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Characterization of partition and thermodynamic properties of cephalosporins using micellar electrokinetic chromatography in glycodeoxycholic acid solution

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Abstract

Micellar electrokinetic chromatography (MEKC) was introduced to evaluate the hydrophobicity of cephalosporins (cefpim, cefpirom, cefazolin, ceftazidim, cephradin, cefuroxim, cefotaxim, cephapirin and cephalothin) (Fig. 1). Partition coefficients of cephalosporins were calculated between a micelle and an aqueous phases from the measurement of the migration time, provided the critical micelle concentration and the phase ratio are known. Thermodynamic quantities such as enthalpy and entropy changes of micellar solubilization were calculated from the temperature dependence on the partition coefficients. Sodium glycodeoxycholate in low-salt aqueous solutions was employed to prepare a micellar solution. Substances for pharmaceutical purposes have to meet several requirements to be well-tolerated. Therefore, they are often derived from naturally occurring ones, e.g., from the bile salts in bile juice. The electrophoretic velocity of a micelle and the phase ratio between the micelle of the glycodeoxycholic acid and the aqueous phase were calculated. Partial specific volumes at different temperatures (from 20 to 45°C) were measured using dynamic light scattering. The logarithm of the partition coefficients and the migration factor in the micellar system were correlated with the logarithm of the 1-octanol–water partition coefficients.

Keywords: Thermodynamic parameters; Partition coefficients; Cephalosporins; Glycodeoxycholic acid; Antibiotics

1. Introduction

Cephalosporin antibiotics are extensively used to control bacterial infections in both humans and animals. In addition to therapeutical use, the drugs are widely used as a feed supplement in animal husbandry. Bioavailability and pharmacokinetics of oral administered drugs are strongly influenced by solubilization effects in the gastro-intestinal fluids. Especially micelle forming bile salts can solve even hydrophobic substances and partition coefficients of drugs between micellar and aqueous phases control the amount and rate of intestinal uptake. Micellar electrokinetic chromatography (MEKC) is a field of high-performance capillary electrophoresis [1,2]. The separation principle is based on different partitioning

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of solutes. A drug is distributed between a micelle and an aqueous phases. 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]. Micellar liquid chromatography (MLC) was used to calculate the distribution coefficient between the micelle and the aqueous phase [11,12]. Therefore, MEKC is more suitable for calculation of the distribution coefficient than MLC [13]. The low amount of sample required, the relatively short analysis time and the costs (very low costs compared to LC) were the main advantages of the method. In addition, MEKC provides high efficiency separations. The partitioning behaviour of solutes in micelles was also evaluated by electrokinetic chromatography [14–16]. Only a few papers report on MEKC and microemulsion electrokinetic chromatography (MEEKC) methods for the calculation of partition coefficients and thermodynamic quantities such as enthalpy and entropy changes of micellar solubilization. Terabe et al. calculated the partition coefficients of solutes and the enthalpy and entropy changes of micellar solubilization using MEKC [13]. Ishihama at al. studied the hydrophobicity of anionic and cationic solutes by using the microemulsion electrokinetic chromatography [17,18]. Muijselaar et al. described the calculation of partition coefficients and partial molar volumes of micelles [19]. However, they used the simplification, that the volumes 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 [20]. In this work, the partition coefficients, enthalpy and entropy changes of micellar solubilization for cephalosporins were determined using the MEKC technique in order to evaluate the temperature dependence of separation selectivity and also to investigate the partition mechanism in MEKC. The electrophoretic velocity of a micelle and the phase ratio between the micelle of glycodeoxycholic acid (GDCS) and the aqueous phase were studied.

2. Theory

2.1. Determination of the migration factor k′ and the partition coefficient P_{mw}

The migration factor is defined by

$$k' = n_{\mathrm{mc}}/n_{\mathrm{aq}}$$

where n_{mc} and n_{aq} are the number of the moles of the solute incorporated into the micelle and in the aqueous phase, respectively.

