行政院國家科學委員會補助專題研究計畫成果報告

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※　毛細管電泳對環境污染物及藥物分離之研究 III　※

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□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

☑出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：國立台灣大學化學系

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國科會專題研究計畫成果報告

計畫名稱：毛細管電泳對環境污染物及藥物分離之研究 III

計畫編號：NSC-89-2113-M-002-055

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執行期限：89 **年** 8 **月** 1 **日至** 91 **年** 7 **月** 31 **日**

計劃主持人：林敬二　　執行單位：國立台灣大學化學系

第一部份　毛細管電泳對環境污染物之分離研究

1 A、計劃緣由與目的

Chloropyridines, chlorophenols **及** triazines **皆為常見之環境污染物。對環境造成之污染實在不可忽視，必須加以監測。因此對此類污染物之之分離及分析方法的探討是個相當重要的課題。**

此研究計劃擬以毛細管區帶電泳法(CZE)**及微胞電動法**(MEKC)**分別對此類污染物分離做最佳化條件及電泳遷移行為之探討，並且對分析物與界面活性劑微胞之結合常數，遷移順序，以及分離機制做更進一步的測定與瞭解。分離參數對偵測極限至及解析度之影響以及應用新近發展的** sample stacking **技巧亦將加以探討，期能在** UV **偵測法上降低偵測極限(至** 10^{-8}~10^{-9}M)**。由於分析物與界面活性劑之間有相當程度的交互作用力，因此可利用電泳法加以測定並探討界面活性劑以及添加**β-**環糊精時在電泳緩衝液中臨界微胞濃度**(CMC)**之變化情形，以便能進一步瞭解在微胞化過程中及** CD-CZE **及** CD-MEKC **之分離機制差異之奧秘。**

1 B、結果與討論

第一年之研究工作著重於以 MEKC **法探討** chloropyridines **之分離及分離機制，並對** SDS **微胞與分析物之結合常數、遷移順序加以測定與瞭解。此部份之研究工作已順利完成，研究結果已發表於** *J. Chromatogr. A* 910 (2000) 165 **(詳見附件** 1)。

第二年之研究工作著重於以 CZE **法探討** s-triazine **中性物種之分離機制及以** sweeping **法探討** s-triazine **之線上濃縮及濃縮機制。此部份之研究工作已順利完成，研究結果已發表於** *J. Chromatogr. A* 878 (2000) 137 **及** 916 (2000) 239 **(詳見附件** 3, 4)。

第三年之研究工作著重於利用毛細管電泳法探討 Chlorophenols **與** SDS **界面活性劑之間的相互作用力與分離並探討界面活性劑在添加** ß-**環糊精時界面活性劑的臨界微胞濃度之變化，並期望對** CD-CZE **及** CD-MEKC **分離機制上的差異有所了解。此部分之研究工作已完成，並發表於** *J. Chromatogr. A,* 917 (2001) 297 **(詳見附件** 6)。

第二部份　毛細管電泳對藥物分離之研究

2 A、計劃緣由與目的

抗生素如 cephalosporins **及**

tetracyclines 為臨床上常用的藥物，因此，發展快速而有效的檢驗方法在藥物分析化學上是個重要的研究課題。此研究計劃在於發展以 CZE 及 MEKC 方法分離 cephalosporins 及 tetracyclines 並探討其電泳遷移行為；瞭解分離機制及遷移順序之源由。

2 B、結果與討論

第一年之研究工作著重於以 citrate 及 MES 與 phosphate buffer 做比較，利用 CZE 方法研究 cephalosporins 的分離及電泳遷移行為。此部份之研究工作已順利完成，部分研究結果已發表於 *J. Chromatogr. A*, 879 (2000) 197 (詳見附件 2)。

第二年之研究工作著重於以 acetate buffer 在 pH 5.0-9.0 下添加 SDS 或 SDS+Brij 35 為界面活性劑，利用 MEKC 方法研究 tetracycline 的分離及電泳遷移行為同時，也以 TTAB, CTAB 等陽離子界面活性劑利用 MEKC 方法研究 cephalosporins 的分離及電泳遷移行為。此部分之研究工作已順利完成，部分研究結果已發表於 *J. Chromatogr. A*, 802 (1998) 95 (詳見附件 5)。

附件 1：研究論文六篇

1. C. E. Lin*, C. C. Chen, H. W. Chen, C. H. Lin, (2001), "Optimization of Separation and Migration Behavior of Chloropyridines in Micellar Electrokinetic Chromatography", *J. Chromatogr. A*, 910, 165.

2. C. E. Lin*, H. W. Chen, E. C. Lin and K. S. Lin, (2000), "Optimization of Separation and Migration Behavior of Cephalosporins in Capillary Zone Electrophoresis", *J. Chromatogr. A*, 879, 197.

3. C. E. Lin*, T. Z. Wang, C. C. Hsueh and T. C. Chiu, (2000), "Capillary Zone Electrophoretic Separation and Migration Behavior of Neutral Species of Chloro-s-triazines in the Presence of Cationic Surfactant Monomers", *J. Chromatogr. A*, 878, 137.

4. C. E. Lin*, Y. C. Liu, T. Y. Yang, T. Z. Wang and C. C. Yang, (2000), "On-Line Concentration of s-Triazine Herbicides by Micellar Electrokinetic Chromatography Using Cationic Surfactants", *J. Chromatogr. A*, 916, 239.

5. C. E. Lin*, Y. C. Chen and C. C. Hsueh, (1998), "Migration Behavior and Separation of Tetracycline antibiotics by Micellar Electrokinetic Capillary Chromatography", *J. Chromatogr. A*, 802, 95.

6. C. E. Lin*, H. C. Huang and H. W. Chen, (2001), "Influence of β-Cyclodextrins on the Critical Micelle Concentration of Sodium Dodecyl Sulfate: A Capillary Electrophoresis Study", *J. Chroamtogr. A*, 917, 297.

附件 2：出席國際學術會議心得報告 5 份

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Optimization of separation and migration behavior of chloropyridines in micellar electrokinetic chromatography

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Abstract

The separation and migration behavior of pyridine and eight chloropyridines, including three monochloropyridines, four dichloropyridines, and 2,3,5-trichloropyridine were investigated by micellar electrokinetic chromatography using either sodium dodecyl sulfate (SDS) as an anionic surfactant or SDS–Brij 35 mixed micelles. Various parameters such as buffer pH, SDS concentration, Brij 35 concentration and methanol content that affect the separation were optimized. Complete separation of these chloropyridines was optimally achieved with a phosphate buffer containing SDS (30 m*M*) and methanol (10%, v/v) at pH 7.0. The resolution and selectivity of analytes could be considerably affected by the addition of methanol and/or Brij 35 to the background electrolyte. The migration order of these chloropyridines depends primarily on their hydrophobicity. However, electrostatic interactions may also play a significant role in the determination of the migration order of the positional isomers of chloropyridines.

Keywords: Buffer composition; Micelles, mixed; Chloropyridines; Pyridines

1. Introduction

Pyridine and chloropyridines are widely used as intermediates or insecticides in some chemical and agricultural industries [1–5]. For example, chlorpyrifos used as an insecticide is derived from chloropyridines [1]. In the production process of chloropyrifos, pyridine is first chlorinated to pentachloropyridine. During the chlorination reaction of pyridine, other lower chlorinated pyridine isomers in addition to pentachloropyridine are also present as by-products. Moreover, chloropyridines are of environmental concern. Thus, the development of an efficient method for separating these pyridine compounds is desirable.

Although various chromatographic methods and hyphenated techniques, including GC [6–8], GC–MS [9] and HPLC [7,10] have been applied to separate some mixtures of chlorinated pyridine, complete separation of chloropyridine isomers is still not achieved by aforementioned methods.

Capillary electrophoresis (CE) has become a popular and powerful technique to separate diverse analytical samples [11–14]. This technique provides high resolution, great efficiency, rapid analysis and small consumption of solvent in comparison with HPLC. In recent years, the application of this

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technique to the analysis of environmental pollutants has gained considerable attention. However, to our knowledge, a systematic study on the separation of chloropyridines is still lacking. In this study, micellar electrokinetic chromatography (MEKC) using sodium dodecyl sulfate (SDS) as an anionic surfactant was employed to obtain baseline separation of a mixture of pyridine and eight chloropyridines, including three monochloropyridines, four dichloropyridines and 2,3,5-trichloropyridine. The optimization of various parameters that may affect the separation is taken into consideration. Moreover, factors that influence the migration order of these chloropyridines are examined.

2. Experimental

2.1. Chemicals

Pyridine, three monochloropyridines and Brij 35 were obtained from Tokyo Kasei Kogyo (TCI, Tokyo, Japan); four dichloropyrines were purchased from Sigma-Aldrich (St. Louis, MO, USA); 2,3,5-trichloropyridine and sodium dodecyl sulfate were supplied from Merck (Darmstadt, Germany). All other chemicals were of analytical grade obtained from various suppliers. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

A stock solution containing 1000 μg/ml of each analyte dissolved in methanol was prepared. Standard sample solutions were prepared by mixing the stock solution with methanolic solutions to obtain a desired concentration.

2.2. Apparatus

Capillary electrophoretic experiments were carried out on a Beckman Coulter P/ACE System Model 5500 (Fullerton, CA, USA) equipped with a photodiode array detector for absorbance measurements. Uncoated fused-silica capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were used. The dimensions of the capillary were 67 cm× 50 μm I.D. The effective length of the capillary is 60 cm from the injection end of the capillary. Analytes were detected by on-column measurements of UV absorption at 214 nm or 200 nm as specified. The CE systems were interfaced with a microcomputer and a laser printer using MDQ software for data acquisition. All CE experiments were performed at 25°C, unless otherwise specified. For pH measurements, a pH meter (Suntex model SP-701, Taipei, Taiwan) with an accuracy of 0.01 pH unit was used.

2.3. Procedures

When using a new capillary column, the capillary was washed for 60 min with NaOH solution (1.0 *M*) at 60°C, followed by washing for 10 min with water at the same temperature and for 10 min with water at 25°C. Before each injection, the capillary was flushed with the buffer solution for 5 min. The capillary was washed with NaOH solution (0.1 *M*) and water to keep the electroosmotic flow (EOF) to normal when needed.

The buffer solutions were prepared by mixing stock solutions of NaH_2PO_4 (100 m*M*), SDS and methanol at varied ratios and then adjusted to the desired pH value with KOH solution (0.1 *M*).

All solutions were degassed by sonication and passed through a membrane filter (0.22 μm) before use. The electroosmotic mobility (μ_{eo}) was determined with methanol as a neutral marker and the electrophoretic mobility of the micelles was determined with Sudan III as a micelle marker. Samples were injected in a hydrodynamic mode during 2 s. Analytes were identified by the spiking technique.

2.4. Calculations

The electrophoretic mobility of analytes were calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V} \cdot \left(\frac{1}{t_m} - \frac{1}{t_{eo}} \right) \quad (1)$$

in which μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time of a solute peak measured directly from the electropherogram, t_{eo} is the migration time for an uncharged solute, L_t is the total length of capillary, L_d is the length of capillary between injection and detection, and V is the applied voltage.

3. Results and discussion

3.1. Effect of buffer pH

The effects of buffer pH on the migration behavior and separation of pyridine and eight chloropyridines in the absence and presence of SDS micelles was investigated. Fig. 1 shows the variations of the electrophoretic mobility of pyridine and chloropyridines as a function of buffer pH in the range 1.5–8.0 without the addition of SDS surfactant to the buffer solution. The pK_a values of pyridine and chloropyridines can be determined from the variation of the effective electrophoretic mobility of each analyte as a function of buffer pH, according to the following equation [15–17]:

$$\mu_{\text{eff}} = \alpha_{\text{BH}^+}\mu_{\text{BH}^+} = \frac{[\text{H}_3\text{O}^+]}{[\text{H}_3\text{O}^+] + K_a} \cdot \mu_{\text{BH}^+} \qquad (2)$$

where μ_{eff} is the effective electrophoretic mobility of a protonated basic analyte (BH^+), μ_{BH^+} and α_{BH^+} are the limiting electrophoretic mobility and mole fraction of the protonated form of a basic analyte, and K_a is the acid dissociation constant of BH^+. The pK_a values determined for pyridine, 4-chloropyridine, and 3-chloropyridine are 4.95, 3.65, and 2.70 respectively, and the pK_a values of 2-chloropyridine and dichloropyridines are estimated to be less than 1.0. Therefore, all analytes are practically in the neutral form at pH 7.0 and complete separation of these analytes is experimentally impossible without the addition of surfactant molecules or electrolyte modifiers to the background electrolyte at pH 7.0.

