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Research Article

Effect of Complexes and Microemulsions on the Permeability of Drugs: Determination Using a New Biomimetic Artificial Membrane

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Abstract. The aim of this work was to predict the permeability of two model drugs, sulfamerazine (SMR) and indomethacin (INM), and to determine the effect on their apparent permeabilities by complexation with cyclodextrins and/or meglumine or incorporation in microemulsions. Permeation experiments were performed using two-chamber diffusion cells with a new composition of bio-mimetic membrane composed of 80% of Lipoid® S100 and 20% of cholesterol in *n*-octanol 10% *w*/*w* solution, at 37 ± 0.5°C and 14,000 rpm. The predictive capacity of the permeability of passive diffusion absorbed compounds was evaluated using 20 drug standards and showed an exponential correlation between the apparent permeability coefficients (P_{app}) and the fraction absorbed percentages in humans (Fa%), with an R^2 value of 0.67942 and a constant value of − 4.1 ± 0.8. SMR and INM were classified as Class II and I, respectively, according to the Biopharmaceutical Classification System. These drugs were complexed and incorporated in microemulsions. The Fa% from all the drug products was higher than 90%. SMR in the complexes and both drugs in microemulsions were classified as highly soluble. Thus, SMR and INM incorporated in these pharmaceutical products could be classified as Class I.

KEY WORDS: permeability; microemulsions; complexes; artificial membranes; passive diffusion absorbed compounds.

INTRODUCTION

Solubility, dissolution rate, and intestinal permeability are the major biopharmaceutical factors that affect the rate and extent of absorption of an oral drug product. The FDA has developed a guidance document that reflects the impact of these processes on drug product performance (1). The Biopharmaceutical Classification System (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. According to the BCS, drug substances are classified as follows:

Class I: High Solubility–High Permeability
Class II: Low Solubility–High Permeability
Class III: High Solubility–Low Permeability
Class IV: Low Solubility–Low Permeability

The solubility class boundary is based on the highest dose strength of an immediate-release product that is the subject of a biowaiver request. A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1–6.8 at 37 ± 1°C. The volume estimate of 250 ml is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with an 8 fluid ounce glass of water.

The pH-solubility profile of the test drug substance should be determined at 37 ± 1°C in aqueous media with a pH in the range of 1–6.8. The solubility class should be determined by calculating the volume of an aqueous medium sufficient to dissolve the highest strength in the pH range of 1–6.8. A drug substance should be classified as highly soluble when the highest strength is soluble in ≤ 250 ml of aqueous media over the pH range of 1–6.8.

The permeability class boundary is based indirectly on the extent of absorption (fraction of dose absorbed, not systemic bioavailability (BA)) of a drug substance in humans and directly on measurements of the rate of mass transfer across human intestinal membrane. A drug substance is considered to be highly permeable when the systemic BA or the extent of absorption in humans is determined to be 85% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose. To demonstrate

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suitability of a permeability method intended for application of the BCS, a rank-order relationship between test permeability values and the extent of drug absorption data in human subjects should be established using a sufficient number of model drugs. For demonstration of suitability of a method, model drugs should represent a range of low (*e.g.*, < 50%), moderate (*e.g.*, 50–89%), and high (≥ 90%) absorption (1).

Intestinal drug absorption is essential for the therapeutic success of oral administered drugs, and the increasing discovery of new pharmaceutical ingredients has raised the need of reliable high-throughput screening methods for rapid evaluation and prediction of the biopharmaceutical properties of such molecules in early stages (2). Biological membranes mainly consist of three classes of amphipathic lipids: phospholipids, glycolipids, and steroids. The amount of each depends upon the type of cell, but, in general, phospholipids are the most abundant, being phosphatidyl choline an important component. The arrangement of hydrophilic polar heads and hydrophobic nonpolar tails of the lipid bilayer allows for passive diffusion of hydrophobic molecules (3). However, passive diffusion is driven by high drug concentration (and high drug activity) in the aqueous membrane exterior or in the aqueous drug vehicle (4).

Galenic formulations can optimize drug solubility or improve membrane permeability and, therefore, increase the bioavailability of the drug (5–8).

Previous works reported by our group demonstrated increased solubility of sulfamerazine (SMR) by binary (9) and ternary (10) complex formation with cyclodextrins (CD) and meglumine (MEG), and for SMR and indomethacin (INM) by the incorporation of the plain and complexed drugs in soy oil-based microemulsions (ME) (11,12). However, the permeability behavior of these drugs in the free forms or contained in drug products has not been well described.

Methods to assess absorption rely on *in situ* (7,13), *in vivo* (14–16), *in silico* (14–17), or *in vitro* models (18–20) used alone or in combination. Even though using *in vivo* models would predict better the intestinal absorption of drug molecules, it would not be able to satisfy industrial production requirements in terms of mechanical stability and productivity (3). As an *in vitro* method, Caco-2 cell monolayers are widely used to evaluate the intestinal absorption of compounds (21–23). Caco-2 cell monolayers have the advantage that they incorporate both passive transport (transcellular or paracellular route) and active transporters. In contrast, the method using Caco-2 cells is rather labor intensive and not easily applicable to high-throughput screening (24). The *in silico* techniques, based on pure calculations of some physicochemical properties of drug molecules to estimate their oral absorption potential, are very attractive, but their predictive power is at present unsatisfactory (2).

