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Review

Biopartitioning micellar separation methods: modelling drug absorption

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Abstract

The search for new pharmacologically active compounds in drug discovery programmes often neglects biopharmaceutical properties as drug absorption. As a result, poor biopharmaceutical characteristics constitute a major reason for the low success rate for candidates in clinical development. Since the cost of drug development is many times larger than the cost of drug discovery, predictive methodologies aiding the selection of bioavailable drug candidates are of profound significance. This paper has been focussed on recent developments and applications of chromatographic systems, particularly those systems based on amphiphilic structures, in the frame of alternative approaches for estimating the transport properties of new drugs. The aim of this review is to take a critical look at the separations methods proposed for describing and predicting drug passive permeability across gastrointestinal tract and the skin.

Keywords: Reviews; Biopartitioning; Drug absorption

Contents

Abbreviations: BMC, biopartitioning micellar chromatography; Brij35, polioxyethylene (23) lauryl ether; CE, capillary electrophoresis; Chol, cholesterol; CTAB, cetyltrimethylammonium bromide; CV, coefficient of variation; ED_{50}, effective dose of drug causing a given effect to the 50% of the population; EPC, egg phosphatidylcholine; EPL, egg phospholipid liposomes; GI tract, gastrointestinal tract; HBD, number of hydrogen bond donors per molecule; HPLC, high performance liquid chromatography; IC_{50}, inhibition concentration of drug displacing 50% of a marker from a binding site of receptor; IAM, immobilised artificial membrane; ILC, immobilised liposome chromatography; LC, liquid chromatography; LEKC, liposome electrokinetic chromatography; MEEKC, microemulsion electrokinetic chromatography; MEKC, micellar electrokinetic chromatography; MI, migration index; ML, membrane lipid liposomes; MLC, micellar liquid chromatography; MLR, multiple linear regression; MP, melting point; MR, molar refractivity; MV, molar volume; MW, molecular weight; NN, neural networks; PA, phosphatidic acid; PAMPA, parallel artificial membrane permeability assay; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PLS, partial least squares; PS, phosphatidylserine; PSA_d, dynamic polar molecular surface area; QRAR, quantitative retention–activity relationship; QSAR, quantitative structure–activity relationship; RP-HPLC, reversed-phase high performance liquid chromatography; SDS, sodium dodecyl sulfate; SPR, surface plasmon resonance; α_1-AGP, α_1-acid glycoprotein

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1. Introduction

Today with the development of combinatorial chemistry hundreds and hundreds of drugs that have potential biological activity are synthesized. The studies involved from drug discovery to market, which include the selection of drug candidates and the study of their pharmacological properties are very expensive, time consuming and usually require the use of experimentation animals. For ethical and/or economical reasons, a great deal effort is currently being made to develop in vitro systems to avoid or reduce the use of experimentation animals and provide primary information about the capability of new compounds in the first steps of drug development.

The drug's overall activity cannot be considered to result only from the specific interaction of a drug molecule at the action site (receptor) in a tissue or cellular substrate. Several fundamental processes determine drug action: release of the active agent from dosage form, absorption into general circulation, binding to blood proteins, distribution to the various tissues where receptor site–drug interaction itself occurs, drug biotransformation into its metabolites and excretion of the unaltered drug or its biotransformation products.

Drugs can be administered by two major routes, enteral and parenteral. Enteral route involves drug administered via the gastrointestinal (GI) tract, also includes buccal, sublingual and rectal. Parenteral applies to drugs given by other routes, i.e. intravenous and intramuscular injections, absorption via the skin (percutaneous, intradermal and subcutaneous injections), inhalation, etc.

Regardless of the route of exposure, drug absorption is a requirement for a substance to be capable of producing a pharmacological effect. The absorption of a chemical substance from any site of exposure involves its passage across cellular membranes [1]. Despite the diversity in membrane functions, there is a consensus regarding their basic structure. The basic cell membrane comprises a bimolecular lipid leaflet containing phospholipids, cholesterol and fatty acid esters oriented with their hydrophobic portions inside and their hydrophilic portions facing the outside aqueous environment. Associated with the lipid molecules are globular protein molecules embedded into or passing through the membrane.

Compounds can cross cell membranes in several ways: passive permeation (diffusion) through the lipid bilayer, passive transport through membrane channels or pores, active transport, facilitated transport (carrier-mediated transport) and phagocytosis. However, most of the drugs substances cross cells by passive permeation. In this process, a substance dissolves in the membrane lipid bilayer, permeates through the membrane, and enters into the cytoplasm of the cell. To establish an adequate concentration gradient for passive permeation the substance not only must be soluble in lipids but also must be sufficient soluble in water due to the aqueous nature of the extracellular and intracellular spaces. Therefore, lipid–water partitioning is the more important factor governing a substance's ability to diffuse through cell membranes [1,2].

The drug transport rate across membranes is quantified by the membrane permeability coefficient, P_m. P_m is actually the linear velocity of drug movement through the membrane and it is lineally related with the drug membrane partition coefficient, K_m, and its membrane diffusion coefficient or drug diffusivity, D_m:

$$P_m = \frac{D_m K_m}{L} \quad (1)$$

being L the thickness of the bilayer membrane. K_m is the major source of variation of drug permeability, though drug passive diffusion through cell membranes also depends on D_m. It has been experimentally demonstrated that solute diffusivity through a lipidic membrane depends on molecular size or molecular weight of the drugs according to the equation:

$$D_m = D_0 e^{-\beta MV} \quad (2)$$

where β is a constant, D_0 the diffusivity of a hypothetical molecule having molecular volume equal to zero, and MV is the molar volume. For drugs with similar molecular volume, the differences in their permeability will be only due to differences in membrane partition coefficients, K_m [3].

The extent of absorption, expressed as percentage, is defined as:

$$\%A = \frac{[\text{drug}]_1}{[\text{drug}]_1 + [\text{drug}]_2} \times 100 \quad (3)$$

being $[drug]_1$ and $[drug]_2$ the absorbed and non-absorbed drug concentration in the absorption site, respectively.

$\%A$ is related with drug membrane partition coefficient K_m as an hyperbolic function:

$$\%A = \frac{K_m}{1 + K_m} \times 100 \quad (4)$$

$$K_m = \frac{[drug]_1}{[drug]_2} \quad (5)$$

Physico-chemical properties are critical determinants of a substance's ability to be absorbed. Quantitative structure–activity relationships (QSARs) are mathematical models that statistically relate the biological activity of a compound to its physico-chemical properties. Several studies have shown their importance for the prediction of drug permeability [4–10].

