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Non-ionic micellar affinity capillary electrophoresis for analysis of interactions between micelles and drugs

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Abstract

Micellar affinity capillary electrophoresis (MACE) was introduced to evaluate the affinity of various kinds of drugs as benzoic acid, salicylic acid, trinitrophenol, *p*-hydroxybenzoic acid and *o*-acetylsalicylic acid. Non-ionic micelles as Brij 35 (polyethylenglycol dodecylether), Tagat (polyoxyethylene (20) glycerol monooleate) and Tween 20 (polyoxyethylen sorbitan monolaurate) were used as a pseudostationary phase in capillary electrophoresis. For polyvinyl alcohol (PVA) coated capillary was used in this examinations. The drugs had negative electrophoretic mobilities at a pH value of pH 7.2. The negatively charged drugs migrated toward the anode and were related by their interaction with the micelles. The difference in the mobility of the drugs owing to the presence of the micelles reflected the interaction between these drugs and the micelles. Equations were derived to calculate the capacity factor k' from the migration times in the presence of micelles t' and in the absence of micelles t, the partition coefficients P_{wm} and the Gibbs free energy. The drugs show different interaction and affinity with the micelles in the systems. Strong interaction was observed between benzoic acid and the micelles. Furthermore, a linear relationship ($R = 0.999$) was obtained between $\Delta G°$ and ln P_{wm} in the micellar solubilization of drugs. These results show that $\Delta G°$ can give us information on the affinity and on the partition behaviour of the drugs in these systems.

Keywords: Non-ionic micellar affinity capillary electrophoresis; Partition coefficients; Free energy

1. Introduction

Micellar electrokinetic chromatography (MEKC) [1,2] is a mode of capillary electrophoresis (CE), but its separation principle is based on different partition and differential migration of the drugs. Capillary electrophoresis is a powerful tool for determining physicochemical properties, such as the dissociation constants [3,4], protein-ligand binding constants [5] and aggregation constants [6,7]. Furthermore, electrostatic interaction and hydrogen bonding effects between solutes and surfactants were studied [8–10]. The partitioning behaviour of solutes in micelles was evaluated by

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electrokinetic chromatography [11–13]. The determination of hydrophobic parameters by reversed-phase liquid chromatography, which uses a micellar solution as a mobile phase, also allows calculation of the partition coefficient between the micelle and the aqueous phase [14]. Hydrophobicity plays an important role in the biological and physicochemical behaviour of drugs. The hydrophobic effect is the driving force for the water micelles partitioning processes and biological activities [15]. Terabe et al. calculated the partition coefficients of solutes and the enthalpy and entropy changes of micellar solubilization using MEKC [16]. Ishihama at al. studied the hydrophobicity of anionic and cationic solutes by using micellar and microemulsion electrokinetic chromatography [17,18]. Muijselaar et al. described the calculation of partition coefficients and partial molar volumes of micelles [19]. Furthermore, the partitioning behaviour of various drugs in microemulsions was evaluated using non-ionic surfactants [20]. In this work, the partition coefficients and the standard Gibbs free energy in micellar solubilization for drugs were determined using the new non-ionic micellar affinity capillary electrophoresis (MACE) technique. The electrophoretic mobility of drugs and the phase ratio between the micelle and the aqueous phase were studied.

2. Theory

2.1. Determination of the apparent solute mobility μ_a

The apparent mobility of the solutes were determined at pH 7.2 using the following equation:

$$\mu_a = L_g L_t / tV \quad (1)$$

where V is the applied voltage, L_g the effective capillary length (to the detector), L_l the total capillary length, t the migration time of the solute.

2.2. Determination of the capacity factor k'

The capacity factor, k' is defined similar to that used in immobilized liposome chromatography (ILC) [21,22]

$$k' = (t - t_0)/t_0 \quad (2)$$

where t is the migration time of a drug in the presence of surfactants and t_0 is that in the absence of surfactants.

2.3. Determination of the partition coefficients P_{wm}

The capacity factor is related to the partition coefficient, P_{wm}, by the following equation:

$$k' = P_{wm}[V_c/V_q) = P_{wm}\phi \quad (3)$$

where ϕ is the phase ratio and V_c and V_q are the volumes of the micelle and the remaining aqueous phase.

