

PHARMACEUTICAL PREFORMULATION AND FORMULATION

**A Practical Guide from
Candidate Drug Selection to
Commercial Dosage Form**

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Editor



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Biopharmaceutical Support in Formulation Development

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The pharmaceutical formulation plays an important role in the delivery of a drug to the body. The clinical benefit of a drug molecule can thereby be optimised by delivering the right amount at the right rate to the right site at the right time. For example, extended-release (ER) formulations have been used for a long time to control the rate of absorption and thereby keep drug levels within the therapeutic interval during an entire dosage interval. More examples of biopharmaceutical properties that can be provided by oral formulations are given in Table 7.1. In the future, the pharmaceutical possibilities for improving clinical utility may be extended to include site-specific drug delivery systems that reach systemic targets, such as cancer cells and the central nervous system (CNS), or gene delivery to cell nuclei. Such areas of drug delivery are, however, outside the scope for the present chapter.

In order to achieve the potential clinical benefits that can be provided by a formulation, as exemplified in Table 7.1, biopharmaceutical input is needed from the start of preformulation, through formulation development, to documentation for regulatory applications. The main objective is to obtain and verify desirable drug delivery properties for a pharmaceutical formulation. The key activities are as follows:

- Characterisation of relevant physicochemical, pharmacokinetic/dynamic prerequisites provided by the drug molecule
- Identification of the relevant biopharmaceutical targets and hurdles in formulation development

- Definition of test methods/study designs needed to obtain the biopharmaceutical targets in the formulation development and correct interpretation of the study results obtained
- Choice of suitable drug form, formulation principles and excipients

In addition, understanding of the physiological processes that may interact with the biopharmaceutical function of the dosage form is crucial.

Successful biopharmaceutical input during development can make a significant contribution to clinical efficiency and tolerability of a drug product. In certain cases, such as poorly absorbable drugs or drugs that are degraded in the gut, the biopharmaceutical aspects can make the difference between a new useful product or an aborted development programme of a potentially very useful drug compound. Additionally, appropriate use of biopharmaceutics will also contribute to a time and cost-efficient development process.

The present chapter is limited to presentations and uses of different biopharmaceutical test methods in formulation development, such as

- *in vitro* dissolution testing,
- bioavailability studies,
- *in vitro/in vivo* (IVIVC) correlation of drug dissolution,
- use of animal models in *in vivo* studies of formulations and
- *in vivo* imaging of formulations by gamma scintigraphy.

This chapter is strongly focussed on oral drug delivery. The relevant principles and methods involved in biopharmaceutical characterisation of a drug molecule, mainly applied in the preformulation phase, are described in Chapter 4, "Biopharmaceutical Support in Candidate Drug Selection".

Table 7.1
Examples of biopharmaceutical properties of oral dosage forms.

Biopharmaceutical Target	Formulation Function
Increase amount absorbed/ reduced variability of amount absorbed	Dissolution or permeability enhancement Protection from degradation in GI tract
Control rate of absorption	Extended release Pulsed release
Control site of delivery	Gastric retention Colon release Mucoadhesive

IN VITRO DISSOLUTION

In vitro dissolution testing of solid dosage forms is the most frequently used biopharmaceutical test method in formulation development. It is used from the start of dosage form development and in all subsequent phases. Examples of different purposes of dissolution testing in research and development are as follows:

- Investigation of drug release mechanisms, especially for ER formulations
- To obtain a predefined target release profile and robust formulation properties regarding influences of physiological factors (e.g., pH and food) on the drug release
- Generation of supportive data to bioavailability studies as an aid in interpretation of *in vivo* results
- Validation of manufacturing processes
- Investigation of effects of different storage conditions
- Batch quality control (QC)
- A surrogate for bioequivalence studies

An *in vitro* dissolution method for batch QC is always defined for a new solid dosage form product. However, this method may not be sufficient for all the different aims of dissolution testing that might arise. The choice of dissolution method and test conditions should therefore be adapted to best serve their purpose. For example, simplicity and robustness are crucial properties of a QC method; whereas physiological relevance may overrule these factors when a method is used for *in vivo* predictions.

Standard *in vitro* dissolution testing models two processes; the release of drug substance from the solid dosage form and drug dissolution. Drug release will be determined by formulation factors such as disintegration/dissolution of formulation excipients or drug diffusion through the formulation. Drug dissolution will be affected by the physicochemical substance properties (e.g., solubility, diffusivity), solid-state properties of the substance (e.g., particle surface area, polymorphism) and formulation properties (e.g., wetting, solubilisation). *In vitro* dissolution testing should thus provide predictions of both the drug release and the dissolution processes *in vivo*. Therefore, in most situations, the use of *in vitro* dissolution will be meaningless if the method used does not provide some correlation with *in vivo* data or resemblance with the physiological conditions in the gastro-intestinal (GI) tract. In order to reach this goal, the choice of dissolution apparatus and test medium should be carefully considered. Another important aspect in the development and definition of a new method is that it must be designed and operated in such a way that drug release and dissolution are not sensitive to minor variations in the operating conditions.

This chapter will provide some practical considerations for developing and using *in vitro* dissolution methods. Aspects of study design and evaluation of *in vitro* dissolution data will also be discussed. For additional information on *in vitro* dissolution testing, the "FIP Guidelines for Dissolution Testing of Solid Oral Products" (1997), *Handbook of Dissolution Testing* (Hansson 1991), pharmacopoeias and regulatory guidelines are recommended.

Dissolution Apparatus

The most well-established apparatuses are those described in the pharmacopoeias. Four methods, mainly intended for oral solid dosage forms, are described in the U.S. Pharmacopeia (USP) XXIV: the rotating basket method (USP I), the rotating paddle method (USP II), the reciprocating cylinder (USP III) and the flow-through method (USP IV). All of these methods, except for the reciprocating cylinder, are also described in the European Pharmacopoeia (EP), although the equipment specifications are not identical to those in the USP. These methods are schematically presented in Figures 7.1 a–d.

- **USP I.** The dosage form is placed in a cylindrical basket that is covered by a mesh. The basket is immersed in the dissolution medium and rotated at a speed of between 25 and 150 rpm. The standard beaker has a volume of 1 L, but 4 L vessels are also available. The mesh size in the basket wall can also be varied.
- **USP II.** The dosage form moves freely in the same type of glass beaker as used for USP I. A paddle is rotated at a speed of 25 to 150 rpm. The dosage form may be placed in a steel helix in order to avoid floating.
- **USP III.** The formulation is placed in a cylindrical glass tube with steel screens in the bottom and the top. The mesh size of the tubes may vary. This tube is moved up and down in a larger tube that contains the dissolution fluid. The amplitude of the inner tube movements is 5–40 dips/min, and the volume of the outer tube is 300 mL. Tubes containing 100 mL and 1 L are also available. The inner tube can be moved during the dissolution process between different outer tubes, which may hold different dissolution fluids.
- **USP IV.** The formulation is placed in a thermostated flow-cell. The dissolution fluid is pumped through the cell in a pulsating manner at a constant rate, typically between 4 and 16 mL/min. Before the inlet flow reaches the formulation, it is passed through a bed of glass pellets to create a laminar flow. A filter is placed in the cell at the outlet side of the formulation. The cell is available in different sizes/designs, and tablet holders are available as an option.

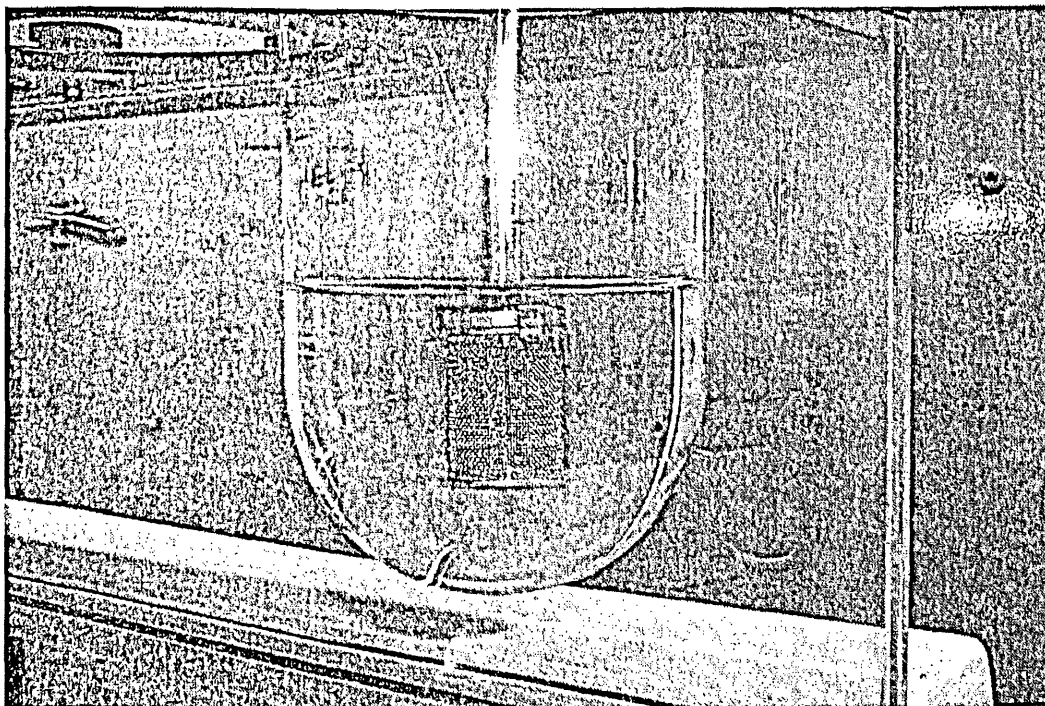
USP XXIV and the EP describe four additional apparatuses mainly intended for transdermal or dermal delivery: the paddle over disc (USP V, EP), the extraction cell method (EP), the cylinder method (USP VI, EP) and the reciprocating holder method (USP VII). A large number of other non-compendial methods have been described. Most of them could be categorised as

- modified USP methods,
- rotating flask methods (Koch 1980) and
- dialysis methods (El-Arini et al. 1990).

An example of a commercially available (VanKel, Cary, N.C., USA) alternative to the standard USP II method is one that consists of a glass vessel that has been modified by introducing a peak in the bottom (see Figure 7.2). This modification has been introduced to create appropriate stirring in all parts of the vessel and thereby avoid formation of poorly agitated heaps of undissolved material.

Figure 7.1 Different dissolution apparatuses:

(a) the rotating basket (USP I) dissolution apparatus.



(b) The rotating paddle (USP II).

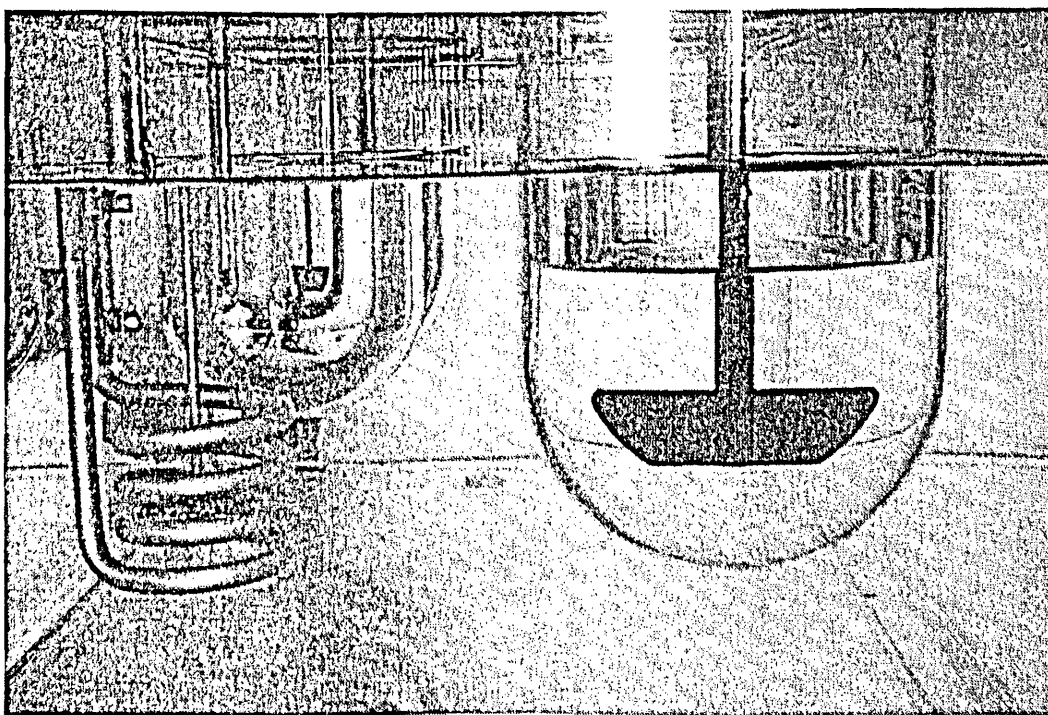
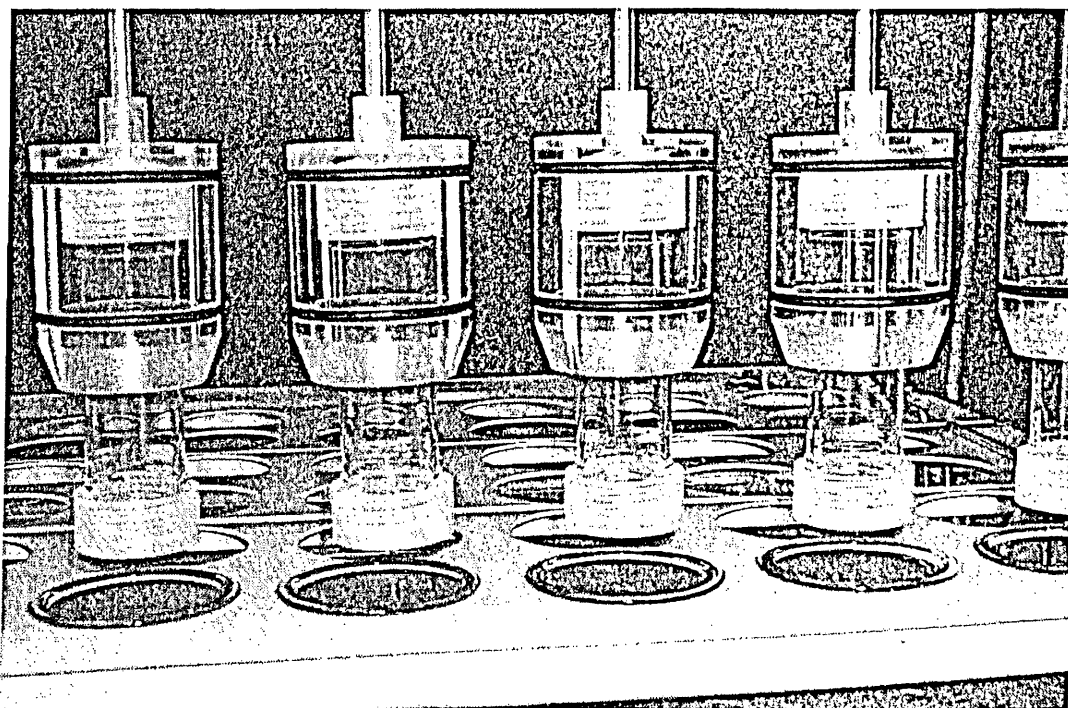


Figure 7.1 continued

(c) The reciprocating cylinder (USP III) dissolution apparatus.



(d) Flow-through cell (USP IV).