The dynamic processes of drug action are considered to have much in common with the basic processes of chromatographic/electrochromatographic separations. Under adequate experimental conditions, the same basic properties-hydrophobic, electronic and steric-determine the behaviour of chemical compounds in both the biological and chromatographic/electrophoretic environments. In addition, none of the essential chromatographic/electrophoretic or pharmacokinetic/pharmacodynamic processes except metabolism implies the breaking or the formation of bonds in the drug [11]. Therefore, chromatography and electrophoresis can be used as powerful techniques for estimating physico-chemical parameters and biological activities. In addition, chromatographic/electrophoretic techniques are dynamic systems that permit the strict control of experimental conditions thus very reproducible retention/migration data can be obtained. The application of retention parameters to obtain descriptive and predictive models of pharmacological responses gives rise to a new field, quantitative retention–activity relationships (QRARs).

In this paper the separation methods based on amphiphilic structures developed for the obtaining of models that describe gastrointestinal and dermal drug absorption are reviewed.

2. In vitro methods for predicting oral drug absorption

Oral drug delivery is the preferred route of drug administration. The major absorption barrier to orally administered drugs is the intestinal mucosa where drugs are generally absorbed by a passive diffusion mechanism.

In order to obtain models for predicting oral drug absorption different predictive variables have been used which include the use of physico-chemical parameters, permeability data obtained from cell culture lines or artificial membranes, liposome–water partition coefficients and chromatographic/electrophoretic retention data of drugs.

In general, univariate hyperbolic models for oral drug absorption have been obtained when structural diversity is introduced which agrees with the absorption-drug membrane partition coefficients dependence (see Eq. (4)). Their main usefulness is as fast primary screening tools that can provide key information about the potential transport properties of new compounds during the drug discovery processes. However, they fail when factors that decrease the absorption of drugs exist, like poor dissolution of the compound, drug precipitation at the absorption site, chemical and bacterial degradation at the absorption site, and the first pass metabolism in the intestinal cells and the liver and also if compounds are actively transported or transported by paracellular pathway.

2.1. QSAR models

Physico-chemical parameter-based estimations methods [4,6,12–19] are attractive because of their throughput capacity, reproducibility and because they do not involve cumbersome cell cultivation [20]. However, single physico-chemical descriptors are not reliable predictive parameters of drug absorption, as the correlations often break down when structural diversity is introduced [12,21,22]. For this reason more complex models involving several molecular descriptors such as hydrophobic parameters ($\log P$ or $\log D$), hydrogen-bonding ability descriptors, molecular size (e.g. molecular weight), solubility and computational parameters derived from three-dimensional structures of drugs (e.g. dynamic polar molecular surface area (PSA_d)), have been developed using different multivariate techniques, e.g. multiple linear regression (MLR) [6,15,17], partial least squares (PLS) [13,16,19] and artificial neural networks (NN) [4].

2.2. Membrane-based permeability assays

Most of the in vitro studies examining drug uptake and transport in the intestinal epithelium have utilised different anatomical structures as everted sacs, brush border membrane vesicles, isolated cells, and intestinal rings [23]. More recent works have focused on Caco-2 cells, a colorectal adenocarcinoma cell line of human origin, as a model for studying intestinal transport [21–32]. 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. Caco-2 cells to a certain degree mimics additional transport mechanism such as paracellular transport trough tight-junctions, active transport via transporters, as well as efflux phenomenum induced by P-glycoproteins, the latter of which work against the permeability process and can complicate data interpretation for some compounds. However, the use of Caco-2 cell lines has several drawbacks [32]: (i) the lack of standardisation in cell culturing and experimental procedures makes very difficult

to compare inter-laboratory permeability data, (ii) Caco-2 cell monolayers have a laborious cultivation [20,28], the experiments requires up to 20 days for the preparation of stable monolayers, and the cells must be maintained in protective environments, free from contamination, and examined for tight-junction formation prior to use [32], (iii) the method requires careful sample analysis to calculate permeability correctly, (iv) its use as high-throughput tool is limited by the long membrane growth cycle and high implementation cost [33]. The use of Caco-2 continues, but the future of its role as a primary screen is coming into question [32].

As an alternative to Caco-2 cell lines, the use of parallel artificial membrane permeability assay (PAMPA) is gaining acceptance. PAMPA use synthetic membranes prepared from single or mixtures of phospholipids [32–36]. Advantages of PAMPA versus Caco-2 cells are its wider pH-range and higher dimethylsulfoxide content tolerability, which allows for better coverage of intestine pH range and higher sample solubility, respectively, low cost and easy implementation. However PAMPA technique also present some drawbacks, the methodology is time consuming because in addition to the incubation time, the determination of permeability data of each compound requires the accurate determination of drug concentration in the donor and acceptor wells. The results and the permeation rate depends on the nature of support material for phospholipid membrane.

2.3. Chromatographic methods

Chromatographic models to predict drug absorption are experimentally easier than membrane-based permeability assays. Shared advantages of chromatographic models to predict drug absorption are their experimental simplicity, low cost, accuracy and high-throughput. Different chromatographic systems have been proposed to predict oral drug absorption. The use of conventional reversed-phase columns only has proven to provide adequate correlations for homologous series of compounds. The inclusion of amphiphilic structures in the stationary and/or mobile phases is a pre-requisite to emulate interactions of drugs with the phospholipids bilayers in the membranes.

2.3.1. Immobilised artificial membrane columns

Immobilised artificial membrane columns (IAM columns) contain different types of phospholipid monolayers that are covalently bonded to silica particles [37–39]. IAMs may contain a single or a mixture of phospholipids mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidic acid (PA) and phosphatidylserine (PS) ligands.

Pidgeon et al. [38] used an ^{ether}IAM.PC$^{C10/C3}$ column to predict drug absorption of 11 structurally cephalosporin analogs ($r^2 = 0.89$). A linear correlation was obtained between the permeability coefficients through Caco-2 cells measured by Artursson et al. [21] and the retention factors obtained at pH 7.4 ($\log k_{IAM}$) for 11 unrelated drugs ($r^2 = 0.58$). This relationship was slightly improved when corrections for the size of molecules were made ($\log k_{IAM}$/MW) ($r^2 = 0.73$). A linear relationship was also found between the percentage of absorption in perfused rat small intestine and $\log k_{IAM}$ at pH 5.4 ($r^2 = 0.63$), this model was also improved when $\log k_{IAM}$/MW was used instead ($r^2 = 0.74$).

IAM methodology is experimentally simple, and large-volume screening of experimental compounds for drug absorption is possible. However, for hydrophobic compounds it is necessary to obtain retention factors of compounds at different organic modifier concentrations to calculate by extrapolation the retention data at 0% modifier concentration.

2.3.2. Immobilised liposome chromatography

Drug-liposome partitioning has also been used as a tool for predicting human passive intestinal absorption [20]. Traditionally, drug-liposome studies have been carried out with free liposomes suspended in aqueous solutions where the partitioning of solutes between the liposome bilayers and water is determined [20,40,41].

Immobilised liposome chromatography (ILC) uses stationary phases where liposomes are steric, hydrophobic, electrostatic or covalently immobilised into gel beads. The preparations of the columns is very simple and they are stable during long time. A great advantage of ILC chromatography respect to IAM chromatography is the absence of organic solvents in mobile phase for eluting the most hydrophobic compounds [42]. ILC turned to be a good approach to study drug-membrane interactions since the phospholipids ratios used to prepare the liposomes can be modulated to get liposomes with phopholipid, protein and cholesterol ratios very similar to the composition of the membrane of study.

