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Review

Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms

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Dissolution tests are used for many purposes in the pharmaceutical industry: in the development of new products, for quality control and, to assist with the determination of bioequivalence. Recent regulatory developments such as the Biopharmaceutics Classification Scheme have highlighted the importance of dissolution in the regulation of post-approval changes and introduced the possibility of substituting dissolution tests for clinical studies in some cases. Therefore, there is a need to develop dissolution tests that better predict the *in vivo* performance of drug products. This could be achieved if the conditions in the gastrointestinal tract were successfully reconstructed *in vitro*. The aims of this article are, first, to clarify under which circumstances dissolution testing can be prognostic for *in vivo* performance, and second, to present physiological data relevant to the design of dissolution tests, particularly with respect to the composition, volume, flow rates and mixing patterns of the fluids in the gastrointestinal tract. Finally, brief comments are made in regard to the composition of *in vitro* dissolution media as well as the hydrodynamics and duration of the test.

KEY WORDS: dissolution tests; prediction of *in vivo* performance; dissolution test conditions; composition of dissolution media.

INTRODUCTION

An important aspect of the development of a pharmaceutical product is to find an *in vitro* characteristic of potential formulations that reflects their *in vivo* performance. Although immediate release solid dosage forms are routinely subjected to tests such as content uniformity, weight, hardness, friability and disintegration, the test that is most often associated with the assessment of *in vivo* performance is the dissolution test. Currently there are about 500 tablet and capsule monographs in the USP which have dissolution requirements (1), and dissolution testing is an integral component of new drug applications to regulatory bodies worldwide.

In vitro dissolution testing provides useful information at several stages of the drug development process. Formulation scientists use dissolution to assess the dissolution properties of the drug itself and thereby select appropriate excipients for the formulation. Dissolution testing is also employed to assist in choosing among candidate formulations, with the aim of selecting the dosage form with the most suitable and reproducible release profile. However, if these tests are not performed under appropriate conditions, the prediction of which drugs and which dosage forms will exhibit the desired release profiles *in vivo* may be completely erroneous.

Clinical scientists rely on dissolution tests to establish *in vitro/in vivo* correlations between release of drug from the dosage form and drug absorption. When *in vitro* results fail to adequately predict the *in vivo* performance of a drug product, more and larger clinical studies are needed to assess product bioavailability, thus adding substantially to the cost of product development. For drugs and formulations that have release rate limited absorption, it is also of particular interest to know whether the drug will be better absorbed when the product is given with food. Current pharmacopeial tests do not address this need.

From the regulatory scientist's point of view, the evaluation of preclinical and clinical data would be greatly facilitated by the availability of validated, prognostic dissolution methodology for the product. In certain cases, it may be appropriate to use dissolution test results to evaluate the biopharmaceutical implications of a product change, rather than to automatically require a bioequivalence study (2).

Important aspects of the quality assurance of a drug product include the ability to confirm that the correct manufacturing procedures have been followed for a given batch, that batch-to-batch reproducibility of the product meets regulatory requirements, and that the product performs adequately throughout its

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shelf life. Insofar as possible, the *in vitro* test conditions should bear a meaningful relationship to the conditions in the gastrointestinal tract (3). In the case of very poorly soluble drugs, however, the ability to test whether the product is able to release all of the active drug within the desired time-frame under physiologically relevant test conditions may be difficult to achieve with current apparatus.

In summary, there is a real need to develop dissolution tests that better predict *in vivo* performance of drug products. This could be achieved if the conditions in the gastrointestinal tract were successfully reconstructed in *in vitro* test systems. The development of prognostic *in vitro* tests should lead not only to a reduction in the work needed for formulation development, but also in the number and size of clinical studies required, and to more meaningful quality assurance tests.

In this article, we seek first to clarify under which circumstances dissolution testing can be prognostic for *in vivo* performance, then to present physiological data relevant to the design of dissolution tests, particularly with respect to the composition of the medium, the hydrodynamics employed and the duration of the test. In a companion article, examples of drugs for which dissolution is highly dependent on test conditions will be used to illustrate the importance of selecting physiologically relevant test conditions for *in vitro* performance tests.

RATE LIMITING FACTORS TO DRUG ABSORPTION

Essentially, there are four possible sources of incomplete drug absorption following the oral administration of a solid dosage form (4):

1) The drug is not delivered from its formulation over an appropriate time frame in solution form to those sites in the GI tract where it is well absorbed,

2) The drug is decomposed in the gastrointestinal tract or forms a nonabsorbable complex,

3) The drug is not transported efficiently across the gut wall in the apical to basal direction, and/or

4) The drug is metabolized and/or eliminated *en route* to the systemic circulation. These possibilities are illustrated in Figure 1.