Since the majority of drugs are mainly absorbed through passive transport, the use of artificial membranes, which mimic passive diffusion, offers a potentially effective, high-throughput approach for the assessment of drug absorption potential (2). The parallel artificial membrane permeability assay (PAMPA) was developed for the rapid determination of passive membrane permeability of molecules (25,26). PAMPA consists of hydrophobic filters coated with lecithin in an organic solvent solution, and it is completely artificial without pores and active transporter systems (24,27). The technique has received considerable attention in the pharmaceutical industry since it offers a simple physicochemical measure of permeability for research compounds with low cost and high throughput (26,28). However, PAMPA has some disadvantages, such as the impossibility of maintaining sink conditions during permeation experiments due to the very small volume of the donor and receptor compartments (2). Among these models, the permeability assays using Franz diffusion cell systems are the most commonly used methods (18,27,29–31) that may constitute a simple, fast, and useful technique to predict the oral absorption of drugs with good reproducibility and low cost, if used together with a proper bio-mimetic artificial membrane. Moreover, the use of Franz cell allows the temperature to be controlled and the donor and receptor compartments to be higher in volume compared to PAMPA. It is also possible to continuously stir the donor and receptor solutions, allowing the reduction of the thickness of the water layer that exists adjacent to the membrane surface in unstirred conditions, which may act as a diffusion barrier for rapid drug penetration (32).

To evaluate the confidence of an *in vitro* oral absorption predictive method, an *in vivo–in vitro* correlation can be performed (2,28,33–35). For all these reasons, this method could turn into a routine technique for the control quality of drug products in terms of oral absorption and may also contribute to reduce and/or replace the use of animals in research.

Previously in our laboratory, an *in vitro* permeability assay was developed, using a modified lipid membrane, obtained by impregnating a cellulose ester support (0.22 μm pore size) with 10% (*w*/*v*) *n*-octanol solution containing a mixture of Lipoid 75. The predictability of the method was established by correlating the obtained apparent intestinal permeability coefficients (P_{app}) and the oral dose fraction absorbed in humans (Fa) of 16 drugs with different absorption properties. The P_{app} values correlated well with the absorption rates and the method showed high predictability and good reproducibility (35). However, this modified lipid membrane did not contain cholesterol in its formula, which presence is important to better mimic the composition of the artificial membrane.

For this reason, we aimed to evaluate a different membrane model to predict the permeability of drugs that are absorbed by a passive diffusion pathway. For this, 80% of Lipoid® S100 and 20% of cholesterol in an *n*-octanol solution impregnated on a cellulose ester membrane was mounted between a two-chamber side-by-side diffusion cell system, with good stirring and constant 37°C temperature. Furthermore, in order to evaluate the applicability and efficacy to predict the permeability of drugs that are absorbed by passive diffusion, the study was carried out with 20 drug compounds (Fig. 1a) of known percentage of fraction of dose absorbed in humans (Fa%) to find a correlation between the *in vitro* drug permeability coefficient (P_{app}) and the fraction absorbed determined *in vivo*, using the approach described above. Moreover, two model drugs, SMR and INM (Fig. 1b), were classified according to the BCS using the current method, and the effect on the apparent permeability by complexation with cyclodextrins and/or meglumine (Fig. 1c) or by the incorporation inside microemulsions as solubilizing strategies was determined.

MATERIALS AND METHODS

Materials

Antipyrine, atenolol, allopurinol, verapamil, ketoprofen, naproxen, furosemide, propranolol, chloramphenicol,

