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Review Article

Theme: Pharmacokinetics, Biopharmaceutics and Bioequivalence: History and Perspectives
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Application of Method Suitability for Drug Permeability Classification

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Abstract. Experimental models of permeability in animals, excised tissues, cell monolayers, and artificial membranes are important during drug discovery and development as permeability is one of several factors affecting the intestinal absorption of oral drug products. The utility of these models is demonstrated by their ability to predict a drug's *in vivo* intestinal absorption. Within the various permeability models, there are differences in the performance of the assays, along with variability in animal species, tissue sources, and cell types, resulting in a variety of experimental permeability values for the same drug among laboratories. This has led to a need for assay standardization within laboratories to ensure applicability in the drug development process. Method suitability provides a generalized approach to standardize and validate a permeability model within a laboratory. First, assay methodology is optimized and validated for its various experimental parameters along with acceptance criteria for the assay. Second, the suitability of the model is demonstrated by a rank order relationship between experimental permeability values and human extent of absorption of known model compounds. Lastly, standard compounds are employed to classify a test drug's intestinal permeability and ensure assay reproducibility and quality. This review will provide examples of the different aspects method suitability for *in situ* (intestinal perfusions), *ex vivo* (everted intestinal sacs, diffusion chambers), and *in vitro* (cell monolayers, artificial membranes) experimental permeability models. Through assay standardization, reference standards, and acceptance criteria, method suitability assures the dependability of experimental data to predict a drug's intestinal permeability during discovery, development, and regulatory application.

KEY WORDS: artificial membranes; drug permeability; *ex vivo* perfusion; *in situ* perfusion; *in vitro* cell monolayers; method suitability.

INTRODUCTION

The physicochemical properties of a drug substance and its product, the physiological functions of gastrointestinal tract, and the biochemical and physical properties of the epithelial barrier all influence the complex process of intestinal drug absorption. Since oral delivery is the preferred route of administration, successful therapy requires sufficient intestinal absorption to ensure that the drug is available at its intended target site. Good oral bioavailability takes place when the drug has maximal permeability and solubility at the site of absorption. Consequently, *in vivo* extent of absorption (f_a) can be predicted based on solubility and permeability measurements (1). The fundamental relationship between the rate of drug absorption measured as a permeability coefficient and extent of absorption has led to the use of experimental models as a surrogate for predicting the absorption of oral drug products (2,3).

Permeability models can assist in the drug candidate selection for *in vivo* clinical studies at early stages in drug discovery and development, along with submission of regulatory applications (4–7). They serve as tools in the decision-making process to prevent the loss of drug candidates in later, more costly, clinical phases due to poor pharmacokinetics (8). Since drug absorption is an important selection criterion in drug discovery and development, there is a need for reliable and appropriate screening methods to assess intestinal permeability (4–6).

The dependability of an experimental permeability model is revealed by its ability to accurately predict a drug's *in vivo* intestinal absorption. Experimental conditions need to be first optimized and controlled for the physiological environment that drugs encounter in the intestinal tract to obtain satisfactory *in vitro–in vivo* correlations (IVIVC) (8–12). To make the models useful in drug discovery and development, there is a need to establish a correlation between experimental and *in vivo* absorption with standardized methods for the quantification of permeability data (9,13). Standardization of the assays reduces intra-laboratory variations in permeability results and thus improves the predictive potential of the assay. The validity of the assays is reflected in their ability to predict the behavior of a drug at the *in vivo* intestinal barrier (14).

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METHOD SUITABILITY

Different experimental effective (P_{eff}) or apparent (P_{app}) permeability values for the same drug between laboratories are the result of differences in the performance of *in situ*, *ex vivo*, and *in vitro* permeability assays, along with variability in animal species, tissue sources, and cell types (15–20). Numerous factors influence the performance of the permeability models from the choice of animal species, tissue source, and cell line, how the permeation experiment is conducted within a laboratory, and how the data are analyzed (7,21,22). Method suitability provides a practical and generalized approach to standardize and validate a permeability model within a laboratory. It also accounts for intra- and inter-laboratory variability, allows for improvements in technology, and is applicable to a variety of tissues, cell lines, and membranes (23,24).

Method suitability provides a framework to utilize different permeability models and protocols involving human studies, intact animals, intestinal tissue, or epithelial cells (23–25). There are three stages which compromise method suitability (Table I), including method development (optimization, standardization), demonstrating assay suitability (IVIVC), and permeability classification of new drugs (23). The assay is first optimized and standardized for the parameters that influence its experimental outcome to increase predictivity and throughput (26–29). Additionally, the assay can be characterized for the presence of functional active transporters (*e.g.*, amino acids, di/tripeptides, monocarboxylic acids, organic anions and cations) and drug efflux mechanisms. Acceptance criteria are defined for selected standard compounds and measurements (*e.g.*, viability, resistance, integrity). These criteria are used to assess the permeability assay's functionality (29).

