Partition Behaviour of Drugs in Microemulsions Measured by Electrokinetic Chromatography

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INTRODUCTION

Capillary electrophoresis (CE) is a powerful tool for determining physicochemical properties, such as the aggregation constants (1), dissociation constants (2) and protein-ligand binding constants (3). Furthermore, electrostatic interaction and hydrogen bonding effects between solutes and surfactant were observed (4). The partitioning behaviour of solutes in micelles was evaluated by electrokinetic chromatography (EKC) (5–6). Microemulsion electrokinetic chromatography was investigated by Watarai *et al.* (7). O/W microemulsions (ME) of water/ sodium dodecyl sulfate (SDS)/ 1-butanol/heptane were used to separate different solutes (7). Terabe et al. compared microemulsion electrokinetic chromatography with the micellar electrokinetic chromatography (8). Ishihama et al. studied the hydrophobicity of cationic and anionic solutes by using the microemulsion electrokinetic chromatography (9–10). However, to date, no direct method for characterizing partition behaviour of drugs in ME exists. Information concerning drug partition in ME is very useful to design optimal ME for controlled drug delivery. Therefore, in this work, the partitioning behaviour of various drugs in ME was evaluated by EKC using ME consisting of non-ionic surfactants. Furthermore, this system was compared with the octanol/water system.

EXPERIMENTAL

Apparatus

Capillary electrophoresis experiments were performed on a Hewlett Packard Model G1600A (Waldbronn, Germany) ^{3D} CE system with diode-array detector from 190 to 600 nm. CE ChemStation equipped with a HP Vectra 486/66U workstation was used for instrument control, data acquisition, and data analysis. The system was controlled by windows software, which was modified to the HP system. The detection wavelengths were 200 nm. The capillaries (fused- silica) obtained from Hewlett Packard (Waldbronn, Germany) with a total length (48.5 cm), length to detector (40 cm) and internal diameter (50 μm) were used for the determination of the capacity factor.

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Chemicals

Ceftazidim, oxacillin, dicloxacillin, diclofenac, chinine and propranolol were obtained from Sigma-Aldrich Chemie (Deisenhofen, Germany). Acetone, n-propanol, n-butanol, potassium-hydrogenphosphate, potassiumdihydrogenphosphate and octanol were obtained from Merck (Darmstadt, Germany). Thiamin was purchased from Fluka Chemie AG (Switzerland). Tween 80 was supplied by Serva (Heidelberg, Germany). Isopropylmyristate (IPM) was obtained from Caelo (Hilden, Germany). Tagat (polyoxyethylene (20) glycerol monooleate) was obtained from Franken-Chemie (Germany).

Sample Preparation

Standard solutions of the drugs were prepared at 500 μg/ ml in 50 mM buffer solution at pH 7. These samples were filtered through a 0.45 μm syringe filter and injected immediately into the apparatus.

Buffer Preparation

For capillary electrophoresis, 50 mM phosphate buffer solution (pH 7.0) was prepared by dissolving 5.29 g potassium-hydrogenphosphate and 2.61 g potassiumdihydrogenphosphate in water, filling up to a volume of 1000 ml. The pH of the buffer was measured at 25°C using a HI 9321 microprozessor pH meter (HANA instruments). The buffer solutions were filtered through a 0.45 μm syringe filter and degassed by ultrasound for at least 10 min before use.

Determination of Distribution Coefficient

The distribution coefficients of the drugs were determined between water (buffer) and octanol (Table 1). Buffer at pH 7.0 was used as aqueous phase. These two phases were saturated with each other. The compounds were dissolved in the water phase (500 μg/ml). These solutions were filled into suitable vials and shaken for 12 h at room temperature. After separation of the samples into both phases, the drug content was analysed by CE.

The distribution coefficients at pH 7.0 were calculated using the following equation:

$$D = a_{oct} / a_{aq} \quad (1)$$

where a_{oct} and a_{aq} were the concentrations of the drugs in the octanol and in the aqueous phases.

Preparation of Microemulsion

The desired amounts of the surfactants, alcohol and IPM were mixed for 15 min. 50 mM phosphate buffer was added slowly to this mixture under stirring until a clear solution was formed. The solution was left to stand for 1 h at room temperature. Then, solution was filtered through a 0.45 μm syringe filter.