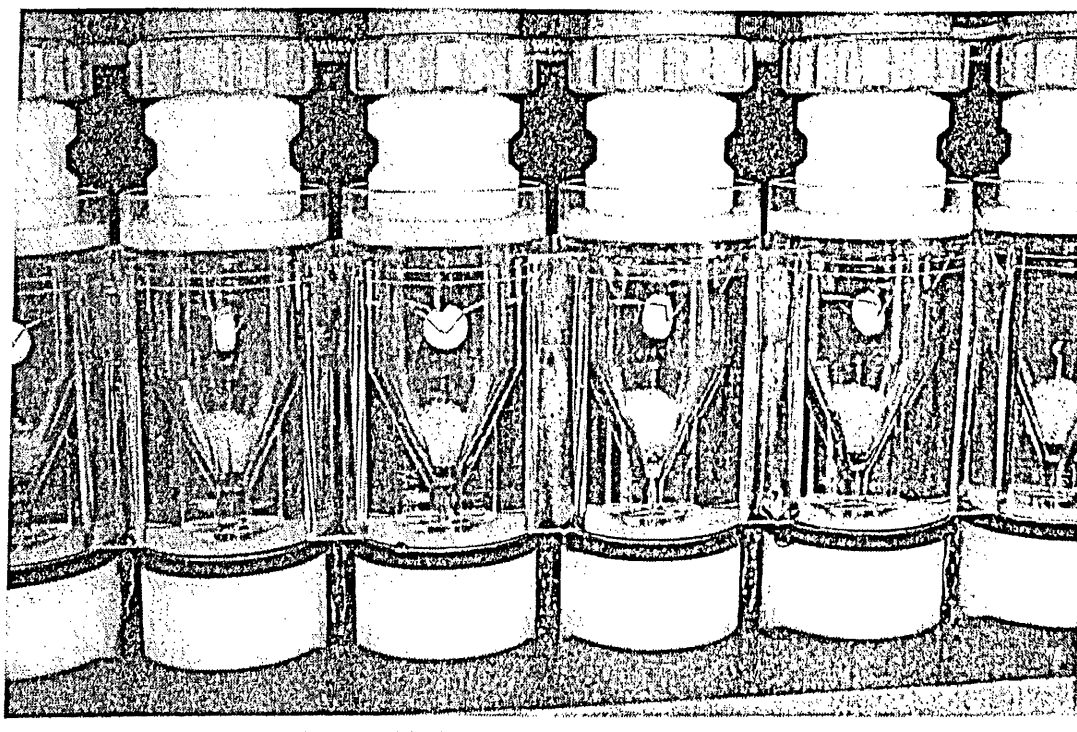
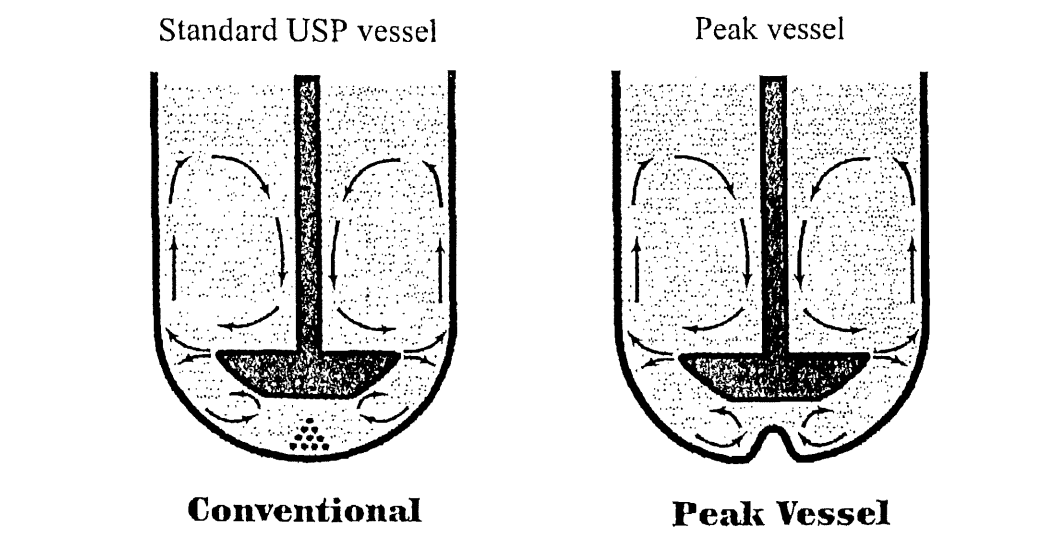


Figure 7.2 Standard and modified (peak vessel) USP II dissolution apparatuses including illustrations of the different flow patterns within the beakers and photographs taken at a paddle stirring rate of 100 rpm showing a heap of pellets beneath the paddle in the standard method compared to the desirable dispersion of pellets in the modified method.



The choice of dissolution apparatus will be specific for each formulation, and the following factors should be considered:

- Correlation to *in vivo* data
- Risk for hydrodynamic artefacts
- Regulatory guidelines
- Drug solubility
- Need to change the dissolution medium during dissolution testing
- Ease of operation, in-house know-how and suitability for automation

As a general guideline in the choice of dissolution test apparatus, the simplest and most well-established method should be chosen, with respect to both in-house know-how and regulatory aspects. In most cases, this is the USP II paddle method or the USP I rotating basket method. However, if satisfactory performance cannot be obtained by these methods, others should be considered. Primarily, the USP III and USP IV methods, and non-compendial methods could also provide relevant advantages.

Correlation of the *in vitro* dissolution to the *in vivo* dissolution is a crucial property of a dissolution test. The major difference in this respect between different apparatus is the hydrodynamic conditions. It has been argued for some of the methods, such as the USP IV flow-through cell or a rotating flask with baffles, that an *in vivo*-like situation is created in the

vitro test. However, this hypothesis has not been verified by any experimental means for any method, and it is clear that no apparatus mimics the full complexity of the motility patterns in the GI tract. A recommended approach is therefore to evaluate different apparatuses on a case-by-case basis using IVIVC studies (see "Bioavailability Studies", p. 257) to reveal which method provides the most desirable results.

The potential for hydrodynamic artefacts (e.g., floating, clogging of material to screens, adhesion to equipment of the formulation or variable flow conditions in the vicinity of the formulation due to other reasons) is strongly formulation dependent and thus has to be evaluated for each type of formulation. In order to detect artefacts, careful visual inspection of the dissolution test equipment is crucial. Video recordings can be used to aid such investigations.

The present regulatory guidelines in the United States and Europe propose the use of USP I and USP II as the methods of choice. Other methods, both compendial and non-compendial, could be acceptable, but the rationale for not using USP I and II must be clearly stated and supported by experimental data. In generic product development, complete dissolution methods, including the apparatus, are provided for many products in the USP and should thus be a first choice in a regulatory context. It should be noted, however, that this is not applicable in all cases. A dissolution method that is well functioning for a certain formulation type may provide high variability, artefacts or poor IVIVC for other dosage forms. Thus, in particular, for ER formulations or dosage forms containing dissolution enhancing principles, different dissolution tests may be needed for different formulations, although the drug substance is the same.

For sparingly soluble substances, the volume in standard vessel methods may not be sufficient to dissolve the dose. In this case, the USP IV flow-through method is beneficial, since it provides a continuous renewal of the dissolution fluid. However, the maximum flow rate will limit the apparent solubility in this procedure. Sufficient solubility will not be obtained for a rapidly releasing formulation of a drug with very low solubility in relation to the dose.

In certain cases, it is desirable to change the dissolution medium during the dissolution test. For example, a more physiologically relevant medium is desired with changes of the conditions (e.g. pH) corresponding to the differences along the GI tract. Both USP III and USP IV permit such changes without significant interruptions of the dissolution process.

Irrespective of the chosen apparatus, the equipment must be set up and handled in a way that both minimises the variability of the dissolution and avoids artefacts. The most common source to such variability or artefacts is hydrodynamic factors, but unwanted chemical reactions or temperature shifts could also occur. Alterations of hydrodynamics, as well as changes of temperature, can both affect the dissolution of a drug substance and the release of a substance from the dosage form. Chemical reactions in the test medium may cause degradation of the drug substance or some formulation excipient which may affect the dissolution, or may lead to misinterpretation of the results. Examples of different sources to variability for the USP apparatuses are summarised below:

- USP I—clogging of basket screen, positioning of basket
- USP II—adherence of formulation to the beaker wall, floating, "entrapment" of solid material in the stagnant area beneath the paddle, positioning of the paddle
- USP III—floating, adherence to tube wall or bottom screen, clogging of screens, disappearance of undissolved material through the screens
- USP IV—clogging of filter, variations in flow rate

Some other factors that potentially could cause problems are not specific for a certain apparatus. These general factors include vibrations, variations in agitation rate, impurities due to poor cleaning or to trace amounts of metals from dissolution equipment, variations in dissolution fluid components and poor quality of dissolution media components. Another factor relevant for lipophilic compounds is migration of the drug substance into fillers and plastic material.

Choice of Agitation Intensity

All compendial dissolution apparatus can be operated at different agitation intensities. The three most outstanding aspects to consider when deciding at which level the tests should be performed are

1. correlation to *in vivo* data,
2. variability of dissolution results and
3. regulatory guidelines and pharmacopoeial recommendations.

The U.S. regulatory agency recommends a stirring rate of 50–100 rpm for USP I and 50–75 rpm for USP II.

The above-proposed agitation intensities should be used if IVIVCs cannot be improved or the variability in dissolution data can be improved by other settings. The major problem associated with a too low agitation is that solid material is not sufficiently well dispersed, which will delay the dissolution. On the other hand, the possibility to discriminate between different formulations/batches with different dissolution properties might be lost at a too intensive agitation.

Sometimes a dissolution test is performed with the aim to investigate the robustness of the release properties towards potential changes of the physiological conditions *in vivo*. In this case, tests at different agitation intensities should be considered to model different intestinal motilities. The use of more than one apparatus may also be considered.

Choice of Dissolution Test Media

The choice of dissolution medium is highly dependent on the purpose of the dissolution study, but the following aspects should always be considered:

- Correlation to *in vivo* data
- Resemblance of physiological conditions in the GI tract
- Regulatory and pharmacopoeial recommendations
- Drug solubility and stability properties at different pH values
- Known sensitivity of the formulation function for different medium factors

Attainment of IVIVC is a key aspect in the choice of dissolution test medium. However, it is not recommended to choose a test medium based only on correlation to *in vivo* data. The dissolution test medium should also be relevant for the physiological conditions in the GI

tract. Components and physicochemical characteristics of the GI fluids that might be considered in the choice of dissolution medium were discussed in Chapter 4. The *in vivo* conditions are not static, but the fluids are constantly changing along the GI tract due to absorption of water and nutrients, secretions of enzymes, carbonate, salts and bile and digestive processes. It is therefore clear that it is not realistic to reconstitute the full complexity of the *in vivo* conditions in an *in vitro* test. An approach has to be taken where the most relevant factors are included, based on knowledge of the solubility of the drug substance and the release mechanism of the dosage form. Examples of some dissolution test media that have been proposed to be physiologically relevant are given in Table 7.2 (USP 2000; Dressman et al. 1998).

Another important aspect in the selection of a dissolution test medium is the need to consider the saturation solubility of drug in the test medium in relation to the drug dose tested. Drug dissolution will depend on the amount of drug in the solution if the dissolved amount of drug in the test medium approaches the saturation solubility. This can be understood from the Noyes-Whitney equation (see equation 1 in Chapter 4, "Biopharmaceutical Support in Candidate Drug Selection"); the dissolution rate will be affected by the drug concentration in the dissolution medium (C_t) if C_t is not much less than the saturation solubility (C_s). This is not a desirable situation since, if C_t controls the rate of dissolution, the test may not be discriminative for factors related to the formulation performance. Another disadvantage, if C_t is significant in relation to C_s , is that the dissolution rate will be dose dependent, and different results will be obtained for different strengths of the same formulation. Finally, it can be assumed, in most cases, that C_t does not affect the *in vivo* dissolution rate due to the continuous removal of drug from the GI lumen by the drug absorption process that keeps the drug concentrations in the GI tract at a level far below C_s . Consequently, it is desirable to choose a

Table 7.2
Examples of dissolution test media including physiological components.

USP simulated gastric fluid	2 g/L NaCl
	3.2 g/L pepsin
	0.06 M HCl
USP simulated intestinal fluid	0.05 M KH_2PO_4
	0.015 M NaOH
	10.0 g/L pancreatin
	pH adjusted to 6.8 by HCl or NaOH
Simulated gastric fluid—fasted state (Dressman et al. 1998)	0.01–0.05 M HCl
	2.5 g/L Na lauryl sulphate
	2 g/L NaCl
Simulated intestinal fluid—fasted state (Dressman et al. 1998)	0.029 M KH_2PO_4
	5 mM Na taurocholate
	1.5 mM lecithin
	0.22 M KCl
	pH adjusted to 6.8 by NaOH

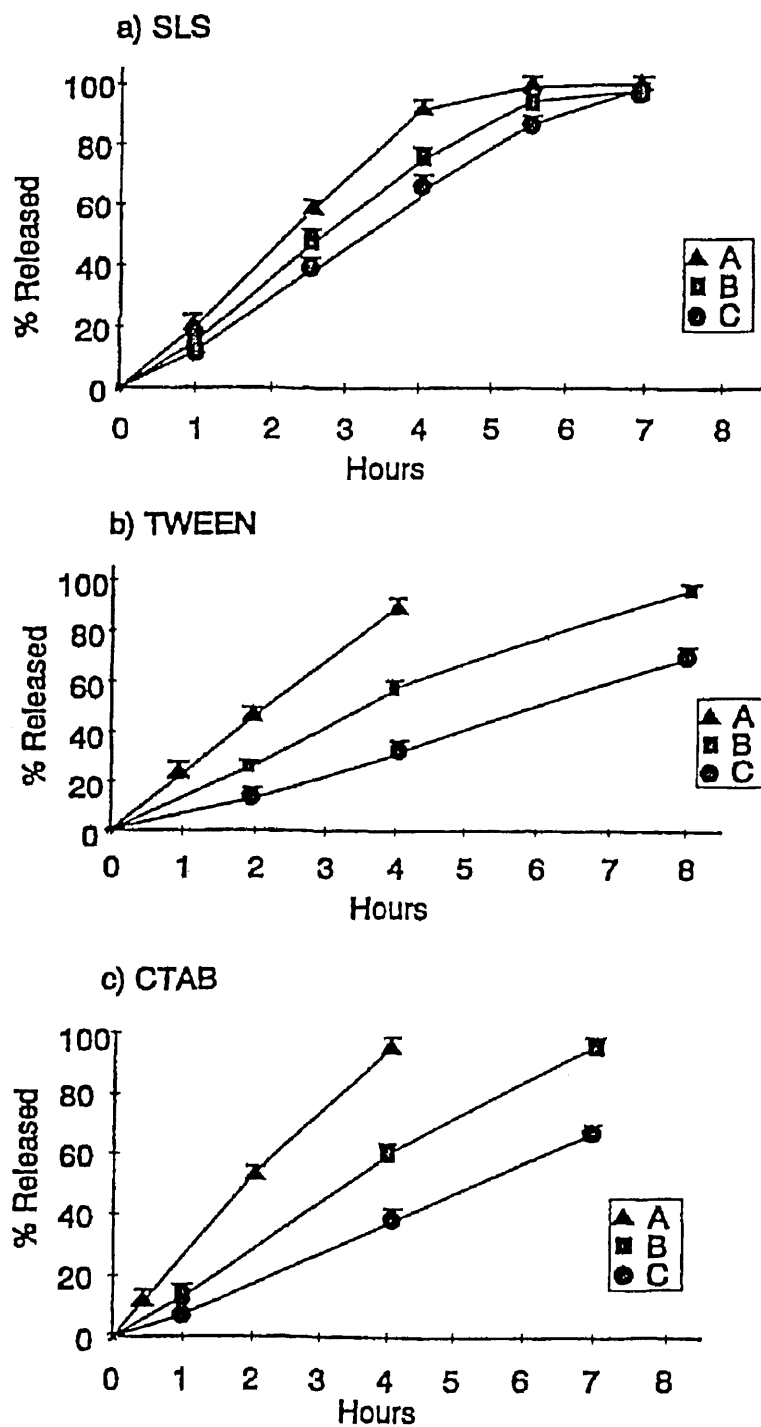
dissolution test method that provides a high enough saturation solubility to avoid dependence on C_t . C_s should be at least 3–5 times higher than C_t when the total dose has been dissolved. Such conditions have been termed “sink conditions”. In the first instance, maximum volume or flow of test medium should be used to obtain sink conditions. Adjustment of the pH to a level that provides optimal solubility should be considered for proteolytic drugs without neglecting the aspects of physiological relevance. For drugs with a very low solubility in relation to the administered dose, the above-described approaches will not provide sufficient drug solubility in the test media. In those cases, a surfactant should be added to the test medium in amounts above its critical micelle concentration (CMC) in order to solubilise the drug. The solubility can thereby be increased several hundred orders of magnitude. This approach is also favoured due to the occurrence *in vivo* of drug solubilising micelles formed in the presence of bile acids. However, the use *in vitro* of bile acids in standard methods is not recommended due to variations in quality and high costs, and it will still be almost impossible to simulate the *in vivo* complexity. Therefore, synthetic surfactants are the first choice, and sodium lauryl sulphate (SLS) has been especially recommended (Shah et al. 1989). Due to the risk of specific interactions between the formulation and SLS (of no *in vivo* relevance) or poor solubilisation capacity for certain drugs, other synthetic surfactants may be considered on a case-by-case basis. An example of the first case is illustrated in Figure 7.3 where the *in vitro* dissolution-time profiles of a poorly soluble compound, felodipine, are shown for three different hydrophilic matrix ER tablets (A–C) when three different surfactants (SLS, CTAB [cetyltrimethylammonium bromide], Tween) were used to obtain sink conditions (Abrahamsson et al. 1994). SLS interacted with the gel forming excipient, which led to much less of a difference in drug dissolution rate between the three different tablets, compared to the use of the other surfactants. It is also important to realise that attainment of sink conditions does not guarantee that the *in vitro* results correlate to the *in vivo* performance, due to other effects. The discriminating power of a dissolution test may be lost if the solubility of the drug is too favourable in the dissolution medium. For example, an *in vitro* dissolution method including SLS in amounts providing sink conditions was used to test three other felodipine ER tablets. No difference was obtained *in vitro* for the three tablets, which contained different forms of the drug substance, whereas one of the tablets provided almost no bioavailability *in vivo* due to poor dissolution (Johansson and Abrahamsson 1997). Thus, general recommendations of the amount and type of solubiliser to be used in an *in vitro* test medium may be misleading, and the test medium should preferably be based on correlation to relevant *in vivo* data for poorly soluble substances.

Based on the knowledge of the substance solubility, release mechanisms from the dosage forms and known interactions with key excipients, certain components may be of special importance to include or exclude in the dissolution test medium. This has to be considered on a case-by-case basis. Two examples are given below.

