REVIEW

Keeping a Critical Eye on the Science and the Regulation of Oral Drug Absorption: A Review

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ABSTRACT: This review starts with an introduction on the theoretical aspects of biopharmaceutics and developments in this field from mid-1950s to late 1970s. It critically addresses issues related to fundamental processes in oral drug absorption such as the complex interplay between drugs and the gastrointestinal system. Special emphasis is placed on drug dissolution and permeability phenomena as well as on the mathematical modeling of oral drug absorption. The review ends with regulatory aspects of oral drug absorption focusing on bioequivalence studies and the US Food and Drug Administration and European Medicines Agency guidelines dealing with Biopharmaceutics Classification System and Biopharmaceutic Drug Disposition Classification System. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

In 1855, when Fick developed what is known today as Fick's laws of diffusion, he could not imagine that these fundamental relationships would become, after 100 years or so, the basis for the description of diffusion processes in pharmaceutical systems. Fick's first law states that the flux J (which represents the amount of material flowing through a unit cross section of a barrier or a membrane in unit time) is proportional to the concentration gradient dC/dx

$$J = -D\frac{dC}{dx} \tag{1}$$

where D is the diffusion coefficient of the diffusing species (penetrant or diffusate) in cm^2/s, C is its concentration, and x is the distance moved by the species perpendicular to the surface of the barrier or the membrane.[1] The negative sign in Eq. 1 denotes that the species diffuse in a direction opposite to that of the increasing concentration. Forty-two years later, the physical chemists Noyes and Whitney working in the Massachusetts Institute of Technology studied the dissolution of lead chloride and benzoic acid and found that the rate of dissolution, dC/dt, is proportional to the concentration gradient (C_s–C):

$$\frac{dC}{dt} = K(C_s - C) \tag{2}$$

where K is a composite first-order dissolution rate constant, C_S is the solubility of the compound in the dissolution medium at the temperature of the experiment, and C is the concentration of solute in the bulk solution at time t.[2,3] Equation 2 signifies, in accord with Fick's first law (Eq. 1), that the driving force for the rate of dissolution of solid particles is the concentration gradient.

In mid-1950s, pioneer pharmaceutical scientists started studying gastrointestinal (GI) absorption phenomena and rediscovered Eqs. 1 and 2. They found that GI uptake involves a simple diffusion, driven by differences in drug concentration on both sites of the membrane.[4–7] Since then, the term "passive diffusion" was coined and the corresponding theoretical principles were formulated, namely, that the fraction of undissociated drug and the pH of the intestines are the principal factors for drug uptake. According to this "pH-partition hypothesis," only the nonionized (i.e., lipid soluble) form of drug species passes easily through biological membranes. Nowadays, our

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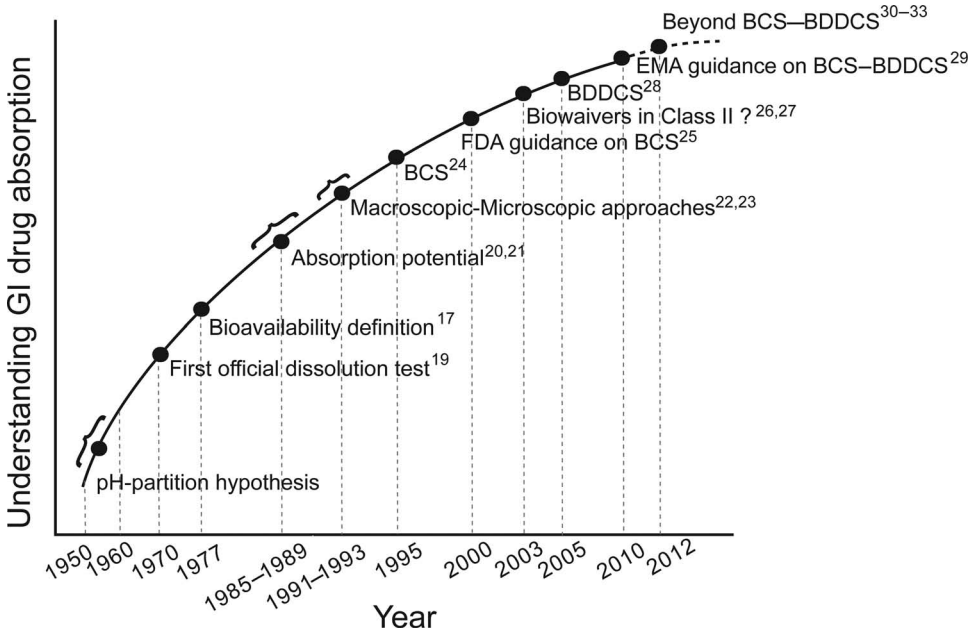

Figure 1. The evolution of gastrointestinal (GI) drug absorption analysis: science and regulations. The numbers depicted in figure correspond to references.

views on the transport of molecules across membranes have changed; a great variety of transporters (proteins) are involved in moving various species such as nutrients, drugs, and xenobiotics through biological membranes.[8,9] From the mid-1950s to mid-1960s, it was realized that drug's dissolution is the rate-limiting step for the oral absorption of sparingly soluble drugs, and Eq. 2 was used as the basis for the analysis of dissolution data. Actually, Edwards,[10] in 1951, was the first who realized that the rate of dissolution of a solid drug in the GI fluids can be the rate-limiting step controlling its appearance in the blood stream, provided that the absorption process is rapid. In the same period, another pioneer scientist Eino Nelson strongly believed that "the rate of GI absorption of most drugs, when administered in solid form as tablets or capsules, is determined by their rate of dissolution in GI fluids."[11] In 1957, Nelson[12] first described the relationship between blood levels of orally administered theophylline derivatives and their dissolution rate *in vitro*. Inspired by Nelson, his student Gerhard Levy[13–16] decided to extend his work. Realizing that the mixing of GI contents has a great influence on *in vivo* drug dissolution, he tried to relate *in vivo* behavior to the *in vitro* dissolution test by optimizing the *in vitro* stirring rate. Eventually, his beaker dissolution test evolved into the widely used United States Pharmacopeia (USP) dissolution apparatus II. In fact, the rotating basket method of dissolution testing was the first official dissolution test for solid dosage forms adopted by the USP 18 back in 1970. A few years later, on January 7, 1977, the US Food and Drug Administration (FDA) finalized the bioavailability and bioequivalence (BE) regulations (Federal Register).[17]

This period (from mid-1950s to late 1970s) marked the rise of biopharmaceutics–pharmacokinetics both scientifically and in the regulatory setting.[18] These advances not only reshaped pharmacy but also pharmacy studies in particular in the United States. Thus, the relevant absorption, distribution, metabolism, and excretion studies progressively became the core of drug development. Since the mid-1980s, scientists placed particular emphasis on the study of oral drug absorption; these studies resulted in the publication of a number of regulatory guidelines, by the drug agencies, which greatly affected the pharmaceutical companies.

In this work, we critically review the scientific and regulatory evolution of oral drug absorption during this modern era. The most important steps toward the better understanding of GI absorption phenomena together with the most relevant regulatory guidelines can be seen in chronological order in Figure 1.

