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Determination of drug lipophilicity by phosphatidylcholine-modified microemulsion high-performance liquid chromatography

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ABSTRACT

A new biomembrane-mimetic liquid chromatographic method using a C_8 stationary phase and phosphatidylcholine-modified (PC-modified) microemulsion mobile phase was used to estimate unionized and ionized drugs lipophilicity expressed as an *n*-octanol/water partition coefficient (logP and logD). The introduction of PC into sodium dodecyl sulfate (SDS) microemulsion yielded a good correlation between logk and logD ($R^2 = 0.8$). The optimal composition of the PC-modified microemulsion liquid chromatography (PC-modified MELC) mobile phase was 0.2% PC—3.0% SDS—6.0% *n*-butanol—0.8% ethyl acetate—90.0% water (pH 7.0) for neutral and ionized molecules. The interactions between the analytes and system described by this chromatographic method is more similar to biological membrane than the *n*-octanol/water partition system. The result in this paper suggests that PC-modified MELC can serve as a possible alternative to the shake-flask method for high-throughput unionized and ionized drugs lipophilicity determination and simulation of biological processes.

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1. Introduction

During the early stages of drug discovery, a large number of newly discovered molecules are typically screened for their pharmacological effects. Those that show activity may be considered as candidates for further development. Often this involves identification of those compounds more likely to be satisfactorily absorbed and appropriately distributed in the human body. While many routes exist for absorption of drugs through membranes and tissues, the one of most common transport route is passive diffusion. To be absorbed by this route, drugs must be sufficiently lipophilic to penetrate the biomembrane lipid, while drugs with poor lipophilicity remain on the outside of the membrane. Therefore, it is prudent to identify and discontinue development of molecules with hydrophilicity at an early stage. Lipophilicity is usually expressed by the *n*-octanol/water partition coefficient (logP) for neutral molecules and the distribution coefficient (logD) for ionized molecules. The general logP and logD can be written as (Eq. (1) (Hansch and Fujita, 1964), (2) and (3) (Scherrer and Howard, 1977):

$$\log P_{n-\text{octanol/water}} = \log \frac{C_{n-\text{octanol}}}{C_{\text{water}}} \quad (1)$$

$$\log D^{\text{pH}}{}_{\text{acid}} = \log P + \log\left[\frac{1}{1 + 10^{\text{pH}-\text{p}K_a}}\right] \quad (2)$$

$$\log D^{\text{pH}}{}_{\text{basic}} = \log P + \log\left[\frac{1}{1 + 10^{\text{p}K\text{a}-\text{pH}}}\right] \quad (3)$$

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Table 1
The compositions of the mobile phases used in this paper

Mobile phase	The compositions and percentages (w/w, %)				
	PC	SDS	Oil phase	*n*-Butanol	Water phase
MP1	–	3.0	0.8 (Ethyl acetate)	6.0	90.20
MP2	0.08	3.0	0.8 (Ethyl acetate)	6.0	90.12
MP3	0.08	3.0	0.8 (Ethyl acetate)	6.0	90.12 (pH 7.0)
MP4	0.2	3.0	0.8 (Ethyl acetate)	6.0	90.00
MP5	0.2	1.0	0.8 (Ethyl acetate)	2.0	96.00
MP6	0.2	1.0	0.8 (*n*-Octanol)	2.0	96.00
MP7	0.2	3.0	0.8 (Ethyl acetate)	6.0	90.0 (pH 7.0)

where C is the concentration of analyte in *n*-octanol or water phase.

Although logP/logD is a surrogate for the interaction of drugs with membranes, it has remained an enduring concept in medicinal chemistry because it is relatively easy and quick to measure. Moreover, it has been proven to provide a useful approximation of a drug's behavior. The classical procedure to determine logP/logD is the shake-flask method. Because of its clear relationship to the partitioning phenomenon, the shake-flask method is the benchmark by which other methods are validated. However, compared to chromatographic methods, the shake-flask method is time consuming and generally requires a large amount of pure analytes that must completely dissolve in water and *n*-octanol. One frequently encountered problem is the formation of microemulsions that remain stable for hours or days, preventing the two solvent layers from separating. Determination of logD by high-performance liquid chromatography–mass spectrometry (HPLC–MS) using automatic sampling from 96-well plates has been reported in an effort to minimize sample and reduce analysis time (Dohta et al., 2007). This modified method required a volume of just 10 μL of a 10 mmol/L sample and small amounts of organic solvent for each assay.

