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Biopartitioning micellar chromatography: an in vitro technique for predicting human drug absorption☆

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Abstract

The main oral drug absorption barriers are fluid cell membranes and generally drugs are absorbed by a passive diffusion mechanism. Biopartitioning micellar chromatography (BMC) is a mode of micellar liquid chromatography that uses micellar mobile phases of Brij35 under adequate experimental conditions and can be useful to mimic the drug partitioning process in biological systems. In this paper the usefulness of BMC for predicting oral drug absorption in humans is demonstrated. A hyperbolic model has been obtained using the retention data of a heterogeneous set of 74 compounds, which shows predictive ability for drugs absorbed by passive diffusion. The model obtained in BMC is compared with those obtained using the well-known systems (Caco-2 and TC-7) that use intestinal epithelium cell lines. The use of BMC is simple, reproducible and can provide key information about the transport properties of new compounds during the drug discovery process.

Keywords: Biopartitioning micellar chromatography; Oral drug absorption; Intestinal epithelium cell lines

1. Introduction

The costs of drug product from discovery to market have been recently estimated those exceed US$400 million. These studies include the selection of drug candidates and the study of their pharmacokinetic and pharmacodynamic properties. In the early stage of drug discovery, the pharmacokinetic studies have traditionally been conducted in living systems such as mice, rabbits, dogs, etc., but this methodology is expensive and time consuming. For ethical and/or economical reasons, a great deal of effort is currently being made to develop in vitro systems in order to avoid or reduce the use of experimental animals.

Oral drug delivery is the preferred route of drug administration. It is well known that the major absorption barrier to drugs given orally is the intestinal mucosa and generally drugs are absorbed by a passive diffusion mechanism.

To circumvent the problems associated with screening experimental compounds in animals, a number of in vitro models for predicting oral drug

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absorption have been developed [1–15] which include the use of physicochemical parameters of drugs, permeability data obtained from cell culture lines and chromatographic models.

Maximum drug absorption occurs when the drug has maximum permeability and maximum concentration (saturation solubility) at the absorption site. Solubility, dissolution rate, particle size, hydrophobicity, p*K*, stability, gastrointestinal motility patterns volume and flow-rate of gastrointestinal contents as well as membrane permeability have been found to influence drug absorption from the gastrointestinal tract.

Hydrophobicity is a key factor in oral drug absorption. The hydrophobicity of a solute, measured as its partition coefficient between octanol and water (log *P*), has been commonly used as predictor of its transmembrane permeability [5], but good correlations were only found within homologous series of compounds [6].

Most of the in vitro studies examining drug uptake and transport in the intestinal epithelium have utilized different models such as everted sacs, brush border membrane vesicles, isolated cells, and intestinal rings [2]. More recent works have focused on the Caco-2 cell, a colorectal adenocarcinoma cell line of human origin, as a model for studying intestinal transport [1–12]. In recent years the use of Caco-2 cell monolayers has gained in popularity as an in vitro human absorption surrogate, moreover the Caco-2 cell monolayers are generally accepted as a primary absorption screening tool in several pharmaceutical companies. However, the lack of standardization in cell culturing and experimental procedures makes impossible to compare inter-laboratory permeability data.

Chromatographic models to predict drug absorption are experimentally easier than cell culture models. The retention data of 11 structural unrelated drugs in a gel bed with immobilized liposomes (ILs) (IL chromatography) [14] and the retention data of 12 drugs in immobilized artificial membranes (IAMs) (IAM chromatography) [15] have been shown to correlate with oral drug absorption.

Our research group has demonstrated that the use of retention data obtained in a chromatographic system constituted by polioxyethylene (23) lauryl ether, Brij35, micellar mobile phases and C_{18} reversed stationary phase under adequate experimental conditions is helpful in describing the biological behavior of different kinds of drugs [16–24]. We have called this drug biopartitioning simulation chromatographic system biopartitioning micellar chromatography (BMC). The retention of compounds in this chromatographic system depends on its interactions with modified reversed stationary phase and micelles presents in the mobile phase (Fig. 1A). These interactions are governed by hydrophobic, electronic and steric properties of compounds.

The usefulness of BMC in constructing good models could be attributed to the fact that the characteristics of the BMC systems are similar to biological barriers and extracellular fluids. Firstly, the stationary phase modified by the hydrophobic adsorption [25,26] of Brij35 surfactant monomers structurally resembles the ordered array of the membranous hydrocarbon chains. In addition, the hydrophilic/hydrophobic character of the adsorbed surfactant monomers resembles the polar membrane regions.

In addition, Brij35 micellar mobile phases prepared at physiological conditions could also mimic the environment of drug biological partitioning. The extracellular and intracellular fluids are basically composed of water, salts, glucose, amino acids, cholesterol, phospholipids, fatty acids and proteins [27]. Phospholipids, cholesterol, fatty acids and triglycerides form micellar complexes with proteins (lipoproteins) (critical micelle concentration, cmc$<10^{-6}$ *M*) [28].