The migration factor k' of analytes was calculated from the observed migration times using the following equation [1]:

$$k' = \frac{t_{\mathrm{m}} - t_0}{t_0\left(1 - \frac{t_{\mathrm{m}}}{t_{\mathrm{mc}}}\right)} \tag{1}$$

where t_{m} is the migration time of a solute measured from the electropherogram, t_0 is the migration time in the absence of micelles and t_{mc} is the migration time of the micelle.

In MEKC, the migration factor k' is directly related to partition coefficients between the aqueous and micellar phases as [1,3]

$$k' = P_{\mathrm{mw}}[V_{\mathrm{mc}}/V_{\mathrm{aq}}] = P_{\mathrm{mw}}\phi \tag{2}$$

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 migration factor according to Eq. (3):

$$k' = P_{\mathrm{mw}}\upsilon(C_{\mathrm{t}} - \mathrm{CMC})/[1 - \upsilon(C_{\mathrm{t}} - \mathrm{CMC})] \tag{3}$$

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. (3) becomes negligible and Eq. (3) can be rewritten as follows:

$$k' = P_{\mathrm{mw}}[\upsilon(C_{\mathrm{t}} - \mathrm{CMC})] \tag{4}$$

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

$$\ln P_{mw} = -\Delta H^\circ/RT + \Delta S^\circ/R \quad (5)$$

where ΔH° is the enthalpy associated with the micellar solubilization or the transition of the solute from the aqueous phase to the micelle, ΔS° is the corresponding entropy, R is the gas constant and T is the absolute temperature.

The Gibbs free energy, ΔG°, for the micellar solubilization can be calculated according to

$$\Delta G^\circ = \Delta H - T\Delta S^\circ \quad (6)$$

2.2. Determination of the partial specific volumes υ

With the experimental technique of static and dynamic light scattering it is possible to determine the aggregation number and the hydrodynamic radius of micelles [21]. Light scattering measurements and theoretical evaluations the partial specific volumes were derived as follows [22]:

$$\upsilon = \frac{4\pi}{3} \cdot R_h^3 \cdot \frac{N_A}{\check{n}} \quad (7)$$

where υ is the specific volume, $\check{n}$ the aggregation number, R_h the hydrodynamic radius of the micelles and N_A the Avogadro number (Table 1).

2.3. Determination of partition coefficient

The partitioning coefficients of the drugs were determined between water and 1-octanol (Table 3). These two phases were saturated with each other. The compounds were dissolved in the water phase (200 μg/ml). The 1-octanol–water solutions were filled into suitable vials and shaken for 12 h at 35°C. After separation of the samples into both phases, the drug content was analysed by capillary zone electrophoresis (CZE) [23,24] and LC [25].

The partitioning coefficient was calculated using the following equation:

$$P_{ow} = a_{oc}/a_{aq} \quad (8)$$

where a_{oc} and a_{aq} are the concentrations of the drugs in the 1-octanol and in the aqueous phases, respectively.

Table 1
Micellar parameters, aggregation number $\check{n}$, hydrodynamic radius R_h, phase ratio between the micelle and aqueous phases Q, partial specific volumes υ studied at different temperatures using dynamic light scattering

T (°C)	$\check{n}$	R_h (nm)	υ (ml mol^{-1})	Q (V_{mc}/V_{aq})
20	11.6	1.50	808.53	0.0331
25	11.1	1.48	735.92	0.0301
30	10.8	1.44	695.66	0.0284
35	10.3	1.41	687.56	0.0281
40	10.0	1.36	634.20	0.0258
45	9.5	1.30	583.10	0.0237

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 264 nm. Fused-silica capillaries from Hewlett-Packard of 48.5 cm (length to the detector 40 cm)×50 μm I.D. were used.