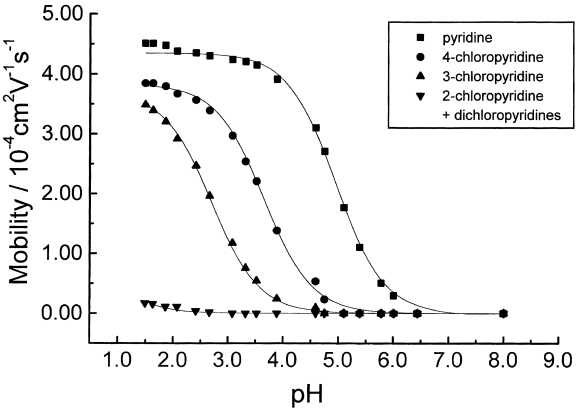

Fig. 1. Variations of the electrophoretic mobility of pyridine and chloropyridines as a function of buffer pH without the addition of SDS micelles to the background electrolyte. Experimental results are represented by data points; predicted mobility curves are shown by solid lines. Background electrolyte: 10 m*M* phosphate buffer containing 10% (v/v) methanol at varied buffer pH. Capillary: 67 cm×50 μm, I.D. Other operating conditions: 30 kV, 25°C. Sample concentration, 50 μg/ml. Detection wavelength, 214 nm.

The effect of buffer pH in MEKC was then examined in the range 5.6–10.0 for these analytes in a phosphate buffer containing 30 m*M* SDS. In this pH range, the EOF increases almost linearly with increasing pH at pH<7, but in a lesser extent at pH>7, whereas the electrophoretic mobility of analytes, except pyridine and 4-chloropyridine, does not show significant change with increasing buffer pH, especially at pH>7. The electrophoretic mobility of 4-chloropyridine decreases slightly from $-6.6\cdot10^{-5}$ to $-4.0\cdot10^{-5}$ $\text{cm}^2\,\text{V}^{-1}\,\text{s}^{-1}$, whereas that of pyridine decreases appreciably with increasing buffer pH in the range 5.6–7.0. This is probably resulted from the micelle-shifted pK_a values of pyridine and 4-chloropyridine. It is found that pyridine and 2-chloropyridine are barely separated at pH 5.6, but they are well resolved at pH in range 6.0–7.0. Hence, effective separation of these analytes is optimally achieved in MEKC at pH 7.0.

3.2. Effect of SDS concentration

Fig. 2 shows the variations of the electrophoretic mobility of pyridine and chloropyridines obtained in a phosphate buffer containing varied concentration of SDS and 10% (v/v) methanol at pH 7.0. The SDS concentration was varied in the range 0–75 m*M*. As expected, the partition of a solute into SDS micelles is favored with increasing SDS concentration as the electrophoretic mobility of each analyte increases with increasing SDS concentration. On the other hand, the EOF decreases comparatively to a lesser extent with increasing SDS concentration. Consequently, the separation with an increase in SDS concentration results in a longer analysis time. As shown in Fig. 3, a baseline separation of a mixture of pyridine and eight chloropyridines was optimally achieved in 10 min using a phosphate buffer (10

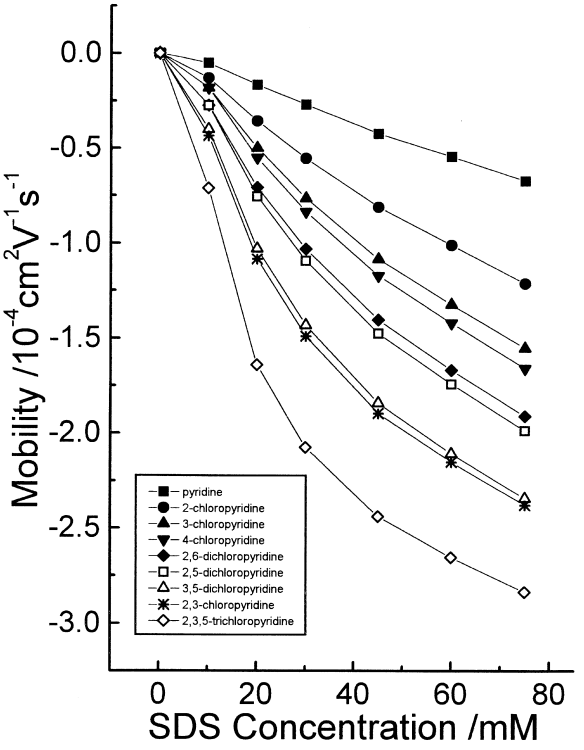

Fig. 2. Variations of the electrophoretic mobility of pyridine and chloropyridines as a function of SDS concentration in MEKC. Background electrolyte: 10 m*M* phosphate buffer containing 10% (v/v) methanol at pH 7.0. Other operating conditions as for Fig. 1.

m*M*) containing 30 m*M* SDS and 10% (v/v) methanol at pH 7.0 with an applied voltage of 30 kV. With this buffer electrolyte, the electrophoretic mobility of SDS micelles using Sudan III as a micelle marker was determined to be $-3.46 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}.

3.3. Effect of methanol content

A background electrolyte (BGE) solution composed of NaH_2PO_4 (10 m*M*) and SDS (30 m*M*) containing various proportions of methanol (0–20%, v/v) at pH 7.0 was used to examine the effect of methanol content on the EOF and electrophoretic mobility of each solute. The EOF declines almost linearly and the electrophoretic mobility of each analyte also decreases, but to a lesser extent, with increasing methanol content. The decrease in the EOF is attributed to the interaction between methanol and the silanol groups on the capillary wall,

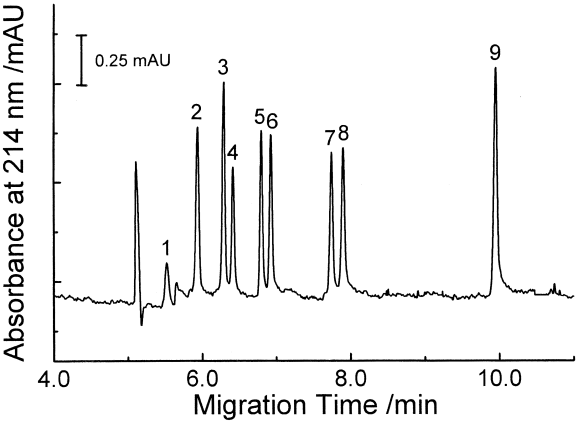

Fig. 3. A typical electropherogram of pyridine and eight chloropyridines obtained with a phosphate buffer (10 m*M*) containing 30 m*M* SDS and 10% (v/v) methanol at pH 7.0. Other operating conditions as for Fig. 2. Peak identification, 1, pyridine; 2, 2-chloropyridine; 3, 3-chloropyridine; 4, 4-chloropyridine; 5, 2,6-dichloropyridine; 6, 2,5-dichloropyridine; 7, 3,5-dichloropyridine; 8, 2,3-dichloropyridine; 9, 2,3,5-trichloropyridine.

resulting in the lowering of the zeta potential of the silica surface. Furthermore, the dielectric constant of the BGE decreases linearly and the viscosity of the BGE also increases in the presence of methanol, thus causing the decrease in the EOF [18]. The migration time of analytes separated in a BGE without the addition of methanol is shorter than that separated in a BGE containing methanol. Unfortunately, 2,3-dichloropyridine and 3,5-dichloropyridine elute together in a BGE without the addition of methanol.

As shown in Fig. 4, the resolution of the peaks of 2,3- and 3,5-dichloropyridine can be improved with addition of methanol. In fact, the peaks of these two analytes are effectively separated with addition of 10% (v/v) methanol, and the peaks are well separated with addition of 20% (v/v) methanol. On the contrary, the separations for the peaks between 2,5- and 2,6-dichloropyridine and for the peaks between 3- and 4-dichloropyridine become worse as methanol content in the buffer electrolyte increases from 10 to 20% (v/v).

3.4. Effect of Brij 35 concentration

A mixed micelle composed of SDS and Brij 35 has been reported to improve separation selectivity [19–23] and to increase the elution range in MEKC

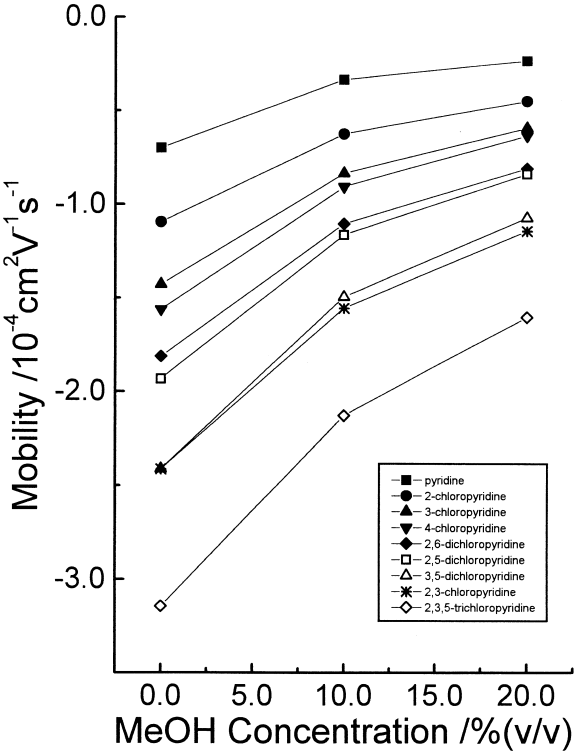

Fig. 4. Variations of the electrophoretic mobility of pyridine and chloropyridines as a function of methanol content using SDS (30 m*M*)–phosphate (10 m*M*) buffer system at pH 7.0. Other operating conditions as for Fig. 2.

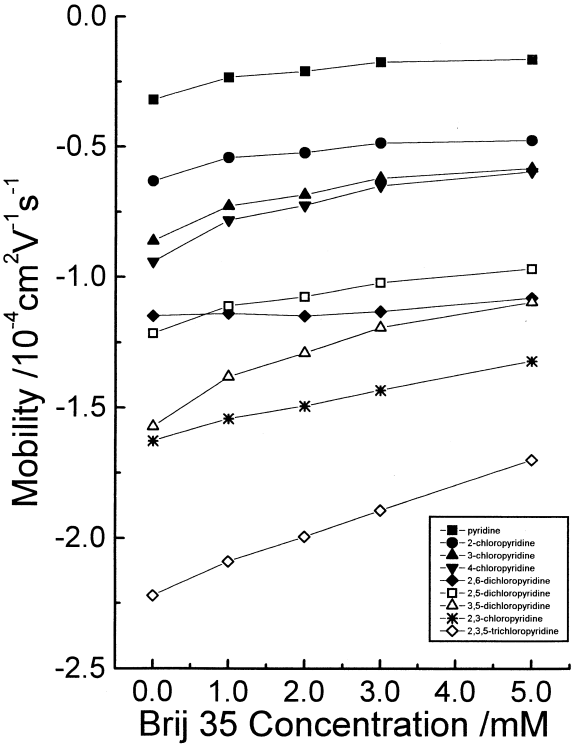

Fig. 5. Variations of the electrophoretic mobility of pyridine and chloropyridines as a function of Brij 35 concentration using SDS (30 m*M*)–phosphate (10 m*M*) buffer system at pH 7.0. Other operating conditions as for Fig. 2.

[24]. Fig. 5 shows the variations of the electrophoretic mobility of pyridine and chloropyridines as a function of Brij 35 concentration in a phosphate buffer (10 m*M*) containing 30 m*M* SDS 10% (v/v) methanol, and varied concentration of Brij 35 in the range 0–5 m*M* at pH 7.0. As can be seen, the resolution of the peaks of 2,3- and 3,5-dichloropyridine is greatly enhanced with further addition of Brij 35 to the SDS–phosphate buffer system. With addition of Brij 35, the electrophoretic mobility and the selectively of the analytes studied, except 2,3-dichloropyridine, are considerably affected. The migration of 2,6-dichloropyridine behaves differently from the other analytes, thus causing the reversal of the migration order of the peaks of 2,5- and 2,6-dichloropyridine. Moreover, the peaks of 3- and 4-chloropyridine become even unresolvable when the concentration of Brij 35 exceeds 3 m*M*.

As a result, the separation of these chloropyridines is optimally achieved with addition of 2–3 m*M* Brij 35 in the SDS–Brij 35 mixed micellar buffer system. Fig. 6 shows the electropherogram of a mixture of chloropyridines obtained with addition of 3 m*M* Brij 35 to the SDS–phosphate buffer system.