SCIENTIFIC ASPECTS OF ORAL DRUG ABSORPTION

Oral intake is the most commonly used route of drug administration and the most convenient for patients resulting in high therapy compliance. Orally administered drug compounds should possess biopharmaceutical properties that enable them to achieve therapeutic concentrations at their site of action. The absorption of orally administered drugs is complex and depends not only on drug properties but also on

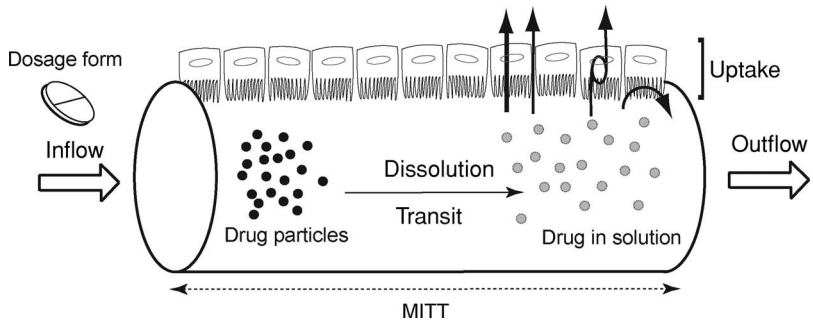

Figure 2. A schematic representation of drug absorption processes. The term MITT refers to mean intestinal transit time. Four uptake mechanisms are depicted: transcellular, paracellular, carrier-mediated transport, and efflux transport.

physiological aspects of the GI tract.[34] The various factors that may influence the rate and extent of intestinal drug absorption can be classified as drug/formulation dependent and system dependent as follows: (a) drug/formulation-dependent factors—drug physicochemical properties [e.g., aqueous solubility, permeability, molecular size, aggregation/complexation, charge (pK_a), H-bonding potential, hydrophobicity, and crystal lattice energy] and formulation composition (e.g., dosage form, absorption enhancers, and drug release); and (b) system-dependent factors—physiological parameters (e.g., gastric emptying, intestinal motility, intestinal pH, site-dependent permeability, intestinal content composition, and disease state) and biochemical parameters (e.g., metabolism, efflux transporters, and active uptake transporters). Some of the most important drug absorption processes are depicted in Figure 2.

Fundamental Processes in Oral Drug Absorption

The Complex Interplay Between Oral Drugs and GI System

From the early days of gamma scintigraphy,[35,36] until the recent reviews on the *in vivo* imaging of drug delivery systems in the GI tract,[37] the complexity of the structure, function, and composition the GI system, as well as its critical impact on drug absorption processes has been justified. A pictorial view of the main absorption processes in the GI tract shown in Figure 2 provides only a small insight into its complexity. For example, the gastric emptying kinetics of drugs as well as the transit of dosage forms through the small intestine and the colon are all critical parameters contributing to the intra- and intersubject variability in GI transit.[38–40] Obviously, the bioavailability of drugs is highly dependent on their transit through the GI tract. Gastric emptying influences the performance of delayed release dosage forms when the latter are designed to release the drug upon reaching the higher pH of the small intestine. Also, the effect of meals on gastric emptying is pronounced. Although the mean intestinal transit time is around 3–4 h, the colonic transit time varies enormously.[40] The interested reader can find a plethora of studies in review articles[39,40] dealing with the methods used for the transit of dosage forms through the GI tract.

One of the most important contributions relating to the understanding of the volume and distribution of intestinal fluid within the lumen of the GI tract was published in a nonpharmaceutical journal in 2005.[41] The results of this study clearly showed that fluid is not homogeneously distributed along the gut; moreover, extremely variable fluid volumes between 45 and 319 mL in the small intestine and between 1 and 44 mL in the colon were observed in fasted subjects. "Fluid pockets" and "dry segments" were also observed along the GI tract and the authors calculated the number of nondisintegrating capsules at various time points surrounded by liquid, partly surrounded by liquid, and not in contact with liquid (Fig. 3). According to the authors, inhomogeneous distribution (fluid-filled pockets and "dry segments") in the intestines causes erratic absorption. Most importantly, the authors argued against the homogeneous conditions of the official dissolution tests. In full agreement with these observations, similar explanations for the failure of *in vitro - in vivo*correlations (IVIVC) of modified release formulations have been proposed recently.[42]

The understanding of the effect of the heterogeneous structure, composition, and function of the GI tract on the kinetics of drug absorption processes has been a goal for several decades in the pharmaceutical sciences. However, a paper published in 1997 questioned the validity of the diffusion theory in the complex medium of the GI tract for the description of the drug absorption processes.[43] It was proposed that "fractal like kinetics"[44] is more suitable for processes taking place in heterogeneous media. According to this type of kinetics, time-dependent rate coefficients and note rate constants govern the GI processes:

$$k = k_1 t^{-h} \quad (t \neq 0) \tag{3}$$

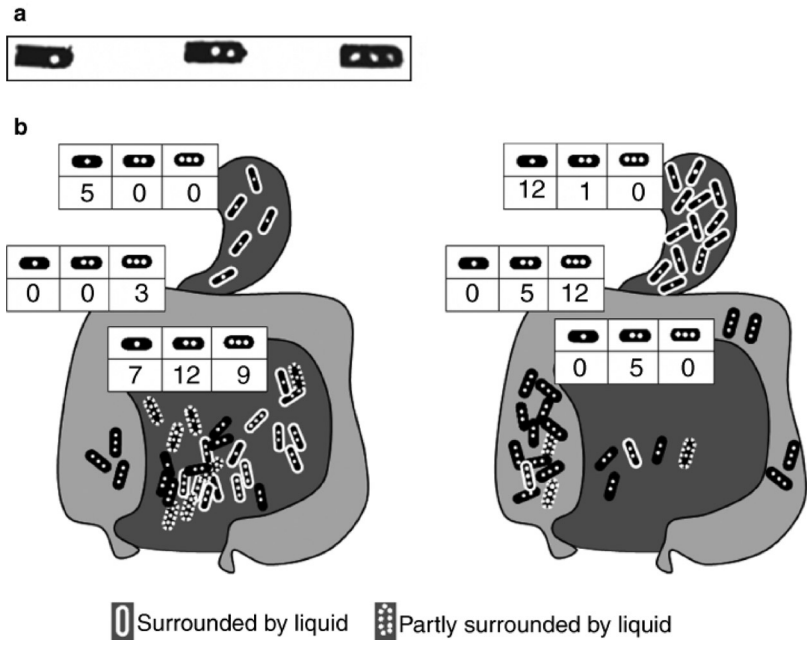

Figure 3. (a) Image of the indigestible capsules, with watery gel pellets, and (b) Location of capsules in the stomach, small and large intestine under fasting conditions (left) and 1 h after ingestion of meal (right). The number of the labeled capsules located in the respective organ is listed in the tables. Adopted from Ref. 41.

where k_1 is a constant not dependent on time with units $(\text{time})^{h-1}$, h is a pure number different than zero, and k the time-dependent rate coefficient. The exponent, h, arises from two different phenomena, namely, the geometric disorder of the medium and the imperfect mixing of the GI contents.

Dissolution

Although the official pharmacopeial dissolution tests have been designed as quality control procedures, these tests are also used as surrogates of *in vivo* bioavailability for the past 20 years or so. However, the wide use of Biopharmaceutics Classification System (BCS)[24] and the publication of the relevant FDA guidance[25] in 2000 increased the possibility of substituting dissolution testing for clinical studies and the need for dissolution tests designed to better predict the *in vivo* performance. Accordingly, the advances made in the various aspects of drug dissolution were mainly focused on the understanding of and/or resemblance to *in vivo* drug dissolution. The principal features–components of drug dissolution, namely, dissolution mechanism(s), hydrodynamics, solubility, and composition of the dissolution medium were recently studied in the context of the complex interplay between drug delivery system and GI physiology.

