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Potential of biopartitioning micellar chromatography as an in vitro technique for predicting drug penetration across the blood–brain barrier

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Abstract

The blood–brain barrier (BBB) is considered to be the main barrier to drug transport into the central nervous system (CNS). The BBB restricts the passive diffusion of many drugs from blood to brain. The ease with which any particular drug diffuses across the BBB is determined largely by the molecular features of drugs, and it is therefore possible to predict the BBB permeability of a drug from its molecular structure. Biopartitioning micellar chromatography (BMC), a mode of micellar liquid chromatography that uses micellar mobile phases of Brij35 in adequate experimental conditions, can be useful in mimicking the drug partitioning process into biological systems. Retention in BMC depends on the hydrophobicity, electronic and steric properties of drugs. In this paper, the usefulness of BMC for predicting the BBB penetration ability of drugs expressed as the brain/blood distribution coefficient (BB) is demonstrated. A multiple linear regression (MLR) model that relates the BB distribution coefficients data with BMC retention data and total molar charge is proposed. The model is obtained using 44 heterogeneous drugs including, neutral, anionic, and cationic compounds. A comparison with other reported methodologies to predict the BBB permeability is also presented.

Keywords: Drug penetration; Blood–brain barrier; Biopartitioning micellar chromatography

1. Introduction

The development of combinatorial chemistry has made possible to synthesize hundreds of potentially active drugs. The need to find a tool for estimating the biopharmaceutical parameters of new compounds supports the postulation of predictive models as a complement to conventional assays that can reduce the use of animal experimentation, the cost and can save time.

To directly affect central nervous system cells a drug must appear in the fluid environment of these cells. The distribution of many drugs to brain is quite different from that to other organs. The major factor creating this difference is the blood–brain barrier (BBB). The blood–brain barrier is formed by high resistance tight junctions between adjacent endothelial cells in the cerebral micro-vessel walls and between the epithelial cells of the chord plexus. This tight junction inhibits the passage of hydrophilic compounds excluding molecules with a diameter larger than 20 Å. Thus, the hydrophobicity of compounds has long been considered to determine the rate at which they are capable of entering the brain via the lipid-mediated pathway [1].

Although some drugs use transporters, most drugs enter the brain by passive diffusion through the endothelial cells, which depends on their hydrophobicity, degree of ionization, molecular weight (MW), relative brain tissue, and plasma bindings. The blood brain barrier can be the major impediment for the treatment of central nervous system diseases, for many drugs are unable to reach this organ at therapeutic concentrations [2,3]. Prediction of passage across the blood brain barrier is of great importance for centrally acting drugs or for peripherally acting drugs that should be devoid of CNS side-effects. Convenient and reliable methods for predicting distribution of these agents between blood and brain are highly desirable.

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Several techniques have been utilized to quantify the BBB permeability that can be classified as in vivo and in vitro techniques [4]. In vivo methods include brain homogenization, cerebrospinal fluid (CSF) sampling, voltammetry, autoradiography, nuclear magnetic resonance (NMR) spectroscopy, positron emission tomography (PET), intracerebral microdialysis, and brain uptake index (BUI) determination. In most of them chromatographic techniques are used as adjunct methods. In general, in vivo methods are complicated, time-consuming and require the synthesis of test compounds in radiolabeled form [1,5,6].

To circumvent the problems associated with screening experimental compounds in animals, a number of in vitro models for predicting brain penetration have recently appeared which include computational methods that correlate physicochemical parameters or molecular descriptors with brain–blood distribution [7–15]. Cell culture models such as bovine brain micro-vessel endothelial cell (BMECs) lines [16,17] have also been used for transport studies.

The hydrophobicity of a solute as measured by its partition behavior between octanol and water ($\log P$) has been widely used to model transmembrane permeability and the brain–blood equilibrium distribution ratio. This kind of correlation was graphically represented by Pardridge [18]. However, the octanol/water partition coefficient is unfortunately an unreliable predictor for drug penetration across cellular barriers. The observations that $\log P$ alone is unable to account for differences between drugs' brain–blood concentrations ratios was demonstrated by Young et al. [5]. A better correlation was found by Abraham and Chadka [19] using the logarithm of the cyclohexane–water partition coefficient ($\log P$cyh). Upon reanalyzing the data of Young et al., Van de Waterbeemd and Kansy [20] obtained a two-parameter regression equation describing the brain uptake in terms of the calculated molar volume (VM) of the molecule and a descriptor derived from the measurements of the alkane–water partition coefficient.

Levin [21] and Conford et al. [22] found good correlations when they assumed that permeability is a function of the diffusion coefficient and that variability in diffusion coefficients of small molecules approximates the square root of the molecular weight.

Chromatography is a powerful technique for measuring of the physicochemical parameters of drugs. Different chromatographic strategies have been proposed in order to model drug-membrane transport. For instance, phospholipids have been covalently immobilized to silica propyl amide particles [23] and liposomes have been immobilized in capillary continuous beds with covalently linked C_4 or C_8 alkyl ligands [24]. The relationship between immobilized artificial membrane chromatographic retention and the brain penetration of drugs has been reported by Salminen et al. [25] and Reichel and Begley [26].