2.4. Determination of the free energy $\Delta G°$

The free energy of the interaction between drugs and surfactants was defined by [23,24]:

$$\Delta G° = -RT \ln\{\phi^{-1}[(v_0 - v_c)(v_{net} - v_E - v_c) - 1]^{-1}\} \quad (4)$$

where R is the gas constant, and T is the absolute temperature, ϕ is the phase ratio, v_0 and v_{net} are the velocities of the drugs in the absence and presence of the surfactants, v_c is the velocity of the surfactants, and v_E is the electroosmosis velocity. In our work we used coated capillary. In the case, v_E and v_c were equal zero.

$\Delta G°$ can be described by the following equation:

$$\Delta G° = -RT \ln[\phi^{-1}(v_0/v_{net} - 1)] \quad (5)$$

By introducing the migration time t and t_0 corresponding to the drug velocities v_{net} and v_0 in the presence and absence of the surfactants, respectively, the following equation is obtained:

$$\Delta G° = -RT \ln[(t/t_0 - 1)/\phi] \quad (6)$$

3. Experimental

3.1. 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. A polyvinyl alcohol coated (PVA) capillary obtained from Hewlett Packard (Waldbronn, Germany) with a total length (64.5 cm), length to detector (54 cm) and internal diameter (50 m) was used in this study.

3.2. Chemicals

Benzoic acid, salicylic acid, trinitrophenol, *p*-hydroxybenzoic acid and *o*-acetylsalicylic acid were obtained from Sigma-Aldrich Chemie (Deisenhofen, Germany). Brij 35 (polyethylenglycol dodecylether), Tagat (polyoxyethylene (20) glycerol monooleate) and Tween 20 (polyoxyethylen sorbitan monolaurate) were purchased from Fluka Chemie AG (Switzerland). Potassium hydrogenphosphate and potassium dihydrogenphosphate were obtained from Merck (Darmstadt, Germany).

3.3. Sample preparation

Standard solutions of the drugs were prepared at 100 μg ml^{-1}. These samples were filtered through a 0.45 μm syringe filter and hydrodynamically injected into the apparatus.

3.4. Buffer preparation

For capillary electrophoresis, a 10 mM phosphate buffer solution (pH 7.2) was prepared by dissolving 1.05 g potassium hydrogenphosphate and 0.53 g potassium dihydrogenphosphate in water, filling up to a volume of 1000 ml. The pH of the buffer was measured at 25°C using a HI 9321 microprocessor pH meter (HANA instruments). The buffer solution was filtered through a 0.45 μm syringe filter and degassed by ultrasound for at least 10 min before use.

4. Results and discussion

The affinity of drugs using the micellar electrokinetic chromatography (MEKC) method was determined only for cationic and anionic surfactants. Most of these systems do not have importance for pharmaceutical purposes. In this paper, three different systems with non-ionic surfactants were studied. In pharmaceutics it is important to know the affinity of drugs between aqueous and micelle phases. In the literature a direct method for the investigation of the affinity of a drug using the non-ionic MACE is not known. Using the new MACE technique we tried to characterize the affinity between drugs and micelles. In the electroosmosis free capillary ($\mu_E = 0$) the non-ionic micelles were stationary in the electrophoretic field ($\mu_C = 0$). The negatively charged drugs migrated toward the anode and were retarded by their partition and their interaction with the micelles. Because of the interaction of drugs with the micelles the ionic mobility and the migration time of the drugs changed. This led to a change of the partition behaviour of the drugs in these systems. These drugs had negative electrophoretic mobilities and migrated in the direction of the anode. The apparent mobility of the drugs were calculated using Eq. (1). It was observed that the mobility changed with change of the concentration of the surfactant (Figs. 1 and 2).

