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BIOPHARMACEUTICAL CONSIDERATION IN DRUG PRODUCT DESIGN AND *IN VITRO* DRUG PRODUCT PERFORMANCE

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ABSTRACT

Physiological, physicochemical, and biopharmaceutical aspects can be used to classify the variables that determine how well oral medications are absorbed and metabolised. The physical, chemical, and biological factors influencing permeability and solubility the purpose of this study was to elucidate the rate-limiting mechanism in oral biopharmaceutical classification system absorption. Due to its particular anatomical and physiological activities, designing the ocular surface and evaluating its performance for ophthalmic ointment medicinal solutions prepared with a range of bases provide specific challenges. Drug assay, content homogeneity, API, rheological characteristics, in vitro drug release, and in vitro drug permeation were among the quality and performance parameters examined. the physicochemical and release parameters, the in vitro drug release constant, and quality and performance qualities. drug dissolution vessel to conduct experiments using a USP paddle apparatus, drug release characteristics for products with various formulations and manufacturing processes to gauge the effects of this altered vessel environment while still keeping the vessel dimensions within the required specifications. In vitro dissolution/release testing of innovative administrations will be thoroughly reviewed in this article, including both compendial and non-compendial approaches. This work proposes a novel type of in vitro-in vivo correlation (IVIVC): the relationship between the in vitro parent drug dissolution data and the in vivo pharmacokinetic data of the medication's metabolite. Stability tests that ensure the maintenance of the product's quality, safety, and efficacy over the shelf life are necessary for any pharmaceutical product to be accepted and licenced.

INTRODUCTION

Finding and creating therapeutically effective drugs is a labor-intensive, expensive, and difficult process. Recent statistics show that developing a new drug in the US takes 10–15 years and costs

$8 billion on average [1]. The majority of drugs available on the market are administered via the oral route since it is convenient and affordable [2, 3]. Oral administration does not require the assistance of trained medical personnel, unlike intravenous dosing, and it carries no risk of infection [3,4]. Therefore, oral bioavailability is one of the key factors that is often considered in drug discovery when determining whether to continue developing a new chemical entity (NCE). Low oral bioavailability is well documented to be a significant cause of therapeutic variability [5]. A significant negative correlation between the coefficient of inter-individual variability and the oral bioavailability of drugs from a number of therapeutic groups was demonstrated by Hell Riegel et al. [6]. If a patient's oral bioavailability is poor, they may be at risk for dangerous or subtherapeutic plasma drug concentrations, as well as drug-drug or food-drug interactions [7]. This is essential for drugs like cytotoxic drugs and antibiotics [8] that have a short therapeutic window or a high risk of acquiring resistance. The preclinical evaluation of an NCE's pharmacokinetic profile is routinely done during the drug discovery process. It is common practice to use this data, coupled with details from in vitro and in silico studies, to project the pharmacokinetic profile of individuals. Rats are frequently employed for in vivo pharmacokinetic experiments, including the assessment of oral bioavailability, due to their short lifespan and ability to produce a large number of animals quickly. These studies on larger animal species cannot be carried out because NCEs are produced in such small quantities. The outcomes of such evaluations are then used to project the bioavailability of humans. It is not usually accurate to extrapolate an in vivo rat study to human oral bioavailability, though. For instance, Cao et al. examined the oral bioavailability of 48 drugs with different physicochemical properties in rats and humans and found no correlation between the oral bioavailability levels in the two species [9]. This is a prime example of how challenging it is to extrapolate a human pharmacokinetic profile from data collected in rats, which can result in inaccurate inferences about the pharmacokinetic properties of the NCE and/or inaccurate conclusions about its potential as a useful therapeutic drug [10–12]. Theoretical foundations of factors affecting oral bioavailability; a summary of species-level similarities and differences in the absorption and metabolism mechanisms affecting oral bioavailability of NCEs in rats and humans; and reviews of physicochemical and biopharmaceutical factors influencing drug absorption and metabolism in both rats and humans are all covered in this report. Overall, this study provides a rational perspective on how differences in species and similarities in the absorption and metabolic pathways that affect oral bioavailability in rats and humans interact with the physicochemical and biological properties of drugs. Using in-vivo data from rats, this will make it easier to predict how well a drug will work when taken by mouth in humans.

Drug bioavailability factors influenced by biopharmaceutical factors

Oral bioavailability estimations based on theory

The amount that an active moiety is digested from such a pharmacological dose form and then becomes present in the systemic circulation is known as bioavailability (F percent) [1. There are two categories of bioavailability as a parameter: absolute bioavailability and relative bioavailability [5]. Absolute bioavailability is the percentage of an extravascular dose (such as an

oral dose) that, in comparison to an intravenous dose, enters the systemic circulation unmodified [5]. It is often found by adding up the total area under the medication plasma concentration versus time curve (AUC) after oral and intravenous delivery.

(Equation 1):

$$\text{Absolute bioavailability} = AUC_{oral}/AUC_{IV} \times dose_{IV}/dose_{oral}.$$

When administering a medicine intravenously is not an option, the bioavailability of a drug is assessed by comparing the proportion of a dose that enters the systemic circulation to a reference standard [5,13]. The relative bioavailability of this parameter is often calculated as.

(Equation 2):

$$\text{Relative bioavailability} = AUC_{test}/AUC_{ref} \times dose_{ref}/dose_{test}.$$

The bioavailability of a drug is evaluated by comparing the percentage of a dose that enters the systemic circulation to a reference standard when giving a medication intravenously is not an option [5,13]. This parameter's relative bioavailability is frequently determined using the formula in (Equation 3):

$$F = F_{abs} * F_g * F_h * F_I$$

For Equation 3: F denotes oral drug bioavailability; F abs denotes the portion of the dose absorbed into intestinal enterocytes; F g denotes the portion of the dose that avoids intestinal first pass elimination; F h denotes the portion of the dose that avoids pre-systemic liver first pass elimination; and F l denotes the portion of the dose that occurs before pulmonary first pass elimination. To be clear, F l only contributes to a drug's oral bioavailability when the oral plasma exposure is contrasted with the plasma exposure following intra-arterial rather than intravenous delivery. Because the intravenous dose should travel through the lung before reaching the systemic circulation in this instance, identical to the oral dose, F l cancels out [14]. As a result, only F abs, F g, and F hare products when the oral bioavailability of a drug is compared to its intravenous administration.

(Equation 4):

$$F = F_{abs} * F_g * F_h$$

As shown in Equation 4: The fraction of the dose that escapes first-pass elimination across the intestines (F g) and liver (F h) can be estimated experimentally via the comparison of systemic exposures (AUC ratios) where the dosing routes are selected to isolate the contribution by a particular organ. When doses are given orally and through a cannulated hepatic portal vein (h. p. v.), the fraction absorbed (F abs) is either assumed to be 100% or is known and can be accounted for as in:

(Equation 5):

$$F_{abs} \cdot F_g = AUC_{oral}/AUC_{h.p.v} \times dose_{h.p.v.}/dose_{oral.}$$

In the same way, FH can be found for a substance when doses are given intravenously and through an h. p. v. that has been cannulated.

(Equation 6):

$$F_h = AUC_{h.p.v.}/AUC_{I.V.} \times dose_{I.v.}/dose_{h.p.v.}$$

Additionally, F g and F h can be calculated using a chemical and the organ extraction ratio. (E). (Equations 7):

$$F_g = 1-E_g$$

(Equations 8):

$$F_h = 1-E_h$$

Equation 9, CL h can be found by scaling in vitro data or from in vivo data (if you know the relationship between CL h and the systemic clearance for a given compound) [15,16]. Both the hepatic blood flow (Q h) and the hepatic blood clearance (CL h) can be used to estimate the hepatic extraction ratio.

(Equation 9):

$$E_h = CL_h / Q_h$$

Equations 10 and 11: Following adopting a linear one-compartment model, the following can be used to express the drug plasma concentration (Cp) and AUC of a drug after oral administration:

(Equations 10):

$$C_p = K_a \cdot F \cdot dose \div V_d (K_a - K_e) \times (e^{-K_e \cdot t} - e^{-K_a \cdot t})$$

(Equations 11):

$$AUC = F \cdot dose / CL$$

For Equations 10 and 11: V d is the drug's volume of distribution, and Ka and K e are the first order rate constants for absorption and elimination, respectively [13,17]. The rate of absorption and elimination has a big effect on two pharmacokinetic parameters called C max and T max. C max and T max are the highest drug concentrations in the systemic circulation after an oral dose. T max is the amount of time it takes to reach the maximum drug concentration in the systemic circulation after an oral dose.

Equations 10 and 11: The results clearly demonstrate that, in addition to the drug absorption rate constant (Ka), the drug elimination rate constant (Key), bioavailability (F), and distribution also have an impact on the medication plasma levels following oral dosage (Vd). However, when calculating the degree of plasma exposure, AUC only considers the drug dose, clearance (CL), and bioavailability. As a result, a medication's plasma exposure following an oral dose may vary greatly based on a species' unique drug metabolism and absorption patterns.

FACTORS THAT AFFECT ORAL BIOAVAILABILITY

1. The first law of Fick's absorption

can be used to show the interaction between a medicine's rate of absorption and its solubility in the biological fluid in which it is dissolved (absorptive flux). (Equation 12) [18]

(Equation 12):

$$J = P_e \cdot \Delta C \cdot SA$$

According to Fick's first law, J equals the intestinal drug absorptive flux across a homogenous intestinal membrane, P e equals the effective intestinal permeability (cm/s), C equals the concentration gradient across the intestinal mucosa, "drug solubility in the luminal fluid," and SA equals the intestinal surface area that is open to oral absorption (cm^2).

Equation 12: shows how permeability and solubility impact absorptive flow, which has an impact on the rate of drug absorption and the percentage of the oral dose that is absorbed [19]. Physicochemical, pharmacological, and physiological variables [20–24] may have an impact on the rate and amount of oral medication absorption.

Physiological variables affecting the absorption of oral medicines

Below is a brief summary of the extensive review that has been done on the morphological and physiological differences between the GI tracts of rats and humans [25,26].

2. Anatomy and physiology of the gastrointestinal tract

In both rats and humans, the stomach, small intestine (which comprises the duodenum, jejunum, and ileum), and large intestine make up the majority of the gastrointestinal tract (cecum, colon, and rectum). Between the two species, the sizes of these components and their surface areas differ. The major GI tract subdivisions' physical lengths and percentage contributions in rats and humans are compared in Table 1 [25]. The human GI tract is just 8.35 m long, or 5.5 times shorter than the rat GI tract, despite weighing 70 kg, compared to 0.25 kg for rats (1.5 m). Rats' small intestine, which is thought to be the principal location of medicine absorption, has an 83 percent length to total gastrointestinal tract length ratio compared to 81 percent in humans. The size of rats' big intestines is between 17 and 19% larger than that of humans. Another thing to keep in mind is that the cecum, which is where the majority of microbial digestion occurs, makes up around 26% of the length of the large intestine in rats. It only accounts for about 5% of the length of the human large intestine [27,28].