3.5. Binding constants versus migration order

In MEKC, the effective electrophoretic mobility (μ_{eff}) of a neutral solute can be expressed by the following equation [25,26]:

$$\mu_{\text{eff}} = \frac{K_{\text{B}\cdot\text{S}}(\text{CMC})\mu_{\text{B}\cdot\text{S}} + K_{\text{B}\cdot\text{M}}[\text{M}]\mu_{\text{M}}}{1 + K_{\text{B}\cdot\text{S}}(\text{CMC}) + K_{\text{B}\cdot\text{M}}[\text{M}]} \quad (3)$$

where μ and K denote the electrophoretic mobility and binding constant, B·S represents the complexes formed between neutral analytes (B) and surfactant monomers (S), and B·M represents the complexes

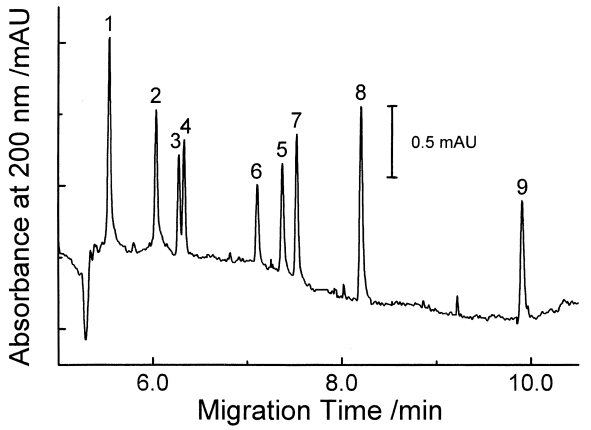

Fig. 6. An electropherogram of pyridine and eight chloropyridines obtained in a phosphate buffer (10 m*M*) containing 30 m*M* SDS, 3 m*M* Brij 35 and 10% (v/v) methanol at pH 7.0. Detection wavelength, 200 nm. Other operating conditions and peak identification are the same as for Fig. 3.

formed between neutral analytes (B) and surfactant micelles (M), [M] is the concentration of surfactant micelles which is defined as the total concentration of surfactant molecules minus the critical micelle concentration (CMC). The CMC value of SDS in 10 m*M* phosphate buffer containing 10% (v/v) methanol in the presence of chloropyridines is determined to be 4.8 m*M* [27]. As the magnitude of the term involving $K_{B\cdot S}$ is very small because the binding between chloropyridines and SDS monomers is very weak, Eq. (3) can be simplified to

$$\mu_{eff} = \frac{K_{B\cdot M}[M]\mu_M}{1 + K_{B\cdot M}[M]} \quad (4)$$

Accordingly, the binding constant of each individual solute to SDS micelles can be calculated by varying the parameter ($K_{B\cdot M}$) through the utilization of Excel software until the simulated mobility curve is best fitted to the observed data points. Table 1 lists the magnitudes of the binding constants evaluated for these chloropyridines, together with the log P_{ow} (logarithm of octanol–water partition coefficient) values available in the literature.

As the log P_{ow} values of pyridine, monochloropyridines, dichloropyridines, 2,3,5-trichloropyridine, 2,3,5,6-tetrachloropyridine, and pentachloropyridine reported in the literature [28,29] are in the range

Table 1
Binding constants evaluated for pyridine and eight chloropyridines in a SDS–phosphate buffer system at pH 7.0[a]

Peak no.	Analytes	Binding constant (M^{-1})	Log P_{ow}[b]
1	Pyridine	3.2	1.04
2	2-Chloropyridine	7.5	1.45
3	3-Chloropyridine	11.2	1.43
4	4-Chloropyridine	13.0	1.28
5	2,6-Dichloropyridine	17.0	2.15
6	2,5-Dichloropyridine	18.5	2.40
7	3,5-Dichloropyridine	28.5	2.56
8	2,3-Dichloropyridine	30.0	2.11
9	2,3,5-Trichloropyridine	58.0	3.11

[a] Binding constants are evaluated according to Eq. (4) with the CMC value of SDS equal to 4.8 m*M*.
[b] Refs. [28,29].

1.04, 1.28–1.45, 2.11–2.56, 3.11, 3.32, and 3.53, respectively, the hydrophobicity of chloropyridines increases with increasing the number of chlorine substituents on the pyridine ring. Moreover, as indicated in Table 1, the binding constants of pyridine, monochloropyridines, dichloropyridines and 2,3,5-trichloropyridine evaluated are in the range 3.2, 7.5–13.0, 17.0–30.0, and 58.0 M^{-1}, respectively. The binding constant of chloropyridines increases as the number of chlorine substituents on the pyridine ring increases. The results clearly indicate that the migration order of these chlopopyridines depends primarily on their hydrophobicity. However, the magnitudes of the binding constants of positional isomers of monochloropyridines and those of dichloropyridines do not follow the same order as for their hydrophobicity. The results reveal that the migration behavior of those chloropyridines is not solely governed by the hydrophobic interaction. It also implies that electrostatic interactions may play a significant role in the determination of the migration order.

3.6. Reproducibility and detection limits

The migration times of these analytes were quite reproducible, with relative standard deviation (RSD) varying in the range 0.6–0.8% ($n = 6$). The limits of detection (LOD) at a signal-to-noise ratio (S/N) equal to 3 determined with UV detection at 214 nm

for 2- and 3-chloropyridine, 2,5- and 3,5-dichloropyridine, and 2,3,5-trichloropyridine were in the range 0.5~1.1 μg/ml, depending on the molar absorbtivity of each individual analyte. It is believed that much lower levels of the LOD values of these analytes can be obtained with UV detection by using sweeping technique [30–32]. Currently, on-line concentration of chloropyridines by sweeping in MEKC is undertaken and the results of the analysis will be reported later.

4. Conclusion

The separation of pyridine and eight chloropyridines is effectively achieved by MEKC with the use of either SDS micelles or SDS–Brij 35 mixed micelles. Various separation parameters are optimized. In addition to buffer pH and SDS concentration, methanol content and Brij 35 concentration also affect the selectivity of analytes considerably. The migration order of those analytes depends primarily on the hydrophobic interaction of each analyte with the SDS micelles. However, electrostatic interactions may also play a significant role.

Acknowledgements

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Optimization of separation and migration behavior of cephalosporins in capillary zone electrophoresis

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Abstract

The influences of buffer pH, buffer concentration and buffer electrolyte on the migration behavior and separation of 12 cephalosporin antibiotics in capillary zone electrophoresis using three different types of buffer electrolyte, including phosphate, citrate, and 2-(*N*-morpholino)ethanesulfonate (MES), were investigated. The results indicate that, although buffer pH is a crucial parameter, buffer concentration also plays an important role in the separation of cephalosporins, particularly when cefuroxime and cefazolin, cephalexin and cefaclor, or cefotaxime and cephapirin are present as analytes at the same time. The electrophoretic mobility of cephalosporins and electroosmotic mobility measured in citrate and MES buffers are remarkably different from those measured in phosphate buffer. With citrate buffer, optimum buffer concentration is confined to a small range (35–40 m*M*), whereas buffer concentrations up to 300 m*M* can be used with MES buffer. Complete separations of 12 cephalosporins could be satisfactorily achieved with these three buffers under various optimum conditions. However, the separability of 12 cephalosporins with citrate or MES buffer is better than that with phosphate buffer. As a consequence of a greater electrophoretic mobility of cephalosporins than the electroosmotic mobility with citrate buffer at pH below about 5, some cephalosporins are not detectable. The cloudiness of the peak identification and of the magnitudes of the electrophoretic mobility of cefotaxime and cefuroxime reported previously are clarified. In addition, the pK_a values of cephradine, cephalexin, cefaclor, and cephapirin attributed to the deprotonation of either an amino group or a pyridinium group are reported, and the migration behavior of these cephalosporins in the pH range studied is quantitatively described.

Keywords: Buffer composition; Cephalosporins; Antibiotics

1. Introduction

Cephalosporins are antibiotics for the treatment of Gram-positive and Gram-negative infections. These antibiotics derived from the 7-aminocephalosporanic acid composed of a β-lactam ring fused with a dihydrothiazine, but differ in the nature of the substituents attached at the 3- and/or 7-positions of the cephem ring. These substituents affect either the pharmacokinetic properties or antibacterial spectrum. For the past two decades, these antibiotics are generally separated and determined by high-performance liquid chromatography (HPLC). Numerous HPLC procedures have been proposed for the separation and/or quantification of these antibiotics [1–5].

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In recent years, capillary electrophoresis (CE) has become an important separation technique owing to its advantageous features, such as extremely high column efficiency, small sample volumes and rapid analysis, in comparison with HPLC [6–11]. The applications of this technique to the separation and/or determination of cephalosporins have previously been demonstrated using either micellar electrokinetic chromatography (MEKC) [12–18] or capillary zone electrophoresis (CZE) [18–21].

The separation of nine cephalosporins was performed by MEKC using sodium dodecyl sulfate (SDS) and sodium *N*-lauroyl-*N*-methyltaurate as anionic surfactants with phosphate buffer at pH 9.0 [12]. Twelve cephalosporins were separated under similar electrophoretic conditions, but at pH 6.0 [13]. Five cephalosporins were analyzed with a borate buffer containing SDS at an alkaline pH [15]. The determinations of cefuroxime in human serum [16] and of cefotaxime in plasma [17,18] by MEKC were demonstrated.

On the other hand, the determinations of cefotaxime in plasma with phosphate buffer or with borate buffer were first illustrated by CZE [18,19]. Recently, the separation and determination of nine cephalosporins in urine and bile sample solutions [20] and in plasma sample solution [21] were performed by CZE using either a citrate buffer at pH 6.0 or a phosphate buffer at pH 7.2. Dissociation constants of nine cephalosporins were determined using citrate, acetate, and phosphate buffers in three different pH regions ranging from 2 to 9 [22]. However, the influence of the buffer pH on the migration behavior and separation of the cephalosporins by CZE were less rigorously investigated and the effects of buffer concentration and buffer type were incompletely examined or even lacking.

It should be pointed out that some of the results reported previously by Mrestani et al. [20–22] seem to be not very consistent. For instance, the pH-dependence of the electrophoretic mobility curves of cefotaxime and cefuroxime level off at pHs greater than about 4.2 [22], but the curves of the migration time versus buffer pH level off at pHs greater than about 7.5 [21]; the electrophoretic mobility of cefotaxime and cefuroxime reported previously [22] are two orders of magnitude smaller than that of cefamandole, but cefamandole migrates in between cefotaxime and cefuroxime [20]. The difference in pK_a values between these two cephalosporins may be less than 0.2, as was determined from the potentiometric measurements [22] because the mobility curves of cephalexin and cefaclor shown in [22] are very close to each other. Moreover, the peak identification of cefotaxime and cefuroxime [20–22] may be questionable because their peak identification was not confirmed by our preliminary results. Furthermore, the cause of the disappearance of the signals of some cephalosporins with a citrate buffer at a pH below about 5 is speculated, but not clearly understood [22].

In order to clarify all this cloudiness, a more thorough investigation with regard to the effects of buffer pH and buffer concentration on the migration behavior and separation of cephalosporins is certainly needed. Furthermore, in view of the modification on the capillary surface with the use of a zwitterionic buffer such as sodium 2-(*N*-morpholino)ethanesulfonate (MES), an investigation on the migration behavior and separation of cephalosporins using such a buffer electrolyte is desirable. A comparative study on this matter using these three different types of buffer electrolyte is also worthy for investigation. In this work, 12 cephalosporins, which have a similar basic structure but with different substituents attached to the cephem ring, are selected. These cephalosporins are prescribed antibiotics and are commercially available. Here, we present the results of our investigations.

2. Experimental

2.1. Apparatus

All CE experiments were performed on a Beckman P/ACE System 5500 equipped with a UV detector for absorbance measurements at 214 nm. The dimensional spectral scan of the CE separation was performed on a Beckman P/ACE System MDQ with a photodiode array detector (Beckman Coulter, Fullerton, CA, USA). Uncoated fused-silica capillaries, purchased from Polymicro Technologies (Phoenix, AZ, USA), were used. The dimensions of the capillary were 57 cm×50 μm I.D. for the Beckman P/ACE System 5500 instrument and 60.2

cm×50 μm I.D. for the Beckman P/ACE System MDQ. The effective length of the capillary is 50 cm from the injection end of the capillary. The CE system was interfaced with a microcomputer and a laser printer. The System Gold software of Beckman was used for data acquisition. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of ±0.01 pH unit.

2.2. Chemicals and reagents

Twelve cephalosporins were obtained from Sigma (St. Louis, MO, USA). MES and mesityl oxide (used as a neutral marker) were purchased from Tokyo Kasei Kogyo (TCI, Tokyo, Japan). All other chemicals were of analytical-grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of cephalosporins at various concentrations ranging from 10 to 50 μg/ml were prepared by dissolving analytes in an aqueous solution. The pH of a phosphate buffer was adjusted to the desired pH value by mixing various proportions of a certain concentration of sodium dihydrogenphosphate solution with the same concentration of disodium hydrogenphosphate solution. Similar procedures were followed to adjust the pH of a citrate buffer by mixing various portions of a certain concentration of trisodium citrate solution with the same concentration of citric acid solution, and to adjust the pH of a MES buffer solutions by mixing salt solutions with the corresponding acid solutions. All buffer solutions, freshly prepared weekly and stored in a refrigerator before use, were filtered through a membrane filter (0.22 μm).