3.2. Chemicals

Cefpim, cefpirom, cefuroxim and cefotaxim were obtained from Hoechst, Germany. Cephalothin, cephradin, cefazolin, cephapirin and ceftazidim were obtained from Sigma–Aldrich, Germany. Acetone was obtained from Merck, Germany. The sodium salt of GDCS and NaCl were purchased from Fluka, Switzerland. See Fig. 1 for structures of the cephalosporins.

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, 0.03 M NaCl was dissolved in water. Then 2% GDCS was dissolved in

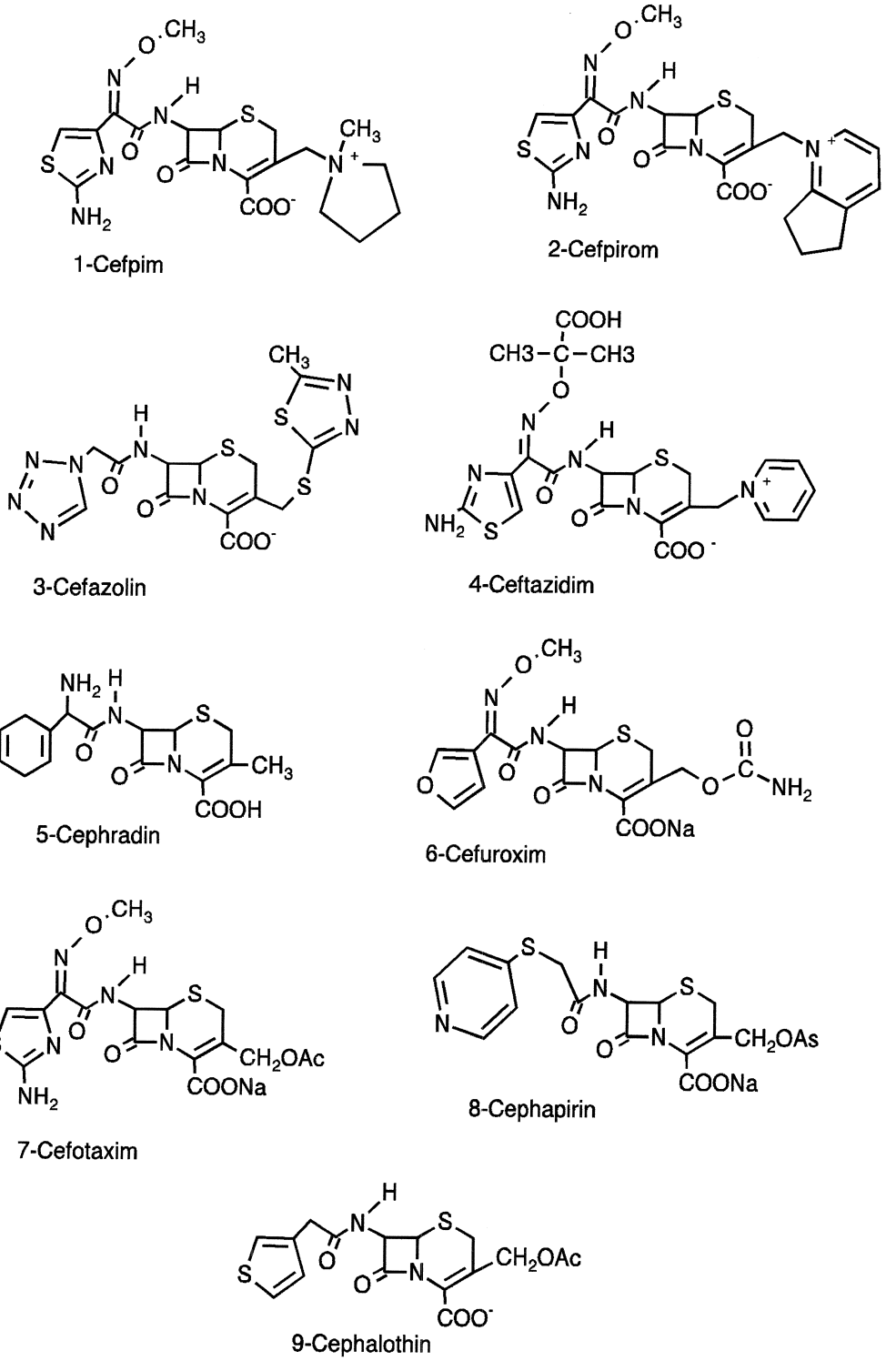

Fig. 1. Chemical structures of cephalosporins.

the NaCl solution. 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 20, 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. 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.

