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Journal of Chromatography A, 802 (1998) 89–93

JOURNAL OF CHROMATOGRAPHY A

Characterization of interaction between cephalosporins and charged surfactants using capillary zone electrophoresis

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Abstract

Capillary electrophoresis offers a new way of characterizing the interaction between surfactants and drugs. This interaction was studied using capillary zone electrophoresis (CZE). It was observed that addition of tetraalkylammonium bromide to the electrolyte buffer at different concentrations of dodecyl trimethylammonium bromide (DTAB) led to a change of the migration time (t_m) and of the ionic mobility (μ) of cephalosporins. For quantitative evaluation, a physicochemical model was developed to calculate the aggregation constants (k) and the stoichiometric coefficients (m) between DTAB and cephalosporins. The relative standard deviation of the effective mobilities and of the EOF were estimated. © 1998 Elsevier Science B.V.

Keywords: Stoichiometric coefficients; Association constants; Cephalosporins; Surfactants; Tetraalkylammonium bromide; Antibiotics

1. Introduction

The influence of tetraalkylammonium bromide (dodecyl trimethylammonium bromide) on the transport of cephalosporins such as cefuroxim, cefotaxim, cephapirin and cefazolin (Fig. 1) through a dodecanol collodium membrane has been investigated. No membrane transport of cephalosporins was observed when they were used without tetraalkylammonium bromide. It turned out that the transport of cephalosporins through artificial lipid membranes occurred only in the presence of tetraalkylammonium bromide [1]. Obviously, the enhancement of cephalosporin transport is based on the interaction of tetraalkylammonium bromide with these drugs. The interaction between drugs and proteins was studied by fluorescence quenching, difference spectrophotometry and ultracentrifuge sedimentation [2,3]. Avial and others have recently demonstrated that affinity capillary electrophoresis (ACE) is a useful and sensitive method for measuring binding constants of ligands to proteins [4,5]. Schwarz et al. have studied the interaction between drugs and bile salts using the micellar electrokinetic capillary electrophoresis (MECC) [6,7]. Furthermore, binding stoichiometries of protein–ligand interactions were determined using ACE [8]. The present paper reports the use of capillary zone electrophoresis (CZE) as a technique to determine the association constants and the stoichiometric coefficients between dodecyl trimethylammonium bromide and cephalosporins. The effects of various concentrations of dodecyl tri-

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0021-9673/98/$19.00
PII S0021-9673(97)01072-8

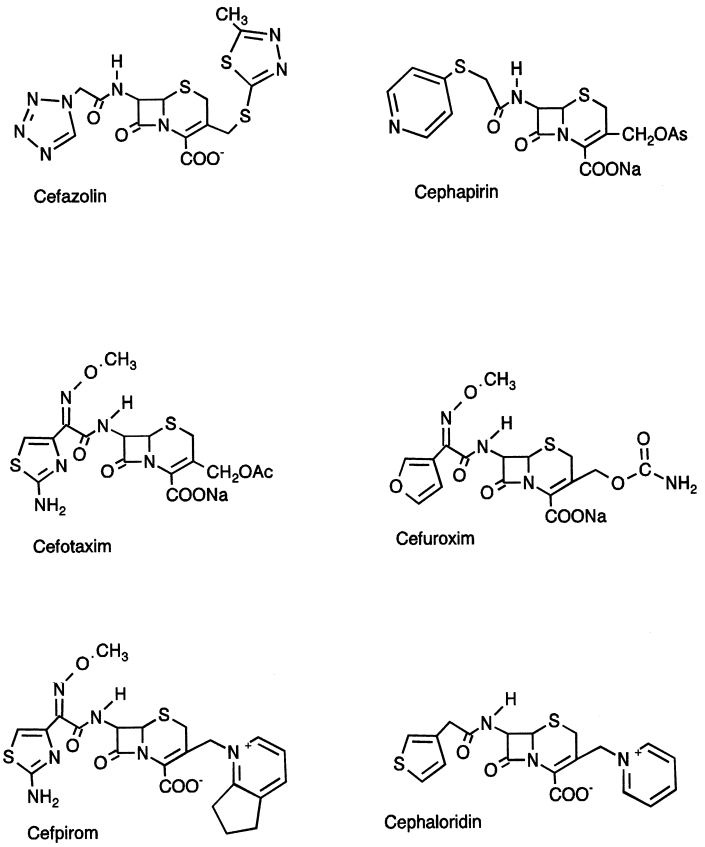

Fig. 1. Chemical structure of the compounds.

methylammonium bromide (DTAB) in the separation buffer on the migration time of cephalosporins were used to obtain a quantitative measure for the strength of interaction between DTAB and cephalosporins.