The composition of these ME was as follows: ME 1: 6% n-butanol/ 9% Tween, 2% IPM/ buffer. ME 2: 8% n-propanol/ 9% Tween, 2% IPM/ buffer. ME 3: 8% n-propanol/ 12% Tagat, 2% IPM/ buffer.

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Table 1. Capacity Factors and Distribution Coefficient Value is of the Drugs Used

	k_{cf} (ME 1)[a]	k_{cf} (ME 2)	k_{cf} (ME 3)	D^b
Thiamin	0.86	0.34	0.23	0.06
Propranolol	2.05	3.10	3.19	0.83
Chinine	3.73	3.38	3.26	0.53
Diclofenac	9.20	14.5	16.4	18.0
Dicloxacillin	6.82	5.17	6.90	0.55
Oxacillin	1.94	2.21	2.51	0.22
Ceftazidim	0.52	0.55	0.44	0.02

[a] At pH 7.0.
[b] pH of the EKC buffer was 7.0.

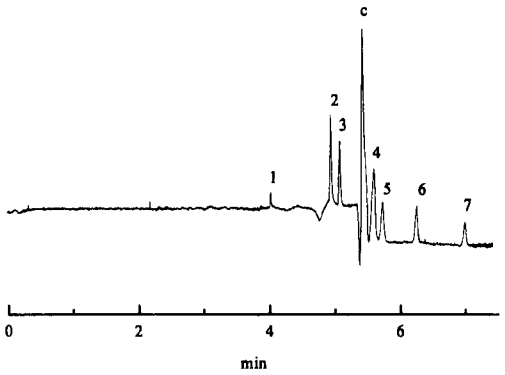

Fig. 2. Electropherogram of the various drugs of ME 3, other conditions as in Figure 1.

Analysis Conditions

A new capillary was washed for 15 min with NaOH (1.0 M) at 40°C, followed by washing for 10 min with water at the same temperature and for 5 min with water at 25°C. Before each injection, the capillary was flushed with 0.1 M NaOH for 5 min and with the actual buffer solution for 5 min. The temperature was kept at 25°C, 50 mM phosphate buffer (pH 7.0) and a separation potential of 30 kV were used. Acetone was used as a marker substance for the determination of the electroosmotic mobility. The samples [buffer-acetone (99:1)] were injected at a pressure of 50 mbar for 9 s (hydrodynamic injection) with a sample volume of 18.8 nl.

Detailed experimental conditions are listed in Figs. 1–2.

RESULTS AND DISCUSSION

Determination of the Capacity Factor

ME are transparent systems that generally consist of surfactant/cosurfactant, oil and water. They are thermodynamically stable. Previous investigations using microemulsion electrokinetic chromatography were carried out only with cationic (cetyl trimethylammonium chloride) and anionic surfactants (sodium dodecyl sulfate). However, these ME-systems do not have importance for medical and pharmaceutical purposes. In this paper, three different ME-systems with non-ionic surfactants were studied because these ME are used for pharmaceutical applications (11). Furthermore, in pharmaceutics it is important to know the partition of drugs between aqueous and oily phases of a ME. In the literature a direct method for the investigation of the partition of a drug between these two phases is not known. Using the new EKC technique we tried to characterize drug partition in ME.

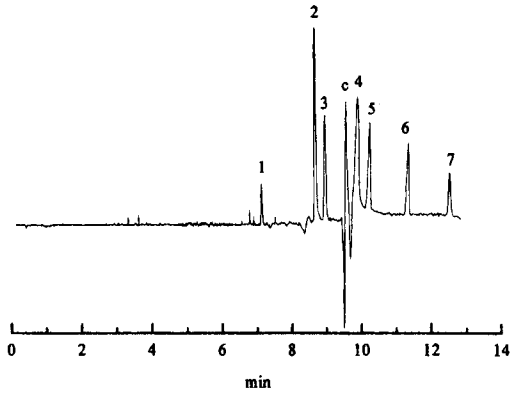

Fig. 1. Electropherogram of the various drugs of ME 2. Capillary: 48.5 (40 cm to detector) × 50 μm i.d., field strength: 30 kV, temperature: 25°C, pressure injection: 9 s at 50 mbar, detection: 200 nm, buffer: 50 mM phosphate.

