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Characterization of cephalosporin transfer between aqueous and colloidal phases by micellar electrokinetic chromatography

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Abstract

A new electrokinetic chromatographic method was applied to the determination of the partition coefficient between water and micelle for a group of cephalosporins (cefmetazol, cephradin, cefaclor, ceftazidim, cefodizim, cephapirin, cephalothin and ceftriaxon) using sodium dodecyl sulphate as an anionic surfactant in microemulsion and in micellar systems. In the new method, the running buffer contains both the micelles and the drug, and the injected solution contains the same concentration of micelles as the running buffer but not the drug. The mobility of the drug can be measured from a negative peak recorded the chromatogram. The required parameters for the determination of the capacity factor (μ_{aq} and μ_{me} are the electrophoretic mobilities of the solutes in the aqueous and the micelle phases, μ_{eff} is the effective mobility in the micellar system or in the microemulsion) were measured by the new micellar and microemulsion electrokinetic chromatography technique. Linear log–log relationships were found between both the micelle–water partition coefficient and the capacity factor and the *n*-octanol–water partition coefficient.

Keywords: Partition coefficients; Cephalosporins

1. Introduction

Micellar electrokinetic chromatography, which is based on micellar solubilization and electrokinetic migration, extends the power of capillary zone electrophoresis to the separation of both neutral and charged species [1–3]. The partitioning behaviour of drugs in different systems (micelle, microemulsion, liposome) plays an important role in the pharmaceutical and physicochemical behaviour. The hydrophobic effect is the driving force for the liquid–liquid partitioning processes and biological activities such as biomembrane transport, bioaccumulation of pollutants, soil sorption [4]. The partitioning behaviour of solutes in micelles has been evaluated by electrokinetic chromatography (EKC) [5–7]. Only a few papers report the micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) methods for the calculation of the partition coefficients. Terabe et al. [8] calculated the partition coefficients of solutes and the enthalpy and entropy changes of micellar solubilization using EKC. Ishihama at al. studied the hydrophobicity of anionic and cationic solutes by using the MEEKC [9,10]. Muijselaar et al. described the calculation of partition coefficients and partial molar volumes of micelles [11]. Furthermore, the partitioning behaviour of various drugs in microemulsions was evaluated using non-ionic surfactants [12]. Katsuta et al. determined the solubilization isotherms of neutral solutes using the new MEKC [13]. In the

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present work, the partition coefficients for cephalosporins were determined using the new MEKC and new MEEKC techniques. In this new method, the running buffer contains SDS and solute and the injected solution contains only SDS. Information concerning drug partition in microemulsion (ME) is very useful to design optimal MEs for controlled drug delivery. In this work, the partitioning behaviour of cephalosporins (Fig. 1) was evaluated by EKC using ME and micellar (MC) systems consisting of anionic surfactants. The partitioning behaviour of cephalosporins using MEEKC was compared with that using MEKC. Furthermore, the log k_{mw} and the log P_{mw} of the cephalosporins in ME and MC