Employing an optimized assay protocol, a rank order relationship is established between P_{eff} or P_{app} permeability values and the extent of intestinal absorption in humans with a sufficient number of passively absorbed model drugs that are not subject to active or efflux transport (20,25) (Fig. 1). This relationship should clearly differentiate between high (HP, $f_a \geq 90\%$) and low (LP, $f_a < 90\%$) permeability drug substances such as that according to the Biopharmaceutics Classification System (BCS) (25) (Fig. 1). Model drugs, evaluated at clinically relevant concentrations (27,28), should ideally represent an extent of absorption range of <50%, 50–89%, and ≥90%. Even though investigators have utilized a wide variety of drugs to demonstrate assay suitability, many include similar HP (*e.g.*, antipyrine, ketoprofen, naproxen, metoprolol, propranolol) and LP (*e.g.*, atenolol, ranitidine, hydrochlorothiazide) model drugs in their experiments. An evenly distributed data set according to absorption improves permeability predictions since bias toward completely absorbed model compounds in the assay tend to predict HP well but lack precision to predict LP drugs (5). A highly permeable internal standard (HP-IS), with permeability in close proximity to the high/low class boundary, is selected from the model drugs (Fig. 1) (30).

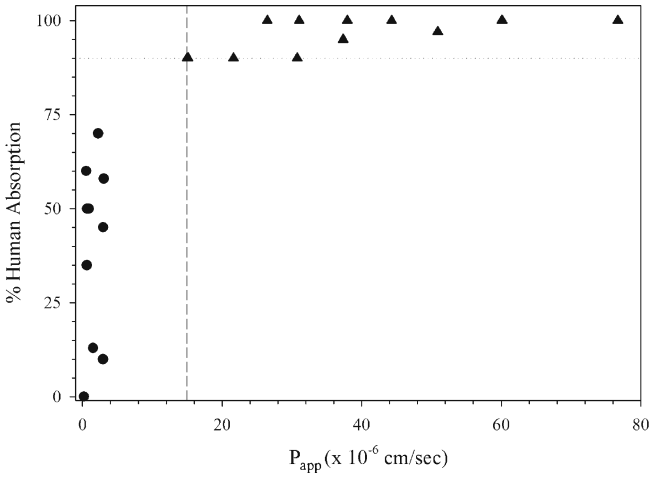

Fig. 1. Demonstration of method suitability from a 21-day Caco-2 assay in 12-well format (30). *Triangle* HP drugs, *Circle* LP drugs, *Square* HP-IS, *Dashed line* LP/HP boundary, *Dotted line* 90% absorption

Employing the same assay protocol to demonstrate assay suitability, test drugs are classified as HP or LP. Standard compounds (reference, internal, marker) are used to monitor intra-laboratory variation, active and efflux transporters, and barrier integrity in the experimental assays (31,32). The standards are also used to establish permeability class membership, assure reproducibility, and enhance the rank order of a drug series (24,26,27). The routine use of standard compounds generates acceptance criteria which can be monitored on a regular basis (4,10,14,26,33–36). Furthermore, repeated investigation of the transport of standards at regular time intervals facilitates intra- and inter-laboratory comparisons (26,37).

The reference standards should be known LP and HP drugs, along with substrates for active transporters and/or efflux mechanisms to monitor intra-laboratory variability (25). Internal standards are used to classify a test drug as low or high permeability. The flux of a low or zero permeability paracellular marker molecule (*e.g.*, mannitol, polyethylene glycol (PEG), dextran, inulin, Lucifer yellow) is used in each study to provide evidence for tissue or cell layer integrity. These markers demonstrate the presence of an intact physical barrier for drug transport (38,39). The permeability results for standard compounds (reference, internal, marker) should not differ considerably over multiple experiments, including those that demonstrated assay suitability. Along with each reference compounds, the laboratory sets in-house specifications and acceptance criteria for each assay

Table I. Method Suitability

Method development	• Establish assay protocol • Optimize and standardize assay parameters • Set acceptance criteria
Assay suitability	• Rank order relationship between experimental permeability values and human intestinal absorption • Define HP-IS
Permeability classification	• HP-IS to classify new compound • Reference standard to demonstrate assay reproducibility • Molecular markers for tissue, cell or membrane integrity

Table II. Advantages and Limitation of Permeability Assays

In situ perfusion	
Advantages	Closest to *in vivo* anatomy; retains blood flow and innervation; assay requires surgery and anesthesia; low throughput
Limitations	Animal usage; not a screening tool
Ex vivo tissue diffusion	
Advantages	Retains gut architecture; regional differences; human or animal tissue; mechanistic and directional transport
Limitations	Limited tissue viability; suboptimal stirring conditions
In vitro cell monolayers	
Advantages	Transcellular and paracellular passive diffusion, active transport, and efflux; mechanistic studies; human or animal cell lines; can be automated
Limitations	Inter-laboratory variability due to culture conditions; labor-intensive; low expression of transporters; lack of mucus layer
Artificial membranes	
Advantages	Relatively simple and high throughput; can be automated; tolerates wider pH ranges and higher solubilizer concentrations
Limitations	Transport dependent upon lipid composition and pH; membrane retention of lipophilic compounds; no active transport

(4,6,8,19,22)

system. Lastly, the HP-IS is utilized to facilitate the classification of a test drug substance. If the test drug's experimental P_{app} or P_{eff} value is equal to or greater than that of the HP-IS, it is classified as highly permeable (25,40).

Drug stability and solubility are other considerations in the permeability models (27). It is important that the drug is stable in the aqueous buffer solutions for the time, temperature, and pH conditions of the transport experiment to ensure that the P_{app} or P_{eff} results are not biased from drug loss due to instability. Another issue is that the drug is not lost due to adherence to the experimental apparatus or retained in the cells or tissues (27,41). Drug solubility can be limiting since the experiments are conducted in an aqueous buffer.