4. Results and discussion

For the calculation of k' (Table 2) the knowledge of both t_0 and t_{mc} is indispensable. The micelle migration can be determined by compounds which are completely solubilized by the micelles, such as Sudan III, anthracene or timepidium bromide. In our work, a tracer which is perfectly incorporated into the lipid phase is employed for estimation of the lipid phase. In every run dodecylbenzoyl propionic acid [13] was used as tracer of the micelle. Dodecylbenzoyl propionic acid exhibited higher UV absorption and a better symmetrical peak compared to Sudan III. For the determination of the electroosmotic flow (EOF) acetone was used as marker substance. To control the reproducibility of the micelle migration and the EOF three injections of the solute were made. The relative standard deviation of the migration times was between 0.1% and 1%. Furthermore, the electrophoretic velocities of the micelles of GDCS were determined at different temperatures. It was observed that the electrophoretic velocities increased with an increase of the temperature (Fig. 2). We assume that three factors influence the electrophoretic velocities of the micelle. First, an increase in temperature leads to a decrease of viscosity of the aqueous solution. Second, an increase in temperature leads to a decrease of the aggregation number and the radius of micelles. Third, a decrease in aggregation number leads also to a decrease of the charge of the micelle.

In this paper, we studied a micellar system and a drug class that are very important for medical and pharmaceutical purposes. Our results demonstrate that a combination of bile salt with cepalosporins leads to an improvement of the membrane transport and the bioavailability of cephalosporins about 40-fold. Furthermore, in pharmaceutics it is important to know the partition of drugs between aqueous and micelle phases. A direct method for the determination of the partition of drugs between these two phases is not known. Using the MEKC technique we tried to characterize drug partition in the micellar system. To calculate the partition coefficient from the capacity factor (Table 2) according to Eq. (4), the CMC of the GDCS micelle (1.48 m*M*) was calculated at six different temperatures as described by Kratohvil and Dellicolli [26]. The temperature of

Table 2
Migration factors (k') of cephalosporins at different temperatures in GDCS solutions

	Temperature (°C)					
	20	25	30	35	40	45
(1) Cefpim	0.065	0.061	0.054	0.050	0.044	0.039
(2) Cefpirom	0.152	0.128	0.116	0.113	0.088	0.070
(3) Cefazolin	0.185	0.243	0.317	0.356	0.387	0.462
(4) Ceftazidim	0.429	0.435	0.449	0.465	0.480	0.487
(5) Cephradin	0.183	0.337	0.437	0.508	0.586	0.767
(6) Cefuroxim	0.550	0.560	0.579	0.588	0.664	0.722
(7) Cefotaxim	0.550	0.576	0.600	0.632	0.644	0.657
(8) Cephapirin	0.644	0.652	0.683	0.684	0.719	0.719
(9) Cephalothin	0.760	0.777	0.782	0.809	0.820	0.856