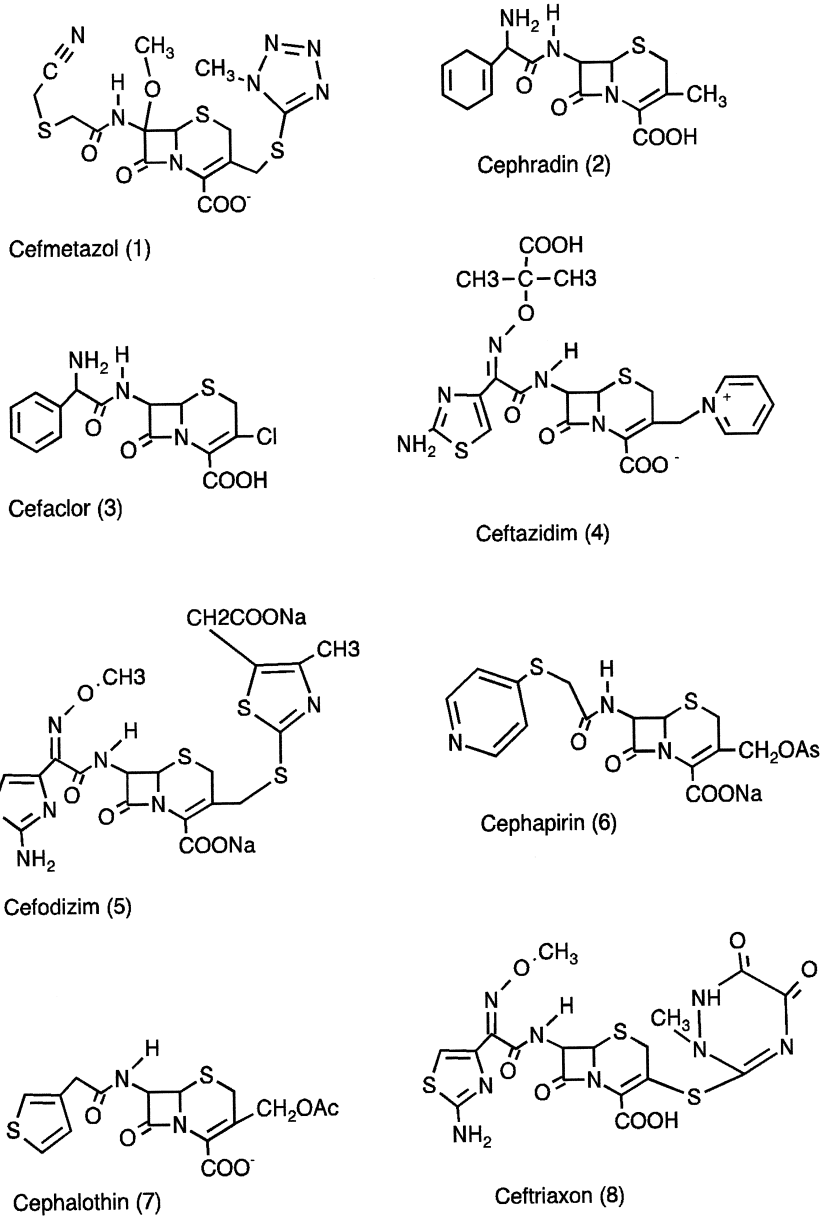

Fig. 1. Chemical structure of cephalosporins.

systems were correlated with the log P_{ow} in the *n*-octanol–water system.

2. Theory

2.1. Determination of the capacity factor k_{mw} and the partition coefficients P_{mw}

The capacity factor is defined by

$$k_{mw} = n_{mc}/n_{aq} \quad (1)$$

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

The migration factor k_{mw} of analytes was calculated from the observed ionic mobility using the following equation [1]

$$k_{mw} = (\mu_{aq} - \mu_{me})/(\mu_{me} - \mu_{mc}) \quad (2)$$

where μ_{aq} and μ_{mc} are the electrophoretic mobilities of the solute in the aqueous and in the oily phases of the ME, μ_{me} is the effective mobility in the ME. μ_{me} has been obtained by measuring the migration times of acetone (tracer for the aqueous phase) and of the solutes in the ME systems. μ_{mc} has been obtained by measuring the migration times of 3-(4-dodecylbenzoyl) propionic acid (tracer for the oily phase). μ_{aq} has been obtained by capillary zone electrophoresis (CZE) with phosphate–borate buffer, pH 7.0, including 8% *n*-butanol [10,11].

In MEKC, capacity factor is directly related to partition coefficients between the aqueous and micellar phases as in [2,3]

$$k_{mw} = P_{mw}\,[V_{mc}/V_{aq}] \quad (3)$$

where V_{mc} and V_{aq} are the volumes of the micelle and the remaining aqueous phase. Therefore, we can calculate the partition coefficients from the capacity factor according to Eq. (3)

$$k_{mw} = P_{mw} v[(C_t - \mathrm{CMC})/(1 - (C_t - \mathrm{CMC})] \quad (4)$$

where *v* 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. (4) becomes negligible and Eq. (4) can be rewritten as follows

$$k_{mw} = P_{mw} v[(C_t - \mathrm{CMC})] \quad (5)$$

3. Experimental

3.1. Apparatus

Capillary electrophoretic experiments were performed on a Hewlett-Packard Model G1600A (Waldbronn, Germany) ^{3D}CE system. The detection wavelength was at 264 nm. Fused-silica capillaries from Hewlett-Packard of 48.5 cm (effective length 40 cm)×50 μm I.D. were used.