Apart from the official dissolution tests, various dissolution apparatuses have been designed during the past 15 years to mimic what happens to a drug once ingested.[45–51] Some of these innovative approaches have been patented and for this reason have not been used extensively. Most of these approaches attempt to incorporate one or more of the dynamical physiological processes, for example, GI peristalsis, physiological mixing, hydrodynamics, mechanical conditions, and food materials.

Shortly after the heterogeneous consideration of GI drug absorption,[43] the population growth model of dissolution was developed[52]; this model does not require the presuppositions of Fick's law of diffusion and does not rely on the concept of time continuity because the mass dissolved is a function of a discrete time index specifying successive dose-dissolved generations. This approach has been also used for the modeling of supersaturated dissolution data.[53] The continuous analog of the population growth model of dissolution[52] was introduced by Lánský and Weiss[54] wherein the dissolution rate is considered as a decreasing function of the dissolved amount controlled by the solubility—dose ratio. This work should be considered as the first reaction-limited model of drug dissolution of the modern era; the study shows that the mean dissolution time (MDT) is dependent on the solubility–dose ratio contrary to the common belief that MDT is equal to the reciprocal of the dissolution rate constant. These findings prompted Rinaki et al.[55] to reexamine the classical diffusion layer model of Noyes and Whitney (expressed in terms of the amount of drug dissolved) and found that the MDT is explicitly related to the solubility–dose ratio when the entire dose can be dissolved. Interestingly, the MDT is equal to the reciprocal of the dissolution rate constant only when the

dose is equal to the amount needed to saturate the dissolution medium. Because of the predominant role of the solubility–dose ratio in drug dissolution, branched versions of the Noyes and Whitney equation and of the Weibull function were developed for the analysis of dissolution data that reach the plateau either at infinite or finite time.[56] Moreover, particular emphasis was placed on the random effects of drug dissolution as well as the role of heterogeneity in the deterministic models of drug dissolution.[57] These concepts were further developed by the introduction of fractional kinetics to model nonclassical drug dissolution, release, and absorption.[58–60]

Solubility, Supersaturated Solutions, and Precipitation. According to Eq. 2, the saturation solubility (C_s) drives the dissolution rate. This fact is mirrored in the dominant role of solubility for biopharmaceutical classification purposes.[24] Traditionally, equilibrium solubility measurements using the so-called "shake flask method" are carried out to get estimates of the thermodynamic solubility. The latter corresponds to the highest concentration of the substance under investigation that can be achieved in a liquid medium of specified composition at a given temperature, for example, 25°C or 37°C.

Since the mid-1980s, the interest of scientists was focused on drug solubility measurements and dissolution tests in media that are more akin to *in vivo* conditions. In this context, a series of studies[61–65] revealed that the solubility of drug in milk increases with lipophilicity while the dissolution results in milk were found to be drug dependent for immediate release formulations or product dependent for controlled release formulations. Upon the publication of the BCS article,[24] these type of studies triggered off the development of biorelevant media in particular for solubility–dissolution studies of class II compounds.[66] A comprehensive complete review article on the use of biorelevant media for the *in vitro* testing of orally administered dosage forms was published recently.[67]

Several years ago, scientists developed methods for the measurement of what we call today kinetic solubility.[68,69] The kinetic aspects of solubility are related to supersaturated solutions and precipitation phenomena. In fact, kinetic solubility has been defined as the concentration where precipitation first starts to appear during the process of inducing precipitation. Because supersaturated solutions are frequently formed in the GI tract[70,71] and drug precipitation can affect the bioavailability of drug, kinetic solubility measurements and crystallization rates have been the subject of several recent publications.[72,73] In a recent study,[74] two patterns of pH-induced precipitation behavior for weakly basic compounds were observed. The duration of supersaturation was short-lived for compounds that precipitate in crystalline forms. Prolonged supersaturation and amorphous precipitates were found for compounds that did not readily crystallize.

Overall, three approaches are utilized for the study of (gastro) intestinal precipitation. The first approach relies on *in vitro* multicompartment systems where the studied substance is dissolved in simulated GI media in the different compartments and the incomplete dissolution/precipitation is monitored.[75] Important factors for all *in vitro* methodologies are the hydrodynamic conditions used during the experiment as well as the stirring mechanism.[73] According to Carlert et al.,[73] the simple *in vitro* methods of *in vivo* precipitation of BCS class II bases overpredict the crystalline intestinal precipitation in humans. The second approach is based on pharmacokinetic studies in humans.[73] Here, the linearity of maximum observed plasma concentration (C_{max}) and area under the concentration—time curve (AUC) versus dose plots as well as the comparison of the plots, obtained under various experimental conditions, allow the investigator to assess the *in vivo* precipitation of drug. This approach has been applied to the study of the intestinal precipitation of a basic BCS class II drug in the presence or absence of omeprazole.[73] It is interesting to note that this type of analysis (AUC vs. dose plots) has been used for the development of dose-dependent BCS (DDBCS).[32] Finally, the third approach relies on human aspirates.[70] The results obtained in this type of study should be interpreted with caution because they provide a very specific regional estimate of the extent of precipitation at the site of measurements. Because of spatiotemporal changes in the composition–hydrodynamics of the GI system,[41,43] the estimates for the percentage of precipitation observed cannot be extrapolated to the whole physiological region of interest, for example, the upper small intestine. In parallel, the extreme variability and time-dependent solubilizing capacity of human intestinal fluids[76] in the fasted, fed, and fat-enriched fed state coupled with the stress effect of the volunteers participating in the study are additional reasons for the cautious interpretation of *in vivo* precipitation results. It can be concluded that the intersubject differences in intestinal luminal contents coupled with their time-dependent character are the main reasons for the enormous variability in the *in vivo* dissolution/release precipitation studies based on human aspirates.[76,77]

Various attempts have been made to model supersaturated dissolution–precipitation data. A classical supersaturated dissolution curve shows a rise to a concentration maximum followed by a decline to a steady-state level. This behavior cannot be captured by the conventional diffusion layer model. The models proposed for the interpretation of supersaturated dissolution data are the population growth model of dissolution,[52] and two versions of a

