REVISITING BLOOD-BRAIN BARRIER: A CHROMATOGRAPHIC APPROACH

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Abstract

Drugs designed to reach a pharmacological CNS target must be effectively transported across the blood-brain barrier (BBB), a thin monolayer of endothelial cells tightly attached together between the blood and the brain parenchyma. Because of the lipidic nature of the BBB, several physicochemical partition models have been studied as surrogates for the passive permeation of potential drug candidates across the BBB (octanol-water, alkane-water, PAMPA…). In the last years, biopartition chromatography is gaining importance as a noncellular system for the estimation of biological properties in early stages of drug development. Microemulsions (ME) are suitable mobile phases, because of their ease of formulation, stability and adjustability to a large number of compositions mimicking biological structures. In the present work, several microemulsion liquid chromatographic (MELC) systems have been characterized by means of the Abraham's solvation parameter model, in order to assess their suitability as BBB distribution or permeability surrogates. In terms of similarity between BBB and MELC systems (dispersion forces arising from solute non-bonded electrons, dipolarity/polarizability, hydrogen-bond acidity and basicity, and molecular volume), the passive permeability surface area product (log PS) for neutral (including zwitterions), fully and partially ionized drugs was found to be well correlated with the ME made of 3.3% SDS (w/v; surfactant) 0.8% heptane (w/v; oil phase) and 6.6% 1-butanol (w/v; co-surfactant) in 50 mM aqueous phosphate buffer, pH 7.4.

Keywords

Blood-brain barrier; LFER; log BB; log PS; microemulsion; liquid chromatography

Abbreviations

BB: plasma-to-brain distribution ratio; BBB: blood-brain barrier; CNS: central nervous system; LFER: linear free energy relationships; ME: microemulsion; MELC: microemulsion liquid chromatography; PS: permeability-surface area product; SP: solute property; SDS: sodium dodecylsulfate

1. Blood-brain barrier

1.1. Experimental models: log BB and log PS

The blood–brain barrier (BBB) plays a fundamental role in the pharmacological activity of drugs targeting the central nervous system (CNS). It is a thin monolayer of endothelial cells, tightly attached together, that separates the circulating blood and the brain parenchyma.

Two different *in vivo* BBB experimental models have been considered in the present work, the plasma-to-brain distribution ratio (log K_p, also known as log BB) and the permeability-surface area product (PS). K_p accounts for the concentration of drug present in the brain at steady state in relation to that in plasma. This is, in fact, a partition coefficient between the concentrations of both bound and unbound drug in brain (intracellular and interstitial fluids) and plasma. *In vivo*, log BB is determined at a specific time point after drug administration. It should be pointed out that bound drug molecules (for instance, to plasma and cytoplasmic proteins) are not expected to be pharmacologically active [1]. Therefore, besides BBB equilibration of unbound drug molecules, log BB measures nonspecific binding to brain tissue and plasma proteins. Consequently, in the case of drug molecules significantly bound to cytoplasmic proteins in brain, log BB might fail to indicate the effective extent of BBB penetration [2]. However, log BB is a widely used parameter in BBB studies, especially for *in silico* predictions of BBB *in vivo* data [3,4].

In contrast to log BB, *in situ* brain perfusion experiments, mainly performed on rodents, allow the measurement of the initial and unidirectional rate of brain penetration from blood, or usually from saline, to brain across the luminal BBB membrane, even in the case of solutes strongly bond to proteins. Perfusion time is about 30 to 180 s [5], and it ends before any equilibrium state can be reached. In this way, the clearance or K_{in} (mL g^{-1} s^{-1}, mL of perfusate per gram of brain tissue and second of net perfusion time) is determined. However, this parameter depends on the perfusion flow velocity and, therefore, K_{in} is corrected by the flow of the perfusion fluid in brain, measured by an appropriate flow calibrant, such as radioactive iodoantypirine, microspheres or diazepam [6]. Thus, PS is obtained, by the product of luminal permeability (cm s^{-1}) and the endothelial surface area per gram of brain tissue (cm^2 g^{-1}).

1.2. Factors affecting the distribution and permeation between blood and brain: a LFER approach

log BB was extensively studied by Abraham and coworkers [7,8] by means of linear free energy relationships (LFER) in order to point out the factors that influence the distribution of

solutes between blood and brain. According to the solvation model for unionized molecules [9], a solute dependent variable (log SP) is linearly related to specific interactions between solute and surrounding phase, mainly dispersion ($e \cdot E$), dipole-dipole or dipole-induced dipole plus some polarizability interactions ($s \cdot S$), solute hydrogen-bond acidity and basicity ($a \cdot A$ and $b \cdot B$, respectively), and a volume term ($v \cdot V$) related to the work of separating solvent molecules to provide a cavity of suitable size for the solute molecule and solute-solvent general dispersion interactions:

$$\log \mathrm{SP} = c + eE + sS + aA + bB + vV \quad (1)$$

where E, S, A, B, and V are solute descriptors, and e, s, a, b, and v are system constants reflecting differences between the two condensed phases being studied, in the present case blood and brain. Thus, a set of 157 substances with directly measured and indirectly determined log BB values was studied yielding the following equation [8]:

$$\log \mathrm{BB} = 0.044 + 0.511E - 0.886S - 0.724A - 0.666B + 0.861V \quad (n = 148,\ R^2 = 0.710,\ \mathrm{SD} = 0.367,\ F = 71) \quad (2)$$

At the time of its publication in 2001, due to the size of the set and chemical diversity of the selected molecules, this was a good general blood-brain distribution model, which revealed the factors of brain uptake. Provided that solute descriptors are zero or positive, large and positive coefficients increase log BB, which means, in turn, a higher affinity for brain. Thus, according to Eq. (2), solutes interacting through π- and n-electron pairs ($e \cdot E > 0$) and large molecules ($v \cdot V > 0$) show higher brain uptakes, whereas dipolar or polarizable solutes ($s \cdot S < 0$) with hydrogen-bond interactions ($a \cdot A$, $b \cdot B < 0$) tend to remain in the blood phase. The relatively low determination coefficient in Eq. (2) might be due to the difficulty of accurate experimental determination of log BB values, and the molecular descriptors used, either experimentally measured or calculated, referred to neutral solutes.

In a later study in 2004 [10], Eq. (1) was applied to 30 log PS values of neutral compounds, leading to the following equation for permeation from saline (standard deviations of the coefficients are reported in brackets):

$$\log \mathrm{PS} = -0.639(0.408) + 0.312(0.515)E - 1.009(0.158)S - 1.895(0.385)A - 1.636(0.410)B + 1.709(0.392)V \quad (n = 30,\ R^2 = 0.870,\ \mathrm{SD} = 0.52,\ F = 32.2) \quad (3)$$

It should be stressed that acidic or basic compounds that could be totally or partially ionized at the physiological pH of 7.4 were not included in that analysis, although carboxylic acids could be included in the log BB model of Eq. (2) by introduction of a correction factor [8]. In a later work, acids and bases totally ionized were also included in log PS correlations

[11]. A comparison of the coefficients in Eqs. (2) and (3) reveals that, qualitatively, blood-brain distribution and permeation are ruled by the same factors.

1.3. MELC as a physicochemical method for the determination of biological activity

Beyond ethical concerns in animal experimentation, in early stages of the drug discovery process an accurate *in vivo* determination of biological activity for a large number of potential candidates is unaffordable. Thus, isotropic organic solvent/water partition models (octanol, hexadecane…) were studied as physicochemical surrogates of BBB [5]. However, simple partition coefficients like octanol-water were unable to model the desolvation (breaking of the hydrogen-bounds between a solute and the solvating water molecules) involved in the transfer of compound from aqueous solution into a phospholipid bilayer. The combination of partition coefficients measured in octanol-water and alkane-water allowed the inclusion of hydrogen-bonding interactions, improving the prediction capacity of the model, but increasing the time required to carry out the determination. For screening purposes the measurement of several partition coefficients for a single molecule is excessively time consuming, and thus faster approaches are desirable.

Microemulsion liquid chromatography (MELC) is a very interesting technique, especially in the field of pharmaceutical analysis, because of the ability of the microemulsions (ME) used as mobile phases to solubilize both lipophilic and hydrophilic compounds and its separation capabilities [12,13]. Oil-in-water ME are made of oil droplets (octane, heptane…) stabilized by a surfactant (SDS, sodium cholate, Brij 35…) and a cosurfactant (a short-chain alcohol as 1-butanol, 1-pentanol…) and dispersed in an aqueous buffer. The anionic SDS is commonly used as surfactant in a concentration range of 2-3%, and typically the amount of oil is frequently below 1% [12,13]. When linear alkanes are involved in the ME, the mass ratio between SDS and the cosurfactant is suggested to be 0.5 [14]. For such systems, the oil-in-water ME strongly depends on the salt concentration and it can only exist in a relatively small water-rich range of compositions [15,16]. Once prepared, ME are stable and variations in their composition (pH, buffer nature, surfactant type and concentration…) do not significantly change their functionality [17]. However, retention mechanisms in MELC systems are complex, since solutes are expected to partition at least between the bulk aqueous phase, the oil droplet, and the surfactant-coated stationary phase [18].