Our research group has demonstrated that in adequate experimental conditions the use of polyoxyethylene(23)lauryl ether (Brij35) micellar mobile phases and C_{18} reversed stationary phases permits drug biopartitioning simulation. This technique, that we have called biopartitioning micellar chromatography (BMC), has demonstrated to be useful in describing the biological behavior of different kinds of drugs [27–33] and to predict human oral drug absorption, skin permeability, and ocular tissue permeability of drugs [34–38]. The success of BMC in constructing these models could be attributed to the fact that the characteristics of the BMC systems show similarities with the biological barriers and extracellular fluids. Therefore, the retention of a drug in BMC, which depends on its hydrophobic, electronic, and steric properties, reflects adequately the extension of the biopartitioning process.

In this paper, the usefulness and limitations of biopartitioning micellar chromatography for predicting drug penetration across the blood–brain barrier are studied.

2. Experimental

2.1. Instrumental and measurement

A Hewlett-Packard 1100 chromatograph with an isocratic pump, an UV-Vis detector and a HP Vectra computer, were used (Palo Alto, CA, USA). Data acquisition and processing were performed on a HP Vectra XM computer (Amsterdam, The Netherlands) equipped with HP-Chemstation software (A0402, 1996). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA), with a 20 μl loop. Kromasil octadecyl-silane C_{18} columns (5 μm, 150 mm × 4.6 mm and 50 mm × 4.6 mm i.d.) (Scharlab, Barcelona, Spain) were used. The mobile phase flow rate was 1 ml min^{-1}. All the assays were carried out at 36.5 °C.

2.2. Reagents and standards

Micellar mobile phases of 0.04 M polyoxyethylene(23) lauryl ether (Brij35, Acros Chimica, Geel, Belgium, critical micellar concentration, CMC, 1×10^{-4} M) were prepared by dissolving Brij35 in 0.05 M phosphate buffer at pH 7.4. The phosphate buffer solutions were prepared with disodium hydrogen phosphate and sodium di-hydrogen phosphate (analytical reagent, Panreac, Barcelona, Spain). In order to reproduce the osmotic pressure of biological fluids, NaCl (9.20 g/l, Panreac) was added to the micellar mobile phase.

The compounds used in this study were obtained from different sources: ethanol, benzene, 1-propanol, and toluene (Scharlab, Barcelona, Spain); amobarbital, atenolol, carbamazepine epoxide, codeine, desipramine, haloperidol, hexobarbital, promazine, pyrilamine, thioridazine, and trifluoperazine (Sigma, St. Louis, MO USA); acetaminophen, acetylsalicic acid, amitriptyline, caffeine, carbamazepine, cimetidine, clonidine, diazepam, fluphenazine, hydroxizine, imipramine, ranitidine, theophylline, and estrone (Guinama, Valencia, Spain); salicylic acid, antipyrine (Panreac, Montplet & Esteban S.A., Barcelona, Spain), bromperidol

Table 1
Logarithm of the brain–blood distribution coefficient (log BB), logarithm of the retention factor in BMC ($\log k_{BMC}$) and physico-chemical and structural descriptor values tested for log BB modeling