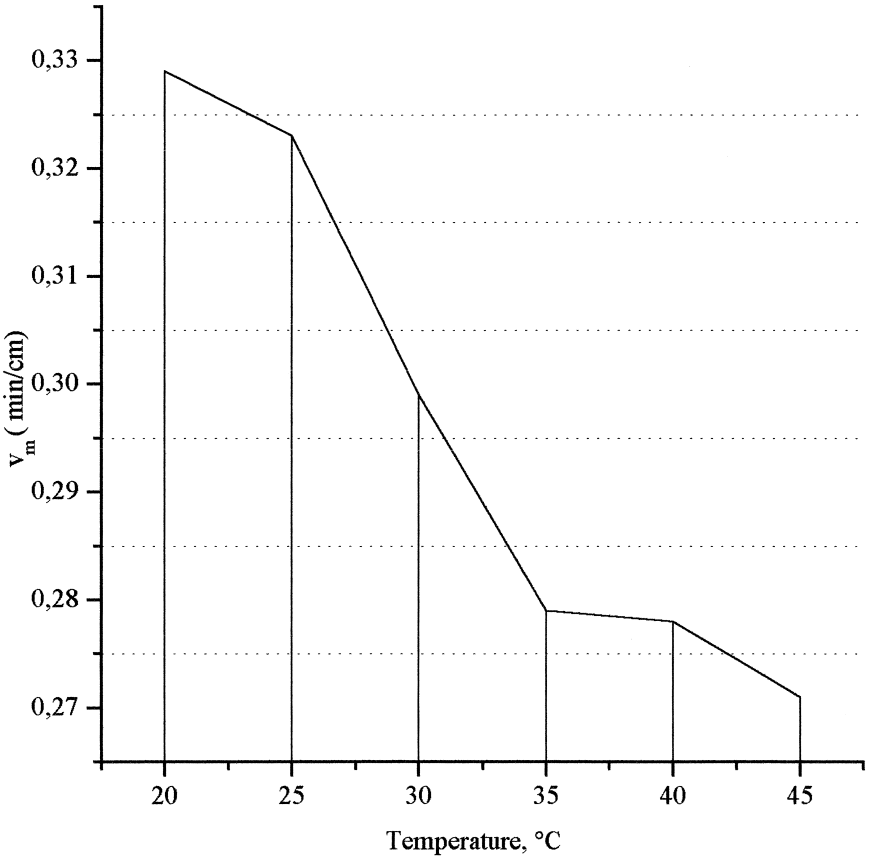

Fig. 2. Electrophoretic velocities of the micelles (v_m) of GDCS at different temperatures.

the capillary was controlled by a high velocity air stream. No change of the CMC was observed at different temperatures. However, the aggregation number, the phase ratio between the micelle and aqueous phases, the hydrodynamic radius as well as the partial specific volumes of the dihydroxy bile salt (Table 1) have changed. Although cephalosporins are hydrophobic drugs and have several similar polar groups they show different partition behaviour in the system. We assume that the structure of cephalosporins, electrostatic and hydrogen bonding interactions play also a role in the present study. The partition coefficient values of the cephalosporins in the micellar system and in the 1-octanol–water system are given in Table 3. As can be seen in Table 3, the micellar system gave higher P_{mw} values than the 1-octanol–water system. For the micellar system in

Table 3
Partition coefficients (P_{mw}) of cephalosporins at different temperatures in GDCS solutions

	Temperature (°C)						P_{ow} [a]
	20	25	30	35	40	45	
1	1.99	2.03	1.90	1.78	1.69	1.63	0.01
2	4.59	4.25	4.08	4.02	3.40	2.95	0.02
3	5.59	8.10	11.16	12.67	14.94	19.50	0.41
4	13	14.90	15.81	16.55	18.53	20.55	0.55
5	5.53	11.20	15.35	18.11	22.63	32.36	0.57
6	16.61	18.66	20.38	20.93	24.86	30.46	0.68
7	16.61	19.20	21.12	22.49	24.86	27.72	0.74
8	19.50	21.73	24.04	24.34	27.76	30.34	0.70
9	22.96	25.90	27.53	28.79	31.66	36.12	1.19

[a] Partition coefficients in the 1-octanol–water system at 35°C.

which hydrophobic interactions play an important role in influencing the migration and selectivity of solutes, one may expect a linear relationship between the logarithm of the partition coefficient and the logarithm of the 1-octanol–water partition coefficient. The P_{ow} can be compared with the P_{ow} in the 1-octanol–water system which is mostly used to characterize the hydrophilic–lipophilic properties of the drugs. The P_{mw} in the micellar system turns out to be an evident parameter because it shows a better diversification than P_{ow}. The plots of log P_{mw} and of the log k' vs. log P_{ow} of cephalosporins were studied. A linear relationship was obtained between log P_{mw} ($R=0.987$) and log k' ($R=0.906$) vs. log P_{ow} in the micellar system and in the 1-octanol–water system (Fig. 3). The results obtained indicate that the partition coefficient determined by MEKC could be used as parameter to characterize the partition behaviour of a drug in micellar systems and as hydrophobic parameter instead of log P_{ow}.