Furthermore, and this is the main point of this study, ME can be used as physicochemical surrogate models of biological processes, such as lipophilicity [19–21] or BBB [22–24], since ME mimic, to some extent, the properties of cell membranes. Liu and

coworkers [22], following a LFER approach, characterized several MELC systems and compared them to biological ones. The authors concluded that a C18 stationary phase and a ME mobile phase consisting of 3.3% SDS, 6.6% butanol, 1.6% heptane and 88.5% 50 mM phosphate buffer pH 7.0 (all percentages in weight) was a good surrogate of BBB distribution, particularly log BB. However, Liu and coworkers [22] studied only 37 compounds, six of which were left out as outliers.

The purpose of this study is the comparison of several MELC systems to BBB systems by means of the Abraham model in order to find appropriate MELC systems for surrogation of BBB systems. Since in principle the Abraham model was derived for non ionic compounds, a further goal is to check the performance of MELC surrogation for drugs that should be totally or partially ionized drugs at the blood physiological pH.

2. Material and methods

2.1. Instrumentation

pH measurements were taken with a Crison (Barcelona, Spain) 5014 combination electrode (glass electrode and a reference electrode with a 3.0 M KCl solution in water as salt bridge) in a Crison GLP22 pH meter. MEs were sonicated in a J.P. Selecta (Barcelona, Spain) ultrasonic bath with a power of 360 W.

HPLC measurements were performed on a Shimadzu (Kyoto, Japan) HPLC system consisting of two LC-10ADvp pumps, a SIL-10ADvp auto-injector, an SPD-M10Avp diode array detector, a CTO-10ASvp oven at 37 °C and a SCL-10Avp controller. A 5 μm 150 x 4.6 mm Gemini C18 column and a 4 x 3.0 mm guard cartridge from Phenomenex (Torrance, CA, USA) were used at a flow rate of 1.0 mL min^{-1}. Each compound was analyzed at least in triplicate and injection volumes were set to 10 μL. Retention factors were expressed as log k = log $((t_R - t_0)/t_0)$, where t_R and t_0 were the retention times of analyte and potassium bromide (Merck, for analysis) as dead timer marker, respectively.

2.2 Mobile phase and sample preparation

Water was deionized to a resistivity of 18.2MΩ cm by the Milli−Q plus system from Millipore (Billerica, MA, USA). Aqueous buffer was prepared from sodium dihydrogenphosphate (Merck, 99%) and sodium hydrogenphosphate (J. T. Baker, 99.5%) to a final concentration of 50 mM and pH 7.4. Under magnetic stirring and at room temperature, 3.3% w/v of SDS (Sigma-Aldrich, > 99%) was dissolved in aqueous buffer until a transparent colorless solution was obtained. Then pH was adjusted to 7.4 by the addition of small

186 volumes of a 3 M NaOH solution prepared shortly before use from pellets (Merck, > 99%),
187 followed by the addition of 6.6% w/v 1−butanol (Sigma−Aldrich, 99.8%) and the desired
188 amount of heptane (0%, 0.8% or 1.6% w/v; Merck, for analysis). At this point, the solution
189 became white and turbid. Magnetic stirring was maintained for 10 min and the desired ME
190 volume was adjusted with aqueous buffer (in order to compensate the volume contraction of
191 the mixture). Then the ME was sonicated for about 30 min until it became clear again, and
192 finally the solution was left to stand at room temperature for at least 12 h. Immediately before
193 use, ME was vacuum filtered using a Büchner funnel and a 0.45 μm nylon membrane
194 (Teknokroma, Spain).
195 Injected compounds were provided by Abbott Laboratories (Abbot Park, IL, USA),
196 Acros Organics (Geel, Belgium), Astrazeneca (London, UK), Baker (Center Valley, PA,
197 USA), Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT, USA), Carlo Erba (Milano,
198 Italy), Esteve (Barcelona, Spain), Janssen (Beerse, Belgium), Merck (Billerica, MA, USA),
199 Roche (Basel, Switzerland), Scharlau (Barcelona, Spain), Sigma–Aldrich (St. Louis, MO,
200 USA), and Toronto Research Chemicals (Toronto, ON, Canada); all of high purity grade
201 (≥97%). 10 mg mL^{-1} stock solutions were prepared in methanol (Fisher, HPLC grade) and
202 ten-fold diluted with ME before injection.
203
204 **2.3 HPLC and column cleaning**
205 After a working session, in order to avoid the precipitation of SDS, the HPLC instrument and
206 column were washed at a flow rate of 1 mL min^{-1} with water/methanol 95:5 followed by
207 water/methanol 5:95, 30 min each.
208
209 **3. Results and discussion**
210 **3.1. LFER characterization of BBB permeability**
211 A new LFER characterization study according to Eq. (1) was conducted which broadens the
212 chemical diversity of test compounds in relation to Eq. (3). The study was based in the *in situ*
213 rodent brain perfusion permeability data referred to permeation from saline at pH 7.4 and
214 corrected for ionization, compiled by Avdeef [5]. Molecules were selected that exhibited BBB
215 passive permeation only, avoiding carrier-mediated or actively transported processes.
216 Therefore, the solvation property selected for this study was the so called intrinsic passive
217 permeability (log P_0^{BBB}). In fact, log P_0^{BBB} is just a correction of log PS for ionized
218 compounds and therefore log P_0^{BBB} = log PS in the case of non-ionized species. Observed log
219 P_0^{BBB} values obtained from experiments with rats were correlated with measured (when

220 available) or calculated molecular descriptors [25] (see Table 1), according to Eq. (1) then the
221 fitted coefficients were used to back calculate $\log P_0^{BBB}$ values, and finally a linear regression
222 was established between observed and predicted $\log P_0^{BBB}$ values. In this work, compounds
223 with residuals higher than twice the standard deviation of the regression were considered as
224 outliers. After excluding these values from correlations, the final coefficients obtained are
225 those in Eq. (4) and the corresponding plot is presented in Figure 1:

226 $$\log P_0^{BBB} = -4.048(0.139) + 0.213(0.133)E - 0.947(0.126)S - 0.438(0.150)A - 1.497(0.163)B + 1.953(0.133)V \quad (n = 141,\ R^2 = 0.833,\ \mathrm{SD} = 0.64,\ F = 135) \qquad (4)$$

227 It is noteworthy that Eq. (4) covers a wide range of permeability values (about 7 log
228 units) and includes molecules with different chemical properties as reflected by their
229 descriptors (Table 1).
230
231 **3.2. LFER characterization of MELC systems**
232 With the aim of exploring the predictive capacity of MELC systems for the prediction of BBB
233 distribution or permeability, three different mobile phases were prepared from 50 mM
234 phosphate buffer pH 7.4 containing the same SDS and 1-butanol concentration (3.3% and
235 6.6% w/v, respectively) but with different amounts of heptane (0, 0.8, and 1.6% w/v). Test
236 compounds (Table 2) were selected to present different chemical characteristics (hydrogen-
237 bonding interactions, dipolarity/polarizability…) and to be unionized at the desired pH in
238 order to build the correlations between log *k* and neutral molecular descriptors. The column
239 temperature was set to 37ºC because this is the physiological temperature. One additional
240 advantage of 37ºC over room temperature is the higher the temperature, the lower the mobile
241 phase viscosity and consequently the instrumental backpressure. Once outliers were excluded
242 (Figure 2), the following equations were obtained:

243 $$\log k_{1.6\%\ \mathrm{heptane}} = 0.179(0.059) - 0.011(0.052)E - 0.418(0.072)S - 0.283(0.099)A - 1.148(0.074)B + 1.203(0.095)V \quad (n = 46,\ R^2 = 0.938,\ \mathrm{SD} = 0.15,\ F = 122) \qquad (5)$$

244 $$\log k_{0.8\%\ \mathrm{heptane}} = 0.186(0.053) - 0.010(0.046)E - 0.411(0.062)S - 0.237(0.086)A - 1.133(0.064)B + 1.231(0.082)V \quad (n = 45,\ R^2 = 0.952,\ \mathrm{SD} = 0.13,\ F = 153) \qquad (6)$$

245 $$\log k_{0.0\%\ \mathrm{heptane}} = 0.197(0.052) - 0.015(0.039)E - 0.353(0.056)S - 0.167(0.086)A - 1.196(0.059)B + 1.202(0.081)V \quad (n = 41,\ R^2 = 0.959,\ \mathrm{SD} = 0.11,\ F = 163) \qquad (7)$$

246 Interestingly, both ME (Eq. (5) and (6)) show nearly identical system coefficients
247 despite the different concentration of heptane, and they are even similar to the micellar system
248 without heptane (Eq. (7)). Apparently the oil phase slightly favors interactions with

dipolar/polarizable solutes with hydrogen-bonding acidity properties, whereas the micellar phase shows somewhat affinity for molecules with hydrogen-bonding basicity.

