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CORRELATION OF OCTANOL-WATER PARTITION COEFFICIENTS WITH CAPACITY FACTORS MEASURED BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Micellar electrokinetic chromatography (MEKC) was used to measure hydrophobicity of 18 aromatic compounds. The capacity factors, which are proportional to the micelle-water distribution coefficients, were closely correlated with the octanol-water partition coefficients. The addition of Brij 35 to the charged micelle was effective in improving the correlation because the surface charges of the micelle were shielded.

KEYWORDS micellar electrokinetic chromatography; hydrophobicity; capacity factor; partition coefficient; octanol-water

Hydrophobicity of pharmaceuticals is of great importance in predicting biological activity because it may be correlated with properties such as absorbability and transportation. The logarithm of partition coefficients between 1-octanol and water, log P_{ow}, has been used as a hydrophobic parameter and was applied to quantitative structure-activity relationship (QSAR) studies.[1] However, the measurement of log P_{ow} by the shake-flask technique is quite time-consuming. In addition, purity of samples and skilled operations are required.

Alternative techniques have been developed, such as a reversed-phase HPLC method.[2] This method, however, is also problematic because the scale of hydrophobicity obtained was dependent on analytical conditions such as the column and the aqueous organic mobile phase.

Micelles and biomembranes are different from octanol in terms of their amphiphilic and anisotropic properties . Thus, micelles had been investigated as models of biomembranes.[3] However, it was difficult or tedious to monitor the partitioning between micelles and water.

Micellar electrokinetic chromatography (MEKC) is a mode of capillary electrophoresis, and the principle of the separation is based on the distribution of the solute between an aqueous phase and a micellar phase[4,5]. Therefore, the distribution ratio between the two phases, i. e., the capacity factor, can be easily obtained from the migration velocities of the solute, the aqueous phase and the micellar phase. Terabe et al. reported thermodynamic constants in the distribution between water and micellar phases using MEKC[5,6]. For congeneric compounds such as alkyl p-hydroxybenzoates and alkyl phthalates, the logarithm of the capacity factors was correlated with the carbon number of the alkyl chains[7] or log P_{ow}.[8] In this paper, for various compounds such as esters, ethers, amides and so on, the correlation of the capacity factor in MEKC using various ionic micelles with log P_{ow} is presented.

In MEKC, the capacity factor k' is defined as the ratio of the number of the solute in the micellar phase to the number in the aqueous phase. When the solute is neutral, k' can be calculated as follows:

$$k' = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} \qquad (1)$$

where t_0, t_R and t_{mc} are the migration times of the electroosmotic flow, the solute and the micelle, respectively. When the volume of the micellar phase, V_{mc}, is constant, k' is proportional to the distribution coefficient between two phases, K_{mw}, as follows:

$$k' = K_{mw} \cdot \frac{V_{mc}}{V_{aq}} \qquad (2)$$

where V_{aq} is the volume of the aqueous phase. Therefore, if the partitioning behavior in the micelle-water system and the octanol-water system are both subject to hydrophobicity of the solutes, the logarithm of k' can be represented by

$$\log k' = a \cdot \log P_{ow} + b \qquad (3)$$

This linear relationship is based on the change in the free energy in the partitioning process between two phases.

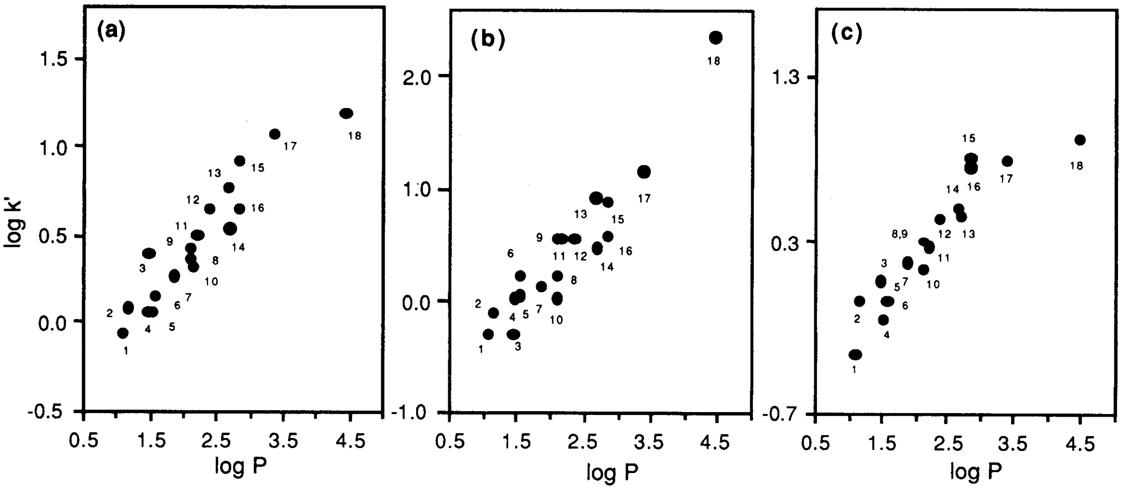

Fig. 1. Correlations of log k' with log P_{ow} Using (a) 50 mM CTAB, (b) 50 mM SDS and (c) 25 mM Brij 35 and 25 mM SDS

Conditions: system, Beckman P/ACE 2100; capillary, 50 µm I.D. x 57 cm (50 cm to the detector), uncoated; applied voltage, 20 kV; detection, 214 nm; separation solution, surfactants dissolved in 50 mM phosphate-100 mM borate buffer (pH 7.0); temperature, 25°C.