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 cephalosporins on the temperature in MEKC, experiments were carried out at different temperatures between 20 and 45°C with background electrolyte containing 2% GDCS in 0.03 *M* NaCl solution. At each temperature, a constant current of 57 μA was applied in order to exclude differences in Joule heating. The enthalpy and the entropy for the micellar solubilization of cephalosporins can be calculated according to Eq. (5). The Van 't Hoff plots for all sample compounds are shown in Fig. 4. The slope for the Van 't Hoff plot gives $\Delta H°$, and the intercept gives $\Delta S°$. $\Delta G°$ at 35°C was calculated from Eq. (6) (Table 4). The correlation coefficient of the T^{-1} vs. ln P_{mw} plots for cephalosporins were between 0.958 and 0.999 (Fig. 4).

The slope for cefpim and cefpirom is positive because cefpim and cefpirom had a zero electrophoretic mobility (neutral) in the solution. Here, it is not necessary to warm up to dissolve the drug in the micelles (exothermal process). The residual cephalosporin represents the sodium salt. Table 3 shows that the partition coefficient of cefpim and cefpirom decreases with increasing of the temperature (exothermal process).

As shown in Fig. 5, ln P_{ow} correlated well with

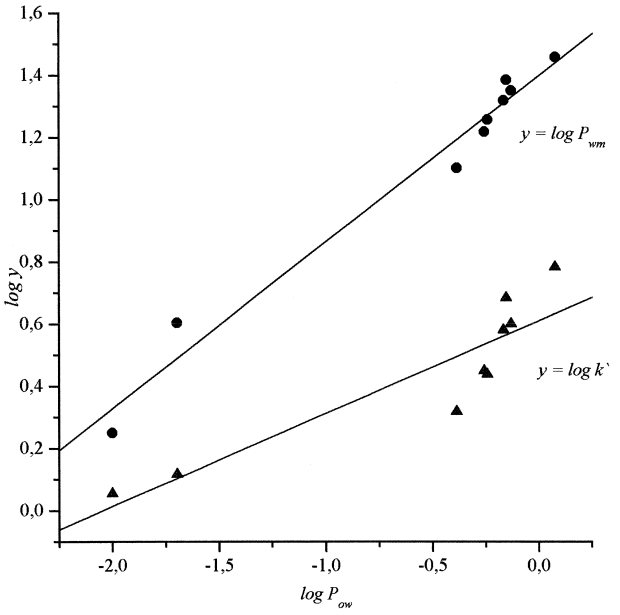

Fig. 3. Relationship between log P_{ow} and log P_{mw} and log k' at 35°C. For conditions see text.