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed 30 min with 1.0 *M* NaOH solution, followed by 20 min with deionized water at 25°C. Before each injection, the capillary was prewashed for 5 min with running buffer and postwashed for 2 min with deionized water, 5 min with 1.0 *M* NaOH and 5 min with deionized water to maintain proper reproducibility of run-to-run injections. Sample injections were done in a hydrodynamic mode over 4 s under a pressure of 0.4 p.s.i. The measurements were run at least in triplicate to ensure reproducibility. Applied voltages of 20 kV for the phosphate buffer and 30 kV for the citrate and MES buffers were selected to keep the total current less than 100 μA in order to avoid experimental complications resulting from Joule heating. For instance, the current measured for a phosphate buffer at 150 m*M* with an applied voltage of 20 kV is 78.7 μA. The detection wavelength was set at 214 nm. The relative standard deviation of migration time is less than 0.6% ($N=5$).

For peak identification, on-column UV spectra (200–300 nm with a 2-nm wavelength increment) of cephalosporins were recorded simultaneously during the electrophoretic separation. Spiking with the analyte to be identified was also employed.

2.4. Mobility calculations

The electrophoretic mobility of analytes was calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V} \cdot \left(\frac{1}{t_m} - \frac{1}{t_{e_o}}\right)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an unchanged solute, L_t is the total length of capillary, L_d is the length of capillary between injection and detection and V is the applied voltage.

3. Results and discussion

The optimization of the separation of cephalosporins in CZE can be achieved by manipulation of separation parameters, such as buffer pH and buffer concentration, of a selected buffer electrolyte. In this work, the combined effects of buffer pH and buffer concentration are taken into consideration to obtain the optimized separation of 12 cephalosporins. Three different buffer electrolytes, including phosphate, citrate and MES are selected to examine the effects of buffer electrolytes on the migration behavior and separation of cephalosporins. The structures of the

12 cephalosporins selected are shown in Fig. 1. According to the nature of the carboxyl group of cephalosporins, these analytes are categorized into two classes. The cephalosporins belonging to class I, possessing their carboxyl groups in the acid-form, consist of cephradine (**1**), cephalexin (**2**) and cefaclor (**3**), whereas cephalosporins belonging to class II, used as sodium salt, include the rest of the nine cephalosporins.

3.1. Influence of buffer pH

The pH of the buffer plays an important role in the separation of ionizable analytes since it determines the extent of the ionization of the analytes [23,24]. Moreover, the charge of the capillary wall surface and the zeta potential are influenced by buffer pH. Thus, manipulation of buffer pH becomes a key strategy to optimize the separation in CZE. For minimizing any possible degradation due to β-lactam hydrolysis of cephalosporins, the separations were performed in a weak acidic, neutral or a weak basic medium.

Fig. 2 shows the influence of buffer pH on the electrophoretic mobility of 12 cephalosporins with a phosphate buffer (100 m*M*) in the pH range of 5.5–7.8. The electrophoretic mobility (migrating in the opposite direction to the electroosmotic flow) of cephalosporins belonging to class I increases sigmoidally with the pH of the buffer, whereas the electrophoretic mobility of cephalosporins belonging to class II, except cephapirin (**7**), remains essentially constant with increasing the pH of the buffer.

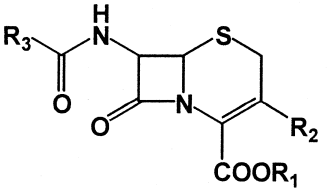
As shown in Table 1, the class I cephalosporins possess a carboxylic acid group with pK_{a1} values in the range 1.5–3.1 and an amino group with pK_{a2} values in the range 6.8–7.4 [22,25,26]. Thus, these cephalosporins exist as zwitterionic species in the pH range studied. As the carboxylic acid group is fully dissociated at pH>5, the variation in the electrophoretic mobility of these three cephalosporins in the pH range from 5 to 9 is a result of the increase in the degree of deprotonation of an amino group at the 3-position of the cephem ring. The migration behavior of these cephalosporins is predictable, once the pK_{a2} value and the limiting electrophoretic mobility at $pH \geq pK_{a2}+2$ (or adequate mobility data) are available. In fact, the pK_{a2} values of cephradine (**1**), cephalexin (**2**) and cefaclor (**3**), which will be described later in Section 3.4, are determined to be 7.27, 6.96 and 6.92, respectively.

The electrophoretic mobility of cephapirin (**7**) increases with increasing buffer pH from pH 5.5 to 7.8. The variation of the electrophoretic mobility of cephapirin (**7**) in this pH range is believed due to the deprotonation of its pyridinium group at the 7-position of the cephem ring. The pK_{a2} value of this pyridinium group, which will be described later in Section 3.4, is determined to be 4.72. Thus, the results that cephapirin (**7**) appears before cefsulodin (**5**) at a pH below 5.9 and it elutes together with cefazolin (**10**) at a pH above 7.0 is predictable.

As shown in Fig. 2, the electrophoretic mobility of cefotaxime (**6**) and cefuroxime (**9**) is essentially pH-independent because these two cephalosporins do not possess any acidic or basic functional groups with pK_a values lying in the pH range studied. With the phosphate buffer (100 m*M*) at pH 6.2, the observed magnitudes of the electrophoretic mobility of cefotaxime (**6**) and cefuroxime (**9**) are $-9.03 \cdot 10^{-5}$ and $-9.65 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$, respectively, which are greater than those reported previously [22] by a factor of about 45. In addition, the observed electrophoretic mobility of cefamandole (**8**) is $-9.34 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$, which is in between the values of cefotaxime (**6**) and cefuroxime (**9**). Evidently, the results indicate that the correctness of mobility data of these two cephalosporins reported previously [22] are questionable.

As the primary amino group of class I cephalosporins contribute to their migration behavior in the pH range studied, all members of class I cephalosporins migrate at a slower velocity toward the anode than those of class II cephalosporins at pH below 7.0. Thus in the pH range 5.5–7.0, excluding cephapirin (**7**), the migration of the 11 cephalosporins selected follows the order: cephradine (**1**)< cephalexin (**2**)<cefaclor (**3**)<cefoperazone (**4**)< cefsulodin (**5**)<cefotaxime (**6**)<cefamandole (**8**)< cefuroxime (**9**)<cefazolin (**10**)<cephalothin (**11**)< ceftriaxone (**12**). Compared with other members of class II cephalosporins, it is expected that ceftriaxone migrates with the greatest mobility towards the anode because it carries an extra negative charge in the R_2 substituent.

It is noted that, with phosphate buffer at a



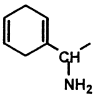
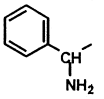
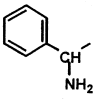
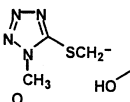
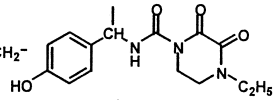
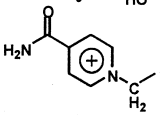
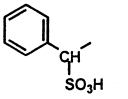
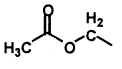
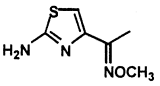
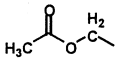
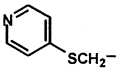
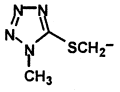
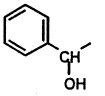
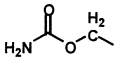
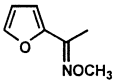
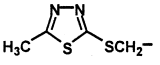
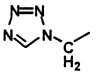
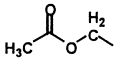
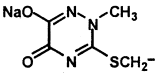
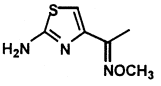
No.	Solutes	R_1	R_2	R_3
1	Cephradine	H	CH_3	
2	Cephalexin	H	CH_3	
3	Cefaclor	H	Cl	
4	Cefoperazone	Na		
5	Cefsulodin	Na		
6	Cefotaxime	Na		
7	Cephapirin	Na		
8	Cefamandole	Na		
9	Cefuroxime	Na		
10	Cefazolin	Na		
11	Cephalothin	Na		
12	Ceftriaxone	Na		

Fig. 1. Structures of cephalosporins selected.

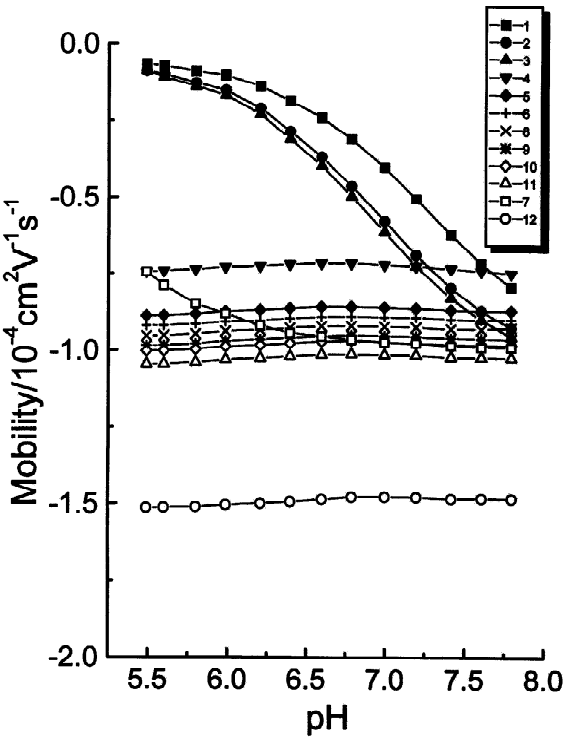


Fig. 2. Electrophoretic mobility of 12 cephalosporins obtained with phosphate buffer (100 m*M*) at varied pH in the range 5.5–7.8. Capillary: 57 cm×50 μm I.D.; the length between column inlet and detector, 50 cm. Injection method: hydrodynamic mode; pressure, 0.4 p.s.i., 1 p.s.i.=6894.76 Pa; injection time, 4 s. Other operating conditions: 20 kV, 25°C and current, 47~91 μA. Peak identification: 1=cephradine, 2=cephaloxin, 3=cefaclor, 4=cefoperazone, 5=cefsulodin, 6=cefotaxime, 7=cephapirin, 8=cefamandole, 9=cefuroxime, 10=cefazolin, 11=cephalothin, 12=ceftriaxone.

concentration of 100 m*M*, the resolution of peaks between cephalexin (**2**) and cefaclor (**3**) improves as the pH of the buffer increases from 5.8 to 7.0. In fact, the peaks of these two cephalosporins are barely resolved at pH 5.8, but they are well resolved at pHs above 6.2. Since all of the cephalosporins, except cephapirin (**7**), are well separated, complete separations of the 12 cephalosporins can be achieved by careful manipulation of buffer pH at a pH above 6.2. Fig. 3 shows the electropherograms of cephalosporins obtained with a phosphate buffer (100 m*M*) at pHs 5.8, 6.2, 6.4 and 7.0. As shown in Fig. 3B and C, complete separations of these cephalosporins were achieved with phosphate buffer (100 m*M*) at pHs 6.2 and 6.4. As ceftriaxone (**12**) elutes relatively far behind cephalothin (**11**), the peak of ceftriaxone which appears at about 20–23 min, depending on the pH of the buffer, is not shown in Fig. 3.

3.2. Influences of buffer concentration

It is well known that, for a given type of buffer electrolyte, the magnitude of the electroosmotic flow (μ_{eo}) depends mainly on the zeta potential which decreases with decreasing buffer pH and/or increasing ionic strength (or buffer concentration) of the buffer solution. Hence, at a given buffer pH, it is expected that an increase in the ionic strength (or buffer concentration) results in a decrease in the zeta potential, thus leading to a decrease in the value of $\mu_{eo.}$ Similar arguments can be applied to account for the variation in the electrophoretic mobility (μ_{ep}) of analytes.

At a particular buffer pH, buffer concentration plays a significant role in the separation of cephalosporins. This is particularly true when cefuroxime (**9**) and cefazolin (**10**) are simultaneously present as analytes because the resolution of the peaks between these two cephalosporins is concentration-dependent. They are hardly resolved with the phosphate buffer at a concentration of 20 m*M*, partially resolved at 50 m*M* and are well separated at a concentration above 100 m*M*. On the other hand, the separation of cephalexin (**2**) and cefaclor (**3**) and that of cefotaxime (**6**) and cephapirin (**7**) depend also on the concentration of a buffer electrolyte. At pH 6.0, the peaks of cefaclor (**3**) and cephalexin (**2**) are barely resolved with phosphate buffer at 50 m*M*. These two peaks are incompletely resolved at 100 m*M* and are baseline separated at 150 m*M* with an applied voltage of 20 kV. Similarly, the peak of cephapirin (**7**) almost merges with cefotaxime (**6**) at 50 m*M* and is merely resolved at 100 m*M*. Fig. 4 shows such electropherograms of cephalosporins obtained with a phosphate buffer at pH 6.0. Thus, to achieve optimum separation of these 12 cephalosporins, a phosphate buffer at a concentration of at least 100 m*M* at pH 6.2 or at a concentration above 150 m*M* at pH 6.0 is required.