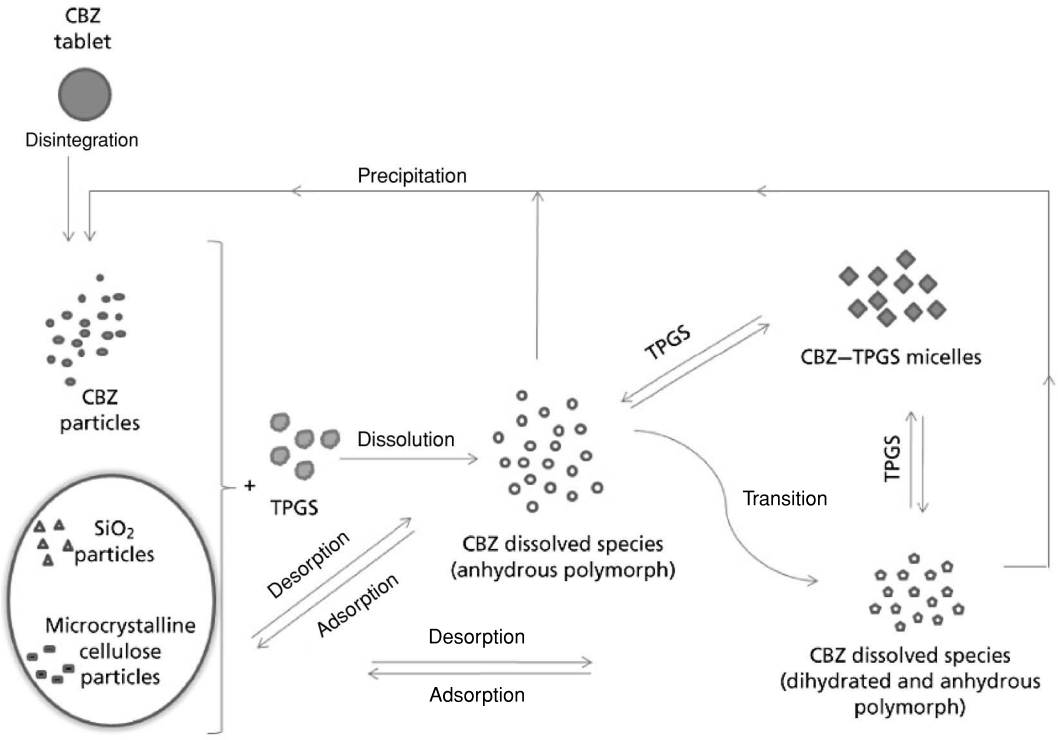

Figure 4. Processes involved in the dissolution of carbamazepine (CBZ) tablets in the presence of D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS). Adopted from Ref. 79.

reaction-limited model of dissolution.[54,78] Another approach employs a time-dependent rate coefficient instead of a dissolution rate constant; it has been used to describe the complex dissolution–precipitation of carbamazepine tablets in the presence of D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS) (Fig. 4).[79,80] Several studies have recently focused on the modeling of the precipitation process as well as the assessment of precipitation rates. For modeling purposes, the classical theory of crystallization involving the nucleation step and the particle growth step is used.[81,82]

Dissolution Mechanisms—Hydrodynamics. As mentioned in the *Introduction*, the first dissolution study was published in 1897 and since then Eq. 2 has become the landmark in the quantification of dissolution studies. As a matter of fact, Eq. 2 relies on the "diffusion layer model" or film model.[83] According to this model, a thin diffusion layer of high concentration fluid is formed around the surface of the solid particles; the transfer of the dissolved species through the diffusion layer to the bulk aqueous phase is the rate-limiting step of the dissolution process. According to Eq. 2, the C_s is the predominant parameter because it drives the dissolution rate. Equation 2 has not only been used as the basic tool for the analysis of dissolution data and the interpretation of the effect of the various factors involved on the rate of dissolution but also it has been used to emulate the dissolution process under *in vivo* conditions.

However, the drug dissolution mechanism(s) is closely related to the hydrodynamics prevailing under *in vitro* or *in vivo* conditions. In this vein, Scholz et al.[84] questioned whether the USP paddle method can be used to represent *in vivo* conditions. Although the authors concluded that the USP paddle method can be used to reflect variations in hydrodynamic conditions in the upper GI tract provided an appropriate composition is chosen, the results of recent studies based on computational fluid dynamics revealed the high complexity of the fluid flow in the *in vitro* systems.[85–88] Moreover, the detailed analysis of the relationship between the diffusional layer thickness and particle size under a defined set of hydrodynamic conditions revealed different functions dependent on paddle speed.[89] In the most recent work on this topic of research, Wang et al.[90] elegantly described the classical dissolution models and showed that the models of Brunner–Tolloczko[91] and Hixson–Crowell[92] are quite similar to the Noyes–Whitney model (Eq. 2) and all of them rely on an unphysical constant diffusion layer thickness assumption. Because of confinement of particles as a result of either the intestinal geometry or an impermeable rigid container, the authors[90] developed a time-dependent "infinite domain model" and a "quasi steady-state model," which both take into account the change with time of the diffusion layer

thickness. The "confinement effects" justified in the latter study are in full agreement with the concept of "topological constraints" used to interpret the kinetics of drug dissolution[93] on the basis of time-dependent rate coefficients and not rate constants. The latter approach provides a theoretical interpretation of the extensive use of the Weibull function in dissolution and release studies.[94]

Permeability

Permeability is a parameter that shows the degree of a solute penetration into a membrane in a specific time. It is, therefore, expressed in velocity units length per time. It can be derived from Fick's first law of diffusion[1] (Eq. 1) and is extensively used for the quantification of drug's penetration ability through biological membranes.

Drug absorption through the gut membrane is a complex process, achieved either by passive diffusion or by active transport (Fig. 2). In addition, the presence of various efflux transporters such as P-glycoprotein (P-gp), multidrug resistance protein 2, and breast cancer resistance protein may limit intestinal absorption by drug extrusion from the cells, thus preventing several drug molecules to enter the blood circulation.[9] Drugs that are passively transported may cross the GI membrane via either the paracellular or trancellular route (Fig. 2). Paracellular absorption involves gut wall permeation through the tight junctions. Accordingly, drugs with special physicochemical characteristics (e.g., small size, positive charge, and hydrophilicity) may enter blood circulation through this route. On the contrary, transcellular penetration requires drugs of medium size, nonionized and of adequate lipophilicity, to pass through epithelial gut membrane. Drugs that cannot pass either paracellularly or transcellulary may enter blood circulation via influx transporters.

The first attempts to simulate cell membranes and measure intestinal permeability for passively transported molecules were made in the early 1960s[95,96] Thin teflon sheets were used with a small hole of 0.5 mm diameter. When placing a small quantity of phospholipids over the hole, a thin film was formed and excess lipid flowed to the perimeter of the hole. The central film eventually formed a single bilayer lipid membrane (BLM) over the hole. Lipids able to form this BLM were found to be phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and so forth, mostly coming from natural sources. These BLMs, although not easy to handle because they are very fragile, were considered as adequate models to simulate the complex physiological membranes.[96] Using these BLM systems, a linear relationship was found between the determined permeability coefficients, $\log P_0$, of various carboxylic acids[97] and *p*-toluic acid derivatives,[98] and the values of the respective oil/water partition coefficients, $\log K_p$.

Various *in vitro* methods have been developed to study drug permeation across intestinal epithelial cells. Methods that are based on the physicochemical parameters of the drug and measurement of their ability to pass through artificial lipid phases that mimic *in vivo* cell membranes can be roughly referred as physicochemical or "cell-free" methods. Methods that are based on the measurement of drug transport through cultured cell lines can be referred as "cell-based" methods. The most often applied cell-based *in vitro* methods use culture cells such as the adenocarcinoma cell line derived from human colonic epithelia (Caco-2 cells) and Martin–Darby canine kidney (MDCK) cells.[99,100] Culture cell-based models can be used not only to study drug partition into a membrane but also to elucidate the mechanisms of drug absorption and the possible binding to epithelial proteins, for example, influx and efflux transporters and enzymes.[99,100] Alternatively, cell-free *in vitro* methods using artificial membranes have been proposed, with reduce cost, such as the "parallel artificial membrane permeability" approach (PAMPA),[96,101–112] mainly to study and predict intestinal permeability of drugs that are passively absorbed.