Samples: (1) benzylalcohol (2) acetanilide (3) phenol (4) benzaldehyde (5) benzonitrile (6) acetophenone (7) nitrobenzene (8) anisole (9) methyl benzoate (10) benzene (11) propiophenone (12) p-nitrotoluene (13) butyrophenone (14) toluene (15) 2-naphthol (16) chlorobenzene (17) naphthalene (18) anthracene.

Table I. Linear Relationships between log P_{ow} and log k'

	log k' = a log P_{ow} + b, r = correlation factor								
Samples	CTAB			SDS			Brij 35-SDS		
	a	b	r	a	b	r	a	b	r
n = 18^a	0.423	-0.433	0.940	0.712	-1.140	0.947	0.406	-0.598	0.928
n = 17^b	0.461	-0.545	0.935	0.596	-0.916	0.909	0.505	-0.788	0.954
n = 15^c	0.467	-0.587	0.963	0.554	-0.819	0.902	0.497	-0.792	0.961

a) Samples listed in Fig. 1.
b) Same samples without anthracene.
c) Same samples without anthracene, phenol and 2-naphthol.

For eighteen aromatic compounds, correlations of log k' with log P_{ow} were examined using three different ionic micelles. A good linear relationship was obtained in a micellar solution of sodium dodecyl sulfate (SDS), but deviation from the linearity in the larger log P_{ow} region was observed in a micellar solution of cetyltrimethylammonium bromide (CTAB) and a mixed micellar solution of Brij 35 (polyoxyethylene (23) dodecyl ether) and SDS (Fig. 1). In MEKC, the tracer of the micelle, which is perfectly incorporated in the micelle, is necessary for determination of k'. In the case of the anionic SDS micelle, Sudan III[4)] or quinine was employed as the tracer. In the cases of the cationic CTAB and mixed Brij 35-SDS micelles, however, Sudan III and anthracene migrated together. Therefore, unknown impurities, which were observed as a small peak after the peak of Sudan III in every run, were used as the tracer of the micelles. It was considered that the deviations of the linear relationships especially in the larger log P_{ow} region were caused by the inexact t_{mc} values, although the SDS micelles were exactly traced by Sudan III. In fact, in the Brij 35-SDS system, the linearity was improved by eliminating anthracene from the samples, although it was not effective in the CTAB micelle owing to the deviation of phenol as mentioned below (Table I).

The effect of the surfactant structures on the separation selectivity in MEKC has been studied, and it was found that the hydrophilic group was more important than the hydrophobic group, especially for compounds with polar functional groups.5,9) In this study, the migration orders were quite different among three micellar systems, although Brij 35 and SDS have the same hydrophobic chain. Therefore, selection of surfactants was quite important for measuring hydrophobic parameters.

The CTAB micelle displayed a better linear relationship than the SDS micelle, as reported previously3,10). Though the reason was not clear, it may be considered that the cationic charge center of CTAB, which is located at the end of the hydrocarbon chain, is shielded more than the anionic charge center of SDS11). However, electrostatic interactions between the CTAB micelle and polar solutes such as phenol and 2-naphthol, both of which possess acidic characteristics, were observed, and elimination of these solutes provided better regression in the case of CTAB, as shown in Table I. On the other hand, Brij 35, which has a long polyoxyethylene chain as a hydrophilic group, was added to SDS for the purpose of shielding the anionic charges located at the surface of the micelle. The addition of Brij 35 was quite effective in improving the linearity, although the migration window, which is the range from t_0 to t_{mc} related to the separation efficiency, became narrow owing to the decrease in the anionic charges of the micelle5,9).

The slopes of the regression lines about SDS and CTAB were fairly consistent with the slopes of the lines between log P_{ow} and log K_{mw} of some aromatic compounds as identified by micellar liquid chromatography (MLC) (0.518 for SDS and 0.466 for CTAB).10) This meant that both MEKC and MLC were based on the same separation principle: that is, differences in K_{mw} of the solutes, whereas measurement of K_{mw} by MLC was tedious and lacked reproducibility.

This method provided good reproducibility and rapid separation with high efficiency. For example, for 18 samples listed in Fig.1, the relative standard deviation of k' was 0.60 ± 0.47 % (n = 5) and theoretical plate numbers were 271000 ± 82000 in MEKC with SDS.

In conclusion, it was found that capacity factors obtained by MEKC could be used as a hydrophobic parameter for QSAR instead of log P_{ow}. In this rapid, reproducible and micro-scale technique, purity and stability of the samples do not have to be considered. In addition, MEKC provides a single, continuous and universal scale independent of the operator, the capillary and the instrument due to the pseudo-stationary phase nature of micelles; however, exact tracers of micelles must be sought, and further optimization of the micelle and MEKC conditions employed such as the pH of the micellar solution are required to minimize the electrostatic interactions. Further studies are already in progress in our laboratory.

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