2. Theory

It was assumed that the drugs (L) were formed with DTAB (D) following complex equilibrium:

$$D + mL \rightarrow DL_m \tag{1}$$

The complex forming equilibrium between drug and DTAB was described as follows:

$$K = \frac{DL_m}{DL^m} \tag{2}$$

where K is aggregation constant, m the stoichiometric coefficients, D the drug concentration, and L the ligand (DTAB) concentration.

The effective mobility of the drugs was related to the effective mobility of the free drug according to its degree of dissociation:

$$\mu = \alpha\mu_D + (1 - \alpha)\mu_{DL_m} \tag{3}$$

where μ is effective mobility, α rate of dissociation.

The degree of dissociation of the complex was yielded from the concentration of the free drugs (D) to the used total concentration (c_0):

$$\alpha = \frac{D}{c_0} = \frac{D}{D + DL_m} \tag{4}$$

Combining Eq. (2) and Eq. (4) α is given as:

$$\alpha = \frac{1}{1 + KL^m} \tag{5}$$

α depends here on K, L and m.

Calculated from Eq. (3) and Eq. (5), the effective mobility is given by

$$\mu = \frac{1}{1 + KL^m} \cdot \mu_D + \left(1 - \frac{1}{1 + KL^m}\right) \cdot \mu_{DL_m} \qquad (6)$$

μ_D is the experimental value at $L = 0$ and μ_{DL_m} is the experimental value at the highest concentration of L.

Furthermore, the values K and m were optimized in two steps.

3. Experimental

3.1. Apparatus

Capillary electrophoresis experiments were performed using a Hewlett-Packard Model G1600A (Waldbronn, Germany) ^{3D}CE system with a diode-array detector from 190 to 600 nm. A 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 was 264 nm. The fused-silica capillaries obtained from Hewlett-Packard of 48.5 cm (length to detector, 40 cm)×50 μm I.D. were used for the determination and for the separation of cephalosporins.

3.2. Chemicals

Cefotaxim, cefuroxim, and cefpirom were obtained from Hoechst (Frankfurt/M, Germany). Cephapirin, cefazolin and cephaloridin were obtained from Sigma–Aldrich (Deisenhofen, Germany). Acetone for chromatography, potassium hydrogenphosphate, potassium dihydrogenphosphate and DTAB were obtained from Merck (Darmstadt, Germany).

3.3. Sample preparation

Standard solutions of cephalosporins were prepared at 500 μg/ml in buffer containing different concentrations of DTAB. The samples were filtered through a 0.2-μm syringe filter and injected immediately into the apparatus.

3.4. Buffer preparation

For capillary electrophoresis, 20 m*M* phosphate buffer solutions (pH 7.4) were prepared by dissolving 0.788 g potassium hydrogenphosphate and 2.47 g potassium dihydrogenphosphate in water filling up to a volume of 1000 ml. The pH of the buffer was measured at 25°C using an HI 9321 Microprocessor pH meter (Hanna Instruments). The buffer solutions were filtered through a 0.2-μm syringe filter.

3.5. Analysis conditions

A new capillary was washed for 10 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, a separation potential of 30 kV was 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 5 s (hydrodynamic injection). Detailed experimental conditions are listed in the figures.

4. Results and discussion

The effective mobilities of cephalosporins were calculated from the measured apparent mobilities at pH 7.4 [9]. Interaction of cefpirom and cephaloridin with DTAB at increasing concentrations of DTAB was not observed. These compounds exhibited a zero electrophoretic mobility at this pH value, and moved only with the electroosmotic flow. We assume that this is not an electrostatic interaction between the unloaded compounds and DTAB. Cefazolin, cephapirin, cefotaxim and cefuroxim had negative electrophoretic mobility at this pH value ($L = 0$) and migrated in the direction of the cathode. On increasing the concentration of DTAB, a large change of electrophoretic mobility of the compounds was observed (Figs. 2–5).