Table 2 GI tracts' absolute surface areas in humans and rats are compared. A human's small intestine has 200 times the surface area of a rat's, which is an interesting finding [25]. Only two of the three structural changes that greatly expand the human small intestine's surface area are present in the rat small intestine. This observable variation in surface area is explained by these three architectural modifications in the human small intestine. The plicae circularis, or folds of kecking, which quadruple the mucosa's surface area in the human small intestine but are missing in the rat intestine, are the most visibly apparent mucosal folds. The surface area of rats and humans is increased by 5 and 10 times greater, respectively, by microscopic projections from the plicae circularis known as villi, which resemble tiny finger-like tissue projections. Each villus' surface area is 20 times greater thanks to the microvilli that cover it. The human small intestine's relative surface size is barely triple that of the rat when compared to the total body surface area [25]. The surface of the great intestine lacks villi and is segmented into sections by transverse furrows, in contrast to the small intestine. The large intestine also has slightly different enterocytes than the small intestine and has microvilli that are less tightly spaced apart [26]. When everything is taken into account, this dramatically shrinks the size of the large intestine in both rats and humans (Table 2), which is consistent with the fact that the small intestine is where most medications are absorbed in both species.

Table 1. Comparison of the anatomical lengths of the intestinal tract and its major subdivision in rats and humans [25]

Region of intestinal tract	Human		Rat	
	Length (m)	% of total	Length (m)	% of total
Small intestine	6.80	81	1.25	83
Large intestine	1.55	19	0.25	17
Total intestinal tract	8.35		1.50	

Table 2. comparison of the absolute surface area of the gastrointestinal tract in rats and humans [25]

Region of intestinal tract	Human Absolute surface area (m^2)	Rat Absolute surface area (m^2)
Stomach	0.053	0.000624
Small intestine	200	1
Large intestine	0.35	0.034

3. A layer of stagnant water adjacent to the intestinal membrane may prevent numerous medication molecules from passing through [31,32]. This layer is present in rats and is about 100 m thicker than in humans [33]. The effects of the unstirred water layer along the intestinal membrane on the pace and volume of passively absorbed pharmaceuticals with different membrane absorption half-lives were scientifically examined by Chou et al. in humans, dogs, rabbits, rats, and mice (10–300 min). The results of the experiment show that it is normal to think that the layer of water that hasn't been stirred will have little or no effect on the rate and total amount of absorption [34].

4. Transit periods in the digestive system

The amount of a pharmaceutical molecule that is absorbed overall depends on how long it stays in the GI tract and how much of it is absorbed in each segment [35]. Drug absorption and digestion into the body might be influenced by stomach transit time in general. However, intestinal transit time affects a variety of items, including those with reduced mucosal permeability, carriers that aid in absorption, medications that are prone to intestinal degradation, or goods whose dissolution is the rate-limiting stage for systemic absorption [36]. The physical properties of the contents of the colon have little impact on intestinal transit time, in contrast to how food is ingested [37]. Rats and people both have gastrointestinal transit times of between three and four hours [25,38]. According to several studies, the proximal intestine of both rats and humans has a smaller small intestinal transit than the distal intestine [38,39]. Human large intestine transit periods range from 8 to 72 hours, but the average for rats is 15 hours [25,40,41].

5. The pH of the digestive system

The rate at which medications dissolve and the GI tract's passive permeability are both significantly influenced by the level of ionization. As a result, based on the pH at the absorption site, various ionizable medicinal components can dissolve and be absorbed in different ways [25,42]. Luminal secretions have an impact on the pH of the luminal material, which is crucial to note (chyme). The pH readings for the GI tracts of the rat and humans are shown in Figure 1 [36]. Human chyme can have a pH as low as 2.3, making it more acidic than the chyme of rats, which has a pH of 3.3. As soon as the chyme enters the duodenum, bile and pancreatic bicarbonate are released, quickly neutralizing it. In the distal small intestines of rats and humans, the chyme pH steadily rises. The pH of chyme in both species is often higher in the large intestine than in the small intestine. This could be because of fermentation caused by the microbial flora [26].

6. Bile created

Through the many bile channels that enter the liver, which is expelled by the hepatocytes [25]. During this process, the bicarbonate-rich watery solution is continuously added by the epithelial cells, raising the alkalinity of the solution. Human gall bladders can concentrate and store up to five times as much bile when at rest. On the other hand, the absence of gall bladders in rats may indicate that bile enters the duodenum continually as a diluted solution. It is important to note that the human gall bladder secretes bile between 2 and 22 ml/kg each day. The rat liver, on the other hand, secretes 48 ml/kg daily into the duodenum [26]. Rats and people use bile as a detergent to emulsify lipids by increasing the surface area to promote enzyme activity, which then makes them easier to absorb in the small intestine. Bile salts, such as those consisting of taurocholic acid and deoxycholic acid, are another component of bile. These salts help phospholipids and bile emulsion break down fat globules by linking their hydrophobic and hydrophilic surfaces with lipids and water, respectively. Bicarbonate solution can also be found in bile. The subsequent organization of emulsified droplets into many micelles improves their absorption. Bile facilitates the breakdown of fats and is essential for the absorption of steroids and fat-soluble vitamins [43–44].

7. Both in rats and in people

Bacterial microflora makes up the bulk of the GI tract and has grown to account for a sizable component of the luminal composition. Rats' stomach, small intestine, and large intestine all contain a significant number of microorganisms. The human stomach and upper small intestine do not have any bacterial microflora. The low pH of the gastrointestinal contents is the primary culprit here. However, the distal small and large intestines of humans contain a wide variety of bacterial microflora [45]. By hydrolyzing, dehydroxylating, deamidating, decarboxylating, and reducing azide groups, these bacterial microflorae participate in the metabolism of many chemicals and xenobiotics [45–47]. The most crucial metabolic process among them is the hydrolysis of the glucuronide conjugates, which is accomplished by the -glucronidase enzyme and is produced by the bacterial microflora that is prevalent in the GI tracts of rats and humans. The quantity of the enzyme glucuronidase in the rat GI tract is noteworthy since it is substantially higher than that in the human GI tract [26]. Another element that is known to downregulate CYP isoforms is the bacterial microflora [26]. Conventional laboratory animals have a limited capacity to simulate the metabolism of the human gut microbiota *in vivo*. A germ-free rat model with microorganisms from

the human colon that are similar to those in the rat was used to make an in vivo model for human projections [48,49].

8. Highly lipophilic medications' lymphatic absorption

according to the intestinal lymphatic pathway. Benefits include a change in the rate of oral drug input to the systemic circulation, allowing for controlled drug formulation; increased oral bioavailability of highly lipophilic drugs by avoiding the hepatic first pass effect; direct targeting of lymphoid tissue; indirect targeting of specific sites linked to low-density lipoprotein receptors; and targeting of lymphoid tissue directly. The variations in drug absorption by animal lymphatic systems are poorly understood. This is a result of the bias present in the methods used to evaluate the lymphatic medication absorption in animals [53]. On the other hand, the similarities and differences in medication lymphatic uptake are based on lymph flow to different absorption sites, drug lymphatic absorption strategies, lipid digestion mechanisms, and bile composition and secretion pattern [36].

9. How oral absorption works After oral delivery

A variety of active or passive transport pathways can be used to move medication molecules through the luminal membrane. Passive diffusion occurs along the transcellular (lipophilic) pathway as well as the paracellular pathway. While the transcellular (lipophilic) pathway necessitates drug diffusion via the enterocyte's lipid cell membrane, the paracellular pathway necessitates drug diffusion through the tight junctions' aqueous pores. Transporters mediate the active drug inflow and efflux pathways of the transporter-mediated active transport pathway. It's critical to remember that each route's application is governed by the physicochemical characteristics of the molecule and potential affinities for different transport proteins [1]. gastrointestinal transit times 3.

The total amount of a drug's absorption depends on how long the drug stays in the GI tract and how much of it is absorbed in each segment [35]. The speed and efficiency with which medications are metabolized and taken into the body are often influenced by stomach transit time. Drugs with lower mucosal permeability, carriers that aid in absorption, medications that are prone to intestinal breakdown, and products whose dissolution is the rate-limiting step for systemic absorption are all affected by intestinal transit time, though [36]. Contrary to how food is ingested, the physical properties of the contents of the intestine have no impact on intestinal transit time [37]. For both humans and rats, the intestinal transit time lasts between three and four hours [25,38]. According to several investigations, the small intestinal transit in the proximal intestine was faster than that in the distal intestine in both the rat and human small intestines [38,39]. Human transit periods range from 8 to 72 hours, but the average for rats' large intestine is 15 hours [25,40,41].

Factors affecting oral medication absorption that are physicochemical and biopharmaceutical

The impact of physicochemical and biological parameters on drug permeability and solubility is examined in detail in a recent study [1], which is outside the purview of this investigation.

Five-point rule of Lipinski

The physicochemical properties of the substance have a big impact on how well an oral medicine is absorbed. The rule of five (RO5), created by Lipinski et al., is one of the most widely used

concepts for qualitatively estimating oral medicine absorption. The team looked at 2245 compounds from the World Drug Index (WDI) database that were either being considered for or included in Phase II clinical studies. The results show that the following medication molecules have higher chances of being effectively absorbed when taken orally: MW 500, calculated lipophilicity (clog P), 5, 5 hydrogen bond donors (specified as NH or OH groups), and 10 hydrogen bond acceptors (defined as oxygen or nitrogen atoms, including those that are part of hydrogen-bond donors).

The polar surface area (PSA)

The molecular surface (Van der Waals or solvent accessible) that is caused by oxygen and nitrogen atoms or hydrogen atoms connected to them, is another extensively used physicochemical component to predict oral drug absorption. It is assumed that it has something to do with the drug molecule's ability to form hydrogen bonds. Along with polar hydrogen atoms, several workers also added phosphorus, sulphur, and other elements. [57] The finding that PSA corresponds with passive molecular transport via membranes makes it possible to predict the intestinal transport properties of many pharmaceutical drugs. In 1996, Palm et al. showed a substantial correlation between the PSA of pharmaceutical drugs and their permeability using two well-known in vitro intestinal permeability models (Caco-2 monolayers and rat intestinal segments). A homologous series of-adrenoreceptor antagonists were used as model pharmacological compounds. PSA and permeability of these compounds correlated strongly in Caco-2 cells and the rat ileum (R2: 0.99 and 0.92, respectively) [58]. In a further study, Palm et al. found that 20 pharmaceutical substances with a variety of physicochemical characteristics and PSA and human F abs values (0.3 to 100 percent) showed excellent sigmoidal association (R2: 0.94). The majority of these medicinal chemicals' absorption was due to passive diffusion [59]. Regression research revealed that drug molecules with a PSA value of > 140 2 would have poor oral absorption (10%), whereas drug molecules with a PSA of 60 2 might be anticipated to have full oral absorption (> 90%). Similar findings were reported by Kielder et al., who calculated the PSA for 2000 oral medications that had advanced to Phase II studies. The chemicals were divided into two groups: oral CNS drugs (n = 776) and oral non-CNS drugs (n = 1590). They discovered that the PSA value for orally active non-CNS drugs that are passively given through the transcellular route is generally 120. On the other hand, oral CNS medicines often have a PSA score of between 60 and 70 [60].

Drug metabolism

It is one of the most important factors that influences oral bioavailability, along with absorption. An oral medication's initial passage through the liver and gut is referred to as "first-pass metabolism" when it is processed between the GI tract (site of delivery) and the systemic circulation (location of measurement) [61]. Despite being completely absorbed from the GI tract, medications with moderate to high hepatic extraction ratios (0.3 E h 1.0), such as lidocaine, may nonetheless have low absolute oral bioavailability due to the effects of first-pass metabolism [62–63]. For a drug undergoing full absorption (F abs = 1), Figure 2 illustrates the relationship between F h and F g and absolute bioavailability. Either the liver or the intestines, or both, can participate in first-pass metabolism. Studies that used ciclosporin A and midazolam [64, 65] showed how

important first-pass metabolism is in the body. First-pass metabolism is governed by physicochemical and biopharmaceutical properties. much as how physiological factors, such as metabolizing enzymes, regulate drug absorption.