In a previous paper [24] the similarity between MLC systems with Brij35 (BMC systems) and other well recognized natural systems to emulate the absorption of drugs in gastrointestinal barriers [29] [red cell membrane lipid liposomes (MLs), human red cell membranes vesicles (vesicles), native membranes of adsorbed red cells (ghosts) and egg phospholipids liposomes (EPLs)] was demonstrated. Regression models for the prediction of passive drug absorption for barbiturates and β-blockers were also obtained.

In this paper, the usefulness and limitations of BMC to predict oral drug absorption is studied. The model obtained in BMC is compared with those

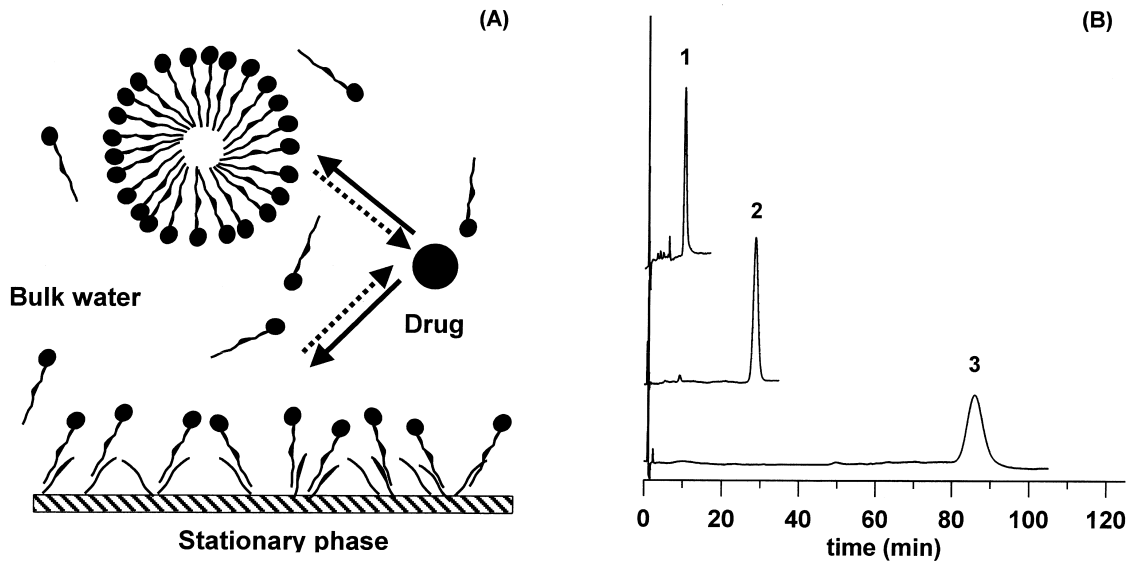

Fig. 1. Schematic representation of drug partitioning in BMC (A). Chromatograms corresponding to fenbufen (1), lorazepam (2) and quazepam (3) eluted using 0.04 *M* Brij35 at pH 7.4 (B).

obtained using the well-known systems that use intestinal epithelium cell lines.

2. Experimental

2.1. Reagents and standard

The chromatographic system uses as micellar mobile phases aqueous solutions 0.04 *M* of polyoxyethylene (23) lauryl ether (Brij35; Acros, Geel, Belgium) at pH 6.5 and 7.4 adjusted with 0.05 *M* phosphate buffer, prepared with disodium hydrogenphosphate and sodium dihydrogenphosphate (analytical reagent; Panreac, Barcelona, Spain).

The drugs were obtained from different sources (see Table 1). Stock standard solutions were prepared by dissolving 10 mg of the compound in 10 ml of mobile phase solution or acetonitrile. Working solutions of 50 mg/l were prepared by dilution of the stock standard solutions using the mobile phase solution.

Barnstead E-pure, deionized water (Sybron, Boston, MA, USA) was used throughout. The mobile phase and the solutions injected into the chromatograph were vacuum-filtered through 0.45- and 0.22-μm nylon membranes, respectively (Micron Separations, Westboro, MA, USA).

2.2. Instrumental and measurement

A Hewlett-Packard HP 1100 chromatograph with an isocratic pump, an UV–visible detector, a column thermostat and a HP Vectra computer (Amsterdam, The Netherlands) equipped with HP-Chemstation software (A.07.01 [682] ©HP 1999) was used. The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20-μl loop. A Kromasil octadecyl-silane C_{18} (5 μm, 150×4.6 mm i.d.) column and the corresponding guard column of similar characteristics (35×4.6 mm I.D.) (Scharlau, Barcelona, Spain) were used. In all cases the mobile phase flow-rate was 1 ml/min. Detection of compounds was performed at 220 nm, except for anxiolytics, hypnotics and lamotrigine that were detected at 240 nm. The column was thermostatted at 36.5°C for all assays. The k_{BMC} values determined in this study were averages of at least triplicate determinations. The retention data were highly reproducible, the relative standard deviation (RSD) values were <1% for intra-day and <5% for inter-day assays. Fig. 1B shows chromatograms