3.2. Chemicals

Cefodizim was obtained from Hoechst (Germany). Cefmetazol, cepharadin, cefaclor, ceftazidim, cephapirim, cephalothin and ceftriaxone were obtained from Sigma-Aldrich (Germany). Acetone, *n*-butanol, *n*-heptane, potassium hydrogenphosphate, potassiumdihydrogen phosphate and *n*-octanol were obtained from Merck (Germany). Sodium dodecylsulfate (SDS) was purchased from Fluka (Switzerland).

3.3. Sample preparation

Standard solutions of the drugs were prepared at 200 μg/ml at pH 7.0. 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 0.05 *M* phosphate + 0.1 *M* borate, pH 7.0 buffer solution was prepared. The pH of the buffer was measured at 25°C using a HI 9321 microprocessor 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.

3.5. Determination of partition coefficient

The partitioning coefficients of the drugs were determined between water and *n*-octanol (Table 1). These two phases were saturated with each other. The compounds were dissolved in the water phase (200 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 [14,15] and LC [16].

The partitioning coefficient was calculated using the following equation

$$P_{ow} = a_{oc}/a_{aq} \quad (6)$$

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

3.6. Preparation of surfactant solutions in the micellar systems (MC)

Surfactant solutions of 50 m*M* SDS (1.44%, wt%) were prepared in buffer solution.

3.7. Preparation of microemulsions (ME)

The desired amounts of the surfactants, alcohol and *n*-heptane were mixed for 10 min at room temperature. Buffer solution containing definite concentrations of surfactants 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 and then filtered through a 0.45-μm syringe filter. The composition of these ME was as follows: 6.49% *n*-butanol–0.82% *n*-heptane, 1.44% SDS (wt%)/buffer.

3.8. Analysis conditions

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, a separation potential of 30 kV was used. 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 (v/v)] were injected at a pressure of 50 mbar for 5 s (hydrodynamic injection) (Tables 1 and 2).

Table 1
Capacity factors k_{mw} of cephalosporins in MC and ME systems[a]

	k_{mw} (MC)	k_{mw} (ME)	P_{ow}
1-Cefmetazol	0.09	0.29	0.01
2-Cephradin	0.15	0.22	0.01
3-Cefaclor	0.29	0.76	0.02
4-Ceftazidim	0.15	0.72	0.03
5-Cefodizim	0.69	0.80	0.06
6-Cephapirin	0.63	1.80	0.07
7-Cephalothin	3.82	11.22	0.43
8-Ceftriaxon	0.21	0.47	0.04

[a] P_{ow}, partition coefficients in the *n*-octanol–water system. Concentration, 200 μg/ml.

4. Results and discussion

Fig. 2 presents the principles of the newly proposed EKC as well as conventional EKC for an anionic solute in an anionic micelle solution. In the conventional method (Fig. 2a), 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 new method (Fig. 2b), the running solution contains both the micelles and the solute. The injected solution con-

Table 2
Partition coefficients P_{mw} of cephalosporins in MC and ME systems[a]

Cephalosporin	P_{mw} (MC)	P_{mw} (ME)
1	7.37	23.77
2	12.29	18.03
3	23.77	62.29
4	12.29	59.01
5	56.55	65.57
6	51.63	147.54
7	313.11	919.67
8	17.21	38.52

[a] Concentration: 200 μg/ml.