3.3. Influences of buffer electrolytes

It was found that the migration behavior of

Table 1
The pK_a values and mobility data of cephalosporins

Peak	Analytes	pK_{a1}	pK_{a2}			Limiting mobility[a]: this work
		Literature values	Literature values	This work		
				Curve-fitting	Inflection point	
1	Cephradine	2.63[c]	7.30[c]	7.27	7.27	0.95
2	Cephalexin	3.11[b](2.34)[b] 2.56[c]	6.79[b](7.08)[b] 6.88[c]	6.96	6.96	0.99
3	Cefaclor	2.69[b] 1.5[d]	7.38[b](7.19)[b] 7.2[d]	6.92	6.92	0.01
4	Cefoperazone	2.6[d]				
5	Cefsulodin					
6	Cefotaxime	2.09[b](2.9)[b] 3.4[d]				
7	Cephapirin			4.65	4.72	1.13
8	Cefamandole	2.46[b](2.60)[b] 2.7[d]				
9	Cefuroxime	2.04[b](2.17)[b]				
10	Cefazolin	2.1[d]				
11	Cephalothin	2.5[d]				
12	Ceftriaxone	3.2[d]	3.2[d]			

[a] Mobility in units of 10^{-4} cm^2 V^{-1} s^{-1}.
[b] Ref. [22], values in parathesis determined from potentiometric measurements.
[c] Ref. [25].
[d] Ref. [26].

cephalosporins in citrate buffer was significantly different from that of cephalosporins in phosphate buffer at pH 6.0 [20,21], and the separation of cephalosporins was remarkably improved on addition of tetraalkylammonium salts to the SDS solution [13]. We thought that the surface modification of the capillary wall with the use of a zwitterionic buffer, such as MES, and that the electrostatic forces of the attraction and repulsion between cephalosporins and zwitterionic buffer electrolytes, would influence significantly the migration behavior and separation of cephalosporins. Thus, it would be of interest to examine the effects of separation parameters using these two types of buffer electrolyte.

3.3.1. Citrate buffer

Fig. 5 shows the effect of the buffer pH on the migration and separation of cephalosporins in the pH range 5.3–6.7 using citrate buffer at a concentration of 40 m*M*. As can be seen, the trends in the variation of the electrophoretic mobility of these 12 cephalosporins as a function of buffer pH with citrate buffer are similar to those observed with the phosphate buffer shown in Fig. 2. The electrophoretic mobility of class I cephalosporins, as well as cephapirin (**7**), increases considerably, but the electrophoretic mobility of class II cephalosporins decreases about 10% of their magnitudes with increasing buffer pH from 5.3 to 6.7. The electrophoretic mobility of cephalosporins measured in citrate buffer has a 1.2–1.3 fold increase, compared with the corresponding mobility measured in a phosphate buffer, whereas the electroosmotic mobility has a 1.1–1.3 fold increase with increasing buffer pH from 5.5 to 7.0. With the citrate buffer at a pH below 5.3, the absolute magnitudes of the electrophoretic mobility of class II cephalosporins, expect cephapirin (**7**) and cefoperazone (**4**), exceed that of the electroosmotic mobility, thus leading to the disappearance of the signals of the cephalosporins that elute later than cefoperazone (**4**). Similar phenomena were also observed with a citrate buffer at a lower concentration, but at a lower buffer pH. The disappearance of the signals of these cephalosporins occurred at pH below 5.0 with citrate buffer at a concentration of 35 m*M*.

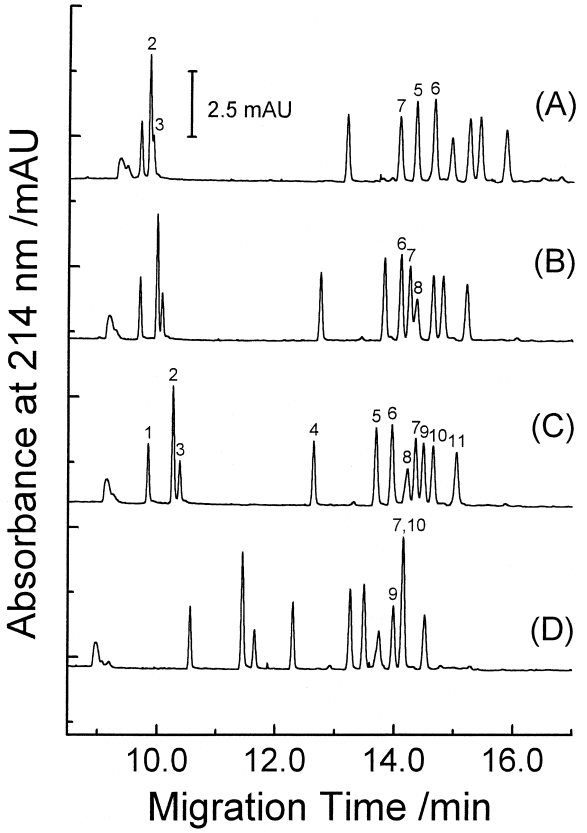


Fig. 3. Electropherograms of cephalosporins obtained with phosphate buffer (100 m*M*) at varied pH: (A) 5.80, (B) 6.21, (C) 6.4, (D) 7.00. Operating conditions and peak identification as for Fig. 2.

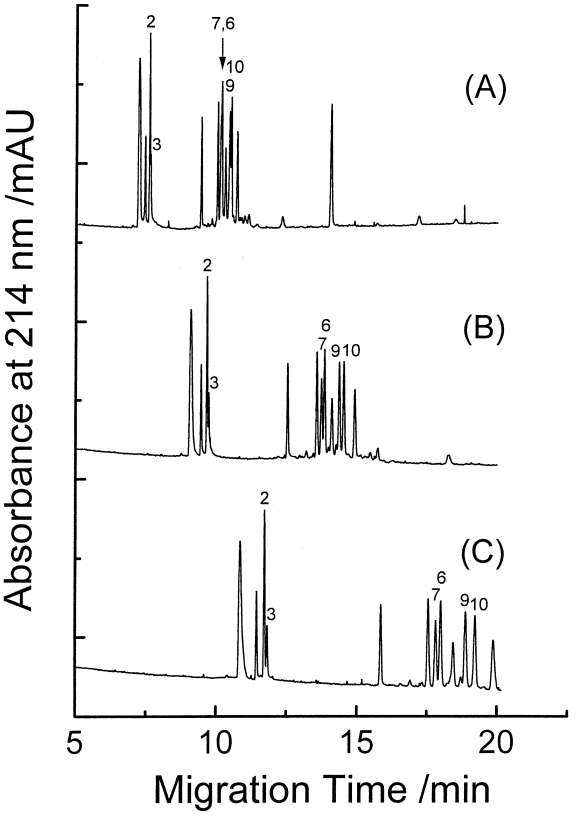


Fig. 4. Electropherograms of cephalosporins obtained with phosphate buffer at varied concentrations at pH 6.0: (A) 50 m*M*, (B) 100 m*M*, and (C) 150 m*M*. Operating conditions and peak identification as for Fig. 2.

As indicated in Fig. 5, with the citrate buffer at a concentration of 40 m*M*, the peaks of cephalexin (**2**) and cefaclor (**3**) are well separated in the pH range studied. A similar situation is encountered to the peaks of cefuroxime (**9**) and cefazolin (**10**). Hence, by manipulating the buffer pH in the range 5.5–6.2, the overlapping of the peak of cephapirin (**7**) with the others may be avoided and complete separations of the 12 cephalosporins selected can be easily achieved. As a matter of fact, complete separations of 12 cephalosporins can be achieved at pHs 5.5, 5.8, 6.0 and 6.2. Fig. 6 shows the electropherograms of the 12 cephalosporins obtained with the citrate buffer at these pH values.

The optimum concentration of the citrate buffer is confined in a small range. With the citrate buffer at a concentration of 50 m*M*, the current of the electrophoretic system exceeds 100 μA at pHs above 6.0. Hence, in order to avoid experimental difficulties due to excessive Joule heating, the use of the citrate buffer at concentrations greater than 50 m*M* is not recommended. On the other hand, at buffer concentrations below 35 m*M*, the peaks of cephalexin (**2**) and cefaclor (**3**) are baseline separated only at a pH above 6.2. Unfortunately, the peaks of cefuroxime (**9**) and cefazolin (**10**) can not be well resolved at pHs above 5.6. Thus complete separation of the 12 cephalosporins is impossible to achieve with citrate buffer at concentrations below 35 m*M*.

As the extent of the increase in the magnitude of the electroosmotic mobility (μ_{eo}) and the extents of the increase in the electrophoretic mobility of class I

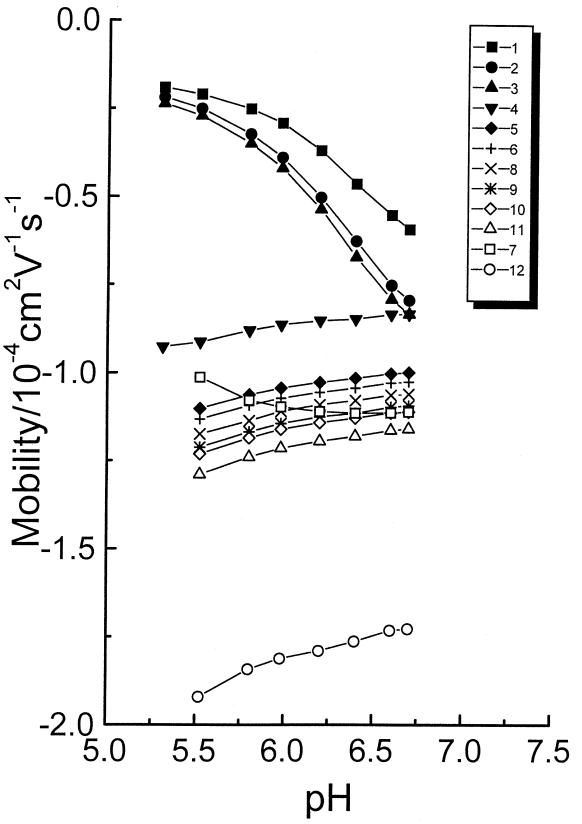


Fig. 5. Electrophoretic mobility of cephalosporins obtained with citrate buffer (40 m*M*) at varied pH in the range 5.5–6.7. Operating conditions and curve identification as for Fig. 2, except that the applied voltage is 30 kV.

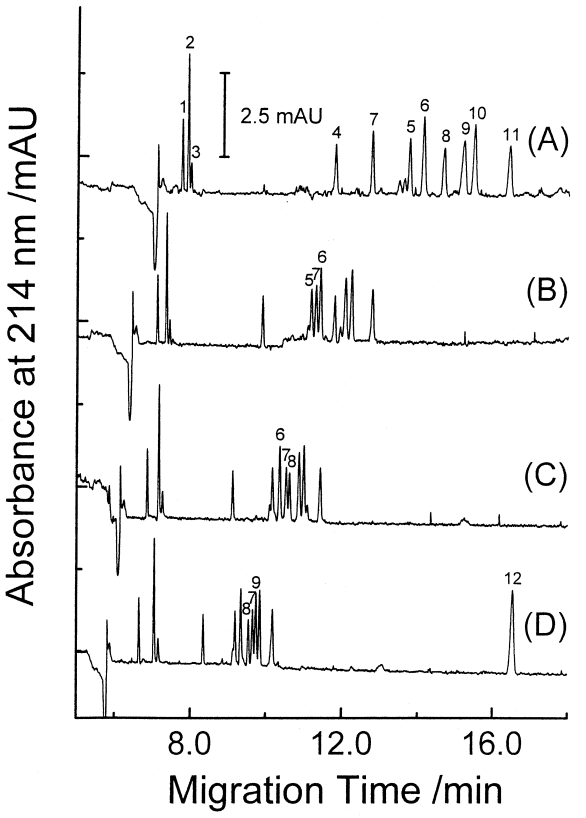


Fig. 6. Electropherograms of cephalosporins obtained with citrate buffer (40 m*M*) at varied pH: (A) 5.5, (B) 5.8, (C) 6.0, (D) 6.2. Operation conditions and peak identification as for Fig. 5.

cephalosporins and the decrease in the electrophoretic mobility of class II cephalosporins with the citrate buffer are much greater than those of the corresponding mobility with the phosphate buffer when the pH of the buffer increases from 5.3 to 6.7, the difference between the electrophoretic mobility of each analyte and electroosmotic mobility at a particular buffer pH is greater with the citrate buffer than with the phosphate buffer. Comparing Fig. 6 with Fig. 3, better separability and shorter analysis time is clearly illustrated when using the citrate solutions as a buffer electrolyte.