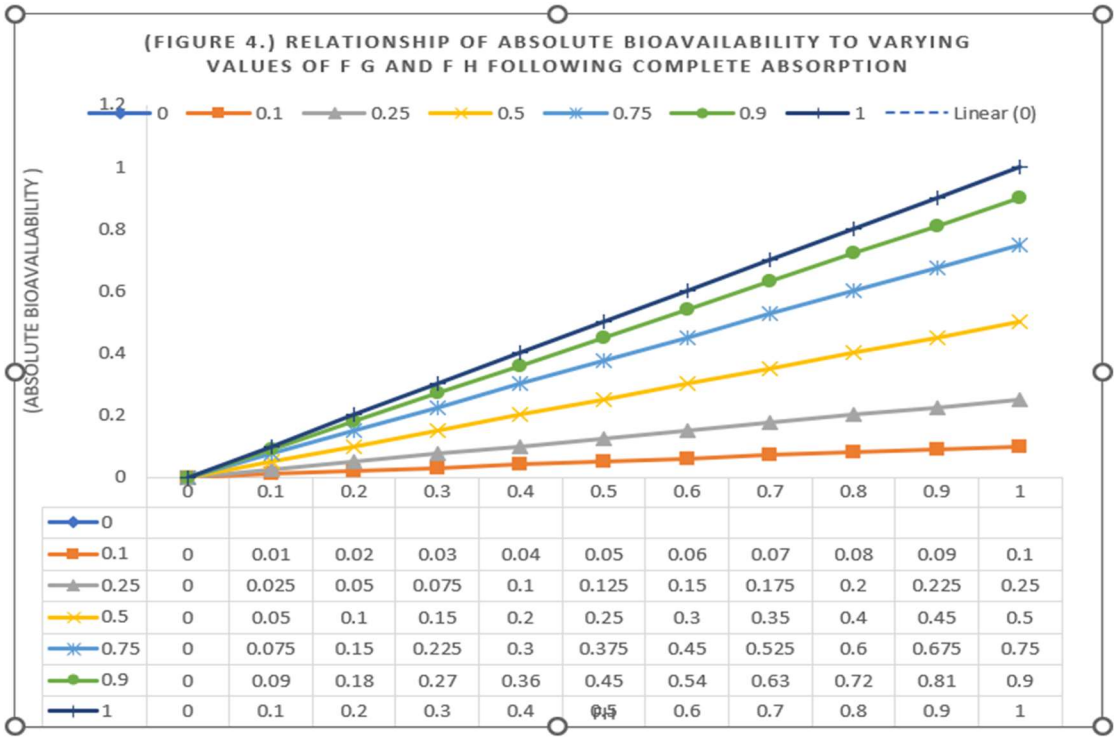

Figure2. Relationship of absolute bioavailability to varying values of F g and F h following complete absorption (Fabs = 1). Fabs: Fraction of the dose that is absorbed from the intestinal lumen to the intestinal enterocytes; F g: Fraction of the dose that escapes pre-systemic intestinal first-pass elimination; F h: Fraction of the dose that passes through the liver and escapes pre-systemic liver first-pass elimination

Factors in physiology that influence how medicines are metabolized

When determining if preclinical data is appropriate to use for human projections, it is vital to know the rate and metabolic destiny of the chemical in both species in vitro and ensure that the intrinsic metabolic clearances or extraction ratios are similar. The primary subject of the following section is examples of interspecies comparisons for two significant drug metabolizing enzyme families.

Differences in metabolic enzymes among species

1. Chromatin P450s:

The CYP enzyme superfamily of drug-metabolizing enzymes requires a cofactor called NADPH. Furthermore, the CYP superfamily is divided into families (CYP1, CYP2, and so on; > 40% amino acid identity), subfamilies (CYP2C, CYP2D; > 55% identity), and specific enzymes (CYP3A4, CYP3A5) [66, 67]. The liver, small intestine, kidney, brain, skin, lung, nasal epithelium, and testis

are just a few of the organs that contain CYPs in both humans and animals. However, CYP medicines are primarily removed from the liver and stomach [68,69].

2. Physical-chemical variables affecting drug metabolism:

The relationship between lipophilicity and clearance is a critical measure for evaluation in early drug development at the critical phase of chemical template optimization [70] [184]. For neutral pharmaceuticals with Log D values > 0, hepatic clearance tends to contribute more to overall clearance, and for particularly lipophilic medications, hepatic clearance may wind up being the primary route of elimination [70]. This relationship between unbound hepatic intrinsic clearance and drug Log D has been demonstrated by a number of chemical classes, including a series of -adrenergic antagonist substances and a series of calcium channel antagonists [70]. The drawback is that even very lipophilic compounds need to have access to the active site of the metabolising enzyme. Therefore, steric hindrance as well as the lipophilicity of the persistent should be considered while building NCEs [71].

3. The percentage of dosage absorbed (Fa):

The solubility of drugs with poor water solubility was theoretically computed [72] using variables for dissolution and penetration. The Noyes-Whitney model was used to explain how solid medications dissolve [73]. Extraction of Dissolution Parameter z from an In Vitro Dissolution Curve In order to simulate drug dissolution in vivo from the in vitro dissolution profile in a miniscale dissolution test, we first defined parameter z, a hybrid parameter derived from the formula 3D/hr0, where D is the diffusion coefficient, is the drug density, h is the diffusion layer thickness, and r0 is the initial particle radius. The z values from the in vitro data from Equation were estimated using the mathematical tool SAAM II version 1.2 (SAAM Institute Inc., University of Washington, WA, USA).

$$Dx_{d,\,vivo}(t)/dt = 3D/_{\varrho}hr_0 * x_{o,\,s\,vitro} * (x_{s,\,vitro}(t)/x_{0,\,s,\,vitro})^{2/3} * (c_s - x_{d,\,vitro}(t)/v_{vitro})$$

$$= z * X_{0,\,s,\,vitro} * (X\,s,\,vitro * (x_{s,\,vitro}(t)/x_{0,\,s,\,vitro})^{2/3} * (C_S - X_{d,\,vitro}(t)/v_{vitro}) \quad (1)$$

Xd, vitro (t) is the mass of the dissolved drug at time t, X s, vitro (t) is the mass of the solid drug at time t, Cs is the drug's saturation solubility, and V vitro is the volume of the dissolution medium. Calculating the Permeability of the Unstirred Water Layer Highly permeable medicines cannot pass through the intestinal epithelial barrier because of diffusion via the unstirred water layer (UWL) [74–79]. UWL permeability (PUWL) is defined as the diffusion coefficient and the thickness of UWL [80] using the Stokes-Einstein equation for small, spherical molecules. UWL permeation can be modelled as a straightforward diffusion process in a water layer.

$$P_{UWL} = D/\delta = k_B\,T/6\pi\eta\,\delta * 1/R \qquad \text{EQ. (2)}$$

The Boltzmann constant (kB), the Kelvin temperature (T), the UWL viscosity, and the molecular radius (R) are all present. Using the rate-limited penetration of glucose through the effective intestinal membrane in dogs (16104 cm/s) [81], Eq. 3 below expresses the thickness of the diffusion layer of UWL (6). The MW of glucose, which is 180, can also be used to figure out the diffusion coefficient, because the molecular radius is a function of the cube root of the molecular weight (MW1/3), assuming that the molecule is round.

$$\boldsymbol{\delta} = K_B T/6\pi\eta * 1/(3/4\pi_{\varrho} * MW)^{1/3} * 1/P_{UWL}$$

$$=K_BT/6\pi\eta*1/(3/4\pi_{\varrho}*180)^{1/3}*1/16*10^{-4} \quad \text{EQ. (3)}$$

The PUWL for each drug is given by the following equation.

$$P_{UWL}=16*10^{-4}*(180/MW)^{1/3} \quad \text{EQ. (4)}$$

Drug Absorption Simulator in Canine Small Intestine:

The model for a drug's dissolution and passive permeation in the small intestine makes the following assumptions: (2) the diffusion layer simulates the dissolution process; (3) the drug is absorbed in the small intestine rather than the stomach or colon; (4) a lipophilic drug's membrane permeability is high and is thus limited by diffusion through the UWL; and (5) the intestinal transit time in dogs is 2 hours [82]. In Beagle dogs, the small intestine measures 225–290 cm and has a diameter of 1 cm, while in humans, it measures on average 625 cm and has a diameter of 5 cm [83]. Using these figures, it is determined that the overall intestinal surface area in dogs (16–20 cm^2/kg) is two to three times lower than that in humans (45 cm^2/kg). The mass balances of medicines that are both liquid and solid in the GI tract can be found in the following equations:

$$Dx_{s,\,vivo}(t)/dt=-z*X_{0,\,s,\,vivo}*(X_{s,\,vivo\,(t)}/X_{0,\,s,\,vivo})^{2/3}*(c_s-X_{d,\,vivo(t)}/V_{vivo}) \quad (5)$$

$$DX_{d,\,vivo}(t)/dt=z*X_{0,\,s,\,vivo}*(X_{s,\,vivo}(t)/X_{0,\,s,\,vivo})^{2/3}*(cs-X_{d,\,vivo}(t)/v_{vivo}) \quad (6)$$

$$-P_{UWL}*S*X_{d,\,vivo}(t)/V_{vivo}$$

X s, vivo (t) is the amount of drug in the small intestine that is solid at time t and X d, vivo (t) is the amount of drug that is dissolved at the same time. The dose given was used as the value for the function X 0, s, vivo, which is a function of the quantity of particles in vivo. In vitro testing yielded the z and Cs values that were employed. The formula for calculating the absorption rate is:

$$dx_{a,\,vivo}(t)/dt=P_{UWL}*S*X_{d,\,vivo}(t)/v_{vivo} \quad (7)$$

The mass of drug absorbed at time t is represented by X a, vivo (t). The ratio of X 0, s, vivo to X a, vivo (t) at 2 h is the expected fraction of the dosage absorbed (Fa).

$$Fa(t)=X_{a,\,vivo}(t)/X_{0,\,s,\,vivo}*100 \quad (8)$$

The Runge-Kutta method was applied with STELLA® 5.1.1 software to create simulated profiles (Cognitus Ltd., North Yorkshire, UK).

1. Estimation of Oral Absorption Rate-Limiting Steps:

The percentage C ratio (t) represents the ratio of the intestinal drug concentration at time t to the drug saturation solubility in the drug absorption simulation.

$$C_{ratio}(t) = (X_{d,\,vivo}(t)/v_{vivo})/c_s*100 \quad (9)$$

The solid surface's diffusion layer (h) in equations 5 and 6 has a concentration gradient across it that represents [Cs (X d, vivo (t)/V vivo)]/h and regulates the rate of dissolution over time. The medication dissolves in sink circumstances if C ratio (t) is low (Cs > > X d, vivo (t)/V vivo). The oral absorption in this instance might be dissolution rate-limited. Due to the solubility limit of the medication, drug dissolution diminishes when C ratio (t) increases (non-sink conditions).