Method suitability is a process to optimize and validate permeability assays for drug classification (23). The use of acceptance criteria demonstrates the functionality of the assay, while reference standards ensure reproducibility and high-quality results. Such assays become tools for decision making in the discovery and early development of new drugs. The advantages of method suitability is that it accounts for inter-laboratory variability and differences in assay protocols, allows for the incorporation of improved technologies, and is applicable to *in situ*, *ex vivo*, and *in vitro* permeability assays (23,24,30).

PERMEABILITY ASSAYS

There is a variety of methods that have been developed to assess drug permeation across the gastrointestinal tract. The methods include *in situ* perfusion through isolated intestinal segments, *ex vivo* diffusion across tissues, and *in vitro* permeation through cell monolayers or artificial membranes. Each assay has it own advantages and limitations to be considered when developing models for permeability classification (Table II). Furthermore, due to inter-assay differences in the experimental P_{app} or P_{eff} values, cross-system permeability comparison ought to rely solely upon evaluations generated relative to specific standard compounds that have been well characterized across the model systems.

In Situ Perfusion

The *in situ* perfusion model allows for the measurement of drug permeability in the intact intestine by single-pass and recirculating methods (42–44) (Table III). For the single-pass (open loop) perfusion method, a section of intestine in an anesthetized animal is cannulated proximally and distally, rinsed with buffer solution, and then perfused with a drug solution. The amount of drug in the perfusate is measured at defined time points. The perfusion assays are normalized for inlet drug concentration, flow rate, and drug aqueous diffusion coefficient. Intestinal permeability (P_{eff}) is calculated from the difference between solute concentration entering and leaving the cannulated region. Falgerholm *et al.* (9) reported on a single-pass *in situ* perfusion study in rats with comparison to *in vivo* perfusion values in humans, finding that jejunal P_{eff} estimates for passively absorbed compounds correlated well and could be used to predict human *in vivo* absorption. Alternatively, carrier-mediated transport required scaling between models as there were differences in transport maximums and/or substrate specificity (9).

Kim *et al.* (20) demonstrated the suitability of a rat single-pass perfusion method to classify 20 drugs according to the BCS (Fig. 2a). In this study, the initial drug concentration was based on the highest dose strength (HDS) in 250 mL, and PEG 4000 was utilized as a non-absorbable marker to measure water flux (20). This rat single-pass perfusion method categorized the selected test drugs into the proper permeability class in comparison to human absorption with metoprolol at the HP/LP boundary (Fig. 2a) (20). Zakeri-Milani *et al.* (45) determined P_{eff} values in ligated jejunal segment in rats with a similar assay to classify compounds

Table III. *In Situ* Perfusion

Parameters to standardize	• Animal species and age • Fed/fast status of animal • Anesthesia regimen • Time to equilibrium • Intestinal region • Perfusion buffer composition, osmolarity and pH • Perfusion rate • Drug analysis and P_{eff} calculation
Acceptance criteria	• P_{eff} of non-absorbable marker • P_{eff} of HP marker • P_{eff} of active transport marker

(6,8,21,27,28)

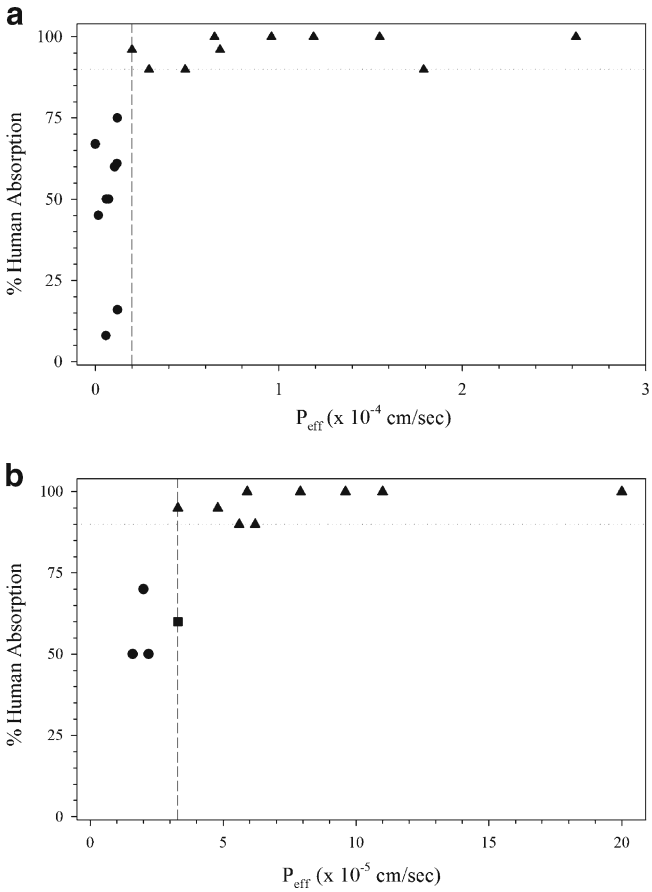

Fig. 2. Rat *in situ* perfusion assays from **a** Kim *et al.* (20) and **b** Zakeri-Milani *et al.* (45). *Triangle* HP drugs, *Circle* LP drugs, *Dashed line* LP/HP boundary, *Dotted line* 90% absorption, *Square* outlier drug

based upon permeability (Fig. 2b). Metoprolol was also at the HP/LP boundary in this smaller data set (45). Furosemide, a low permeability drug, had a P_{eff} value the same as metoprolol. It is possible that the efflux of furosemide was decreased in this system, increasing its absorption.