The research group directed by Lundahl has developed various techniques for immobilisation of liposomes, proteoliposomes, membrane vesicles and red cells/ghosts. Beigi et al. [42] used to prepare ILC columns, egg phosphatidylcholine (EPC) liposomes immobilised by freeze-thawing in small agarose-dextran gel beds (Superdex 200) which were packed into columns. The authors found a hyperbolic relationship between oral absorption in humans (%) and the specific capacity factors obtained in ILC ($\log K_s$) for a set of 12 unrelated drugs.

In another paper, Beigi et al. [43] compared the retention of a set of 17 drugs on EPL (egg phospholipid liposomes) with the retention on egg phosphatidylcholine (PC), membrane lipid liposomes (ML), vesicles and ghosts and, except PC, gave similar results. From the results, the authors concluded that the essential feature of good biomembrane model for drug partitioning analyses is a bilayer heterogeneity mimicking that of natural membranes. The authors also compared the retention of the set of compounds on an IAM-PC column and on the PC liposome column finding a moderate rectilinear correlation ($r^2 = 0.83$). The fact that no better relationship was found despite the head-group identity between both columns supports the idea that the structure of

the hydrophobic region is of key importance on drug partition into such lipid layers. Finally, drug retention on vesicles was related to oral absorption in humans finding low oral absorption at the outskirts of the range $0.6 < \log K_s < 3.0$, whereas drug oral absorption was nearly complete at the intermediate $\log K_s$ values (1.5–2.5). Later, the authors removed several drugs showing non passive diffusion mechanisms from the original model, leading to a slight decrease of absorption for high $\log K_s$ values. Österberg et al. [44] observed that many drugs with $\log K_s$ values >2.5 on PC liposomes were almost completely absorbed in humans, making a dramatic absorption decrease for drugs with such high $\log K_s$ values more improbable. Drugs with $\log K_s$ values higher than 2.5 and low absorption values were found to have efflux transport mechanism and exceptionally strong interactions with the gel beads of the ILC columns.

Liu et al. [45] immobilised unilamellar liposomes in the pores of gel beads by avidin–biotin binding. The membrane partition coefficients values ($\log K_{LM}$) of 29 structurally diverse drugs obtained on an EPC-PS-PE-chol column (EPC: egg yolk phosphatidylcholine; PS: phosphatidylserine; PE: phosphatidylethanolamine; chol: cholesterol) was used to describe drug intestinal absorption obtaining a qualitative sigmoidal relationship. Compounds showing paracellular diffusion, efflux or active transport were found as outliers. The $\log K_{LM}$ values correlated well with those obtained using surface plasmon resonance (SPR) biosensor [46], which is another technique also used to predict oral drug absorption.

2.3.3. Micellar chromatographic methods

Another chromatographic approach used to develop oral drug absorption models is micellar liquid chromatography (MLC). MLC is a mode of reversed-phase liquid chromatography which uses a surfactant solution above the critical micellar concentration as mobile phase [47–50]. The use of micellar solutions produces the adsorption of surfactant monomers to the stationary phase, thus providing it with both hydrophobic and electronic sites of interaction. The retention of compounds in MLC depends on their interactions with the modified reversed stationary phase and micelles present in the mobile phase. In MLC different surfactants-anionic, cationic, zwitterionic and non-ionic-can be used. The adequate selection of the nature of surfactant and mobile phase composition is a key point to emulate biopartitioning process. For structural related compounds and experimental conditions in which all compounds present the same ionisation degree, adequate correlations can be obtained regardless the nature of surfactant and mobile phase composition [51]. However, the use of ionic surfactants like SDS (anionic) or CTAB (cationic) fails to describe biopartitioning process for structurally unrelated compounds [52] and only non-ionic surfactants are able to give good QRAR models.

Detroyer et al. [53] found linear QRAR models by correlating the retention factors in MLC of six β-blocking agents (alprenolol, atenolol, metoprolol, oxprenolol, pindolol and practolol) and the permeability coefficients through Caco-2 monolayers (P_c) and rat intestinal segments (P_{app}). The micellar mobile phase used was 0.1125M SDS (pH 3) + 10% *n*-propanol. The MLC QRAR models were compared with those obtained with $\log P$ values and the literature retention factors on an IAM column concluding that the use of MLC provided better correlation coefficients for that set of compounds.

More recently, Detroyer et al. [54] performed a comparative chemometric study on the retention behaviour of 21 basic pharmaceutical substances (seven psychotropics, one α-adrenoreceptor agonist, eight β-adrenolytics and five antihistaminic drugs) in 10 chromatographic/electrophoretic systems. The systems studied were: MLC using 0.15 M SDS (pH 7.4) + 15% *n*-propanol as mobile phase, MEKC using mixed micelles taurodesocycholate-phosphatidylcholine in borate buffer at pH 8.0 as electrophoretic buffer, IAM, a chiral α_1-acid glycoprotein column (pH 6.5), a Suplex pK_b-100 column (pHs 2.5 and 7.4), a RP-Spheri column (pHs 2.5 and 7.0), an Aluspher RP-select B column (pH 7.3) and a Unisphere PBD column (pH 11.7).

The authors observed that for all chromatographic systems the main retention mechanism was hydrophobicity but some differences were observed in secondary retention mechanisms. The use of amphiphilic structures in either mobile and/or stationary phase introduced an extra interaction respect to classical chromatographic systems. The amphiphilic structures used showed to play a more important role than the difference in technique (LC versus CE, use of adsorbed versus permanently bound amphiphilic structures).

The capability of the chromatographic techniques studied as tools for predicting permeability through Caco-2 monolayers (P_c) of five β-blocking agents (alprenolol, atenolol, metoprolol, oxprenolol and pindolol) was studied. Linear $P_c - k$ or $\log k$ relationships were obtained in all cases (see Table 1). The authors concluded that the use of k instead of $\log k$ as independent variable provided better correlations and the MLC system exhibited the best results.

2.3.4. Biopartitioning micellar chromatography

Our research group has demonstrated that the retention data obtained in a chromatographic system constituted by a C_{18} reversed stationary phase and a polyoxyethylene (23) lauryl ether (Brij35) micellar mobile phase in adequate experimental conditions are helpful in describing the biological behaviour of different kinds of drugs [51,52,55–67]. We call this drug biopartitioning simulation chromatographic system biopartitioning micellar chromatography (BMC).