2. Drug Choice:

Danazol, griseofulvin, and prepatent were selected as the models for poorly water-soluble drugs. Table I. The oral absorption of neutral drugs such as danazol and griseofulvin largely depends on dissolution in the small intestine because neutral drugs mainly dissolve in the small intestine and not in the stomach or colon due to the presence of bile salts. Prepatent, a free weak base (pKa

4.38), will likely dissolve not only in the small intestine but also in the stomach due to the low gastric pH. However, when the gastric pH is elevated, prepatent will dissolve mainly in the small intestine and not in the stomach because of the presence of bile salts. In the present study, prepatent was administered after the dogs had received the gastric acid blocker famotidine and therefore dissolved mainly in the small intestine. Because the present prediction system assumes that drug dissolution and absorption occur in the small intestine and not in the stomach or colon, the drugs selected were adequate models for establishing an *in vitro–in vivo* correlation. Prepatent, griseofulvin, and danazol were chosen as the prototypes for ineffective water-soluble medications Figure 1: Because neutral medications usually dissolve in the small intestine rather than the stomach or colon due to the presence of bile salts, the oral absorption of neutral drugs like danazol and griseofulvin depends heavily on this process. Due to the low gastric pH, prepatent, a free weak base with a pKa of 4.38, will probably dissolve both in the small intestine and the stomach. However, when the gastric pH is raised, prepatent will dissolve mostly in the small intestine and not the stomach due to the presence of bile salts. In the current study, prepatent was given to the dogs after they had taken the gastric acid-blocking medication famotidine, so it dissolved primarily in the small intestine. Because the current prediction system assumes that drug breakdown and absorption happen in the small intestine and not in the stomach or colon, the drugs chosen were good models for building a link between in vitro and in vivo.

3. Data on Canine Oral Absorption Collected:

The value of Fa in dogs was necessary to verify the soundness of the current system. As an alternative to Fa, we used the relative bioavailability of a solid dose form and an oral solution (rel. For medicines that are lipophilic, the rel. BA solid/solution is almost equivalent to the Fa of the solid dosage form (Fa, solid). The ratio of the area under the curve for solid dosages (AUC solid) to solution dosages (AUC solution), where AUC solid is the product of Fa, solid, Fg, solid, and Fh, solid, and AUC solution is the product of Fa, solution, Fg, solution, and Fh, solution, was used to compute the relative BA (subscript notation includes administration form). The letters Fg and Fh stand for the amount of dose-escaping metabolism done by the liver and the GI mucosa, respectively.

$$\text{rel.BA}_{\text{solid/solution}} = \text{AUC}_{\text{solid}}/\text{AUC}_{\text{solution}} * 100$$

$$= F_{\text{a, solid}} * F_{\text{g, solid}} * F_{\text{h, solid}} / F_{\text{a, solution}} * F_{\text{g, solution}} * F_{\text{h, solution}} * 100 \quad (10)$$

The relative bioavailability is defined as the ratio of Fa, solid to Fa, solution under the assumption that drug metabolism follows a linear kinetics following either delivery. Since lipophilic drugs are given as solutions and can be fully absorbed because they have a high permeability, Fa, solution is taken to be 1.

$$F_{\text{a, solid}} = \text{rel.BA}_{\text{solid/solution}} \quad (11)$$

4. Supplies and methods:

Materials: Danazol, griseofulvin, and cremophor EL were purchased from Sigma Chemical (St. Louis, MO, USA). Aprepitant was isolated from Emend® capsules (Merck & Co., Inc., Whitehouse Station, NJ, USA). Sodium taurocholate was purchased from Wako Pure Chemical Industries (Osaka, Japan). L--phosphatidylcholine was purchased from Nippon Oil and Fats

Corporation (Tokyo, Japan). Vitamin ETPGS was purchased from Eastman Chemical Company (Kingsport, TN, USA). PEG400 was purchased from Dai-Ichi Kogyo Seiyaku Co., Ltd. (Kyoto, Japan). The 0.45-m polyvinylidene fluoride membrane filters (25 mm Automation Certified Filter Unit) for dissolution tests and the 0.4-m polycarbonate filter plates (Multiscreen® Solubility Plate) for the solubility studies were purchased from Millipore Corporation (Billerica, MA, USA). The logD values and pKa values were calculated from the chemical structures of the drugs using Pallas 3.0 software (Comp Drug, Budapest, Hungary). Sigma Chemical was used to acquire the materials danazol, griseofulvin, and cremophor EL (St. Louis, MO, USA). Emend® pills included an appetent that was isolated (Merck & Co., Inc., Whitehouse Station, NJ, USA). Wako Pure Chemical Industries sells sodium taurocholate (Osaka, Japan). The Nippon Oil and Fats Corporation was paid for the L-phosphatidylcholine (Tokyo, Japan). From Eastman Chemical Company, vitamin ETPGS was purchased (Kingsport, TN, USA). The supplier of PEG400 was Dai-Ichi Kogyo Seiyaku Co., Ltd. (Kyoto, Japan). Both the 0.4-m polycarbonate filter plates (Multiscreen® Solubility Plate) and the 0.45-m polyvinylidene fluoride membrane filters (25 mm Automation Certified Filter Unit) were acquired from Millipore Corporation (Billerica, MA, USA). The Pallas 3.0 software (Comp Drug, Budapest, Hungary) was used to determine the logD values and pKa values from the chemical structures of the medicines.

Creating Solid Drug Model Particles for *In Vitro* and *In Vivo* Studies:

By recrystallizing danazol (melting point (MP) of 224 °C) and griseofulvin (MP of 219 °C) in acetone and a combination of tetrahydrofuran and heptane, respectively, crystals were produced that were then filtered and vacuum-pumped dried. Using conventional sieves, sieve classification was used to generate sieve fractions of 100–150 m with a volume means diameter (VMD) of 229 m for danazol and 118 m for griseofulvin (Tokyo Screen Co., Ltd., Tokyo, Japan).

Table 3. Drug and its parameters

Drug name	Ionization property [a]	MW	LogD (6.5) [a]
Danazol	Neutral	337.5	4.0
Griseofulvin	Neutral	352.8	2.9
Aprepitant	Weak base ($_p$Ka=4.38)	534.4	5.3

"$_p$Ka and log D values were calculated using pallas 3.0 software".

Fluid energy milling was used to micronize the materials, producing a VMD of 5 m for danazol and 7 m for griseofulvin (one cycle at 600 MPa, Jetmill A-O JET, Seishin Enterprise Co., Ltd., Tokyo, Japan). Neither the differential scanning calorimetry nor powder X-ray diffraction revealed any changes in the crystal shape or crystallinity during the milling operation. In order to increase the medication's wettability prior to the in vitro and in vivo investigations, one part of the drug was combined with nine parts of lactose as an excipient. Appetent crystals (MP 252°C) were produced by recrystallizing in methanol and drying by vacuum pump after filtration. The result was a sieve

fraction of 100–150 m (VMD 26 m). Using a high-pressure emulsifier (Nanomizer System YSNM2000AR, Yoshida Kikai Co., Ltd., Aichi, Japan), appetent crystals were milled in water by high-pressure homogenization under two distinct settings before being lyophilized. The two milling conditions for the aprepitant crystals—ten cycles at 50 MPa and one hundred cycles at 100 MPa—produced VMDs of 5 and 2 m, respectively. The milling operation did not result in the formation of any additional crystal types or changes in crystallinity. Before the in vitro and in vivo tests, the aprepitant particles were mixed with 0.5 percent methylcellulose in water at a concentration of 2 mg/ml Particle size analysis includes microscopic examination (VH-8000, VH-Z450, KEYENCE, Osaka, Japan) and image processing utilising software (Image-Pro Plus 5.1J, Media Cybernetics, MD, USA) to calculate the diameter of the ferret of more than 1,000 particles.

1. Tests for Miniscale Dissolution

As previously reported, dissolving tests were performed using the paddle method (50 rpm, 50 ml) with a 100-ml glass jar (42 mm diameter, 105 mm, Takao Manufacturing Co., Ltd., Kyoto, Japan) (6). In order to examine the impact of dissolving media on prediction, Fasted-state simulated canine intestinal fluid (Fassi dog) and phosphate buffer (PB) were utilized for the solubility study and the miniscale dissolution test. PB is a typical phosphate buffer (50 mM, pH 6.5) devoid of bile salt and lecithin, while Fassi-Do is a physiologically biorelevant medium comprising 5 mM sodium taurocholate and 1.25 mM lecithin in 29 mM of phosphate buffer (pH 6.5) [84, 85].

2. Study of Saturated Solubility Study

After 24 h of equilibration in media at 37°C, the saturated solubility of the medicines was assessed in a shaking incubator. The incubator held a 96-well polypropylene plate with 0.5 mL of media in each well. The wells were then filled with extra medication, and trials were run three times. The aqueous samples were run through a 0.4-m polycarbonate isopore membrane after 24 hours. To prevent medication loss from the sample as a result of adsorption, the first 0.2 mL was eliminated. The remaining sample was diluted with an equivalent volume of tetrahydrofuran along with an internal standard of 0.8 mg/ml of p-hydroxybenzoic acid dodecyl ester.

Analysis of the Miniscale Dissolution Test and Saturated Solubility Study by High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC; Waters 2795 separation module, Waters, Milford, MA, USA) was used to measure the sample concentrations for the dissolution test and solubility research using a UV detector (Waters 2487 dual UV/VI-S detector, Waters). On a C18 column (Cadenza CD-C18 3 m 3.0 mm, Intake Corporation, Kyoto, Japan), diluted samples (10l) were injected. Danazol, griseofulvin, and aprepitant were eluted with a mobile phase of water, acetonitrile, and trifluoroacetic acid at ratios (by volume) of 55:45:0.1, 50:50:0.1, and 30:70:0.1, respectively, and were quantified with variable UV detection at 284, 320, and 230 nm, respectively. The internal standard was eluted with a mobile phase of 5:95. The hydroxybenzoic acid n-dodecyl ester had a 270 nm detection wavelength. By doing a linear regression analysis using Microsoft Excel 2000, a standard curve was created for each drug, and linearity was discovered at a concentration range of roughly 0.02-80 g/ml on a log-log plot (correlation coefficient of $r^2 > 0.999$). (Microsoft, Redmond, WA, USA)

1. Beagle Dog In Vivo Oral Administration Study

Five male Beagle dogs (body weight: 12–15 kg) received oral doses of danazol, griseofulvin, and aprepitant. A week-long washout time was kept between each successive dose. Overnight and for eight hours after the dose, the dogs were fasted and had limited access to water. Dogs were given 4 mL/kg of water after oral treatment. The Institutional Animal Care and Use Committee of Chugai authorised all experimental protocols, and all operations involving the use of animals were carried out in compliance with the ethical standards for animal care that were established by the company. Each dose of 2 mg/kg of danazol solid particles in capsules was given orally to two different weight groups. Smaller particles were also given at a dose of 0.2 mg/kg to examine the dose effect on danazol absorption. Orally delivered at doses of 2 and 0.2 mg/kg was an aqueous danazol solution containing 10% dimethyl sulfoxide (DMSO) and 20% vitamin ETPGS as solubilizers. An intravenous dose of 0.0625 mg/kg of danazol solution (5 percent EtOH, 25% dimethylacetamide, and 70% saline) was administered. Two different sizes of griseofulvin solid particles were given orally at doses of 2 mg/kg each. The 0.2 mg/kg dose of the smaller griseofulvin particles was also given orally. Oral administration of 2 and 0.2 mg/kg doses of an aqueous griseofulvin solution in 10% DMSO and 90% PEG400. A mixture of Danazol Neutral 337.5 4.0 MW and griseofulvin ionisation property (6.5) Pallas 3.0 software was used to calculate the pKa and logD values for griseofulvin, which were determined to be neutral at 352.8 2.9 and a weak base (pKa=4.38) at 534.4 5.3. Predicting Oral Absorption Rate-Limiting Steps 2337 A 0.5 mg/kg intravenous dose of 5% EtOH, 25% dimethylacetamide, and 70% saline was administered Solubilizers of 10 percent DMSO and 5 percent cremophor EL were used in an aqueous solution of prepatent that was taken by mouth at a dose of 2 mg/kg along with three different sizes of aprepitant particles that were mixed in a solution of 0.5 percent methylcellulose. Another aprepitant solution was injected intravenously at a dose of 0.0625 mg/kg. It was made up of 5% EtOH, 25% dimethylacetamide, and 70% saline. Two hours before the aprepitant treatments, dogs received an intravenous dose of famotidine (10 mg/dog) to maintain a high stomach pH. At 0 (pre-dose), 0.25, 0.5, 1, 2, 4, 8, 24, and 48 h after oral delivery and 0 (pre-dose), 0.08, 0.25, 0.5, 1, 2, 4, 8, 24, and 48 h after intravenous treatment, blood samples (1 ml) were taken from a foreleg vein with a heparinized syringe. Plasma samples were made by spinning blood samples in a centrifuge. The plasma samples were then kept at 20 °C until they were needed.