Example 1

Hard gelatine capsules have the potential to be cross-linked during storage, which leads to formation of non-water soluble capsules. However, this does not affect the *in vivo* dissolution due to the presence of enzymes that digest the gelatine. Thus, the presence of pepsin and pancreatin in simulated gastric and intestinal fluids, respectively, may be especially important in the dissolution testing of hard-gelatine capsules (Digenis et al. 1994).

Figure 7.3 *In vitro* dissolution-time profiles of a poorly soluble compound, felodipine, for three different hydrophilic matrix extended release tablets (A-C) when three different surfactants (SLS, CTAB, Tween) were used in the dissolution test medium at levels providing "sink conditions".



Example 2

The ionic concentration in the test medium can affect both the drug solubility and the release mechanism for modified-release formulations. One example of the latter case is hydrophilic gel matrix tablets, a type of ER tablet that forms a gel layer in contact with the GI fluids. Solutes will affect the hydration of the gel matrix and, thereby, affect the drug release rate.

It has been shown for such tablets that the correlation to *in vivo* data can be completely lost by use of inappropriate ionic compositions in the test medium (Abrahamsson et al. 1998a). For ER formulations with osmotically driven drug release, a decreased drug release rate approaching no release will occur for high ionic concentrations in the test medium, and misleading *in vitro* results may be obtained if a relevant ionic composition is not used (Lindstedt et al. 1989).

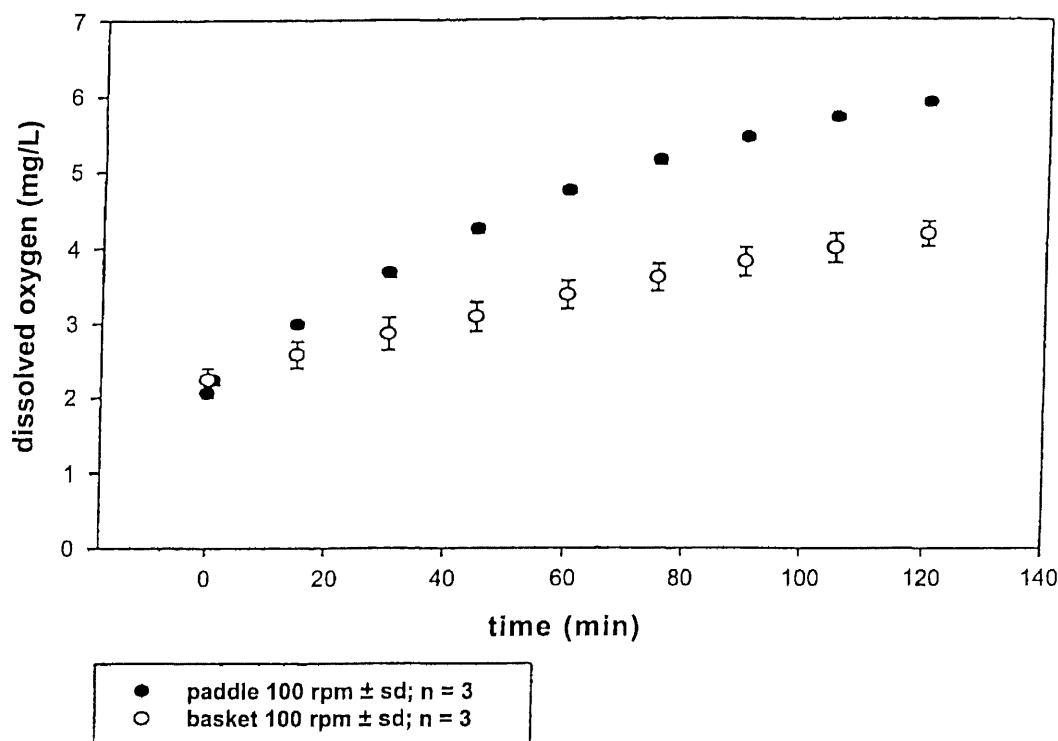
The concern for variability in dissolution results is of special significance when setting up a specification test method to be used for batch release but may also be considered for other tests. In order to reduce variability in dissolution results due to the test medium, the quality aspects of the dissolution media components that could affect the drug dissolution and release must be identified, and appropriate qualities of the components should be defined. This is especially important for the use of surfactants to provide micellar solubilisation in the test medium (Crison et al. 1997). Another potential source of variability is impurities in the components that may alter the solubility or catalyse degradation of labile drugs. It is also important to see that the dissolution test medium is stable, i.e., that the components are not degraded or precipitated during the dissolution test period. This is of no concern for plain buffer systems but is more relevant for complex media including physiological components.

Dissolved air in the dissolution medium could, under certain circumstances, be located as air bubbles on the surface of the dosage form or released solid material. This will clearly affect the dissolution process by reducing wetting and the available surface area for dissolution in an uncontrolled way. In order to avoid this problem, the dissolution media has to be deaerated. A method for deaeration based on heating and filtration can be found in USP XXIV. Other methods have also been described (Diebold and Dressman 1998). It is, however, important to realise that the reaeration of deaerated water is a rapid process. The oxygen content increases significantly during filling of the *in vitro* dissolution test vessel as well as during the dissolution experiment (Diebold and Dressman 1998) (see Figure 7.4).

Other Study Design Aspects

The design aspects of dissolution testing include, primarily, the choice of sampling intervals and number of tablets to be tested. Batch control often includes the testing of 6 individual units, whereas testing for regulatory purposes most often requires the testing of 12 individual units. In batch control of immediate-release (IR) formulations, one test point is most commonly used to assure complete dissolution (e.g., 80 or 85 percent of the total dose) at a specified time point (e.g., after 30–60 min), whereas three time points are required for ER formulations. For more slowly dissolving IR formulations, or poorly soluble drugs, a two-point dissolution test, i.e., sampling at two different times, has been proposed (FDA 1997b). For regulatory purposes, when *in vitro* dissolution is used as a surrogate for bioequivalence studies, multiple time point determinations may also be required for IR formulations; if not, complete dissolution is not obtained within 15 min. For ER formulations, a more frequent sampling schedule than three time points is recommended during development, or as surrogate for *in vivo* bioequivalence studies, to reveal the full character of the dissolution profile. For example, the biphasic release pattern or a significant lag phase may not be detected if too few samples are collected.

Figure 7.4 Oxygen concentration in deaerated dissolution test medium at different times in the USP I and II apparatuses at an agitation speed of 100 rpm.



Another design aspect of dissolution tests occurs when several parameters in the dissolution test method are varied. This could be the situation when looking for the best correlation to *in vivo* data, testing the robustness of the dissolution method or testing the robustness of the dissolution properties of a certain formulation towards different physiological factors. The traditional approach has been to vary one factor at a time, while keeping the others at a constant level. The main disadvantages of this design approach are the numerous experiments needed when many factors have to be investigated, and the risk of suboptimisation when there are interactions between different study variables. Statistical experimental design has been applied to dissolution testing during recent years as a method of reducing these problems. For full information regarding design and evaluation of such experiments, statistical textbooks such as *Statistics for Experimenters* (Box et al. 1978) are recommended, since the aim of the present chapter is only to provide a short introduction to this topic.

The basic principle of experimental design is to vary all factors concomitantly according to a randomised and balanced design, and to evaluate the results by multivariate analysis techniques, such as multiple linear regression or partial least squares. It is essential to check by diagnostic methods that the applied statistical model appropriately describes the experimental data. Unacceptably poor fit indicates experimental errors or that another model should be applied. If a more complicated model is needed, it is often necessary to add further experimental runs to correctly resolve such a model.

An example of a design aimed at validation of a dissolution method is given below (Gottfries et al. 1994). Seven factors were included, and each one was tested at two different levels plus one centre point (i.e., all factors are set at a level in the middle between the high and low levels). In this case, there were $2^7 = 128$ number of unique experiments that could be performed, excluding the centre point, in order to cover all possible combinations of the low and high level settings of the seven different factors. Such a large number of experiments is seldom practically and economically justified. However, in statistical design, it is possible to do fractional designs, i.e., a limited number of all possible experiments is chosen according to balanced design. In the present case, only 16 experiments, excluding the centre point, were performed, and the settings in all experimental runs are presented in Table 7.3. The centre point was replicated four times in order to assess experimental variability. The most common ways to present the results are shown in Figure 7.5 and 7.6. In Figure 7.5, the bars represent the effect on the amount of drug dissolved after 4 h of each variable when varying the setting from the centre to the upper level. The most predominant effects were provided by the stirring rate (St), temperature (T), ionic strength (Ion), the square of T, interaction between St and buffer volume (Buf) and interaction between T and Ion. Figure 7.6 is a surface response plot that displays the dissolution response at different levels of two selected variables while others are held constant at the mid-level. It is also possible to use an obtained model to predict dissolution results for any experimental setting within the tested domain. In this case, dissolution profiles were simulated for all possible combinations of settings within a series of predetermined limits in order to determine acceptable limits for methodological variation.

Examples of applications of statistical designs for optimising correlations with *in vivo* data and for the testing of a formulation under different experimental conditions to elucidate the sensitivity of the drug release towards different physiological factors have also been published (Abrahamsson et al. 1999; Abuzarur-Aloul et al. 1997).

Assessment of Dissolution Profiles

It is often desirable to present the dissolution results by some response variable. The most common descriptor is the amount dissolved at a certain time point. For rapidly dissolving dosage forms, it may be sufficient to provide the amount dissolved, for example, at 15 or 30 min. For dosage forms where it is relevant to study the whole profile, more sophisticated methods are needed, since the use of a single point neglects all other data points.

Any model can be applied to *in vitro* dissolution data and fitted by linear or non-linear regression, as appropriate. Sometimes a first-order model [$A(t) = A - Ae^{-kt}$ where $A(t)$ is the amount dissolved after time t , A is the initial amount and k is the first-order dissolution rate constant] or even a zero-order model ($A(t) = A - Akt$) is sufficiently sophisticated to determine a dissolution rate that is representative for the whole process. However, a more general equation that is commonly applied to dissolution data is the Weibull equation (Langenbucher 1976):

$$A(t) = A_{\infty} \times \left[1 - e^{-\left(\frac{t-t_{\text{lag}}}{\tau_d}\right)^b} \right] \quad (1)$$

where $A(t)$ is the amount dissolved at time t , A_{∞} is the final plateau level of amount dissolved in the dissolution-time curve and t_{lag} is the duration of an initial period of no dissolution. τ_d

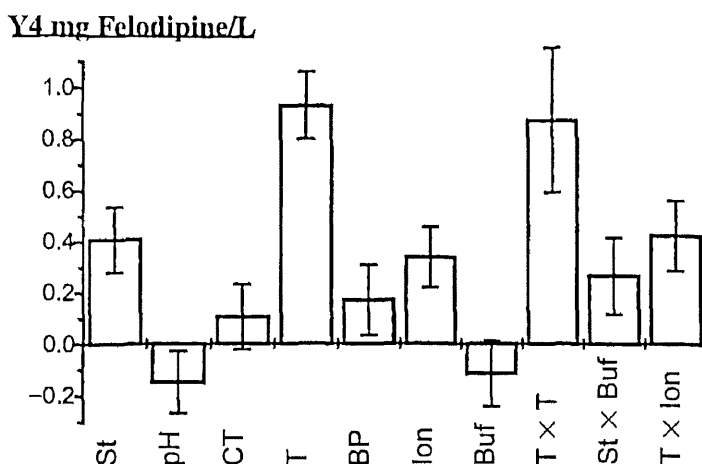
Table 7.3
Worksheet illustrating a statistical experimental design for
evaluating the effect on the dissolution of variations of the
test conditions in an *in vitro* dissolution method.

Exp. No.	Stirring Sp. (rpm)	pH	Conc. of CTAB (%)	Temp. (°C)	Basket Pos. (cm)	Ionic Strength of Buffer (M)	Buffer Vol. (mL)
1	110.0	6.0	0.50	34.0	2.0	0.05	510.0
2	110.0	6.0	0.30	34.0	2.0	0.15	490.0
3	110.0	7.0	0.50	34.0	0.0	0.15	490.0
4	110.0	7.0	0.30	34.0	0.0	0.05	510.0
5	110.0	7.0	0.50	40.0	2.0	0.15	510.0
6	110.0	6.0	0.50	40.0	0.0	0.05	490.0
7	110.0	7.0	0.30	40.0	2.0	0.05	490.0
8	110.0	6.0	0.30	40.0	0.0	0.15	510.0
9	100.0	6.5	0.40	37.0	1.0	0.10	500.0
10	100.0	6.5	0.40	37.0	1.0	0.10	500.0
11	100.0	6.5	0.40	7.0	1.0	0.10	500.0
12	100.0	6.5	0.40	37.0	1.0	0.10	500.0
13	100.0	6.5	0.40	37.0	1.0	0.10	500.0
14	90.0	7.0	0.50	34.0	2.0	0.05	490.0
15	90.0	6.0	0.50	34.0	0.0	0.15	510.0
16	90.0	6.0	0.30	34.0	0.0	0.05	490.0
17	90.0	7.0	0.30	34.0	2.0	0.15	510.0
18	90.0	6.0	0.30	40.0	2.0	0.05	510.0
19	90.0	7.0	0.30	40.0	0.0	0.15	490.0
20	90.0	7.0	0.50	40.0	0.0	0.05	510.0
21	90.0	6.0	0.50	40.0	2.0	0.15	490.0
A1	110.0	7.0	0.50	40.0	0.0	0.15	490.0
A2	90.0	6.0	0.30	40.0	0.0	0.05	490.0
A3	110.0	7.0	0.50	40.0	0.0	0.05	510.0
A4	90.0	6.0	0.50	34.0	0.0	0.05	510.0
A5	90.0	7.0	0.50	34.0	2.0	0.15	490.0
A6	100.0	6.5	0.40	34.0	1.0	0.10	500.0
A7	100.0	6.5	0.40	40.0	1.0	0.10	500.0
A8	100.0	6.5	0.40	37.0	1.0	0.10	490.0
A9	100.0	6.5	0.40	37.0	1.0	0.10	510.0

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is a rate parameter, more specifically it is a scale factor of the time axis. Two curves differing only in τ_d appear as being stretched or compressed along the time axis. Finally, β is a parameter that characterises the shape of the curve. At β values of 0 and 1, the dissolution-time curve follows zero- and first-order kinetics, respectively.

Figure 7.5 Scaled and centred coefficients for felodipine *in vitro* dissolution after 4 h (Y₄). The height of the bars illustrates the change in response estimated for a relative increase of each factor from the mid-point level to the high level in the factorial design. The different factors were stirring speed (St), pH, concentration cetyltrimethylammonium bromide (CT), temperature (T), basket position (BP), ionic strength (Ion), buffer volume (Buf), square of T (T × T), interaction terms between St and Buf (St × Buf) and between T and Ion (T × Ion).



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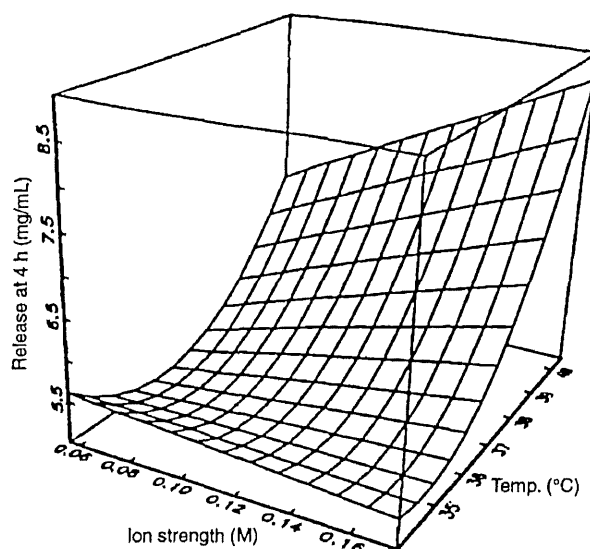
Another approach to obtain a parameter that describes the dissolution rate is to use statistical moments to determine the mean dissolution time (MDT) (von Hattingberg 1984). This method has the advantage of being applicable to all types of dissolution profiles, and it does not require fitting to any model. The only prerequisite is that data points are available close to the final plateau level. The MDT can be interpreted as the most likely time for a molecule to be dissolved from a solid dosage form. In the case of zero- and first-order dissolution processes, the MDT corresponds to the time when 50 and 63.2 percent have been released, respectively. The MDT is determined from:

$$\text{MDT} = \frac{\sum t_i \times \Delta M_i}{M_0} \quad (2)$$

where t_i is the midpoint of the i th time period during which the fraction ΔM_i has been released and M_0 is the final amount released. The length of each time period is given by the sampling intervals.

A special case occurs when two dissolution profiles are compared. This is often the case when a change has been introduced in the composition, manufacturing process or manufacturing site. The aim is then to maintain the same dissolution properties as for the original version. Such comparisons of dissolution profiles are performed by calculating a similarity factor, f_2 , which is calculated as follows from cumulative mean data (Shah et al. 1998):

Figure 7.6 Response surface of felodipine dissolved *in vitro* after 4 h expressed as drug concentration at different ionic strengths and temperatures.