The usefulness of BMC in describing the biological behaviour of drugs could be attributed to the following features: (i) the characteristics of the BMC systems are similar to biological barriers and extracellular fluids. First, the stationary phase modified by hydrophobic adsorption of Brij35 surfactant monomers structurally resembles the ordered array of the membranous hydrocarbon chains. In addition, the hydrophilic/hydrophobic character of the adsorbed surfactant

Table 1
Reported oral absorption QRAR models for short data series of structurally related compounds ($y = a + bx$)

Chromatographic system	Independent variable	Dependent variable (n)	$a \pm$ ts (p-value)	$b \pm$ ts (p-value)	r^2	S.E.	F (p-value)	RMSEC	RMSECV	RMSECVi
MLC[a]	k	P_c (Caco-2) (5)	n.a.	n.a.	0.98	n.a.	n.a.	n.a.	n.a.	n.a.
	$\log k$	P_c (Caco-2) (5)	n.a.	n.a.	0.91	n.a.	n.a.	n.a.	n.a.	n.a.
MEKC[a]	k	P_c (Caco-2) (5)	n.a.	n.a.	0.88	n.a.	n.a.	n.a.	n.a.	n.a.
	$\log k$	P_c (Caco-2) (5)	n.a.	n.a.	0.85	n.a.	n.a.	n.a.	n.a.	n.a.
IAM[a]	k	P_c (Caco-2) (5)	n.a.	n.a.	0.88	n.a.	n.a.	n.a.	n.a.	n.a.
	$\log k$	P_c (Caco-2) (5)	n.a.	n.a.	0.74	n.a.	n.a.	n.a.	n.a.	n.a.
RP-HPLC[a,b]	k	P_c (Caco-2) (5)	n.a.	n.a.	0.79–0.93	n.a.	n.a.	n.a.	n.a.	n.a.
	$\log k$	P_c (Caco-2) (5)	n.a.	n.a.	0.74–0.95	n.a.	n.a.	n.a.	n.a.	n.a.
BMC[c]	$\log k$	$\log(K_a)$ (9)	-1.54 ± 0.07 (<0.0001)	0.55 ± 0.05 (<0.0001)	0.99	0.02	618.61 (<0.0001)	0.0202	0.0255	0.0270
	$\log k$	log(%A) (9)	0.84 ± 0.07 (<0.0001)	0.42 ± 0.05 (<0.0001)	0.98	0.02	366.88 (<0.0001)	0.0199	0.0297	0.0174
	$\log k$	log(%FA) (8)	1.63 ± 0.04 (<0.0001)	0.23 ± 0.04 (<0.0001)	0.97	0.03	195.28 (<0.0001)	0.0257	0.0339	0.0344

[a] Results taken from [54].
[b] Seven chromatographic systems.
[c] Results taken from [68]—n: number of molecules included in the model; ts: 95% confidence interval for coefficient estimates; r^2: r-squared statistic; S.E.: standard error of the estimate; F: modelled-to-residual variance ratio; p-value: measure of significance of a model derived from ANOVA; RMSEC: root mean square error of calibration; RMSECV: root mean square error of cross-validation (leave-one-out); RMSECVi: root mean square error of cross-validation (leave-one-out) for interpolated data; n.a.: non available data.

monomers resembles the polar membrane regions. Second, Brij35 micellar mobile phases prepared at the specific physiological conditions could also mimic the environment of drug biological partitioning. (ii) The retention of a drug in this chromatographic system is mainly governed by its hydrophobic, electronic properties and, to a less extent, by its steric properties. These features of compounds also determine their passive permeability across cell membranes.

In order to study the similarity between BMC and other well-recognised natural systems that mimics biomembranes, we correlated [68] the retention data on BMC using 0.02 M Brij35 (pH 7.4) as mobile phase, $\log k_{BMC}$, for a heterogeneous set of 16 compounds (benzodiazepines, β-blockers and phenothiazines) with the retention factors, $\log K_s$, on immobilised liposome or vesicle columns of different nature: lipids extracted from human red cell membrane vesicles (MLs), cytoskeleton-depleted human red cell membrane vesicles (vesicles), human red cell membranes (ghosts) and egg phospholipid liposomes (EPLs). The K_s values were taken from ref. [43]. Excellent linear correlations were obtained in all cases ($r^2 \geq 0.96$) indicating that the BMC system mimics adequately the relative importance of drugs interactions with biomembranes.

The differences between the slopes probably reflect the extent of polar and non-polar interactions that occur in the naturals biomembranes. In this sense the systems BMC, MLs and vesicles show similar hydrophilic/hydrophobic character (slopes near 1). In ghost, the membrane with highest hydrophilic character, the hydrophilic interactions are more important. On the contrary, in EPLs, probably the most hydrophobic system mainly constituted by phosphatidylcholine and phosphatidylethanolamine, the hydrophobic interactions are more important than the hydrophilic [43].

In the same study, the correlation between some parameters related to passive transport through the gastrointestinal barrier, and the retention in BMC were also evaluated. Fig. 1 shows the relationships between the K_a values (absorption rate constant in rat intestine) and %*A* (percentage of absorption in rat intestine in 1 h) for 9 barbiturates, %FA (percentage of the absorbed fraction after oral administration in humans) for eight β-blockers and the $\log k_{BMC}$ data. Table 1 shows the statistical features of these models. Strong correlations were obtained in all cases ($r^2 > 0.97$) and the predictive ability of the models suggested that predictions based on interpolations and extrapolations should be reasonably adequate.

In a recent article the usefulness of BMC in predicting oral drug absorption in humans was evaluated [69]. For this purpose 74 structurally diverse drugs absorbed by a passive process were selected. The model drugs were chosen to cover a wide range of absorption after oral administration (16–100%) as well as a wide range of physico-chemical properties such as hydrophobicity ($\log P$ ranged between 0.34 and 5.20) and charge (cationic, anionic and neutral compounds).

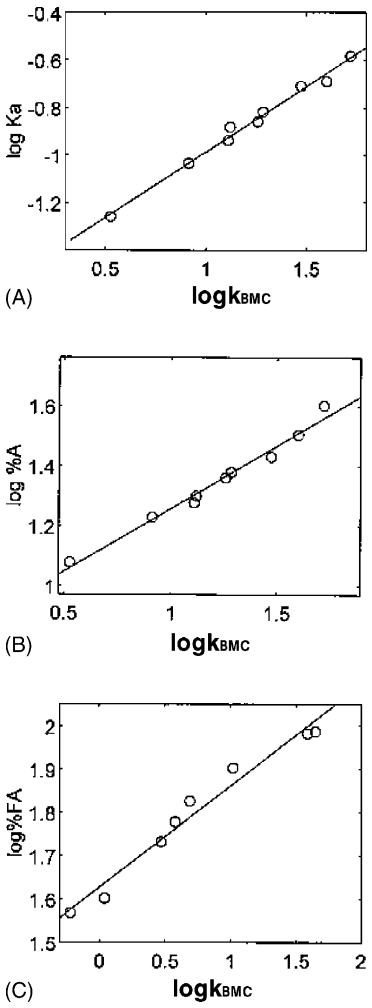

Fig. 1. Relationships between retention data in BMC ($\log k_{BMC}$) and: (A) absorption rate constant in rat intestine (K_a) for barbiturates; (B) percentage of absorption in rat intestine of barbiturates after 1 h (%*A*); (C) percentage of absorbed fraction after oral administration in humans (%FA) for β-blocking agents. The retention was obtained in all cases with a 0.02 M Brij35 mobile phase at pH 7.4.