3.3.2. MES buffer

Fig. 7 shows the variation of electrophoretic mobility of cephalosporins as a function of buffer pH in the range 5.5–7.3 using MES buffer at a concentration of 260 m*M*. In general, the trends in the variation of the electrophoretic mobility of these 12 cephalosporins as a function of buffer pH with MES buffer are similar to those observed with the phosphate and citrate buffers (shown in Figs. 2 and 5, respectively). However, the electrophoretic mobility of each individual cephalosporin increases about 5% of its magnitude when the buffer pH increases from 5.5 to 7.3. The electrophoretic mobility of cephalosporins measured in MES buffer has a slight increase, compared with the corresponding mobility measured in the phosphate buffer. Since the magnitude of μ_{eo} decreases quite rapidly from $2.85 \cdot 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ at pH 5.5 to $2.25 \cdot 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ at pH 6.2, and then gradually decreases further to $2.18 \cdot 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ at pH 7.0, the peaks of

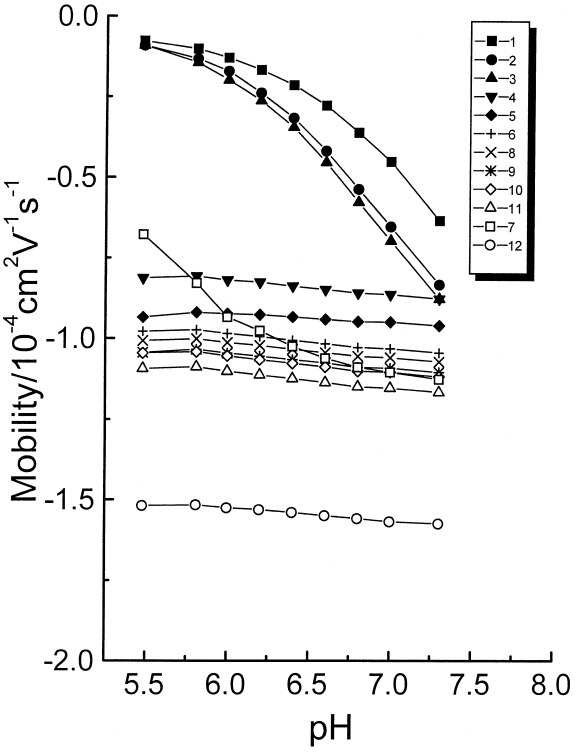


Fig. 7. Electrophoretic mobility of cephalosporins obtained with MES buffer (260 m*M*) at varied pH in the range 5.5–7.3. Operating conditions and curve identification as for Fig. 5.

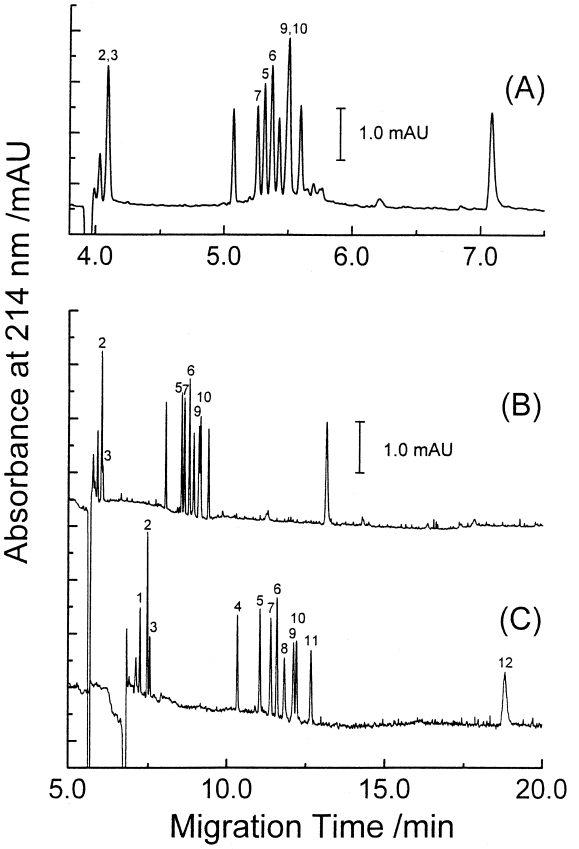


Fig. 8. Electropherograms of cephalosporins obtained at pH 6.21 with MES buffer at varied concentrations: (A) 40 m*M*, (B) 150 m*M*, (C) 260 m*M*. Operating conditions and peak identification as for Fig. 5.

cephalosporins move towards the position of neutral marker as the buffer pH decreases. To achieve complete separation of these 12 cephalosporins, the buffer pH at 6.2 should be selected when using the MES buffer at the concentration of 260 m*M*. With this buffer concentration, the current measured was about 70 μA.

The electrophoretic mobility of cephalosporins increases with increasing buffer concentration. Fig. 8 shows the electropherograms of cephalosporins obtained with the MES buffer at concentrations of 40, 150 and 260 m*M* at pH 6.21. The migration of cephapirin (**7**) is markedly influenced by the concentration of the MES buffer. The peak of cephapirin (**7**) appears before cefsulodin (**5**) with the buffer concentration at 40 m*M*. However, cephapirin (**7**) comigrates with cefsulodin (**5**) at 150 m*M*, but elutes later than cefsulodin (**5**) at or above 260 m*M*. Moreover, the resolution of the peaks between cephalexin (**2**) and cefaclor (**3**) and that of the peaks between cefuroxime (**9**) and cefazolin (**10**) are significantly affected by varying the concentration of the MES buffer. In fact, these two pairs of peaks are well resolved at a concentration greater than 260 m*M* with the MES buffer at pH 6.2.

It should be noted that complete separations are much easier to achieve when cephalexin (**2**) and cefaclor (**3**) or cefuroxime (**9**) and cefazolin (**10**) are not simultaneously present as analytes. As shown in Fig. 8A, most of the cephalosporins studied can be

easily and effectively separated even with the MES buffer at a concentration as low as 40 m*M* at pH 6.21.

3.4. Determination of pK_{a2} value and prediction of migration behavior

The pK_{a2} values of class I cephalosporins in the phosphate buffer (100 m*M*) and that of cephapirin (**7**) in the citrate buffer (35 m*M*) can be determined from the inflection points of the electrophoretic mobility curves which are the plots of electrophoretic mobility versus buffer pH. To determine the pK_a value of an analyte, a simulated mobility curve which is best-fitted to the experimental mobility curve is first obtained through the utilization of the Sigmoidal Fit of Microcal Origin software (version 5.0) so that small differences in the electrophoretic mobility ($\Delta\mu$) for every small increment of buffer pH (ΔpH), say 0.005, in the pH range studied are calculated. The first or second derivative of $\Delta\mu$ with respect to ΔpH is then plotted against buffer pH for each mobility curve and the pH value of the inflection point corresponding to the pK_a value is determined. Fig. 9 shows such plots for cephradine (**1**), cephalexin (**2**), cefaclor (**3**) and cephapirin (**7**). The arrows shown in Fig. 9 indicate the positions of the inflection points of these mobility curves. The pK_a values of cephradine (**1**), cephalexin (**2**), cefaclor (**3**) and cephapirin (**7**) determined are 7.27, 6.96, 6.92 and 4.72, respectively. As the literature values of the pK_{a2} of nicotinic acid attributed to the pyridinium group is 4.81 [27], the determined pK_{a2} value of cephapirin (**7**) would be a reasonable value.

As mentioned earlier, the carboxylic acid group of cephalosporins is fully dissociated at pH>5 and the variation of the electrophoretic mobility of cephradine (**1**), cephalexin (**2**), cefaclor (**3**) and

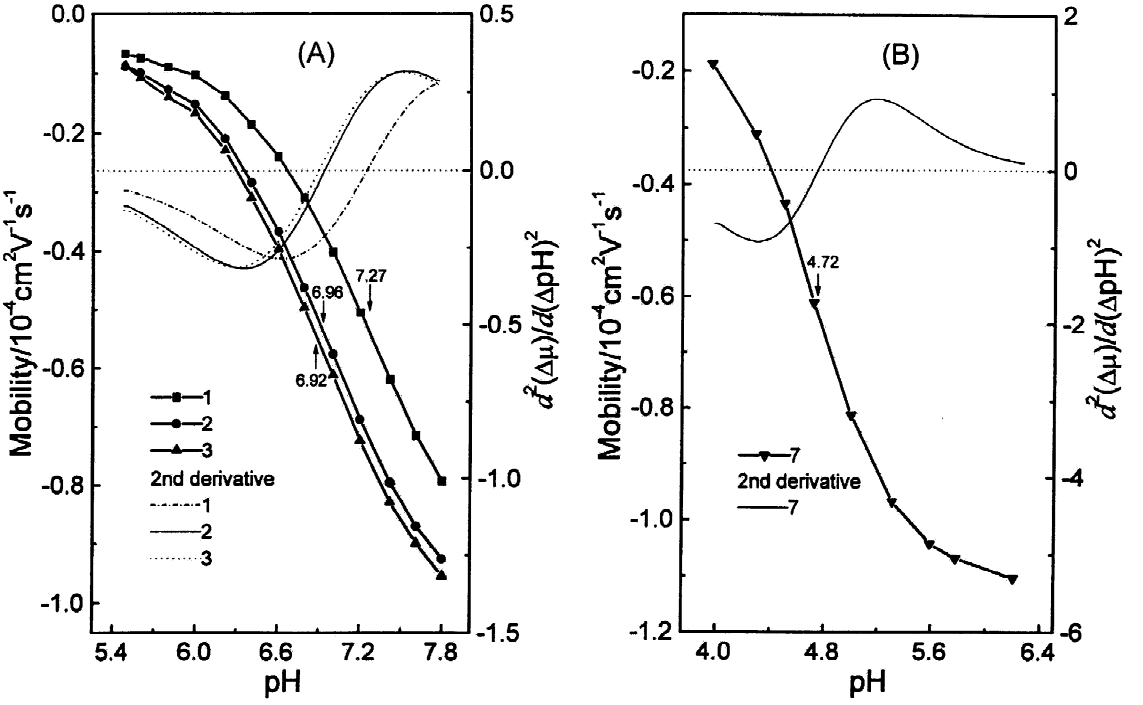


Fig. 9. The pK_{a2} values of cephalosporins determined from the inflection points of pH-dependent mobility curves: (A) cephradine (**1**), cephalexin (**2**) and cefaclor (**3**) in phosphate buffer (100 m*M*), (B) cephapirin (**7**) in citrate buffer (35 m*M*). Other operating conditions in the cases of (A) and (B) are the same as for Fig. 2 and Fig. 5, respectively. Arrows indicate the inflection points of mobility curves.

cephapirin (**7**) in the pH range studied is the result of the increase in the degree of deprotonation of either an amino group or a pyridinium group of the specific cephalosporin. Thus, the effective electrophoretic mobility (μ_{eff}) of these cephalosporins as a function of buffer pH can be described by the following equation modified from the one used for cationic species [28,29]:

$$\mu_{eff} = \frac{[H_3O^+]\mu_{BH^+}}{[H_3O^+] + K_{a2}} - \mu_{BH^+} = -\frac{K_{a2}\mu_{BH^+}}{[H_3O^+] + K_{a2}} \quad (1)$$

where $\mu_{BH^+}$is the electrophoretic mobility of the protonated form of a zwitterionic solute. Accordingly, the migration behavior of these cephalosporins in the pH between p$K_{a2}-2$ and p$K_{a2}+2$ can be predicted using Eq. (1), provided that the pK_{a2} values and the necessary mobility data are available.