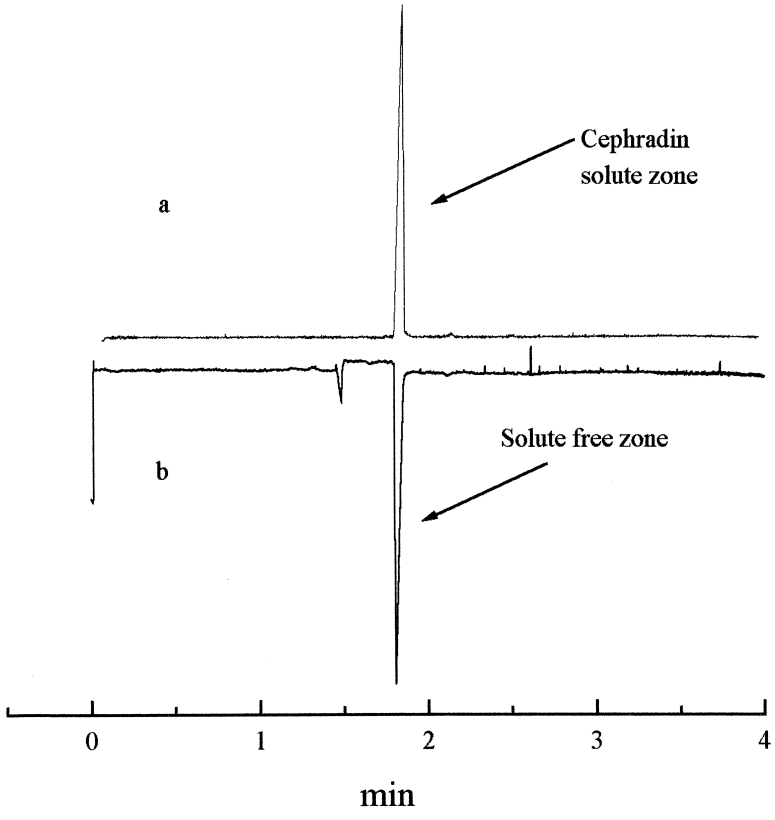

Fig. 2. Electropherogram of the principles of conventional MEKC (a) and newly described MEKC (b). Buffer: 0.05 m*M* phosphate–0.1 *M* borate, pH 7.0; a capillary with 40 cm effective length×50 μm I.D.; 30 kV; temperature: 25°C; pressure injection: 5 s at 50 mbar; detection: 264 nm.

tains only the micelles. In the new method, after injection a solute-free zone was formed, if the solute has absorption at the detection wavelength. Here the solute-free zone was detected as negative peak. In the new method was observed that (1) the mobility of the solute-free zone is identical to that of the solute in the capillary at low concentration (Fig. 2) (2) the migration time of acetone as marker for the determination of the electroosmotic flow and 3-(4-dodecylbenzoyl) propionic acid as marker for the determination of the velocity of the micelles gave a migration time identical to that measured using the conventional method (3) the migration time of the solute in the running buffer depends on the concentration of the solute at high concentrations (Figs. 3 and 4). This leads to a change of the partition behaviour of the solute. In the conventional method the variation of the solute concentration in the injected solution had little effect on the migration times of the solute and micelles.

A number of important drugs show no, or only a poor absorption from the intestinum. Therefore, they have to be applied parenterally. To enable oral administration, one has to search for modifications which improve the absorption of such drugs. Since it is known that a combination of cationic and anionic surfactans enhances the membrane transport of cephalosporins about 40-fold, there is a possibility to improve cephalosporin application by combining these drugs with surfactants. However, 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