3.3. Comparative study

A very interesting tool for the quantification of the similarity between two systems is the euclidean distance (*d*) of their characteristic vectors [26]. *e*, *s*, *a*, *b*, and *v* coefficients on Eq. (1) define the properties of a particular system, and they can be considered as the elements of a five-dimensional vector. When the comparison is established between vectors of different magnitudes, for instance log BB and log *k*, it is convenient to divide the elements by the length of the vector to obtain unit vectors (e_u, s_u, a_u, b_u, and v_u, Table 3), and then calculate the distance (Table 4). Complementarily, a plot of the two principal components (PC) obtained after a PCA analysis of the elements of unit vectors provides an approximate visual representation of similarity between systems.

In this study the comparison was performed between the biological systems of Eqs. (2-4) and the chromatographic surrogates of Eqs (5-7) and that reported by Liu and coworkers [22] mentioned in section 1.3, further referred as MP3 system according to the designation used in the original paper. From the data presented in Table 3, it can be concluded that all biological systems have in common that the larger the molecular volume, the more favored brain uptake, followed in a lesser extent by the capacity of interactions through π- and n-electrons. The coefficients of both permeability parameters, log PS and $\log P_0^{BBB}$, are very similar with the exception of the solute hydrogen-bonding acidity, more negative for log PS. Concerning the comparison of chromatographic systems, differences between log $k_{0.8\%}$ and log k_{MP3} were larger than expected, given that both ME were prepared in a similar way.

Concerning the PCA plot shown in Figure 3, the chromatographic approaches assayed in the present work form a cluster, with the ME systems containing 0.8 and 1.6% of heptane being slightly closer to each other. Interestingly, although the physicochemical system used by Liu et al. (log k_{MP3}) [22] was proposed as a surrogate of biological log BB, according to this PCA results it is much more similar to log PS, and the top left log BB seems to be far from the rest of all other systems, either biological or chromatographic. It must be pointed out that, according to the PCA loadings, the most relevant contribution to PC1 is the hydrogen-bond basicity of the system (-0.33e_u, 0.34s_u, 0.74a_u, -0.34b_u, and 0.34v_u), and therefore the systems with more negative a_u values lead to negative and similar PC1 digits (log BB, log PS,

281 and log k_{MP3}), whereas the opposite trend is obtained for the less negative ones (log P_{0BBB}, log
282 $k_{0\%}$, log $k_{0.8\%}$, and log $k_{1.6\%}$).

283 The quantitative estimation of differences between pairs of systems shown in Table 4
284 confirms the significant difference between log $k_{0.8\%}$ and log k_{MP3} observed on the PCA plot,
285 much larger than initially expected taking into account that both ME were prepared in a
286 similar way. The particular reasons leading to this mismatch are difficult to elucidate, but we
287 provide here tentatively some of the possible explanations. Firstly, the representativity of the
288 compounds used for correlations must be examined. In the present work the number of
289 molecules included in the characterization set was larger than that of Liu (45 vs 26), and the
290 studied log *k* range was wider (-0.848/1.203 vs -0.365/1.212). Another possible reason might
291 lie in the chromatographic column used. Although both stationary phases were C18, the
292 particular support and column technology might affect the retention of analytes (Gemini vs
293 AT Chrom). Finally, the accuracy in the dead time measurement and thus in the determination
294 of retention factors might have had an influence in the characterization (potassium bromide
295 peak vs first significant deviation of the baseline).

296 In relation to the biological systems, there is nearly the same distance from the three
297 studied MELC systems to log BB and to log PS, with the distance to the latter being slightly
298 shorter (Table 4). log PS and log P_0^{BBB} were initially expected to be closer to each other,
299 since the latter is a correction of the former in order not to consider only the permeation of
300 unionized species, which was very convenient in order to increase the number of compounds
301 involved in the LFER characterization, but both of them are related to the BBB penetration. In
302 order to find the possible reasons of this mismatch, a joint PCA was performed with the
303 molecular descriptors (*E*, *S*, *A*, *B*, and *V*) of both sets of compounds included in the
304 correlations of Eqs. (3) and (4), and the scores of the two main PC are plotted in Figure 4.
305 Although the 30 substances included in log PS study show a reasonably good distribution
306 over the two PCs, the higher number of compounds used for log P_0^{BBB} characterization allow
307 a better coverage of the chemical diversity space, including molecules that broadened the
308 range of hydrogen-bonding properties (A, 0.00/0.95 vs. 0.00/2.30; B, 0.48/2.55 vs 0.45/4.04)
309 and π- and n-electrons interactions (E, 0.21/3.48 vs. 0.18/4.63).

310 When comparing the calculated distances between the chromatographic systems
311 characterized in the present work and the biological BBB parameters, the highest similarity
312 (i.e. the lowest distance) was obtained for the ME containing a 0.8% of heptane and $\log P_0^{BBB}$
313 (0.175). In contrast, the shortest distance with log BB was found to be 0.597 in the case of the

ME with a 1.6% of oil. Therefore, according to the LFER characterization, the chromatographic systems here studied seemed to be better models of BBB permeability (log PS/log P_0^{BBB}) rather than distribution (log BB) measurements, particularly the ME containing a 0.8% of heptane.

3.4. MELC system as surrogate model for BBB

The previous section shows that MELC systems can be good surrogate sytems for brain perfusion of non ionized compounds **(**log P_0^{BBB}), but many BBB active drugs are partially or totally ionized at the physiological blood pH. Thus, it would be very convenient to test MELC surrogation for ionized drugs.

With the aim of assessing the predictive capacity of the proposed physicochemical system as a BBB model, several analytes with known log BB (Table 5) or log PS (Table 6) values were injected using as mobile phase the ME with a 0.8% of heptane. About only one-fourth of the injected substances were unionized at pH 7.4, which corresponds to saline solutions employed in the brain perfusion assays, and therefore it was the selected pH for the chromatographic mobile phase, log PS data were used instead of log P_0^{BBB} as a measure of unidirectional brain penetration. Depending on the acid-base properties of the compounds an appropriate mobile phase pH might possibly allow an estimation of the penetration of unionized species, but these results could not be correlated with *in vivo* data since these experiments can be only performed at pH values close to the physiological one. Literature BBB values were plotted against obtained chromatographic retention factors (Figure 5) and after removing outliers from the correlations the following models for log BB and log PS were built:

$$\log \mathrm{BB} = 0.524(0.084)\log k_{0.8\%} - 0.072(0.058) \quad (n = 42,\ R^2 = 0.496,\ \mathrm{SD} = 0.34,\ F = 39) \tag{8}$$

$$\log \mathrm{PS} = 1.149(0.080)\log k_{0.8\%} - 2.286(0.061) \quad (n = 40,\ R^2 = 0.843,\ \mathrm{SD} = 0.39,\ F = 204) \tag{9}$$

As expected from the LFER study, the MELC chromatographic system was not a good surrogate of log BB, since only 50% of the variance in log BB was predictable from retention factors and the slope of the regression is relatively low. In addition, compounds with extreme log BB values, either below -1.10 (ritonavir, flurbiprofen, didanosinec, salbutamol, atenolol) or above 1.15 (metoprolol, promazine, haloperidol, fluphenazine), were considered as outliers and thus the model failed in its modeling capacity. The standard deviation of the regression might appear to be acceptable (0.34), but it must be pointed out that the amplitude between

346 the lowest and the highest log BB values is only 2.25 units. In contrast, the chromatographic
347 system explained log PS variance (84%) better and outliers were distributed along all the
348 biological property range. In this case the standard deviation of the fitting was slightly higher
349 (0.39), but in relation to a wider scale of log PS values (3.66 units). The presence of a
350 relatively high number of outliers might be explained not only because of differences between
351 biological and chromatographic systems, but also as a consequence of the experimental
352 complexity of *in situ* brain perfusion experiments. In fact, from single compounds
353 significantly different log PS values can be found in the literature. For instance, this was the
354 case of the outlier sucrose, with reported log PS values in the range between -5.4 and -3.7, but
355 also quercetin (-3.8 and -2.7) or quinidine (-3.7 and -2.7). In case of different data from single
356 compounds, averaged log PS values were considered in the correlations, providing a rough
357 estimate of its accuracy, but unfortunately for some solutes only single results were reported.
358 It is also noteworthy to mention that the chromatographic system was intended to model
359 passive permeation, and thus it should not be applied to molecules that might present any kind
360 of active transport through the BBB.
361 Application of Eq. (9) to the different forms (neutral, zwitterionic or ionized) of acid
362 and basic drugs of diverse structure means that MELC surrogation of blood-brain perfusion
363 can be extended to all types of drugs regardless of drug charge or structure. Since both solvent
364 media (MELC mobile phase and blood saline plasma) are mainly similar aqueous phases,
365 drugs exhibit similar pK_a values and degrees of ionization, surrogation can be extended to
366 partially ionized drugs. This is an additional advantage of MELC for surrogation of biological
367 systems over other surrogating HPLC mobile phases containing organic solvents.
368
369 **4. Conclusions**
370 MELC systems of SDS+1-butanol+heptane at pH 7.4 have been characterized and
371 compared to blood brain transport by the Abraham model. Increasing the heptane
372 concentration up to 1.6% does not significantly changed the properties of the ME. The most
373 relevant factor for solute retention was the molecular volume, suggesting a high affinity of
374 large compounds for the C18 stationary phase. In contrast, dipolar/polarizable analytes and
375 those with hydrogen-bonding basicity interacted preferably with the ME mobile phase,
376 decreasing retention times. The oil concentration seemed to have a minor effect on
377 interactions through π- and n-electrons and solute acidity by hydrogen-bonding, reducing
378 retention as well but to a much lesser extent.

