than that demonstrated in the oral oestrogen group.

Applications of hydroxypropylated β -CD. Two hydroxypropylated CDs (HP β CDs) have been approved in various world markets (United States and Europe)^{25,27,66–69}. HP β CD (FIG. 5) is available in registered oral, intravenous, buccal, rectal and ophthalmic products, whereas HP γ CD is available in an eye drop formulation that contains the anti-inflammatory agent diclofenac sodium⁷⁰. Of the HP β CD products, the oral and intravenous solutions of itraconazole (Sporanox; Janssen) have the most widespread use⁷¹.

Itraconazole is a triazole-type drug that exerts its effect by inhibiting fungal cytochrome P450 and inhibiting the biosynthesis of ergosterol, an essential component of the fungal membrane. The compound is noteworthy in that it was the first approved orally bioavailable agent with significant clinical activity against both *Candida spp.* and *Aspergillus spp.*, the two most common human fungal pathogens^{72–75}. Formulation development for this drug was complicated by its challenging set of physico-chemical properties, which include a pK_a of 4, a $\log P > 5$ and an aqueous solubility at neutral pH estimated at 1 ng per ml^{71,76}. The production of solid oral dosage forms was eventually made possible by using solid solution technology in which the drug and a polymeric carrier (hydroxypropyl methylcellulose (HPMC)) were sprayed on inert sugar spheres to form a thin film. As the film dissolves in the stomach, the molecularly dissolved drug is released at supersaturated levels. The co-dissolving HPMC inhibits

LOG P
The logarithm of the partition coefficient of a substance in octanol–water.

Table 1 | **Approved and marketed drug–cyclodextrin* complexes in various world markets**

Drug	Administration route	Trade name	Market
<i>α-Cyclodextrin</i>			
Alprostadil (PGE ₁)	Intravenous	Prostavastin, Caverject, Edex	Europe, Japan, United States
Cefotiam hexetil HCl	Oral	Pansporin T	Japan
Limaprost	Oral	Opalmon, Prorenal	
<i>β-Cyclodextrin</i>			
Benexate	Oral	Ulgut, Lonmiel	Japan
Dexamethasone	Dermal	Glymesason	Japan
Iodine	Topical	Mena-Gargle	Japan
Nicotine	Sublingual	Nicorette	Europe
Nimesulide	Oral	Nimedex, Mesulid	Europe
Nitroglycerin	Sublingual	Nitropen	Japan
Omeprazole	Oral	Omebeta	Europe
Dinoprostone (PGE ₂)	Sublingual	Prostarmon E	Japan
Piroxicam	Oral	Brexin	Europe
Tiaprofenic acid	Oral	Surgamyl	Europe
<i>2-Hydroxypropyl-β-cyclodextrin</i>			
Cisapride	Rectal	Propulsid	Europe
Hydrocortisone	Buccal	Dexocort	Europe
Indomethacin	Eye drops	Indocid	Europe
Itraconazole	Oral, intravenous	Sporanox	Europe, United States
Mitomycin	Intravenous	Mitozytrex	United States
<i>Randomly methylated β-cyclodextrin</i>			
17β-Oestradiol	Nasal spray	Aerodiol	Europe
Chloramphenicol	Eye drops	Clorocil	Europe
<i>Sulphobutylether β-cyclodextrin</i>			
Voriconazole	Intravenous	Vfend	Europe, United States
Ziprasidone maleate	Intramuscular	Geodon, Zeldox	Europe, United States
<i>2-Hydroxypropyl-γ-cyclodextrin</i>			
Diclofenac sodium	Eye drops	Voltaren	Europe

*Commercial suppliers of pharmaceutical-grade cyclodextrins are Roquette, Cerestar, Wacker Chemie and CyDex. See figures 2 and 5 for schematic representations of cyclodextrins and functionalized cyclodextrins, respectively.

nucleation and crystallization such that the supersaturated drug solutions are stable for long enough to allow significant absorption and oral bioavailability.