Compounds	Compound number	log BB	$\log k_{BMC}$	log P	pK_{an}	α	MW	MR (cm^3)	MV (cm^3)	Pr (cm^3)	Polarizability ($\times 10^{24}$) (cm^3)
Acetaminophen	9	−0.31	0.66	0.46	9.38 (A)	−0.0104	151.16	42.40	120.90	326.00	16.81
Acetylsalicylic acid	7	−0.50	0.57	1.19	3.5 (A)	−0.9999	180.16	44.52	139.50	370.90	17.65
Alprazolam	19	0.04	1.44	2.12	6.2 (B)	0.0594	308.77	88.22	225.50	606.20	34.47
Amitriptyline	35	0.90	2.37	5.04	9.42 (B)	0.9905	277.41	91.52	257.70	675.10	36.28
Amobarbital	18	0.04	1.58	2.07	7.8 (A)	−0.2847	226.27	57.95	211.40	507.30	22.97
Antipyrine	14	−0.10	0.34	0.38	1.45 (B)	0.0000	188.23	54.55	162.70	416.10	21.62
Atenolol	2	−1.42	−0.34	0.16	9.6 (B)	0.9937	266.34	74.25	236.60	613.00	29.43
Benzene	27	0.37	1.66	2.13	N	0	78.11	26.25	89.40	207.20	10.40
Bromperidol	42	1.38	2.06	4.45	8.65 (B)	0.9468	420.32	103.80	307.50	811.80	41.15
Caffeine	16	−0.06	0.26	−0.07	0.6 (B); 14 (A)	0.0000	194.19	50.38	133.30	364.50	19.97
Carbamazepine	15	−0.07	1.21	2.45	N	0	236.27	69.68	186.50	513.40	27.62
Carbamazepine epoxide	8	−0.34	0.84	n.a.	N	0	252.30	n.a.	186.50	n.a.	n.a.
Cimetidine	1	−1.42	0.28	0.41	6.8 (B)	0.2008	252.34	70.70	198.20	526.00	28.03
Clobazam	25	0.35	1.45	2.12	N	0	300.74	79.86	225.20	606.50	31.66
Clonidine	22	0.11	0.92	1.59	8.05 (B)	0.8171	230.10	57.28	153.10	409.20	22.70
Codeine	32	0.55	1.28	1.14	8.21 (B)	0.8659	299.37	82.85	222.60	620.80	32.84
Chlorpromazine	38	1.06	2.43	5.35	9.3 (B)	0.9876	318.87	92.75	262.90	686.90	36.67
Desipramine	39	1.20	1.79	4.90	10.44	0.9991	266.39	84.16	254.20	639.30	33.36
Diazepam	31	0.52	1.65	2.80	3.3 (B)	0.0001	284.74	80.91	225.80	588.60	37.07
Ethanol	12	−0.16	0.02	−0.31	15.9 (A)	0.0000	46.07	12.84	59.00	128.40	5.09
Flunitrazepam	20	0.06	1.52	2.06	1.8 (B)	0.0000	313.29	81.84	224.80	605.30	32.44
Fluphenazine	44	1.51	1.99	4.36	3.9 (B); 8.1 (B)	0.8340	437.52	114.30	343.80	885.90	45.31
Haloperidol	41	1.34	2.01	3.36	8.3 (B)	0.8882	375.87	101.01	303.20	797.80	40.04
Hexobarbital	21	0.10	1.36	1.49	8.2 (A)	−0.1368	236.27	60.38	192.80	501.80	23.93
Hydroxyzine	29	0.39	1.74	2.36	2.1 (B); 7.1 (B)	0.3339	374.91	105.91	317.10	833.70	41.98
Ibuprofen	11	−0.18	1.19	3.50	5.2 (A)	−0.9937	206.29	60.77	200.30	497.60	24.09
Imipramine	37	1.06	2.49	4.80	9.5 (B)	0.9921	280.42	88.92	269.20	677.50	35.25
Indomethacine	3	−1.26	1.20	4.27	4.5 (A)	−0.9987	357.80	94.59	269.50	707.60	37.49
Mianserin	36	0.99	2.20	4.26	7.1 (B)	0.3339	264.37	82.88	223.60	605.90	32.85
Midazolam	26	0.36	1.70	4.33	6.2 (B)	0.0594	325.78	89.65	239.80	625.00	35.54
Oxazepam	33	0.61	1.43	2.24	1.7 (B); 11.6 (A)	−0.0001	286.72	76.43	201.80	548.80	30.30
Pentobarbital	23	0.12	1.66	2.07	8 (A)	−0.2008	226.28	57.89	209.10	507.30	22.95
Phenytoin	17	−0.04	1.54	2.47	8.3 (A)	−0.1118	252.27	69.58	200.50	531.30	27.58
Promazine	40	1.23	2.24	4.55	9.36 (B)	0.9892	284.43	87.85	250.90	649.70	24.82
1-Propanol	13	−0.16	0.19	0.25	16.1 (A)	0.0000	60.10	17.48	75.50	168.20	6.93
Propranolol	34	0.64	1.62	3.56	9.45 (B)	0.9912	259.36	78.98	237.10	606.10	31.31
Pyrilamine	30	0.50	1.67	3.27	4.02 (B); 8.92 (B)	0.9711	285.39	87.44	262.10	677.30	34.66
Ranitidine	4	−1.23	0.11	0.27	2.3 (B); 8.2 (B)	0.8632	314.41	85.64	265.40	687.50	33.95
Salicylic acid	5	−1.10	0.55	2.26	2.97 (A); 13.4 (A)	−1.0000	138.12	35.06	100.30	284.40	13.90
Theophylline	10	−0.29	0.22	−0.02	3.5 (B); 8.6 (A)	−0.0592	180.17	43.14	122.90	352.40	17.10
Thioridazine	24	0.24	2.48	5.90	9.5 (B)	0.9921	370.58	112.80	299.50	829.10	44.71
Toluene	28	0.37	1.86	2.73	N	0	92.14	31.07	105.70	244.90	12.32
Trifluoperazine	43	1.44	2.55	5.03	8.1 (B)	0.8337	407.50	108.15	328.70	828.80	42.87
Verapamil[a]	6	−0.70	1.91	3.79	8.92 (B)	0.9707	454.61	131.86	429.30	1063.90	52.27

log P, logarithm of the partition coefficient in the *n*-octanol/water system of the neutral form of compounds studied; α, total molar charge; MR, molar refractivity; MV, molar volume; Pr, parachor; MW, molecular weight; n.a., non-available data; (A) pK_{an} value for an acidic and (B) for a basic group. (N) Neutral compound or at least non-ionized ($\alpha = 0$) at pH 7.4.

[a] Verapamil is an inhibitor of *p*-glycoprotein.

(Janssen Pharmaceutica, Beerse, Belgium). Other compounds were kindly donated from several pharmaceutical companies: indomethacine (Llorens, Barcelona, Spain), testosterone (Schering, Madrid, Spain), progesterona (SEID, Barcelona, Spain), propranolol (ICI pharma, Barcelona, Spain), naproxen (Syntex Latino, Madrid, Spain), pentobarbital (B. Braun Medical, Barcelona, Spain), and phenytoin (Laboratorios Rubió, Barcelona, Spain). The following compounds were obtained from pharmaceuticals: alprazolam (Trankimazin®, Pharmacia Spain, Barcelona, Spain), acyclovir (Zovirax®, Glaxo-Wellcome, Madrid, Spain), clobazam, and chlorpromazine (Noiafren®, Largactil® Aventis Pharma, Madrid, Spain); flunitrazepam, and midazolam (Rohipnol®, Dormicum® Roche Farma, Madrid, Spain), ibuprofen (Nurofen®, Boots Healthcare Iberia, Madrid Spain), mianserin (Lantanon®, Organon Española, Barcelona, Spain), oxazepam (Adumbran®, Boerhinger Ingelheim España, Barcelona, Spain), verapamil (Manidon®, Laboratorios Knoll, Madrid, Spain).