A chromatographic system consisting of a Gemini C18 column as stationary phase and a ME made of 50 mM phosphate buffer pH 7.4, 3.3% w/v SDS, 6.6% w/v of 1-butanol, and 0.8% w/v of heptane as mobile phase is proposed as surrogate model for the rate of BBB penetration, particularly the logarithm of the passive permeability surface area product (log PS). Chromatographic retention factors (log *k*) of neutral and ionized drugs are directly and linearly related to log PS, without the need of any additional correction parameter.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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Table 1. Intrinsic permeability values (log P_0^{BBB}) [5] and solute descriptors [25] of the compounds used on Eq. (4)

Compound	log P_0^{BBB}	*E*	*S*	*A*	*B*	*V*
1-Aminocyclohexanecarboxylic acid*	-5.99	**0.56**	**0.98**	**0.78**	**0.93**	1.16
3-Hydroxyanthranilic acid*	-2.72	**1.28**	**1.38**	**1.03**	**0.83**	1.09
3-Hydroxykyunrenine	-6.49	**1.70**	**2.19**	**1.31**	**1.71**	1.63
5-F-Uracil	-5.67	**0.97**	**1.29**	**1.17**	**0.99**	0.77
Acetamide	-5.05	**0.48**	**0.36**	**0.31**	**0.45**	0.51
Adenosine*	-4.80	**2.69**	**2.64**	**0.97**	**2.22**	1.75
Aldosterone	-5.46	**2.13**	**3.35**	**0.48**	**1.91**	2.75
Amantadine*	-1.20	**0.84**	**0.68**	**0.21**	**0.64**	1.29
Aminoguanidine	-5.85	**0.95**	**0.69**	**0.69**	**1.47**	0.61
Aminopyrine	-3.30	1.78	1.78	0.00	1.60	1.87
Amitriptyline	-1.48	2.25	1.78	0.00	1.00	2.40
Amoxapine	-2.75	**2.25**	**1.68**	**0.16**	**1.43**	2.25
Anthranilic acid	-4.91	1.08	1.48	0.74	0.50	1.03
Antipyrine	-4.00	1.32	1.50	0.00	1.48	1.48
Arabinose	-6.63	**0.98**	**1.55**	**0.94**	**1.52**	1.06
Ascorbic acid*	-2.54	**1.23**	**1.68**	**1.12**	**1.65**	1.11
Atomoxetine	-1.27	**1.37**	**1.36**	**0.13**	**0.90**	2.19
Brompheniramine	-1.70	**1.70**	**1.57**	**0.00**	**1.02**	2.26
Bupropion	-2.09	1.14	1.30	0.09	1.02	1.94
Butanediol	-5.03	**0.42**	**0.71**	**0.63**	**0.62**	0.79
Butanol	-2.88	0.22	0.42	0.37	0.48	0.73
Butyric acid*	-2.15	0.21	0.64	0.61	0.45	0.75
Caffeine	-3.90	1.50	1.72	0.05	1.28	1.36
Carbamazepine	-3.74	2.15	2.11	0.53	1.10	1.81
Carmustine	-3.81	**0.83**	**2.06**	**0.16**	**0.77**	1.39
Cetirizine*	-5.80	**2.05**	**2.24**	**0.57**	**1.76**	2.94
Chlorambucil*	-0.80	**1.22**	**1.60**	**0.57**	**0.80**	2.26
Chlorpheniramine	-1.84	1.47	1.34	0.00	1.35	2.21
Chlorpromazine	-1.33	2.20	1.83	0.00	0.94	2.41
Cimetidine	-5.92	1.70	1.73	0.67	2.21	1.96
Citalopram	-2.07	**1.66**	**1.87**	**0.00**	**1.08**	2.53
Clemastine	-0.96	**1.70**	**1.55**	**0.00**	**0.97**	2.76
Clozapine	-2.66	**2.46**	**1.82**	**0.18**	**1.44**	2.43
Colchicine*	-5.20	**2.23**	**2.59**	**0.31**	**1.95**	2.99
Corticosterone	-4.29	1.86	3.43	0.40	1.63	2.74
Creatinine*	-6.60	**1.03**	**0.51**	**0.31**	**1.07**	0.84
DADLE	-6.80	**3.01**	**5.54**	**2.30**	**3.76**	4.41
Daunomycine*	-2.40	**3.59**	**3.53**	**0.93**	**3.06**	3.67
DDEP	-3.60	**2.39**	**2.09**	**0.45**	**0.98**	1.97
DDMP	-3.47	**2.39**	**2.08**	**0.45**	**0.98**	1.83
Dianhydrogalactitol	-5.60	**0.98**	**1.09**	**0.46**	**1.18**	0.97

Diazepam	-3.30	2.08	1.57	0.00	1.25	2.07
Dibromodulcitol	-5.72	**1.44**	**1.65**	**1.23**	**1.26**	1.54
Diphenhydramine	-1.94	**1.36**	**1.43**	**0.00**	**0.95**	2.19
Donepezil	-1.68	**2.12**	**2.49**	**0.00**	**1.50**	3.03
Dopamine*	-2.68	1.35	1.46	1.20	1.04	1.22
Doxepin	-1.24	**1.75**	**1.46**	**0.00**	**0.98**	2.32
Doxorubicin	-4.00	**3.75**	**3.69**	**1.17**	**3.34**	3.73
DPDPE	-5.60	**3.87**	**5.81**	**2.30**	**4.04**	4.77
Ehylene glycol	-5.30	0.40	0.90	0.58	0.78	0.51
Ergotamine	-3.82	**4.63**	**3.87**	**0.85**	**3.56**	4.21
Erythritol	-6.90	0.62	1.20	0.83	1.45	0.91
Estradiol	-3.30	1.80	1.77	0.86	1.10	2.20
Ethanol	-3.40	0.25	0.42	0.37	0.48	0.45
Ethosuximide	-4.46	**0.74**	**0.94**	**0.34**	**0.93**	1.12
Fexofenadine*	-6.60	**2.72**	**2.48**	**1.20**	**2.12**	4.09
Fluoxetine	-1.10	**1.01**	**1.19**	**0.13**	**0.78**	2.24
Fluphenazine	-3.35	2.16	2.30	0.26	1.80	3.09
Flurbiprofen*	-0.58	**1.50**	**1.51**	**0.57**	**0.58**	1.84
Fluvastatin	-2.28	**2.75**	**2.48**	**1.20**	**1.46**	3.13
Formamide	-5.72	0.47	1.30	0.64	0.57	0.37
Fructose	-6.80	**1.30**	**1.61**	**1.31**	**1.83**	1.20
Ftorafur	-5.02	**1.05**	**1.66**	**0.24**	**1.14**	1.28
Gabapentin	-4.56	**0.56**	**0.99**	**0.78**	**0.93**	1.44
Galactitol	-6.70	**1.23**	**1.75**	**1.62**	**1.81**	1.31
Glibenclamide	-3.24	**2.81**	**2.52**	**0.99**	**2.07**	3.56
Glucose*	-4.50	**1.34**	**1.64**	**1.31**	**1.85**	1.20
Glycerol	-5.40	0.51	0.76	0.47	1.43	0.71
Glycine	-5.50	**0.37**	**0.93**	**0.78**	**0.90**	0.56
Grepafloxacin	-4.86	**2.23**	**2.43**	**0.73**	**1.88**	2.59
Guanidine	-5.60	0.60	0.86	0.36	1.24	0.51
Haloperidol	-2.46	1.90	1.39	0.40	1.76	2.80
Hexanoic acid*	-1.31	0.17	0.63	0.62	0.44	1.03
Hispidulin	-3.11	**2.30**	**2.32**	**0.96**	**1.20**	2.05
Hydrocortisone	-5.85	2.03	3.49	0.71	1.90	2.80
Hydroxyzine	-3.04	2.00	2.21	0.10	1.89	2.92
Hypoxanthine	-5.46	**1.65**	**1.68**	**0.44**	**1.04**	0.88
Inulin	-7.35	**2.28**	**2.60**	**2.01**	**3.41**	2.23
Iodoacetamide	-4.10	**1.03**	**1.37**	**0.49**	**0.60**	0.76
Iodoantipyrine	-3.20	**2.01**	**1.98**	**0.00**	**1.31**	1.74
Isocarboxazid*	-3.22	**1.61**	**2.16**	**0.39**	**1.38**	1.74
Isopropanol	-3.66	0.21	0.36	0.33	0.56	0.59
L-Alanine	-5.44	**0.38**	**0.92**	**0.78**	**0.93**	0.71
Lamotrigine	-4.67	2.27	2.03	0.35	0.96	1.65
L-Arginine	-4.64	**1.06**	**1.24**	**1.26**	**1.95**	1.38