Table 1
Therapeutic group and commercial source for the different compounds studied

Compound	Source	Therapeutic group
Acebutalol	Sigma–Aldrich, Madrid, Spain	β-Blocker
Acetylsalicylic acid	Panreac, Barcelona, Spain	NSAID
Acyclovir	Zovirax®, Glaxo Wellcome, Madrid, Spain	Antiviral
Alprazolam	Trankimazin®, Upjohn Farmoquímica S.A., Madrid, Spain	Anxiolytic
Alprenolol	Sigma–Aldrich, Madrid, Spain	β-Blocker
Amiloride	Guinama, Valencia, Spain	Diuretic
Amitriptyline	Tryptizol®, Merck Sharp & Dohme S.A., Madrid, Spain	Antidepressant
Amobarbital	Sigma, St. Louis, MO, USA	Hypnotic
Amoxapine	Demolox®, Cyanamid Ibérica S.A., Madrid, Spain	Antidepressant
Aprobarbital	Sigma, St. Louis, MO, USA	Hypnotic
Atenolol	Sigma–Aldrich, Madrid, Spain	β-Blocker
Bromperidol	Janssen Pharmaceutica N.V., Beerse, Belgium	Antipsychotic
Butabarbital	Sigma, St. Louis, MO, USA	Hypnotic
Chlordiazepoxide	Omnalio®, Estedi S.L., Barcelona, Spain	Antiepileptic
Chlorpromazine	Largactil®, Rhône-Poulenc Rorer S.A., Barcelona, Spain	Antipsychotic
Cimetidine	Guinama, Valencia, Spain	Antiulcer
Clobazam	Noiafren®, Hoechst Farma S.A., Barcelona, Spain	Anxiolytic
Clomipramine	Anafranil®, Lab. Geigy, Barcelona, Spain	Antidepressant
Clonazepam	Rivotril®, Productos Roche S.A., Madrid, Spain	Anxiolytic
Corticosterone	Sigma, St. Louis, MO, USA	Corticosteroid
Desipramine	Sigma, St. Louis, MO, USA	Antidepressant
Dexamethasone	Sigma, St. Louis, MO, USA	Corticosteroid
Diazepam	Valium®, Productos Roche S.A., Madrid, Spain	Anxiolytic
Diclofenac	Novartis Farmacéutica, S.A., Barcelona, Spain	NSAID
Dothiepin	Prothiaden®, Alter Laboratories, Madrid, Spain	Antidepressant
Doxepin	Sinequan®, Pfizer, Madrid, Spain	Antidepressant
Famotidine	Guinama, Valencia, Spain	Antiulcer
Fenbufen	Cincopal®, Cyanamid Ibérica S.A., Madrid, Spain	NSAID
Flunitrazepam	Rohipnol®, Productos Roche S.A., Madrid, Spain	Anxiolytic
Fluphenazine	Guinama, Valencia, Spain	Antipshichotic
Flurbiprofen	Froben®50, Laboratorios Knoll S.A., Madrid, Spain	NSAID
Haloperidol	Janssen Pharmaceutica N.V., Beerse, Belgium	Antipsychotic
Hexobarbital	Sigma, St. Louis, MO, USA	Hypnotic
Hydrochlorothiazide	Guinama, Valencia, Spain	Diuretic
Hydrocortisone	Sigma, St. Louis, MO, USA	Corticosteroid
Ibuprofen	Nurofen® 400, Boots Healthcare S.A., Madrid, Spain	NSAID
Imipramine	Tofranil®, Novartis Farmacéutica, Barcelona, Spain	Antidepressant
Indomethacin	Laboratorio Llorens S.A., Barcelona, Spain	NSAID
Ketoprofen	Rhône-Poulenc, Rorer, S.A., Barcelona, Spain	NSAID
Labetalol	Sigma–Aldrich, Madrid, Spain	β-Blocker
Lamotrigine	Labileno®, Glaxo Wellcome S.A., Burgos, Spain	Antiepileptic
Lidocaine	Seid S.A., Barcelona, Spain	Local anesthetic
Lorazepam	Orfidal®, Wyeth-Orfi S.A., Madrid, Spain	Anxiolytic
Lormetazepam	Loramet®, Wyeth-Orfi S.A., Madrid, Spain	Anxiolytic
Loxapine	Desconex®, Alonga Laboratories, Madrid, Spain	Antidepressant
Mannitol	Merck, Germany	Diuretic
Maprotiline	Ludiomil®, Novartis Farmacéutica, Barcelona, Spain	Antidepressant
Metoprolol	Sigma–Aldrich, Madrid, Spain	β-Blocker
Mianserin	Lantanon®, Organon Española, Barcelona, Spain	Antidepressant
Nadolol	Sigma–Aldrich, Madrid, Spain	β-Blocker
Naproxen	Syntex Latino, Madrid, Spain	NSAID
Nortriptyline	Lilly Laboratories, Madrid, Spain	Antidepressant
Orphenadrine	Sigma, St. Louis, MO, USA	Antihistamine
Oxazepam	Adumbran®, Boeringer Ingelheim S.A., Barcelona, Spain	Anxiolytic

