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Characterization of micellar solubilization of antibiotics using micellar electrokinetic chromatography

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Abstract

Micellar electrokinetic chromatography (nMEKC) method was applied to the determination of the partition behaviour between water and micelle for a group of antibiotics using sodium dodecyl sulphate (SDS) as an anionic model surfactant. In the method, the running buffer contains both the micelles and the drug, and the injected solution contains the same concentration of the micelles as the running buffer but no drug. The mobility of the drug can be measured from a negative peak recorded in the chromatogram. The required parameters for the determination of the capacity factor were measured by the MEKC technique. Thermodynamic properties such as enthalpy and entropy changes of micellar solubilization were calculated from the temperature dependence of the partition coefficients. The critical micellar concentrations (CMC) of the SDS salt were determined in phosphate solutions at pH 7 at different temperatures using the MEKC. The method described in this article based on MEKC is efficient and very fast in order to determine parameters for characterizing micellar solubilization of drugs.

Keywords: Micellar electrokinetic chromatography; Micellar solubilization; Partition coefficients; Thermodynamic quantities; Antibiotics

1. Introduction

Micellar electrokinetic chromatography (MEKC) [1,2] is a field of high performance capillary electrophoresis. The partition coefficient can be obtained from the migration times of the drugs, the micelle, and the bulk solution, provided the phase ratio or the volume ratio of the micelle to the aqueous phase are known. 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 electrokinetic chromatography [11–13]. Only a few papers report on MEKC and microemulsion

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electrokinetic chromatography (MEEKC) methods for the calculation of partition coefficients and thermodynamic quantities such as enthalpy and entropy changes of micellar solubilization [14,15]. Terabe et al. calculated the partition coefficients of solutes and the enthalpy and entropy changes of micellar solubilization using MEKC [16]. Muijselaar et al. described the calculation of partition coefficients and partial molar volumes of micelles [17]. However, they used the simplification, that the volume of the micellar phase is negligible compared to the volume of the aqueous phase. Furthermore, the partitioning behaviour of various drugs in microemulsion was evaluated using non-ionic surfactants [18]. The characterization of drug affinities to several vehicle systems were studied using the affinity capillary electrophoresis (ACE) [19]. Katsuta et al. determined the solubilization isotherms of neutral solutes using the nMEKC [20]. In this work we studied the partition behaviour of charged drugs using the nMEKC. Our results show that the migration time of the solute in the running buffer depends on the concentration of the solute. Therefore, the nMEKC gives better and more exact information on the partition behaviour of drugs compared to the conventional method (MEKC) [21]. In this work, the partition coefficients, enthalpy and entropy changes of micellar solubilization of charged drugs were determined using the nMEKC technique. The critical micellar concentrations of the sodium dodecyl sulfate (SDS) salt were determined in phosphate solutions at pH 7 by differing temperature using the capillary electrophoresis system (Fig. 1).

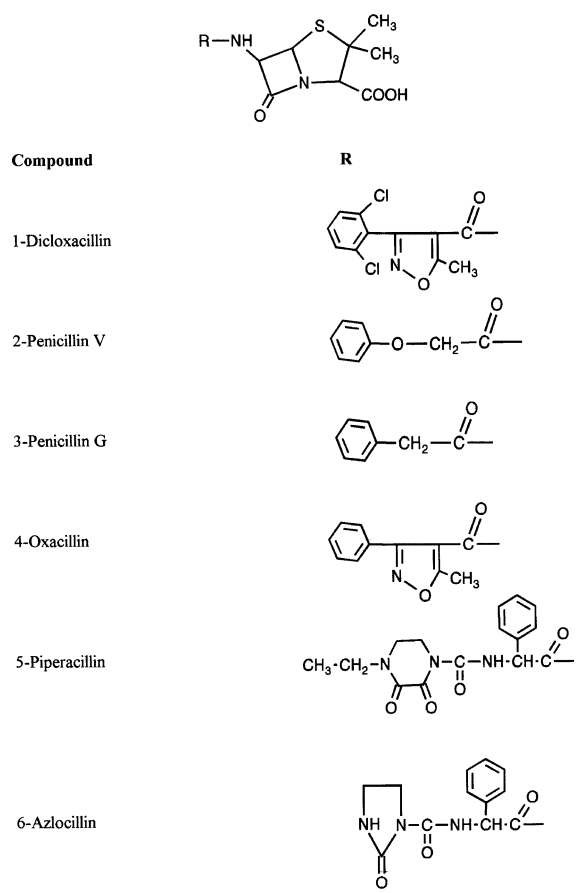

Fig. 1. Chemical structure of the compounds.