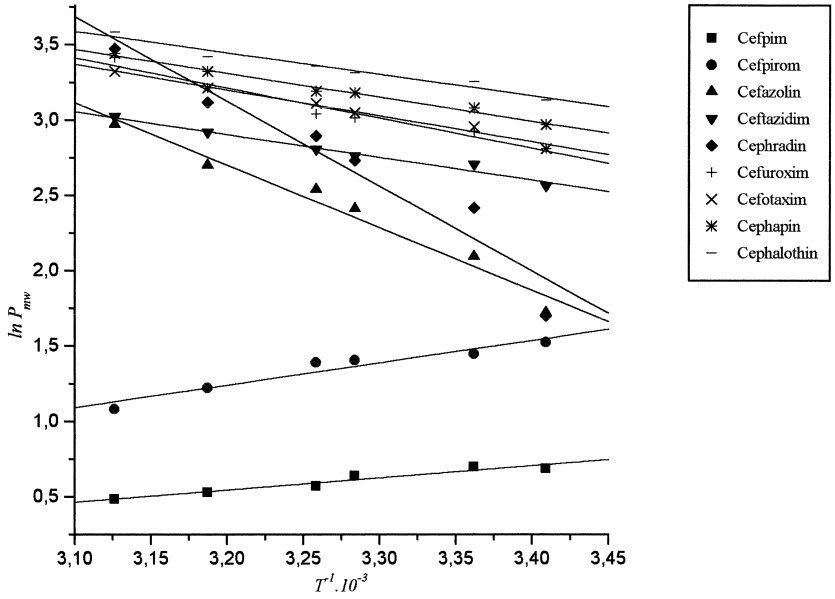

Fig. 4. Van 't Hoff plots of cephalosporins. For conditions see text.

$\Delta G°$, while $\Delta H°$ and $\Delta S°$ showed much less correlation.

The results of the regression analysis were as follows:

$$\ln P_{ow} = 26.9\Delta H° + 102, R = 0.740$$

$$\ln P_{ow} = 6.9\Delta S° + 23, R = 0.668$$

$$\ln P_{ow} = -1.4\Delta G° - 8.3, R = 0.986$$

these results show that $\Delta G°$ can give us better information as $\Delta H°$ and $\Delta S°$ on the partition behaviour of the drugs in this system.

Furthermore, a linear relationship ($R = 0.995$) was obtained between $\Delta H°$ and $\Delta S°$ in the micellar solubilization of cephalosporins (Fig. 6). The compensation temperature β was equal to the slope of the line in Fig. 6. Chen et al. compared this β value with values found in reversed-phase high-performance liquid chromatography (RP-HPLC) [14].

The characterization of the different thermodynamic properties of polar cephalosporins is very difficult compared to nonpolar solutes that were only

Table 4
Standard enthalpy $\Delta H°$, standard entropy $\Delta S°$ and standard Gibbs fee energy $\Delta G°$ at 35°C in micellar solubilization of cephalosporins by the GDCS micelle

	$\Delta H°$ (kJ mol^{-1} K^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	$\Delta G°$ (kJ mol^{-1} K^{-1}, 25°C)
1	−6.57	−16.47	−1.49
2	−12.07	−28.26	−3.37
3	33.97	130.98	−6.37
4	12.34	63.59	−7.24
5	45.97	172.93	−7.29
6	16.31	78.86	−7.98
7	14.04	71.48	−7.97
8	12.97	68.99	−8.28
9	11.67	65.96	−8.64

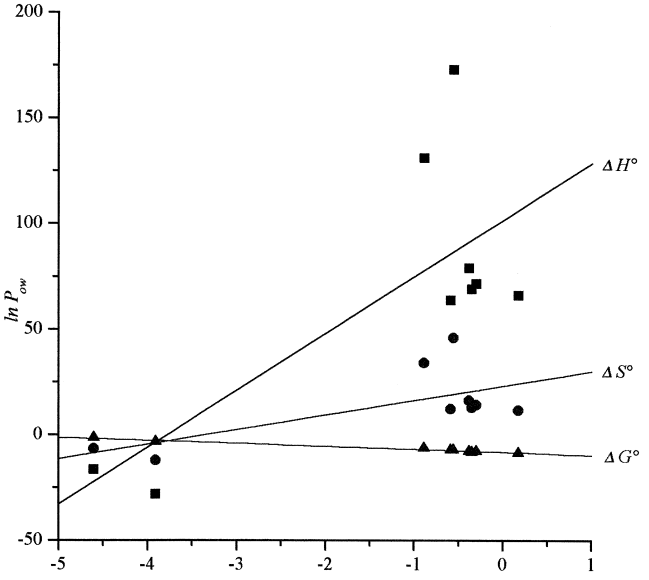

Fig. 5. Relationship between ln P_{ow} and the thermodynamic parameters $\Delta H°$, $\Delta S°$, $\Delta G°$.