L-Aspartic acid	-6.66	**0.55**	**1.37**	**1.18**	**1.26**	0.92
Levodopa*	-3.90	**1.33**	**1.77**	**1.56**	**1.44**	1.43
L-Glutamic acid	-6.26	**0.55**	**1.37**	**1.35**	**1.26**	1.06
L-Glutamine	-5.28	**0.86**	**1.12**	**1.09**	**1.35**	1.10
L-Histidine*	-4.28	**1.02**	**1.74**	**1.13**	**1.41**	1.13
Lidocaine	-3.24	1.01	1.50	0.12	1.21	2.06
L-Isoleucine	-4.16	**0.39**	**0.92**	**0.78**	**0.97**	1.13
L-Kynurenine	-6.16	**1.50**	**2.06**	**0.96**	**1.60**	1.57
L-Lysine	-4.93	**0.58**	**1.26**	**0.99**	**1.48**	1.23
L-Methionine	-4.39	**0.72**	**1.08**	**0.78**	**1.06**	1.15
Lomustine	-4.00	**0.93**	**2.00**	**0.16**	**0.79**	1.72
Loratidine*	-4.00	**2.19**	**2.09**	**0.00**	**1.14**	2.87
L-Ornithine	-4.68	**0.58**	**1.25**	**0.99**	**1.48**	1.09
Lovastatin acid	-2.53	**1.39**	**1.84**	**1.20**	**1.62**	3.45
Lovastatin*	-3.42	**1.38**	**2.34**	**0.31**	**1.44**	3.29
Loxapine	-3.36	**2.30**	**1.67**	**0.00**	**1.49**	2.39
L-Threonine	-5.21	**0.61**	**1.14**	**1.03**	**1.33**	0.91
L-Tryptophan	-4.22	**1.62**	**1.80**	**1.09**	**1.23**	1.54
L-Tyrosine	-3.90	**1.18**	**1.60**	**1.28**	**1.29**	1.37
L-Valine	-4.68	**0.39**	**0.92**	**0.78**	**0.97**	0.99
Mannitol	-6.90	0.84	2.26	0.86	1.79	1.31
Maprotiline	-0.40	**1.76**	**1.27**	**0.13**	**0.68**	2.33
Melphalan*	-5.27	**1.43**	**1.90**	**0.78**	**1.37**	2.22
Meprobamate	-5.09	**0.71**	**1.62**	**0.89**	**1.12**	1.73
Mesoridazine*	-1.41	**2.87**	**2.97**	**0.00**	**1.69**	2.96
Methanol	-3.66	0.28	0.44	0.43	0.47	0.31
Methotrexate	-5.40	**3.51**	**4.23**	**1.85**	**2.82**	3.22
Methylurea	-5.70	**0.53**	**1.14**	**0.59**	**0.70**	0.61
Metoclopramide	-2.86	**1.59**	**1.57**	**0.54**	**1.50**	2.34
Midazolam	-3.11	2.57	2.01	0.00	1.38	2.26
Mirtazapine	-2.75	**2.08**	**1.67**	**0.00**	**1.22**	2.11
Naproxen*	-0.77	1.51	1.98	0.60	0.68	1.78
Naringenin	-3.96	**2.23**	**2.19**	**1.30**	**1.14**	1.89
Nicotinamide	-4.88	1.01	1.09	0.63	1.00	0.93
Octanoic acid*	-1.14	0.15	0.65	0.62	0.45	1.31
Olanzapine	-2.73	**2.30**	**1.59**	**0.13**	**1.45**	2.37
Oxycodone	-3.40	**2.18**	**2.28**	**0.23**	**1.80**	2.26
PCNU	-4.86	**1.47**	**2.72**	**0.50**	**1.66**	1.71
Pemoline	-5.45	**1.48**	**1.45**	**0.21**	**1.22**	1.26
Pentazocine*	-3.69	**1.54**	**1.13**	**0.50**	**1.04**	2.45
Pergolide	-1.14	**2.22**	**1.48**	**0.31**	**1.01**	2.54
Perphenazine	-2.61	**2.87**	**2.33**	**0.23**	**1.84**	3.02
Phenelzine	-4.32	**0.98**	**1.02**	**0.34**	**0.99**	1.20
Phenytoine	-4.09	1.71	2.19	0.85	1.00	1.87

Pramipexole		-2.70	**1.35**	**1.35**	**0.36**	**0.97**	1.68
Procarbazine		-4.62	**1.22**	**1.79**	**0.52**	**1.59**	1.88
Progesterone		-3.74	1.45	3.29	0.00	1.14	2.62
Propranolol*		-1.30	1.88	1.43	0.17	1.42	2.15
Propylene glycol		-4.49	0.37	0.90	0.58	0.80	0.65
Pyrilamine		-2.90	1.82	1.92	0.00	1.59	2.39
Pyrimethamine		-3.57	**1.90**	**0.98**	**0.34**	**1.36**	1.85
Quercetin		-4.70	**2.68**	**2.64**	**1.88**	**1.63**	1.96
Quetiapine		-3.06	**2.72**	**1.93**	**0.23**	**2.01**	2.91
Quinidine		-3.90	**2.40**	**1.71**	**0.23**	**1.81**	2.55
Quinine		-3.45	2.47	1.23	0.37	1.97	2.55
Quinolinic acid		-6.26	**0.99**	**1.59**	**1.14**	**1.06**	1.11
Rimantadine*		0.13	**0.84**	**0.67**	**0.21**	**0.68**	1.57
Rimonabant		-3.60	**3.38**	**3.13**	**0.26**	**1.55**	3.21
Risperidone		-2.94	**2.59**	**2.23**	**0.00**	**1.70**	3.04
Rizatriptan		-4.43	**2.21**	**2.05**	**0.31**	**1.28**	2.14
Salicylic acid*		-1.02	0.89	0.84	0.71	0.38	0.99
Selegiline		-3.12	**1.00**	**1.00**	**0.09**	**0.71**	1.72
Sucrose		-6.90	1.97	2.50	2.10	3.00	2.23
Sumatriptan		-5.06	1.87	2.28	0.85	1.88	2.27
Tacrine		-1.51	**1.61**	**0.94**	**0.13**	**0.61**	1.60
Temazepam		-3.35	2.29	1.55	0.12	1.70	2.13
Terfenadine		-0.92	**2.55**	**2.04**	**0.63**	**1.80**	4.01
Testosterone		-3.40	1.54	2.59	0.32	1.19	2.38
Theobromine		-5.00	1.50	1.60	0.50	1.38	1.22
Theophylline		-5.00	1.50	1.60	0.54	1.34	1.22
Thioridazine		-1.95	2.70	2.10	0.00	1.30	2.90
Thiothixene		-2.35	**2.94**	**2.59**	**0.00**	**2.19**	3.36
Thiourea		-5.50	0.84	0.82	0.77	0.87	0.57
Thymidine		-5.84	**1.78**	**2.01**	**0.81**	**2.11**	1.66
Thymine		-3.93	**1.09**	**1.23**	**1.00**	**1.01**	0.89
Tiagabine*		-4.45	**1.77**	**1.60**	**0.57**	**1.02**	2.89
Tolbutamide		-2.64	**1.44**	**1.61**	**0.68**	**1.33**	2.06
Trazodone		-3.13	**2.64**	**2.47**	**0.00**	**1.92**	2.73
Trifluoperazine		-3.00	2.00	1.80	0.00	1.50	2.89
Trimethylene glycol		-5.40	0.40	0.91	0.77	0.85	0.65
TYR-MIF-1		-5.78	**2.59**	**4.80**	**1.71**	**3.30**	3.48
Urea		-6.00	**0.74**	**0.57**	**0.52**	**0.87**	0.46
Valproic acid		-2.00	0.18	0.60	0.61	0.45	1.31
Warfarin*		-1.56	2.30	2.18	0.35	1.49	2.31
Xanthine		-5.62	1.50	1.60	0.97	1.07	0.94
Zaleplon		-4.25	**2.36**	**2.60**	**0.00**	**1.42**	2.31
Ziprasidone		-3.25	**3.38**	**2.67**	**0.48**	**1.65**	2.92
	Minimum	*-7.35*	*0.15*	*0.36*	*0.00*	*0.38*	*0.31*

Maximum	*0.13*	*4.63*	*5.81*	*2.30*	*4.04*	*4.77*
Median	*-3.90*	*1.50*	*1.63*	*0.52*	*1.28*	*1.84*
Average	*-3.89*	*1.58*	*1.76*	*0.60*	*1.36*	*1.89*
SD	*1.66*	*0.85*	*0.87*	*0.50*	*0.64*	*0.92*

Average $\log P_0^{BBB}$ is reported in case of different literature values for the same compound.

Experimental molecular descriptors marked in bold.

*Compounds excluded from correlation.