The interaction between the anionic cephalosporins and the cationic DTAB led to aggregation of DTAB on the cephalosporin compounds. Therefore,

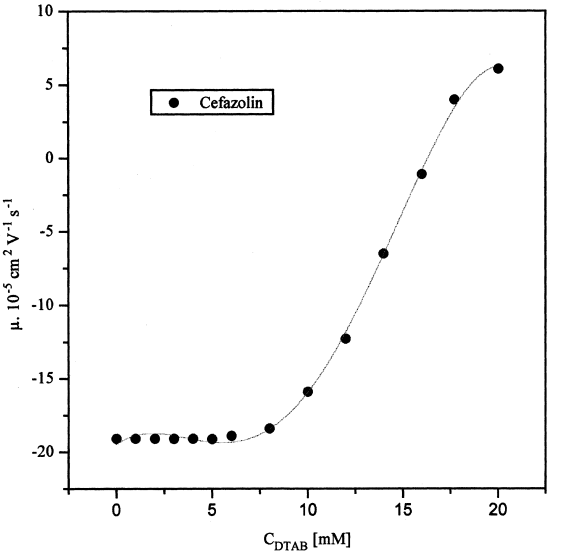

Fig. 2. Electrophoretic mobility of cefazolin in dependence of various concentrations of DTAB buffer: pH 7.4, 20 m*M* phosphate containing from 0.0 to 20 m*M* DTAB; capillary, 48 cm (40 cm to detector)×50 μm I.D.; field strength, 30 and −30 kV at a concentration of more than 3 m*M*; temperature, 25°C; pressure injection, 5 s at 50 mbar; detection, 256 nm.

the electrophoretic mobility of the compounds changed from negative to zero and afterwards to positive. The relative standard deviation of the

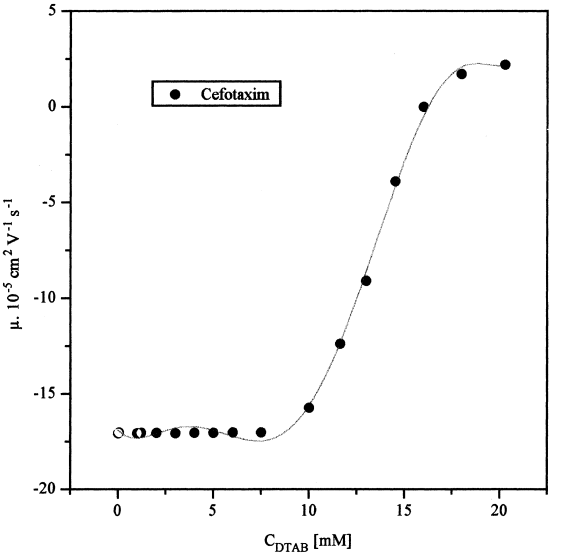

Fig. 4. Electrophoretic mobility of cefotaxim in dependence of various concentrations of DTAB; other conditions as in Fig. 2.

effective mobilities ($n=4$) is between 2 and 3%. Acetone was used as a marker substance (high absorbance and symmetrical peaks [10]) for the calculation of the electroosmotic mobility. We have observed here that the increase of the DTAB concentration resulted not only in a change of the

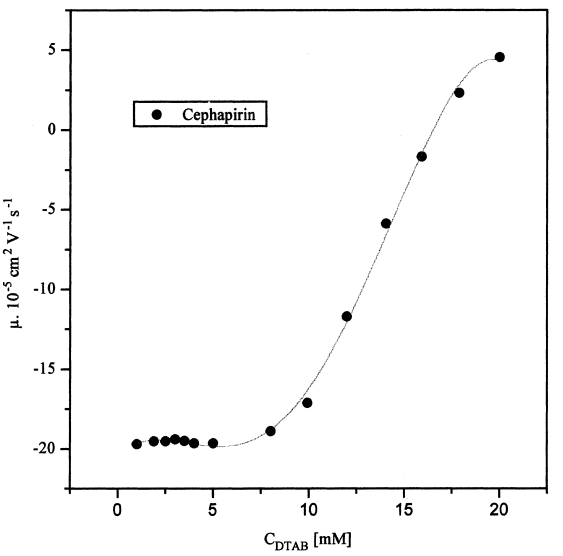

Fig. 3. Electrophoretic mobility of cephapirin in dependence of various concentrations of DTAB; other conditions as in Fig. 2.