Drug's physicochemical makeup Dissolution and drug formulation of factors influencing drug product performance *in vitro*:

Only eye drop solutions (drugs) account for more goods than the 10% used for ocular delivery of medicines in ointment bases, which is fairly common across all US-approved drug products. Clinically, ophthalmic ointments are a good dose form for treating localized eye illnesses such as glaucoma, infections, and inflammatory conditions. [86] Because (1) it is the outermost part of the eye, the organ responsible for vision, and fully performs three of the most important functions: optical (to transmit light), mechanical (blinks more than 14,000 times per day to moisten for lubrication and to remove foreign objects), and immunological (to provide defence against bacterial and viral infections), the ocular surface poses special challenges for formulation design

and performance assessment of ophthalmic drug products.[87] To avoid precorneal clearance, any medicine that is applied topically must be able to tolerate the strong shear of blinking. [88] In order to achieve its therapeutic effect while not interfering with the eye's normal function; (2) because the majority of ophthalmic drug products' sites-of-action are primarily restricted to the ocular surface and/or interior eye tissues, clinical performance assessment of topically applied ophthalmic drug products, including ointments, is dependent on the interaction of the physicochemical factors governing drug release and physiological elucidation. In addition to posing obstacles to the creation of more efficient drug products for ophthalmic use, these issues significantly raise the regulatory bar for drug approval, especially for generic ophthalmic drug products. To prove that generic pharmaceutical medicine goods are interchangeable and therapeutically equal to those of their associated innovators' products, it is essential to have available product quality measurements. When a company intends to switch excipients, methods, sites, etc., or in some circumstances, when a variant of the original dosage form is converted to a new dosage form, sameness analyses are also required. To establish the bioequivalence (BE) required by regulation, a list of in vivo and in vitro techniques has been supplied [89]. It is neither appropriate nor practical to establish the BE of topically applied ophthalmic medication products using a traditional *in vivo* BE study using pharmacokinetic endpoints like C max and AUC. Since local drug concentrations cannot be readily quantified, determining topical bioequivalence for locally acting medicines in the eye is more difficult. The Committee of Proprietary Medical Products (CPMP) of the European regulatory authorities wrote in their bioavailability and bioequivalence guidance that "the common systemic bioavailability approach cannot be applied for medicinal products not intended to be delivered into the general circulation" (EMA, 2000). With regard to the establishment of BE for such particular items, the US FDA made certain recommendations. [90]. To help sponsors comply with legal and regulatory requirements, the FDA has created draught guidance guidelines on locally acting topical medication products, including acyclovir topical ointments and cyclosporine ophthalmic emulsions [91]. According to the drug-specific guidance, the FDA typically handles the problem on a case-by-case basis. It is therefore vital to determine the fundamental scientific principles for the discovery of bioequivalence approaches for locally acting topical ophthalmic therapeutic products. Currently, where pharmacodynamic endpoint measurements or other suitable alternatives cannot be used to demonstrate BE between topical generic and reference listed drug (RLD) products, clinical endpoint trials must be conducted [92]. Because topical ophthalmic medication products are more variable than other in vivo methods for assessing bioequivalence, clinical endpoint bioequivalence studies with these products are time-and money-consuming [93]. As a result, it is common for these clinical endpoint bioequivalence studies to need the enrolment of several hundred participants in order to attain the required statistical power [94]. In light of the problems and limits of the in vivo method, the goal of this study is to come up with ways to compare things based on how they behave *in vitro*.

Materials and procedures

One substance is acyclovir USP

(> 99%) were obtained from RIA International LLC (East Hanover, NJ, USA). The following substances were purchased from Fischer Scientific, Norcross, Georgia: polyethylene glycol 400 (PEG-400), polyethylene glycol 3350 (PEG-3350), white petrolatum USP, heavy mineral oil USP, phosphate buffer saline (PBS, 10X), glacial acetic acid USP, citric acid anhydrous, sodium chloride, and boric acid. Lubrizol provided the lanolin alcohol (Carolan®) (Cleveland, Ohio). All materials, unless otherwise noted, were of an analytical grade.

Analysis using high-performance liquid chromatography (HPLC)

Acyclovir The HPLC system consists of a binary pump, an online degasser, a column heater, an autosampler, and photodiode array UV/detector Vi's on an Agilent 1260 Series (Agilent Technologies, Wilmington, DE, US). Che Station was used for data gathering and processing (Agilent Technologies). The current approach was validated in accordance with USP and ICH Q2R1 guidelines after being adapted from literature [95]. On a Sun Fire TM C18 column (5 m, 4.6 mm x 150 mm), the separation was accomplished quickly. Elution was isocratic at 1.2 mL/min (pH 2.8) and included 0.5 percent (v/v) acetic acid in the mobile phase.25°C was kept as the column temperature. The UV detection was done at 254 nm, and the injection volume was 5 mL in the concentration range of 0.04 to 10 g/mL, linearity was established ($r^2 = 0.999$).

Drug assay, homogeneity of content

In order to evaluate the homogeneity of the dosage form's medication content and physical features, the ointment was also tested. Three distinct units of packed aluminium tubes were chosen, briefly. In order to reveal the ointment medication product, the bottom seal of each unit was removed, and a vertical cut was made from the bottom to the top of the tube. A visual and/or tactile inspection of the product was performed to look for any changes. To check for drug content, a suitable quantity of precisely weighed ointment (90–110 mg) was taken from the top, middle, and bottom regions of the tube and transferred to a flask with 400 ml of solvent (pH 9.2 borate buffer). The contents were homogenized at 8,000 rpm for 15 min at 50°C, filtered through 0.45-micron PVDF, appropriately diluted, and then examined using HPLC to determine the concentration of acyclovir. To evaluate the uniformity of drug distribution in the ointment, the drug concentration in the top, middle, and bottom parts of the ointment were calculated. For each DoE formulation, the relative standard deviation of drug concentration was computed and employed in the DoE statistical analysis. With the exception of using just ointment from the middle region of the tube, the drug assay was carried out on three more different units of packed aluminium tubes using the same procedures as in the content uniformity test.

Analysis of particle size Samples of ointments:

They were put on a glass slide, and a cover slip was used to disperse them uniformly. Using an Olympus BX51 polarised light microscope, pictures were taken (Olympus America Inc, Melville, New York). For each sample, an average of three microscopy images (200x magnification; 500–2000 particles) were obtained. Using ImageJ software (National Institute of Health, Bethesda, MD), particles were automatically counted and measured in each image to determine the particle size and elongation. This information was then imported into Excel to produce statistical data (i.e., D10, D50, and D90) with a percentile function. An R5 lens (0.5-8.75 m)-equipped HALEOS laser

diffraction device was used to measure the particle size of acyclovir API powder. For each measurement, a controlled feeder (VIBRI/L) with dry dispersal (RODOS) attachments set at a main pressure of 1.0 bar distributed about 100 mg of dry sample at a feed rate of 50%. The triggering condition was a 0.2 percent optical concentration. The data was looked at with WINDOX 5 software and Fraunhofer theory (Symantec, Claustral Zeller Feld, Germany).

The characterisation of rheology Rheological:

A stress-controlled hybrid rheometer (DHR-3, TA Instruments, New Castle, Delaware, USA) with a step-Peltier stage (25°C) and a 25 mm sandblasted parallel plate was used to measure the behaviour of ointments. On the lower plate of each test, roughly 0.3 mL of the ointment sample was deposited before the upper plate was gradually lowered to the current trimming gap of 550 m. Following the removal of extra material, the geometry gap was set at 500 m. Each sample underwent the sequential application of the following techniques to identify its rheological behaviour: 2) Evaluation of yield stress using the strain sweep method (0.005-5 percent strain at 1 Hz), as determined by plotting storage modulus vs. oscillation stress and determining the onset point [96].3) 10 minutes of equilibration to allow the material to recover from the shear applied in the previous step (monitored at 0.005% strain and 1 Hz oscillation); 4) steady-state flow method to characterize the flow property (10-4 to 100 s-1) and obtain viscosity values at low (0.01 s-1), medium (1 s-1) and high (100 s-1) shear rates. For the temperature sweep investigation, the temperature was gradually raised from 16°C to 50°C at 1°C intervals. The sample was first allowed to acclimate for 20 minutes at 16°C (monitored at 0.01 percent strain at 1 rad/s). The oscillation test was conducted after a 30-s soak period (equilibration period) at each temperature (0.01 percent strain at an angular velocity of 1 rad/s). Every rheological study was carried out in triplicate, and the data was presented as Mean SD.

Compendial dissolution technique

For solid oral dosage formulations, dissolution/drug release testing was initially developed. It has been decades since these dosage formulations have undergone extensive dissolving testing. Pharmacopoeias [97, 98] currently describe four ways to test how well solid oral dosage forms dissolve: the paddle apparatus (Apparatus 1), the basket apparatus (Apparatus 2), the reciprocating cylinder (Apparatus 3), and the flow through cell (Apparatus 4).