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$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (3)$$

where n is the number of time points in the dissolution-time curve and R_t and T_t are the cumulative amount dissolved at time t for the reference and test formulation, respectively. The number of time points, n , should be at least 3, but only including one value close to the final plateau level (≥ 85 percent). From a regulatory perspective, f_2 -values greater than 50 ensure that the profiles are similar enough (FDA 1997b) and if $f_2 = 100$, the test and reference products have identical dissolution-time curves. An f_2 -value of 50 corresponds to an average difference between the test and reference curves of 10 percent. This test is not relevant for very rapidly dissolving formulations (i.e., complete release within 15 min), since differences in the dissolution profiles between formulations that are so rapid is of no *in vivo* relevance.

Validation of *In Vitro* Dissolution Methods

High quality and valuable *in vitro* dissolution tests are obtained by a rational design of test method as described above. However, there are different means to validate the method, i.e., to verify that the method functions as intended. These include

- dissolution tests with USP calibrator tablets,
- robustness testing and
- comparison with *in vivo* results.

Disintegrating as well as non-disintegrating USP calibrator tablets are available with a specified dissolution profile for each apparatus. These tablets are used to control the dissolution apparatus and to allow it to operate as intended, i.e., so that the hydrodynamic conditions are satisfactory. However, it should be noted that certain formulations might be more sensitive to such factors than are the calibrator tablets.

Another important aspect in validation of a new dissolution method is to investigate how sensitive the dissolution results of the product, for which the method has been developed, are for minute variations in operating conditions. Examples of factors to consider in such a test are temperature of test medium, rotational speed, volume, sampling procedure, medium compositions and testing performed by different operators. Based on such robustness tests of the method, limits can be defined for acceptable variations of test conditions. Statistical design may be useful to apply in situations such as those demonstrated earlier in this chapter.

Comparison of *in vitro* dissolution results with corresponding *in vivo* data for different formulations in order to verify that the *in vitro* methods predict the *in vivo* dissolution properties (see the section “*In Vitro/In Vivo Correlations*”) is preferably used already in the design of the test method. However, if not so, the *in vivo* validity of the method should be investigated at a later stage, especially for modified release formulations and poorly soluble drugs.

BIOAVAILABILITY STUDIES

In bioavailability studies, the drug plasma concentrations, and potentially the amount of drug/metabolites in urine, are followed over an appropriate time interval after administration in order to derive drug absorption parameters. There are basically two different aspects of the function of the formulation that can be evaluated: (a) rate of drug dissolution and/or release and (b) extent of drug that is made available for absorption.

The drug dissolution or release rate will directly determine the absorption rate in cases where this is the rate-limiting step in the absorption process. The formulation may also affect the extent of absorption, for example

- when dissolution enhancers are used for poorly soluble compounds
- when absorption enhancers are used for poorly permeable drugs
- when the drug release is incomplete from the formulation
- when the formulation provides a means of avoiding degradation in the GI tract.

The importance and need to study these factors increases if the substance has problematic absorption properties, or if the aim is to develop an advanced formulation, such as a modified release product, or if a dosage form affects the biopharmaceutical properties in any other way.

Bioavailability studies are performed during formulation development at different stages and for several reasons:

- to obtain and verify the desirable dissolution and release properties
- to study the influence of physiological factors such as food
- to establish bioequivalence between clinical trial and commercial formulations after changes of a formulation
- to develop and validate IVIVCs for *in vitro* dissolution test methods

Although all of these different types of studies aim to investigate the influence of the dosage form on the rate and extent of absorption, different designs and means to evaluate the data are applied. This chapter describes different ways to assess the formulation function from plasma concentration data obtained in bioavailability studies. Certain study design aspects will also be pointed out. For a more basic understanding of pharmacokinetics, specialised textbooks should be consulted, such as *Clinical Pharmacokinetics—Concepts and Applications* (Rowland and Tozer 1995).

Aspects of Study Design

Single-Dose Studies

Single-dose Studies are most sensitive for evaluation of absorption properties and should generally be used for evaluation of formulations. The main exception is if the regulatory guidelines require repeated dosing studies. The drug should be administered under fasting conditions (overnight) together with 200 mL of water. No food should generally be allowed for 4 h after intake, and the subjects should thereafter follow a standardised meal schedule during the study day.

Cross-Over Designs

In cross-over designs, the same subjects receive test and reference formulations to avoid the influence of any interindividual differences that could affect the plasma concentration-time profile. For drugs with a very long half-life (several weeks), a parallel group design (i.e., each formulation is studied in different groups of subjects) may be applied for practical reasons to shorten the duration of the study. A parallel group design could also be used if the interindividual (between subjects) variability of the bioavailability variables is of the same magnitude as the intraindividual (within-subject) variability. Additionally, other standard design principles such as randomisation should be used, as described in more detail in statistical textbooks.

Wash-out Period

A wash-out period, i.e., a minimum number of days between administration of each formulation, is needed in order to avoid influence of the previous administration on the plasma concentration profile of the following formulation. As a rule of thumb, the wash-out period should be at least five times the elimination half-life of the drug under investigation.

Reference Formulation

In almost all studies, a reference formulation is needed, either as a comparator for assessment of relative performance compared to the test formulation, or as a simple vehicle, to characterise the drug substance pharmacokinetics. If the aim is to investigate the influence of certain formulations on the rate and extent of absorption, which is the case in early development or IVIVC studies, an oral solution of the drug is the first choice as a reference formulation. Stability of the solution, regarding drug compound degradation and precipitation, is an important factor to verify before study start. Inclusion of a parenteral reference formulation, if feasible, provides additional information, as will be further discussed below.

Number of Subjects

The number of subjects to be included in the study will be determined by the inherent variability in drug substance pharmacokinetics, the magnitude of effects that are of interest, the desired confidence in conclusions, costs, time, ethical aspects and where relevant, regulatory guideline recommendations. Three different situations may be identified that require different algorithms to determine the sample size:

1. The aim is to determine the absorption characteristics for a certain formulation, for example, in an IVIVC study. In this case, the question for the pharmaceutical scientist is how precise the mean estimates of primary variables must be, i.e., how wide confidence intervals around the mean are acceptable.
2. The aim is to investigate the difference between two or more formulations. In this case, the question is how large a difference between the formulations can be of interest to detect at a certain statistical significance level.
3. The aim is to establish bioequivalence between two formulations by obtaining a confidence interval for the difference within specified limits. In this case, the main question is how large a risk the investigator is willing to take to obtain non-conclusive results. Inclusion of more subjects will decrease the width of the confidence interval and thereby reduce the risk of not meeting the acceptance criteria.

Calculations of a suitable number of subjects are most often made by statisticians. However, for an understanding of these calculations, any basic statistical textbook is recommended for the first two cases, and in the case of bioequivalence studies, another reference (Hauschke et al. 1999) is suggested.

Plasma Sampling

The plasma sampling schedule has to be designed so that the desired accuracy of the primary bioavailability variables can be obtained. In cases of evaluation of formulation performance, it is crucial to have frequent sampling during the absorption phase. In addition, at least three samples should be obtained during the major terminal elimination phase in order to obtain a relevant measure of the rate constant for this phase, which is needed for a correct estimate of the extent of absorption. Numerous late plasma samples, when the drug concentration is below the limit of quantification of the bioanalytical assay, should be avoided.

Food

Food may not only affect drug substance pharmacokinetics, such as first-pass metabolism or drug clearance; it may also influence drug dissolution, or by other means, the function of the dosage form. For example, with food, drug residence time in the stomach will be increased, the pH will be changed, motility will be altered and bile and pancreatic secretions will increase. All of these factors could potentially affect drug release and dissolution from a solid formulation. It is therefore relevant to study the influence of food on rate and extent of drug dissolution/release during development. Such a study should include an oral solution, to allow for a distinction between the effects of food on formulation and drug substance. The dosage forms are typically administered immediately after intake of the food. Since almost all medications are administered in the morning, studies are usually performed together with a breakfast. The composition of the meal has to be well defined, since variations can introduce unwanted variability.

Generally, a heavy breakfast (approximately 1000 calories and 50 percent of the energy content from fat) should be used, since this is supposed to stress potential food effects. A proposal for a standardised composition of a heavy breakfast is displayed in Table 7.4 (FDA 1997c).

Assessments

Evaluation of drug plasma concentrations is an indirect way of estimating the rate and amount of drug dissolution and/or absorption. Several assumptions are therefore needed, irrespective of which of the metrics described below are applied:

- In cross-over studies, drug substance pharmacokinetics, including first-pass metabolism, distribution and clearance, should be identical within the same subject, between administrations of different formulations.
- Linear pharmacokinetics within the investigated rate of delivery (dose/time units) of the drug to the body, i.e., the plasma concentration–time profile, should be identical for different doses after correction for dose. The most common reason for non-linear pharmacokinetics is dose-dependent first-pass metabolism. This phenomenon does not just occur at high doses, but has also been shown for the slow delivery rates obtained by ER formulations.

In cases where the *in vivo* dissolution/release rate is going to be quantified in some way from plasma concentrations, it is important to emphasise that this is only relevant if this is the rate-limiting step in the absorption process. If other factors are affecting the absorption rate,

Table 7.4
Example of a standardised breakfast to be used in food interaction studies.

2 eggs fried in butter	4 ounces of hash brown potatoes
2 strips of bacon	8 ounces of whole milk
2 slices of toast with butter	

estimations of the *in vivo* dissolution will be confounded. Examples of such confounding factors in determination of the *in vivo* dissolution rate are

- gastric emptying (assuming negligible absorption over the gastric mucosa);
- low drug permeability over the gastric wall;
- degradation in the GI tract;
- complex-binding of the drug to any component in the GI tract; and
- entero-hepatic circulation, i.e., intact drugs are excreted by the bile into the intestine.

In vivo evaluation of formulations thus requires a high level of knowledge regarding the drug substance absorption properties obtained both by *in vitro* and *in vivo* methods described in this chapter and in Chapter 4, and also by basic pharmacokinetic studies, a review of which is outside the scope of the present chapter.

Standard Bioavailability Variables

The standard bioavailability variables after a single-dose administration are the maximum plasma concentration ($C_{\max}$), the time to reach $C_{\max}$ ($t_{\max}$) and the area under the plasma concentration—time curve from time zero to infinity (AUC).

$C_{\max}$ and $t_{\max}$ are used as characteristics of the absorption rate and may thereby be affected by the drug dissolution and release, as discussed above. However, both variables are affected by several pharmacokinetic properties other than the absorption rate (K_a) as shown in equations 4 and 5, which describe $C_{\max}$ and $t_{\max}$, respectively, for a first-order absorption process;

$$C_{\max} = \frac{F \times D \left(\frac{K_a}{K_e} \right)^{-K_e(K_a - K_e)}}{V_d} \quad (4)$$

$$t_{\max} = \frac{2.303 \times \log \left(\frac{K_a}{K_e} \right)}{K_a - K_e} \quad (5)$$

where F is the extent of oral drug bioavailability expressed as a fraction, D is the administered dose, K_e is the first-order elimination rate constant, according to one compartment model, and V_d is the volume of distribution.

Thus, $C_{\max}$ and $t_{\max}$ are not useful as pure measures of the absorption rate but can be used in comparisons between different formulations. Another disadvantage is that they are single-point estimates, which does not take into account all data sampled during the absorption process. In the case of rapid dissolution processes, it is difficult to accurately localise the true maximum, and frequent plasma sampling during the absorption phase is especially crucial in getting reasonably good estimates. The main merit of $C_{\max}$, besides simplicity, is its potential clinical relevance, i.e., when the peak plasma concentration is related to maximal pharmacological effects or adverse effects.

AUC is used to evaluate the extent of absorption which may be affected by the formulation, as discussed above. AUC is a composite variable determined not only by the

extent of bioavailability (F) but also by the systemic drug clearance (Cl) and the dose (D) as described by:

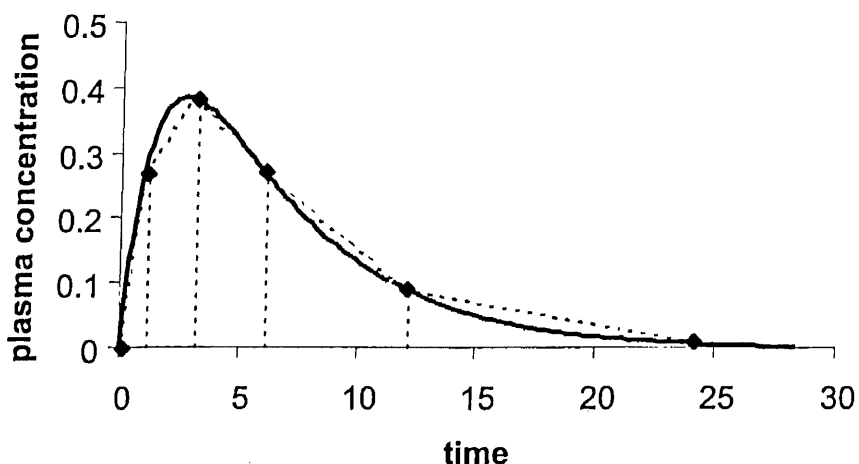
$$AUC = \frac{D \times F}{Cl} \quad (6)$$

Thus, AUC is only of interest as a relative variable, where the AUC for a test formulation is related to a reference. If the AUC-ratio is determined for a formulation in relation to an oral solution, the influence of the dosage form on the extent of absorption can be determined, that is, if $AUC_{solid}/AUC_{solution} \neq 1.00$, the deviation from 1 can be attributed to the formulation, provided that the above-stated assumptions hold. AUC is also a primary variable in bioequivalence studies.

The calculation of AUC is commonly determined by the linear trapezoidal rule. In this procedure, the sum of the areas of a series of trapezoids which are formed between the data points for two adjacent times is calculated (see Figure 7.7). This approximate method requires that blood sampling be frequent enough so that the curvature of the plasma concentrations between two data points is negligible. For example, in the ascending part, the plasma concentration curve has a convex profile, and a straight line will provide an overestimation of the AUC. Applying a modified version, the log trapezoidal rule, can reduce this problem. The area under each segment between two data points for the linear trapezoidal (equation 7) and logarithmic trapezoidal (equation 8) methods are determined by:

$$AUC(t_n, t_{n+1}) = \left(\frac{C_n + C_{n+1}}{2} \right) \times (t_{n+1} - t_n) \quad (7)$$

Figure 7.7 Drug plasma concentration–time curve illustrating formation of trapezoids, in calculation of the area under the curve (AUC).



$$\text{AUC}(t_n, t_{n+1}) = \left(\frac{C_n - C_{n+1}}{\ln \frac{C_n}{C_{n+1}}} \right) \times (t_{n+1} - t_n) \quad (8)$$

where C_n is the drug concentration for the n th plasma sample obtained at time t_n . It is imperative in single-dose studies, which are generally most suitable for evaluation of dosage form performance, to determine the AUC from time zero to infinity. This requires that the remaining area from the last measured data point ($C_{t_{\text{last}}}$) to infinity be extrapolated, and is made from the following equation:

$$\text{AUC}_{t_{\text{last}}-\infty} = \frac{C_{t_{\text{last}}}}{K_e} \quad (9)$$

where K_e is the elimination rate constant which is determined from the slope of the terminal log-linear plasma concentration–time profile (see Figure 7.8) by linear regression. In order to obtain a reliable estimate of AUC, the extrapolated area ($\text{AUC}_{t_{\text{last}}-\infty}$) should not exceed 10 per cent of the total AUC.

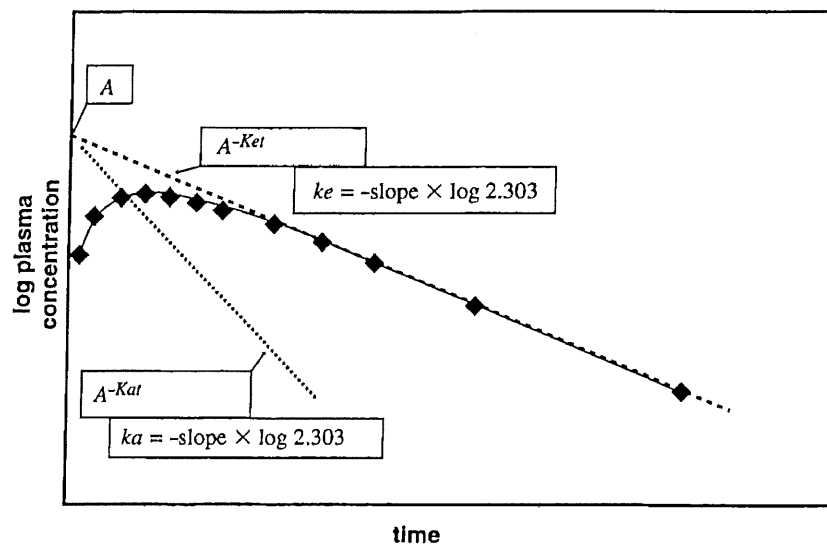
C_{max} and AUC are also used as primary variables in bioequivalence studies, i.e., studies aiming to establish that a test and a reference product provide similar enough plasma concentration–time profiles to be exchanged without altering the clinical effects. Such studies are, for example, performed in NDA programmes to compare the clinical trial formulations with the “to-be-marketed” product, at post-approval changes of composition, manufacturing process or site of a product and to document a generic versus the original product. More details regarding performance and evaluation of such studies can be found in appropriate guidelines (FDA 1999; EMEA 1998).