Given the weakly basic nature of itraconazole, the pH of the stomach must be sufficiently low to ensure good dissolution and the formation of a stable solution^{78,79}. In certain subpopulations, stomach pH can be a limiting factor for bioavailability. AIDS patients, for example, often suffer from hypochlorhydria and so treating opportunistic fungal infections with itraconazole in the patients can be problematic⁸⁰. Similarly, the chemotherapeutic agents given to cancer patients can reduce gastrointestinal function by inducing mucositis, and an individual undergoing autologous bone marrow transplants can experience graft-versus-host gut disease⁸¹.

To better prevent or treat fungal infections in these conditions, a liquid oral formulation of itraconazole was developed, in addition to a parenteral dosage form for treating disseminated systemic infection, as well as for use in administering loading doses. HPβCD was chosen as the functional excipient to enable both of these

formulations⁷¹. It was reasoned that the oral solution might improve bioavailability in the described subpopulations because no phase transition is required.

The oral and intravenous formulations that were developed proved to be safe and effective in numerous clinical trials, and this resulted in their market introduction in the United States and Europe (the oral solution in 1997 and the intravenous product in 1999)^{81–83}. The oral product is indicated in empiric therapy of febrile patients with suspected fungal infections, as well as for the treatment of oropharyngeal and oesophageal candidiasis^{81,84}. The aqueous 40% w/v HPβCD solution contains 10 mg per ml itraconazole, which represents an increase in apparent solubility of five to six orders of magnitude. The oral bioavailability of itraconazole is more consistent in the subpopulations described (fraction absorbed 85% and oral bioavailability 55%)^{81,85}. On the basis of an oral dose of 200 mg itraconazole, the dose of HPβCD is 8 g per day. The intravenous product is also approved for empiric therapy as well as for blastomycosis (pulmonary and

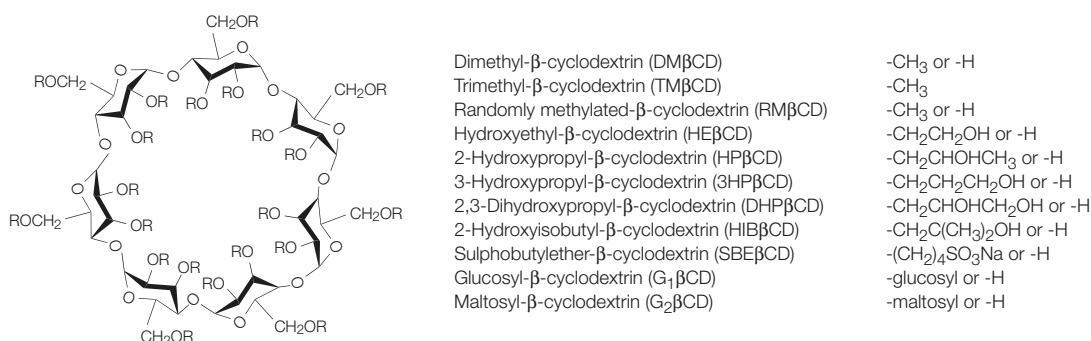


Figure 5 | Graphical representations of β-cyclodextrin and its pharmaceutically relevant chemical derivatives.

extra-pulmonary), histomycosis (pulmonary and disseminated, non-meningeal) and aspergillosis (pulmonary and non-pulmonary), in the latter case in individuals refractory or intolerant to amphotericin B^{81,84}. Based on intravenous doses of itraconazole between 200 and 400 mg and a formulation containing 10 mg itraconazole in a 40% HPβCD solution, the intravenous dose of HPβCD is between 8 and 16 g per day.