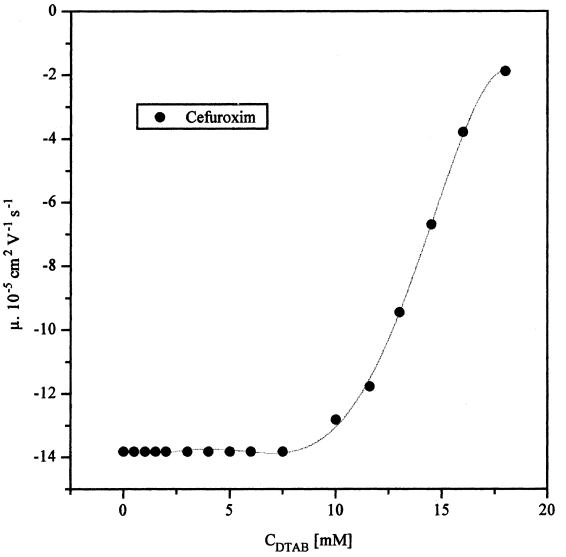

Fig. 5. Electrophoretic mobility of cefuroxim in dependence of various concentrations of DTAB; other conditions as in Fig. 2.

electrophoretic mobility of the compounds but also in a large change of the EOF. The interaction between DTAB and the capillary wall at concentrations of more than 3 m*M* led to reversal of the EOF in the direction of the anode. The relative standard deviation of the EOF is between 0.2 and 2%. In this paper we studied the determination of the aggregation constants (k) and the stoichiometric coefficients (m) using CZE as a new technique. For the determination of k and m by CZE some factors are important: (a) the interaction between cephalosporins and DTAB was investigated below the critical micellar concentration of DTAB (The critical micellar concentration, CMC = 18.103, was determined by conductivity measurements.); (b) there is no interaction between cephalosporins and capillary wall; (c) the influence of DTAB concentration on the electrophoretic mobility of the drugs was discussed as a complex formation. Table 1 summarizes the calculated aggregation constants and the stoichiometric coefficients estimated by application of the fitted function (Eq. (6)). m and k give information about the strength of the interaction on the one hand and about the affinity of drugs to the surfactants on the other hand. Also, the strength of the interaction depends both on the degree of the ionization of the drug and on the hydrophobic interaction. Cefpirom and cephaloridin ($\mu = 0$) show no ionic or hydrophobic interaction with DTAB. These results show that only interaction between anionic cephalosporins and DTAB exits. The strength of the interaction between anionic cephalosporins and DTAB was found to be as follows: cefuroxim > cefotaxim > cefazolin > cephapirin.

That is, the higher the interaction of cephalosporins with DTAB is the higher the values of k.

Table 1
Aggregation constants and stoichiometric coefficients estimated by application of a fitted function (Eq. (6))

Compounds	k $(\mathrm{l/mol})^m$	m
Cefazolin	$10.6\times10^{-5}\pm7.3\times10^{-7}$	4.99 ± 0.25
Cephapirin	$13.5\times10^{-5}\pm1.0\times10^{-7}$	4.98 ± 0.28
Cefotaxim	$6.0\times10^{-5}\pm6.0\times10^{-7}$	3.31 ± 0.34
Cefuroxim	$1.2\times10^{-5}\pm5.0\times10^{-7}$	2.19 ± 0.39
Cefpirom	0.0	0.0
Cephaloridin	0.0	0.0

5. Conclusion

The present study shows that it is possible to characterize interactions between DTAB and cephalosporins quantitatively using CZE. This aggregation model gives quantitative information about the strength of the interaction and about the maximum stoichiometric coefficients of the aggregates. We assume that the interaction between cephalosporins and DTAB depends on the charge of the compounds. The calculation of the stoichiometric coefficients resulted in values from 2 to 5. Here, aggregation of several molecules of DTAB was considered as complex formation. This aggregation model gives reasonably quantitative information.

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