The more the migration time is, the more is the partition in the micelle (Figs. 3 and 4). Here, the solutes migrate with the micelles more slowly. Benzoic acid changed its mobility from -10.5×10^{-4} cm^2 V^{-1} s^{-1} in the absence of Brij to -6.58×10^{-4} cm^2 V^{-1} s^{-1} in the presence of 3% Brij. Fig. 3 shows the changes of the migration time and of the resolution of the used drugs at different concentrations of Brij. Very strong affinity and interaction with increasing of the concentration of Brij was observed for benzoic acid compared to other solutes.

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.

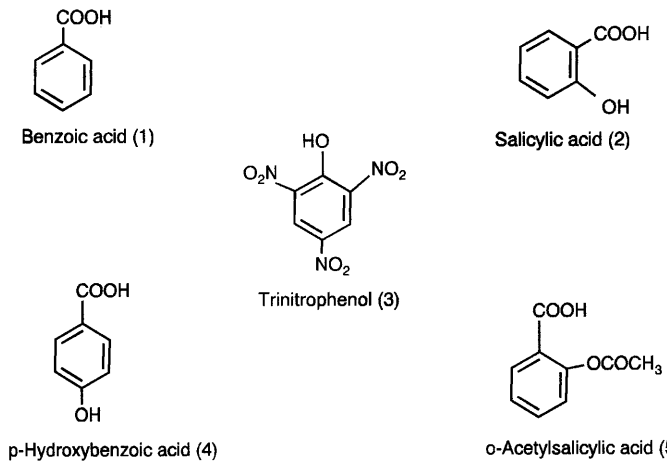

Fig. 1. Chemical structure of the drugs.

Similar interaction was also observed for both Tagat and Tween (Fig. 4).

The capacity factor values of the drugs in these systems given in Tables 1 and 2 were calculated using Eq. (2).

Furthermore, the influence of the concentration of Brij on the capacity factor of the drugs was studied. The k' of benzoic acid in the system increased with increasing of the concentration of the surfactant. The k' values of benzoic acid rise from 0.31 at a concentration of 0.5% Brij to 0.59 at a concentration of 3% Brij. In the case of the other substances the k' values decreased with increasing of the concentration of Brij (Fig. 5).

Depending on the chemical structure of the drugs and the surfactants, various chemical interactions additional to hydrophobic interactions, such as dipole interaction and hydrogen bonding, may occur between them in the partitioning process. The strength of the affinity of the micelles to the drugs was found to be as follows:

Tagat > Brij > Tween

The partition coefficients of the drugs (Tables 1 and 2) were also calculated from the capacity factor according to Eq. (3). The accuracy of the P_{wm} values obtaind from Eq. (3) obviously depends on the phase ratio ϕ which is calculated from V_c/V_q, where V_c (volumes of the micelle) is 3 ml and V_q (volumes of the aqueous phase) is 97 ml. The phase ratio ϕ is 0.03. The results obtained indicated that the partition coefficients determined by MACE could be used as parameter to characterize the partition behaviour of a drug in micellar systems and as hydrophobic parameter. Using this technique it is possible to characterize

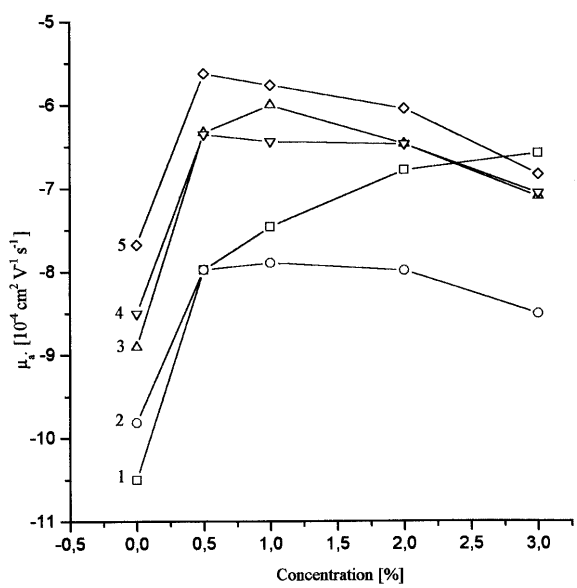

Fig. 2. Influence of different concentrations of Brij on the apparent mobility of the drugs. Polyvinyl alcohol (PVA) coated capillary with 54 cm effective length × 50 μm I.D.; 15 kV; temperature: 25°C; pressure injection: 5 s at 50 mbar; detection: 200 nm.