Table 2. Molecular descriptors [25] and measured retention factors of the compounds used for the characterization of the chromatographic systems containing 0, 0.8, and 1.6% of heptane (w/v).

Compound	Molecular descriptors[a]					log k[b]		
	E	S	A	B	V	0%	0.8%	1.6%
1,2,4-trimethylbenzene	**0.68**	**0.56**	**0.00**	**0.19**	1.14	1.119	1.116	1.071
2-nitroanisole	**0.97**	**1.34**	**0.00**	**0.45**	1.09	0.419	0.425	0.380
4-chloroacetanilide	**0.98**	**1.47**	**0.64**	**0.51**	1.24	0.358	0.346	0.199
Acetamide	0.46	1.30	0.54	0.68	0.51	-1.200	-0.848	-0.888
Acetanilide	**0.90**	**1.39**	**0.48**	**0.67**	1.11	0.057	0.070	-0.022
Acetophenone	**0.82**	**1.01**	**0.00**	**0.48**	1.01	0.410	0.449	0.440
Aminopyrene	**1.78**	**1.78**	**0.00**	**1.60**	1.87	-0.162	-0.120	-0.182
Anisole	**0.71**	**0.75**	**0.00**	**0.29**	0.92	0.788	0.827	0.832
Anthracene	**2.29**	**1.34**	**0.00**	**0.28**	1.45	1.144	1.081	1.027
Antipyrine	**1.32**	**1.50**	**0.00**	**1.48**	1.48	-0.365	-0.281	-0.366
Benzaldehyde	**0.82**	**1.00**	**0.00**	**0.39**	0.87	0.390	0.436	0.442
Benzamide	**0.99**	**1.50**	**0.49**	**0.67**	0.97	-0.120	-0.106	-0.192
Benzene	**0.61**	**0.52**	**0.00**	**0.14**	0.72	0.866	0.908	0.928
Benzofuran	**0.89**	**0.83**	**0.00**	**0.15**	0.91	0.934	0.936	0.919
Benzyl alcohol	**0.80**	**0.87**	**0.39**	**0.56**	0.92	0.104	0.124	0.046
Bromobenzene	**0.88**	**0.73**	**0.00**	**0.09**	0.89	1.000	0.991	0.977
Butanone	0.17	0.70	0.00	0.51	0.69	-0.297	-0.178	-0.162
Butylbenzene	**0.60**	**0.51**	**0.00**	**0.15**	1.28	1.170	1.149	1.096
Butyrophenone	**0.80**	**0.95**	**0.00**	**0.51**	1.30	0.839	0.838	0.815
Caffeine	**1.50**	**1.72**	**0.05**	**1.28**	1.36	-0.663	-0.533	-0.606
Carbamazepine	**2.15**	**2.11**	**0.53**	**1.10**	1.81	0.212	0.197	0.068
Celecoxib	2.51	2.43	0.44	1.22	2.47	0.732	0.653	0.447
Cortisone	1.96	3.50	0.36	1.87	2.76	0.008	-0.004	-0.105
Coumarin	**1.06**	**1.76**	**0.00**	**0.43**	1.06	0.219	0.224	0.163
Diazepam	**2.08**	**1.57**	**0.00**	**1.25**	2.07	0.459	0.424	0.274
Ethylbenzene	**0.61**	**0.51**	**0.00**	**0.15**	1.00	1.061	1.052	1.037
Flunitrazepam	**2.10**	**2.15**	**0.00**	**1.48**	2.14	0.333	0.314	0.180
Hydrocortisone	**2.03**	**3.50**	**0.71**	**1.90**	2.80	0.041	0.026	-0.109
Lamotrigine	**2.27**	**2.03**	**0.35**	**0.96**	1.65	0.153	0.186	0.074
Loratadine	2.19	2.09	0.00	1.14	2.87	0.857	0.772	0.548
N,*N*-dimethylacetamide	**0.36**	**1.35**	**0.00**	**0.77**	0.79	-0.954	-0.710	-0.782
Naphthalene	**1.34**	**0.92**	**0.00**	**0.20**	1.09	1.043	1.019	0.997
Nitrobenzene	**0.87**	**1.11**	**0.00**	**0.28**	0.89	0.621	0.631	0.631
N-phenylurea	**1.11**	**1.33**	**0.79**	**0.79**	1.07	0.005	0.020	-0.084
Omeprazole	2.67	3.18	0.35	2.05	2.52	0.349	0.346	0.186
Paracetamol	**1.06**	**1.63**	**1.04**	**0.86**	1.17	-0.609	-0.461	-0.547
Pentachloronitrobenzene	1.47	1.70	0.00	0.01	1.50	1.248	1.203	1.113
Phenanthrene	**2.06**	**1.29**	**0.00**	**0.29**	1.45	1.132	1.095	1.018
Prednisolone	**2.21**	**3.10**	**0.71**	**1.92**	2.76	0.066	0.034	-0.083
Pregnenolone	**1.36**	**3.29**	**0.32**	**1.18**	2.67	0.778	0.700	0.519
Progesterone	**1.45**	**3.29**	**0.00**	**1.14**	2.62	0.671	0.606	0.473
Propiophenone	**0.80**	**0.95**	**0.00**	**0.51**	1.16	0.666	0.683	0.685
Propylbenzene	**0.60**	**0.50**	**0.00**	**0.15**	1.14	1.127	1.119	1.073

Pyrene	2.60	1.52	0.00	0.25	1.59	1.156	1.111	1.021
Pyrrole	**0.61**	**0.91**	**0.22**	**0.25**	0.58	-0.039	0.012	-0.031
Riluzole	1.36	1.45	0.23	0.67	1.32	0.584	0.588	0.422
Rofecoxib	1.66	2.43	0.00	1.15	2.23	0.093	0.120	0.000
Theophylline	**1.50**	**1.60**	**0.54**	**1.34**	1.22	-0.812	-0.587	-0.680
Toluene	**0.60**	**0.52**	**0.00**	**0.14**	0.86	0.985	0.998	0.996
Valerophenone	**0.80**	**0.95**	**0.00**	**0.50**	1.44	0.957	0.940	0.897
Minimum	*0.17*	*0.50*	*0.00*	*0.01*	*0.51*	*-1.200*	*-0.848*	*-0.888*
Maximum	*2.67*	*3.50*	*1.04*	*2.05*	*2.87*	*1.248*	*1.203*	*1.113*

[a]Experimental molecular descriptors marked in bold.

[b]Mean log *k* values obtained from triplicate injections, with SD below 0.01 in all cases.

Table 3. LFER system coefficients of unit vectors.

	e_u	s_u	a_u	b_u	v_u
log BB	0.308	-0.534	-0.436	-0.401	0.519
log PS	0.097	-0.314	-0.590	-0.510	0.532
log P_0^{BBB}	0.080	-0.353	-0.163	-0.558	0.728
log k_{MP3}	0.059	-0.373	-0.533	-0.550	0.521
log $k_{1.6\%}$	-0.006	-0.241	-0.163	-0.660	0.692
log $k_{0.8\%}$	-0.006	-0.237	-0.136	-0.652	0.708
log $k_{0.0\%}$	-0.009	-0.203	-0.096	-0.687	0.691

Table 4. Distances between pairs of studied systems.

	log BB	log PS	log P_0^{BBB}	log k_{MP3}	log $k_{1.6\%}$	log $k_{0.8\%}$
log BB	0	-	-	-	-	-
log PS	0.358	0	-	-	-	-
log P_0^{BBB}	0.477	0.474	0	-	-	-
log k_{MP3}	0.345	0.100	0.425	0	-	-
log $k_{1.6\%}$	0.597	0.497	0.178	0.447	0	-
log $k_{0.8\%}$	0.612	0.523	0.175	0.475	0.033	0
log $k_{0\%}$	0.661	0.570	0.230	0.522	0.082	0.066

Table 5. Biological log BB values [8] and their corresponding measured retention factors in the chromatographic system containing 0.8% of heptane (w/v).