Ex Vivo Diffusion Chambers

In *ex vivo* diffusion chambers, a small section of the intestine is removed from an animal or human and then opened to form a flat epithelial sheet which is mounted between two chambers containing oxygenated buffer. Drug solution is added to the buffer in the donor chamber and its appearance is measured over time in the receiver chamber. The method allows for the measurement of drug transport across the tissue as a function of time in the absorptive (mucosal to serosal) and secretive (serosal to mucosal) directions. Flux, or apparent permeability (P_{app}), is defined as the rate of drug accumulation in the receiver chamber normalized for tissue surface area. Acceptance criteria for this assay can include electrical measures of barrier function (*e.g.*, potential difference, short circuit current, resistance), along with markers of passive and active transport and tissue viability (Table IV) (6,18,46).

Lennernäs (47) found that rat permeability in jejunal segments was comparable to human *in vivo* perfusion. There was a similar rank order for passively absorbed drugs in the two assays, although human *in vivo* P_{eff} was five to six times higher than rat *ex vivo* P_{app} due to differences in available intestinal surface area, experimental methods (*e.g.*, oxygenation), tissue viability, and stirring (47).

Ungell *et al.* (18) were able to classify compounds as HP or LP according to method suitability in stripped rat proximal jejunum, ileum, and colon tissue (Fig. 3). Tissue viability was monitored by potential difference and transsegmental electrical resistance as a quality control measure. Hydrophilic LP drugs had P_{app} values of $0.9–8.3 \times 10^{-6}$ and $11.4–100.3 \times 10^{-6}$ cm/s for HP hydrophobic drugs (18). Erythritol (f_a=90%) was at the HP/LP boundary for the three tissues, although the P_{app} for erythritol decreased from the jejunum to colon. Creatinine, raffinose, and foscarnet may be considered as outliers in the colon, but these were only slightly greater than the HP-IS. Creatinine was properly classified in the jejunum (18) and both foscarnet and raffinose were correctly classified in the same assay in the ileum and jejunum (18). Regional differences in drug permeability were observed as the P_{app} of LP drugs decreased down the intestinal tract (jejunum > ileum > colon), while it increased for HP drugs (jejunum < ileum < colon) (18).

Ex Vivo Gut Sacs

In the everted gut (or intestinal) sac model, a section of the intestine is removed from an anesthetized animal, flushed with buffer, and everted over a rod or tube. The intestine is divided into 2- to 4-cm sacs which are tied at each end, filled with oxygenated buffer, and placed in a container of well-mixed oxygenated buffer containing the test drug. After a specified time period, the amount of drug in the sac is measured and P_{app} is normalized based on sac protein content. Alternatively, the intestine is not everted and the drug solution is placed in the sac. The sac is then placed in a container with oxygenated buffer and the drug is measured over time from the container. The applicability of a non-everted rat intestinal sacs method was validated and demonstrated for 11 marketed compounds (46). The study showed a

Table IV. *Ex Vivo* Tissue Diffusion

Parameters to standardize	• Animal species and age • Fed/fast status of animal • Anesthesia regimen • Stripped or unstripped tissue • Intestinal region • Time to equilibrium • Diffusion buffer composition, osmolarity, and pH • Monitoring of viability and integrity • Oxygenation of buffer and mixing process • Sink conditions and sampling method • Drug analysis and P_{app} calculation
Acceptance criteria	• Measure of tissue viability/integrity • Potential difference, tissue resistance, and/or short circuit current • P_{app} of non-absorbable marker • P_{app} of HP and LP markers • P_{app} of active transport marker

(6,8,18,21,22,28,46)