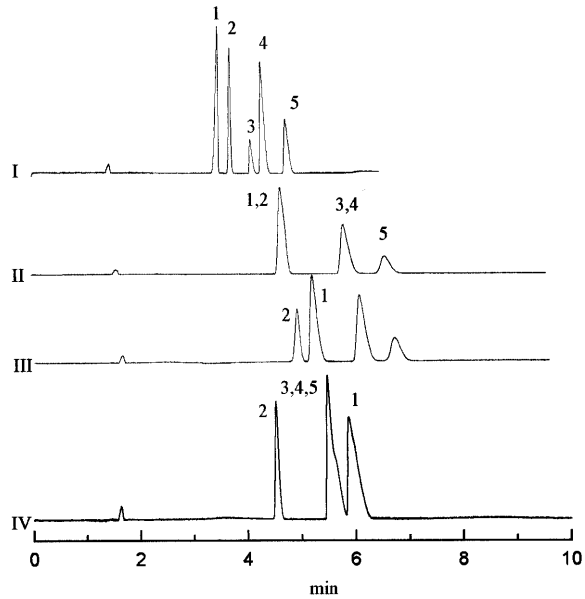

Fig. 3. Electropherogram of the drugs at different concentrations of Brij (I) in 10 mM phosphate buffer, pH 7.2; (II) in 0.5% Brij; (III) in 2% Brij; and (IV) in 3% Brij; conditions see Fig. 2.

the affinity of a drug to micellar systems. The strength of the affinity of the drugs to the non-ionic micelle was found to be as follows:

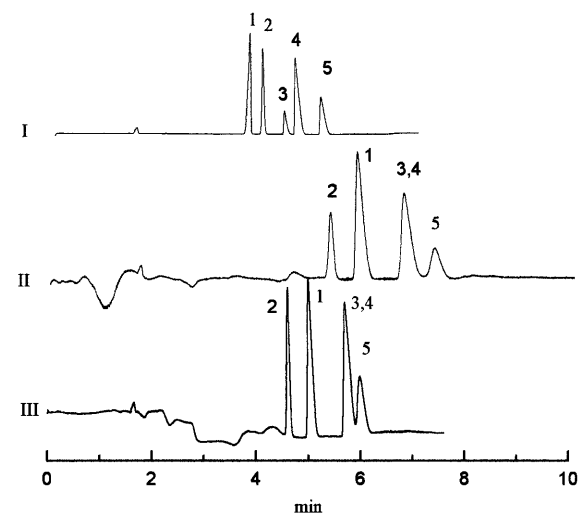

Fig. 4. Electropherogram of the drugs (I) in 10 mM phosphate buffer, pH 7.2; (II) in 3% Tagat; and (III) in 3% Tween. For conditions see Fig. 2.

Table 1
Capacity factor (k'), partition coefficients (P_{wm}), ionic mobility (μ_a) and standard Gibbs free energy $\Delta G°$ in micellar solubilization by the Tween micelle[a]

Compound	k'	μ_a	P_{wm}	$\Delta G°$ (kJ mol^{-1} K^{-1})
1	0.21	−8.6	7.0	−4.78
2	0.06	−9.27	2.0	−1.56
3	0.16	−7.64	5.4	−4.10
4	0.11	−7.64	3.7	−3.12
5	0.05	−7.32	1.7	−1.06

[a] Concentration of Tween is 3%.

benzoic acid > trinitrophenol
> $p-$ hydroxybenzoic acid
> salicylic acid
> $o-$ acetylsalicylic acid

It is known, that the Gibbs free energy $\Delta G°$ gives also information on the partition equilibrium between drugs and micelles. The Gibbs free energy calculated from Eq. (6) is given in Tables 1 and 2. The more negative $\Delta G°$ is, the more is the equilibrium moved to the micelle side. As shown in Fig. 6, ln P_{wm} correlated well with $\Delta G°$ [$R = 0.999$ (for Tween), $R = 0.998$ (for Tagat)]. The results obtained indicate that $\Delta G°$ determined by MACE could be used as parameter to characterize the affinity and the hydrophobicity/hydrophilicity properties of drugs.