Since the gastrointestinal tract is not a static system, the rate at which release, decomposition, complexation and gut wall transport occur must additionally be weighed against the transit rate of the dosage form/drug through the gastrointestinal tract. In order for a drug to be well absorbed, release and uptake must be completed within the time taken for the dosage form/drug to traverse that part of the gastrointestinal tract up to and including the sites at which the drug is absorbed, whereas decomposition and complexation must occur more slowly than either release/uptake or transit.

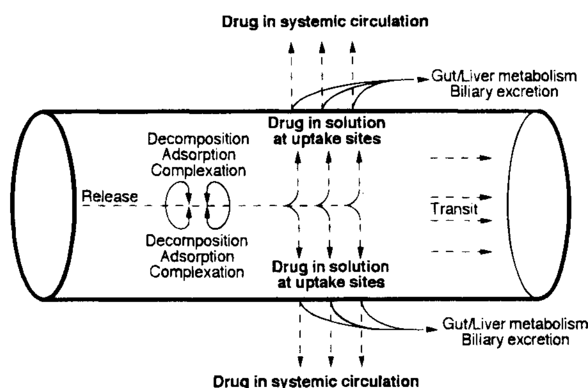

Fig. 1. Steps in drug absorption and sources of incomplete bioavailability following oral administration of a solid dosage form.

THE BIOPHARMACEUTICS CLASSIFICATION SCHEME

Recently, a Biopharmaceutics Classification Scheme (BCS) has been proposed (5). Under this scheme (Table 1), drugs can be categorized into four basic groups according to their solubility properties and their ability to penetrate the gastrointestinal mucosa.

Thus, the BCS addresses two of the potential four limitations to oral drug bioavailability. Of these two, drug solubility recognizes the physicochemical limitations of the drug as a potential source of incomplete release from the dosage form. It is important to understand that the classification is based on the solubility properties of the drug substance throughout the upper GI tract. In the Commentary Section, appropriate media for such studies are suggested. The results of dissolution studies with the drug in the same media can be subsequently used in the development process to assess the influence of formulation on the release rate. Permeability studies are needed to locate the main sites of drug absorption in the gastrointestinal tract, as well as assessing the efficiency of drug transport across the gut wall. A variety of cell culture, tissue and animal models are available for assessing permeability; currently there are also several groups studying permeability of drugs directly in humans (6,7). In principle, studies addressing drug stability problems in the lumen of the gastrointestinal tract should be run in media that reproduce the conditions to which the drug is likely to be subjected. In this respect, considerations for design of stability test media parallel those applicable to release and dissolution studies. In the case of drugs that undergo metabolism in the gut wall and/or liver, the rate and extent of the effect must be assessed using tissue preparations or from pharmacokinetic analysis.

Although the BCS is limited to two of the four important factors, it nonetheless provides a useful starting point for recognizing when and how dissolution tests can aid in the design and evaluation of oral dosage forms. Compounds belonging to Class I, i.e. compounds with high solubility and permeability, should go into solution quickly when they are housed in immediate release dosage forms, and also be rapidly transported across

Table 1. The Biopharmaceutics Classification Scheme

Class I: HIGH SOLUBILITY HIGH PERMEABILITY	**Class II:** LOW SOLUBILITY HIGH PERMEABILITY
Class III: HIGH SOLUBILITY LOW PERMEABILITY	**Class IV:** LOW SOLUBILITY LOW PERMEABILITY

Note: From Ref. 5.

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the gut wall. Therefore, it is expected that they will be well absorbed unless they are unstable, form insoluble complexes, are secreted directly from the gut wall, or undergo first pass metabolism. Dissolution tests for immediate release formulations of Class I drugs, therefore, need only to verify that the drug is indeed rapidly released from the dosage form under mild aqueous conditions.