Stock standard solutions of compounds were prepared by dissolving 10 mg of compound in 10 ml of mobile phase, methanol or acetonitrile. Working solutions were prepared by dilution of the stock standard solutions using the mobile phase. Solutions were stored at 4 °C.

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 μm Nylon membranes (Micron Separations, Westboro, MA, USA).

2.3. Data sources, software, and data processing

A total of 44 values of logarithm of blood brain distribution, log BB, have been collected from a number of sources [5,6,8,10,11,13] including directly measured and indirectly determined values, they are presented in Table 1. These values were the brain/blood concentration ratios (BB) obtained in the rat at steady state. The values of log BB are ranged between −1.5 and 1.5. A basic assumption of the study was that BBB permeation was via purely passive diffusion.

Structural parameters (molar refractivity (MR), molar volume, parachor (Pr), and polarizability) were calculated using the ACD/ChemSketch software (ACD labs demo version). The logarithm of octanol–water partition coefficients (log *P*) and acidity constants (pK_a) were taken from references [39–41].

Microsoft Excel (Microsoft Corporation, v. 2000), Statgraphics (Statistical Graphics Cor. V. 2.1), and Matlab (The Mathworks v 4.2c.1) were used to perform the statistical analysis of the regressions. The Unscrambler Version 7.6 by CAMO was used to perform multivariate analysis.

2.4. BMC data

The chromatographic data of the compounds listed in Table 1 were obtained in our laboratory using a micellar solution of 0.04M Brij35 at pH 7.4 as mobile phase and a C_{18} Kromasil column as stationary phase. All retention factor values were averages of at least triplicate determinations. The retention factors were calculated using acetanilide as external standard for hold-up time estimations [42].

3. Results and discussion

3.1. Brain–blood distribution coefficients-retention relationships

The compounds studied are structurally diverse drugs with different pharmacodynamic and pharmacokinetic properties and varying degrees of ionization at pH 7.4, resulting in either positive or negative charge.

Table 1 shows the BMC retention factors measured using 0.04 M Brij35 pH 7.4 micellar mobile phases for the compounds used in this study together with their literature "in vivo" values of brain/blood concentration ratio in rats [5,6,8,10,11,13]. In the same table, dissociation constants and some molecular descriptors of compounds are shown. The molecular descriptors used were the logarithm of octanol–water partition coefficients (log *P*), molecular weight, molar refractivity (MR), molar volume, parachor (Pr), polarizability, and molar total charge (α). The α values were calculated as in reference [29]:

$$\alpha = \sum_{j=0}^{n} a_j \delta_j \quad (1)$$

where a_j is the value with its sign of the net charge of the considered specie (i.e. +1, −1, 0, +2) and δ_j the molar fraction of the considered specie at the desired pH value.

In order to study the importance of variables in the construction of a regression model for predicting log BB, a partial least squares analysis (PLS) was performed. The log BB values were used to construct the **y**-block and the descriptor variables log k_{BMC}, molecular weight, molar refractivity, molar volume, parachor, polarizability, and molar total charge (α), were used to construct the **X**-block. Descriptor variables (**X**-block) and response variable (**y**-block) were autoscaled before the PLS.

Four latent variables (LVs) account for the 75.5% of the total variance of the original log BB data. The loading plot corresponding to the first two latent variables (Fig. 1A) indicates that there is a high correlation between the steric descriptors molecular weight, molar volume, molar refractivity, parachor, and polarizability and low correlation is observed with log BB values. On the other hand a direct correlation between log BB, log k_{BMC}, and α is observed.

The PLS-model regression coefficients together their uncertainty limits were obtained for LV 4 (Fig. 1B). As can be observed the regression coefficients of some molecular descriptors were not statistically significant (MW, MV, MR, and polarizability). Non-significant variables were

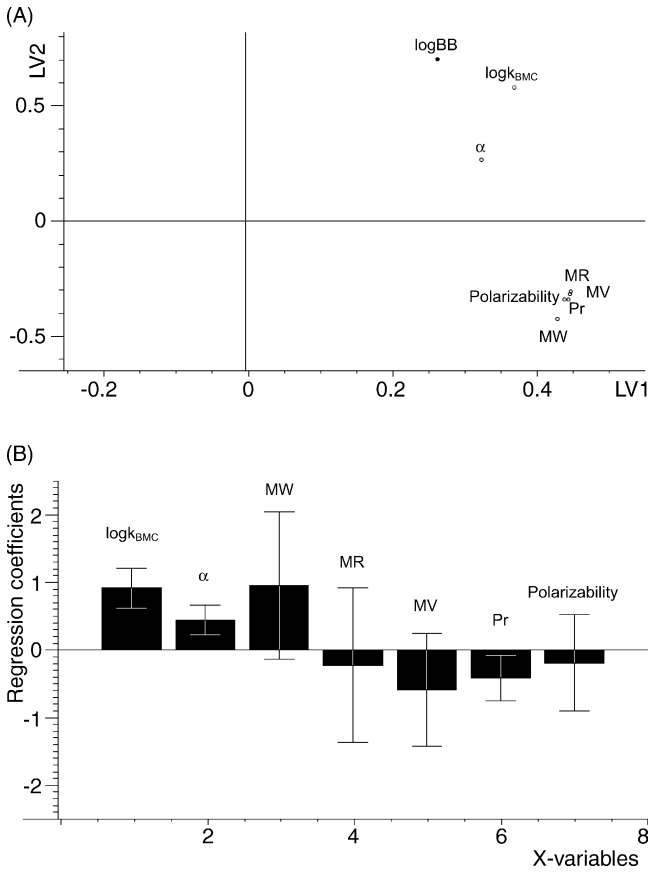

Fig. 1. PLS results. (A) Loading plot (B) regression coefficients for LV 4. See text for details.

eliminated step by step, re-analyzing each time the PLS model. Finally a PLS model was obtained by using the two significant original variables, $\log k_{BMC}$ and α.