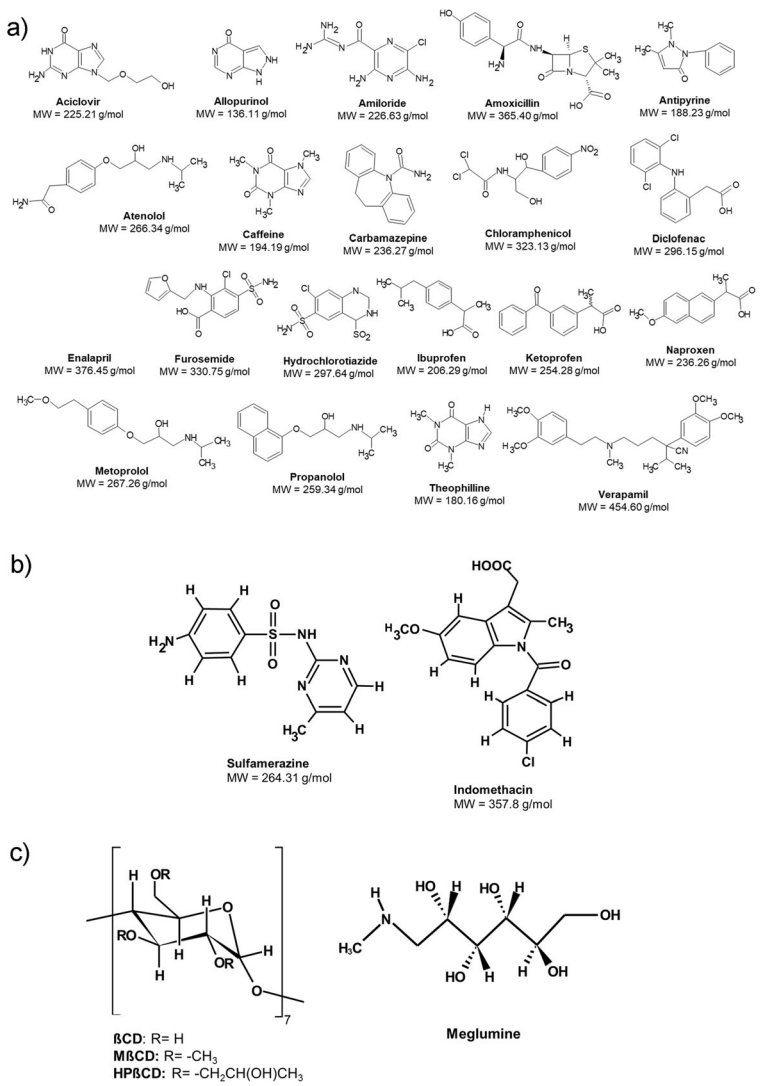

Fig. 1. Chemical structures of **a** standard drugs used for the membrane validation; **b** sulfamerazine and indomethacin; **c** cyclodextrins and meglumine

hydrochlorotiazide, ibuprofen, diclofenac, amiloride, enalapril, sulfamerazine, and indomethacin were purchased from Parafarma®, Argentina. Acyclovir was obtained from ELEA®, Argentina, and caffeine, theophylline, carbamazepine, and amoxicillin from Unifarma®, Argentina. Meglumine and metoprolol were purchased from Sigma Aldrich®, USA; βCD (MW = 1135), methyl-β-CD (MβCD) (MW = 1190), and hydroxypropyl-β-CD HPβCD (MW = 1325) were kindly supplied by Ferromet® (agent in Argentina of Roquette®). Microemulsions used in this work were chosen according to a previous study. Soya phosphatidylcholine was purchased from Degussa Texturants Systems Deutschland GmbH & Co. (Hamburg, Germany); polyoxyethylenglycerol (PEG)-40 hydrogenated castor oil (Eumulgin® HRE 40) (EU) (Sigma Aldrich®); soy oil (Liza®) (O). Sodium oleate (SO) was obtained from the stoichiometric reaction of oleic acid with 1 M NaOH solution for 30 min. The precipitate was filtered and washed with three portions of 100 ml of acetone. All the other materials and solvents were analytical grade or better. Purified water was obtained from Millipore Milli-Q Water Purification System.

Methods

In Vitro Permeability Studies

Permeation experiments were carried out using a two-chamber side-by-side diffusion cell system (active diffusion area of 1.44 cm^2). In order to mimic the lipophilic properties of biological membranes, 0.45 μm cellulose ester membrane (Gamafil S.A., Argentina) was used as support and impregnated by immersion for 30 min, with 80% of Lipoid® S100, whose main component (> 94%) is soybean phosphatidylcholine, and 20% of cholesterol (Sigma Aldrich®) dispersed in 2 ml of *n*-octanol in a 10% *w*/*w* solution (Table I). Then, the membrane was mounted between the donor and receptor compartments. Both phases were thermostated at 37.0 ± 0.5°C and continuously circulated on two sides of the diffusion cell. A phosphate buffer solution (pH 7.4; 0.01 M) (PBS) was used as the diffusion medium in the donor and receptor cells. The test formulation sample in a higher oral dose (32) (see Table II) in 250 ml equivalent quantity was suspended in PBS 7.4 and then loaded into the

Table I. Composition of the Artificial Membrane Used in the Permeability Studies

n-Octanol (%):	90		
Lipids (%):	10	Cholesterol (%):	20
		Lipoid S100 (%):	80

donor compartment. Solutions inside the cell compartments were mechanically stirred at 14,000 rpm (Auto Science®). Samples (2.0 ml) were withdrawn from the receiver compartments at fixed time intervals and replaced with an equal volume of previously warmed PBS. The drug concentration was measured spectrophotometrically at specific wavelengths using an Agilent Technologies® Cary 60 UV–Vis spectrometer (see Table II). All standard curves and dilutions were performed in PBS. All experiments were run in triplicate. To ensure that the experiment was maintained under sink conditions, the relation between the drug concentrations in the receptor and donor compartments at all the time points was monitored, considering appropriate a 0.1 or lower ratio. The apparent permeability coefficients (P_{app}) were calculated from the slope of the linear region of the permeation profile according to the following equation:

$$P_{app} = dQ/dt \times V/(A.C_0.60) \quad (1)$$

where dQ/dt is the amount of drug permeated per unit of time (mg/min), V is the volume of the receiver compartment, A is the surface area of the membrane, C_0 is the initial drug concentration in the donor compartment, and 60 is the conversion factor from minute into second.