Table 1. Continued

Compound	Source	Therapeutic group
Oxprenolol	Sigma–Aldrich, Madrid, Spain	β-Blocker
Pentobarbital	B. Braun Medical	Hypnotic
Phenobarbital	Bayer, Barcelona, Spain	Hypnotic
Phenytoin	Rubió S.A., Barcelona, Spain	Antiepileptic
Pindolol	Sigma–Aldrich, Madrid, Spain	β-Blocker
Primidone	Zeneca Farma, Pontevedra, Spain	Antiepileptic
Propranolol	Sigma–Aldrich, Madrid, Spain	β-Blocker
Quazepam	Quiedorm®, Laboratorios Menarini S.A., Barcelona, Spain	Anxiolytic
Quinine	Guinama, Valencia, Spain	Antimalarial
Salicylic acid	Panreac, Barcelona, Spain	NSAID
Secobarbital	UCB, Barcelona, Spain	Hypnotic
Sulindac	Sulindal®, Merck Sharp & Dohme S.A., Madrid, Spain	NSAID
Sulpiride	Sigma, St. Louis, MO, USA	Antisychotic
Terbutaline	Sigma, St. Louis, MO, USA	Bronchodilator
Testosterone	Sigma, St. Louis, MO, USA	Androgen
Tetrazepam	Myolastan®, Sanofi Winthrop S.A., Barcelona, Spain	Anxiolytic
Tolmentin	Laboratorio Estedi S.L., Barcelona, Spain	NSAID
Trimipramine	Surmantil®, Rhône-Poulenc, Rorer, S.A., Barcelona, Spain	Antidepressant
Warfarin	Aldocumar®, Aldo-Unión, Barcelona, Spain	Anticoagulant
Zopiclone	Aventis Pharma S.A., Madrid, Spain	Antiepileptic

corresponding to fenbufen, lorazepam and quazepam eluted using 0.04 *M* Brij35 at pH 7.4.

2.3. Software and data processing

Excel 7.0 from Microsoft Office and SPSS 8.0 software were used to perform the statistical analysis of the regressions.

3. Results and discussion

3.1. Potential of biopartitioning micellar chromatography for predicting oral drug absorption

The usefulness of BMC in predicting oral drug absorption in humans was evaluated. For this purpose 74 structurally diverse drugs were selected (Table 1). The model drugs were chosen to cover a wide range of absorption after oral administration (16–100%) as well as a wide range of physicochemical properties such as hydrophobicity (log *P* ranged between 0.34 and 5.20) and charge (cationic, anionic and neutral compounds). Stringent inclusion criteria were used in the selection of the model drugs. These included: (1) the availability of reliable data on the absorbed fraction in humans, and (2) clear indications that the drugs were predominantly absorbed by a passive process.

The retention data of the compounds studied were obtained using 0.04 *M* Brij35 micellar solutions buffered at pH 6.5 and 7.4 as mobile phases. The pH value 6.5 is considered as the average pH of the small intestine and 7.4 is the plasmatic pH value. Table 2 shows the retention data of compounds studied obtained at pH 6.5 together with their oral absorption values in humans (%) reported in bibliography. As can be expected, at pH 7.4 the retention of acidic compounds with pK_a values larger than 4.5 (i.e., barbiturates) decreased, while the retention of basic compounds (i.e., antidepressants, β-blockers drugs, local anesthetics, etc.) increased.

Fig. 2A and 2B show the relationships between the retention factor of drugs at pH 6.5 and 7.4, respectively, and their oral drug absorption values. As can be observed similar hyperbolic relationships were obtained. Eqs. (1) and (2) showed the models obtained at pH 6.5 and 7.4, respectively:

Table 2
Retention data using 0.04 *M* Brij35 at pH 6.5 and, human oral absorption literature and predicted values using the proposed training set