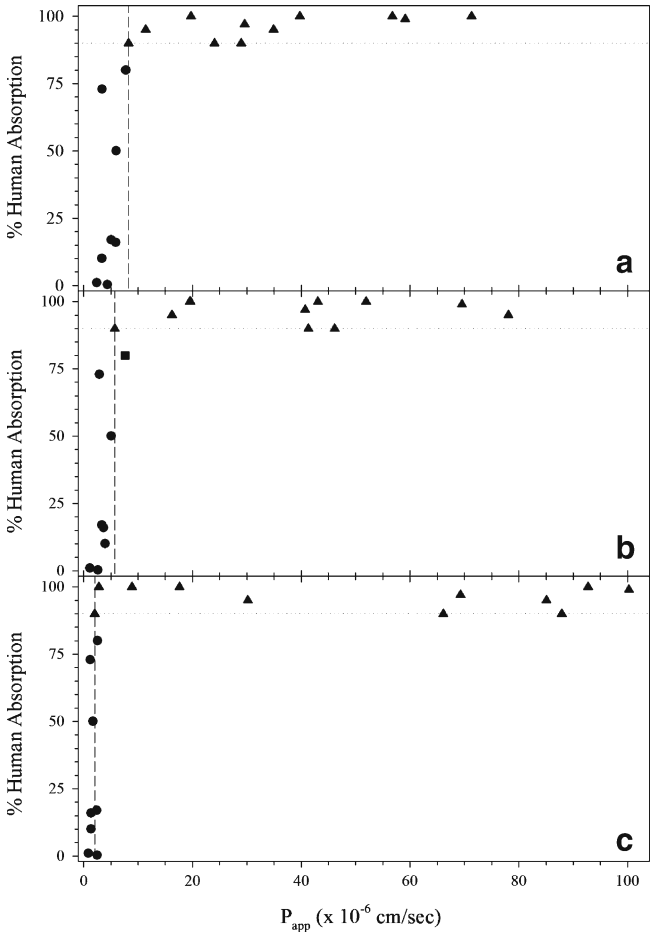

Fig. 3. Rat *ex vivo* diffusion assay from Ungell *et al.* (18) in the **a** jejunum, **b** ileum, and **c** colon. *Triangle* HP drugs, *Circle* LP drugs, *Square* outlier drugs, *Dashed line* LP/HP boundary, *Dotted line* 90% absorption

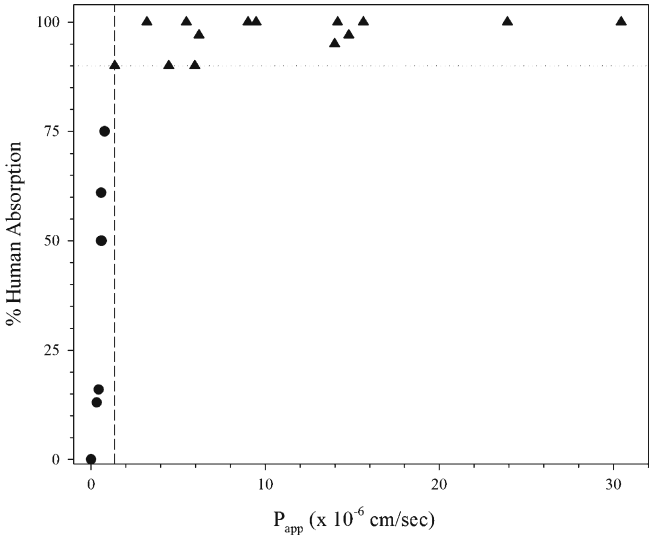

Fig. 4. Frog *ex vivo* everted gut sac assay from Trapani *et al.* (16). *Triangle* HP drugs, *Circle* LP drugs, *Dashed line* LP/HP boundary, *Dotted line* 90% absorption

good relationship between the permeability of the model drugs and their corresponding human f_a data. The mean permeability values of the series of drugs examined range from 1.08×10^{-6} cm/s for acyclovir to 15.66×10^{-6} cm/s for caffeine (46).

Trapani *et al.* (16) found a rank order relationship for BCS classification between human extent of absorption and P_{app} calculated from a non-everted frog intestinal sac assay (Fig. 4). The assay used a 5-cm segment from the intestinal tract in frog Ringer's solution at pH 8.2 without oxygenation for the assays which was agitated during the experiment. Metoprolol was used as a reference standard. In this assay with method suitability, HP drug had P_{app} values $>1.1\times10^{-6}$ cm/s and with LP drug P_{app} values $<1\times10^{-6}$ cm/s based on propranolol as the HP-IS (16).

In Vitro Cell Monolayers

Various cell lines are grown on a semi-porous filter to form monolayers that morphologically and functionally resemble the intestinal epithelium with barrier properties (Table V). The monolayer is placed in diffusion apparatus containing apical (AP) and basolateral (BL) chambers that represent the mucosal (lumen) and serosal (blood) surfaces of the intestine, respectively. Drugs are added to the AP or BL chamber and its appearance measured over time in the BL (absorption) or AP (efflux) chamber, respectively. P_{app} is measured as the rate of drug accumulation in the receiver chamber normalized for the filter area and chamber volumes. A number of researchers have found a correlation between Caco-2 and Madin–Darby canine kidney (MDCK) cell assay with human *in vivo* drug absorption (50–52).

Volpe *et al.* (30) validated a traditional 21-day, 12-well Caco-2 cell assay with over 20 model drugs with an initial drug concentration based on the HDS in 250 mL. Acceptance criteria were set for TEER values of the monolayers and metoprolol, FITC-dextran, and rhodamine 123 were standards to monitor high permeability, monolayer integrity, and efflux, respectively. There was good IVIVC between the drugs' P_{app} values and extent of human absorption, resulting in a Spearman rank correlation coefficient of 0.89 (Fig. 1) (30). Labetalol was determined to be the HP-IS which was

Table V. *In Vitro* Cell Monolayers

Parameters to standardize	• Cell clone and passage number • Culture media composition • Filter type, diameter, pore size • Initial seeding density • Feeding regimen • Monolayer age • Transport buffer composition and pH • Transport temperature and time • Co-solvent effects on cells • Sink conditions and stirring process • Sampling method • Drug analysis and P_{app} calculation
Acceptance criteria	• *M*easure of monolayer integrity • P_{app} of non-absorbable marker • P_{app} of HP and LP markers • Efflux of substrate compound

(6,14,19,21,26,48,49)