Model Dependent Analysis

An absorption rate, which could reflect dosage form performance, as discussed above, could be determined from plasma concentration data by fitting the data to a pharmacokinetic model. The absorption rate could be a zero-order or first-order rate constant, i.e., the amount absorbed is constant over time, or, the absorption rate decreases exponentially over time, respectively. The choice of model also depends on how many phases in the log-plasma concentration–time profile are identified after the peak concentration, i.e., how many compartments are needed to describe the plasma concentration–time profile. The model may also include a lag time (t_{lag}) before absorption starts. A comprehensive description of different models can be found in Gabrielsson and Weiner (1997). Model dependent analysis could also be used to determine the standard bioavailability variables such as C_{max} , t_{max} and AUC. Fitting the plasma concentration data to the suitable model is done by non-linear regression. Computer programs designed for pharmacokinetic modelling are available, such as NONLIN (Pharsight Corp., Mountain View, Calif., USA). More detail regarding performance and evaluation of non-linear regressions for this purpose can be found elsewhere (Gabrielsson and Weiner 1997).

In the case of one-compartment disposition pharmacokinetics, i.e. when the declining part of the plasma concentration–time curve can be approximated by a one log-linear phase, a first-order rate constant (K_a) can be determined according to:

Figure 7.8 Logarithmic drug plasma concentration–time curve for an oral administration illustrating the elimination rate constant (K_e) and first-order absorption rate constant (K_a).



$$Ae^{-K_a t} = Ae^{-K_e t} - Ct \quad (10)$$

where A is determined from the intercept with the y axis of the extrapolated log-linear elimination phase (see Figure 7.8), K_e is the first-order rate constant that describes the elimination phase, and C is the plasma concentration at time t . The difference between the extrapolated elimination phase and the actual plasma concentration ($Ae^{-K_e t} - Ct$) is plotted versus time, which thus equals $Ae^{-K_a t}$ and describes the absorption phase. The K_a can then be determined from the slope of the line in a log-linear plot as shown in Figure 7.8.

Moment Analysis

Moment analysis provides the means to determine a model independent characteristic of the absorption rate or dissolution rate (Riegelman and Collier 1980). A single value characterising the rate of the entire dissolution or absorption process is obtained, which is called the mean absorption or dissolution time (MAT and MDT, respectively). These parameters can be determined without any assumptions regarding absorption or disposition pharmacokinetics, apart from the general prerequisites of linear pharmacokinetics and absence of intraindividual variability described above. MAT/MDT can be interpreted as the most probable time for a molecule to become absorbed/dissolved, based on a normal Gaussian distribution.

If these processes follow zero- or first-order kinetics, MAT/MDT corresponds to 50 and 63 percent absorbed/dissolved, respectively. MDT is especially useful for correlations with *in vitro* data since this *in vivo* variable corresponds to the MDT determined from *in vitro* dissolution data, as described in the section “*In Vitro* Dissolution”.

MAT and MDT for a test formulation are determined from drug plasma concentrations–time data obtained in a single dose study according to the following equations;

$$\text{MRT} = \frac{\text{AUMC}_{0-\infty}}{\text{AUC}_{0-\infty}} \quad (11)$$

$$\text{MAT} = \text{MRT}_{\text{test}} - \text{MRT}_{\text{IV}} \quad (12)$$

$$\text{MDT} = \text{MRT}_{\text{test}} - \text{MRT}_{\text{oral solution}} \quad (13)$$

where MRT is the mean residence time, which includes the complete time from intake to elimination of the drug. $\text{AUMC}_{0-\infty}$ is the area under the curve of the product of time t and the plasma concentration versus time from zero to infinity, and $\text{AUC}_{0-\infty}$ is the area under the plasma concentration–time curve, as defined above. Thus, administration of an oral or intravenous (IV) reference administration is needed to calculate MAT and MDT, respectively, except when the drug substance follows one-compartment pharmacokinetics. In this case, the MAT can be determined without a reference according to the following formula:

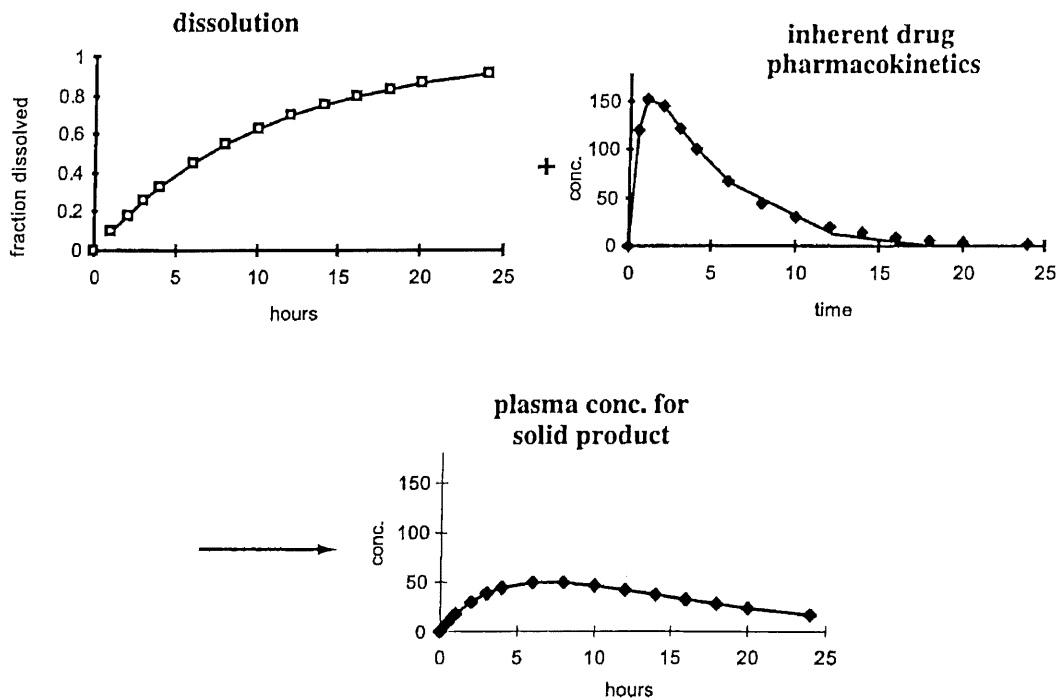
$$\text{MAT}_{\text{test}} = \text{MRT}_{\text{test}} - \left(\frac{1}{K_e} \right) \quad (14)$$

i.e., MRT and K_e are determined from the plasma concentration–time profile for the test formulation according to the procedures described above.

In Vivo Dissolution/Absorption-Time Profiles

A full-time course *in vivo* dissolution- or absorption-time profile is generally the most informative way of evaluating formulation performance from the plasma concentration–time data. The methods described in the present chapter all have the advantage over the modelling of zero- or first-order rate constants, in that no assumptions have to be made regarding the kinetics of the absorption phase. Some of the methods provide an estimate of the absorption-time profile, rather than the *in vivo* dissolution-time profile. However, in the context of evaluating formulation performance, the former measure is fully valid, provided that the drug dissolution/release is the rate-determining step in the absorption process.

The method of choice for determination of *in vivo* dissolution- or absorption-time profiles is *deconvolution*. In deconvolution, three components are defined: input, weighting and response functions, as exemplified in Figure 7.9. The input function corresponds to the entry of drug into the body, i.e., the *in vivo* dissolution- or absorption-time profiles for the oral test formulation. The weighting function corresponds to the time course of the drug within the body, as described by the plasma concentration–time profile after administration of an oral or IV reference solution, and the response function is described by the plasma concentration–time curve for the test formulation. In deconvolution, the input function is determined from data that describe the weighting and response functions, i.e., the *in vivo* dissolution- or absorption-time profile is calculated from the plasma concentration–time data of the test formulation and a reference solution. It is also possible to calculate the response function from available data of the input and weighting functions, i.e., the plasma concentration–time profile for a solid oral dosage form is simulated from *in vitro* dissolution data and plasma concentration–time data for a solution. This latter procedure is called *convolution*. The three

Figure 7.9 Illustrations of the different functions included in deconvolution/convolution.

most common definitions of the input, weighting and response functions in deconvolution/convolution are presented in Table 7.5.

Different deconvolution algorithms have been described (Tucker 1983). However, further descriptions in the present chapter will be limited to numerical deconvolution (Langenbucher 1982). The merits of this equation compared to others are the relative simplicity of the algorithm and the lack of any modelling of the plasma concentration–time data to be used in

Table 7.5
Definitions of the different functions in deconvolution/convolution.

Input (I)	Weighting (W)	Response (R)
	Plasma concentration–time profile for:	Plasma concentration–time profile for:
Dissolution–time profile or solid formulation	Oral solution	Solid formulation
Absorption–time profile or solid formulation	IV solution	Solid formulation
Absorption–time profile for oral solution	IV solution	Oral solution

the assessment of the input profile. The fractional amount dissolved or absorbed (I) is calculated by the following deconvolution algorithm:

$$\begin{aligned}
 I_1 &= \frac{R_1 T}{W_1} \\
 I_2 &= \frac{\frac{R_2}{(T - I_1) W_2}}{W_1} \\
 I_3 &= \frac{\frac{R_3}{(T - I_1)(W_3 - I_2) W_2}}{W_1} \\
 I_n &= \frac{\frac{R_n}{(T - I_1)(W_n - I_2)(W_{n-1} - \dots - I_{n-1}) W_2}}{W_1}
 \end{aligned} \tag{15}$$

where W_i and R_i are the plasma concentrations for the i th time representing the weighting and response functions, respectively. These plasma concentration-time profiles are divided into finite intervals of equal time T before the plasma concentrations can be entered into the equation described above. Interpolations are generally needed between the measured data points to obtain such data. A common method for performing such interpolations is the point-area method (Vaughan and Dennis 1978).

A drawback of deconvolution algorithms is that they are not stable. Small inconsistencies or noise in the data could, in some cases, lead to input time profiles that oscillate by increasing amplitude, i.e., no meaningful interpretation of such results can be made. Such problems could be overcome by changing the time interval T , by suitable interpolation or by smoothing/modelling of the plasma concentration data used as weighting and response functions.

The plasma concentration for a solid product can be estimated from *in vitro* dissolution data and plasma concentration-time data for a reference formulation by rearranging equation 15 to a convolution algorithm:

$$\begin{aligned}
 R_1 &= I_1 W_1 T \\
 R_2 &= (I_1 W_2 + I_2 W_1) T \\
 R_3 &= (I_1 W_3 + I_2 W_2 + I_3 W_1) T \\
 R_n &= (I_1 W_n + I_2 W_{n-1} + \dots + I_n W_1) T
 \end{aligned} \tag{16}$$

The convolution algorithm (equation 16) is stable, in contrast to deconvolution procedures.

Two other methods have been described that require modelling of the disposition pharmacokinetics but not of the absorption phase. For drugs with disposition pharmacokinetics that can be approximated by a one-compartment model, i.e., the declining part of the plasma concentration-time curve can be approximated by a one log-linear phase, the following equation can be applied to determine the fractional amount absorbed (A) at different times t after administration (Wagner and Nelson 1963):

$$A(t) = \frac{Ct + (Ke \times AUC_{0-t})}{(Ke \times AUC_{0-\infty})} \quad (17)$$

where Ke and AUC have been defined previously in this chapter. This equation is known as the *Wagner-Nelson method*. The main advantage of this method is that the absorption-time profile can be determined without administration of any reference formulation, provided that the elimination rate constant can be accurately determined from the post-absorption phase of the plasma concentration-time curve of the test formulation. Another merit of the Wagner-Nelson method is the simple calculation procedure compared to other methods. An important limitation of the Wagner-Nelson method is that the amount absorbed is expressed as the fraction of the total amount absorbed, i.e., the asymptotic plateau level of the absorption-time profile will always be 1.0. Thus, no conclusions can be drawn from such data regarding completeness of drug release or dissolution.

For drugs that are best described by a two-compartment pharmacokinetic model, the absorption-time profile can be determined by the *Loo-Riegelman method* (Loo and Riegelman 1968). However, this method requires administration of an IV bolus dose to determine the disposition pharmacokinetics. Therefore, this method does not provide any clear advantages compared to deconvolution.

Fractional Amount Absorbed

The fractional *amount of drug absorption* (Fa) can be defined as the amount of intact drug that permeates the gastric wall. It is related to the absolute bioavailability (F), i.e., the fraction of the administered drug that reaches the systemic circulation, according to the following equation:

$$F = Fa \times Fh \times Fg \quad (18)$$

where Fh and Fg are the fractions of the administered drug that escape first-pass metabolism, before entering the systemic circulation, in the liver and the gut wall, respectively. Fa is of special relevance for elucidation of formulation performance, because it is affected by the completeness of dissolution/release, the success in avoiding degradation in GI tract and/or potential effects of the formulation on drug permeability.

Fa can be estimated from plasma concentration-time data and urine data. In the former case, administration of a reference IV solution is needed, in addition to administration of the test formulation. Fa can be approximately determined from the following series of algorithms:

$$Fa = \frac{F}{1 - E_h} \quad (19)$$

$$F = \left(\frac{AUC_{oral}}{AUC_{IV}} \right) \times \left(\frac{dose_{IV}}{dose_{oral}} \right) \quad (20)$$

$$E_h = \frac{Cl_{h_{blood}}}{Q_h} \quad (21)$$

$$Cl_{h_{blood}} = Cl_{h_{plasma}} \times \text{blood:plasma concentration ratio} \quad (22)$$

$$Cl_{h_{\text{plasma}}} = Cl_{\text{tot}} - CLr \quad (23)$$

$$Cl_{\text{tot}} = \frac{\text{dose}_{\text{IV}}}{\text{AUC}_{\text{IV}}} \quad (24)$$

$$CLr = fe \times Cl_{\text{tot}} \quad (25)$$

$$fe = \frac{Au}{F \times \text{dose}} \quad (26)$$

where E_h is the hepatic elimination ratio, $Cl_{h_{\text{blood}}}$ is the hepatic blood clearance and Q_h is the hepatic blood flow, which is assumed to be 1.5 L/min in man. The partition of drug between blood and plasma has to be experimentally determined. Cl_{tot} is the total systemic plasma clearance, CLr is the renal plasma clearance, fe is the fraction of drug excreted unchanged in urine and Au is the total amount of drug excreted unchanged in urine. It should be realised that this is only an approximate method since neither the blood flow nor the blood/plasma drug ratio is measured during the absorption process. In addition, it has to be assumed that all first-pass metabolism is performed by the liver. Systemic clearance is only provided by the liver and kidneys, and hepatic metabolism is linear within the concentration range encompassed by high levels during the first-pass through the liver and lower levels returned from the systemic circulation.

If a radioactively labelled drug is administered intravenously and orally, the fraction absorbed (Fa) can be more simply estimated from the oral/intravenous ratio, corrected for dose, of total radioactivity in the urine. This requires that urine collection continue over a time period long enough to provide almost complete emptying of the radioactivity. Furthermore, in order to get reasonable precision, a significant amount of metabolites and/or unchanged drug has to be excreted by the kidneys. Finally, it has to be assumed that the parent drug molecule is not degraded in the GI tract and is subsequently absorbed as the metabolite.

IN VITRO/IN VIVO CORRELATIONS

The correlation between *in vitro* and *in vivo* dissolution, or other *in vivo* characteristics, is useful during several stages of formulation development. For example, in early formulation development, it may be desirable to establish the *in vivo* relevance of the dissolution method used for screening of different candidate formulations. During later development phases, IVIVCs can be used to validate the batch control dissolution test method, or as a guide when setting the dissolution specification limits. IVIVC may also form a basis for replacing bioequivalence studies with simpler *in vitro* dissolution studies for modified release formulations, both during development and in connection with later post-approval changes (EMEA 1999; FDA 1997a).

It should be possible to establish IVIVCs provided that the following prerequisites are fulfilled:

- Drug release or dissolution is the rate-limiting step in the absorption process.
- The *in vitro* dissolution method provides *in vivo* relevant results.
- Unbiased estimates of the *in vivo* dissolution-time profiles are obtained from plasma concentration–time data (see “Bioavailability Studies” section).

The drug release or dissolution can only be expected to fulfil the first prerequisite given above in cases of modified release formulations and poorly soluble drugs. In other cases, such as rapidly dissolving formulations of highly soluble drugs, or drugs with low permeability over the GI wall, gastric emptying and transport over the gastric mucosa, respectively, will determine the absorption kinetics. Thus, IVIVCs are not possible to obtain in those cases, since permeability and gastric emptying are not at all modelled by an *in vitro* dissolution test method.

It is important to realise that an established IVIVC is not a general characteristic of a drug compound that can freely be applied to all kinds of oral formulations. An IVIVC is, rather, specific for a certain type of formulation. The main limitation of IVIVCs is the problem of defining how large the changes made to a specific formulation can be without affecting the applicability of an established correlation.

Reports have been published in the case of ER tablets, showing that the relationship between *in vitro* and *in vivo* data could be altered by changing the quality of a critical excipient (Abrahamsson et al. 1994). The most conservative approach is to apply IVIVC only to formulation changes that are covered by the formulations included in the establishment of a correlation. However, provided that good knowledge exists regarding drug absorption properties, function of the dosage form and role of critical excipients, as well as the *in vitro* dissolution under various physiologically relevant conditions, somewhat wider applications are possible with reasonable confidence.