Cell-Free In Vitro Methods. For passively absorbed drugs, permeability characteristics across the GI epithelium can be predicted using the physicochemical parameters of a drug, for example, molecular weight, aqueous solubility, partition or distribution coefficients, log *P* and log *D*, polar surface area, and so forth.[101–105] Ungell[106] found good correlation between log *D* values and drug permeability across intestinal epithelial cell monolayers (Caco-2), whereas Camenisch et al.[104] studied drug permeation through artificial membranes composed of millipore cellulose ester filters impregnated with octanol and isopropylmyristate. They found an S-shaped curve relating the measured P_{eff} values and the respective octanol/water partition coefficients, $K_{d,7.4}$, within the range of lipophilicity values studied. Phospholipid vesicles composed of phosphatidylcholine analogs (the main phospholipid component of the cell membrane) have been used to study drug uptake into the cellular lipid bilayer.[107] In parallel, immobilized artificial membrane columns[108,109] have also been used to predict membrane permeability. These artificial membranes are composed of phospholipids (e.g., phosphatidylcholine analogs or unilamellar liposomes)[110] immobilized on silica surfaces and measurements of permeability are based on the different retention of drugs on the column, depending on their lipophilicity.

In 1998, Kansy et al.[111] introduced the "parallel artificial membrane permeability assay." The PAMPA assay, as extended by Avdeef et al.[102,103] possesses

an easy way to measure membrane retention. The PAMPA assay is composed of a "sandwich" system formed from a 96-well microtiter plate and a 96-well microfilter plate. Each composite well is divided into two chambers (a donor at the bottom and an acceptor at the top), separated by a 125-μm-thick microfilter disc with 0.45 μm pores, coated with a 2% (w/v) dioleoylphosphatidylcholine–dodecane solution, under conditions that form multilamellar bilayers inside the filter channels when the system is in contact with an aqueous solution. This method and its modifications[102,103,112] are very suitable to assess passive transcellular permeability during early stages of drug discovery.

Cell-Based In Vitro Methods. Cell-based *in vitro* methods are also applied to investigate intestinal drug absorption. These methods offer more physiological conditions, whereas absorption mechanisms (i.e., passive diffusion, active transport) and transport rate and uptake into cells can also be studied. The most commonly applied cell-based *in vitro* models for intestinal absorption use intestinal adenocarcinoma cell lines, Caco-2, HT29, HT29-18, HT29-H, and T84, for permeability experiments because of their ability to differentiate in culture.[99,113–116] Alternatively, cell lines from animals, such as MDCK, CHO (Chinese hamster ovary), and 2/4/A1 (rat), can be also used as *in vitro* models for intestinal absorption.[117,118]

The Caco-2 cell line was first produced in 1977 by Fogh et al.[113] using well differentiated colon adenocarcinoma cells taken from a 72-year-old patient. The main advantages of Caco-2 cells are their high degree of spontaneous differentiation under standardized culture conditions, similar to human intestinal enterocytic differentiation, as well as their ability to be maintained in culture.[99,113,119,120] Differentiation is completed within 20 days.[119,120] Caco-2 differentiated cells form an epithelial membrane that exhibits barrier function similar to the human colon, expresses carrier proteins similar to the small intestine and have high levels of alkaline phosphatase, sucraseisomaltase, and aminopeptidase activity, characteristic to enterocyte brush border microvilli.[99,121,122]

Caco-2 cell models have been used to study the permeability of drugs that are passively transported through the intestinal epithelium by either paracellular[99,120,123] or transcellular mechanisms,[99,114,120] whereas carrier-mediated transport of various compounds has also been studied.[99,120–125] In the last decade, Caco-2 cell lines have also been applied to investigate the effect of carrier-mediated efflux and enterocyte metabolism on the intestinal absorption of several drugs because a variety of transporters and enzymes (such as efflux transporters of the ABC-transporter family) are expressed in Caco-2 cells.[99,120,126–133]

Good correlation between the fraction of dose absorbed after oral administration in humans and the predicted *in vitro* apparent permeability in Caco-2 cells has been observed,[134,135] whereas structure permeability relationships using permeability measurements in Caco-2 cells have been established for drugs that are either passively or actively transported.[136–138] However, because of their poor paracellular permeability properties, Caco-2 cells[122,123,139] lead to very poor permeability predictions for hydrophilic drugs that are absorbed mainly paracellularly.

Martin–Darby canine kidney cell lines are also commonly used epithelial cell lines for studying intestinal drug absorption and investigating the underlying mechanism.[117] MDCK cells differentiate to columnar epithelial cells when cultured on semipermeable supports, showing well formed tight junctions at the apical surface.[140] Culture MDCK cell lines are well characterized (e.g., morphology, electrical resistance, and polarization) and provide a good *in vitro* model for drug transport and interaction studies with drugs.[141–145] Permeability data for highly and mainly passively diffused drugs correlate well with permeability data obtained from experiments with other cell cultures.[140] However, poor correlation was found for compounds showing low permeability, probably because of differences in tight junctions and active transport properties.

The main advantage of MDCK cells is their small culturing time that varies from 3 days for the parent line to 7–10 days for subclone 2 MDCK, in comparison with Caco-2 cells that need about 21 days to form a fully differentiated polarized cell monolayer.

It should also be mentioned that apart from the various *in vitro* methods used to predict intestinal permeability of drugs, the interplay of various factors, such as cytochrome P450 enzymes and P-gp transporters, pH microclimate and P-gp transporters, concentration dependence of P-gp substrates permeability, on GI drug absorption has been extensively discussed.[146–148]

Mathematical Modeling of Oral Drug Absorption

The Absorption Potential Concept

The introduction of the absorption potential (AP) concept[20] marked the commencement of the modern era of oral drug absorption analysis. A pseudo-steady-state model was utilized to emulate GI drug absorption; the AP of each drug was defined in terms of the dose (D), the n-octanol–water partition coefficient, P, the intrinsic solubility (S_0), the volume of the intestinal fluids (V_L), and the fraction of the nonionized form

of the drug (F_{non}) at pH 6.5 as follows:

$$AP = \log PF_{non}\frac{S_0V_L}{D} \quad (4)$$

The use of log in Eq. 4 is made simply for numerical reasons. The AP concept relies on Fick's first law of diffusion and the "pH-partition hypothesis" principles because the parameters S_0, log P, and F_{non} are related proportionally to AP. For the first time, the dose D was expressed explicitly in this type of calculations while the use of the luminal volume V_L makes the AP dimensionless; the latter was assigned a value of 250 mL. The article[20] provided conclusive evidence of a sigmoid relationship between the fraction of dose absorbed F_{abs} and AP for nine drugs examined. A quantitative approach for the prediction of F_{abs} as a function of AP was published a few years later,[21] based on a pharmacokinetic version of the pseudo-steady-state model used for the development of AP. F_{abs} was defined in terms of the first-order absorption rate constant k_a and a first-order rate constant leading to nonabsorption k_n:

$$F_{abs} = \frac{k_a}{k_a + k_n} \quad (5)$$

k_a was considered proportional to AP, $k_a = \lambda($ AP), whereas k_n was considered proportional to 1/AP, $k_n = \mu$/(AP), and an explicit relationship was derived for F_{abs}

$$F_{abs} = \frac{\lambda \times (AP)^2}{(AP)^2 + \mu \times F_{non} \times (1 - F_{non})} \quad (6)$$

The last equation reveals that F_{abs} approaches one asymptotically as AP is increased.