2. Theory

2.1. Determination of the partition coefficients P_{mw}

In the MEKC, the capacity factor k' is directly related to partition coefficients between the aqueous and micellar phases as [22,23]

$$k' = P_{\mathrm{mw}}[V_{\mathrm{mc}}/V_{\mathrm{aq}}] = P_{\mathrm{mw}}\phi \quad (1)$$

where ϕ is the phase ratio and V_{mc} and V_{aq} are the volumes of the micelle and the remaining aqueous phases. Therefore, we can calculate the partition coefficients from the capacity factor according to Eq. (2):

$$k' = P_{\mathrm{mw}}[\upsilon(C_{\mathrm{t}} - \mathrm{cmc})/(1 - (C_{\mathrm{t}} - \mathrm{cmc})] \quad (2)$$

where υ is the partial molar volume of the surfactant, C_{t} is the surfactant concentration, cmc is the critical micelle concentration, and P_{mw} is the partition coefficient of the solute between the micellar phase and the aqueous phase. At low micelle concentrations, the second term in the denominator of Eq. (2) becomes negligible and Eq. (2) can be rewritten as follows:

$$k' = P_{\mathrm{mw}}[\upsilon(C_{\mathrm{t}} - \mathrm{cmc})] \quad (3)$$

Table 1
Determination of CMC by SDS at different temperatures using CZE (current) in 10 mM phosphate buffer at pH 7.0

T (°C)	25	30	35	40
CMC (mM)	3.5	3.18	3	3.62

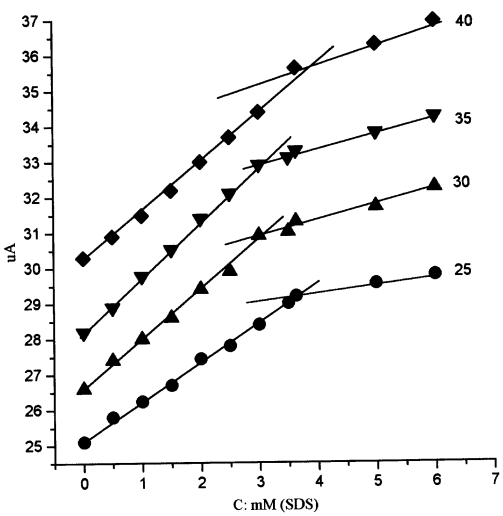

Fig. 2. Electric current, I, vs. the concentration of SDS in 10 mM phosphate buffer at pH 7.0.

Partition coefficients at different temperatures should follow the van't Hoff equation

$$\ln P_{mw} = -\Delta H°/RT + \Delta S°/R \quad (4)$$

where $\Delta H°$ is the enthalpy associated with the micellar solubilization or the transition of the solute from the aqueous phase to the micelle, $\Delta S°$ is the corresponding entropy, R is the gas constant and T is the absolute temperature. The Gibbs free energy, $\Delta G°$, for the micellar solubilization can be calculated according to

$$\Delta G° = \Delta H - T\Delta S° \quad (5)$$

3. Experimental

3.1. Apparatus

Capillary electrophoresis experiments were performed on a Hewlett Packard Model G1600A (Waldbronn, Germany)^{3D} CE system. The detection wavelength was at 200 nm. Fused-silica capillaries from Hewlett Packard (Waldbronn, Germany) with a total length of 48.5 cm, a length to the detector of 40 cm and an internal diameter of 50 μm were used.

3.2. Chemicals

Dicloxacillin, penicillin V, penicillin G, oxacillin, piperacillin and azocillin were obtained from Sigma-Aldrich Chemie, Germany. Acetone and SDS were obtained from Merck, Germany.