Applications of sulphobutyl ether βCD. The sulphobutyl ether derivatives of β-CD (SBEβCD; FIG. 5) represent the newest CD derivative to be approved^{25,86–88}. In addition, γ-CD derivatives of these useful excipients are also available. Two products were introduced in 2002 in the United States and Europe, including an intravenous formulation of the antifungal agent voriconazole (Vfend; Pfizer) and an intramuscular dosage form for the antipsychotic agent ziprasidone (Zeldox; Pfizer). In addition, a number of compounds are under development in areas including parenteral, oral sustained release, pulmonary and ophthalmological drug delivery. Voriconazole is a triazole antifungal agent that, unlike itraconazole, which evolved from the miconazole-ketoconazole series, is structurally related to fluconazole^{89–91}. Voriconazole is effective against *Candida spp.* as well as *Aspergillus spp.*, and is also reported to be effective against such emerging pathogens as *Scedosporium* and *Fusarium spp.*^{89–91}. As with itraconazole, it can participate in numerous drug interactions. The compound is also poorly water soluble (~0.2 mg per ml at pH 3) and is not stable in aqueous solutions because it forms an inactive enantiomer. Unlike itraconazole, which has a log P > 5, voriconazole is more amphiphilic and has a log P of 1.8. These factors complicate the preparation of a parenteral dosage form⁹².

SBEβCD was used in this formulation problem and resulted in a dosage form containing 3.2 g of the CD and 200 mg of the active principle⁸⁴. The supplied vial is diluted with water for injection to give a 10 mg per ml solution of voriconazole in a 16% w/v SBEβCD solution; this is then further diluted such that the administration solution contains 5 mg per ml or less of the active principle. The intravenous dose suggested is 3–6 mg per kg, which means that for a 70-kg individual, between 3.4 and 6.7 g of SBEβCD are administered.

The regulatory status of CDs is evolving. α- and β-CDs are listed in a number of pharmacopeal sources, including the United States Pharmacopeia (USP), European Pharmacopeia (EP) and Japanese Pharmacopeia (JP). A monograph for HPβCD has recently appeared in the EP and will be listed in the USP in the near future. Other derivatives are not yet compendial, but efforts are underway for their inclusion. β-CD is also listed in the Generally Regarded As Safe list of the US FDA⁹³ for use as a food additive.

Future uses of CDs. There are a number of exciting possibilities for future applications of CDs, including new uses for existing derivatives, as well as the development of new derivatives. Although a number of drug-CD complexes have been marketed (TABLE 1), these have been associated with low-molecular-mass drugs. A body of evidence also indicates that CDs might be useful for formulation improvements or drug delivery with protein, peptide and oligonucleotide dosage forms^{93–97}. HPβCD, and other hydrophilic and lipophilic CD derivatives, prevent agitation-associated aggregation in solution of a number of proteins, including human growth hormone, as well as lyophilization-related aggregation of interleukin-2 (IL-2)^{94,98–100}.

Solutions of insulin can also be stabilized against physical and chemical degradation through the use of CDs⁶⁷. The basis for these improvements seems to be related to the ability of CDs to stabilize protein conformation through interactions with accessible hydrophobic amino-acid residues. CDs have also been found to behave as artificial chaperones, meaning that they can interact with aggregated/denatured proteins to result in natural refolding^{101–104}. The endogenous GroE chaperone system involves binding of the GroEL 14-mer to the non-native state of a protein, which triggers refolding by binding to additional substrates, including MgATP, potassium and, in some cases, the co-chaperone GroES¹⁰³. The artificial systems involve the sequential addition of a detergent to complex with the protein, followed by β-CD or a β-CD-derivative, to strip away the detergent in such a manner that natural refolding is allowed. Heat-inactivated carbonic anhydrase, for example, was reactivated by treating the protein with solutions of cetyltrimethylammonium bromide and β-CD¹⁰². The material that is produced is similar to the