Permeability Assay Validation

In order to demonstrate the applicability and efficacy of the applied technique to predict the permeability of drugs that are absorbed by passive diffusion, 20 drug compounds of known percentage of fraction of dose absorbed in humans (Fa%) were tested. The standard drugs were chosen in accordance with the FDA recommendation 1 to represent a wide range of absorption characteristics, which are absorbed by a passive diffusion pathway, and represent a diversity of structures, acid–base behavior, and solubilities to evaluate the predictability of the model (2,20,28,32,36,38–44). Predicted values of polar surface area and experimental solubilities were obtained from DrugBank (38). The correlation of the Fa% and the P_{app} values obtained with the developed method were carried out using the model proposed by Amidon *et al.* (33), utilizing the following equation:

$$Fa\% = \left(1-e^{A.P_{app}}\right) \times 100 \quad (2)$$

where A is the constant value to predict the Fa% from the P_{app} obtained by the permeability assay. *Classification of Model Drugs According to BCS.* In order to classify two model active ingredients according to the BCS, the permeability coefficients of the plain drugs were determined by the incorporation of oral doses in 250 ml equivalent quantities suspended in PBS 7.4 into the donor compartment. The initial concentrations of SMR and INM were held at 0.22 and 0.11 mg/ml, respectively, and the drug concentrations were measured spectrophotometrically at 240 and 267 nm, respectively. The P_{app} values of plain SMR and INM were determined from the slope of the dQ *vs.* t plot by using Eq. 1 to evaluate the permeability behavior of the drugs from the permeability limit for this method.

Considering that the BCS states that a drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1–6.8 at 37 ± 1°C (1), the solubility of the drugs in water, in PBS pH 2, and in PBS pH 8 was experimentally determined, and the needed volumes to solubilize the dose of each model drug were calculated in order to classify the drugs in terms of solubility.

Effect of Different Formulation Systems on the Apparent Permeability of the Drugs. In order to evaluate the effect of different formulations on the permeability of the carried drugs, the permeability coefficients of SMR and INM contained in binary and ternary complexes with CD and/or MEG and in microemulsions with and without the ligands were determined by suspending the same initial concentration of the pure drugs in PBS. The initial concentrations of SMR and INM were held at 0.22 and 0.11 mg/ml, respectively. The P_{app} values of the pharmaceutical formulations were determined from the slope of the dQ *vs.* t plot by using Eq. 1, and the same procedures to classify the plain drugs in terms of permeability and solubility were applied for the drug products.

RESULTS AND DISCUSSION

Permeability Assay Validation

The purpose of the present study was the development and validation of a fast, efficient, reproducible, and low-cost method to appropriately predict the permeability of structurally diverse drugs that are absorbed by a passive diffusion pathway. Permeation experiments were carried out using a two-chamber side-by-side diffusion cell system with a biomimetic artificial membrane composed of 80% of Lipoid® S100 and 20% of cholesterol dispersed in *n*-octanol 10% *w*/*w* solution impregnated on a cellulose ester filter membrane and mounted between the donor and receptor compartments, at 37.0 ± 0.5°C with continuous stirring at 14,000 rpm. In order to demonstrate the applicability and efficacy of the technique to predict the permeability of drugs that are absorbed by passive diffusion, the experiment was carried out with 20 drug compounds to obtain the P_{app} values from Eq. 1, and the fraction absorbed percentages in humans (Fa%) were collected from the literature and are summarized in Table II. These substances are suitable to evaluate the predictability of the model since they are absorbed by a passive diffusion pathway and represent a wide range of absorption characteristics, diversity of structures, acid–base behavior, and solubilities. In all experiments, the calculated ratios between the drug concentration in the receptor and donor compartments at all the time points were equal or lower than 0.1, indicating that the drugs were not retained in the membrane over the whole procedure, and the low standard deviation values

demonstrated the reproducibility of the proposed method for a diversity of compounds. The correlation between the Fa% and the P_{app} values obtained with the developed method was performed by the exponential adjustment of the plot presented in Fig. 2 utilizing Eq. 2, proposed by Amidon *et al.* (1). A correlation was observed for the 20 compounds revealed by an R^2 value of 0.64938 with a correlation scalar "a-value" of -5.09283 ± 0.87486.

It is important to highlight that metoprolol can be regarded as a permeability marker standard because it has demonstrated to have almost complete absorption (~100%) and its intestinal permeability is passive and does not involve carrier-mediated absorption (39). Nevertheless, in a previous work reported by our research group, metoprolol showed a pH-dependent permeability, which might be explained by the presence of a secondary amine moiety in the structure, which may be the reason why metoprolol P_{app} value was under the regression line (35).

Chloramphenicol, diclofenac, ibuprofen, naproxen, furosemide, and hydrochlorothiazide share the characteristic of presenting ionized percentages higher than 45% at the used conditions with negative charge (Table II). Also, the P_{app} of the drugs presenting a carboxylate acid moiety, with a Fa of 100%, increased in the following order: ketoprofen < diclofenac < naproxen < ibuprofen. For this reason, the permeability behavior seems to be determined by the degrees of polarization of the molecule since the drug with higher polar surface area value showed lower P_{app} values. Besides, furosemide and hydrochlorotiazide, whose molecules present similar polar surface area values, presented similar P_{app} values, even though they present different Fa%. Chloramphenicol is the drug with the highest polar surface area value and this may be the reason why the P_{app} value of this compound was under the regression line.

In the low permeability range (Fa < 85%), the rate of permeation was limited by the membrane permeation, as it was previously reported by Sugano *et al.* (26). However, in the high permeability range (Fa% ≥ 85%), an upper limit was bounded by the Fa = 100% line and a wide range of P_{app} values ($1.1–33 \times 10^{-6}$ cm/s) were recorded, indicating an aqueous permeability limitation since going through the aqueous boundary layer around the particles is now the rate-limiting step (33).