Drug	k_{BMC}	Oral absorption[a] (%)	Predicted oral absorption[b] (%)
Acebutalol	2.1	90 [12]	80
Acetylsalicylic acid	2.9	100 [3]	84
Acyclovir	0.7	20 [12]; 23±8 [34]	56
Alprazolam	24.8	95±5 [34]; 88±16 [33]	96
Alprenolol	16.2	93 [12]	95
Amiloride	3.5	50 [13]	86
Amitriptyline	80.9	95 [30]	98
Amobarbital	31.0	95 [30]	97
Amoxapine	38.0	95 [30]	97
Aprobarbital	14.8	85 [31]	95
Atenolol[c]	0.4	50 [3]; 54±17 [8]; 50±5 [34]; 56±30 [33]	–[c]
Bromperidol	50.0	95 [30]	97
Butabarbital	18.8	95 [30]	96
Chlordiazepoxide	32.0	100 [30]	97
Chlorpromazine[c]	94.2	95 [30]	–[c]
Cimetidine	1.9	95 [12]; 84±13 [33]	78
Clobazam	22.3	100 [30]	96
Clomipramine	83.1	95 [30]	98
Clonazepam	28.4	98±31 [33]	96
Corticosterone	31.6	100 [3]	97
Desipramine	30.5	95 [12]	97
Dexamethasone	24.8	100 [3]; 78±14 [33]	96
Diazepam	36.2	100 [12]; 100±14 [33]	97
Diclofenac	21.4	90 [31]	96
Dothiepin	45.1	95 [30]	97
Doxepin	40.8	95 [30]	97
Famotidine	3.4	45 [13]; 45±14 [33]	86
Fenbufen	11.8	80 [31]	94
Flunitrazepam	26.3	100 [30]	96
Fluphenazine	49.4	95 [30]	97
Flurbiprofen	19.7	95 [31]	96
Haloperidol	44.8	95 [30]	97
Hexobarbital	19.3	95 [32]	96
Hydrochlorothiazide	11.3	90 [12]	94
Hydrocortisone[c]	14.7	89 [3]	–[c]
Ibuprofen	30.0	95 [31]	97
Imipramine[c]	64.6	95 [30]	–[c]
Indomethacin	23.1	100 [12]; 98±21 [21]	96
Ketoprofen	9.6	90 [31]	93
Labetalol	16.0	90 [12]	95
Lamotrigine	12.3	98 [32]	94
Lidocaine	6.8	90 [35]	91
Lorazepam	24.4	93±10 [33]	96
Lormetazepam	25.2	98 [34]	96
Loxapine	124.8	95 [30]	98
Mannitol[c]	0.2	16 [3]	–[c]
Maprotiline	31.8	100 [30]	97
Metoprolol[c]	2.3	95 [3]; 102±5 [8]	–[c]
Mianserin	62.1	95 [30]	97
Nadolol	0.7	40 [30]; 40±10 [34]	57
Naproxen	11.4	99 [33]	94

Table 2. Continued

Drug	k_{BMC}	Oral absorption[a] (%)	Predicted oral absorption[b] (%)
Nortriptyline	34.2	95 [30]	97
Orphenadrine	50.3	95 [34]	97
Oxazepam	23.4	97±11 [8]; 97±11 [33]	96
Oxprenolol	5.4	97±13 [8]	90
Pentobarbital	47.3	100 [30]	97
Phenobarbital	17.4	100±11 [33]	95
Phenytoin	26.5	98 [31]	96
Pindolol	2.8	95 [12]; 92±11 [8]; 75±9 [33]	84
Primidone	7.7	90±6 [32]; 92±18 [33]; 93±3 [34]	92
Propranolol[c]	17.0	90 [3]	–[c]
Quazepam[c]	88.4	100 [30]	–[c]
Quinine	17.2	95 [30]	95
Salicylic acid	2.8	100 [3]	84
Secobarbital	37.9	100 [30]	97
Sulindac	6.0	90 [30]	91
Sulpiride	0.7	36±20 [8]	57
Terbutaline[c]	1.2	73 [3]	–[c]
Testosterone[c]	44.7	100 [3]	–[c]
Tetrazepam	51.4	100 [30]	97
Tolmentin	7.0	99 [31]	92
Trimipramine	64.4	95 [29]	97
Warfarin	13.3	98 [3]; 93±8 [33]	95
Zopiclone	11.0	100 [30]	94

[a] Literature values. When different literature values are indicated, only the first one has been used to build the model.
[b] Predicted oral absorption values using the retention data at pH 6.5. Confidence limits±16.
[c] Training set.

$$\%\ \text{Oral absorption} = 100k_{BMC}/[0.7(0.2) + 1.02(0.03)k_{BMC}]\ \text{ENTER}\ n = 74,\ \text{S.E.} = 9.8,\ r^2 = 0.72,\ F = 3185 \quad (1)$$

$$\%\ \text{Oral absorption} = 100k_{BMC}/[1.0(0.3) + 1.00(0.03)k_{BMC}]\ \text{ENTER}\ n = 74,\ \text{S.E.} = 9.8,\ r^2 = 0.72,\ F = 3174 \quad (2)$$

where the numbers in parentheses are the asymptotic confidence intervals at a 95% probability level. The standard error (S.E.) can be used to construct prediction limits for new observations and the correlation coefficient is a measure of the fit degree to the equation. The *P*-values obtained for the models ($P \leq 0.0001$) indicate that the relationships between % oral drug absorption and the k_{BMC} values was statistically significant at the 95% confidence level. Coefficients were also significant at the same confidence level (*P*-value$\leq$0.0001).

The residual plots of the proposed models showed a random distribution of the residuals and practically all were statistically equal to zero, which suggest the adequacy of the hyperbolic model.