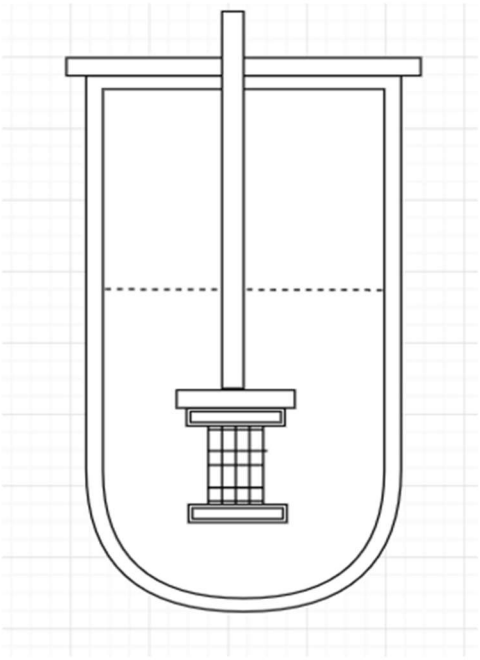

Fig-A

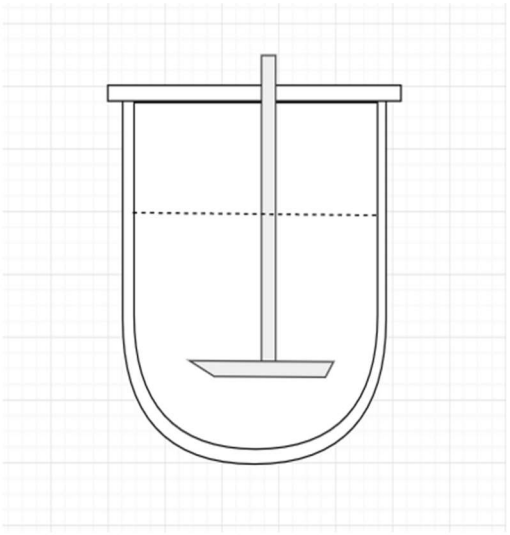

Fig-B

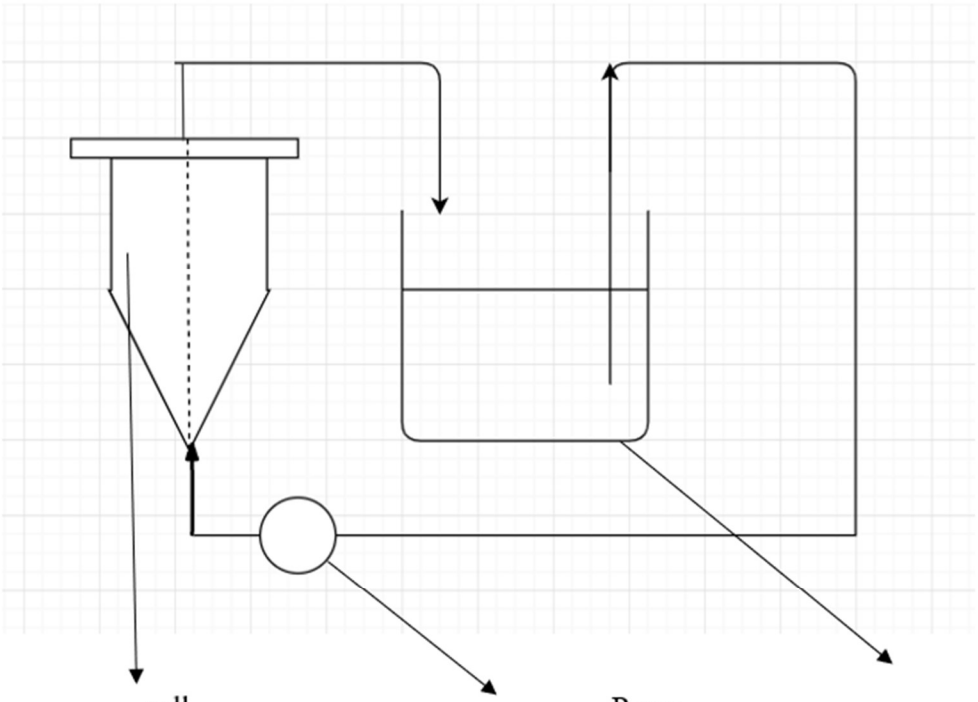

Fig-C

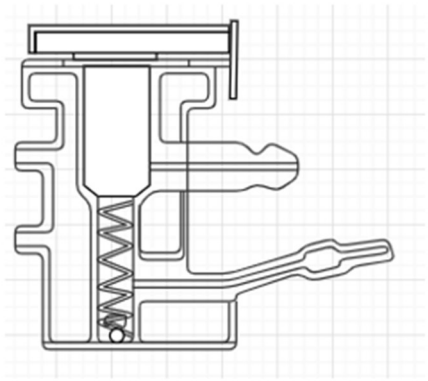

Fig-D

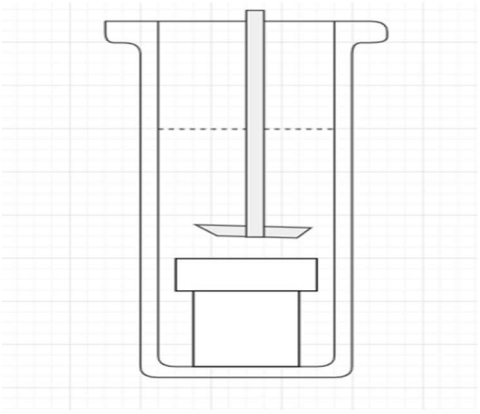

Fig-E

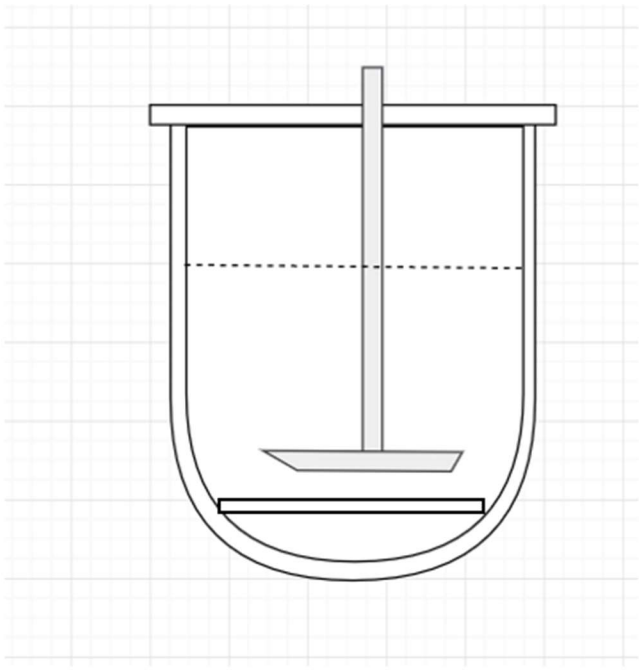

Fig-F

Figure captions Figure 1The chosen compendial dissolution/drug release apparatuses for mucosal delivery systems are shown schematically in the following order: (a) basket apparatus; (b) paddle apparatus; (c) flow through cell; (d) vertical diffusion cell; (e) immersion cell combined with paddle apparatus; and (f) paddle over disc apparatus. Reprinted with permission from Elsevier is Figure 1e. The use of in vitro release testing has grown over time to include a range of additional dosage forms, such as topical (dermal and mucosal) dosage forms. Recent publication of new compendial monographs on this issue reflects the growing interest in topical dosage form drug release testing (for example [11] and [10]). A review of recent compendial (USP and Petur.) monographs on assessing the drug release of mucosal dosage forms is provided. In addition to general compendial monographs, the FDA Dissolution Database and the USP Dissolution Database were investigated in order to gain a better understanding of the types of apparatuses and procedures currently used for drug release testing of mucosal drug delivery systems. Dissolution devices for mucosal dosage forms are listed in the monographs and techniques of the USP, Ph. Eur., and FDA.

Table 4 Apparatuses used/recommended for in vitro release testing of mucosal formulations according to monographs and methods published by USP, Ph. Eur. and FDA

FORMULATION TYPE	APPRATUS
Semisolid dosage form (creams, gels, ointment, lotions)	Vertical diffusion cell Immersion cell Flow through cell with adapter for semisolid dosage forms Paddle apparatus
oral, buccal, sublingual films	Paddle over disk appratus Basket apparatus (standard and small volume configuration)
Sublingual and buccal tablet	Paddle apparatus (standard and small volume configuration) Basket apparatus (standard and small volume configuration)
lozenges	Basket apparatus Paddle apparatus Reciprocating apparatus
Medicated chewing gums	Dissolution apparatus for chewing gums
Suppositories(hydrophilic)	Paddle apparatus Basket apparatus (standard or Palmieri type basket) Flow through cell
Suppositories(lipophilic)	Dual chamber flow through cell
Vaginal tablet and vaginal inserts	Paddle apparatus Basket apparatus
Vaginal rings	Incubator shaker
Mucosal suspensions	Paddle apparatus (standard or small volume configuration)
Mucosal emulsion	Paddle apparatus Vertical diffusion cell
Ocular systems	Reciprocating shaker
Periodontal systems	Tube rotator

The drug release testing is carried out utilizing the apparatuses created for oral formulations for several types of mucosal formulations (such as sub lingual and buccal tablets, vaginal tablets and inserts, mucosal suspensions and emulsions) [99,98]. However, specific dissolving apparatuses have been created and added to the pharmacopoeias for several mucosal dosage forms, such as semisolids, medicated chewing gum, and lipophilic suppositories [99,100,101,102]. These devices are

created, taking into account the unique physicochemical characteristics of the mucosal delivery systems and the circumstances at the mucosal administration locations. Three different types of diffusion cells, including vertical diffusion cells (also known as Franz diffusion cells), immersion cells, and flow through cells with an adapter for semisolid dosage forms, can be used to calculate the drug release rate in the case of semisolid dosage forms (creams, ointments, gels, and lotions) [100]. The medication diffuses into the dissolution medium over an inert, highly permeable support membrane in all three types of diffusion cells from the semisolid dosage form. The slope of the resulting line, which represents the release rate, is determined by the amount of medicine released after a brief lag time, where it becomes proportional to the square root of time. Chewing on medicated gum is necessary for the drug release to begin and continue. In the European Pharmacopoeia [101], two different kinds of chewing gum disintegration machines are specified. By mechanically kneading a piece of gum put in a tiny chamber intended to mimic the chewing motion, the release rate is ascertained. The USP [10] has also made reference to the procedure outlined in the European Pharmacopoeia, and it is listed in the FDA Database. A unique dual chamber flow through cell that is created expressly to prevent analytical interference from the lipophilic excipients is advised for use with lipophilic suppositories [99,102]. The chosen compendial dissolution/drug release devices, which are most frequently utilized for mucosal drug delivery systems, are schematically represented in Fig. 1 a–f. We respectfully suggest consulting pharmacopoeias for a more thorough overview of these devices. A few methods, like the reciprocating shaker, tube rotator, and incubator shaker, use non-compendial apparatuses in addition to compendial apparatuses, which are used in the majority of the methods listed in the USP and FDA databases (Table 1). The FDA Database also lists 14 medicinal items for mucosal use for which the producer is asked to create an in vitro release technique. These medications come in a variety of dose forms, including vaginal rings, ophthalmic suspensions, optic suspensions, rectal suppositories, and vaginal suppositories [103]. The devices typically employed for oral formulations are also utilized for mucosal administration systems, as was already mentioned. However, it should be noted that the volume of the dissolving media and the hydrodynamics offered by these apparatuses are frequently not consistent with the in vivo circumstances at mucosal administration locations. For instance, when performing dissolution tests using paddle or basket apparatuses (in conjunction with conventional compendial dissolution vessels), a large volume of dissolution medium (500–1000 ml) is used, which does not accurately represent the unique circumstances at the administration site of these dosage forms. However, even these amounts are far more than those available to mucosal drug delivery systems at their administration site. The small-volume devices can be utilized with volumes of dissolving media of 50–200 ml. In all advised drug delivery strategies for mucosal formulations, aqueous dissolving media are employed, occasionally with the addition of a surfactant. The pH of 6 of the dissolving mediums in some of the suggested approaches matches the physiological pH at the administration site. Selection of the dissolution medium is based on different factors for other therapeutic products, such as the drug substance's pH solubility profile [103,104]. Pharmacopoeias offer general advice on developing dissolution methods (selection of apparatus, dissolve medium, agitation, etc.),

validating those methods, automating those processes, and managing and interpreting data [105,106]. Although the solid oral dosage forms are the main focus of these comprehensive chapters, many of the ideas are transferable to other dosage forms and administration methods.