Thioridazine and verapamil showed high residual variance and leverage values, therefore they were considered as outliers. These compounds have been omitted in the development of other QSAR models [8,12,13,25]. Excluding these compounds, the model using one latent variable accounts for 73.7 and 70.3% of variance in log BB in calibration and cross-validation (Venetian blinds, random), respectively.

A multiple linear regression (MLR) analysis using non-scaled data was also performed using the selected variables by the PLS approach. The equation of the fitted MLR model was:

$$\log \mathrm{BB} = (-0.84 \pm 0.12) + (0.76 \pm 0.08)\log k_{\mathrm{BMC}} + (0.26 \pm 0.11)\alpha \quad (2)$$

$$r^2 = 0.75;\ \mathrm{S.E.} = 0.39;\ F = 60;\ N = 42;\ P < 0.0001$$

The model explains 75% of variance in the data; this variability can be considered adequate taking into account the inherent difficulty in the BB estimations. In addition the uncertainty of experimental log BB values is unknown.

The *r*-squared statistic and standard error of estimates values are similar to those reported in literature (see Table 2). All the regression coefficients and the model were statistically significant at the 99% of confidence level, ($P < 0.01$). The residual plot of the model showed a random distribution of the residuals (see Fig. 3A) with an average value practically equal to zero, which from a qualitative point of view suggests the adequacy of the model.

The analysis of the coefficients of the proposed model implies that the blood brain barrier favors the passage of neutral or cationic and hydrophobic compounds. The model derived is consistent with other reported models that indicate that the brain–blood distribution coefficients depend directly on the hydrophobic character of compounds which is directly related with retention in BMC. Additionally the retention in BMC depends on the electronic and steric properties of compounds which also have importance in the brain–blood drug distribution.

The inclusion of the total molar charge is of particular importance for compounds containing acidic groups. For these compounds large discrepancies, between the predicted values according to their hydrophobic character and the experimental ones, have been observed. In fact, several indicator variables have been introduced in QSAR models in order to correct them [13]. When molar total charge was removed from the model (Eq. (1)), a significant linear model was obtained:

$$\log \mathrm{BB} = (-0.87 \pm 0.12) + (0.84 \pm 0.08)\log k_{\mathrm{BMC}} \quad (3)$$

$$r^2 = 0.74;\ \mathrm{S.E.} = 0.39;\ F = 109.6;\ N = 41;\ P < 0.0001$$

only indomethacine ($\alpha = -1$) was detected as outlier. This model is simpler than Eq. (2) and presents similar statistical parameters. More log BB experimental data for anionic compounds would be necessary in order to confirm Eq. (1) or Eq. (2), although in both cases adequate results are obtained.

3.2. Potential of the model based on biopartioning micellar chromatography retention for predicting brain penetration

The model developed can be used for qualitative and quantitative purposes. From a qualitative point of view, drugs can be classifying according to their log BB values in poor brain penetrators (BBB−) and easy brain penetrators (BBB+). Different criteria have been used in literature bear in mind that the cut-off is arbitrary and would have to be defined with a specific pharmacology. Indeed the cut-off value will depend on which are more damaging to the classification, false positives or false negatives [12]. 82% of the BBB− compounds and 100% of BBB+ compounds were correctly classified with a cut-off of 0. This criteria implies that drugs with log BB > 0 accumulate preferably in the brain (>50%). If more restrictive cutoffs are chosen to define BBB− compounds (log BB < −0.5) and BBB+ compounds (log BB >

Table 2
Statistical characteristics of the some of literature QSAR models

Model, ref.	Descriptor variables	Number of descriptor variables	log BB range studied	N	r^2	r^2_{CV}	S.E.
Platts et al. [13]	Solvatochromic parameters	6	−1.82 to 1.38	148	0.74	0.715[a]	0.34
Luco [8]	Computacional	18	−2.15 to 1.04	58	0.85	0.75[a]	0.32
Ooms et al. [12]	Computacional	31	−1.42 to 1.23	79	0.76	0.65[b]	n.a.
Rose et al. [15]	Computacional	3	−2.15 to 1.44	102	0.66	0.62[b]	0.45
Salminen et al. [25]	Retention in IAM chromatography and other descriptors	3	−0.7 to 1.3	21	0.85	n.a.	0.27
This work	Retention in BMC and total molar charge	2	−1.42 to 1.51	42	0.75	0.70[a]	0.39
	Retention in BMC	1	−1.42 to 1.51	41	0.74	0.69[a]	0.39

[a] Venetian blinds cross-validation.
[b] Leave-one-out cross-validation.

0.5) [12], the model assign correctly the 71% of BBB− compounds and 80% of the BBB+ compounds.

The quantitative use of the models imply the evaluation of their predictive ability. For this purpose, the fit error (the root-mean-squared error of calibration, RMSEC) and the prediction error based on random Venetian blinds cross-validation (root-mean-squared error of cross-validation, RMSECV) were obtained: RMSEC = 0.3792; RMSECV = 0.4208, Eq. (2) and RMSEC = 0.3784; RMSECV = 0.4138, Eq. (3). As can be observed, the RMSEC and RMSECV values were similar in both cases, which suggest the robustness of the models is reasonably adequate.