The Mixing Tank Model

In 1986, the mixing tank model was introduced by Dressman and Fleisher[149] to describe the absorption process in the intestine. The model described the intestine as a well stirred compartment (mixing tank), where dissolution and absorption take place simultaneously and a first-order decrease of drug is considered because of transfer out of the intestinal tank. Although in its simplest form, the mixing tank model does not consider the intestinal transit process, it can be modified to include the mean intestinal transit time as a time constraint after which absorption is terminated.[22,23]

From the Microscopic Tube Model to BCS

In 1993, Amidon and coworkers[23] developed the microscopic tube model for drug absorption that was based on mass balance considerations. In this work, three fundamental parameters, the "dissolution," "dose," and "absorption" numbers, were described to control the extent of oral drug absorption.[23] According to the microscopic model for drug absorption, C_s is the driving force for drug dissolution because dissolution follows the diffusion layer model, whereas drug permeation follows passive diffusion and drug uptake is governed by drug permeability. Based on this work, in 1995, Amidon et al.[24] developed the BCS. Drug classification according to the BCS is based on drug aqueous solubility and intestinal permeability. Four drug classes were defined on this basis, namely, class I or high solubility/high permeability, class II or low solubility/high permeability, class III or high solubility/low permeability, and class IV or low solubility/low permeability (Fig. 5). These drug substance properties were also combined with the dissolution characteristics of the drug formulation to predict the possibility to establish *in vitro–in vivo* correlations for each BCS class.

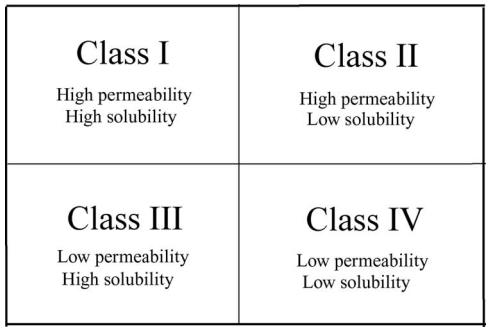

Figure 5. The Biopharmaceutics Classification System as proposed by Amidon and co-workers.[24]

The Compartmental Absorption and Transit Model

The compartmental absorption and transit (CAT) model was developed by Yu et al.[150] and by Grass.[151] This model describes the intestine as a number of compartments in series, each one corresponding to a specific intestinal region. The mathematical description of the model is given by the following equation:

$$\frac{dM_n}{dt} = K_tM_{n-1} - K_tM_n - K_{an}M_n \quad (7)$$

where M_n and M_{n-1} are the amount of drug in compartments n and the previous compartment n–1, respectively, K_t is the transit rate constant that is same for all compartments, and K_{an} is the absorption rate constant for the n^{th} compartment. K_{an} is connected to the effective permeability P_{eff}, by the relation $K_{an} = 2P_{eff}/R$, where R is the radius of the intestinal lumen. Seven compartments have been proposed by Yu et al.[150] as the best model to describe the small intestine. Regarding the physical meaning of the compartments, the first is considered to correspond to the duodenum, the second and the third to the jejunum, and

the last four to the ileum. For model completion, two additional compartments are considered to describe the stomach and the colon and the final model consists of a total of nine compartments. Gastric emptying is considered to be first- or zero-order process describing drug transit from the stomach compartment to the first intestinal compartment, whereas dissolution and release processes can also be incorporated.[152]

In a modified CAT model, the so-called advanced CAT (ACAT), larger values were given to the transit rate constants to simulate the physiologic flows of the actual segments of the intestinal tube.[153] The ACAT model in its total form is the basis of the commercial software package GastroPlus™ (Simulations Plus, Lancaster, CA) and incorporates a number of additional processes, for example, release, dissolution, precipitation, gut metabolism, and influx and efflux transports. Similarly, the advanced dissolution, absorption, and metabolism (ADAM) mechanistic absorption model is the basis of the commercial software package SimCyp® (Certara Inc., St. Louis, MO).

The Dispersion Models

The dispersion model, developed in 1980 by Ni et al.,[154] is an alternative to the compartmental mathematical description of intestinal absorption. The intestine is described by a tube and within it a continuous concentration spatial profile is considered. The time evolution of the spatial profile of the concentration, $C(z,t)$, is described mathematically by a partial differential equation incorporating dispersion, transport, and absorption as follows:

$$\frac{\partial C(z,t)}{\partial t} = \alpha \frac{\partial^2 C(z,t)}{\partial z^2} - \beta \frac{\partial C(z,t)}{\partial z} - \gamma C(z,t) \qquad (8)$$

where α is the dispersion coefficient, mainly because of geometrical dispersion; β corresponds to the velocity of the intestinal fluid; and $\gamma = 2P_{\text{eff}}/R$ is the drug absorption rate constant, expressed in terms of effective permeability, P_{eff}, and the radius of the intestine, R. This equation can be solved numerically by setting appropriate initial profile and boundary conditions to be fully defined, which for some special cases, has analytical solutions.[154]

A dispersion-like model was also proposed in 2003 by Willmann et al.,[155] first for the rat model and later for humans. The model considers a continuous drug concentration profile in the intestine tube, to mimic the dispersion and transport procedure in the intestine, described by a Gaussian function with a transiently moving center and varying width. This model became part of the commercial, physiologically based, pharmacokinetic simulation software PK-Sim® (Bayer Technology Services, Leverkusen, Germany). Another model was proposed, which was based on a heterogeneous GI tube. The latter relies on probabilistic approaches for drug transit, dissolution, and permeation.[156]

Absorption Models Based on Structure

Computer-based models, using calculated molecular descriptors, have also been developed to predict the extent of absorption from chemical structure. These models are mainly used in the early stages of the drug discovery process to facilitate the screening and selection of new drug molecule candidates. The physicochemical descriptors of drug molecules are useful tools for predicting absorption for passively absorbed drugs. On the contrary, for sparingly soluble drugs, dissolution is the rate-limiting step, whereas for polar drugs, permeability becomes the rate-limiting step. Therefore, computer-based models are using molecular descriptors that are closely related to important drug properties such as aqueous solubility and permeability across the intestinal epithelium.

The most popular model for rapid screening of compounds that are likely to be poorly absorbed is the so-called Lipinski's "rule of 5,"[157] according to which intestinal absorption of a drug candidate is more likely to be poor when its structure is characterized by:

- molecular mass > 500 Da,
- log$P$ > 5,
- more than five H-bond donors expressed as the sum of OHs and NHs, and
- more than 10 H-bond acceptors expressed as the sum of Ns and Os.

Apart from the various computational approaches for the prediction of intestinal drug permeability and solubility, which have been reported,[158] computer-based absorption models utilize a large number of topological, electronic, and geometric descriptors to take into account both aqueous drug solubility and permeability. To this end, descriptors of "partitioned total surface areas,"[159] molecular descriptors (e.g., Clog$P$, molecular polar surface area, number of hydrogen acceptors and donors, and Abraham molecular descriptors),[160–162] and various structural descriptors with neural networks[163]were found to be good determinants of oral drug absorption. For example, radial basis function artificial neural networks and theoretical descriptors were used by Turner et al.[163] to develop a quantitative structure–pharmacokinetic relationship for structurally diverse drug compounds. The proposed model may be used as a tool for preliminary selection of potential drug candidates eliminating expensive laboratory experiments. On the contrary, the descriptors included in the final model may provide useful information on the structural requirements for the design of new adequately bioavailable drug molecules.