Hyperbolic relationships between the retention factor of drugs in 0.04 M Brij35 at pH 6.5 (the average pH of the small intestine) (Fig. 2) and 7.4 (the plasmatic pH value) and their oral drug absorption values were obtained. The initial steep-slope of the model limits the prediction accuracy for low to medium absorption-drugs. This fact has been also observed in Caco-2, PAMPA and in situ or in vivo permeability models. However, retention in BMC can be used to classify drugs into two categories according to their transport properties if passive diffusion is the mechanism responsible of absorption:

1. For drugs with retention factors ranged between $0.2 < k_{BMC} < 3$ at pH 6.5 absorption problems can be expected. 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

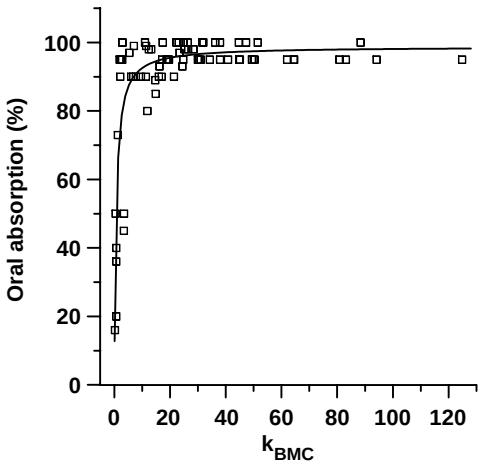

Fig. 2. Oral drug absorption-k_{BMC} model obtained using a 0.04 M Brij35 at pH 6.5.

solubility are poorly absorbed and therefore pose significant problems for effective oral delivery [30].

2. For drugs which show retention factors higher than 3 at pH 6.5 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 [30].

The oral absorption-BMC retention models obtained at pH 6.5 and 7.4, respectively, are:

$$\text{oral absorption (\%)} = \frac{100k_{BMC}}{(0.7 \pm 0.2) + (1.02 \pm 0.03)k_{BMC}} \quad (6)$$

where $n = 74$, $r^2 = 0.72$, S.E. $= 9.8$, $F = 3185$

$$\text{oral absorption (\%)} = \frac{100k_{BMC}}{(1.0 \pm 0.3) + (1.00 \pm 0.03)k_{BMC}} \quad (7)$$

where $n = 74$, $r^2 = 0.72$, S.E. $= 9.8$, $F = 3174$ and the numbers in parenthesis are the asymptotic confidence intervals at a 95% confidence level.

Similar models can be obtained using the retention data of a training set with a reduced number of compounds (atenolol, chlorpromazine, hydrocortisone, imipramine, mannitol, metoprolol, propranolol, quazepam, terbutaline and testosterone), that allow us to check the model along the time. These models can be used to predict the oral absorption of new drugs. The confidence limits for predictions are ±16%, which are in the usual range of the reported absorption values.

The comparison between the oral drug absorption models obtained using the retention data in BMC and apparent permeability in Caco-2 cells [31] and PAMPA membranes [33] for the same set of compounds (Fig. 3) showed similar trends in both cases. In addition, better statistically models were obtained using the retention in BMC.

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

3. In vitro methods for predicting drug skin permeability

The penetration of chemicals through the skin is an area of increasing interest to the pharmaceutical and cosmetic industries, as well as in dermal exposure and risk assessment processes. However, measurement of the penetration of chemicals through skin is laborious and can involve ethical difficulties with either human or animal experiments. Hence there is a need for in vitro methods capable of predicting dermal absorption.

The skin has two basic layers, the epidermis and the dermis, and contains some appendages (hair follicles, sebaceous glands and sweat glands) that provide aqueous channels into the skin. For a substance to be absorbed into the body following dermal exposure, it must initially dissolve in the stratum corneum (the outermost sub-layer of the skin), and then diffuse through the remaining sub-layers of the epidermis and into the dermis, where it will eventually diffuse into the blood capillaries. The stratum corneum, which consists of densely packed, dead, keratinized cells, is thought to provide the major barrier in solute penetration. Diffusion through the highly lipophilic stratum corneum, can occur only by passive diffusion. Passage through the remaining sub-layers of the skin, progressively less lipophilic, is much more rapid [1].

Measurements of the percutaneous absorption of chemicals, expressed either as percent of absorption or as permeability coefficient (K_p), has been achieved using numerous in vivo experiments, by monitoring in vivo drug release in live animals or human volunteers, ex vivo, by employing excised skin from human or animal sources, and in vitro, using techniques such as synthetic model membranes as diffusion barriers [70,71]. Schmook et al. compared various types of skin that could serve as a replacement for human skin in in vitro penetration studies [72]. They studied the penetration properties of human, pig and rat skin, a living skin equivalent (Graftskin^{TM} LSE^{TM}) and a human reconstructed epidermis Skinethic^{TM} HRE. The authors found that among all these skin types, pig skin is the most suitable model for substituting human skin.

