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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.

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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 30–180 s [5], and it ends before any equilibrium state can

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.

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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 iodoantipyrine, 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:

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

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]:

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

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):

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

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.

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