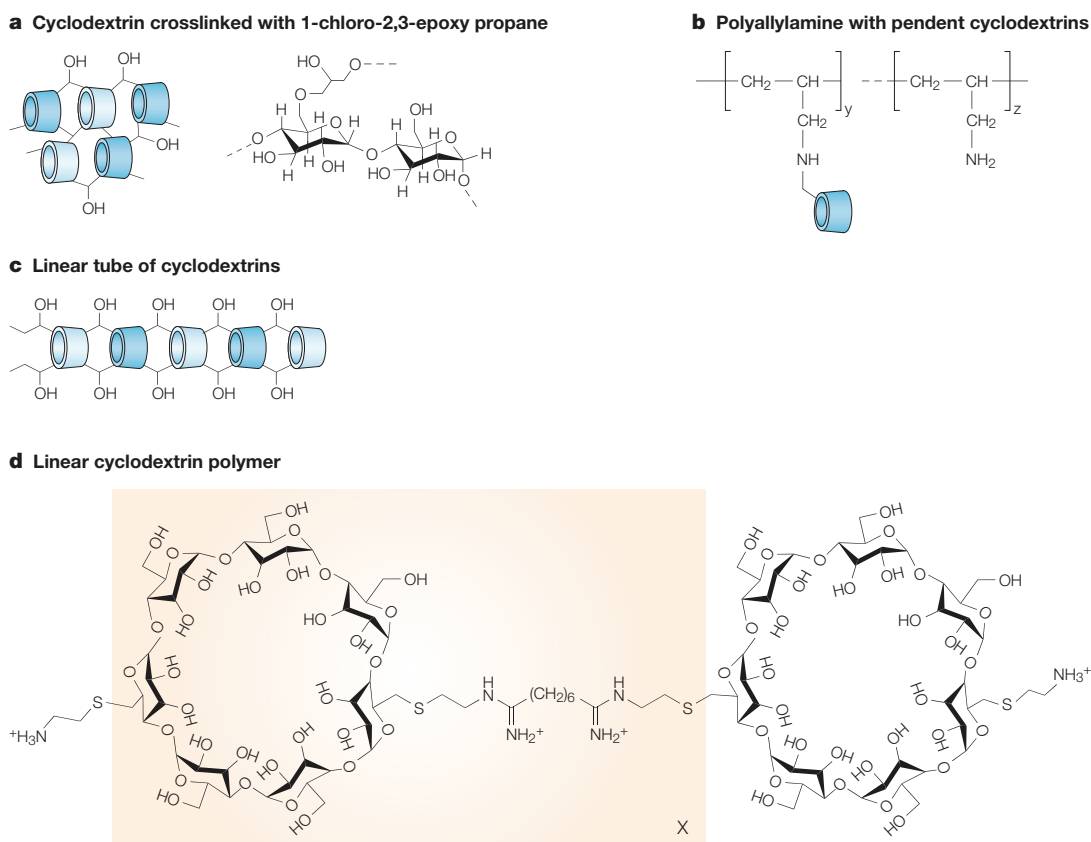


Figure 6 | **Classes of cyclodextrin-containing polymers.**

original protein before inactivation. Similarly, thermally denatured creatine kinase can be reactivated using sodium diocylsulphosuccinate and HP β CD and lysozyme with guanidinium chloride and various CDs¹⁰⁴. Another potential application for CDs is in improving the cellular delivery of oligonucleotides. HP β CD increases the uptake of phosphorothiolate oligodeoxynucleotides by human T-cell leukaemia cells (H9) by several fold^{96,97}. In addition, CD complexation is associated with reduced immunogenicity of oligonucleotides and can reduce other unwanted side effects of oligonucleotide administration, including effects of the macromolecules on platelet count^{94,97}.