Using the retention data of a reduced number of compounds, selected in order to cover a wide absorption and retention ranges (set training, atenolol, chlorpromazine, hydrocortisone, imipramine, mannitol, metoprolol, propranolol, quazepam, terbutaline and testosterone), similar models were obtained. Eq. (3) shows the model obtained for pH 6.5:

$$\%\ \text{Oral absorption} = 100k_{BMC}/[0.5(0.2) + 1.02(0.08)k_{BMC}]\ \text{ENTER}\ n = 10,\ \text{S.E.} = 8.0,\ r^2 = 0.92,\ F = 550 \quad (3)$$

As can be observed, coefficients were statistically

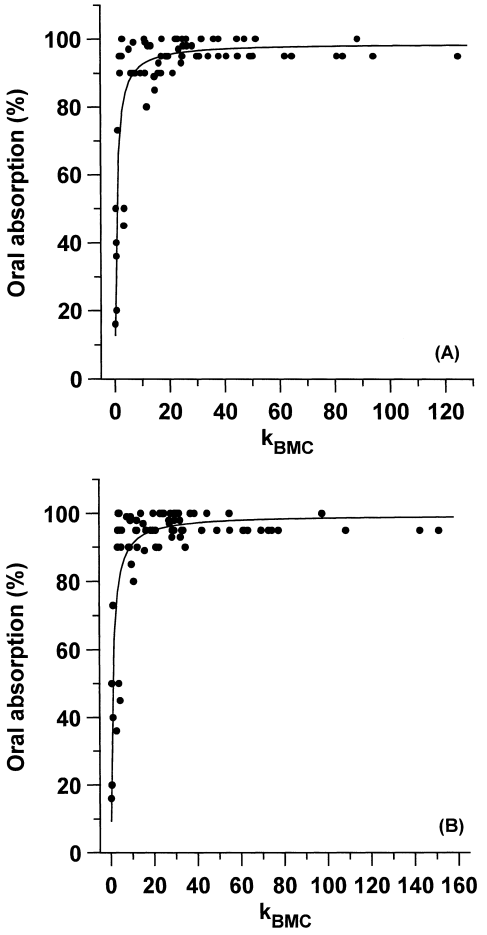

Fig. 2. % Oral drug absorption–k_{BMC} model obtained using 0.04 *M* Brij35 concentration as mobile phase at pH 6.5 (A) and pH 7.4 (B).

significant. In addition, they were not statistically significant different at the 95% confidence level with respect to those obtained in Eq. (1). Table 2 shows the predicted oral drug absorption values obtained using Eq. (3). The confidence limits for predictions were ±16%, which are in the usual range of the reported absorption values (see Table 2). From the results obtained it can be deduced:

(1) For drugs which show retention factors ranged between $0.2<k<3$ at pH 6.5 absorption problems can be expected, but it is not possible to perform accurate predictions of drug absorption with the model. The limiting factor in the absorption process for these drugs is their diffusion through lipid bilayer mainly controlled by the hydrophobicity and ionization of drugs, in a similar way that retention in BMC. These drugs show low permeability and high variability in the rate and extent of absorption because of physiological factors rather than dosage form related factors. In addition drugs in this class which have low solubility are poorly absorbed and therefore pose significant problems for effective oral delivery [11].

(2) For drugs which show retention factors at pH 6.5 higher than 3 maximal oral absorption can be expected. This drugs have high permeability and are rapidly and completely absorbed with extents of absorption >90%. However, if drugs have high solubility, their systemic bioavailability may be limited due to first pass metabolism (i.e., propranolol). For drugs which present low solubility, the dissolution in the gastrointestinal tract is the rate-limiting of the absorption processes and variability in the absorption of this drugs may be due to differences in formulations and physiological variables that may influence the drug dissolution process [11].

3.2. Limitations of the oral absorption–BMC model

The developed oral drug absorption–BMC model could be very useful in the drug discovery process because drug retention in BMC can predict whether the compound has favorable transport properties if passive diffusion is the mechanism responsible of absorption. Other factors that decrease the absorption of drugs as chemical and bacterial degradation at the absorption site and the first pass metabolism in the intestinal cells and the liver or other mechanisms as membrane passage via paracellular routes or any active transport mechanism are not included in this model.

3.3. Comparison between retention data in BMC– and permeability across human epithelial intestinal cell lines–oral drug absorption models

Table 3 shows the retention factors in BMC at pH 7.4, the apparent drug permeability P_{app} into monolayers of cultured epithelial cells lines (Caco-2 and TC-7) obtained at pH 7.4 for a set of compounds together the oral drug absorption values in humans reported by Artursson et al. [3], Yazdaninan et al.