Three different types of correlation have been defined, levels A, B and C, as described in Table 7.6. The following part of this chapter will describe how these levels are established and their respective roles, merits and disadvantages.

Valuable additional information on IVIVCs can be found in Brockmeier (1986) and in Young et al. (1997).

Level A

Level A correlation is generally the most desirable form, since the *in vitro* method completely mimics the *in vivo* results, and a direct correspondence exists at each time point. The achievement of an *in vitro* method that models the entire *in vivo* process confers confidence in the method's capability as a surrogate for *in vivo* studies. This allows for predictions from *in vitro* data of complete absorption-time and plasma concentration-time profiles in early formulation development as well as in later phases, when relevant *in vitro* specification limits are settled. In addition, it is only level A correlations that are accepted by regulatory agencies as a basis for replacing *in vivo* bioequivalence studies with *in vitro* dissolution tests.

The development and evaluation of a level A correlation consists of the following steps:

- I. Design and assessment of *in vitro* dissolution studies and human bioavailability studies
 - choice/development of formulations
 - study design aspects
 - assessment of *in vivo* absorption/dissolution-time data
- II. Establishment of an IVIVC
 - relationship between *in vitro* and *in vivo* data

Table 7.6
Different types of *in vitro/in vivo* correlations of drug dissolution.

Type	Description	Example
Level A	<i>In vitro</i> and <i>in vivo</i> dissolution-time profiles are superimposable	
Level B	Relationship established between MDT_{vitro} and MDT_{vivo} determined by statistical moment analysis	
Level C	Relationship established between single-point <i>in vitro</i> dissolution rate characteristic and bioavailability variables	

III. Evaluation of predictability

- prediction of drug plasma concentrations from *in vitro* model
- comparisons of predicted and measured bioavailability variables by calculation of the prediction error

Design and Assessment of In Vitro Dissolution Studies and Human Bioavailability Studies

It is possible to establish a level A correlation based on data from only one formulation. However, this will not be sufficient for regulatory purposes in most cases, and an established correlation should preferably be validated by additional data. The most common approach is therefore to include several formulations with different *in vitro* release rates. Formulations with different release rates for IVIVC studies may be obtained by (a) choosing batches with

different rates due to batch-to-batch variation, (b) altering the manufacturing process in a way that affects dissolution rate, (c) altering the amount or quality of critical excipients or (d) altering the particle size or crystal form of the active drug substance. The ideal formulations for an IVIVC study should provide differences in the release rate of a magnitude that could be detected *in vivo* on top of the inherent pharmacokinetic variation. Furthermore, the mechanism of the variation of release rate should be known, and the modifications performed to obtain the different batches should be relevant for variations that may occur in normal production.

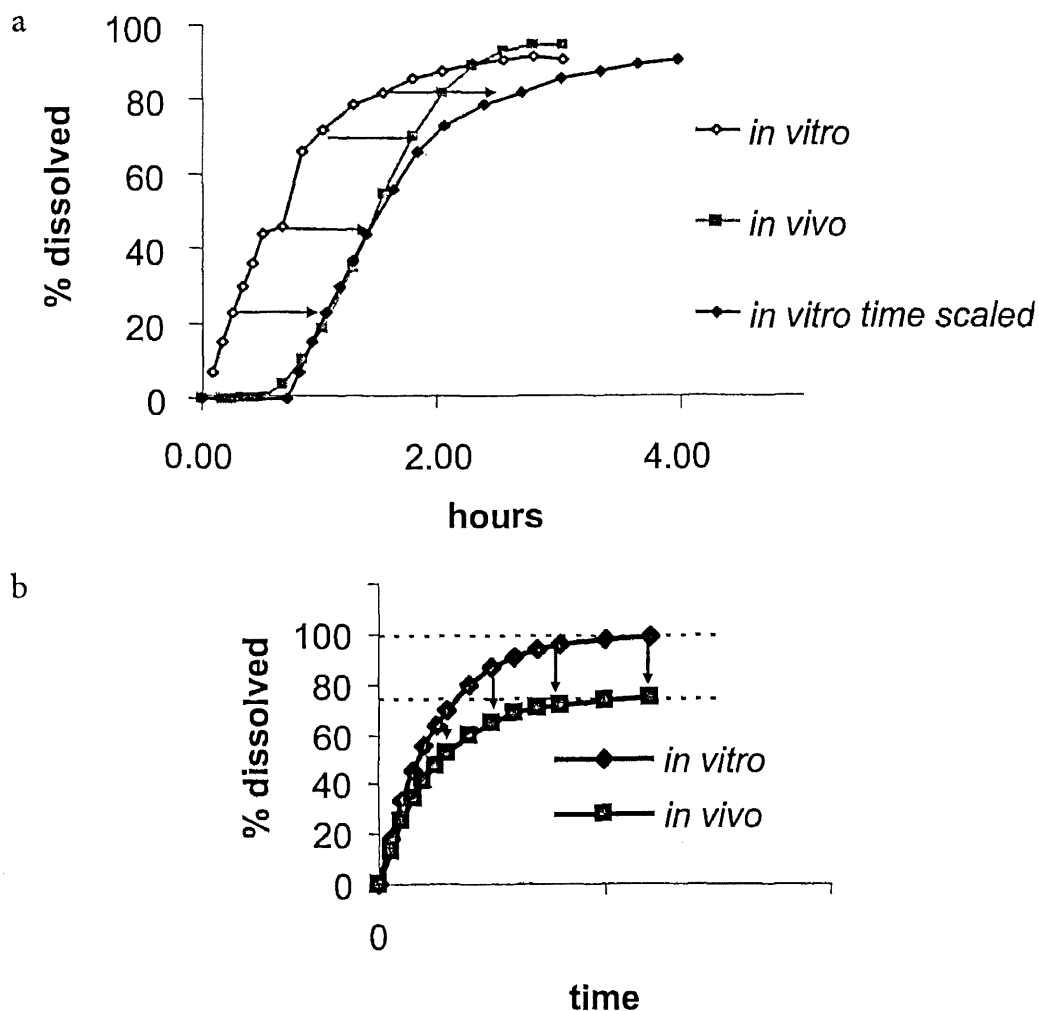
The *in vivo* bioavailability studies should generally be single dose, cross-over studies with administration under fasting conditions, of formulations with different release rates, and a reference solution. The primary response variable in bioavailability studies aimed at establishing IVIVCs is the assessment of an *in vivo* dissolution-time or an absorption-time profile. Further details regarding assessment of such data are given in the section "Bioavailability Studies". The number of subjects to be included should be based on the desired precision in the mean estimate of the dissolution-time profile.

The *in vitro* dissolution programme may comprise tests under several conditions to find the optimal method (see the section "In Vitro Dissolution") or may only include testing by use of a tentative production control method. It is crucial that frequent sampling be applied to appropriately characterise the entire dissolution-time curve, since tentative test points in a product specification are generally not sufficient. An adequate number of individual tablets should be tested. For regulatory purposes, 12 individual dosage units from each batch are usually required.

Establishing an IVIVC

When the obtained *in vitro* and *in vivo* dissolution-time curves for all included formulations are superimposable without further mathematical modelling, no additional steps are needed to establish an IVIVC. However, at times, some scaling of the *in vitro* data must be done to obtain superimposable curves. The same scaling factor should be used for all formulations. Linear scaling is recommended, especially in cases where few formulations (<3) are included in the IVIVC, although the use of non-linear scaling functions has also been proposed. The scaling may be performed in two dimensions, either as a time scaling or as a scaling of the amount dissolved, at a certain time point. In the first case, the *in vitro* and *in vivo* time curves are made superimposable by the scaling of the time axis (see Figure 7.10a). The rationale for this approach is that the drug release/dissolution follows the same type of kinetics *in vitro* and *in vivo*, but the differences between the *in vitro* milieu and physiological conditions affect the rates. Another reason for time scaling may be lag times *in vivo* due to gastric emptying which cannot be reflected by an *in vitro* test. An easy way to obtain a scaling function is to plot the time points from the *in vitro* and *in vivo* dissolution-time curves when equal amounts of drug have been dissolved, and subsequently fit a linear function to the data by linear regression. This function should then be used to transform the *in vitro* dissolution-time points to obtain an *in vivo* relevant dissolution-time curve. Another principle for scaling between *in vitro* and *in vivo* dissolution data is to adjust the y axis, i.e., the *in vitro* dissolution data, by the linear scaling factor ($y = kx$) to reach the same level when the dissolution process has been completed (see Figure 7.10b). Thus, the scaling factor k is determined from the ratio of the asymptotic plateau levels obtained in the *in vitro* and *in vivo* dissolution-time curves. The rationale for using this approach is that there may be differences in the extent of bioavailability between test

Figure 7.10 (a) Cumulative *in vitro* and *in vivo* dissolution-time curves illustrating the time-scaling procedure of an *in vitro* dissolution-time curve to obtain an *in vivo* predictive *in vitro* model. (b) Cumulative *in vitro* and *in vivo* dissolution-time curves illustrating a case where an *in vivo* predictive *in vitro* model can be obtained by scaling of the amount dissolved based on the ratio between the asymptotic plateau levels (dashed lines).



and reference formulations that cannot be attributed directly to the formulation performance, but more to indirect effects (e.g., first-pass metabolism) due to the differences in the rate of administration or site of absorption.

Evaluation of IVIVCs

The *in vivo* predictability of an *in vitro* model with or without a scaling function should be evaluated. A subjective assessment can be made by comparing the modelled *in vitro* dissolution data and the corresponding *in vivo* dissolution-time curves in a graph. However, a more stringent approach, applied in regulatory contexts, is to predict the plasma concentration-time profiles from the *in vitro* model and compare them to measured *in vivo* data. The latter step is performed by comparing the estimated and measured primary bioavailability variables, $C_{\max}$ and AUC.

The plasma concentration-time data of a test formulation can be predicted from a dissolution-time profile, obtained from the *in vitro* model, and measured plasma concentrations for a reference formulation in the IVIVC bioavailability study (e.g., an oral solution), by use of convolution (see previous section). The difference in $C_{\max}$ and AUC based on predicted and measured plasma concentrations can be numerically assessed for each formulation by a simple estimation of the relative difference, denoted prediction error (PE), as follows:

$$\%PE = \left(\frac{\text{observed value} - \text{predicted value}}{\text{observed value}} \right) \times 100 \quad (27)$$

This evaluation may be performed either by use of the same data, as included in the establishment of an IVIVC (internal predictability), or by use of other data sets (external predictability). The criteria for concluding a level A IVIVC in a regulatory context requires, in the case of internal predictability, an average percentage PE of ≤ 10 percent for $C_{\max}$ and AUC, respectively, and the percentage PE for each formulation regarding these two bioavailability variables should not exceed 15 percent (FDA 1997a).

Level B

A level B correlation is based on comparisons between MDT *in vitro* and MDT *in vivo*, or MAT. MDT and MAT are average rate characteristics, which take into account all data points. They are determined by statistical moment analysis, as described in the sections "In Vitro Dissolution" and "Bioavailability Studies" for *in vitro* and *in vivo* data, respectively.

In order to establish a level B correlation, *in vitro* and *in vivo* data for at least three formulations with different release properties are required. Each formulation provides one pair of *in vitro* and *in vivo* MDT values. A level B correlation can be concluded if a linear relationship is obtained between the *in vitro* and *in vivo* MDT values by use of linear regression.

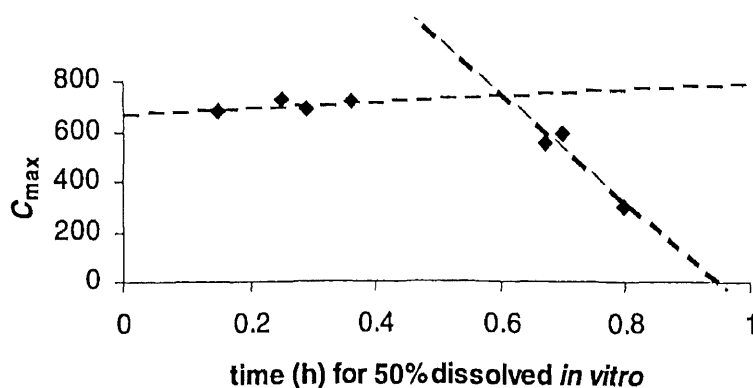
Level B correlations can be used when level A correlations are not possible, due to different dissolution profiles *in vitro* and *in vivo*. The establishment of a level B correlation implies that the dissolution method can discriminate between formulations that are different *in vivo*. This provides an increased confidence in the suitability of a product control method or a method to be used for optimisation during development. It is also possible to use a level B correlation for the scaling of the *in vitro* dissolution rate to obtain a more *in vivo* relevant estimate of the dissolution process. For example, if $MDT_{\text{vivo}} \propto 2MDT_{\text{vitro}}$, the dissolution

process is on average twice as fast *in vivo* compared to *in vitro*. The main disadvantage of level B correlations compared to level A correlations is lack of the possibility to predict the entire *in vivo* dissolution- and plasma concentration-time profiles from *in vitro* data. Therefore, level B correlations have very limited use in regulatory contexts.

Level C

A level C correlation establishes a single-point relationship between a measure of the *in vitro* dissolution rate, e.g., the time when 50 percent of the dose has been dissolved (t_{50} percent), and a bioavailability variable, e.g., $C_{\max}$ or AUC. Thus, a corresponding approach is applied as for level B correlations. An advantage of level C correlations, compared to level A and B correlations, is that no reference formulation is needed in the *in vivo* studies. Another advantage of a level C correlation is that the *in vitro* dissolution data are directly related to a bioavailability variable that can be more easily interpreted in a clinical context, compared to dissolution-time profiles and MDT. Level C correlations could also be useful for rapidly releasing formulations to assess at which level a formulation variable, such as tablet disintegration time or drug particle size, becomes a rate-limiting step in the absorption process and starts to affect the bioavailability variables. An example of such usage of a level C correlation is given in Figure 7.11. In this case, an initial phase is found where the *in vivo* variable is independent of the *in vitro* dissolution rate, followed by a linear relationship at slower dissolution rates. This could be useful in establishing the discriminating power of the dissolution test,

Figure 7.11 Mean maximal plasma concentrations ($C_{\max}$) versus time when 50 percent has been dissolved *in vitro* for seven different immediate release formulations. Two linear relationships have been identified, and the cross-section between the two lines indicates the critical dissolution rate at which the dissolution becomes the rate-limiting step in the absorption process.



optimising the formulation during development and establishing biopharmaceutically relevant specifications for drug particle size or other critical manufacturing variables.

The disadvantages of single-point level C correlations are; that

- it is not possible to predict entire plasma concentration–time profiles from *in vitro* data
- the single-point estimates, such as t_{50} percent and C_{max} , do not take into account the majority of data points obtained in the *in vitro* and *in vivo* studies
- the bioavailability variable is influenced by several other pharmacokinetic drug properties that are not intended to be modelled by the dissolution test.

Single-point level C correlations, therefore, also have a limited use for regulatory purposes.

ANIMAL MODELS

Man is the ultimate model for *in vivo* evaluations, such as bioavailability studies or other investigations of new formulations. However, the use of animal models can provide some important advantages:

- The possibility to perform early *in vivo* studies during preclinical drug development before studies in man are possible
- The use of advanced sampling techniques and other manipulations of experimental conditions not possible in man
- Studies which are cheaper and faster than studies in man

Animal models may thus not only be used as a screening tool before studies are performed in humans, but also for later mechanistic evaluations of findings obtained in human studies.

The main limitation of the usage of animal models is that no single species resembles all physiological properties of man. This introduces a risk that the results obtained in the animal model are not relevant for the situation in man. In the evaluation of oral dosage forms, the main aspects to consider are physiological features of the GI tract, such as dimensions, residence times in different segments, motility patterns, secretions, physical and physicochemical characteristics of GI fluids, the presence of enzymes that could metabolise drugs, and critical excipients, since these factors could directly affect the formulation performance. In cases where the main study variable is the drug plasma concentration, other factors such as the presence of active drug transporters over the GI wall or species differences in first-pass metabolism may also confound the extrapolation of animal data to man. These problems can be handled by choosing the best possible animal model and an appropriate study design and by integrating knowledge of differences between animal and man in the interpretation of obtained results.

Choice of Animal Model

The great majority of studies are performed in dogs. They are, together with other animals such as pigs and monkeys, generally the most suitable species due to many anatomical and physiological similarities with man. Pigs and monkeys, however, have some practical disadvantages compared to dogs, such as being more difficult to train, the need for larger space, ethical concerns and high costs. Rabbits are also used to some extent, despite some fundamental physiological differences to humans. Smaller animals such as rodents, common in other pre-clinical experiments, are often too small to allow administration of solid formulations.

The initial approach should be to first identify which formulation properties are critical for its *in vivo* function. Thereafter, all potential physiological factors that may influence this function should be identified and the correlation between the animal and man regarding those factors should be considered. For example, in the case of a pH-dependent enteric-coat formulation, the dissolution of the coating layer will clearly be a critical formulation variable. The pH in the stomach and small intestine, as well as gastric emptying, will all be critical variables. If the correlation of such parameters with man is poor for all available animal models, there is no rationale for performing such studies if the deviations cannot be accounted for when interpreting the results.