REGULATORY ASPECTS OF ORAL DRUG ABSORPTION

Bioequivalence Studies

The historical aspects of the evolution of bioavailability and BE concepts in the FDA have been reviewed recently.[164,165] The landmark of these advances in regulatory science is the introduction of the term "bioavailability" used to describe the extent and rate of drug entrance in the systemic circulation.[17,166–168] Building on the concept of bioavailability, FDA scientists developed the science of comparative bioavailability studies that under the regulatory framework are called BE studies.[17,166–168] According to the regulatory authorities, two drug products of the same active moiety and at the same molar dose are bioequivalent if their rate and extent of absorption are so similar to ensure comparable *in vivo* performance.[29,169] Extent of absorption is usually expressed by the AUC, whereas rate of absorption is assessed by the C_{max} of the drug.[170–172] A large number of papers published in the literature either question the adequacy of C_{max} as a rate parameter or propose other approaches.[170,173,174] However, C_{max} is still used as the sole parameter for the assessment of the drug absorption rate.[29,169]

Classically, assessment of BE is based on the concept of average BE[169] where the test product (T) is considered bioequivalent to the reference product (R) if the calculated 90% confidence interval for the difference in their logarithmic-transformed mean measures of bioavailability lies between prespecified limits.[169,175] By general consensus, physicians suggested that for most drugs, a 20% difference in the means of pharmacokinetic parameters (AUC, C_{max}) between the two formulations does not lead to significant clinical differences.

Bioequivalence of Highly Variable Drugs

According to the concept of average BE, the estimated residual variability is considered to be a measure of within-subject variability (WSV). When this quantity has a coefficient of variation higher than or equal to 30%, the drug or drug product are called highly variable drugs (HVDs).[176,177] However, in cases of HVDs, there is an increased rejection rate of BE even for truly equivalent drugs.[178–180] In other words, as WSV increases, it becomes more difficult to prove BE, unless a large number of subjects are recruited. To overcome the limitations of high variability, several methods have been proposed such as an increase in sample size, steady-state studies, widening of acceptance limits, scaled BE methods, and the use of replicate clinical designs.[178,180–189] Recently, scientists from the European Medicines Agency (EMA) and the FDA have proposed reference-scaled approaches using only the WSV of the reference formulation.[29,190–196] EMA proposes a reference-scaled BE criterion, for a coefficient of variation of WSV up to 50%, applied only to C_{max}.[29,195] In case of the FDA, a similar approach was proposed with two main differences: (a) scaling is allowed for both AUC and C_{max} and (b) no upper variability limit is considered. This implies that, in case of the FDA's approach, BE limits scale continuously with WSV of reference product.[190–194]

It should be highlighted that even though both the FDA and EMA currently allow the use of reference-scaled BE approaches, the two methods do not exhibit similar performances always. These two procedures are only identical when variability is lower than 30% and in essence no scaling takes place. When scaling is in effect, the FDA's approach appears to be more permissive than the EMA's method. It has been shown that the major discrepancy, between the FDA's and EMA's scaling approaches, is observed for coefficients of variation of WSV values higher than 50% and when sample size is less than 50.[196] In addition, the GMR constraint is essential for the FDA's approach, but it is of limiting importance in case of the EMA's scaling approach.[196] Comparison of these two scaling approaches reveals that the increase of sample size affects the EMA limits more than the FDA's approach.[196]

It is widely acknowledged that the main advantage of scaled BE methods relies on the fact that fewer subjects are required to achieve adequate statistical power comparing with the classic 2×2 BE studies. In turn, this finding is considered to reflect less human exposure. However, it should not be disregarded that human exposure does not only refer to the number of subjects participating in the study, but also to the number of drug administration each subject receives. In this vein, replicate designs include more periods of drug administration, which imply increased exposure of each subject to the drug.

Biowaivers

As has already been mentioned, BE assessment is usually based on *in vivo* studies in accord with the drug agencies guidelines. Nevertheless, other types of evidence can also be used to demonstrate BE such as pharmacodynamic data, clinical studies with safety and/or efficacy endpoints, or even *in vitro* data.[169]

Of special importance are the situations wherein BE studies can be substituted with another type of evidence, such as *in vitro* data to save time, reduce cost, and unnecessary human exposure in clinical trials. These conditions are included under the general term "biowaivers," which refer to all exceptions from the necessity to perform clinical studies. The most common type of biowaivers adopted by the regulatory agencies refer to the application of *in vitro–in vivo* correlations, BCS, and Biopharmaceutic Drug Disposition

Classification System (BDDCS).[24,25,28–31,33,197–201] Granting of a biowaiver can be acceptable if certain prerequisites are upheld.[169]

The FDA Guidance on BCS and Relevant Concerns

This guidance[25] was issued in 2000 provides regulatory benefit for highly permeable drugs that are formulated in rapidly dissolving solid immediate release formulations. A drug is defined as highly soluble "when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1.0–7.5" while a drug product is defined as rapidly dissolving when no less than 85% of the dose is dissolved within 30 min using USP Apparatus I at 100 rpm in a volume of 900 mL in 0.1 N HCl, as well as in pH 4.5 and 6.8 buffers.

However, the BCS[24] and the criteria used for solubility and permeability classification in the FDA guidance [25] have been criticized several times. In this vein, the high solubility definition of the FDA guidance was criticized as too strict for acidic drugs such as non-steroidal anti-inflammatory drugs because they exhibit extensive GI absorption.[26] These observations have been explained on the basis of the dynamics of the two consecutive processes, dissolution and GI wall permeation.[27] Moreover, the solubility–dose ratio was proposed[202] as a more meaningful parameter for biopharmaceutic classification purposes.

The "rule of unity" was proposed in 2006 by Yalkowsky et al.[203] They introduced a new absorption parameter, Π, to predict the absorption efficiency of orally administered drugs that are passively transported. In reality, the "rule of unity" is a semiempirical model based on the AP concept.[20,21] According to this model, drugs are classified as "well absorbed" when their absorption values, Π, correspond to more than 50% of the administered dose, whereas those with absorption values corresponding to less than 50% of the dose are classified as "poorly absorbed." The fraction of dose absorbed was related to parameter Π,[204] allowing experimentally based estimates for the volume of intestinal contents; moreover, scientifically based changes for the current BCS were suggested. Dissolution-based instead of solubility-based classifications have also been proposed for new molecular entities[204,205] and marketed drugs.[204]

Also, Box and Comer[206] used pK_a, log$P$, and intrinsic solubility values (S_0) to investigate supersaturation and predict the BCS class of 84 marketed ionizable drugs. They used plots of log$P$ versus logS_0, divided in four segments corresponding to the BCS classes. For BCS class II drugs that could form supersaturated solutions, it was observed that the use of kinetic solubility instead of S_0 moved them into the region of BCS class I drugs. The authors suggest that supersaturation provides a way to keep a drug temporarily in solution and could be useful when assessing the possibility of improving solubility to obtain a biowaiver.[206]