Table 3
Retention factors in BMC at pH 7.4, oral absorption in humans (%) and apparent permeability coefficients ($\cdot 10^6$ cm/s) in human epithelial intestinal cell lines of the compounds studied reported in the bibliography

Compound	k_{BMC}	Oral absorption (%)	$P_{Caco\text{-}2}$ [a]	$P_{Caco\text{-}2}$ [b]	$P_{Caco\text{-}2}$ [c]	$P_{TC\text{-}7}$ [c]
Acebutalol	3.4	90	0.51	–	–	–
Acetylsalicylic acid	3.9	100	9.09	2.4	–	–
Acyclovir	0.5	20	0.25	–	–	–
Alprenolol	32.4	93	25.3	40.5	–	–
Atenolol	0.6	50	0.53	0.2	1.16	0.34
Cimetidine	3.6	95	1.37	–	–	–
Corticosterone	31.6	100	21.2	54.5	–	–
Desipramine	28.7	95	24.4	–	–	–
Dexamethasone	24.0	100	12.2	12.5	–	–
Diazepam	38.8	100	33.4	–	–	–
Hydrochlorotiazide	12.5	90	0.51	–	–	–
Hydrocortisone	15.9	89	14.0	21.5	12.19	7.7
Indomethacin	23.0	100	20.4	–	–	–
Labetalol	21.5	90	9.31	–	–	–
Mannitol	0.2	16	0.38	0.18	1.17	0.93
Metoprolol	5.3	95	23.7	27	18.0	21.69
Pindolol	4.5	95	16.7	–	–	–
Propranolol	34.8	90	21.8	41.9	34.43	34.68
Salicylic acid	4.2	100	22.0	11.9	–	–
Terbutaline	1.3	73	0.47	0.38	1.04	1.28
Testosterone	44.7	100	24.9	51.8	44.5	43.83
Warfarin	9.3	98	21.1	38.3	–	–

[a] Literature data taken from Ref. [12] for compounds of set I.
[b] Literature data taken from Ref. [3] for compounds of set II.
[c] Literature data taken from Ref. [10] for compounds of set III.

[12] and Grèss et al. [10]. The data shown by the authors indicated that the absorption of orally distributed doses increased monotonically with increasing P_{app} values and reached 100% above $P_{app} \approx 10^{-6}$ cm/s. Since large differences exist in the determination of apparent permeability coefficient from one laboratory to another, the authors indicated it is of the utmost importance to determine permeability coefficients of new chemical entities in relation to a reference curve established with compounds exhibiting a large range of permeability coefficients and for which absorption from an orally administered dose in humans is known [10].

Fig. 3 shows the relationships obtained between permeability coefficients in human epithelial intestinal cell lines and oral drug absorption together the corresponding obtained using the retention in BMC at pH 7.4 as independent variable for the same compounds. As can be observed the absorption data also increased with increasing k_{BMC} values that indicates that the retention in BMC provides a comparable in vitro system to those shown by the above cited authors for estimating the oral drug absorption.

Table 4 shows the statistical analysis of the hyperbolic oral absorption models obtained using the retention in BMC, the apparent permeability in Caco-2 cells and TC-7 cells as independent variables. As can be observed, the use of the retention data provided the higher correlation coefficients, the fitting parameters were similar and the models were statistically significant. The models obtained using the data set III and $P_{Caco\text{-}2}$ or $P_{TC\text{-}7}$ as independent variables were statistically non significant.

In comparison with the cell line systems, the retention in BMC offers several advantages: the preparation of the chromatographic system is rapid, simple and economical, the reproducibility intra-day and inter-day of the retention data is very high (RSD lower than 5%) which permits the oral absorption estimation without need of a previous system calibration.