Compounds	log BB	log $k_{0.8\%}$[a]	Ionization at pH 7.4[b]			
			Neutral	Zwitterionic	Negative	Positive
1,2,4-trimethylbenzene	0.16	1.116	100%	0%	0%	0%
1,2-dimethylbenzene	0.30	1.064	100%	0%	0%	0%
1,3-dimethylbenzene	0.29	1.049	100%	0%	0%	0%
1,4-dimethylbenzene	0.31	1.045	100%	0%	0%	0%
Acetazolamide	-0.52	-0.934	43%	0%	57%	0%
Acyclovir	-0.50	-0.906	78%	0%	0%	22%
Alprenolol	-0.23	0.779	1%	0%	0%	99%
Aminopyrene	0.00	-0.120	100%	0%	0%	0%
Amiodarone[c]	-1.08	1.002	4%	0%	0%	96%
Amitriptyline	0.90	0.860	4%	0%	0%	96%
Amprenavir	-0.56	0.271	100%	0%	0%	0%
Antipyrine	-0.10	-0.281	100%	0%	0%	0%
Atenolol[c]	-1.12	-0.012	1%	0%	0%	99%
Atropine	-0.06	0.383	1%	0%	0%	99%
Barbital	-0.14	-0.221	100%	0%	0%	0%
Benzene	0.37	0.908	100%	0%	0%	0%
Butanone	-0.08	-0.178	100%	0%	0%	0%
Caffeine	-0.06	-0.533	100%	0%	0%	0%
Carbamazepine	-0.11	0.197	100%	0%	0%	0%
Celecoxib[c]	-1.00	0.653	100%	0%	0%	0%
Clobazam	0.35	0.274	100%	0%	0%	0%
Codeine	0.55	0.142	13%	0%	0%	87%
Diazepam	0.48	0.424	100%	0%	0%	0%
Didanosine[c]	-1.30	-0.832	97%	0%	3%	0%
Ethylbenzene	0.20	1.052	100%	0%	0%	0%
Flunitrazepam	0.06	0.314	100%	0%	0%	0%
Fluphenazine[c]	1.51	0.851	25%	0%	0%	75%
Flurbiprofen[c]	-1.68	-0.011	0%	0%	100%	0%
Haloperidol[c]	1.32	1.090	5%	0%	0%	95%
Ibuprofen	-0.18	0.061	0%	0%	100%	0%
Imipramine	1.01	0.841	2%	0%	0%	98%
Lamotrigine	0.36	0.186	0%	0%	0%	100%
Lidocaine	0.34	0.585	14%	0%	0%	86%
Metoprolol[c]	1.15	0.536	1%	0%	0%	99%
Nevirapine	0.00	0.151	100%	0%	0%	0%
Nicotine	0.56	0.129	11%	0%	0%	89%
Paracetamol	-0.42	-0.461	100%	0%	0%	0%
Pindolol	-0.15	0.312	1%	0%	0%	99%
Promazine[c]	1.23	0.786	2%	0%	0%	98%
Propranolol	0.88	0.685	1%	0%	0%	99%
Pyrene	0.23	1.111	100%	0%	0%	0%
Pyrilamine	0.49	0.632	4%	0%	0%	96%
Quinidine	-0.32	0.733	6%	0%	0%	94%
Riluzole	0.30	0.588	100%	0%	0%	0%

Ritonavir[c]	-1.82	0.831	100%	0%	0%	0%
Salbutamol[c]	-1.17	0.100	2%	1%	0%	97%
Salicylic acid	-1.10	-1.064	0%	0%	100%	0%
Saquinavir[c]	-0.86	0.901	87%	0%	0%	13%
Stavudine	-0.48	-0.793	93%	0%	7%	0%
Terfenadine	1.15	1.300	1%	0%	0%	99%
Theophylline	-0.31	-0.587	95%	0%	5%	0%
Toluene	0.37	0.998	100%	0%	0%	0%
Trazodone	-0.22	0.484	83%	0%	0%	17%
Zidovudine	-0.77	-0.457	99%	0%	1%	0%

[a]Mean log *k* values obtained from triplicate injections, with SD below 0.01 in all cases.
[b]Neutral, zwitterionic, negative and positive fraction calculated from GALAS algorithm [25].
[c]Excluded from correlation on Eq. (8).

Table 6. Biological log PS values [5] and their corresponding measured retention factors in the chromatographic system containing 0.8% of heptane (w/v).

Compounds	log PS	log $k_{0.8\%}$[a]	Ionization at pH 7.4[b]			
			Neutral	Zwitterionic	Negative	Positive
5-F-Uracil	-3.77	-0.916	72%	0%	28%	0%
Acetamide	-3.05	-0.848	100%	0%	0%	0%
Aminopyrine[c]	-1.30	-0.120	100%	0%	0%	0%
Amitriptyline	-1.02	0.860	4%	0%	0%	96%
Anthranilic acid[c]	-2.92	-1.389	0%	1%	0%	99%
Antipyrine	-1.94	-0.281	100%	0%	0%	0%
Caffeine[c]	-1.83	-0.533	100%	0%	0%	0%
Carbamazepine	-1.74	0.197	100%	0%	0%	0%
Corticosterone	-2.28	0.125	100%	0%	0%	0%
Diazepam	-1.27	0.424	100%	0%	0%	0%
Diphenhydramine	-1.24	0.739	5%	0%	0%	95%
Estradiol	-1.08	0.393	100%	0%	0%	0%
Fluphenazine	-1.87	0.851	25%	0%	0%	75%
Flurbiprofen	-1.80	-0.011	0%	0%	100%	0%
Formamide	-3.72	-0.818	100%	0%	0%	0%
Glibenclamide	-2.77	0.117	7%	0%	93%	0%
Glycine	-3.49	-0.930	0%	100%	0%	0%
Haloperidol	-1.45	1.090	5%	0%	0%	95%
Hydrocortisone[c]	-3.85	0.026	100%	0%	0%	0%
Ibuprofen	-2.03	0.061	0%	0%	100%	0%
Indinavir[c]	-3.73	0.559	96%	0%	0%	4%
L-Alanine[c]	-3.44	-0.109	0%	100%	0%	0%
Lamotrigine	-2.68	0.186	0%	0%	0%	100%
L-Arginine	-2.64	-0.127	0%	3%	0%	97%
L-Aspartic acid	-4.66	-1.856	0%	0%	100%	0%
L-Glutamic acid	-4.26	-1.898	0%	0%	100%	0%
L-Glutamine	-3.28	-0.963	0%	99%	1%	0%
Lidocaine	-1.90	0.585	14%	0%	0%	86%
L-Lysine	-2.92	-0.115	0%	0%	100%	0%
L-Tryptophan	-2.23	-0.260	0%	98%	2%	0%
L-Tyrosine[c]	-1.91	-0.777	0%	98%	2%	0%
L-Valine	-2.68	-0.113	0%	100%	0%	0%
Maprotiline	-1.35	0.889	0%	0%	0%	100%
Naringenin	-1.98	0.053	59%	0%	41%	0%
Nicotinamide	-2.88	-0.635	98%	0%	0%	2%
Perphenazine	-1.25	0.805	25%	0%	0%	75%
Progesterone	-1.74	0.606	100%	0%	0%	0%
Propranolol	-1.00	0.685	1%	0%	0%	99%
Pyrilamine	-1.82	0.632	4%	0%	0%	96%
Quercetin[c]	-3.05	0.122	64%	0%	36%	0%
Quetiapine	-1.31	0.543	99%	0%	0%	1%
Quinidine[c]	-2.92	0.733	6%	0%	0%	94%
Salicylic acid	-3.40	-1.064	0%	0%	100%	0%
Sucrose[c]	-4.52	0.271	100%	0%	0%	0%

Terfenadine	-1.39	1.300	1%	0%	0%	99%
Testosterone	-1.31	0.387	100%	0%	0%	0%
Theophylline	-2.96	-0.587	95%	0%	5%	0%
Thiourea	-3.52	-0.768	100%	0%	0%	0%
Thymine[c]	-1.93	-0.632	99%	0%	1%	0%
Trazodone	-1.46	0.484	83%	0%	0%	17%
Verapamil	-1.76	0.644	5%	0%	0%	95%

[a]Mean log *k* values obtained from triplicate injections, with SD below 0.01 in all cases.
[b]Neutral, zwitterionic, negative and positive fraction calculated from GALAS algorithm [25].
[c]Excluded from correlation on Eq. (9).
PS in units of 10^{-4} mL g^{-1} s^{-1}

FIGURE CAPTIONS

Figure 1. Plot of observed vs. calculated intrinsic permeability values. Empty symbols show compounds excluded from correlation.

Figure 2. Plot of observed vs. calculated retention factors of the assayed chromatographic systems. Empty symbols show compounds excluded from correlations (loratadine, *N*,*N*-dimethylacetamide, omeprazole, and rofecoxib were excluded in all systems).

Figure 3. Plot of the first two scores of the PCA of the compared biological (Eqs. 2-4) and chromatographic BBB systems (Eq. 5-7 and log k_{MP3} [22]).

Figure 4. Joint PCA analysis of compounds used in log PS (empty squares) and log P_0^{BBB} (full circles) correlations.

Figure 5. Plot of biological BBB distribution (log BB) and permeation (log PS) values vs. retention factors obtained for the chromatographic system containing a 0.8% of heptane at pH 7.4. Legend: (●) unionized, (▼) zwitterionic, (■) totally or partially negatively charged, (▲) totally or partially positively charged, and (x) compounds excluded from correlations.