In addition to their expanding role as drug carriers, CDs can also be used to extract compounds. CDs can alter lipid distribution *in vivo* and affect with cell signalling through the alteration of lipid raft systems^{105,106}. Hildreth *et al.* found that HP β CD could extract virion-associated cholesterol as well as modulate cholesterol associated with HIV-infected cells. Such cholesterol extraction dramatically lowered HIV viral release and those virions released from cholesterol-depleted cells were minimally infectious. These results suggested that the vaginal application of HP β CD could be used to reduce HIV transmission¹⁰⁶. Additionally, CDs can reverse the *in vivo* neuromuscular blocking of rocuronium by forming inclusion complexes with this skeletal muscle relaxant¹⁰⁷. This application of CDs is currently in Phase II clinical trials.

CDs have been combined with polymers and small-molecule drugs to control the amount of CD used¹⁰⁸ and the release rates of the drug¹⁰⁹. Biodegradable polymers that contain complexes of CDs and antibiotics have been prepared and show promise for the fabrication of bacteria-resistant films and fibres^{110–112}. On the basis of initial findings, it is likely that further polymer–CD combinations will be produced. An extension of these combinations would be to use CD-containing polymers, which are described in the next section.

New CD derivatives continue to be reported¹⁰⁷. Recently, CDs that contain quaternized amine groups have been investigated as multifunctional agents in ophthalmic formulations¹¹³. Previously, CDs have been used in eye drops (TABLE 1). These solutions contain quaternary ammonium compounds as antibacterial agents. The CDs can bind to these agents and render them less effective. The CDs that contain quaternized amines have the effect of solubilizing the drug and function as an antibacterial agent¹¹³.

CD-containing polymers

CD-containing polymers have been around for decades, and their use in pharmaceutical applications has been explored since the 1980s^{114–118}. FIGURE 6 illustrates the different classes of CD-containing polymers.

The initial polymer types to be prepared were those that possessed a crosslinked structure (FIG. 6a), and CDs

Table 2 | **Examples of cyclodextrin pendent polymers**

Type of polymer	Cyclodextrin	Preparation method	References
Polyacrylic esters	Alpha, beta	Polymerization of vinyl cyclodextrin derivatives	124,125
Poly(allylamine)s	Beta, gamma	Grafting of cyclodextrin to preformed polymer	126,127
Polymethacrylates	Alpha, beta, gamma	Polymerization of cyclodextrin methacrylate monomers	128
Chitosan	Alpha, beta	Grafting of cyclodextrin to preformed polymer	129,130
Polyester	Beta	Grafting of cyclodextrin to preformed polymer	131
Polyethylenimine	Beta	Grafting of cyclodextrin to preformed polymer	132,133
Dendrimers*	Alpha, beta, gamma	Grafting of cyclodextrin to preformed dendrimer	134,137

*Polyamidoamine and polyethylenimine.

were first crosslinked with epichlorohydrin¹¹⁹. Some of these randomly crosslinked polymers are water soluble; for example, epichlorohydrin-crosslinked β -CD has higher aqueous solubility than β -CD itself. These materials were the initial CD-containing polymers to be investigated for drug delivery applications^{115–117}. In addition to epichlorohydrin, diepoxides and diisocyanates have been used as crosslinking agents. Work continues on these types of polymers for inclusion complex formation and drug delivery applications. Randomly crosslinked polymers were recently prepared using β -CD and diisocyanates, and resulted in inclusion formation constants as high as 10^9 M^{-1} for guest molecules that typically have values around 10^3 M^{-1} with individual CDs¹²⁰. These nanoporous polymers, which demonstrated large formation constants, illustrated the dramatic enhancements that can be obtained when CDs are organized into supramolecular entities.

Disinfecting drugs have been incorporated into CD polymer beads for use in wound and burn treatments¹¹⁶. The CD polymer in this case enables controlled release of the drug without causing any inflammation of the surrounding tissue. Recently, *in situ* forming gels that consist of alkyl-chain-modified dextrans and epichlorohydrin-crosslinked, CD-containing polymers loaded with tamoxifen were reported¹²¹. The gel forms when the two polymers come into contact with one another and the alkyl side chains on the dextran form inclusion complexes with the CDs. A zero-order release profile for tamoxifen was achieved with these polymers¹²¹. Cationic forms of these types of crosslinked, CD-containing polymers have also been investigated for drug delivery applications¹²². Crosslinked polymers containing drugs have also been formulated with other polymers (dextrans with grafted alkyl chains) that can form gels through inclusion complex formation (alkyl chain-CD) for slow release of the drug¹²³.