To determine the pK_{a2} values of cephalosporins properly from the curve-fitting method, the trial values of pK_{a2} and limiting mobility of each individual cephalosporin are first estimated from the mobility curve. The best-fitted pK_{a2} value and limiting mobility are then determined by varying the trial values of these two parameters until the predicted mobility curve calculated by using Eq. (1) is best fitted to the experimental mobility curve through the utilization of Microcal Origin software. Fig. 10 shows the mobility curves obtained for these four cephalosporins. As can be seen, the agreement between the predicted mobility curves (represented by solid or dash lines) and the observed mobility curves (shown by data points) is very good. Table 1 lists the pK_{a2} values and the mobility data evaluated, together with the pK_a values reported in the literature [22,25,26]. The pK_{a2} values determined from the inflection-point and curve-fitting methods agree very well. It is noted that a small correction to the effective mobility is sometimes necessary for the best fit of mobility curves for class I cephalosporins. For instance, for the mobility curves shown in Fig. 9A, a small correction of -0.04 was made. This is perhaps due to a small contribution to the electro-

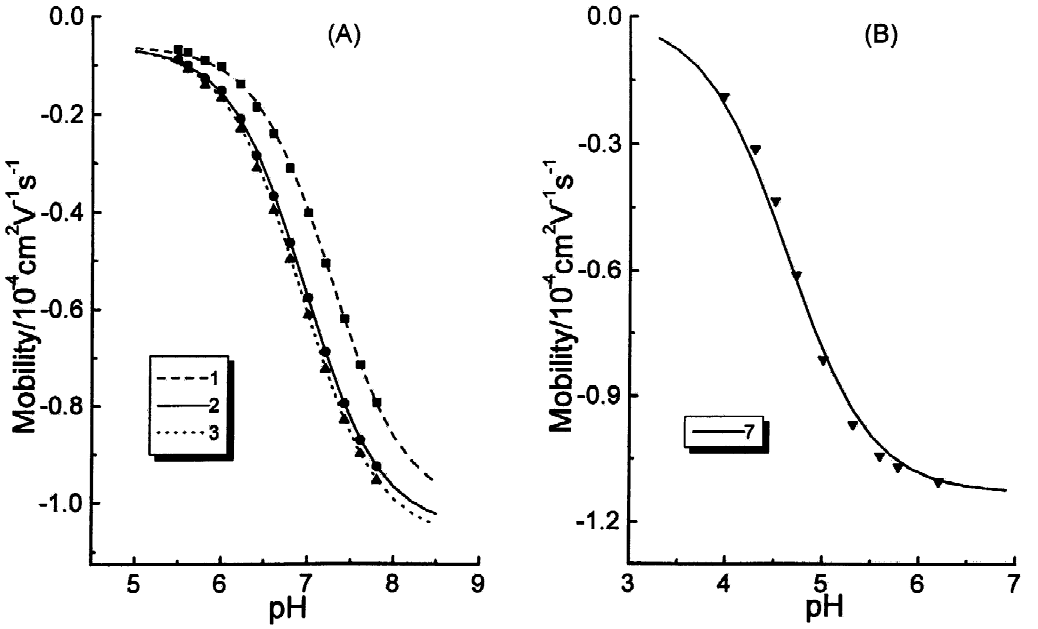


Fig. 10. The agreement between the predicted mobility curves (represented by solid and dash lines) and observed mobility curves (shown by data points) for cephalosporins: (A) cephradine (**1**), cephalexin (**2**) and cefaclor (**3**), (B) cephapirin (**7**).

phoretic mobility from other structural moieties of cephalosporins.

It should be pointed out that the pK_{a2} value of cefaclor (**3**) reported previously by Mrestani et al. [22] was not accurate enough because the mobility curves of cephalexin (**2**) and cefaclor (**3**) shown in Fig. 3 from [22] are very close to each other and the difference in the pK_{a2} values of these two cephalosporins determined by the CE method may be less than 0.2, as was determined from the potentiometric measurements [22]. The results shown in Figs. 9A or 10A clearly demonstrate that the difference in pK_{a2} values between cephalexin (**2**) and cefaclor (**3**) is only 0.04, with the pK_a value of cefaclor (**3**) being smaller than that of cephalexin (**2**). Therefore, a more accurate pK_{a2} value of cefaclor (**3**) than that reported previously [22] is obtained in this work.

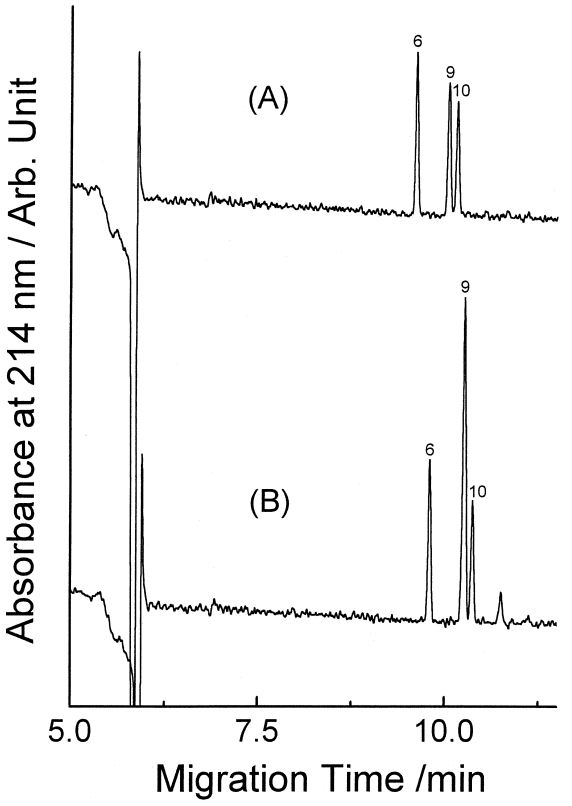


Fig. 11. Electropherograms of cefotaxime, cefuroxime and cefazolin obtained with citrate buffer (50 m*M*) at pH 6.0: (A) a mixture of standards (50 μg/ml each), (B) a standard solution of (A) spiking with Lifurox (50 μg/ml). Operation conditions and peak identification as for Fig. 5.

3.5. Peak confirmation

In the course of our investigation, the migration order of cephalosporins between cefotaxime (**6**) and cefuroxime (**9**) is contradictory to that reported by Mrestani et al. [20]. In order to confirm the correctness of our assignments for these two peaks, a real sample of cefuroxime (Lifurox) obtained from Lilly (Taiwan) was injected into the CE system. Fig. 11A shows the electropherogram obtained for a standard solution containing cefotaxime (**6**), cefuroxime (**9**) and cefazolin (**10**) (50 μg/ml each dissolved in an injection fluid). A amount of 20 μg/ml of urea was added to the sample solutions in order to examine whether urea contained in urine samples can affect the migration order of cefotaxime (**6**) and cefuroxime (**9**) using citrate buffer (50 m*M*) at pH 6.0. Fig. 11B shows the electropherogram of these three analytes obtained after spiking with Lifurox (50 μg/ml). As can be seen, the relative migration time of the cefuroxime standard and that of Lifurox to cefazolin (**10**) or to cefotaxime (**6**) is agreeable. Evidently, the result confirms that our assignments for these peaks should be beyond doubt. On the contrary, the assignments made by Mrestani et al. [20] are questionable.

4. Conclusions

Although manipulation of buffer pH is a key strategy to optimize the separation of cephalosporins in CZE, combined effects of buffer pH and buffer concentration should be taken into consideration to optimize the separation of cephalosporins, particularly when cefotaxime and cephapirin, cephalexin and cefaclor, or cefuroxime and cefazolin, are simultaneously present. Complete separations of 12 cephalosporins are achievable with citrate, MES and phosphate buffers under various optimum conditions. However, citrate and MES buffers are superior to the phosphate buffer in the CZE separation of the cephalosporins. The migration behavior of these cephalosporins in the pH range studied is described.

Acknowledgements

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Capillary zone electrophoretic separation of neutral species of chloro-*s*-triazines in the presence of cationic surfactant monomers

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Abstract

Chloro-*s*-triazines are difficult to separate by capillary zone electrophoresis (CZE), due to their low pK_a values. However, these analytes can be effectively separated by CZE in the presence of cationic surfactant monomers, such as tetradecylammonium bromide (TTAB) and dodecyltrimethylammonium bromide (DTAB). The separation mechanism based on a 1:1 binding of analytes to cationic surfactant monomers is proposed. The binding constants of chloro-*s*-triazines to cationic surfactant monomers are estimated. The results show that the strength of the interactions of these analytes with TTAB monomers is considerably strong, whereas that of the corresponding analyte with DTAB monomers is about 12- to 14-fold weaker. A linear correlation of binding constants with log P_{ow} (the logarithm of the partition coefficient of analytes between 1-octanol and aqueous phases) indicates that the migration order of these chloro-*s*-triazines depends primarily on their hydrophobicity. Moreover, the skewed peaks of chloro-*s*-triazines observed may reveal the occurrence of adsolubilization of these analytes in the adsorbed cationic surfactant layer on the capillary surface.

Keywords: Chlorotriazines; Triazines; Surfactants; Pesticides

1. Introduction

Capillary zone electrophoresis (CZE) has been successfully applied for the separation of diverse analytical samples [1–4]. The separation of analytes in CZE is often accomplished by adding electrolyte modifiers or complexing agents to the background electrolyte in order to vary the extent of chemical interactions so that the electrophoretic mobility of each individual solute can be effectively differentiated from the others.

In the normal mode of CZE separation, an electroosmotic flow (EOF) from the anode to the cathode is generated so that sample solutes can be carried to the detector. However, when the electrophoretic mobility of anionic species is close to or greater than the electroosmotic mobility, these anionic species are difficult or even unable to be detected. Under these circumstances, the use of reversed EOF is advantageous for the separation [5,6].

The reversal of the EOF is induced by adding cationic surfactants, such as long-chain alkylammonium salts, to the background electrolyte at an appropriate concentration either below the critical micelle concentration (CMC) in the case of CZE or above the CMC in the case of micellar electrokinetic

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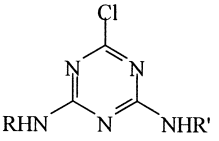
chromatography (MEKC). This is resulted from the adsorption of cationic surfactants onto the charged silica surfaces of the capillary. The adsorption occurs primarily in two successive steps [7,8]. The first step corresponds to the adsorption of individual cationic surfactant monomers by direct adsorption or ion-exchange mechanism, driven by electrostatic interactions. The second step, driven by hydrophobic interactions between the alkyl chains of oncoming surfactants and of surfactants adsorbed in the first step, corresponds to the formation of a bilayer of adsorbed surfactants or surface aggregate on the capillary wall, thus effectively making the surface charge positively [9,10]. Consequently, the electro-osmotic mobility can be varied by altering the concentration of a cationic surfactant at a particular concentration of the background electrolyte.

Chloro-*s*-triazines are important selective pre- and post-emergence herbicides used widely for the control of broadleaf and grassy weeds [11]. Because of their extensive use, toxicity, and relatively high persistence in environmental matrices [12–14], chloro-*s*-triazines are of great environmental concern. Numerous articles on the separation of *s*-triazine herbicides and their degradation products using various modes of capillary electrophoresis (CE), including CZE [15–18], isotachophoresis (ITP) [19,20], MEKC [20–24], and its hyphenated techniques [25–28], have been reported. However, the effective separation of chloro-*s*-triazines using CZE technique under the conditions of normal EOF has not been achieved [18]. This is probably due to the experimental difficulties encountered, as resulted from their low pK_a values. The pK_a values of chloro-*s*-triazines reported in the literature are in the range 1.3–2.0 [12,29–33]. Table 1 presents the pK_a values, along with log P_{ow} values (the logarithm of the partition coefficient of a solute between 1-octanol and aqueous phases) and some characteristics of chloro-*s*-triazines.

In the course of the investigation of the migration behavior and separation of *s*-triazines in MEKC using a cationic surfactant [24], we found that strong interactions were involved between chloro-*s*-triazines and tetradecyltrimethylammonium bromide (TTAB) micelles. In view of this, effective separation of chloro-*s*-triazines may be achievable by CZE in the presence of cationic surfactant monomers. Recently, an effective separation of three chloro-*s*-triazines by CZE under the conditions of reversed EOF using cetyltrimethylammonuim bromide was reported [28]. However, the separation mechanism was not discussed at all by these authors. In this paper, we report the results of our investigation on the sepa-

Table 1
Some characteristics of *s*-triazines studied



s-Triazines	Compound No.	Substituents		pK_a [a]	λ_{max} and ε_{max} [b]				Solubility [c] ($\mu g ml^{-1}$)	Log P_{ow} [d]
		R	R′		λ_1 (nm)	ε_1 ($l\ mol^{-1}\ cm^{-1}$)	λ_2 (nm)	ε_2 ($l\ mol^{-1}\ cm^{-1}$)		
Simazine	1	C_2H_5	C_2H_5	1.65	222	36 000	263	3100	5	2.26
Atrazine	2	C_2H_5	$CH(CH_3)_2$	1.68	222	41 000	263	3900	33	2.61
Propazine	3	$CH(CH_3)_2$	$CH(CH_3)_2$	1.85	221	32 000	268	3100	5	2.91
Terbuthylazine	4	C_2H_5	$C(CH_3)_3$	1.94	223	19 500	263	1500	5	3.06

[a] Refs. [12,28–32].
[b] Ref. [12].
[c] Ref. [13].
[d] Refs. [12,44].

ration of four chloro-*s*-triazines using TTAB and dodecyltrimethylammonium bromide (DTAB) as cationic surfactant monomer. The separation mechanism based on a 1:1 interaction of an analyte with cationic surfactant monomers is discussed. In addition, the binding constants of the analytes to cationic surfactant monomers are evaluated.