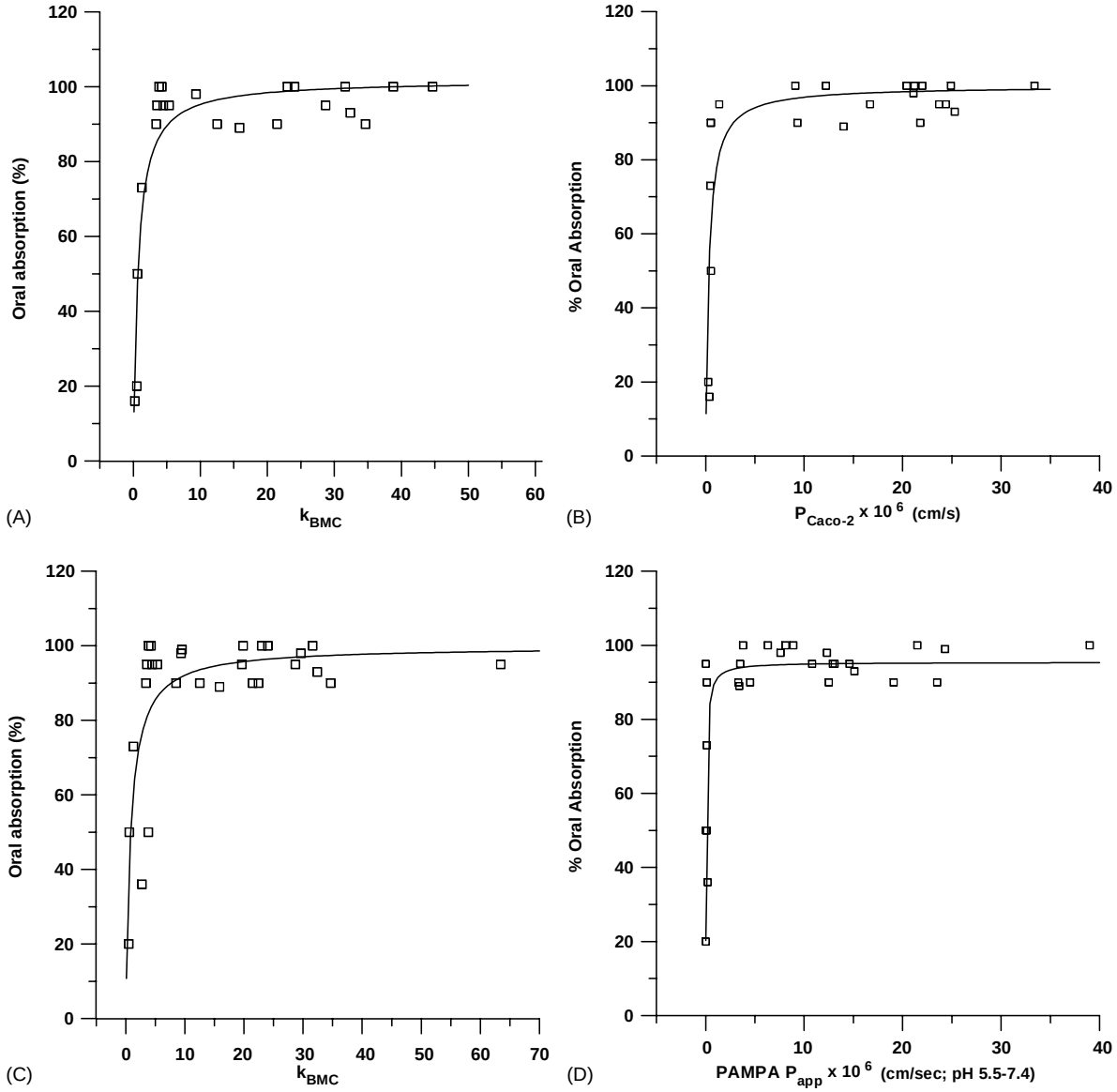

Fig. 3. Comparison between the oral drug absorption models obtained using: (A) retention data in BMC using a 0.04 M Brij35 mobile phase at pH 7.4 (B) and apparent permeability in Caco-2 cell lines for the same set of compounds. A similar comparison is made between the BMC (C) and the PAMPA (D) models. The PAMPA P_{app} values represent the highest permeability value obtained between pH 5.5 and 7.4.

3.1. QSAR models

The rate, K_p, and amount of percutaneous absorption of a compound highly depends on both the physiologic characteristics of the skin (e.g. skin thickness, hydration and temperature) and the physico-chemical nature of the compound (e.g. hydrophobicity, polarity, physical state, water solubility and molecular weight or size) [73].

From the current literature, two general types of structure-activity models, empirical and theoretical, have been proposed to estimate skin permeability coefficients of chemicals [73]. Theoretical skin permeability models are deduced taking into account the possible routes of penetration and the interactions between the permeating chemicals and the skin constituents. On the other hand empirical models attempt to relate experimental permeability constant values, $\log K_p$, to several physico-chemical parameters as octanol–water partition coefficient [74–77], molecular size descriptors [73,78,79] (i.e. molecular weight, molecular volume, molar refractivity and molecular connectivity indexes) or hydrogen bond descriptors [79–89] (the number of hydrogen bonds that may be formed by a compound (H_b) [80], hydrogen bond donor (H_d) and acceptor (H_a) ability of a compound [81,82], the HYBOT plus series of descriptors introduced by Raevsky et al. [83] and melting point of the chemicals as suggested by Moss et al. [84]. This type of approach can be useful to obtain a first crude estimate of the permeability coefficient of many compounds.

3.2. Chromatographic methods

In comparison with oral drug absorption QRAR models, few chromatographic studies have been carried out to describe and predict drug skin permeability.

3.2.1. Immobilised artificial membrane columns

Nasal et al. [90] correlated the retention data on an IAM column ($\log k_{IAM}$) for several sets of structurally related compounds with their corresponding human skin permeability coefficients ($\log K_p$). For a set of 10 steroids, good linear correlation was obtained ($r^2 = 0.89$). For 14 phenolic compounds a parabolic relationship was obtained, ($r^2 = 0.64$) giving a much better fit when the traditional octanol–water partition coefficient was used ($r^2 = 0.89$). On the contrary, good correlation between $\log K_p$ and $\log k_{IAM}$ for 5 compounds that permeate through human skin in ionised form ($r^2 = 0.82$) was found and no correlation was obtained when the permeability was plotted against $\log P$. This fact could indicate that retention on IAM phases seems to be more adequate approach for the description and prediction of bioactivities for the ionised compounds.

Barbato et al. [91] studied the relationships between the skin permeability of a set of 12 structurally unrelated drugs and their corresponding retention on an IAM column. No correlation was found between K_p and k_w thus indicating that retention on IAM column cannot account for all factors affecting the permeation of these compounds through human skin. A subsequent variable ($\Delta \log k_w$) representing the differences between k_w values experimentally obtained and those expected from their $\log P$ values was derived. The authors indicate that this variable describes electrostatic and hydrogen-bonding interactions between solutes and the phospholipid head-groups in the IAM phase. However, in order to confirm the usefulness of this kind of IAM approach to predict skin permeability, larger dataset of compounds including both charged and neutral compounds at pH 5.5 (dermal pH) should be included.

In order to obtain a chromatographic model of percutaneous permeation closer to skin composition an attempt was undertaken by Turowski and Kaliszan to produce HPLC columns comprising keratin [92] since this protein is present in large amounts in the outermost layers of the epidermis. This approach consist on physically immobilise keratin onto chromatographic silica. When using a combination model of $\log k_{IAM}$ and $\log k_{keratin}$ for 17 structurally unrelated compounds, the predictive model for the human skin permeability, expressed as $\log K_p$, slightly improved comparing to the model using $\log k_{IAM}$ solely as predictive variable:

$$\log K_p = (-6.42 \pm 0.14) + (1.46 \pm 0.14)\log k_{IAM} \quad (8)$$

where $n = 17$, $r^2 = 0.81$, S.E. $= 0.47$, p-value $< 10^{-4}$

$$\log K_p = (-6.56 \pm 0.13) + (1.9 \pm 0.2)\log k_{IAM} - (1.0 \pm 0.4)\log k_{keratin} \quad (9)$$

where $n = 17$, $r^2 = 0.87$, S.E. $= 0.40$, p-value $< 10^{-4}$.

The new keratin based phase strongly retains acidic solutes and may be used to quantify differences in drug interaction with keratin.

3.2.2. Biopartitioning micellar chromatography

Martínez-Pla et al. [93] developed a QRAR model based on BMC to describe and predict skin permeability. For this purpose, a set of 42 unrelated chemicals (cationic, anionic and neutral) that covered a wide range of skin permeability values ($\log K_p$ ranged between -5 to 0, K_p expressed in cm h^{-1}) was selected and their retention in BMC using 0.04 M Brij35 at pH 5.5 was obtained.