Other classes of CD-containing polymers are those that contain CDs as pendent moieties of the polymer backbone (FIG. 6b). These types of polymers have been prepared using various polymer backbones and functionalized CDs. TABLE 2 lists some examples of pendent polymers that have been synthesized. A sub-class of pendent polymers are CDs attached to dendrimers^{134–137}. Single¹³⁵ or multiple CDs^{134,137} can form pendent

groups on dendrimers of varying composition^{134,135} and dendrimer generation¹³⁶. In addition, dendrimers can be synthesized from the surface of CDs^{23,24}. Other types of CD-containing polymers are those that are the core of a star polymer (multi-branch analogues¹⁴⁰ of single arms prepared as dendrimers^{138,139}). These polymers can demonstrate cooperativity in binding guest molecules^{141,142}, and can have binding coefficients greater than, or equal to, those of randomly crosslinked CD polymers¹³⁶.

As a result of the generality in terms of polymer type, flexibility, size and chemistry, the pendent polymers seem to be excellent candidates for drug delivery vectors. Control of molecular mass and the distribution, hydrophobicity, solubility, charge, binding of drug and so on are necessary for use in systemic drug delivery applications. The ability to tune the properties of pendent polymers should facilitate their use in drug delivery applications. As with randomly crosslinked polymers, pendent polymers bind drug molecules via inclusion complex formation; the binding and controlled release of the drug will therefore be dictated by the polymer structure.

Additional types of CD-containing polymers are those that possess a tubular structure (FIG. 6c). The cylinders can be formed by using molecules that organize the CDs into a tubular configuration, such as cholesterol¹⁴³. The polymers are then formed from the tubular configurations by crosslinking between the CDs. The tubular structures need not be isolated as shown in FIG. 6c, but can form bundles because the crosslinking can occur not only in the axial direction of the tube, but perpendicular to that direction as well. The class of tubular polymers is really an ordered, sub-class of crosslinked polymers, and the two types of polymers might therefore show similar behaviour when used as drug delivery vehicles.

Davis and co-workers created a class of polymers that are linear and have CD as part of the backbone (FIG. 6d)^{144–152}. The key to success in preparing this type of CD-containing polymer is the synthesis of a difunctionalized CD-containing co-monomer. FIGURE 7 illustrates the strategy of preparing either cationic or anionic polymers starting from diiodo- β -CD.

Linear β -CD-containing anionic polymers have been used to create conjugates with small-molecule drugs (see FIG. 7 for illustration with camptothecin)^{151,152}. Polymer