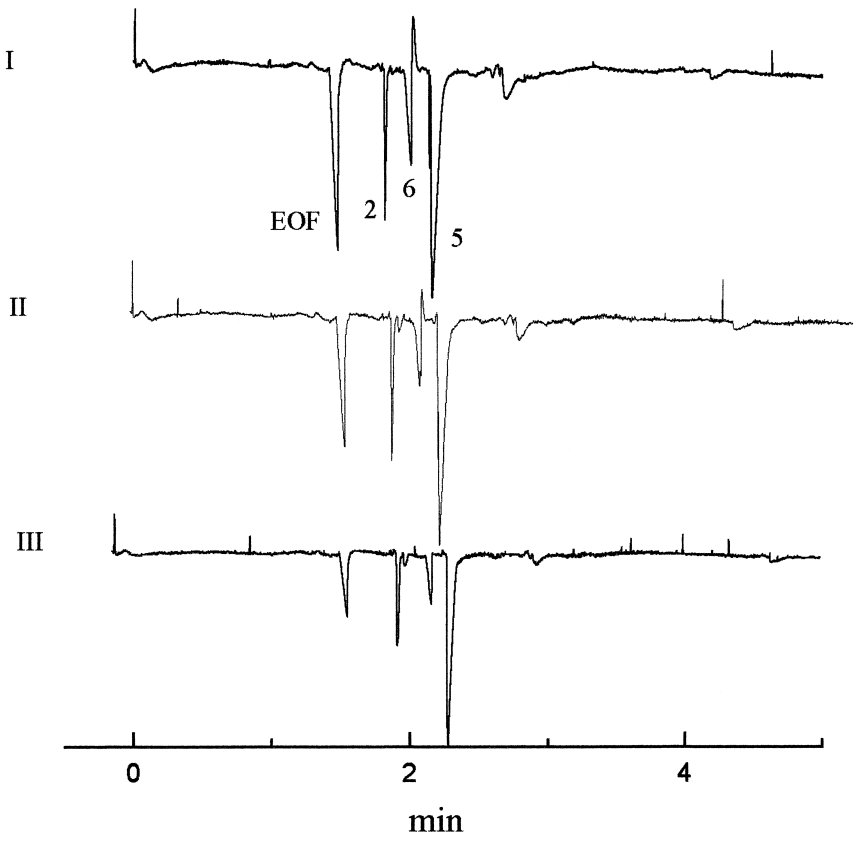

Fig. 3. Electropherogram of cephalosporins at different concentrations, (I) 100 μg/ml (II) 250 μg/ml (III) 500 μg/ml. Conditions see Fig. 2.

the MEKC and MEEKC techniques we tried to characterize drug partition in the micellar system. To calculate the partition coefficient from the capacity factor (Table 1) according to Eq. (5), the CMC of SDS was calculated at 25°C (0.57 m*M*) [17].The partial specific volume of SDS is 0.861 ml/g [8]. However, in the case of the microemulsion, the volume of the microemulsion phase was not known, which was different from the case of the SDS micellar phase. Thus, it was assumed that the concentrations of SDS and *n*-butanol in the microemulsion phase were the same as those in the case of the SDS micellar system containing *n*-butanol. The CMC value of the SDS in the system was 0.57 m*M* and the partial specific volumes were estimated from values given in Ref. [8]. For the calculation of k_{mw} 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 [18]. In our work a tracer, which is perfectly incorporated into the lipid phase, is employed for estimation of the colloidal phase. In every run 3-(4-dodecylbenzoyl) propionic acid was used as tracer of the micelle. 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 0.5 and 2%. Although cephalosporins are hydrophobic drugs and have several similar polar groups they