In order to study the importance of variables in the prediction of skin permeability values (Y-block), a partial least squares analysis (PLS) was performed. The variables of compounds included in the X-block were: $\log k_{BMC}$ using 0.04 M Brij35 at pH 5.5 mobile phase, steric descriptors as molecular weight (MW), molar refractivity (MR), molar volume (MV) and parachor (Pr); electronic parameters as the polarizability, molar total charge (α) and the number of hydrogen bond donors per molecule (HBD) and physical properties as water solubility and melting point (MP). A high correlation between all the steric descriptors, polarizability and melting point was observed. Non-significant variables were eliminated step by step, re-analysing each time the PLS model. Finally a PLS model was obtained by using the three significant predictor variables $\log k_{BMC}$, melting point and molecular weight. The stepwise multiple linear regression method provided a final model where $\log k_{BMC}$ and melting point (MP) were selected as predictive variables. Melting point is a physico-chemical property strongly dependent on molecular weight and hydrogen-bonding. The equation of the fitted MLR model was:

$$\log K_p = (-3.3 \pm 0.3) + (1.3 \pm 0.2)\log k_{BMC} - (0.0080 \pm 0.0014)\mathrm{MP} \quad (10)$$

where $n = 42$, $r^2 = 0.83$, S.E. $= 0.51$, $F = 93$, p-value $< 10^{-4}$.

All the regression coefficients and the model resulted to be statistically significant at the 99% of confidence level, ($P < 0.0001$). The explained variance was 83%, this value is considered adequate for skin permeability data taking into account the intrinsic variability of K_p data available (approximately 25%, [84]), due to inherent variability in the tissue used and experimental conditions.

Fig. 4 shows the experimental versus predicted (fitted and cross-validated) values of permeability constants. The intercept was statistically equal to zero (0.0 ± 0.4) and the slope statistically equal to one (1.00 ± 0.15). Thus, the proposed retention-skin permeability model adequately describes and predicts skin permeability of drugs. In order to check this statement, the predicted permeability values for a set of non-steroidal anti-inflammatory drugs and opioid analgetics non-included in the construction of the model were compared with those reported in bibliography observing a reasonably good concordance.

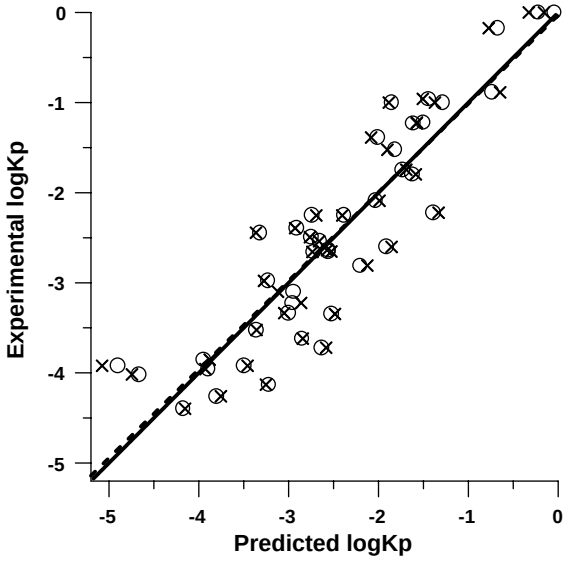

Fig. 4. Validation plot of the QRAR skin permeability model obtained using a 0.04 M Brij35 mobile phase at pH 5.5: experimentally observed vs. predicted log K_p values. Fitted (○, solid line) and cross-validated (×, dashed line) values.

An important advantage of the proposed QRAR model based on BMC is that it can be used for the prediction of skin permeability at any pH. For this purpose it would be enough to measure the retention at the desired pH and interpolate in the proposed QRAR model.

4. Progress on the development of biopartitioning capillary electrophoretic systems for modelling drug absorption

Recently, capillary electrophoresis (CE) has been applied as a nanoscale analytical tool for the study of interactions between drugs and proteins [94], liposomes [95], vesicles [96] and micellar aggregates [97,98]. The migration index (MI) obtained in microemulsion electrokinetic chromatography (MEEKC) has proven to provide a highly reproducible hydrophobicity scale [99]. Good correlations between MI and log *P*, liposome–water partition coefficients, retention data in IAM and α_1-acid glycoprotein (α_1-AGP) columns have been reported [100].

Liposome electrokinetic chromatography (LEKC) is another approach to determine liposome–water partition coefficients. In this technique liposomes are added to the electrophoretic buffers. The liposomes act as a pseudo stationary phase where solutes are separated according to their differences in electrophoretic mobilities and in liposome–water partitioning coefficients. LEKC does not provide any particular advantage as an analytical technique but it is a powerful tool for the study of drug membrane interactions [101,102]. In contrast to IAM and ILC, LEKC is a solution-based technique, i.e. no solid stationary phase is present in the system. Thus, it provides better flexibility and versatility over IAM and ILC.

5. Quantification, validation and evaluation of predictive models

A prior estimation of a drug biological activity or property, avoiding the use of expensive, tedious and sometimes irreproducible experiments (i.e. animals, cell lines), is the objective of some research areas such as QSAR (i.e. physico-chemical or structural descriptors, computer programs) and QRAR (i.e. retention factors, electrophoretic mobility). Two reasons to estimate in these ways a biological endpoint are speed and economy. A third would be the reliability, since in vivo measurements have some practical problems affecting data quality; however, this aspect deserves more attention. For instance, the usual strategy (due the lack of ‘accepted reference’ or ‘conventional true’ biological data) involves the use of some experimental biological activities in the process of estimating future ones; so the problem to solve is included into the process of solution.

Cronin and Schultz have recently pointed-out some usual pitfalls (also bad practices) related to QSAR [103]. They focus the problems in three items: biological data, descriptors and statistics. Part (not all) of these observations, would also be applicable to QRAR models. Particular aspects related to reliability of QRAR (compared to QSAR) models have recently been reviewed [66]. A possible protocol to model biological data, which summarises the information on those reviews, is presented and commented (critical aspects affecting analyst’s decision-making) in Table 2.

Step 1 requires the unambiguous definition of biological endpoint and the selection of a single experimental, preferably standardised, protocol. In step 2, the use of compilation databases from open literature, which provide large endpoint uncertainties, has been not recommended [103]. However, the risk of undetectable laboratory (systematic) errors, intrinsic to the data generated ‘in-house’ (single laboratory data sets) has also to be considered. Probably, data obtained in well-controlled collaborative studies (or peer verified method program) can be a suitable solution to obtain biological data with reasonable quality. When possible, general models based in heterogeneous groups of drugs are preferable, as occurs for instance with a drug oral absorption model [69]. However, in other cases, it is only possible to make individual models for a set of homogeneous drugs. In the later case, the probability of having to work with ‘short data series’, with their corresponding risks [66], increases. In addition, similarities between models for different drugs families or for different properties for a family increase the confidence of the results, as occurs for three models relating neuroleptic effect duration, ED_{50} and IC_{50} of a set of phenothiazines using log *k* in BMC conditions [66].