REFERENCE

1. THOMAS VH, BHATTACHAR S, HITCHINGHAM L et al.: The road map to oral bioavailability: an industrial perspective. Expert Open. Drug Meta. Toxicology. (2006) 2 (4): 591 -608.

2. ABRAHAMSSON B, LENNERNAS H: Application of the biopharmaceutic classification system now and in the future. In: Drug Bioavailability, Estimation of Solubility, Permeability, Absorption, and Bioavailability. Waterweed HVD, Lanners H, Artursson P (Eds), Wiley-VCH, New York, USA (2005): 495 -531.

3. LIU G, FRANSSEN E, FITCH MI, WARNER E: Patient preferences for oral versus intravenous palliative chemotherapy. J. Clin. Oncol. (1997) 15 (1): 110 -115.

4. PFEIFFER P, MORTENSEN JP, BJERREGAARD B et al.: Patient preference for oral or intravenous chemotherapy: a randomised cross-over trial comparing capecitabine and Nordic fluorouracil/leucovorin in patients with colorectal cancer. Eur. J. Cancer (2006) 42 (16): 2738 -2743.

5. TOUTAIN PL, BOUSQUET-MELOU A: Bioavailability and its assessment. J. Vet. Pharmocol. Their. (2004) 27 (6): 455 -466.

6. HELLRIEGEL ET, BJORNSSON TD, HAUCK WW: Interpatient variability in bioavailability is related to the extent of absorption: implications for bioavailability and bioequivalence studies. Clin. Pharmocol There. (1996) 60 (6): 601 -607.

7. BAILEY DG, KREEFT JH, MUNOZ C, FREEMAN DJ, BEND JR: Grapefruit juice-felodipine interaction: effect of naringin and 6 ′ ,7 ′ -dihydroxy bergamottin in humans. Clin. pharmacal. there. (1998) 64 (3): 248 -256.

8. BARDELMEIJER HA, VAN TELLINGEN O, SCHELLENS JH, BEIJNEN JH: The oral route for the administration of cytotoxic drugs: strategies to increase the efficiency and consistency of drug delivery. Invest. N. Drugs (2000) 18 (3): 231 -241.

9. CAO X, GIBBS ST, FANG L et al.: Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model. Pharm. Res. (2006) 23 (8): 1675 -1686.

10. LIN JH: Species similarities and differences in pharmacokinetics. Drug metal. Dispose. (1995) 23 (10): 1008 -1021.

11. BAGGOT JD: Clinical pharmacokinetics in veterinary medicine. Clin. Pharmacokinetic. (1992) 22 (4): 254 -273.

12. MARTIGNONI M, GROOTHUIS GM, DE KANTER R: Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Open. Drug metal. Toxicology. (2006) 2 (6): 875 -894.

13. ROWLAND M, TOZER T: Clinical Pharmacokinetics. Lippincott Williams & Wilkins, Philadelphia, USA (1995).

14. KWON Y: Handbook of Essential Pharmacokinetics, Pharmacodynamics, and Drug Metabolism for Industrial Scientists. Kluwer Academic/Plenum Publishers, New York, USA (2001).
15. OBACH RS: Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half- life approach and nonspecific binding to microsomes. Drug metal. Dispose. (1999) 27 (11): 1350 -1359.
16. RILEY RJ, MCGINNITY DF, AUSTIN RP: A unified model for predicting human hepatic, metabolic clearance from in vitro intrinsic clearance data in hepatocytes and microsomes. Drug metal. Dispose. (2005) 33 (9): 1304 -1311.
17. GIBALDI M, PERRIER D: Pharmacokinetics. Marcel Dekker, Inc., New York, USA (1982).
18. CHIOU WL: New perspectives on the theory of permeability and resistance in the study of drug transport and absorption. J. Pharmacokinetic. BioPharma. (1996) 24 (4): 433 -442.
19. AVDEEF A: Physicochemical profiling (solubility, permeability and charge state). Cur. Top. Med. Chem. (2001) 1 (4): 277 -351.
20. SABNIS S: Factors influencing the bioavailability of peroral formulations of drugs for dogs. Vet. Res. Common. (1999) 23 (7): 425 -447.
21. POUTON CW: Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur. J. Pharm. Sci. (2006) 29 (3-4): 278 -287.
22. ZHOU H: Pharmacokinetic strategies in deciphering atypical drug absorption profiles. J. Clin. pharmocol. (2003) 43 (3): 211 -227.
23. KRAMER SD, WUNDERLI-ALLENSPACH H: Physicochemical properties in pharmacokinetic lead optimization. Formica (2001) 56 (1-2): 145 -148.
24. HORTER D, DRESSMAN JB: Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv. Drug Deliv. Rev. (2001) 46 (1-3): 75 -87.
25. DESESSO JM, JACOBSON CF: Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. Food Chem. Toxicology. (2001) 39 (3): 209 -228.
26. KARARLI TT: Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. BioPharma. Drug Dispose. (1995) 16 (5): 351 -380.
27. HEBEL R, STROMBERG MW: Anatomy and Embryology of the Laboratory Rat. BioMed Verlag, Worth sees, Germany (1986).
28. SNYDER WS, COOK MJ, NASSET ES, KARHAUSEN LR, HOWELLS GP, TIPTON IH: Report of the Task Group on Reference Man. Wiley, New York, USA (1975).
29. ROWLAND M, TOZER TN: Clinical Pharmacokinetics Concepts and Application. Lippincott Williams & Wilkins, Philadelphia, USA (1995).
30. SHARGEL L, YU A: Applied Biopharmaceutics and Pharmacokinetics. Appleton and Lange, Stamford, New York, USA (1999).
31. WINNE D: Unstirred layer thickness in perfused rat jejunum in vivo. Experiential (1976) 32 (10): 1278 -1279.

32. HAYTON WL: Rate-limiting barriers to intestinal drug absorption: a review. J. Pharmacokinetic. biopharma. (1980) 8 (4): 321 -334.
33. STROCCHI A, CORAZZA G, FURNE J: Measurements of the jejunal unstirred layer in normal subjects and patients with celiac disease (1996).
34. CHIOU WL: Effect of 'unstirred' water layer in the intestine on the rate and extent of absorption after oral administration. biopharma. Drug Dispose. (1994) 15 (8): 709 -717.
35. KIMURA T, HIGAKI K: Gastrointestinal transit and drug absorption. Biol. Pharm. Bull. (2002) 25 (2): 149 -164.
36. MARTINEZ M, AMIDON G, CLARKE L, JONES WW, MITRA A, RIVIERE J: Applying the biopharmaceutics classification system to veterinary pharmaceutical products. Part II. Physiological considerations. Adv. Drug Deliv. Rev. (2002) 54 (6): 825 -850.
37. GARANERO G, RAMANATHAN S, AMIDON G: Gastrointestinal dissolution and absorption of drugs. In: Drug Bioavailability: Estimation of Solubility, Permeability, Absorption and Bioavailability. Waterweed HVD, Lanners H, Arturs son P (Eds), Wiley-VCH, Zurich, Switzerland (2005): 191 -210. 38. LENNERNAS H, REGARDH CG: Regional gastrointestinal absorption of the β -blocker pafenolol in the rat and intestinal transit rate determined by movement of 14 C-polyethylene glycol (PEG) 4000. Pharm. Res. (1993) 10 (1): 130 -135.
39. SOERGEL KH: Flow measurements of test meals and fasting contents in human small intestine. Proceedings of the International Symposium on Motility of the Gastrointestinal Tract. Theme, Stuttgart, Germany (1971). 40. ENCK P, MERLIN V, ERCKENBRECHT JF, WIENBECK M: Stress effects on gastrointestinal transit in the rat. Gut (1989) 30 (4): 455 -459.
41. ENCK P, WIENBECK M: Repeated non-invasive measurement of gastrointestinal transit in rats. Physiol. Behave. (1989) 46 (4): 633 -637.
42. CHARMAN WN, PORTER CJ, MITHANI S, DRESSMAN JB: Physiochemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and ph. I J. Pharm. Sci. (1997) 86 (3): 269 -282.
43. HANANO M: Fat-soluble vitamins: Intestinal absorption. Nippon Rinsho (1993) 51 (4): 851 -857. 44. KIRILENKO VN, GREGORIADIS G: Fat soluble vitamins in liposomes: studies on incorporation efficiency and bile salt induced vesicle disintegration. J. Drug Target (1993) 1 (4): 361 -368.
45. CUMMINGS JH, MACFARLANE GT: Role of intestinal bacteria in nutrient metabolism. J. Parenter. Enteral. Nutr. (1997) 21 (6): 357 -365.
46. BLAUT M, SCHOEFER L, BRAUNE A: Transformation of flavonoids by intestinal microorganisms. Int. J. Vitamin. Nutr. Res. (2003) 73 (2): 79 -87.
47. LICHTENSTEIN AH: Intestinal cholesterol metabolism. Ann. Med. (1990) 22 (1): 49 -52.
48. BOWEY E, ADLERCREUTZ H, ROWLAND I: Metabolism of isoflavones and lignans by the gut microflora: a study in germ-free and human flora associated rats. Food Chem. Toxicology. (2003) 41 (5): 631 -636.
49. RUMNEY CJ, ROWLAND IR: In vivo and in vitro models of the human colonic flora. Crit. Rev. Food Sci. Nutr. (1992) 31 (4): 299 -331.