Fig. 2 shows the predicted versus experimental (fitted and cross-validated) values of brain/blood distribution coefficients, obtained from Eqs. (2) to (3) (Fig. 2A and B). As can be observed, the ability of the proposed models to describe and predict brain penetration is adequate. The QSAR models obtained using the log P values instead of log k_{BMC}, showed the same trend described, but worse statistical parameter values were obtained.

$$\log BB = (-0.55 \pm 0.14) + (0.26 \pm 0.05) \log P + (0.47 \pm 0.14)\alpha \quad (4)$$

$$r^2 = 0.59;\ \text{S.E.} = 0.51;\ F = 28;\ N = 41;\ P < 0.0001$$

$$\log BB = (-0.60 \pm 0.12) + (0.35 \pm 0.04) \log P \quad (5)$$

$$r^2 = 0.62;\ \text{S.E.} = 0.47;\ F = 61;\ N = 40;\ P < 0.0001$$

Using the retention data of a reduced number of compounds, selected in order to cover a wide log BB range (−1.42 to 1.44), a similar model to Eq. (3) was obtained. The selected anionic (n = 3), cationic (n = 4), and neutral (n = 3) compounds were: atenolol, salicylic acid, acetylsalicylic, ibuprofen, carbamazepine, alprazolam, midazolam, mianserine, promazine, and trifluoperazine. These compounds showed residual values lower than 0.5 in many QSAR models proposed in literature (see Table 3).

$$\log BB = (-1.3 \pm 0.12) + (1.04 \pm 0.08) \log k_{BMC} \quad (6)$$

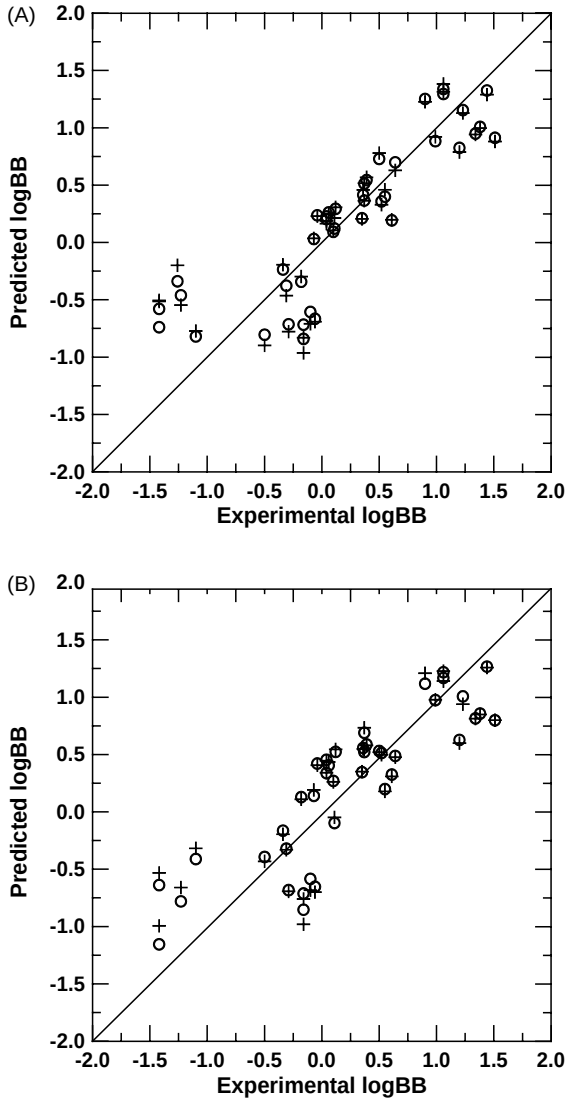

Fig. 2. Validation plots: predicted log BB vs. actual log BB values obtained using (A) Eq. (2) and (B) Eq. (3). (○) Fitted and (+) cross-validated data are shown.