For Class II drugs, by contrast, the rate of dissolution of the drug is almost certain to be the principal limitation to its oral absorption. The limitation can be equilibrium or kinetic in nature. In the case of an 'equilibrium' problem there is not enough fluid available in the gastrointestinal tract to dissolve the dose. This can be checked by calculating the dose:solubility ratio (8). For example, at a dose of 500 mg and an aqueous solubility of 15 μg/ml at 37° C, 33 liters of fluid are required to dissolve one dose of griseofulvin. Since the total volume of fluid entering the gastrointestinal tract in a twenty-four hour period is only about five to ten liters (9), there is clearly insufficient volume present at any given time for the entire dose of griseofulvin to be dissolved. In the case of a 'kinetic' problem, the drug dissolves too slowly for the entire dose to become dissolved before the drug has passed by its sites of uptake. Digoxin, for example, with a dose of 0.25 mg and a solubility of 20 μg/ml, has a dose:solubility ratio of just 12.5 ml. Despite the small volume of fluids required to dissolve the drug, digoxin exhibits dissolution rate limited absorption at particle sizes of greater than 10μ in diameter (8) because the poor driving force for dissolution supplied by the solubility, combined with the low surface area of drug at larger particle sizes, is insufficient to ensure timely dissolution. For Class II drugs, it should therefore be possible to establish a strong correlation between the results of dissolution tests and the *in vivo* absorption rate. Establishment of an *in vitro/in vivo* correlation and the resultant ability to discriminate between formulations with different bioavailabilities will be dependent on how well the *in vitro* tests are designed. In order to be successful, it is necessary to reproduce the conditions extant in the gastrointestinal tract following administration of the dosage form as closely as possible. Adequate comparison of formulations for Class II drugs requires dissolution tests with multiple sampling times in order to characterize the release profile (2), and in some cases the use of more than one dissolution medium may also be worth considering.

Like compounds belonging to Class I, Class III drugs are rapidly dissolving and the test criterion should be that the formulation can release the drug under mild aqueous conditions within a predetermined time. Rapid dissolution is particularly desirable for Class III drugs, in order to maximize the contact time between the dissolved drug and the absorbing mucosa, and consequently the bioavailability of the compound. Therefore, the duration of the dissolution test should be at least as stringent for Class III drugs as for Class I drugs. Class IV drugs are expected to have poor absorption in general, but it is anticipated that, as in the case of Class II drugs, poor formulation could have an additional, negative influence on both the rate and extent of drug absorption. Thus, for all four categories, it is anticipated that well-designed dissolution tests can be a key prognostic tool in the assessment of both the drug's potential for oral absorption and of the bioequivalence of its formulations.

IMPORTANT CONSIDERATIONS IN DISSOLUTION AND THEIR CORRESPONDING PHYSIOLOGICAL PARAMETERS

From the following equation, based on the Nernst-Brunner and Levich modifications of the Noyes-Whitney model (10–12), the factors important to the kinetics of drug dissolution can be identified:

$$\frac{dX_d}{dt} = \frac{A * D}{\delta} * (C_s - X_d/V)$$

where A is the effective surface area of the solid drug, D is the diffusion coefficient of the drug, δ is the effective diffusion boundary layer thickness adjacent to the dissolving surface, C_s is the saturation solubility of the drug under lumenal conditions, X_d is the amount of drug already in solution and V is the volume of the dissolution medium. Some of these factors are primarily influenced by physicochemical properties of the drugs, but most are also influenced by the conditions in the gastrointestinal tract. A summary of the relevant physicochemical and physiological parameters is given in Table 2.

The key factors in the dissolution of drugs in the gastrointestinal tract are thus the composition, volume and hydrodynamics of the contents in the lumen following administration of the dosage form. Only when these factors are adequately reproduced *in vitro* can we expect to accurately predict dissolution limitations to absorption.

In addition to these factors, the permeability of the gut wall to the compound plays a role in the maintenance of sink (less than 20% of saturation concentration) conditions for dissolution, which are required for the fastest possible dissolution rate. For highly permeable drugs sink conditions are likely to be maintained, in which case the dissolution rate per unit surface area will be constant and close to the initial dissolution rate. For less permeable drugs, the dissolution rate per unit surface area will decrease with time, due to the gradual buildup of drug in solution in the lumen.

The lumenal conditions in the gastrointestinal tract vary widely both within and between subjects. Intersubject variabil-

Table 2. Physicochemical and Physiological Parameters Important to Drug Dissolution in the Gastrointestinal Tract

Factor	Physicochemical parameter	Physiological parameter
Surface area of drug	particle size, wettability	surfactants in gastric juice and bile
Diffusivity of drug	molecular size	viscosity of lumenal contents
boundary layer thickness		motility patterns & flow rate
Solubility	hydrophilicity, crystal structure, solubilization	pH, buffer capacity, bile, food components
Amount of drug already dissolved		permeability
Volume of solvent available		secretions, coadministered fluids

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ity arises from normal genetic variation in the population (as in the case of heart rate, liver function and other physiological parameters) as well as from disease states implicating the gastrointestinal tract. Intrasubject variability may occur as the result of circadian rhythm, food ingestion, physical activity level and stress level, among others. This variability notwithstanding, the remainder of this Section is devoted to a summary of representative values for key parameters in the fed and fasted states in different segments of the gastrointestinal tract.