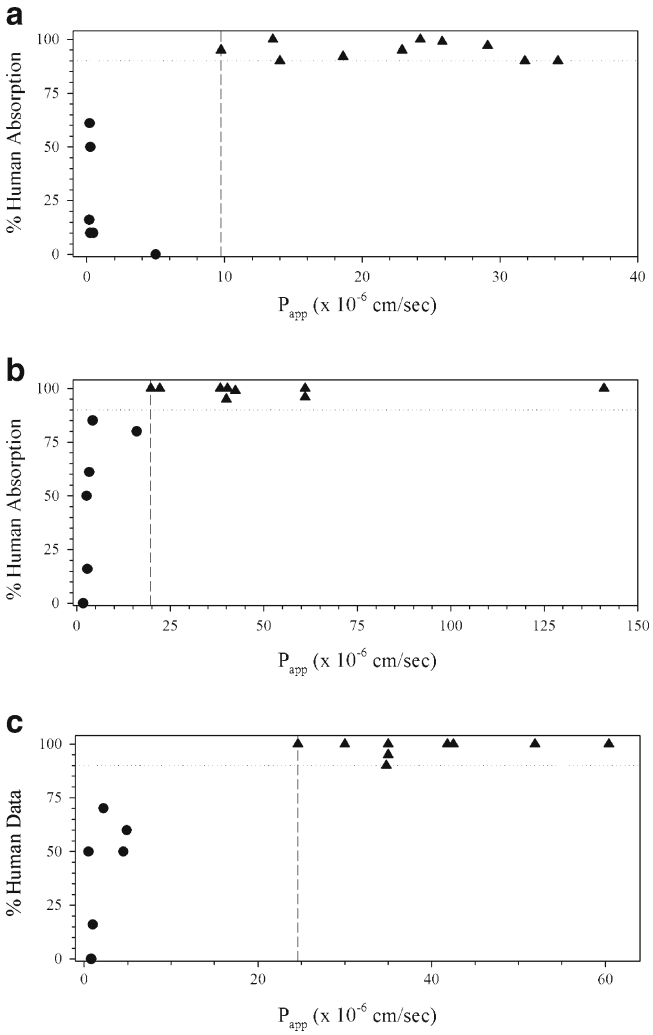

Fig. 5. Caco-2 cell monolayers from **a** Bock *et al.* (14) in a 12-well, 21-day assay, **b** Lentz *et al.* in a six-well, 4-day assay (53), and **c** Withington (54) in a 24-well, 3-day assay. *Triangle* HP drugs, *Circle* LP drugs, *Dashed line* LP/HP boundary, *Dotted line* 90% absorption

then employed to correctly classify four fluoroquinolone drugs in comparison to known human absorption (40). Ciprofloxacin was classified as a LP drug, while levofloxacin, lomefloxacin, and ofloxacin were classified as HP drugs (40).

Bock *et al.* (14) validated another 12-well assay Caco-2 assay for permeability classification. Validation studies included inter-day precision, transport direction, monolayer age, and cell passage number for classification in terms of intestinal permeability. Acceptance criteria were based on passage number, monolayer age, transport of LP (fluorescein) and HP (propranolol) standards, efflux ratio (rhodamine 123), and barrier tightness (TEER) (14). There was a good correlation between human $\%f_a$ and P_{app} for the test compounds with clonidine at the LP/HP boundary (Fig. 5a). Lentz *et al.* (53) developed a rapid, reduced serum assay with Caco-2 cells for application to the BCS. Caco-2 monolayers were grown in a six-well format with 2% iron-supplemented calf serum for 4 days. Cell morphology and differentiation were evaluated along with TEER to characterize the assay. The permeability of mannitol, metoprolol, and taurocholate were monitored along with the presence of efflux pumps (53). The assay classified drug as HP or LP with nicaripine at the HP/LP boundary (Fig. 5b).

Withington (54) compared a 21-day Caco-2 cell assay to an accelerated (3-day) BioCoat™ assay in a 24-well format according to the BCS. The assay was optimized for culture conditions, including cell harvest and cell seeding density, along with investigation of monolayer morphology and biochemistry. The P_{app} values correlated well with human absorption, with theophylline at the HP/LP boundary in this accelerated assay (Fig. 5c) (54). Alsenz and Haenel (55) developed a 7-day, 96-well Caco-2 assay which was optimized for monolayer age, seeding density, feeding conditions, sample analysis, and transport buffer pH effects. Transport was compared with the apical buffer pH at 7.4 or 6.5, while the basolateral buffer remained at pH 7.4. Ketoprofen and desipramine were the HP-IS in the pH 7.4 and 6.5 conditions, respectively (Fig. 6). Highly permeable, but actively transported, cimetidine and amoxicillin were classified as low permeability in both the pH 7.4 and 6.5 assays (55).