Figure 1

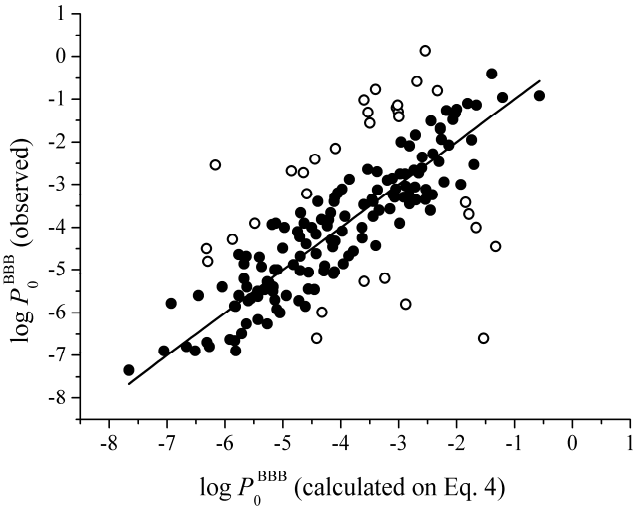

log P_0^{BBB} (observed)
log P_0^{BBB} (calculated on Eq. 4)
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-8
-7
-6
-5
-4
-3
-2
-1
0
1

Figure 2

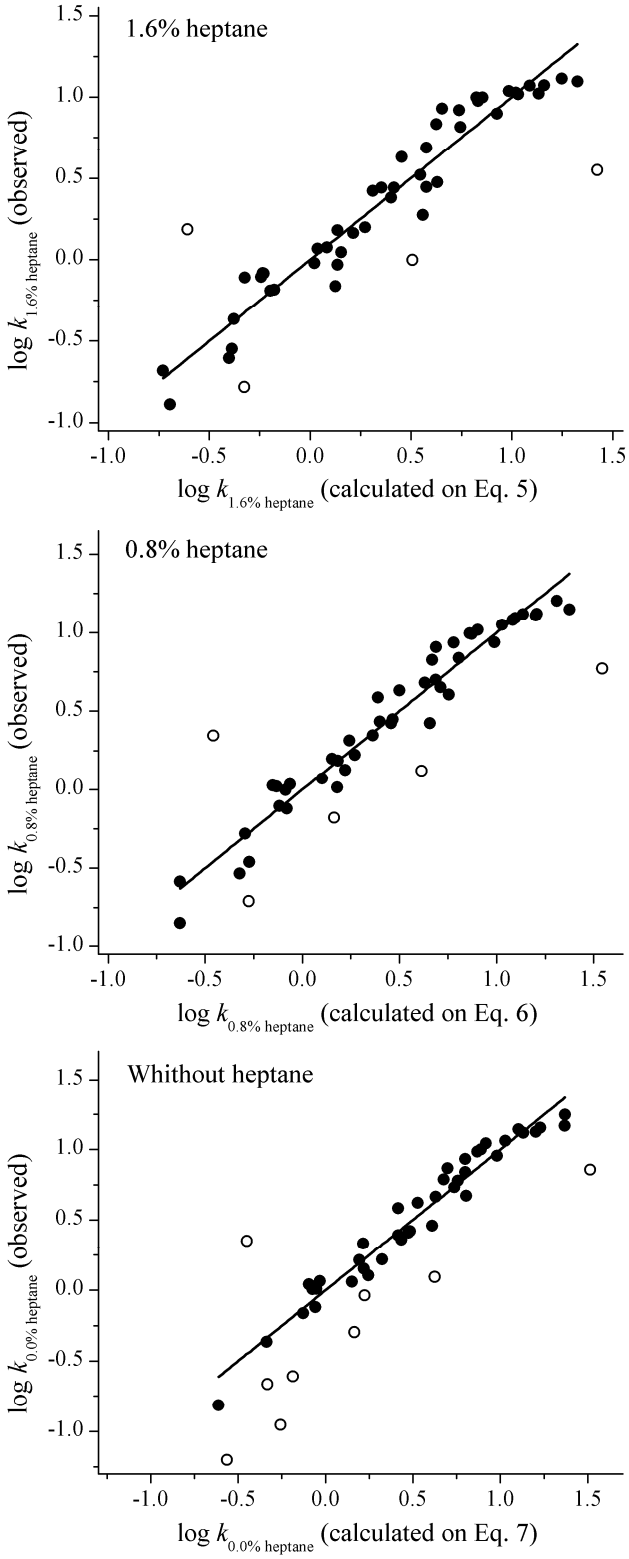

1.6% heptane
log $k_{1.6\%\ heptane}$ (observed)
log $k_{1.6\%\ heptane}$ (calculated on Eq. 5)
0.8% heptane
log $k_{0.8\%\ heptane}$ (observed)
log $k_{0.8\%\ heptane}$ (calculated on Eq. 6)
Whithout heptane
log $k_{0.0\%\ heptane}$ (observed)
log $k_{0.0\%\ heptane}$ (calculated on Eq. 7)

Figure 3

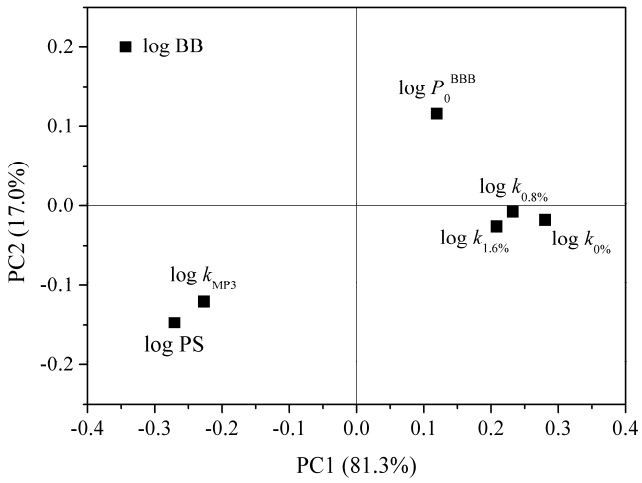

log BB
log P_0^{BBB}
log $k_{0.8\%}$
log $k_{1.6\%}$
log $k_{0\%}$
log k_{MP3}
log PS
PC2 (17.0%)
PC1 (81.3%)
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4

Figure 4

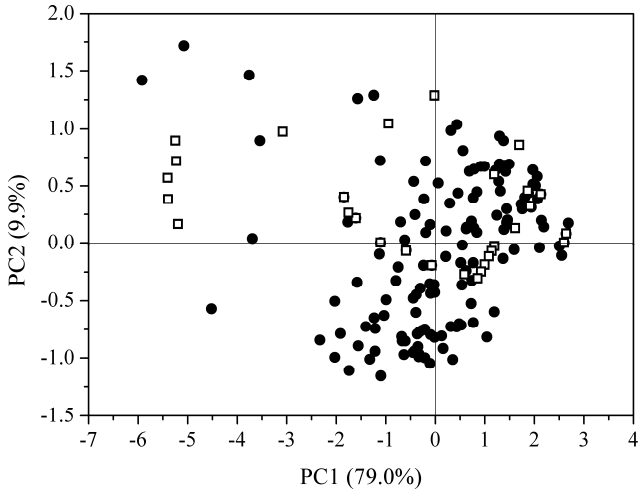
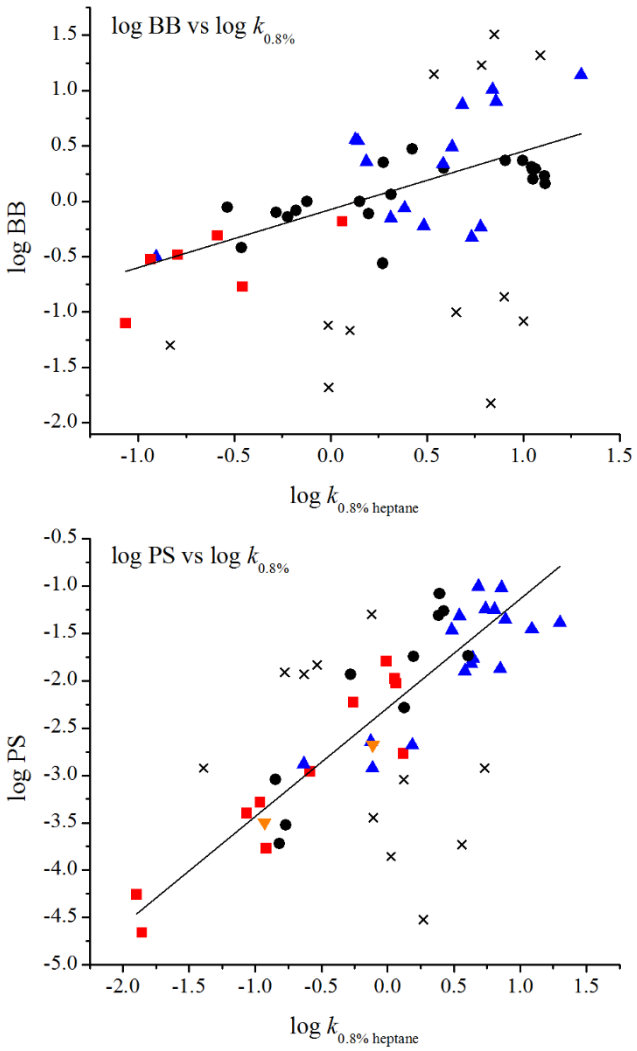


Figure 5


log BB vs log $k_{0.8\%}$
log BB
log $k_{0.8\%\ heptane}$
log PS vs log $k_{0.8\%}$
log PS
log $k_{0.8\%\ heptane}$