3.3. Sample preparation

Standard solutions of the drugs were prepared at 200 μg/ml. These samples were filtered through a 0.45 μm syringe filter and hydrodynamically injected into the apparatus.

3.4. Run solution preparation

For capillary electrophoresis the buffer solutions were filtered through a 0.45 μm syringe filter and degassed by ultrasound for at least 10 min before use.

3.5. Analysis conditions

Before each injection, the capillary was flushed with 0.1 M NaOH for 3 min and with the actual buffer solution for 5 min. The temperature was kept at 25, 30, 35, 40 and 45 °C. The detection was done at the cathodic side. Acetone was used as a marker substance for the determination of the electroosmotic mobility.

3.6. Determination of the cmc

The cmc of SDS in phosphate buffer at pH 7 was determined using the CE by measuring the electric current at different concentrations of SDS (Table 1, Fig. 2).

3.7. Determination of partition coefficients

The partition coefficients of the drugs were determined between phosphate buffer and n-oc-

Table 2
Determination of P_{ow} in n-octanol/water system

	1	2	3	4	5	6
P_{ow}	2.15 ± 0.01	2.16 ± 0.32	2.08 ± 0.12	2.06 ± 0.08	2.04 ± 0.14	1.99 ± 0.04

tanol (Table 2). These two phases were saturated with each other. The compounds were dissolved in the water phase (100 μg/ml). The n-octanol/water 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 [24,25].

The partitioning coefficients were calculated using the following equation:

$$P_{ow} = a_{oc}/a_{aq}$$

where a_{oc} and a_{aq} are the concentrations of the drugs in the n-octanol and in the aqueous phases, respectively.

4. Results and discussion

In pharmaceutics it is important to know the partition behaviour of drugs between aqueous and micelle phases. A direct method for the determination of the partition of drugs in vehicle systems is not available. Until now, only these vehicle systems have been used as separation media. In only a few works the interaction and the partition behaviour of drugs in vehicle systems using the MEKC were studied. Most of these studies do not have importance for pharmaceutical and biopharmaceutical purposes. Using the nMEKC technique it is possible to characterize drug partition in the micellar system. In our work, a tracer which is completely incorporated into the lipid phase is applied for the estimation of the lipid phase. In every run dodecylbenzoyl propionic acid was used as tracer to measure the migration time of the micelles. For the determination of the electroosmotic flow acetone was applied as marker substance. To control the reproducibility of the micelle migration and the EOF three injections of the drug were made. The relative standard deviation of the migration time was between 1.2 and 3%. Fig. 3 presents the principles of the newly proposed nMEKC as well as conventional MEKC for an anionic drug in an anionic micelle solution. In the conventional method (Fig. 3a), the running buffer contains the anionic micelles (SDS) but not the solute, whereas the injection solution contains both the solute and micelles (SDS). In the nMEKC method (Fig. 3b), the running solution contains both the micelles and the drug. The injected solution contains only the micelles. In the new method, after injection a drug-free zone was formed, if the drug had absorption at the detection wavelength. Here the drug-free zone was detected as negative peak.

Using the nMEKC technique it is possible to characterize affinity of drug to micellar systems. To calculate the partition coefficient from the

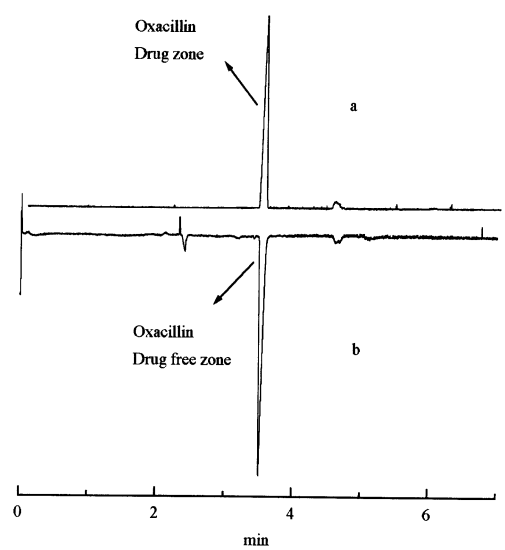

Fig. 3. Electropherogram of the principles of conventional MEKC (a) and newly described MEKC (b). Buffer: 10 mM phosphate, pH 7.0; a capillary with 40 cm effective length × 50 μm I.D.; 30 kV; temperature: 25 °C; pressure injection: 5 s at 50 mbar; detection: 200 nm.