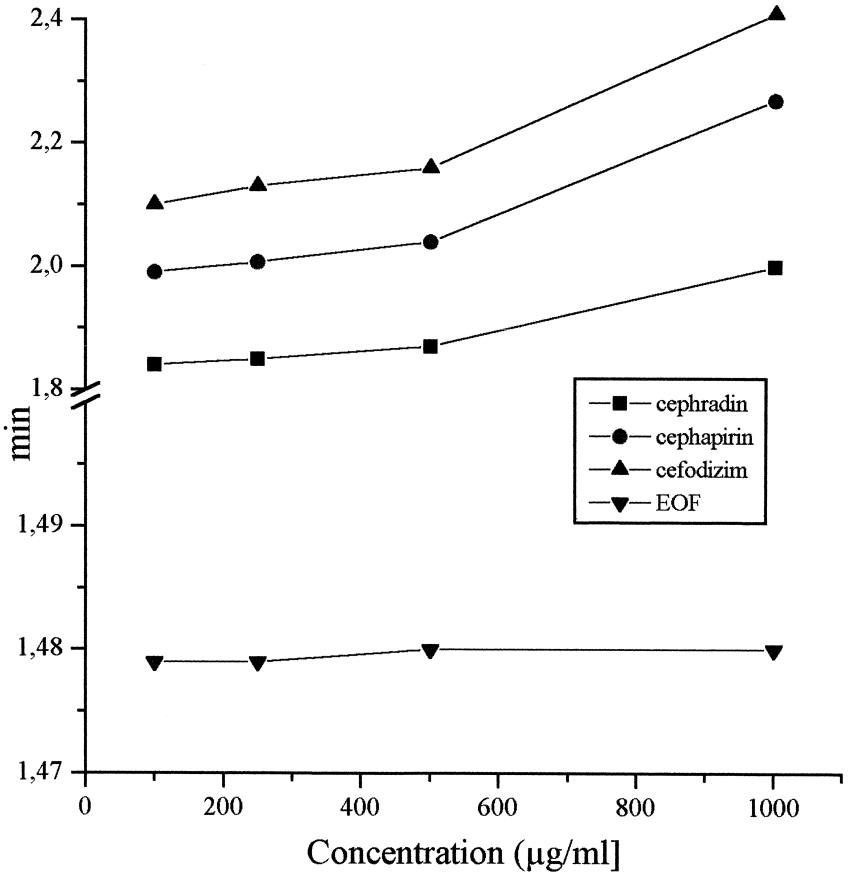

Fig. 4. Dependence of the migration times on the concentration of cephalosporins using the new MEKC.

show different partition behaviour in the system. We assume that the structure of cephalosporins, the hydrophobic interaction and various heteroaromatic compounds containing ring heteroatoms such as nitrogen, oxygen and sulphur, that form strong hydrogen bonds with water and with micelles, play a significant role in the study. The capacity factor and the partition coefficient values of the cephalosporins in MC and ME are given in Tables 1 and 2. ME gave higher k_{mw} and P_{mw} values than MC. The higher k_{mw} and P_{mw} values of cephalosporins in ME indicated that the ME has a stronger affinity than the MC systems. For the ME and MC systems in which hydrophobic interactions play a major 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 *n*-octanol–water partition coefficient. The k_{ow} and P_{ow} can be compared with the P_{ow} in the *n*-octanol–water system which is mostly used to characterize the hydrophilic–lipophilic properties of the drugs. The k_{mw} and P_{mw} in MC and ME turned to be out evident parameters because it show a better diversification than P_{ow}. The plots of log k_{mw} and of log P_{mw} vs. log P_{ow} of cephalosporins were studied. A linear relationship was obtained in MC and ME systems (see Figs. 5, 6). The results obtained indicate that the capacity factor and the partition coefficient determined by the new EKC could be both used as parameters to characterize

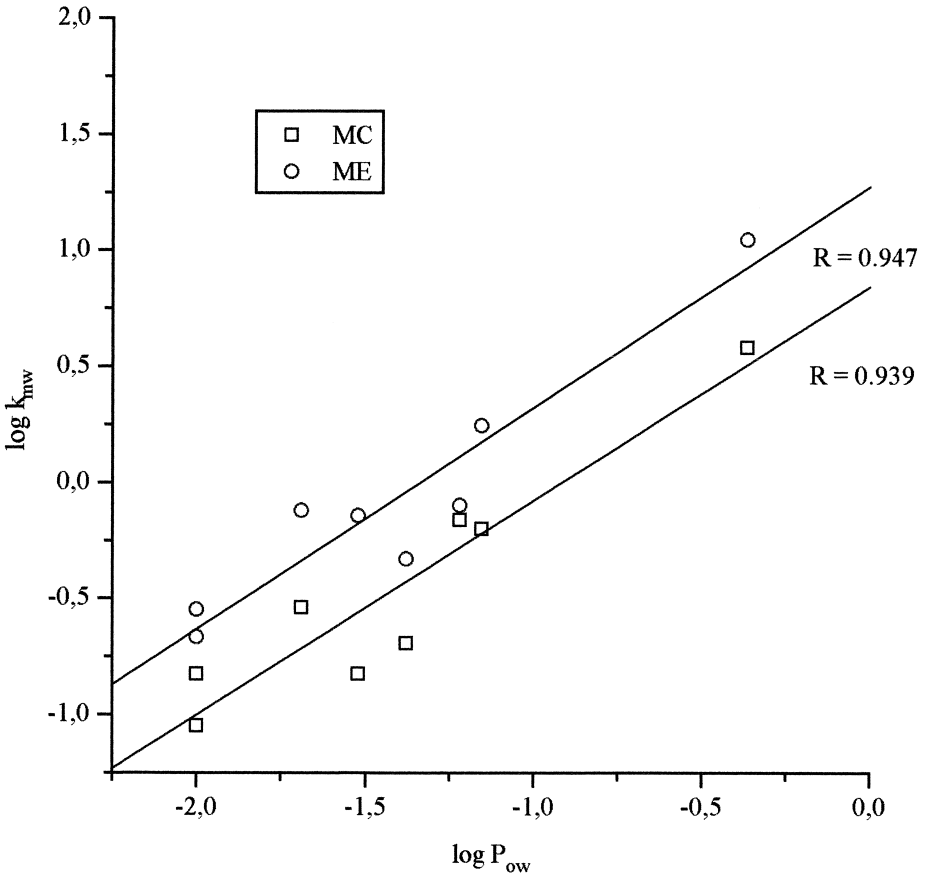

Fig. 5. Relationships between log k_{mw} and log P_{ow} in MC and ME systems.

partition behaviour of drugs in MC and ME systems and as hydrophobic parameters instead of log P_{ow}.