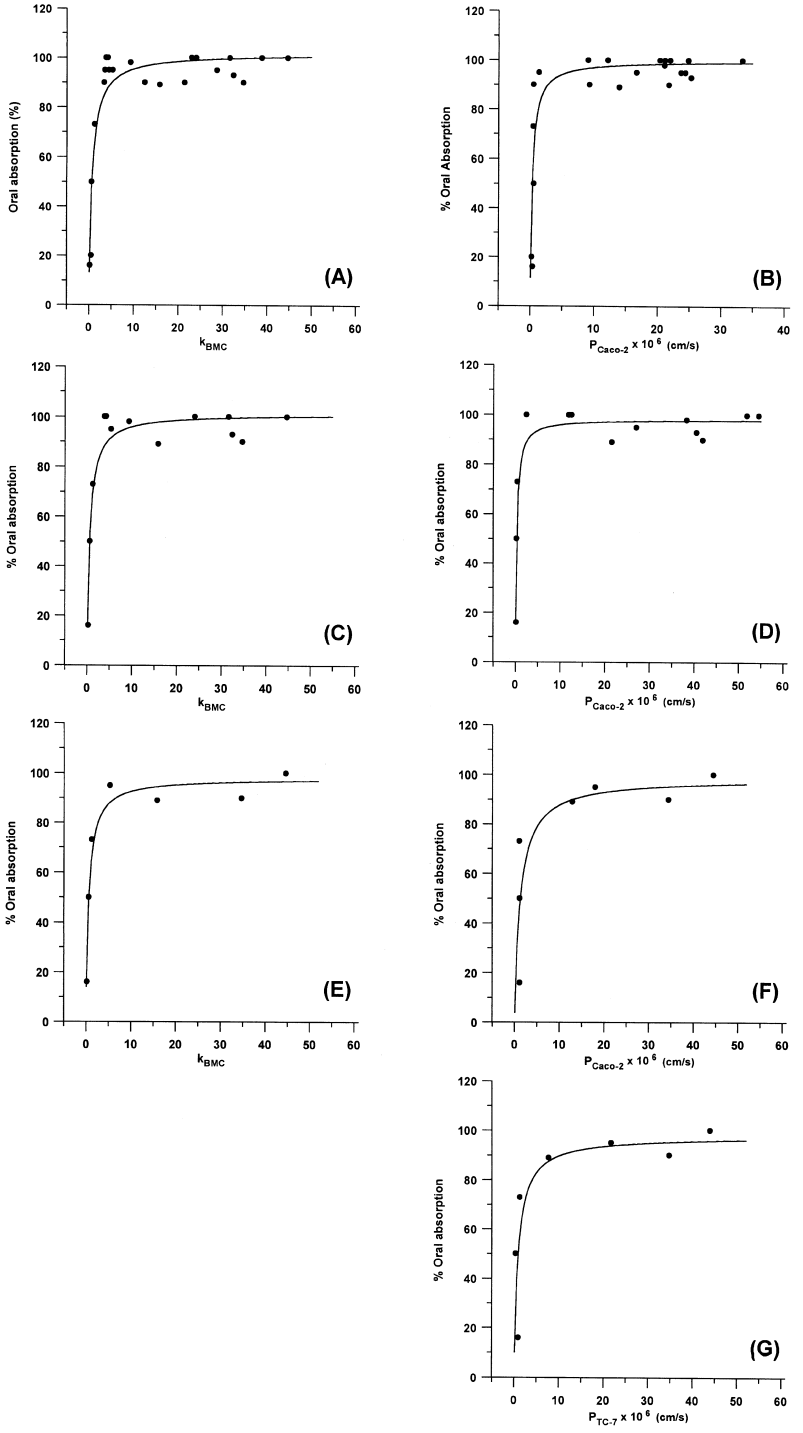

Fig. 3. Comparison between retention data in BMC at pH 7.4 and permeability in human epithelial intestinal cell lines to predict oral drug absorption for different data set of compounds (see Table 3): (A) oral absorption–k_{BMC} model data set I, (B) oral absorption–P_{Caco-2} model data set I, Ref. [12]. (C) Oral absorption–k_{BMC} model data set II, (D) oral absorption–P_{Caco-2} model data set II, Ref. [3]. (E) Oral absorption–k_{BMC} model data set III, (F) oral absorption–P_{Caco-2} and (G) P_{TC-7} model data set III, Ref. [10].

Table 4
Statistical analysis of the oral absorption models [oral absorption (%) = $100x/(a+bx)$]

Data set[a]	Independent variable	$a \pm ts_a$	$b \pm ts_b$	n	r^2	F	S.E.
I	k_{BMC}	0.7±0.3	0.98±0.05	22	0.88	1134	8.7
	$P_{Caco\text{-}2}$ ($\cdot 10^6$, cm/s)	0.3±0.2	1.01±0.09	22	0.65	377	15.0
II	k_{BMC}	0.6±0.3	0.99±0.06	13	0.92	856	7.7
	$P_{Caco\text{-}2}$ ($\cdot 10^6$, cm/s)	0.3±0.2	1.02±0.08	13	0.85	471	10.3
III	k_{BMC}	0.6±0.3	1.02±0.11	7	0.95	390	7.4
	$P_{Caco\text{-}2}$ ($\cdot 10^6$, cm/s)	1.3±1.6[b]	1.0±0.3	7	0.66	56	19.3
	$P_{TC\text{-}7}$ ($\cdot 10^6$, cm/s)	0.9±1.3[b]	1.0±0.3	7	0.65	52	19.8

[a] See Table 3 for details.
[b] Non-statistically significant parameters.

In summary we believe that the BMC offers an easy and reliable model for the study of drug absorption through the intestinal mucosa. The model gives % absorption results which are similar to those reported in the literature, is not time consuming and the uncertainty of predictions is similar of reported values for drug absorption.

4. Conclusions

Today with the development of combinatorial chemistry hundreds and hundreds of drugs that could show potential activity are synthesized. The need to get a tool for biopharmaceutical parameter estimation of new compounds supports the postulation of predictive models as complementary tools to conventional classical assays. The main purpose of these methodologies is the reduction of costs and time from drug discovery to drug market and the use of experimentation animals. The results shown in this paper indicate that the retention of compounds in BMC is capable of describing in vitro human oral drug absorption and it can be used to identify drugs, which can be poorly absorbed at the early stage of the drug discovery process.

The use of BMC is simple, accurate and can provide key information about the absorption properties of new compounds during the drug discovery process. The BMC methodology is a more inexpensive way than cell culture models or in vivo models and minimum experimental effort is required.

This approach can be very useful in medicinal chemistry and pharmaceutical research using BMC as an early predictor of oral absorption in humans. The method could also be applicable in the prediction of passive drug transport across other epithelial barriers, such as the blood–brain barrier.

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