In a 24-well cell assay with MDCKII-MDR1 cells, Thiel-Demby *et al.* (56) demonstrated a rank order relationship for BCS permeability classification. The investigators standardized the method according to cell type, pH conditions, transport direction, incubation time, drug concentration, and reference standards. Labetalol was the HP reference

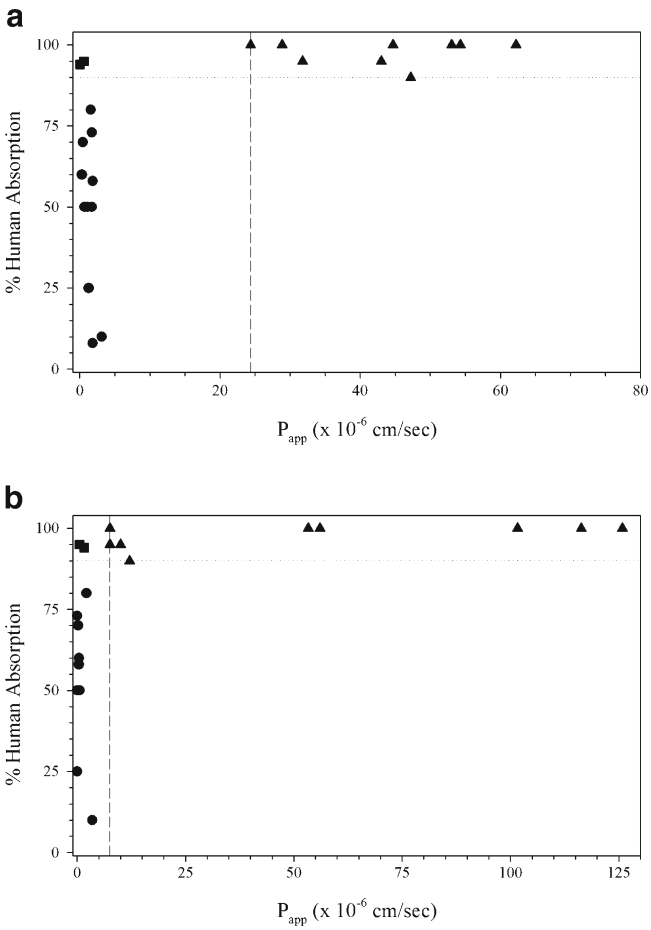

Fig. 6. Caco-2 cell monolayers in a 96-well, 7-day assay from Alsenz *et al.* (55) with AP buffer at **a** pH 7.4 or **b** pH 6.5. *Triangle* HP drugs, *Circle* LP drugs, *Square* outlier drug, *Dashed line* LP/HP boundary, *Dotted line* 90% absorption

standard, Lucifer yellow as a paracellular marker for monolayer integrity, and amprenavir as an efflux substrate. Metoprolol, pindolol, labetalol, and ranitidine were reference standards to show assay reproducibility (56). The assay classified the permeability of model drugs whether the AP buffer pH 6.5 or 7.4 with minoxidil as the HP-IS (Fig. 7) (56). Labetalol, a basic drug, was an outlier in the pH 6.5 assay, which was due to a large decrease in P_{app} at the lower pH as compared to pH 7.4. The actively transported amoxicillin had a lower than expected permeability in pH 7.4 conditions. While the assay was predictive in both pH conditions, it demonstrates how pH conditions during the transport experiment can affect P_{app} values.

In Vitro Artificial Membranes

The non-cellular parallel artificial membrane assay (PAMPA) is comprised of two aqueous buffer solution chambers separated by a porous filter that contains a lipid in an organic solvent. Drug transport is assessed much like the cell monolayer assays where a drug is added to the donor chamber and its appearance measured over time in the receiver chamber. The assay is usually performed in a 96-well microtiter format, allowing for high-throughput screening of passive permeability (Table VI). Kansy *et al.* (57) validated a higher throughput assay in a 96-well plate that categorized drugs as well absorbed (f_a=70–100%), having a flux of 25–100%, intermediate absorption (f_a=30–70%), with a flux of 5–25%, and low absorption (f_a=0–30%), having a flux of <5%. Wohnsland and Faller (58) developed another 96-well model with hexadecane on a polycarbonate filter that had a good correlation for over 30 compounds between *in vitro* permeability and human intestinal absorption.

Flaten *et al.* (59) validated a vesicle membrane PAMPA model where egg phosphatidylcholine liposomes were dis-

Table VI. Artificial Membranes

Parameters to standardize	• Lipid composition • Organic solvent in membrane • Filter type, diameter, and pore size • Transport buffer composition and pH • Transport temperature and time • Co-solvents • Stirring process and sink conditions • Sampling method • Drug analysis and P_{app} calculation
Acceptance criteria	• P_{app} of non-absorbable marker • P_{app} of HP and LP markers

(6,22)