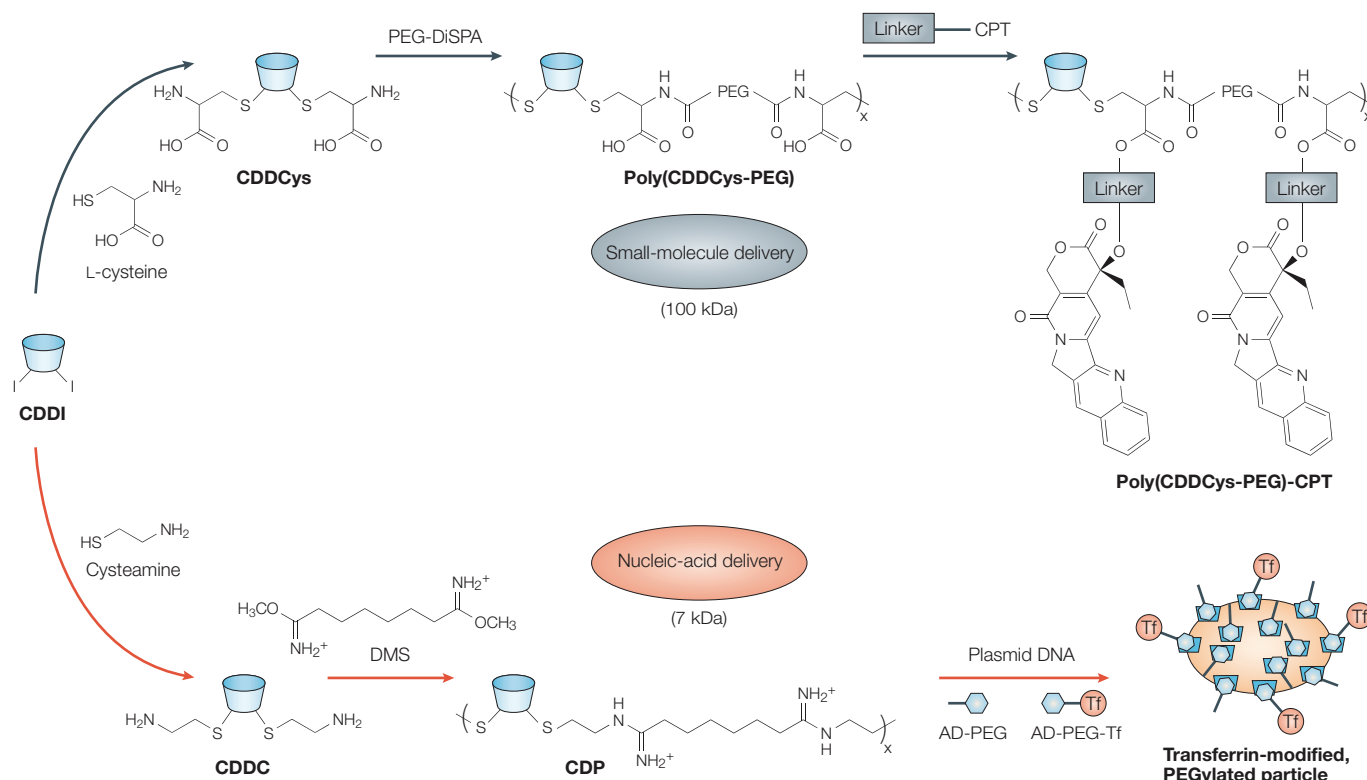


Figure 7 | Examples of linear β -cyclodextrin-containing polymers and their use in compositions with small-molecule (top pathway) and nucleic-acid (bottom pathway) therapeutics. AD-PEG and AD-PEG-Tf denote adamantane conjugated to polyethylene glycol (PEG) and adamantane and transferrin conjugated to PEG, respectively.

size was shown to be crucial to the antitumour efficacy of this conjugate: ~100 kDa polymers outperformed ~40 kDa polymers in mouse xenograph models¹⁵².

Gene delivery

The use of CD-containing polymers for gene delivery began in 1999 (REF. 144). Since that time, numerous CD-containing polymers have been used as delivery vehicles for nucleic acids. A common feature of all CD-containing materials for nucleic-acid delivery is that they are polycations. TABLE 3 summarizes literature data relating to both polymeric and individual CD-containing polycations that have been used to form particles with nucleic acids. So far, the most extensive investigations have been reported on the linear, CD-containing polycations, and a summary of this work has been published¹⁵³. One important feature that is observed with CD-containing polycations is their low *in vitro* and *in vivo*^{133,144} toxicities compared with other non-CD-containing polycations, such as poly-L-lysine (PLL) and polyethylenimine (PEI). Although this feature is not unique to CD-grafted polymers (for example, galactose¹⁵⁹ and other acetylating agents grafted onto PEI^{160,161} reveal *in vitro* toxicities below that of the parent PEI), it is a feature that has been broadly observed and is likely to be germane to other polycations. Although the reduction in charge on the polycations might have some role in lowering the toxicity, it is clear that it cannot be the only factor. That is because polycations with the same charge and separation

distances between the charge centres show increasing toxicity when the backbone contains CDs, trehalose or $-\text{CH}_2-$, respectively^{147,153}. Linear CD-containing polycations have not been observed to enter the nucleus of cells using techniques such as confocal microscopy, and their lack of nuclear localization might contribute to their low toxicity.