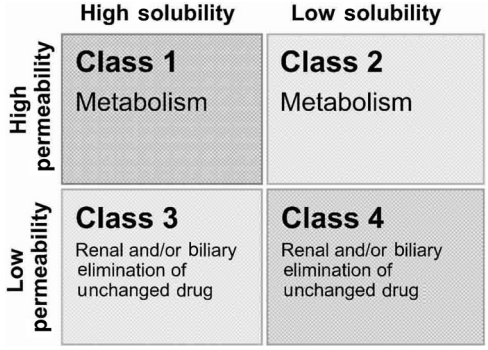

Figure 6. The biopharmaceutics drug disposition classification system as proposed by Wu and Benet.[28]

BDDCS: From Science to Regulation

Professor Leslie Benet questioned the ability of a single permeability estimate to predict the extent of drug absorption, despite the great popularity of BCS and the relevant FDA guidance published in 1995 and 2000, respectively. Based on these concerns, Wu and Benet[28] developed in 2005 the so-called BDDCS, actually, an extended version of BCS that includes drug elimination and the effects of efflux and transporters on oral drug absorption (Fig. 6).[28] Wu and Benet[28] proposed this modified version of BCS as a useful tool in predicting: (1) overall drug disposition when transporter–enzyme interplay will yield clinically significant effects; (2) the direction, mechanism, and importance of food effects; and (3) the transporter effects on postabsorption systemic drug concentration following oral and intravenous dosing. The authors also suggest that the number of class I biowaivers will increase if classified using the BDDCS-based elimination criteria.

However, the effect of transporters is dependent on the dose administered because, for high doses, they may be fully saturated. Thus, absorption may be limited by transporter efflux and gut wall metabolism only for drugs administered at low doses. Irrespectively of the dose utilized, EMA proposed the "≥90% metabolized" as cutoff limit an alternative criterion for the extent of absorption for class I biowaivers.[29] More specifically, in the EMA guideline,[29] it is stated that "following a single oral dose to humans, administered at the highest dose strength, mass balance of phase 1 oxidative and phase 2 conjugative drug metabolites in the urine and feces, account for ≥90% of the dose administered." This is the strictest definition because for an orally administered drug to be at least 90% metabolized by phase 1 and phase 2 processes, it is obvious that the drug must be extensively absorbed.[29]

Recently, computational models have been developed to predict the BDDCS class for new compounds using molecular structure and available molecular descriptors and software.[207] According to the authors, these *in silico* approaches may help the pharmaceutical industry providing drugs to the patient more rapidly and with reduced cost. In addition, these methods may have significant applications in drug discovery for the identification of new molecule candidates to be formulated in dosage forms.[207]

The BDDCS-based permeability classification of marketed drugs was also proposed[200] and the extent of drug metabolism (≥90% metabolized) was used to define class I marketed drugs suitable for a waiver of BE studies. Furthermore, in their recent work, Benet and Larregieu[208] stated that "although FDA-approved BCS class I drugs are designated as high-permeability drugs, in fact, the criterion utilized is high extent of absorption. This ambiguity should be eliminated, and the FDA criterion should explicitly be stated as at least 90% absorption based on absolute bioavailability or mass balance."

Benet et al.[30] classified over 900 drugs using BDDCS criteria and also applied a computational approach to predict BDDCS class of new molecular entities from molecular structures.[31] A very interesting result of both studies is that the solubility–dose ratio is an important parameter for BDDCS classification. Prompted by these advances,[30,31] an in-depth study of the role of the solubility–dose ratio in the biopharmaceutics classification of drugs was performed.[32] The original model[24] used for the development of BCS was modified[27] and used to examine in detail the effect of dose on the fraction of dose absorbed for each one of the four BCS drug classes. Based on this modeling work, the concepts of the critical dose and effective *in vivo* solubility were developed.[32] Moreover, real data were analyzed to derive estimates of effective *in vivo* solubility. These results led to the development of a dose-dependent version of BCS, the so-called DDBCS, according to which class migration as a function of dose may be justified for certain drugs. Finally, in his very recent paper, Benet[33] focuses on the complimentary role of BCS and BDDCS in the improvement, simplification, and speed of drug development.

Locally Acting Drugs in the GI Tract

A waiver for the need to conduct BE studies may also be acceptable in cases of drugs that are locally applied and are intended for local action.[29,209] Plausibly, any systemic action of these products is considered an undesirable effect. Examples of pharmaceutical preparations falling under this category include products for local use after nasal, dermal, ocular administration, and so forth. In the same context, drug products for oral or vaginal administration exerting local action in the GI could be included.

An example of an active moiety with such properties could be mesalamine, which is used to treat inflammatory bowel disease. The rationale for justifying equivalence in these cases is based on the fundamental assumption of BE testing. For example, in the case of an orally administered drug with systemic effect, the measured endpoint (e.g., plasma concentrations) acts as a surrogate for the concentration at the site of action. Likewise, the most critical issue, for mesalamine and its active metabolite, is to achieve effective concentrations in the intestine. Thus, local concentrations of mesalamine in the GI determine local anti-inflammatory action. Accordingly, it is suggested that BE assessment of this type of drugs (e.g., mesalamine) could be based on scintigraphic studies coupled with drugs' measurements in plasma. Specific criteria can be used for the GI performance of the formulations such as the onset of release and transit time through the colon. However, according to the FDA, BE studies with clinical and pharmacokinetic endpoints are proposed.[210–213] In case of the pharmacokinetic studies, plasma mesalamine should be measured not only after oral but also following rectal administration.

CONCLUSIONS

Although the past 30 years or so can be viewed as the golden era of oral drug absorption both in the scientific and regulatory setting, important unanswered problems still exist and many questions relevant to the transformation–implementation of scientific theories and experimental observations of drug absorption to regulatory guidelines are still open.

Generally, classical diffusion principles still apply in most of the published work on GI drug absorption despite the inherent heterogeneity encountered both *in vitro* and *in vivo*. Notable examples are (1) the long dispute on "constant" diffusion layer thickness throughout the dissolution process (see Ref. 90 and references therein); (2) different approaches utilized the most sophisticated software for GI drug absorption processes, namely, the Noyes Whitney (GastroPlus™) equation and the Flanagan relationship (SimCyp®) to mimic *in vivo* dissolution, whereas seven discrete homogeneous compartments with transit rate constants are used to emulate *in vivo* drug transit; (3) permeability estimates that do not properly reflect the extent of drug absorption[33,208,214,215]; and (4) despite the extensive use of the Weibull function and the power law for the analysis of the entire set of dissolution/release data, the time-dependent character of these processes has not been recognized as yet.[93,94,216] Besides, the exact origin and the implications of variability in GI drug lumen concentrations reported in numerous *in vivo* studies have not been elucidated so far. Although

linear IVIVC are routinely applied, nonlinear IVIVC are uncommon in the literature.[59]

As far as the regulatory work is concerned, remarkable differences are found between the BE guidelines of the various agencies. For example, the WHO proposal on *in vivo* BE requirements does not only allow biowaivers for BCS class II substances but also utilizes the solubility–dose ratio for biopharmaceutical classification purposes[217]; these features have not been included in the FDA's and EMA's BE guidelines as yet. Similarly, the leveling off character of the BE limits for C_{max} can only be found in the EMA's and not in the FDA's BE guidelines.[196] In contrast, the first steps toward dealing with the biosimilarity problem have been taken by an FDA board,[218] whereas EMA has not yet taken any steps despite its extensive work on the biologics guidelines.

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