Physiological and anatomical characteristics for different species are summarised in Table 7.7 (Dressman and Yamada 1991). Some additional information of relevance for the use of animal models in formulation studies can be found in other review articles (Kararli 1995; Ritschel 1987).

The most frequently used animal is the dog, which can in many cases be an acceptable model due to its similarity to man regarding anatomy, motility pattern, GI residence times and many secretory components. However, cases exist where favourable results in dogs have not been verified in subsequent human studies. Examples of differences that could lead to pitfalls in formulation development studies are:

- No basal acid secretion in the stomach during fasted state, and thereby often close to neutral pH in contrast to the acidic human pH. However, an acidic pH may be induced in some individuals without intake of food. This has to be specially considered, for example, when drug solubility is pH dependent and for enteric-coated formulations, for which drug release is dependent on the pH in the GI tract.
- The dog has a higher bile salt concentration than man, which could potentially lead to too favourable conditions for dissolution of very low water soluble drugs.
- Fed state could be introduced by coprophagy, which may lead to the tablets and capsules being retained in the stomach for a long time.
- The residence time in the colon is much shorter than in man, making the dog a poor model for long-acting ER formulations, due to the risk of incomplete drug release before the formulation is expelled from the body.
- The microbiological activity in the distal parts of the intestine is much less than in man, which has implications for drugs or excipients that are metabolised by gut flora enzymes, or drug release principles, based on this process.

Table 7.7
Comparison of anatomical and physiological data for the cecum and colon of humans, dogs, swine, and rhesus monkeys (Dressman and Yamada 1991).

Parameter	Human	Dog	Swine	Monkey
Length (autopsy) (m)	7	4	15-20	5 cm (duod.)
(<i>in vivo</i>) (m)	3	1.5		
Duodenal diameter (cm)	3-4	2-2.5	2.5-3.5	1.5-2
Villi: length	0.5-1.5		0.5-1	
shape	Filamentous	Long, slender		Ridges, leaflike
Peyer's patches: location	Ileum	Duodenum, jejunum, ileum	Jejunum, ileum, colon	Ileum
<i>Secretions</i>				
Bile: major acid	Cholic	Cholic	Hyocholeic	
conc. by gallbladder	×5	×8-10	×2	
flow rate (L/day)	0.8-1	0.1-0.4		
Pancreatic juice flow rate (mL/min)	1	2-3	1	
Intestinal pH: fasted	6.1 [5.6-6.4] ^a	6.5-7.5	7.2 (duodenum)	7-9; 5.5-6.0
fed	5.4 [5.0-5.8] ^a	6.5-6.9		
Cecum:				
length (cm)	7	12-15	20-30	5-6
diameter (cm)	6		8-10	
volume (L)	about 1	0.25	1.5-2.2	
Colon:				
length, overall (m)	0.9-1.5	0.6-0.75	4-4.5	0.4-0.5
length, asc. colon (cm)	20		Long, coiled	10
diameter (cm)	6	Similar to SI	8-10	
haustrae	Present	Absent	Present	Present
Microbial metabolism, colon	Volatile fatty acids Vitamins	Very minor	Volatile fatty acids Vitamins	
pH, colon	6 (upper) 7.5 (lower)			

^a Lower duodenum

Reprinted from Animal models for oral drug absorption, by J. B. Dressman and K. Yamada in *Pharmaceutical Bioequivalence*, edited by P. G. Welling, F. L. S. Tse, and S. V. Dighe, pp. 235-266 by courtesy of Marcel Dekker, Inc.

Study Design Aspects in Bioavailability Studies

Most investigations in animals aimed at evaluating new formulations are bioavailability studies, i.e., the rate and extent of dissolution/release is determined from plasma concentration-time data. There may be differences in drug absorption, first-pass metabolism, distribution and elimination between animals and man, which will lead to different plasma

concentration–time profiles. However, this is a minor problem in the evaluation of dosage forms, provided that an oral drug solution is included in the study as a reference. Thereby, it is possible to eliminate the influence of pharmacokinetic factors other than those purely related to the formulation function. The same methods and assumptions should be applied as described in the section “Bioavailability Studies”.

In most animal studies, the dose is given as the amount of drug in relation to body weight. In an evaluation of dosage form, it is preferable to administer the doses intended for humans in order to avoid development of specific low-dose formulations for animal studies. Two prerequisites of specific significance for the usefulness of this approach are (a) no toxicological effects at the given dose and (b) no saturation of any process involved in the drug absorption.

In the *administration of solid dosage forms* to dogs, the formulation is placed into the posterior pharynx followed by closing the dog's mouth. A feeding tube is subsequently inserted into the trachea, and 50 to 75 mL of water is administered. Liquid formulations are administered directly through a feeding tube. For administration to pigs or monkeys, applicators are often used to insert the dosage form. Small solid formulations (<5 mm) may also be administered to rats in this manner.

Many animals, including dogs, rats, rabbits and monkeys eat excrement, which is called *coprophagy*. This could alter the physiological conditions in the GI tract, such as fasting/fed motility, secretions and microflora, and thereby lead to an uncontrolled study situation. Furthermore, drug or metabolites that have been excreted by faeces could be re-absorbed, which will obscure interpretation of bioavailability data. Coprophagy has thus to be prevented by using cages with bottom screens and by placing plastic collars around the animals neck, preventing the animal from reaching its anus.

Additional information on design of bioavailability studies in animals can be found in Ritschel (1987).

IMAGING STUDIES

Non-invasive imaging can be used to study the dosage form after administration in man or animals. The amount of radioactivity administered in such studies is at such a low level that no known hazards exist for study subjects. Several factors of relevance for the function of the formulation can thus be monitored directly *in vivo*. Potential study variables are given below:

- Residence time in different parts of the GI tract
- Location of a formulation within the GI tract at a certain time point
- Transit/emptying times in the GI tract
- Distribution of multiple units or liquid vehicles in the GI tract
- “Pharmacoscintigraphy”—relation of drug plasma concentrations to location of dosage form
- Disintegration/erosion of solid formulations
- Release of a marker substance from the formulation

More detailed overviews of different applications of imaging in *in vivo* studies of formulations can be found in Wilding et al. (1991) and in Digenis et al. (1998a, b).

The imaging data can be used either as the main study variable, for example, in case of regional GI treatments, or to provide complementary information to bioavailability data. In the latter case, the information obtained regarding the location and performance of the formulation provides information that could often be crucial for correct interpretation of the bioavailability data, especially in the case of modified release formulations. Data obtained from imaging studies are generally not required by regulatory agencies, but they may well be used as supportive information in an NDA file.

Gamma scintigraphy has been established as the method of choice for *in vivo* imaging studies of formulations. Other methods such as positron emission tomography (PET), magnetic resonance imaging (MRI), ultrasound and X ray have a much more limited applicability. In gamma scintigraphy, the formulation is visualised by gamma radiation emitted from trace amounts of one or two radionuclides that have to be included in the dosage form. These types of studies in humans or animals are often performed at specialised Contract Research Organisations or at university hospitals. They involve several disciplines, besides traditional biopharmaceutical and pharmaceutical competence, such as radiochemical, nuclear medicinal or other imaging expertise. However, it is crucial that the pharmaceutical/biopharmaceutical scientist, who understands the critical manufacturing variables and the biopharmaceutical function of a dosage form, cooperates with the radiation and imaging expertise in the design and evaluation of such studies. Furthermore, manufacturing and *in vitro* testing of labelled formulations may often be an in-house activity at the company developing the formulation of interest. The present chapter will include an introduction of the basic principles of gamma scintigraphy, different aspects of labelling of formulations and performance of *in vivo* studies.

Basic Principles of Gamma Scintigraphy

The physical principles of gamma scintigraphy are based on the fact that a radionuclide emits electromagnetic gamma rays at decay to stable isotopes. The decay of a radionuclide is logarithmic, and the half-life ($t_{1/2}$) is a key characteristic determined by the following equation:

$$A_t = A_0 e^{-kt} \quad (28)$$

where A_t and A_0 are the radioactivity at time zero and after t time units after time zero, respectively, k is a first-order decay constant, expressed in the same time unit as t . The decay half-life equals $\ln 2/k$. The amount of radioactivity is expressed in Becquerel (Bq), which corresponds to decay/second or Curie (Ci), where $1 \text{ Ci} = 3.7 \times 10^{10} \text{ Bq}$. The gamma rays travel at the speed of light and permeate effectively through the formulation, body tissue and air, although some energy is lost when the photon hits atoms. Such attenuation of the gamma radiation follows the following equation:

$$I_l = I_0 \times e^{-\mu l} \quad (29)$$

where I_l is the intensity of the radiation after travelling the length l through a material with an attenuation coefficient μ , and I_0 is the intensity of the radiation before it hits the material. Typical lengths (l) in water and lead, which reduce the intensity to half of its initial value ($I_0/2$) for radionuclides used in formulation studies, are about 0.5 m and 1 mm, respectively.

A schematic picture of a gamma camera is shown in Figure 7.12. The radiation enters the gamma camera through a collimator, which is a lead shield with many small holes. The purpose of the collimator is to limit the field of view and thereby prevent radiation from non-target areas, such as naturally existing background radiation, from reaching the detectors. When the gamma ray has passed the collimator, it hits an NaI(Tl) crystal and a light impulse is created. An array of photomultipliers is used to determine the position of the light. The signal is then digitalised, and a picture can be produced, or other quantifications can be performed with computer programs. Two radionuclides can be monitored simultaneously if they produce different peak photon energies. This makes it possible to concomitantly administer two formulations with different labellings for head-to-head comparisons under identical conditions. Primarily, an image is obtained which consists of a two-dimensional matrix of, for example, 64×64 units called pixels. The radioactivity gathered during the sampling period within each pixel will provide different colours on an image where most often black-blue-red-yellow-white corresponds to different intensities from very low to maximum. Examples of gamma camera images of a labelled multiple unit formulation at different times after intake are given in Figures 7.13a–c. The data behind the construction of the gamma camera image may be further analysed to obtain various quantifications, as described further below. The spatial resolution, i.e., the minimum distance between two labelled objects that can be distinguished by the camera, is dependent on the radionuclide, collimator and distance from the object to the detector. A normal value of the spatial resolution obtained in phantom studies to mimic the *in vivo* situation is about 0.5 cm.

Figure 7.12 Schematic drawing of a gamma camera.

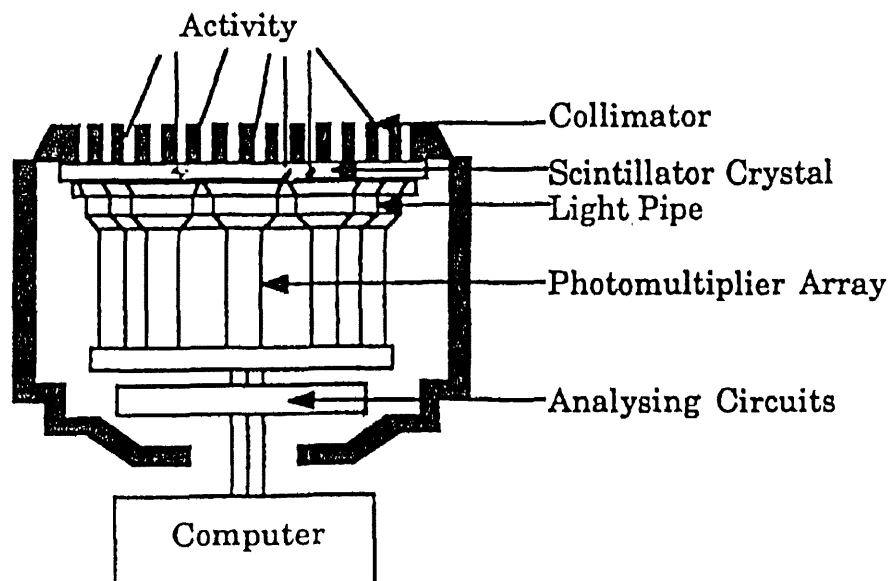
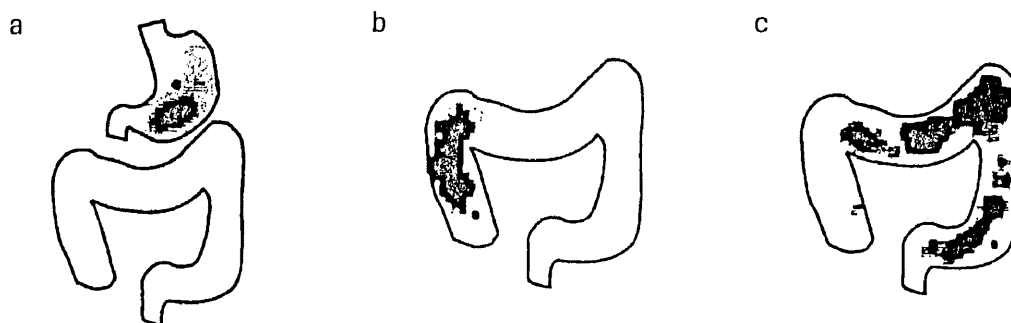


Figure 7.13 Example of a series of gamma camera images of pellet formulation obtained at 0.5 h (a), 10 h (b) and 24 h (c).



Labelling of Formulations

Labelling Principles

Radionuclides are generally introduced in the formulation by physical mixture rather than chemical bonding. The simplest procedure is to mix a radionuclide solution with other excipients, i.e., in a normal granulation step, thereby getting an even distribution of the marker in the formulation. A formulation, or one of its intermediate states, could also be soaked in a solution of the radionuclide. Other methods are including the radiomarker as discrete particles formed by binding to a resin, precipitation as a poorly soluble salt or oxide, coating with non-dissolving material or polymeric material, or by combinations thereof. Such particulate marker material could be included at well-defined positions within the formulation, or by random distribution of a multitude of particles. A formulation may include two radionuclides with different energies in order to follow more than one process at the same time.

The two main aspects in the radiolabelling of a formulation that will guide the choice of labelling principle are (a) that the labelling should provide an unbiased estimate of the decided imaging study variables and (b) that the function of the dosage form is unaltered. For example, if the purpose is to study the location of a solid dosage form at various times in the GI tract, it is critical that the marker is not released from the formulation. In addition, if the formulation is of a disintegrating type, it should be possible to conclude when this has happened to avoid the location of a free marker being interpreted as the position of the formulation. If the aim is to study drug release from the formulation, the radionuclide should be released from the formulation at a rate that corresponds to the drug of interest. The greatest risk for altered formulation performance by the labelling is if "micro-scale" manufacturing equipment has to be used or if other non-standard manufacturing conditions exist in order to minimise radioactive exposure to laboratory personnel.

In Vitro Evaluation

Before performing an *in vivo* study, *in vitro* testing must be used to see if the intended function of the radiolabelling has been met and that the properties of the formulation are unaffected by the labelling. In these tests, the amount of radionuclide released from the

formulation should be determined at physiologically relevant conditions. The amount of released radionuclide could be determined by liquid scintigraphy for assessment of radionuclide in solution and/or by assessing the remaining radioactivity in the solid dosage form by an ionic chamber detector.

Choice of Type and Amount of Radionuclide

Several radionuclides are suitable for use in imaging studies of formulations as outlined in Table 7.8. In the choice of radionuclide, the following aspects should be considered:

- Half-life of radionuclide
 - duration of gamma measurements
 - time needed for manufacturing and QC in advance of study start
- Energy of radionuclide
 - sensitivity of gamma camera crystal to detect radiation
 - combination of two radionuclides with different energies for simultaneous measurements
- Suitability of physicochemical properties of available chemical forms of the radionuclide
 - provide unbiased estimates of desired imaging variables
 - no systemic absorption

Most radionuclides are available as complexes (e. g., ^{99m}Tc -diethylenetriaminepentaacetic acid [DTPA]), or as oxides. The chemical and physicochemical state of the radionuclide should not be altered by either the formulation excipients and/or the physiological conditions in the

Table 7.8
Radionuclides suitable for labelling of formulations, including
decay half-life ($t_{1/2}$) and peak photon energy.

Radionuclide		$t_{1/2}$	Energy (keV)	Comments
Technetium	^{99m}Tc	6.0 h	140	
Indium	^{111}In	2.8 days	173–247	
Iodine	^{131}I	8 days	364	
Chromium	^{51}Cr	28 days	320	
Samarium	^{153}Sm	46.7 h	69–103	Obtained by neutron activation
Erbium	^{170}Er	7.5 h	112–308	"
Ytterbium	^{175}Yb	4.2 days	114–396	"
Barium	^{138}Ba	84 min	166	"

GI tract. Such effects could lead to the radionuclide disappearing from the formulation in an unintended manner, thereby obscuring data evaluation. The amount of radioactivity included in a formulation is determined by the precision and variability that is required in the gamma scintigraphic measurements and by the acceptable level of radiation exposure to study subjects.

Safety Aspects in Manufacturing and Analysis of Radiolabelled Formulations

Radiation exposure is one of the most well-studied hazards to human health, but it has not been possible to link any increased incidence of impaired health at the levels of exposure that are allowed for laboratory personnel. However, higher radiation exposure is harmful, which has several important implications. First, key prerequisites are that personnel involved in the handling of radioactive material are properly educated in how to avoid unnecessary exposure to people and the environment, that appropriate protective and dosimetry devices are used and that a plan is implemented for the handling and disposal of material. Second, it is usually not possible to use normal laboratory or pilot plant-scale equipment, since such batch sizes will require the handling of excessive amounts of radioactivity. Therefore, "micro-scale" equipment may be developed for certain processes.