5. Conclusion

We have developed new MEKC and MEEKC methods for the determination of the partition behaviour of cephalosporins in MC and ME. In the new method the calculated P_{mw} is identical to the calculated using the conventional method at low concentration. At high concentration the migration time of the solute changes and that leading to a change of the capacity factor and the partition coefficient of the cephalosporins. The new method exhibited at high concentration of solute high k_{mw} and P_{mw} values both in MC and in ME systems compared to the conventional method. Therefore, the new method gives better information on the partition behaviour of drugs at high concentration compared to the conventional method. The partition coefficient determined by this method provides fundamental information on 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. Additionally, partitioning coefficient data of the compounds can be used for the determination of several thermodynamic quantities of the micellar solubilization. The method

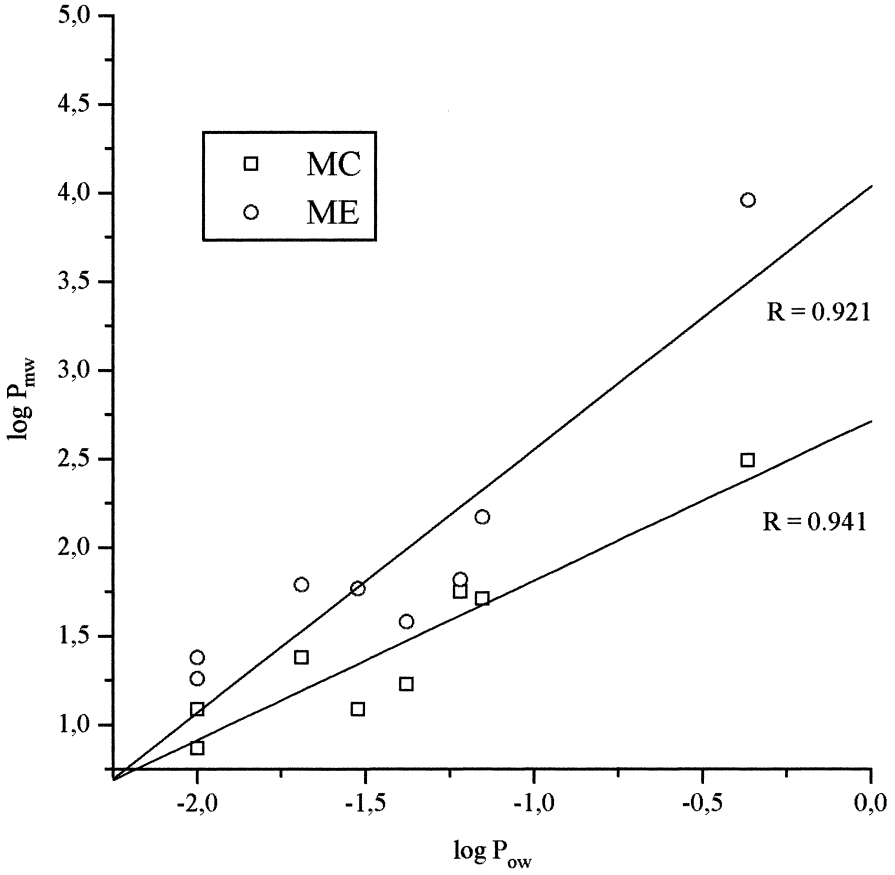

Fig. 6. Relationships between log P_{mw} and log P_{ow} in MC and ME systems.

described is easy, rapid, reproducible and opens a new way for the characterization of the partition behaviour of drugs in different pharmaceutical formulations and for the optimization of the affinity of drugs to relevant vehicle systems.

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