To increase the throughput of logP/logD measurements, various chromatography methods have been researched. The addition of *n*-octanol to the mobile phase was studied, and better correlations were found between the extrapolated logarithm of the chromatographic retention factor ($\log k'_{w(o)}$) and logP (Benhaim and Grushka, 2008; Lombardo et al., 2000). However, the execution of the extrapolated method was tedious and required mobile phases consisting of at least three different percentages of water to determine the k at 100% water. Some fast-gradient RP-HPLC methods was also reported (Henchoz et al., 2008; Valko et al., 1997). In addition to traditional RP-HPLC, biopartitioning chromatography (BPC) techniques, which include micellar/microemulsion electrokinetic chromatography (MEKC and MEEKC) (Bermúdez-Saldaña et al., 2004; Yang and Khaledi, 1995), and micellar/microemulsion liquid chromatography (MLC and MELC) (Detroyer et al., 2004; Martín-Biosca et al., 2003; Stepnik and Malinowska, 2013), are considered useful for estimating logP/logD. BPC can be used to determine pharmacodynamic parameters and simulate the interactions between drugs and biomembranes (Canós-Rius et al., 2005). The micelles or microemulsions are generally comprised of surfactant, water, cosurfactant, and a small amount of organic solvent. The disadvantages of BPC include long retention times for very lipophilic compounds. Moreover, the preparation of the microemulsion phase generally takes a long time to achieve uniformity. The retention of analytes is easy to modify upon evaporation of the organic solvent, hence proper internal standards are required to correct the retention factors (Liu et al., 2008).

Phosphatidylcholine (PC) is the major molecular constituent of human biomembranes. The combination of PC with HPLC, whereby PC molecules are assumed to resemble the biomembrane structure, has been developed. This new type of HPLC includes liposome electrokinetic/liquid chromatography (LEKC and LLC) (Godard and Grushka, 2011; Tsirkin and Grushka, 2001), PC-coated electrokinetic/liquid chromatography (Cunliffe et al., 2002; Hanna et al., 1998, 2000; Kamimori and Konishi, 2002) and immobilized artificial membrane HPLC (IAM-HPLC) (Barbato et al., 2007; Darrouzain et al., 2006; Ducarme et al., 1998; Grumetto et al., 2012; Kaliszan et al., 1994; Lázaro et al., 2006; Stepnik and Malinowska, 2013), all of which use a stationary phase modified by PC molecules. Unfortunately, these methods also have some drawbacks, including the complex and time-consuming preparation of liposomes, the lack of significant differences in retention times prior to and after coating the column with PC because of a lack of PC on the stationary phase and the anisotropic average ensemble orientation of the PC molecules, the expensive IAM column and its significantly short lifetime. As these currently available systems are unsatisfactory, new analogous biomembrane models must be developed.

The BPC model and the model established by introducing PC into the stationary phase are too simple to fully mimic biological membrane behavior although they provide a good prediction of drug lipophilicity. In this paper, we attempted to combine PC with microemulsion as the mobile phase, which resulted in the addition of PC molecules to the mobile and stationary phases. The present study describes the use of PC-modified MELC for accurate logP/logD estimation of unionized and ionized drugs.

2. Experimental

2.1. Materials

The water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). High-purity grade sodium dodecyl sulfate (SDS) was obtained from Sigma–Aldrich (St. Louis, MO, USA). *n*-Octanol, ethyl acetate, *n*-butanol and ethanol were analytical grade and purchased from Baishi Chemical Co., Ltd. (Tianjin, China). Potassium dihydrogen phosphate (HPLC, 99.5%) and sodium hydroxide (HPLC, 96.0%) were analytical grade and purchased

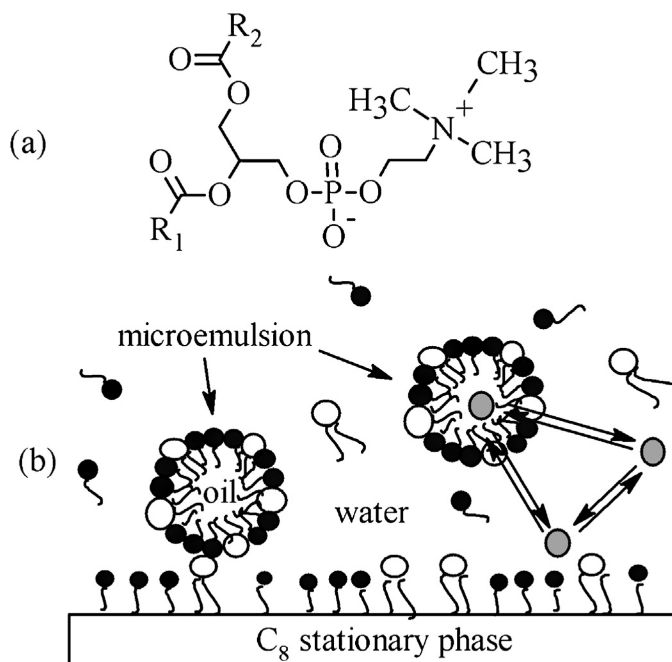

Fig. 1. Schematic drawing of a PC molecule (a) and the proposed inter-phase model for the C_8 stationary phase and mobile phase (b). White = polar head-group of PC; black = polar head-group of SDS; gray = solute.

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