Classification of Model Drugs According to BCS

In order to classify two model drugs according to the BCS and using the developed method, the permeability coefficients of plain SMR and INM were determined from the slope of the dQ *versus* t plot by using Eq. 1. The P_{app} values were 5.9 ± 0.7 and $16.7 \pm 0.7 \times 10^{-6}$ cm/s for SMR and INM, respectively, and the predicted Fa% values from Eq. 2 were 100% in both cases (Table III). According to the high permeability limit for the current method established in "Permeability Assay Validation" section (Fa > 85%), both drugs were classified as high permeating compounds.

The permeability of the model drugs, SMR and INM, can be predicted using the developed method due to the characteristics of these drugs. Both are weak acids and their permeability behavior reflected the behavior of the standard weak acid drugs since SMR has a greater polar surface area (97.97 Å^2) (38) and presented a P_{app} value lower than INM (polar surface area = 68.53 Å^2) (38). These drugs have low aqueous solubility, so the limitation of their permeability is caused by aqueous solubility since they belong to Class II SCB. They are ionized with a negative charge at the experimental pH conditions, as the standard acid drugs.

On the other hand, considering that the BCS states that a drug substance is highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1–6.8 at 37 ± 1°C (1), the solubility of the drugs in water, in PBS pH 2 and in PBS pH 8, were experimentally determined and the needed volumes to solubilize the dose of each model drug were calculated and are presented in Table III. The PBS pH 2 and 8 were chosen since both are weak acid drugs that will present the higher neutral or ionized percentage at these pH values: p$Ka_{(SMR)}$ = 2.06; 6.9 (45); p$Ka_{(INM)}$ = 4.5 (44), thus, encountering the lower and higher aqueous solubility, respectively. As it can be observed, the needed volumes to solubilize INM in pH 2 and in water were higher than 250 ml (375,000 and 3000 ml, respectively). For this reason, INM was classified as a poorly soluble drug. On the other hand, the needed volume to solubilize SMR in water was 227.27 ml; therefore, this compound may be considered as highly soluble according to the BSC criteria. Since compounds presented high permeability, but INM low solubility while SMR high solubility, they were classified as Class II and Class I, respectively, according to the BCS.

Effect of Different Formulation Systems on the Apparent Permeability of the Drugs

In order to evaluate the effect of different formulations on the permeability of the carried drugs, the permeation experiments were performed for SMR and INM contained in binary and ternary complexes with cyclodextrins and/or meglumine and in microemulsions with and without cyclodextrins or meglumine by suspending the same initial concentration of the plain drugs (0.22 and 0.11 mg/ml for SMR and INM, respectively). The P_{app} value of each drug formulation was determined from the slope of the dQ *versus* t plot (Fig. 3) by using Eq. 1 and are collected in Table III. SMR and INM contained in the binary complexes and SMR contained in the ternary complexes presented Fa% values higher than 99%, suggesting that the permeability of the drugs was not affected by complexation, together with the benefit of this solubilizing strategy. However, a decrease in the P_{app} values was observed for most SMR complexes, except for those with HPβCD, while values of INM complexes remained without major variations.

This is due to formation of the drug–cyclodextrin inclusion complex that lowers the free drug concentration (and reduces the drug thermodynamic activity). Comparable results were previously reported with a formulation containing HPβCD by Shaker *et al.* (46). A similar behavior was observed for all the ME systems since they presented Fa% values that were higher than 90%, and decreased P_{app} values were recorded as well for all the formulations. The reasons for the decrease in permeability may be both a reduction in the size of the water channels and an increase in the microviscosity of the formulation, both producing a retention effect

Table II. Wave Length (nm), Higher Oral Dose (mg), Apparent Permeability Constants (P_{app}), Fraction Absorbed (Fa%), p*K*a, Ionized Percentage (%), Charge, and Permeability Classification According to the Biopharmaceutical Classification Systems (BCS) of the Selected Drug Stands for the Membrane Validation Studies

Drug	Wavelength (nm)	Higher oral dose (mg)[a]	P_{app} ($\times 10^{-6}$ cm/s)	%Fa[b]	p*K*a[c]	Ionized %[d]	Charge[e]	Polar surface area (Å^2)[f]	Permeability	Solubility (mg/ml)[g]
Acyclovir	254	500	0.082 ± 0.006	30 (36)	2.3;9.3	1.24	0	114.76	Low	2.50
Allopurinol	251	100	0.64 ± 0.05	90 (36)	7.83	27.09	0	65.85	High	1.22
Amiloride	361	1000	0.07 ± 0.01	50 (37)	3.29; 16.46	0.007	0	159.29	Low	3430
Amoxicillin	271	1000	0.04370 ± 0.00004	50 (36)	3.23; 7.43	51.73	+	132.96	Low	1.33×10^4
Antipyrine	244	400	1.6 ± 0.3	100 (2)	1.5	0.00	0	23.55	High	73.10
Atenolol	274	100	0.29 ± 0.05	52 (2,36)	9.6	99.37	+	84.58	Low	1.64×10^4
Caffeine	272	200	1.8 ± 0.2	100 (2,36)	0.6;14	0.00	0	58.44	High	722.00
Carbamazepine	276	4000	33 ± 3	100 (36)	1.5	0.00	0	46.33	High	21
Chloramphenicol	277	100	21.5 ± 0.8	90 (2)	7.49	44.84	−	115.38	High	2.37
Diclofenac	282	150	6.41 ± 1.03	100 (38)	4; − 2.1	100	−	49.33	High	569
Enalapril	271	40	0.64 ± 0.05	65 (37)	3.67; 5.2	0.627	+	95.94	Low	2500
Furosemide	277	55	0.90 ± 0.06	55 (35)	3.9	99.97	−	122.63	Low	1.69×10^4
Hydrochlorithiazide	276	2000	1.3 ± 0.1	70 (2)	7; 9.2	71.53	−	118.36	Low	7360.00
Ibuprofen	224	800	18.98 ± 0.06	100 (39)	4.85	100	−	37.3	High	15.90
Ketoprofen	262	75	1.1 ± 0.2	100 (2,36)	4.45	99.89	−	54.37	High	4.47
Metoprolol	225	450.00	2.67 ± 0.43	95 (2,36)	14.09; 9.67	99.47	+	50.72	High	5.19×10^4
Naproxen	291	500	23.7 ± 0.3	98 (2)	4.6	99.84	−	46.53	High	2.16×10^4
Propanolol	291	100	4.2 ± 0.4	100 (28)	9.67	99.47	+	41.49	High	17.7
Theophylline	272	100	1.9 ± 0.5	97 (2)	3.5;8.6	5.94	−	69.3	High	51.0
Verapamil	230	480	0.9 ± 0.2	98 (2)	8.9	96.93	+	63.95	High	61.7