Table 3
Migration factors (k') of antibiotics at different temperatures

T	25 °C	30 °C	35 °C	40 °C
1-Dicloxacillin	1.70	2.22	3.14	3.87
2-Pencillin V	1.18	1.57	1.89	2.22
3-Penicillin-G	1.04	1.02	1.00	0.97
4-Oxacillin	0.97	0.80	0.68	0.58
5-Piperacillin	0.88	0.71	0.65	0.60
6-Azlocillin	0.76	0.74	0.70	0.67

Table 4
Partition coefficients (P_{mw}) of antibiotics at different temperatures

T	25 °C	30 °C	35 °C	40 °C
1	147.19	189.26	265.88	330.48
2	102.16	133.84	160.03	189.58
3	90.04	86.96	84.67	83.09
4	83.98	68.2	57.58	49.53
5	76.19	60.34	55.14	51.23
6	65.80	63.08	59.27	57.22

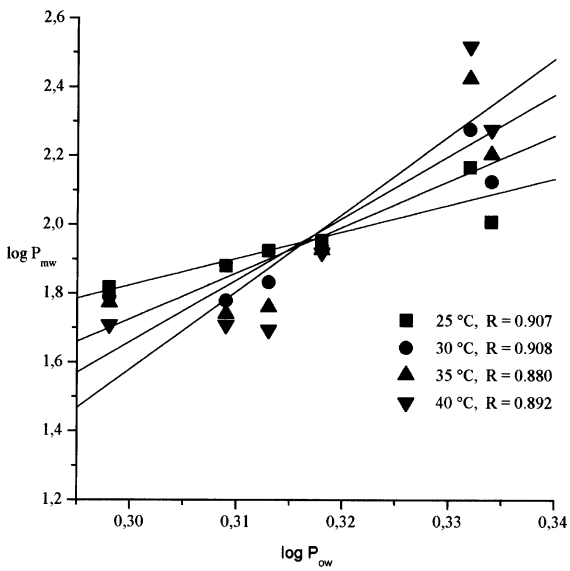

Fig. 4. Relationship between log P_{mw} and log P_{ow}.

capacity factor (Table 3) according to Eq. (3), the CMC of the SDS micelle was calculated at four different temperatures using the CE (Table 1). The partial specific volumes 0.8610, 0.8686, 0.8710 and 0.8758 ml/g at 25, 30, 35 and 40 °C were estimated from values given by Terabe et al. [16]. The temperature of the capillary was controlled with a high velocity air stream. The partition coefficient values of the antibiotics in the micellar system and in the n-octanol/water system were given in Tables 2 and 4. The partition coefficient can be compared with P_{ow} in the n-octanol/water system which is mostly used to characterize the hydrophilic/lipophilic properties of the drugs. A linear relationship was obtained between log P_{mw} versus log P_{ow} in the micellar system as well as in the n-octanol/water system (Fig. 4). The results obtained indicate that the partition coefficients determined by nMEKC could be used as parameter to characterize the partition behaviour of a drug in micellar systems and as hydrophobic parameter.

Partition coefficient data of the compounds at different temperatures can be used for the determination of several thermodynamic quantities of the micellar solubilization. To investigate the dependence of the partition coefficients of antibiotics on the temperature in nMEKC, experiments were carried out at different temperatures between 25 and 40 °C with background electrolyte containing SDS solution. The enthalpy and the entropy for the micellar solubilization of antibiotics can be calculated using Eq. (4). The van't Hoff plots for all sample compounds are shown in Fig. 5. The slope for the van't Hoff plot gives $\Delta H°$, and the intercept gives $\Delta S°$. The slope for pencillin $\Delta G°$, oxacillin, piperacillin and azlocillin is positive because it is here not necessary to warm up to dissolve the drug in the micelles. Table 4 shows that the partition coefficient of penicillin $\Delta G°$, oxacillin, piperacillin and azlocillin decreases with increasing of the temperature (exothermal process). $\Delta G°$ at 25 °C was calculated from Eq. (5) (Table 5). The correlation coefficient of the T^{-1} versus ln P_{mw} plots for antibiotics were between 0.978 and 0.998 (Fig. 5).