Lumenal composition in the GI tract

In addition to food and beverages ingested with the dosage form, various fluids are secreted by the gastrointestinal tract, including hydrochloric acid, bicarbonate, enzymes, surfactants, electrolytes, mucus and, of course, water. Thus, parameters that can profoundly influence the solubility and dissolution rate of a drug, e.g. pH, buffer capacity, presence of surfactant concentration and volume of lumenal contents, may vary widely with position in the gastrointestinal tract and with timing of administration of the drug in relation to meal intake.

pH

Values of gastric pH in the fasted state can fluctuate on a minute-to-minute basis over the range pH 1 to pH 7, but in healthy, young Caucasians gastric pH lies below pH 3 during 90% of the fasted state (13), with an interquartile range of pH 1.4 to pH 2.1. Suitable dissolution media for simulating the fasted state gastric conditions will therefore have pH values between pH 1.5 and pH 2. Fasted state gastric pH values of pH 6 and higher are found in two significant subpopulations: those receiving gastric acid blocker therapy and those over the age of 65 years (about 10–20 % of North Americans (14) and Europeans (15) acquire hypo/achlorhydria; the incidence appears to be much higher in Japan (16)). With ingestion of a meal, the gastric juice is initially buffered to a less acidic pH, which is dependent on the meal composition. Typical gastric pH values immediately following meal ingestion are in the range pH 3 to pH 7. Depending on meal size, the gastric pH returns to fasted state values within two to three hours. Thus, only dosage forms ingested with or soon after meal intake will encounter elevated gastric pH under normal physiological circumstances.

Intestinal pH values (Table 3) are considerably higher than gastric pH values due to the neutralization of incoming acid with bicarbonate ion secreted by the pancreas. Furthermore, there is a pH gradient in the small intestine, with values gradually rising between the duodenum and ileum. pH values in the colon are heavily influenced by products of bacterial exoenzyme reactions. Undigested carbohydrate that is passed into the colon is converted into short chain fatty acids (C_2–C_4) that lower the local pH value to around pH 5 (18). Thus, when suitable carbohydrate substrates are present, the pH in the proximal colon may be 2–3 pH units lower than in the terminal ileum.

Table 3. pH in the Small Intestine in Healthy Humans in the Fasted and Fed States[a]

Location	fasted state pH	fed state pH
mid-distal duodenum	4.9	5.2
	6.1	5.4
	6.3	5.1
	6.4	
jejunum	4.4–6.5	5.2–6.0
	6.6	6.2
ileum	6.5	6.8–7.8
	6.8–8.0 (range)	6.8–8.0
	7.4	7.5

[a] Reproduced from Ref. 17, which summarized results from several studies in the literature.

Table 4. Comparison of Average pH and Buffer Capacity of Chyme Recovered at Midgut From Fistulated Dogs, After Administration of Nonnutrient and Nutrient 'Meals', with Those of Simulated Intestinal Fluid USP, Without Pancreatin

Sample	pH	Buffer capacity (mEq/L/pH unit)
Nonnutrient 'meal' (water)	6.0[a]	0.16 ± 0.16[b]
Nutrient meal (cheeseburger, fries, water)	5.2[a]	76 ± 25[b]
SIFsp (USP)*	7.5	25.8 ± 0.8

Note: Excerpted from Ref. 20.
Shared letters indicate significant differences, [a]$p < 0.05$, [b]$p < 0.005$.
* SIFsp was prepared according to the USP, but without pancreatin. At the time the studies were initiated, the official pH of the medium was 7.5.

Buffer Capacity

The microclimate pH in the diffusion boundary layer adjacent to the dissolving surface is an important determinant to the dissolution of ionizable drugs. In addition to the intrinsic solubility and ionization constant of the drug and the pH of the medium, the buffer capacity of the medium plays an important role in determining the microclimate pH (19). Data obtained in a fistulated dog model (20) suggest that the buffer capacity at midgut is far greater after a cheeseburger/fries/water meal than following administration of water (Table 4).

Surfactants

The surface tension of gastric fluid is considerably lower than that of water, suggesting the presence of surfactants in this region. Usual values in the fasted state lie between 35 and 45 $mN.m^{-1}$ (21). In the small intestine, secretion of bile results in substantial concentrations of bile salts and lecithin, which form mixed micelles even at fasted state concentrations.