50. TREVASKIS NL, PORTER CJ, CHARMAN WN: The lymph lipid precursor pool is a key determinant of intestinal lymphatic drug transport. J. pharmocol. Exp. there. (2006) 316 (2): 881 -891.
51. TREVASKIS NL, PORTER CJ, CHARMAN WN: Bile increases intestinal lymphatic drug transport in the fasted rat. Pharm. Res. (2005) 22 (11): 1863 -1870.
52. CHEEMA M, PALIN KJ, DAVIS SS: Lipid vehicles for intestinal lymphatic drug absorption. J. Pharm. pharmocol. (1987) 39 (1): 55 -56.
53. EDWARDS GA, PORTER CJ, CALIPH SM, KHOO SM, CHARMAN WN: Animal models for the study of intestinal lymphatic drug transport. Adv. Drug Deliv. Rev. (2001) 50 (1-2): 45 -60.
54. LIPINSKI CA: Drug-like properties and the causes of poor solubility and poor permeability. J. pharmocol. Toxicology. Methods (2000) 44 (1): 235 -249. • • A thorough overview describing several discovery approaches for assessing solubility and permeability.
55. LIPINSKI CA: Computational alerts for potential absorption problems: profiles of clinically tested drugs. In: Tools for Oral Absorption. Part Two. Predicting Human Absorption. In: AAPS Meeting. AAPS, Miami, USA (1995).
56. LIPINSKI CA, LOMBARDO F, DOMINY BW, FEENEY PJ: Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. (2001) 46 (1-3): 3 -26. • • A detailed overview of experimental and computational approaches used in the estimation of solubility and permeability.
57. DARVAS F, KESERU G, PAPP A, DORMAN G, URGE L, KRAJCSI P: In silico and ex silico ADME approaches for drug discovery. Cur. Top. Med. Chem. (2002) 2 (12): 1287 -1304.
58. PALM K, LUTHMAN K, UNGELL AL, STRANDLUND G, ARTURSSON P: Correlation of drug absorption with molecular surface properties. J. Pharm. Sci. (1996) 85 (1): 32 -39.
59. PALM K, STENBERG P, LUTHMAN K, ARTURSSON P: Polar molecular surface properties predict the intestinal absorption of drugs in humans. Pharm. Res. (1997) 14 (5): 568 -571.
60. KELDER J, GROOTENHUIS PD, BAYADA DM, DELBRESSINE LP, PLOEMEN JP: Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs. Pharm. Res. (1999) 16 (10): 1514 -1519.
61. POND SM, TOZER TN: First-pass elimination Basic concepts and clinical consequences. Clin. Pharmacokinetic. (1984) 9 (1): 1 -25. • • A thorough overview of the principles of first pass effect and its clinical implications.
62. BOYES RN, SCOTT DB, JEBSON PJ, GODMAN MJ, JULIAN DG: Pharmacokinetics of lidocaine in man. Clin. pharmocol. there. (1971) 12 (1): 105 -116.
63. BRUNTON L, LAZO J, PARKER K: Goodman & Gilman's The Pharmacological Basis of Therapeutics. McGraw Hill, New York, USA (2006).
64. PAINE MF, SHEN DD, KUNZE KL et al: First-pass metabolism of midazolam by the human intestine. Clin. pharmocol. there. (1996) 60 (1): 14 -24.

65. LEHLE K, KIRCHNER G, SEWING KF, RUPPRECHT L, MERK J, PREUNER JG: Intestinal metabolism of cyclosporine A in human heart transplant recipients. Transplant. Proc. (1998) 30 (8): 4044
66. GONZALEZ FJ: The molecular biology of cytochrome P450s. pharmocol. Rev. (1988) 40 (4): 243 -288.
67. NELSON DR, KOYMANS L, KAMATAKI T et al.: P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics (1996) 6 (1): 1 -42.
68. PETERS W, KREMERS P: Cytochromes P-450 in the intestinal mucosa of man. Beachem. pharmocol. (1989) 38 (9): 1535 -1538.
69. SHIMADA T, YAMAZAKI H, MIMURA M, INUI Y, GUENGERICH FP: Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J. pharmocol. Exp. there. (1994) 270 (1): 414 -423. • A study that provides useful information on the abundance of the major CYP enzymes in Japanese and Caucasian subjects.
70. SMITH DA, VAN DE WATERBEEMD H, WALKER DK: Pharmacokinetics and Metabolism in Drug Design. Wiley-VCH, Amsterdam, The Netherlands (2006). • • An excellent reference on drug pharmacokinetics and metabolism.
71. RAEVSKY OA: Physicochemical descriptors in property-based drug design. Mini. Rev. Med. Chem. (2004) 4 (10): 1041 -1052.
72. R. Takano, K. Sugano, A. Higashida, Y. Hayashi, M. Machida, Y. Aso, and S. Yamashita. Oral absorption of poorly water-soluble drugs: computer simulation of fraction absorbed in humans from a miniscale dissolution test. Pharm. Res. 23:1144–1156 (2006). DOI 10.1007/s11095-006-0162-4.
73. A. Noyes, and W. R. Whitney. The rate of solution of solid substances in their own solutions. J. Am. Chem. Soc. 19:930–934 (1987). DOI 10.1021/ja02086a003
74. W. L. Hayton. Rate-limiting barriers to intestinal drug absorption: a review. J. Pharmacokinet. Biopharm. 8:321–334 (1980). DOI 10.1007/BF01059381.
75. J. H. Kou, D. Fleisher, and G. L. Amidon. Calculation of the aqueous diffusion layer resistance for absorption in a tube: application to intestinal membrane permeability determination. Pharm. Res. 8:298– 305 (1991). DOI 10.1023/A:1015829128646.
76. H. Lennernas. Human intestinal permeability. J. Pharm. Sci. 87:403–410 (1998). DOI 10.1021/js970332a.
77. M. D. Levitt, T. Aufderheide, C. A. Fetzer, J. H. Bond, and D. G. Levitt. Use of carbon monoxide to measure luminal stirring in the rat gut. J. Clin. Invest. 74:2056–2064 (1984). DOI 10.1172/ JCI111629.
78. D. Winne, H. Gorig, and U. Muller. Closed rat jejunal segment in situ: role of pre-epithelial diffusion resistance (unstirred layer) in the absorption process and model analysis. Naunyn. Schmiedebergs Arch. Pharmacol. 335:204–215 (1987). DOI 10.1007/ BF00177725.

79. D. Winne. Unstirred layer thickness in perfused rat jejunum in vivo. Experientia. 32:1278–1279 (1976). DOI 10.1007/ BF01953092.
80. K. Obata, K. Sugano, R. Saitoh, A. Higashida, Y. Nabuchi, M. Machida, and Y. Aso. Prediction of oral drug absorption in humans by theoretical passive absorption model. Int. J. Pharm. 293:183–192 (2005). DOI 10.1016/j.ijpharm.2005.01.005.
81. M. D. Levitt, J. K. Furne, A. Strocchi, B. W. Anderson, and D. G. Levitt. Physiological measurements of luminal stirring in the dog and human small bowel. J Clin. Invest. 86:1540–1547 (1990). DOI 10.1172/JCI114873.
82. J. B. Dressman. Comparison of canine and human gastrointestinal physiology. Pharm. Res. 3:123–131 (1986). DOI 10.1023/ A:1016353705970
83. T. T. Kararli. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm. Drug Dispos. 16:351–380 (1995). DOI 10.1002/ bdd.2510160502
84. A. Scholz, E. Kostewicz, B. Abrahamsson, and J. B. Dressman. Can the USP paddle method be used to represent in-vivo hydrodynamics? J. Pharm. Pharmacol. 55:443–451 (2003). DOI 10.1211/002235702946.
85. L. Kalantzi, E. Persson, B. Polentarutti, B. Abrahamsson, K. Goumas, J. B. Dressman, and C. Reppas. Canine intestinal contents vs. simulated media for the assessment of solubility of two weak bases in the human small intestinal contents. Pharm. Res. 23:1373–1381 (2006). DOI 10.1007/s11095-006-0207.
86.Høvding, G., 1989. A comparison between acyclovir and trifluorothymidine ophthalmic ointment in the treatment of epithelial dendritic keratitis. Acta Ophthalmologica 67, 51-54.
87. Doane, M.G., 1980. Interactions of eyelids and tears in corneal wetting and the dynamics of the normal human eyeblink. American journal of ophthalmology 89, 507-516.
88. Gaudana, R., Ananthula, H., Parenky, A., Mitra, A., 2010. Ocular Drug Delivery. AAPS J 12, 348- 360.
89. 21CFR320.1, Title 21: Food and Drugs: Part 320 - Bioavailability and Bioequivalence Requirements, Subpart A- General Provisions. Available at http://www.ecfr.gov/cgi-bin/textidx?SID=301bbb87cfab41036bc82f497f1aa9aa&node=pt21.5.320&rgn=div5#se21.5.320_121 (accessed 30.3.14).
90. FDA, 2010. Guidance for Industry Bioequivalence Recommendations for Specific Products. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072872.pdf (Accessed on March 30, 2014)
91. [FDA, 2012. Draft guidance on acyclovir. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM296733.pdf (accessed on May 19 2015).
[FDA, 2013. Draft guidance on cyclosporine. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM358114.pdf (accessed on March 30 2015).

92.21CFR320.1, Title 21: Food and Drugs: Part 320 - Bioavailability and Bioequivalence Requirements, Subpart A- General Provisions. Available at http://www.ecfr.gov/cgi-bin/textidx?SID=301bbb87cfab41036bc82f497f1aa9aa&node=pt21.5.320&rgn=div5#se21.5.320_121 (accessed 30.3.14).
[21CFR320.24, Title 21: Food and Drugs: Part 320 - Bioavailability and Bioequivalence Requirements, Subpart B—Procedures for Determining the Bioavailability or Bioequivalence of Drug Products. Available at http://www.ecfr.gov/cgi-bin/textidx?SID=301bbb87cfab41036bc82f497f1aa9aa&node=pt21.5.320&rgn=div5#se21.5.320_124 (accessed 30.3.2014)
93. Shah, V.P., Flynn, G.L., Yacobi, A., Maibach, H.I., Bon, C., Fleischer, N.M., Franz, T.J., Kaplan, S.A., Kawamoto, J., Lesko, L.J., Marty, J.P., Pershing, L.K., Schaefer, H., Sequeira, J.A., Shrivastava, S.P., Wilkin, J., Williams, R.L., 1998. Bioequivalence of topical dermatological dosage forms - Methods of evaluation of bioequivalence. Pharmaceutical research 15, 167-171.
94. Bhandari, M., Lochner, H., Tornetta, P., 2002. Effect of continuous versus dichotomous outcome variables on study power when sample sizes of orthopaedic randomized trials are small. Arch Orthop Traum Su 122, 96-98.
95. Parry, G.E., Dunn, P., Shah, V.P., Pershing, L.K., 1992. Acyclovir Bioavailability in Human Skin. J Invest Dermatol 98, 856-863.
96. [Pal, R., 1999. Yield stress and viscoelastic properties of high internal phase ratio emulsions. Colloid Polym Sci 277, 583-588.
97 Dissolution, in: United States Pharmacopeia USP 40/NF 35, The United States Pharmacopeial Convention, Rockville, USA, 2017: pp. 588–598.
98. 9.3. Dissolution test for solid dosage forms, in: European Pharmacopeia 9.0, European Directorate for the Quality of Medicines and HealthCare, Strasbourg, France, 2017: pp. 302– 309
99. 1004> Mucosal drug products – performance tests, in: United States Pharmacopeia USP 40/NF 35, The United States Pharmacopeial Convention, Rockville, USA, 2017: pp. 829–832.
100. Semisolid drug products – performance tests, in: United States Pharmacopeia USP 40/NF 35, The United States Pharmacopeial Convention, Rockville, USA, 2017: pp. 2055–2067
101. 2.9.25. Dissolution test for medicated chewing gums, in: European. Pharmacopeia 9.0, European Directorate for the Quality of Medicines and HealthCare, Strasbourg, France, 2017: pp. 340–344.
102. 2.9.42. Dissolution test for lipophilic solid dosage forms, in: European. Pharmacopeia 9.0, European Directorate for the Quality of Medicines and HealthCare, Strasbourg, France, 2017: p. 377
103.FDA DissolutionMethodsDatabase, https://www.accessdata.fda.gov/scripts/cder/dissolution/index.cfm (accessed March 17, 2017)
104.USP Dissolution Methods Database, http://www.usp.org/usp-nf/overview/compendialtools/usp-dissolution-methods-data
105. The dissolution procedure: development and validation, in: United States Pharmacopeia USP 40/NF 35, The United States Pharmacopeial Convention, Rockville, USA, 2017: pp. 1296–1316.

106. 5.17.1. Recommendations on dissolution testing, in: European. Pharmacopeia 9.0, European Directorate for the Quality of Medicines and HealthCare, Strasbourg, France, 2017: pp. 761– 763.