A feature unique to the CD-containing polycations is that they can be modified by compounds capable of forming inclusion complexes. Pun and Davis showed that the surface of particles formed from linear, CD-containing polycations and nucleic acids (plasmids¹⁴⁶, oligonucleotides¹⁵⁴) could be decorated with polyethylene glycol (PEG)-containing compounds by terminating the PEG chains with adamantane (AD) that form inclusion complexes with CDs on the particle surfaces (FIG. 7, bottom pathway). This approach has been extended to CD-grafted PEI¹³³ and shown to produce delivery vehicles that can target tissues (tumour and liver for instance) in mice with either plasmids^{133,153} or oligonucleotides¹⁵⁴. This type of formulation is completely self-assembled¹⁵³ and is unique to CD-containing polycations. Although the initial demonstrations of this methodology exploited CD-containing polycations, it is also applicable to CD-containing amphiphiles that form vesicles¹⁵⁶, and polycations that are formed from randomly crosslinked CD-containing polymers (FIG. 6a) with inclusion complexes of cationic molecules, such as oleylamine^{157,158}.

Table 3 | **Examples of cyclodextrin-containing polymers used for the delivery of nucleic acids**

Type of polycation	Cyclodextrin type	Nucleic-acid type	References
Linear, cyclodextrin in polycation backbone	Beta	Plasmids	144–150,153,154
Linear, cyclodextrin in polycation backbone	Gamma	Plasmids	149
Linear, cyclodextrin in polycation backbone	Beta	Oligonucleotides	153,154
Linear & branched PEI with grafted cyclodextrin	Beta	Plasmids	133
Polyamidoamine dendrimers with grafted cyclodextrin	Alpha, beta, gamma	Plasmids	135–137
Individual cyclodextrin containing multiple amine groups	Beta	Plasmids	155
Individual cyclodextrin containing amphiphilic molecules that form vesicles	Beta	Plasmids	156
Randomly crosslinked cyclodextrin polymer and cationic molecule	Beta	Plasmids	157,158

Conclusion

Although the existence of CDs and their use in the pharmaceutical industry has been documented for decades, it is only recently that their exploration in applications reaching beyond the solubilization and stabilization of small molecules has occurred. The large-scale commercialization of functionalized CDs has greatly expanded the properties of available CDs, and so it is not surprising that these newer variants are finding widespread application. It will be interesting to see whether CDs will find new commercial applications in the pharmaceutical industry with respect to macromolecules such as proteins and nucleic acids.

CD-containing polymers are used in areas outside of the pharmaceutical industry and have been a research curiosity in drug delivery for quite some time. Recently, it seems that there are serious efforts to

use CD-containing polymers for drug delivery and it has been speculated that they could reach clinical application in 2005.

Gene therapy continues to generate interest. With the reported difficulties of viral gene delivery, non-viral methods are being further explored. CD-containing polycations have unique properties that could be useful in the non-viral delivery of nucleic acids. These materials show promise for gene delivery in animals, although their utility in humans remains to be proven.

The future of CDs and CD-containing polymers in the pharmaceutical industry seems to be bright. There are numerous traditional and non-traditional applications that are on the horizon for commercialization. New uses of CDs are likely to be explored as the properties of CDs are expanded and the number of commercialized and FDA-approved variants increases.

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Competing interests statement

The authors declare **competing financial interests**: See web version for details.

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