In the present study, we investigated a class of drugs that has different physico-chemical properties, different hydrophobic/hydrophilic properties and contains various ring heteroatoms such as nitrogen, oxygen and sulphur, that form strong hydrogen bond with water and with micelles.

From the enthalpy and entropy changes, listed in Table 5, it can be concluded that the hydrophobic interaction plays a significant role in the micellar solubilization of sample compounds in nMEKC. The more negative $G°$ is, the more is the equilibrium moved to the micelle side. Depending on the chemical structure of antibiotics, various chemical interactions additional to hydrophobic interactions, such as dipolar interactions, may occur between them in the partitioning process. The strength of the affinity of antibiotics to the SDS micelle was found to be at room temperature as follows: Dicloxacillin > penicillin V > penicillin G > oxacillin > piperacillin > azocillin

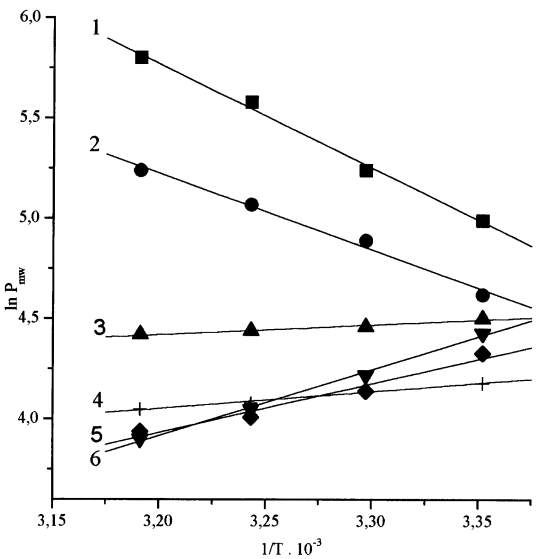

Fig. 5. Van't Hoff plots of antibiotics. Conditions see text.

Table 5
Standard enthalpy $\Delta H°$, standard entropy $\Delta S°$ and standard Gibbs free energy $\Delta G°$ at 25 °C in micellar solubilization of antibiotics

	ΔH° (kJ mol^{-1} K^{-1})	ΔS° (J mol^{-1} K^{-1})	ΔG° (kJ mol^{-1}, 25 °C)
1	42.63	182.26	−11.74
2	31.26	142.34	−11.20
3	−3.97	23.90	−11.09
4	−27.0	−53.78	−10.95
5	−19.95	−31.12	−10.66
6	−6.93	11.45	−3.51

The method described is rapid, reproducible and opens a new way for the characterization of the hydrophobicity-hydrophilicity and of thermodynamic properties of drugs in different pharmaceutical formulations such as micelles in order to optimize the affinity of the drugs to the vehicle systems.

5. Conclusion

In this paper, we investigate a drug class that is very important for medical and pharmaceutical purposes. It is known that a combination of surfactants with drugs leads to an improvement of the membrane transport and the bioavailability of drugs. Furthermore, in pharmaceutics it is important to characterize the hydrophobicity-hydrophilicity of drugs between aqueous and colloidal phases. A direct method for the characterization of this phenomenon is not known. The partition coefficient determined by this method provides fundamental information on the partition behaviour of the drugs between the aqueous and the colloidal phases. Therefore, the partition coefficient could be applied as parameter to characterize the hydrophobicity/hydrophilicity of drugs. Equilibrium and thermodynamic data can be calculated to characterize and opitimize the affinity of drugs to vehicle systems such as micelles. Thermodynamic quantities such as enthalpy and entropy changes of micellar solubilization could be calculated from the temperature dependence of the partition coefficients using the nMEKC technique.

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