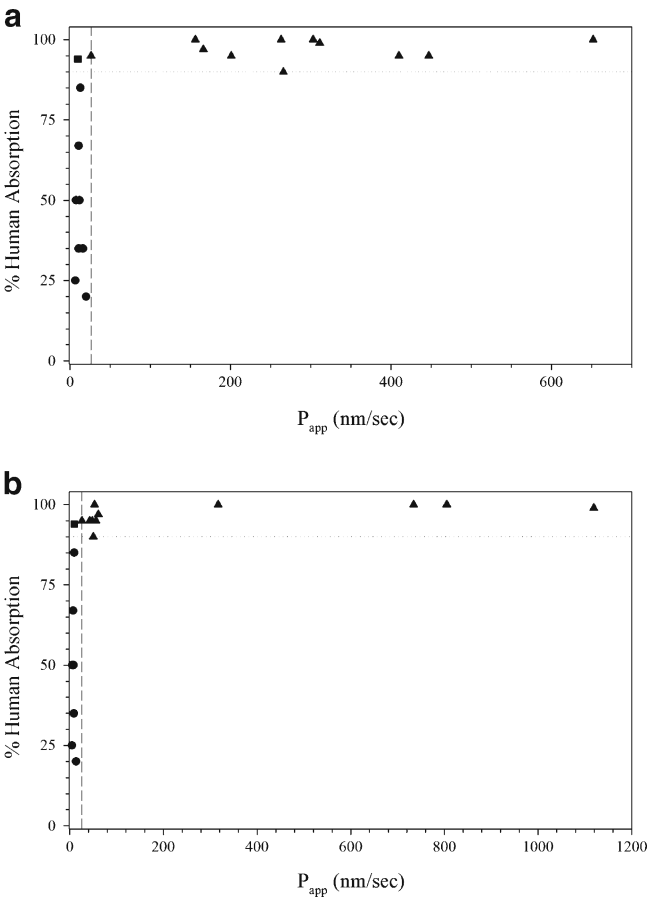

Fig. 7. MDCK-MDR1 cell monolayers in a 3- to 4-day, 24-well assay from Thiel-Demby *et al.* (56) at **a** pH 7.4 or **b** pH 5.5. *Triangle* HP drugs, *Circle* LP drugs, *Square* outlier drugs, *Dashed line* LP/HP boundary, *Dotted line* 90% absorption

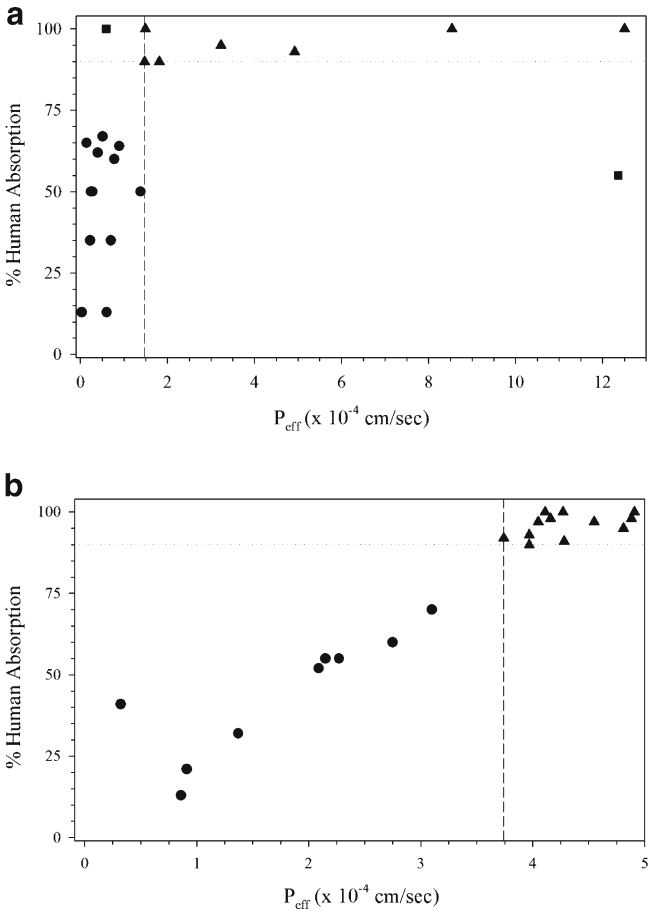

Fig. 8. Artificial membrane assays from **a** Flaten *et al.* (vesicle membrane) (59) and **b** Corti *et al.* (diffusion cell) (60). *Triangle* HP drugs, *Circle* LP drugs, *Square* outlier drugs, *Dashed line* LP/HP boundary, *Dotted line* 90% absorption

persed into the pores and on the surface of 24-well mixed cellulose ester filters. A tight barrier was demonstrated by calcein permeability and TEER measurements followed by model validation with over 30 compounds. Both timolol and acetylsalicylic acid were at the HP/LP boundary (Fig. 8a). The only compounds outside the classification were tranexamic acid and salicylic acid, due to analytical difficulties and active transport, respectively (59). Corti *et al*. (60) demonstrated the method suitability and general applicability of a dynamic diffusion cell artificial membrane method that had been optimized for the filter support (acetate–nitrate cellulose) and lipid phase composition (cholesterol and Lipoid® E80 in *n*-octanol) (61). Lucifer yellow was used as a paracellular marker in this model. There was a linear correlation for 20 drugs between P_{app} and fraction drug absorbed in humans (r^2=0.957) with pindolol as the HP-IS (Fig. 8b) (60).

CONCLUSION

Method suitability is a generalized approach to standardize and validate permeability methods within a laboratory to investigate and classify the intestinal absorption of drugs. It includes the optimization of model methodology to improve permeability predictions (Tables III, IV, V, and VI). Method suitability establishes a correlation between experimental permeability values and the extent of absorption in humans in an assay that is characterized by reference standards and acceptance criteria (23). Literature examples with *in situ* perfusion, *ex vivo* tissue chambers and gut sacs, cell monolayers, and *in vitro* artificial membranes demonstrate the applicability and feasibility of method suitability in devising intestinal drug permeability models (14,16,18,20,30,45,53–56,59,60). Method suitability, with its reliance on assay standardization and validation, reference standards, and acceptance criteria, enhances the consistency of experimental data to predict a drug's intestinal permeability during discovery, development, and regulatory application.

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