Neutron Activation

Neutron activation is an elegant way to avoid restrictions and hurdles introduced by the handling of radioactive material in the manufacturing of formulations. Especially favourable is the possibility to use standard equipment and operation conditions. In this procedure, small amounts of a stable isotope, typically a few milligrams, are included in the formulation. The labelled formulation is subsequently exposed to a neutron flux, and the stable isotope is thereby transformed to a radioactive isotope upon capture of a neutron. The most commonly used isotopes for this purpose are listed in Table 7.8. Several of the listed radionuclides are available in both enriched and natural forms; the use of the former, however, requires a less stable isotope to be included and/or less neutron radiation, at the disadvantage of higher costs. The main potential problem connected with neutron activation of dosage forms is the risk of affecting tablet function or causing degradation of the active compound as well as of excipients. Therefore, this has to be checked on a case-by-case basis before the start of an *in vivo* study. Another potential problem is the creation of radionuclides, other than the intended one, which will obscure data interpretation and increase radiation exposure. If the undesirable isotopes have a significantly shorter half-life than the labelling isotope, this problem can be handled by waiting for a suitable time period such that the activity of the unintended isotope has declined, whereas significant activity remains for the desired radionuclide. One example of a common stable isotope that unintentionally can form radionuclides by neutron flux is sodium (^{24}Na , $t_{1/2} = 15 \text{ h}$).

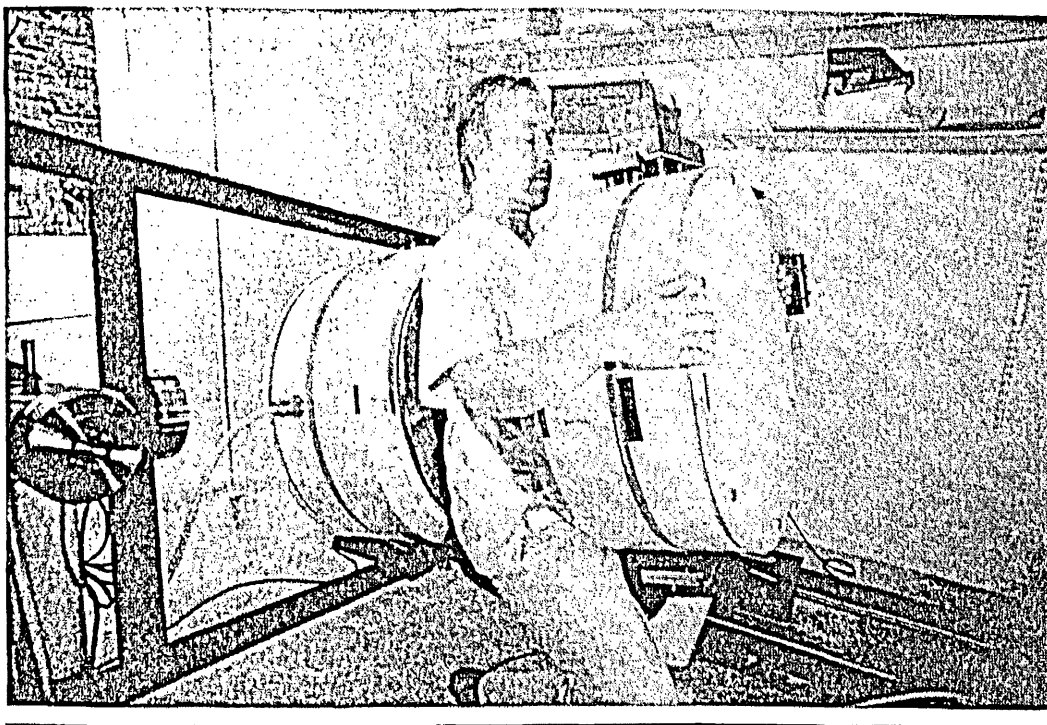
Reviews of different applications of neutron activation in studies of dosage forms can be found in Digenis and Sandefer (1991).

Conduct of In Vivo Studies

Practical Procedures

The subject is positioned in front of a gamma camera, or between two cameras, one in front of the subject and one behind (see Figure 7.14). The latter principle is needed when making quantifications of radioactivity at different positions in the GI tract in order to avoid influence

Figure 7.14 A gamma camera investigation with two opposite cameras.



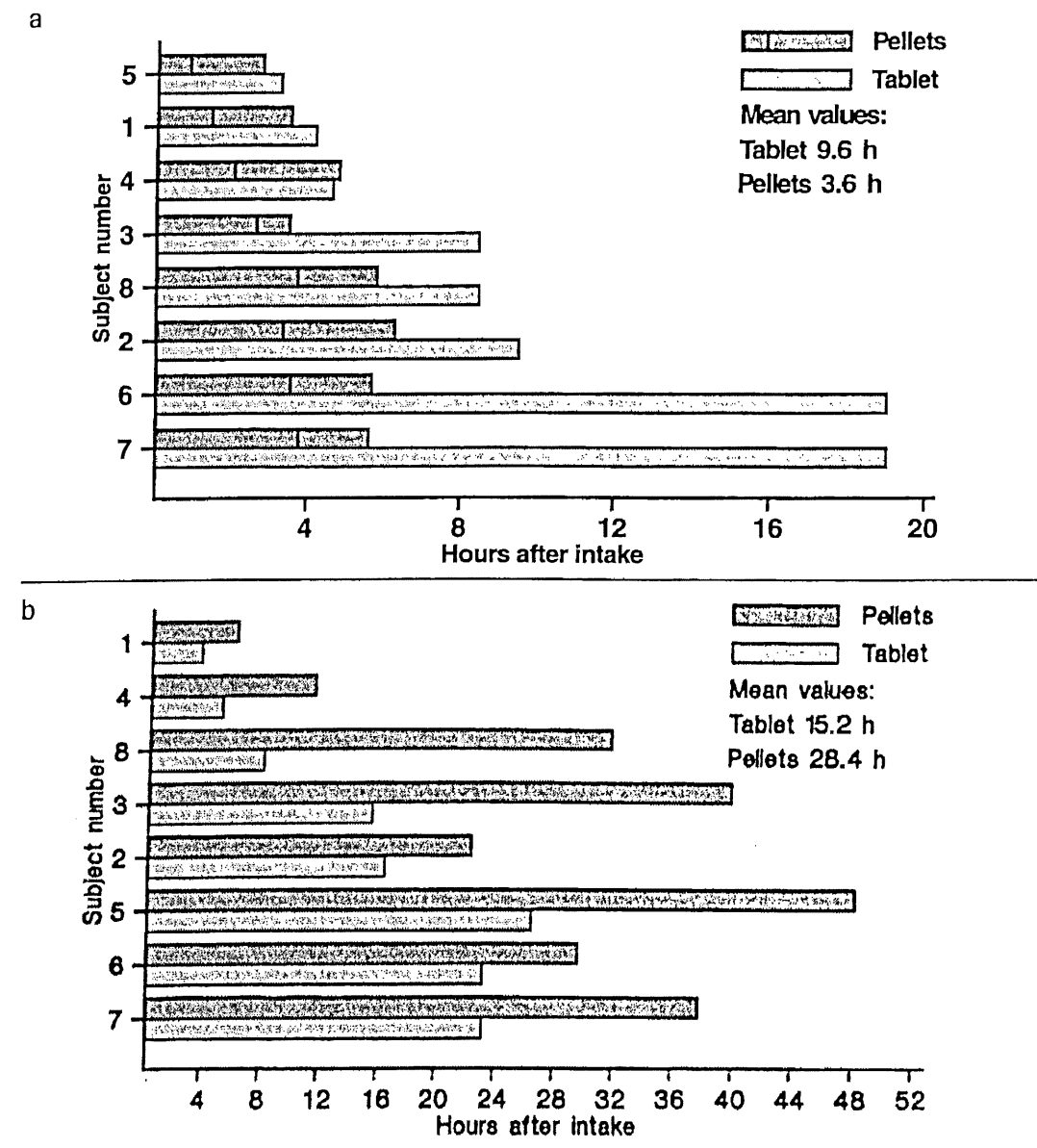
of the position-dependent attenuation of gamma radiation, whereas a one camera system is generally sufficient for locating single units. Systems with cameras that circulate around the subject for creation of three-dimensional images are also available, but their use in formulation studies in man are restricted by the higher amounts of radioactivity needed in the formulation to obtain reliable data. The field of vision usually covers the entire GI tract in most subjects, but sometimes the position of the camera has to be adjusted to cover the lower parts of the abdomen.

The data collection can be dynamic, i.e., the subject is constantly located in front of the gamma camera system, and signals are constantly gathered into images within very short time frames (often <1 min). This procedure may be advantageous to follow very rapid processes. In most cases, radiation is gathered over a finite time period to generate pictures. The time period for collecting radiation to one picture, typically 30 sec to 1 min, is mainly determined by quality requirements on picture/variability in quantitative data and the risk of moving artefacts.

Data Evaluation

It is almost always of interest to determine the localisation, emptying times from one segment to another or transit times through a segment of the formulation in the GI tract. An example of the gastric emptying and colon transit time of a non-disintegrating tablet and small pellets after administration with a breakfast are given in Figures 7.15 a,b. Normally, it is possible to identify the stomach, terminal ileum, caecum, ascending/transversal/descending/sigmoidal

Figure 7.15 Gastric emptying time after administration with breakfast (a) and colon residence time (b) for a non-disintegrating tablet and pellet formulation in eight individual subjects (Abrahamsson et al. 1996).



colon or rectum. In contrast to other parts of the GI tract, it is more difficult to obtain a precise position in the small intestine due to the anatomy.

The location of a single unit in the GI tract is determined from the position of the gamma camera image. Since no image of the GI anatomy is obtainable, an external radioactive marker may be positioned at a well-defined anatomical position as a reference point in the determination of the radiolabelled formulation. Another approach for facilitating determination of

position is to administer a radiolabelled reference solution, which will provide images of certain intestinal parts, such as the stomach. In the case of solid multiple particles, or liquid formulations, the emptying, transit or arrival time is a continuous process over a certain time interval. This is exemplified in Figure 7.16, which shows the amount of food and a floating antacid remaining in the stomach at different times. The emptying or arrival processes from, or to, a certain region are determined by defining a region encompassing the area of interest. The number of counts obtained within this region is determined at several time points after correction for background radiation. The number of counts is often expressed as a percentage, defining the value obtained in the stomach immediately after administration as 100 percent. A rate constant, for example, the gastric emptying time, can then be determined from the emptying-time profile by applying moment analysis (see the section "In Vitro Dissolution"), a model containing a rate constant or by just determining the time point when 50 percent has been emptied, based on the type of data presented in Figure 7.16. The gastric emptying of solids and liquids is often approximated by zero- and first-order kinetics, respectively, although individual data, especially for particles, often shows more discontinuous profiles. The transit times through a segment are determined as the difference between the arrival time to the subsequent compartment and the emptying from the preceding region. For example, the small intestinal transit time is determined from the difference between the colon arrival and the gastric emptying times.

In quantification of the amount of radionuclide remaining in a formulation, for the monitoring of a release process, a region of interest is defined around the formulation, and a quantification of the number of counts within this region is determined at different times after correction for background radiation. Since the formulation may be positioned at different depths in the body at different times, which will affect the number of counts, obtaining simultaneous measurements by a posterior and anterior camera is necessary to avoid artefacts. The geometric mean of the number of counts obtained by the two cameras is used as the characteristic of the release process. The data may be expressed in a percentage, where the number of counts obtained within the formulation immediately after intake is defined as 100 percent. It is important to have frequent data sampling, since a certain number of images have to be discarded due to formulation movements during data collection or other anomalies and arte-

Figure 7.16 The gastric emptying of a meal and a floating antacid formulation after concomitant administration.

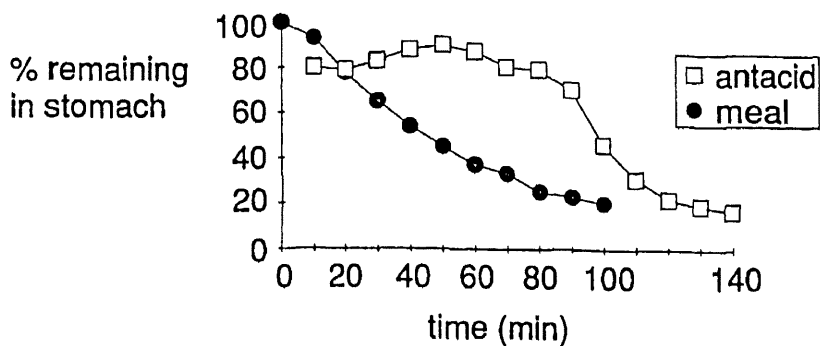
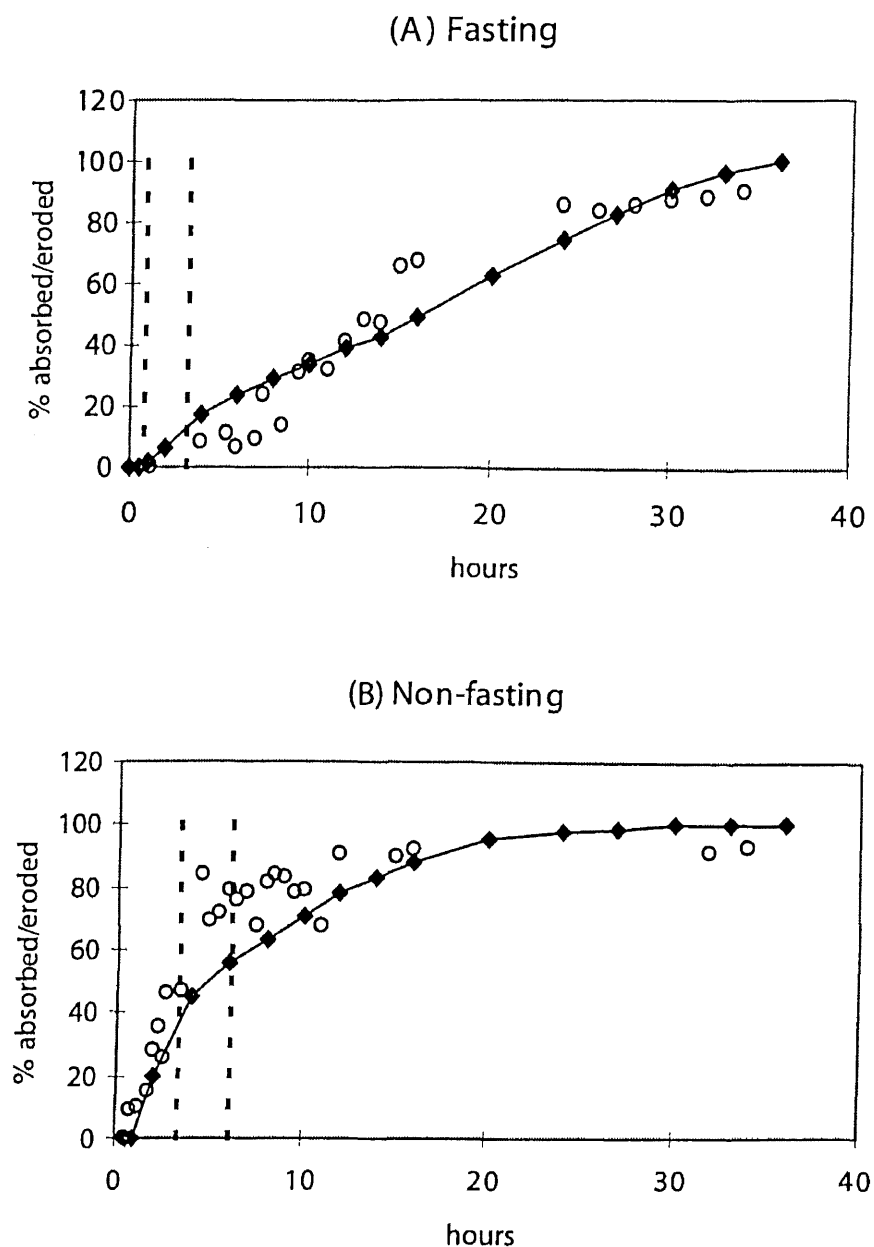


Figure 7.17 Tablet erosion (circles) and cumulative drug absorption (diamonds) for a hydrophilic matrix ER tablet after administration under fasting (a) and non-fasting (b) conditions in one subject. The two dashed vertical lines represent gastric emptying and colon arrival, respectively. The figures illustrate the correspondence between tablet erosion and drug absorption. In addition, the figures also show that the more rapid absorption after intake with food is caused by an increased erosion rate (Abrahamsson 1998b).



facts. A special problem in data evaluation could occur if release of radiolabelled material gather together with the remaining formulation, which, for example, may happen in the terminal ileum due to normal physiological function.

An example of determination of tablet erosion of a hydrophilic ER matrix tablet is given in Figures 7.17 a, b, where determinations were made after administration under fasting and non-fasting conditions. The scintigraphic erosion data revealed the underlying reason for an increase of the drug absorption rate obtained after non-fasting compared to fasting administration.

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