Table 3
Experimental and predicted log BB values

Compound number	Name	Experimental log BB values	Predicted log BB values					
			Eq. (2)	Eq. (3)	Ref. [13]	Ref. [8]	Ref. [12]	Ref. [15]
1	Cimetidine	−1.42	−0.58	−0.64	−0.77		−1.19	−1.01
2	Atenolol	−1.42	−0.74	−1.15	−0.93	−1.36		
3	Indomethacin	−1.26	−0.34	0.14	−0.92	−1.03	−1.15	−0.77
4	Ranitidine	−1.23	−0.46	−0.78	−0.02		−0.61	−0.85
5	Salicylic acid	−1.10	−0.82	−0.41	−0.87	−1.21	−0.66	−0.86
6	Verapamil	−0.70	0.90	0.73		−1.11	−0.96	−0.78
7	Acetylsalicylic acid	−0.50	−0.80	−0.39	−0.75	−1.18	−0.52	−0.59
8	Carbamazepine epoxide	−0.34	−0.24	−0.16				
9	Acetaminophen	−0.31	−0.38	−0.32	−1.22			
10	Theophylline	−0.29	−0.71	−0.68	−0.91	−0.51	−0.96	−0.32
11	Ibuprofen	−0.18	−0.34	0.13	−0.23	−0.56	0.22	−0.14
12	Ethanol	−0.16	−0.84	−0.85	−0.41	−0.23		
13	1-Propanol	−0.16	−0.72	−0.71	−0.26	−0.06		−0.14
14	Antipyrine	−0.10	−0.61	−0.58	−0.24	0.47	0.50	0.34
15	Carbamazepine	−0.07	0.03	0.14			0.31	0.17
16	Caffeine	−0.06	−0.67	−0.65	−0.39	−0.22	−0.87	0.06
17	Phenytoin	−0.04	0.24	0.42	−0.55			
18	Amobarbital	0.04	0.20	0.46	0.05			
19	Alprazolam	0.04	0.23	0.34	−0.03	0.33	−0.38	0.64
20	Flunitrazepam	0.06	0.26	0.41	−0.09			
21	Hexobarbital	0.1	0.09	0.27	0.10			
22	Clonidine	0.11	0.12	−0.10	−0.42		0.46	−0.44
23	Pentobarbital	0.12	0.29	0.52	0.12	−0.19	−0.54	−0.76
24	Thioridazine	0.24	1.33	1.21	1.20	1.06	0.78	0.89
25	Clobazan	0.35	0.21	0.35	0.49			
26	Midazolam	0.36	0.42	0.56	0.49	0.40	0.41	0.29
27	Benzene	0.37	0.37	0.52	0.33	0.55		0.57
28	Toluene	0.37	0.51	0.69	0.48	0.69		0.51
29	Hydroxyzine	0.39	0.54	0.59	0.45	0.13	−0.88	0.02
30	Pyrilamine	0.50	0.73	0.53				
31	Diazepam	0.52	0.36	0.51	0.69			
32	Codeine	0.55	0.40	0.20	−0.22	0.27	0.54	−0.29
33	Oxazepam	0.61	0.19	0.33	0.58	−0.48	−0.66	−0.53
34	Propranolol	0.64	0.70	0.49	0.17			
35	Amitriptyline	0.90	1.25	1.12			0.74	0.84
36	Mianserin	0.99	0.88	0.98	0.69		0.91	
37	Imipramine	1.06	1.34	1.22	0.69		0.92	0.83
38	Chlorpromazin	1.06	1.30	1.17	0.38	0.71	0.42	0.88
39	Desipramine	1.2	0.83	0.63	0.71	0.43	0.42	0.44
40	Promazine	1.23	1.15	1.01	0.84	0.83	0.65	0.97
41	Haloperidol	1.34	0.95	0.82	0.80	−0.27		
42	Bromperidol	1.38	1.01	0.86	0.90			
43	Trifluoperazine	1.44	1.33	1.27	1.09	0.46		0.26
44	Fluphenazine	1.51	0.91	0.80	0.45			

$r^2 = 0.96$; S.E. $= 0.20$; $n = 10$; $F = 187$; $P < 0.0001$

This calibration set can be used to check the stability of the proposed model under intermediate precision conditions and to check the reproducibility for interlaboratory comparisons.

3.3. Comparison with other QSAR and in vitro approaches

As it has been indicated in the introduction section different in vitro, mathematical and chromatographic approaches have been developed to estimate brain–blood distribution coefficients. Table 2 summarizes the characteristics of some log BB models reported in literature together with the characteristics of the models proposed in the present paper. As can be observed, in computational approaches the number of descriptor variables and compounds used to perform the model obviously is higher than in the experimental approaches. The range of log BB values studied is very similar in all approaches, except in the model reported by Salminen et al. [25] because they considered as outliers compounds such as cimetidine, ranitidine, indomethacine and salicylic acid which show low log BB values. The R-squared statistic in cross-validation values (R^2_{CV}) and the standard error of estimates are similar in all cases.

In Table 3, the predicted values for the compounds used in this study obtained using Eqs. (2) and (3) and the re-