Fasting bile salt concentrations of about 3–5 mM have been reported for the proximal small intestine (Table 5). Although

Table 5. Fasting Bile Salt Concentrations in the Human Small Intestine[a]

statistic	duodenum	upper jejunum	lower jejunum
mean ± s.d. (mM)	6.4 ± 1.3	5	6
	4.3 ± 1.2		
median		3	5
range		0–14	0–17

[a] Data from Refs. 22–25.

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Table 6. Postprandial Bile Salt Concentrations in the Human Small Intestine

Time	Location	Statistic	Reference Number
0–30 min	duodenum	mean 14.5 ± 9.4	29
		range 5.8–39.6	
	upper jejunum	mean 16.2 ± 1.5	30
		mean 15	23
		range 4–34	
30–60 min	duodenum	mean 5.2 ± 2.3	29
	upper jejunum	mean 9.7 ± 1	30
		mean 8	23
		range 3–17	
120–150 min	upper jejunum	mean 6.5 ± 0.9	30

concentrations vary widely between individuals, average values are similar in the duodenum and jejunum. Levels fall rapidly in the ileum where bile salts are absorbed by an active transport mechanism, and are insignificant in the colon in healthy individuals.

After eating, the bile output and lumenal concentration of bile components (Table 6) peak within thirty minutes (26). Thereafter levels gradually decline, mostly because of dilution with chyme. The peak level averages about 15 mM in the proximal small intestine. Since the gallbladder empties into the upper small intestine, duodenal levels tend to fluctuate more with meal ingestion than levels in the distal small intestine (27,28).

Enzymes

The primary enzyme found in gastric juice is pepsin, an exopeptidase. Lipases, amylases and proteases (Table 7) are secreted from the pancreas (31) into the small intestine in response to meal ingestion; these enzymes are responsible for the bulk of nutrient digestion. Pepsin and the pancreatic proteases pose a particular threat to stability of proteins and peptides in the lumen, while lipases may affect release of drugs from fat/oil containing dosage forms.

Bacteria, which mainly populate the distal ileum and the colon, also secrete diverse enzymes. Table 8 (32) illustrates some of the enzymes available, classified according to the reactions that they catalyze. The ability of bacterial exoenzymes to split certain chemical bonds has been used to design dosage forms intended for colonic delivery, such as azo polymers and some hydrogels (33–35).

Volume

The volume of fluids available in the gastrointestinal tract for drug dissolution is dependent upon the volume of coadministered fluids, secretions and water flux across the gut wall. About 2 liters per day are ingested orally, though this varies considerably with climate, body weight, activity and personal habit (9). The volume of the stomach in the fasted state may be as little as 20–30 mL, mostly present as wet mucus rather than as a fluid pool. At the other extreme, gastric pressure starts to rise when a volume of about 1.5 liters is exceeded (36).

The secretions of the para-gastrointestinal organs (salivary glands, liver, pancreas) as well as the secretion of the stomach, are received by the first portion of the duodenum. These endogenous secretions, totalling about 6 liters per day, are essential for the normal lumenal digestion of foodstuffs. Approximately 1–2 L of pancreatic juice are secreted into the duodenum over a 24 hour period (37) while bile output in a 24 hour period totals about 600 mL. Most of the pancreatic and biliary output is secreted postprandially. In addition, the intestine secretes about 1 liter of water per day, mostly as a component of mucus.

According to the perfusion studies of Dillard et al. (38), the volume of fluid in the jejunum and ileum varies from 120–350 mL, depending on the perfusion rate. In a landmark study by Fordtran and Locklear (39) (Figure 2), electrolyte and volume measurements were compared at different sites within the small intestine after ingestion of hypertonic (milk/doughnuts) and hypotonic (steak and water) meals. Volumes were considerably higher following administration of a hypertonic meal than after administration of a hypotonic meal. In the case of a hypertonic meal, net water efflux across the mucosa into the lumen occurs due to the osmotic pressure difference, while in the case of a hypotonic meal, there is net water absorption from the meal.

Table 7. Characteristics of Some Exocrine Pancreatic Enzymes

(pro)enzyme	% output	substrates	products
trypsin(ogen)	33	proteins/polypep	peptides, amino acids
chymotrypsin(ogen)	16	proteins/polypep	peptides, aminoacids
(pro)carboxypeptidase A	12	proteins/polypep	amino acids
(pro)carboxypeptidase B	9	proteins/polypep	amino acids
(pro)elastase	8	proteins/polypep	amino acids
ribonucleases	1	nucleic acids	mono-nucleotides
lipase 1	8.5	triglycerides	fatty acids monoglycerides
lipase 2	3.4	triglycerides	monoglycerides
amylase	3.6	polysaccharides	disaccharides trisaccharides limit dextrins

Note: Excerpted from Ref. 31.

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