The capacity factor, k_{cf} of the solute in the EKC is defined as follows:

$$k_{cf} = \frac{\mu_{aq} - \mu_{eff}}{\mu_{eff} - \mu_{em}} \quad (2)$$

where μ_{aq} and μ_{me} are the electrophoretic mobilities of the solute in the aqueous and in the oily phases of the ME, μ_{eff} is the effective mobility in the ME. μ_{eff} and μ_{me} have been obtained by measuring the migration times of acetone (tracer for the aqueous phase) and of dodecylbenzene (tracer for the oily phase). By using non-ionic surfactants μ_{me} was zero and dodecylbenzene moved with the electroosmotic flow and was not separated from acetone peak. μ_{aq} was measured by capillary zone electrophoresis (CZE) with phosphate buffer pH 7 including 8% n-propanol or n-butanol (12). Table 1 summarizes the quantitative analytical parameters for the determination of the capacity factor in three different ME-systems.

Correlation with the Logarithm of Distribution Coefficients (log D)

The apparent k_{cf} can be compared with the distribution coefficient of the drugs in the n-octanol/ water system which is mostly used to characterize the hydrophilic/lipophilic properties of the drugs.

The logarithm of k_{cf} can be represented by the following equation (6):

$$\log k_{cf} = a \log D + b \quad (3)$$

The linear relationship is based on the change in the free energy of the partitioning process between the two phases. The regression equation of the curves and the correlation coefficients between different systems presented in Table 2 were in good agreement. No electrostatic interactions between the non-ionic surfactants and the solutes were observed in these systems.

Table 2. Linear Relationships Between log D and log k_{cf} at pH 7.0

	$\log k_{cf} = a \log D + b$	
	a b	r
ME 1	= 0.491 + 0.559	0.978
ME 2	= 0.509 + 0.611	0.981
ME 3	= 0.579 + 0.648	0.987

Therefore, the *log* k_{cf} correlated [propranolol, chinine, diclofenac, dicloxacillin, oxacillin, ceftazidim] well with the log D.

The characterization of these drug compounds according to partition behaviour was studied at pH 7.0. The measured parameters were as follows: 25°C, 30 kV voltage, 9 s injection time at 50 mbar pressure, 485 mm (total length) (0.05 mm internal diameter) capillary. Figs. 1 and 2 show that complete electrophoretical characterization of these seven compounds was readily achieved using only one EKC-run within 13 min (ME 2) and 8 min (ME 3).

The effective mobilities and the electroosmotic mobility were calculated as described by Weinberger (13). Thiamin, propranolol and chinine exhibited positive electrophoretic mobilities, diclofenac, dicloxacillin, oxacillin and ceftazidim negative electrophoretic mobilities. All compounds moved with the electroosmotic flow. For the identification of the individual peaks UV spectra were recorded simultaneously. The comparison of the UV spectra with those of each individual component led to a reliable and unambiguous assignment. The method provided good reproducibility and rapid separation with high efficiency. The relative standard deviations of the migration times were between 0.5 and 2%. The results obtained indicate that the capacity factor determined by EKC could be used both as parameter to characterize partition behaviour of drugs in ME and as hydrophobic parameter instead of log D. k_{cf} appears to be evident parameter because it shows a better diversification than D (see Table 1). In the octanol/water system we found only one high value of the distribution coefficient (for dicloxacillin). In contrast, the ME-systems used show a better characterization of the drugs according to their hydrophilic/lipophoilic properties.

CONCLUSIONS

EKC was introduced to evaluate the partition behaviour of various kinds of compounds in O/W- ME. The capacity factor determined by this method provides fundamental information concerning the partition coefficient of the drugs between the aqueous and the oily phases of a ME. The logarithm of the capacity factors correlated well with the logarithm of the octanol/water distribution coefficients. Therefore, the capacity factor can be applied as parameter to characterize the hydrophobicity/hydrophilicity of drugs or other substances. The method described is easy, rapid, reproducible and opens a new way for determination of the capacity factor in O/W-ME.

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