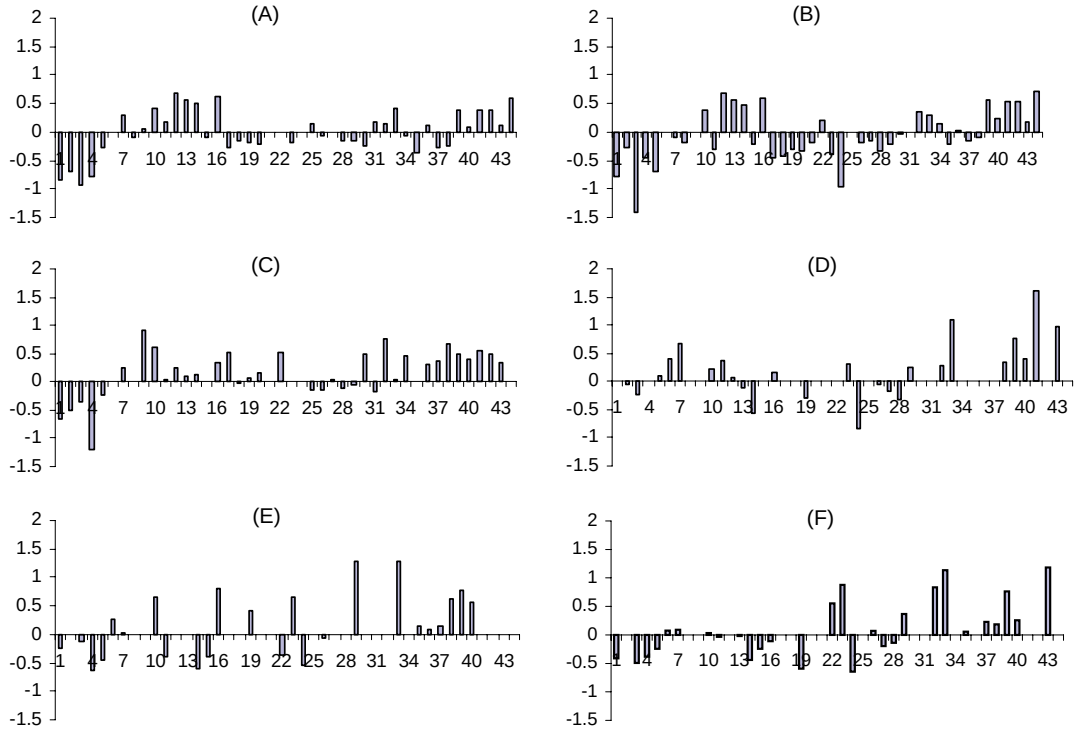

Fig. 3. Residual plots for compounds studied in this work obtained for different QSAR models. (A) BMC model Eq. (2), (B) BMC model Eq. (3), (C) model from reference [13], (D) model from reference [8], (E) model from reference [12], (F) model from reference [15]. Compounds have been ordered according to their experimental log BB values (numbers in Table 1).

ported in literature using different QSAR models are shown. The mean absolute errors for the different models were 0.296 ($n = 42$), 0.316 ($n = 41$), 0.348 ($n = 37$), 0.427 ($n = 25$), 0.459 ($n = 25$), and 0.381 ($n = 28$) for predictions obtained using Eqs. (2) and (3), and reported in refs. [13,8,12,15], respectively. As can be observed, the proposed BMC models show better or at least comparable predictive ability than other reported QSAR models. Fig. 3 shows the residual plots obtained for the compounds studied in this work.

Endothelial cell lines (BBMEC) from bovine brain microvessels have been developed as an in vitro model for transport studies across the blood brain barrier. The relationship between in vitro permeability in BBMEC lines and BMC retention was evaluated. For this purpose, the permeability data (PC $\times$ 10^4 cm/s) of a set of eight drugs (acyclovir, caffeine, antipyrine, propranolol, estrone, progesterone, testosterone, and haloperidol) reported by Lombardo et al. [10] using BBMEC lines were correlated with the retention factors in BMC using a 0.04M Brij35 mobile phase. A linear relationship was obtained (Fig. 4) and the fitted equation to the data was:

$$\mathrm{PC} \times 10^4 = (34 \pm 5) + (32 \pm 3)\log k_{\mathrm{BMC}} \quad (7)$$

$$r^2 = 0.94;\ \mathrm{S.E.} = 8.7;\ n = 7;\ F = 85.5$$

where the numbers in parenthesis are the confidence limits at a 95% probability level. The P-value obtained for the model was lower than 0.05, which indicates that the relationship between PC and the log k_{BMC} values was statistically significant at the 95% confidence level. From the results it can be concluded that the two approaches, BMC, and BBMEC, are comparable and useful in the estimating of drug blood–brain penetration.

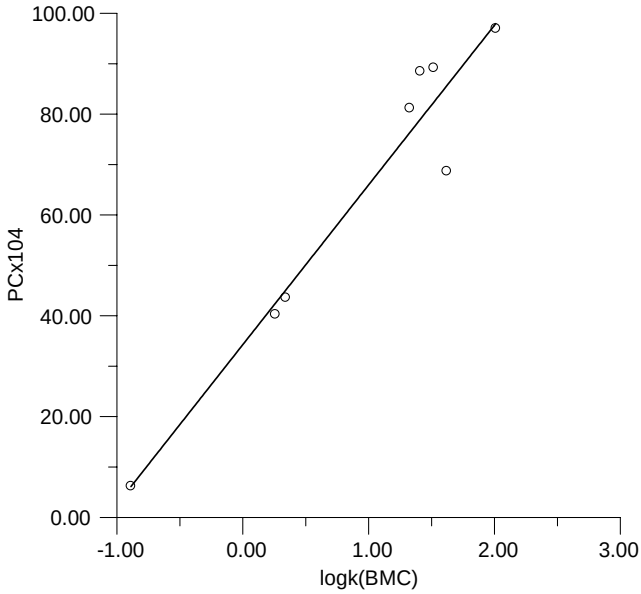

Fig. 4. Relationship between the logarithm of retention factors obtained in BMC using 0.04 M Brij35 as mobile phase for a heterogeneous set of drugs and the PC values obtained in BBMEC.

3.4. Advantages and limitations of the BMC models

The BMC technique is a promising tool for ranking BBB drug penetration, and it offers the practical advantages of being easily automated, time-saving and highly reproducible. However, BMC also has the limitation that it may fail when other processes such as carrier-mediated transport or metabolism limit brain uptake; whereas if the rate-limiting step for permeation across the BBB is the partitioning of the drug into the brain endothelial cell membrane, BMC will be successful.

4. Conclusions

The results presented here indicate that the retention of compounds in biopartitioning micellar chromatography, BMC, is capable of describing and predicting in vitro the blood–brain barrier penetration. Therefore, it can be used to identify the blood brain barrier penetration ability of drugs at an early stage of the drug discovery process.

The comparison with other QSAR models proposed reveals that the BMC models are better or at least comparable from a statistical point of view. In addition, due to the high number of parameters used to develop the QSAR models, the number of experimental data needed to obtain a statistical validated model is also high, mainly when multiple linear regression is used. The use of as few descriptors as possible has been recommended [43]. The BMC methodology is less expensive than cell culture models or in vivo models and minimum experimentation is required.

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