The method described is rapid and reproducible. It is very suitable for the characterization of the partition equilibrium and of thermody-

Table 2
Capacity factor (k'), partition coefficients (P_{wm}), ionic mobility (μ_a) and standard Gibbs free energy $\Delta G°$ in micellar solubilization by the Tagat micelle[a]

Compound	k'	μ_a	P_{wm}	$\Delta G°$ (kJ mol^{-1} K^{-1})
1	0.58	−6.6	19.3	−7.23
2	0.35	−7.25	11.7	−5.98
3	0.55	−5.73	18.3	−7.11
4	0.48	−5.73	16.0	−6.72
5	0.45	−5.28	15.0	−6.62

[a] Concentration of Tagat is 3%.

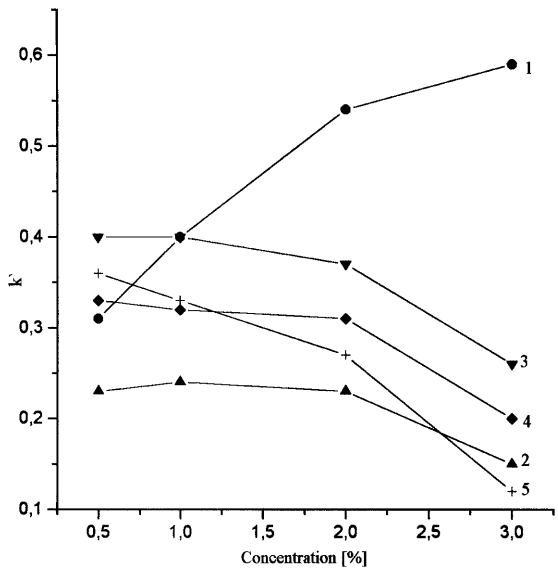

Fig. 5. Influence of different concentrations of Brij on the capacity factor k' of the drugs. Conditions see Fig. 2.

namic properties of drugs in different vehicle systems.

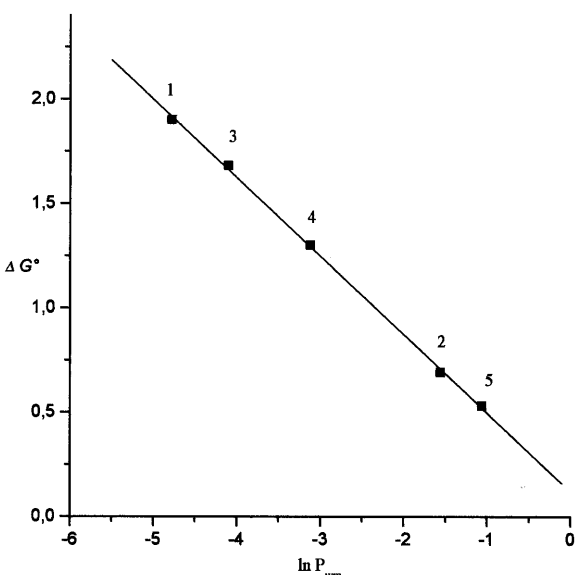

Fig. 6. Relationship between ln P_{wm} and the free energy $\Delta G°$ fot Tween. Conditions see Fig. 2.

5. Conclusion

In pharmaceutics it is important to characterize the hydrophobicity/hydrophilicity of drugs between aqueous and colloidal phases. The capacity factor, the partition coefficients and the free energy $\Delta G°$ determined by this method provides fundamental information on the partition behaviour of the drugs between the aqueous and the colloidal phases.The partition coefficient could be applied as parameter to characterize the hydrophobicity/hydrophilicity of drugs. Therefore, the method described above is a very effective tool not only to characterize the hydrophobicity/hydrophilicity of drugs in several pharmaceutical vehicle systems but also to optimize thees vehicle systems. Because of the simple handling and automation MACE is valuable as a rapid screening method in the development of pharmaceutical formulations.

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