Steps 3 and 4 are the key of the process. The use of as few descriptors as possible, preferably fundamental ones in order to obtain simple transparent models (i.e. in order of preference MLR > PLS > NN), which can generate globally interpretable, sometimes portable (simple equations) models, have been recommended [103]. However, MLR-QSAR

Table 2
Protocol for modelling drug biological data

Step	Critical and decision-making aspects
1. Define unambiguously the endpoint (response variable, vector $\boldsymbol{y}$)	It would be consistent with a single experimental, preferably standardised, protocol
2. Select the appropriate biological data to obtain $\boldsymbol{y}$	Single laboratory data sets vs. compilation databases from open literature Homogeneous vs. heterogeneous (i.e. more than a family of drugs) data sets Number of data available ('large' vs. 'short' data series)
3. Select the appropriate descriptors (predictor variables, matrix $\boldsymbol{X}$)	Large vs. selected number of descriptors
	Descriptors capable of mechanistic evaluation (i.e. $\log P$) vs. descriptors difficult to interpret (i.e. molecular conectivities) Estimated descriptor (software) vs. experimental (i.e. $\log k$)
4. Develop a model to predict future biological data ($\hat{\boldsymbol{y}} = \boldsymbol{Xb} + \boldsymbol{e}$)	Qualitative (SAR, RAR) vs. quantitative (QSAR, QRAR) relationships
	Linear vs. non-linear algorithms Simple transparent (i.e. univariate equation based in $\log P$, $\log k$) vs. complex (i.e. PLS, NN) models
5. Identify outliers	Statistical vs. explainable outliers Outliers associated to $\boldsymbol{y}$ vs. $\boldsymbol{X}$ data Elimination vs. keeping into model
6. Validate the model	Statistical fit (i.e. r^2, or preferably r^2-adj., S.E., F, RMSEC) vs. cross-validation (i.e. RMSECV and RMSECVi) or independent data set-validation (i.e. RMSEP) Significance of coefficients ($\boldsymbol{b}$)
7. Use the model to perform future estimation ($\hat{\boldsymbol{y}}$)	Uncertainty of $\boldsymbol{y}$-estimations ($\hat{\boldsymbol{y}}$) Interpolation vs. extrapolation
8. Update the model	It would be necessary to include new or improved information in $\boldsymbol{y}$ and/or $\boldsymbol{X}$ data to confirm or actualise (or refuse) old models

$\boldsymbol{b}$: vector of coefficients; $\boldsymbol{e}$: vector of $\boldsymbol{y}$-residuals; r^2-adj.: coefficient of determination adjusted for degrees of freedom; RMSEP: root-mean-square error in prediction; MLR: multiple linear regression; PLS: partial least squares; NN: neural networks; SAR: structure–activity relationships; RAR: retention–activity relationships; see Table 1 for further details.

models can fail in the cases of collinearity, non-linearities and 'short data series' [66,103]. QRAR models based on $\log k$ can overcome those problems [103], still conserving simplicity, transparency and interpretability (i.e. $\log k$ in BMC conditions has been related mainly to fundamental properties as $\log P$ and charge [55]).

On the other hand, the obsession for obtaining a nice statistical fit in QSAR or QRAR models, does not justify sacrificing the simplicity of the model, for instance, including more and more descriptors. Particularly when in many situations qualitative relationships are also valuable [66], sometimes inevitable, according with uncertainty in the data, changes in the $\boldsymbol{y}$–$\boldsymbol{X}$ trend, etc. In addition, the reliability of a quantitative model depends on the presence of outliers (step 5) and on the validation (step 6) and predictive ability (step 7) of the model. Outliers that can be explained (i.e. particular mechanism of action or physical effects) can be removed. Otherwise, it is not justifiable their elimination although it improves the statistical fit [103], specially when statistical fit has poor validation capacity and cross-validation or external validation are preferable [66,103]. The use of QRAR models based on a single descriptor (i.e. $\log k$) facilitates the identification of $\boldsymbol{y}$-outliers, since $\boldsymbol{X}$-outliers are improbable (high precision is attributed to chromatography) or at least easily to detect (repeating the experiment due to its rapidity). The more complex the model is (i.e. QSAR) the more difficult is to associate the outlier nature since both $\boldsymbol{y}$ and $\boldsymbol{X}$ (various variables) are subjected to errors. In this case, tools as PLS (better than MLR and NN) can facilitate the identification of outliers.

The indispensable tasks of reliably determining the significance of coefficients and uncertainty of future predictions can fail using the classical MLR-QSAR due to collinearity (PLS can be preferable in such instances). Again, this task is simple in those cases in which a univariate linear or polynomial relationship can be modelled (i.e. using $\log$ k data) [66]. At this point, the modeller do not have to expect a better prediction limits than the own uncertainty in the data (particularly $\boldsymbol{y}$-errors), which is also related to the degree of elimination of outliers [103].

6. Conclusions

Pharmaceutical companies spend hundreds of millions of dollars developing drugs to be administered orally. On the other hand, the penetration of chemicals through the skin is an area of increasing interest to the pharmaceutical and

cosmetic industries. Drug candidates are screened for their absorption potential (mainly oral absorption) early in the discovery and development phase, when investment in a compound is low, as a filter to identify drug candidates.

Different permeability techniques have been described in the literature, but most of them require too much material for analysis or cannot be implemented in a high-throughput environment.

Biopartitioning chromatographic systems which encompasses the main interactions between a drug and biological membranes (hydrophobic, electronic and steric contributions), preserve the intrinsic advantages of HPLC measurements as reproducibility, speed, an easy automation. These features guarantee their progressive incorporation into the drug discovery and development schemes.

7. Nomenclature

%*A*, %FA	percentage of absorbed fraction of drug
D_0	diffusivity of a hypothetical molecule with a molecular volume equal to zero
D_m	membrane diffusion coefficient or drug diffusivity
$\log D$	logarithm of the partition coefficient in the system octanol–water at a given pH
H_a	hydrogen bond acceptor ability
H_b	number of hydrogen bonds that may be formed by a compound
H_d	hydrogen bond donor ability
k	retention factor (k_{IAM} measured in IAM; k_{BMC} measured in BMC; $k_{keratin}$ measured on a column with immobilised keratin)
K_a	absorption rate constant
K_{LM}	membrane partition coefficients (measured in ILC)
K_m	membrane partition coefficient
K_p	skin permeability coefficient
K_s	specific capacity factors (measured in ILC)
k_w	retention factor extrapolated to pure aqueous medium
P_c, P_{app}	permeability coefficients through Caco-2 monolayers or intestinal segments
P_m	membrane permeability coefficient
Pr	parachor
$\log P$	logarithm of the partition coefficient in the system octanol-water

Greek letter

α	molar total charge

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