investigated with sodium dodecyl sulfate (SDS) [11–14]. In the literature only nonpolar homologous series of alkylbenzenes were studied. Here, only hydrophobic interactions between the substances and micelles play a major role and the differences of the thermodynamic properties of the substances were very easy to characterize. The nonpolar and polar compounds are solubilized in the micelles in different ways. In aqueous solutions, it is generally accepted that nonpolar compounds are solubilized in

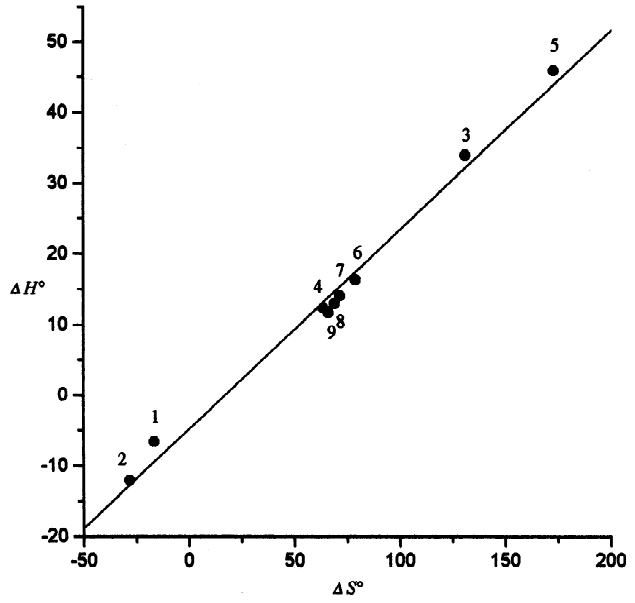

Fig. 6. Relationship between $\Delta H°$ and $\Delta S°$ in the micellar solubilization of cephalosporins by GDCS micelle. For conditions see text.

the hydrophobic interior of the micelle and polar compounds are solubilized by adsorption on the micelle surface. Therefore, the partition coefficients of nonpolar compounds are mainly influenced by the alkyl chain length of the surfactant, whereas the partition coefficients of polar compounds are mainly influenced by the hydrophilic group of the surfactant. In general, nonpolar compounds show a better relationship as polar compounds. In the present study, we investigated a class of drugs that has different physico–chemical properties, different hydrophobic–hydrophilic properties and contains various ring heteroatom such as nitrogen, oxygen and sulphur, that form strong hydrogen bonds with water and with micelles. From the enthalpy and entropy changes, listed in Table 4, it can be concluded that the hydrophobic interactions play a significant role in the micellar solubilization of sample compounds in MEKC. Although cephradin and cefazolin have low partition coefficients (0.57, 0.41) they show high entropy (173, 130) compared to other cephalosporins. The Gibbs free energy, calculated from Eq. (6) is given in Table 4. The more negative ΔG° is, the more the equilibrium is moved to the micelle side. Depending on the chemical structure of cephalosporins, various chemical interactions additional to hydrophobic interactions, such as dipolar interactions, may occur between them in the partitioning process.

5. Conclusion

MEKC was introduced to evaluate the partition behaviour of various kinds of drugs in glycodeoxycholate solution. Equilibrium and thermodynamic data can be calculated for the transfer of the solute from the aqueous to the micellar phases. Thermodynamic quantities such as enthalpy and entropy changes of micellar solubilization were calculated from the temperature dependence of the partition coefficients using the MEKC technique. The partition coefficient determined by this method provides fundamental information about the partition behaviour of the drugs between the aqueous and the micellar phases. Therefore, the partition coefficient 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 the determination of the partition coefficient and thermodynamic quantities of drugs in different pharmaceutical formulations in order to optimize the affinity of the drugs to the vehicle systems.

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