[a] Literature higher oral dose values
[b] Literature fraction absorbed values
[c] Literature p*K*a values
[d] Ionized % were calculated using Henderson–Hasselbalch equation
[e] Charge: of the molecule at PBS 7.4 according to the ionization and p*K*a
[f] Literature polar surface area values
[g] Literature experimental solubilities (except for amiloride, which value was predicted)

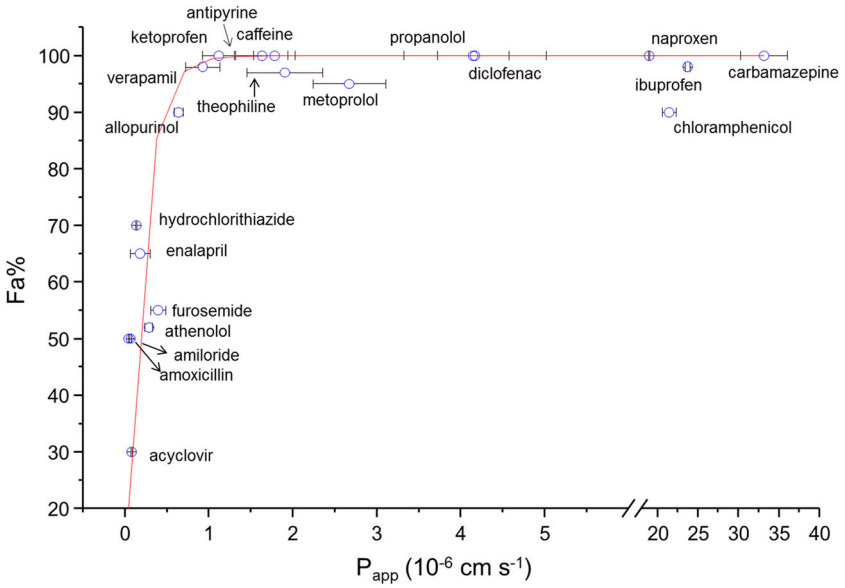

Fig. 2. Correlation between the intestinal absorbed fraction in humans (Fa%) and the apparent permeability values (P_{app}) obtained for 20 drug compounds

Table III. Apparent Permeability Constants (P_{app}), Fraction Absorbed (Fa%), Intrinsic Solubilities (S_0), and Needed Volume to Solubilize the Dose of SMR and INM From:

(a) Binary and ternary complexes								
Sample	P_{app} ($\times 10^{-6}$ cm/s)	Fa (%)	S_0 (mg/ml)			Needed volume (ml)		
			In water	In PBS pH 2	In PBS pH 8	In water	In PBS pH 2	In PBS pH 8
SMR	7 ± 1	100.00	0.22	0.32	1.6	227.27	156.25	31.25
SMR:βCD	4 ± 1	100.00	0.83	1.15	3.768	60.24	43.48	13.27
SMR:MβCD	3.9 ± 0.9	100.00	2.2	3.5	8.1	22.73	14.29	6.17
SMR:HPβCD	6.7 ± 0.9	100.00	0.69	0.99	1.9	72.46	50.51	26.32
SMR:MEG	3.7 ± 0.6	100.00	46.8	0.35	54.8	1.07	142.86	0.91
SMR:βCD:MEG	3.1 ± 0.9	99.99	0.97	1.3	2.36	51.55	38.46	21.19
SMR:MβCD:MEG	3.1 ± 0.9	99.99	6.28	2.8	11	7.96	17.86	4.55
SMR:HPβCD:MEG	3 ± 1	99.99	4.4	1.23	2.5	11.36	40.65	20.00
INM	14 ± 3	100.00	0.010	8.00E−05	0.070	5000	625,000	714.29
INM:βCD	18 ± 5	100.00	0.039	0.0145	5.861	1269	3,445	8.53
INM:MβCD	9.7 ± 0.3	100.00	1.006	0.026	15.801	50	1,947	3.16
INM:HPβCD	12.0 ± 0.8	100.00	0.028	0.0034	0.796	1799	14,902	62.85
(b) Microemulsions								
Sample	P_{app} ($\times 10^{-6}$ cm/s)	Fa (%)	S_0 (mg/ml)			Needed volume (ml)		
			In water	In PBS pH 8		In water	In PBS pH 8	
SMR in ME_1	2 ± 0.1	99.83	6.02203	2.7548		8.3	18.15	
SMR in ME_2	1 ± 0.1	94.91	7.47216	5.0152		6.69	9.97	
SMR in ME_3	1.5 ± 0.2	99.19	5.61844	9.7007		8.9	5.15	
SMR in ME_4	4.7 ± 0.5	100.00	13.346	13.0038		3.75	3.85	
SMR in ME_5	0.8 ± 0.2	92.30	21.05	16.63		2.38	3.01	
SMR in ME_5 + βCD	2.6 ± 0.6	100.00	17.3	20.18		2.89	2.48	
SMR in ME_5 + MβCD	5.2 ± 0.7	100.00	9.62	12.24		5.2	4.08	
SMR in ME_5 + HPβCD	2.3 ± 0.9	99.99	22.53	26.39		2.22	1.89	
SMR in ME_5 + MEG	2.2 ± 0.4	99.91	32.36	35.96		1.54	1.39	
INM in ME_1	5.3 ± 0.3	100.00	18.48	16.17		2.7	3.09	
INM in ME_2	2.6 ± 0.7	99.97	26.82	30.41		1.86	1.64	
INM in ME_3	2.2 ± 0.6	99.88	38.62	41.49		1.29	1.21	
INM in ME_4	4.7 ± 0.3	100.00	48.33	52.12		1.03	0.96	
INM in ME_5	10 ± 2	100.00	67.014	59.91		0.75	0.83	
INM in ME_5 + βCD	12 ± 3	100.00	65.26	73.11		0.77	0.68	
INM in ME_5 + MβCD	7 ± 1	100.00	25.77	36.77		1.94	1.36	
INM in ME_5 + HPβCD	12 ± 2	100.00	64.99	62.06		0.77	0.81	

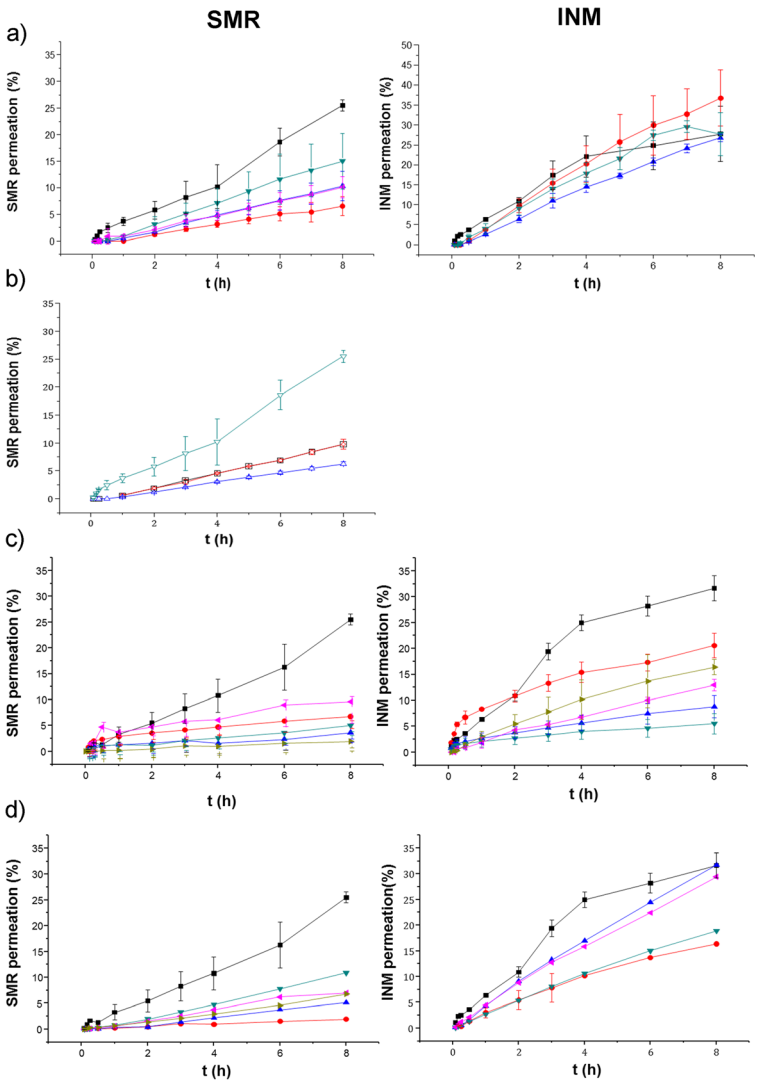

Fig. 3. Permeation profiles of SMR and INM (■) from **a** binary and **b** ternary complexes (for SMR) with βCD (●), MβCD (▲), HPβCD (▼), or MEG (◀); **c** ME_1 (●), in ME_2 (▲), in ME_3 (▼), in ME_4 (◀), and in ME_5 (▶); **d** ME_5 containing 1.8% β-CD (●), 12% M-β-CD (▲), 2.5% HP-β-CD (▼), or 5% MEG (for SMR) (◀)

of the drugs, which was intensified with the increase of oil percentage in the ME.

The P_{app} values were increased when the complexed drugs were used along with the ME system in comparison with the native MEs, but the values were still lower than the values corresponding to the free drugs. This may be because the complexed drugs inside the ME present higher solubility in the aqueous phase of the formulation, enhancing the thermodynamic activity of the drugs in comparison with the native MEs (12). In a previous study of our group, the partition coefficients of the drugs between the oil and aqueous phase of ME, ionization percentages of SMR and INM at pH 7.4 and 37°C, and an apparent solubility increments achieved using the ME were reported (11); SMR and INM presented similar partition coefficients of 2.7 ± 0.8 and 2.8 ± 0.3, respectively, and ionization percentages of 62 and 95% of the weak acid moiety at pH 7.4 and 37°C. The higher P_{app} value was obtained from ME_5 for INM, which presented the highest percentage of oil phase. For SMR, the higher P_{app} was obtained from ME_4, which was the second ME with higher oil quantity. This may be because SMR remains more retained in ME_5 due to its composition, making more difficult the release of the drug, which then passes through the membrane.

As well as with the free active ingredients, the solubilities in water, in PBS pH 2 and in PBS pH 8, of the drugs when contained in the formulations were experimentally determined (Fig. 4) and the needed volumes to solubilize the dose of each model drug were calculated and are presented in Table III. For MEs, the solubility in PBS pH 2 was dismissed since it is not possible to prepare the formulations at this pH value due to the presence of SO in its neutral species (12). It was observed that the needed volumes to solubilize SMR incorporated in the complexes were lower than 250 ml for all the systems at all the tested media. However, the needed

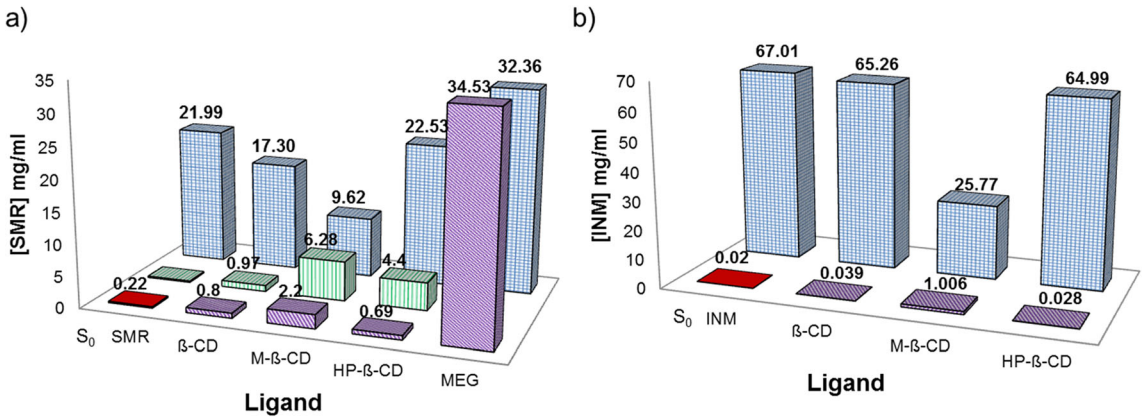

Fig. 4. Solubilities of **a** SMR and **b** INM in water (solid fill), in binary (diagonal lines fill) or ternary [(only SMR), (vertical lines fill)] complexes or in microemulsions (grid fill) containing water, 1.8% βCD, 12% MβCD, 2.5% HPβCD, or 5% MEG as the aqueous phase of the system

volumes to solubilize INM incorporated in the complexes did not reach this goal by this solubilizing strategy. On the other hand, the needed volumes to solubilize SMR and INM were lower than 10 and 2 ml when they were incorporated in the native ME formulations and lower than 5 and 1.2 ml when they were introduced in ME containing ligands, respectively. From these values, all SMR complexes and all the MEs containing both drugs were classified as highly soluble when they were incorporated in these pharmaceutical products. Indeed, since they presented high permeability and high solubility, they can be classified as Class I, according to the BCS.

CONCLUSIONS

A good correlation between the Fa% and the P_{app} values obtained with the developed method was observed for 20 compounds that are absorbed by a passive diffusion pathway and represents a wide range of absorption characteristics, diversity of structures, acid–base behavior, and solubilities. The sink condition was maintained over the whole procedure and the low standard deviation values demonstrated the good reproducibility of the proposed method for a diversity of compounds. The P_{app} value of allopurinol that presents a Fa% of 90% was set as the high permeability limit for the current method, and it was used to classify the model drugs according to the BCS. Therefore, a fast, efficient, reproducible, and low-cost method to appropriately predict the permeability of structurally diverse drugs that are absorbed by a passive diffusion mechanism was successfully developed and validated. INM was classified as high permeating and poorly soluble drug, thus, as Class II, according to the BCS, while SMR was found to be a Class I drug on the base of the BSC criteria, due to its high solubility and permeability characteristics. Even though a decrease in the P_{app} values was observed for most SMR complexes, and values of INM complexes remained without major variations, the predicted Fa% for all the drug products was kept higher than 90%, demonstrating that the permeability of the drugs was not affected by their incorporation in the complexes or the MEs next to the benefit of this solubilizing strategy. All SMR complexes and MEs containing both drugs were classified as highly soluble pharmaceutical products. Since these drug products presented high permeability and high solubility, they can be classified as Class I, according to the BCS.

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