2. Experimental

2.1. Chemical and reagents

Of the four chloro-*s*-triazines selected in this study, terbuthylazine was purchased from Riedel-de Haen (Germany); and simazine, atrazine and propazine were supplied from Supelco (USA). TTAB and DTAB were obtained from Tokyo Kasei Kogyo (TCI, Tokyo, Japan). All other chemicals were of analytical-reagent grade. Deionized water was prepared with the Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of chloro-*s*-triazines were prepared at a concentration ranging from 1 to 20 $\mu g ml^{-1}$ in a methanol aqueous solution containing 10% (v/v) methanol. The pH of the buffer was adjusted by mixing various proportions of 70 m*M* sodium dihydrogenphosphate buffer solution with the same concentration of disodium hydrogenphosphate solution to attain the desired value (pH 6). Methanol was used as a neutral marker.

2.2. Apparatus

Separations were made with a CE system described previously [24,34]. The dimensions of the capillary, purchased from Polymicro Technologies (Tucson, AZ, USA), were 67 cm×50 μm I.D. The UV detection position is 7.0 cm from the anodic end (the polarity of the electrodes is reversed). Sample injection was done in a hydrodynamic mode during 1 s. The CE system was interfaced with a microcomputer and a printer with software CE 1000 1.05 A. For pH measurements, a pH meter (Suntex SP-701, Taipei, Taiwan) calibrated with a precision of 0.01 pH unit was employed.

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed using a standard sequence described previously [34]: that is, 10 min with deionized water at 60°C, 60 min with 1.0 *M* NaOH at 60°C and then 10 min with deionized water at 25°C. To ensure reproducibility, all experiments were performed at 25°C and measurements were run at least in triplicate. The capillary was pre-washed for 5 min with running buffer before each injection and post-washed for 10 min with deionized water at 25°C, followed with 1.0 *M* sodium hydroxide solution at 25°C for 5 min and with 0.1 *M* sodium hydroxide solution at 25°C for 5 min, and then with deionized water at 25°C for 5 min to maintain proper reproducibility for run-to-run injections. The detection wavelength was set at 220 nm. The signs of the electroosmotic and electrophoretic mobilities are defined such that the direction of the migration from anode to cathode is positive.

2.4. Calculation of electrophoretic mobility

The electrophoretic mobility of analytes was calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V} \cdot \left(\frac{1}{t_m} - \frac{1}{t_{eo}} \right) \quad (1)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an unchanged solute, L_t is the total length of capillary, L_d is the length of capillary between injection and detection, and V is the applied voltage.

3. Results and discussion

3.1. Electrophoretic mobility as a function of the concentration of surfactant monomers

Fig. 1 depicts the schematic diagram of the migration of a neutral solute associated with cationic surfactant monomers in CZE. The effective electro-

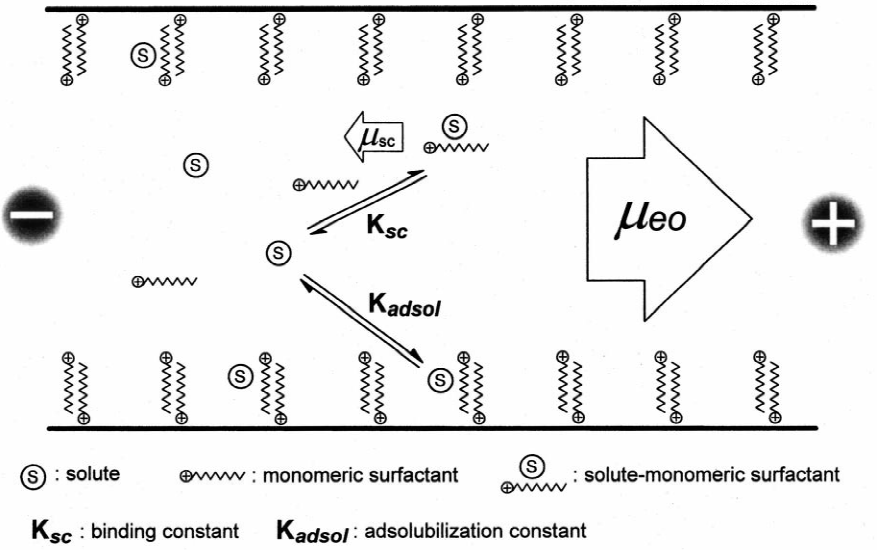


Fig. 1. Schematic diagram of the migration of a neutral solute associated with cationic surfactant monomers in CZE.

phoretic mobility of a neutral solute in CZE can be expressed as:

$$\mu_{\text{eff}} = \alpha_{\text{sc}}\mu_{\text{sc}} \quad (2)$$

where α_{sc} and μ_{sc} are the mole fraction and limiting electrophoretic mobility of the solute associated with surfactant monomers in the aqueous phase, respectively. When cationic surfactant monomers are used as complexing agents, the electrophoretic process involves the following equilibrium between an analyte (A) and surfactant monomers (S),

$$\text{A} + \text{S} \overset{K_{\text{sc}}}{\rightleftharpoons} \text{A}\cdot\text{S}$$

where K_{sc} is the binding constant of a solute to surfactant monomers. The mole fraction (α_{sc}) can be expressed in terms of K_{sc} and [S] as [35–37]:

$$\alpha_{\text{sc}} = \frac{K_{\text{sc}}[\text{S}]}{1 + K_{\text{sc}}[\text{S}]}$$

where [S] is the concentration of surfactant monomers and is defined as:

$$[\text{S}] = \frac{[\text{S}]_{\text{T}}}{1 + K_{\text{sc}}[\text{A}]}$$

where [A] is the analyte concentration and $[\text{S}]_{\text{T}}$ is the total concentration of surfactant monomers which is less than the CMC of the surfactant. Thus, the electrophoretic mobility of a solute can specifically be defined by the following equation [35–37]:

$$\mu_{\text{eff}} = \frac{K_{\text{sc}}[\text{S}]\mu_{\text{sc}}}{1 + K_{\text{sc}}[\text{S}]} \quad (3)$$

The CMC values of TTAB and DTAB determined in a phosphate buffer (70 m*M*) at pH 6.0 are 1.6±0.1 and 11.0±0.2 m*M*, respectively [38]; the CMC values of DTAB determined in pure water and in a phosphate buffer (20 m*M*) at pH 6.0 are 15.6 [8] and 12.4 m*M* [39], respectively. Therefore, the concentration of TTAB and DTAB monomers added to the phosphate buffer should be less than 1.6 and 11.0 m*M*, respectively. Fig. 2 shows the effective electrophoretic mobility of four chloro-*s*-triazines (indicated by the data points) obtained in a phosphate buffer at pH 6.0 with TTAB and DTAB concentrations varying in the range 0.6–1.6 and 4.0–10.0 m*M*, respectively. As expected, the mobility of each individual chloro-*s*-triazines increased with increasing surfactant concentration. According to Eq. (3), the migration behavior of these chloro-*s*-triazines is quantitatively predictable, provided that the binding constant and limiting electrophoretic mobility of the complexes formed between *s*-triazines and surfactant monomers are known.

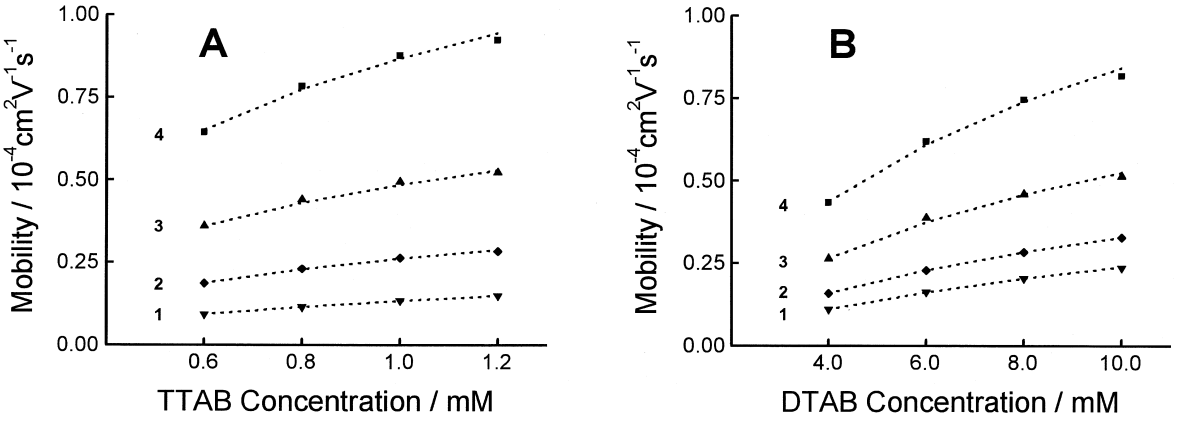


Fig. 2. The variation of observed (indicated by data points) and predicted (represented by dotted lines) electrophoretic mobility curves of chloro-*s*-triazines as a function of the concentration of surfactant monomers (with corrected total surfactant concentration): (A) TTAB, (B) DTAB. Capillary: 67 cm×50 μm, I.D. Buffer: 70 m*M* phosphate buffer at pH 6.0. Other operating conditions: −15 kV, 25°C. Solute: 1=simazine, 2=atrazine, 3=propazine, 4=terbuthylazine.

3.2. Determination of K_{sc} and μ_{sc} values of chloro-s-triazines

The values of K_{sc} and μ_{sc} can be determined either by linear-plot method or by curve-fitting method. In the former case, Eq. (3) can be rearranged and expressed as:

$$\frac{1}{\mu_{eff}} = \frac{1}{\mu_{sc}} + \frac{1}{\mu_{sc}K_{sc}[S]} \quad (4)$$

By plotting $1/\mu_{eff}$ vs. $1/[S]$, the values of K_{sc} and μ_{sc} can be estimated from the slope and intercept of a straight line obtained. The values of K_{sc} and μ_{sc} estimated by the linear-plot method without the correction of total surfactant concentration (assuming that the adsorption of surfactant monomers onto the capillary surfactant does not take place in the electrophoretic process) are listed as the numbers in parentheses in Table 2.

In the curve-fitting treatment, the electrophoretic mobility curves of chloro-*s*-triazines based on Eq. (3) were simulated using Excel software. The binding constants (K_{sc}) and limiting mobility (μ_{sc}) of each individual solute evaluated from the linear-plot method were used as trial values. The most suitable values of K_{sc} and μ_{sc} were then obtained by varying these parameters until the predicted mobility curves were best-fitted to the observed mobility curves. The values of K_{sc} and μ_{sc} evaluated by curve-fitting method without the correction of total surfactant concentration are listed as the numbers in parentheses in Table 3. It should be noted that the agreement between the predicted and observed mobility curves

Table 2
The binding constants and mobility data of chloro-*s*-triazines evaluated by linear-plot method[a]

Chloro-*s*-triazine	Binding constant (M^{-1})		Mobility (10^{-4} cm^2 V^{-1} s^{-1})	
	TTAB	DTAB	TTAB	DTAB
Simazine	877 (546)	69 (25)	0.31 (0.39)	0.62 (1.21)
Atrazine	1176 (773)	86 (37)	0.53 (0.62)	0.75 (1.23)
Propazine	1533 (1039)	105 (51)	0.88 (1.00)	1.08 (1.59)
Terbuthylazine	1642 (1120)	117 (60)	1.53 (1.72)	1.65 (2.30)

[a] The numbers are evaluated with corrected total surfactant concentration by assuming $[S]_{ads}$ equal to 0.085 m*M* for TTAB and 0.85 m*M* for DTAB; the numbers in parentheses are evaluated without correction ($[S]_{ads}=0$).

Table 3
The binding constants and mobility data of chloro-*s*-triazines evaluated by curve-fitting method[a]

Chloro-*s*-triazine	Binding constant (M^{-1})		Mobility (10^{-4} cm^2 V^{-1} s^{-1})	
	TTAB	DTAB	TTAB	DTAB
Simazine	885 (543)	69 (26)	0.31 (0.39)	0.62 (1.18)
Atrazine	1174 (765)	86 (37)	0.53 (0.62)	0.75 (1.23)
Propazine	1525 (1030)	106 (51)	0.88 (1.00)	1.08 (1.56)
Terbuthylazine	1640 (1125)	115 (59)	1.53 (1.70)	1.65 (2.30)

[a] The numbers are evaluated with corrected total surfactant concentration by assuming $[S]_{ads}$ equal to 0.085 m*M* for TTAB and 0.85 m*M* for DTAB; the numbers in parentheses are evaluated without correction ($[S]_{ads}=0$).

is satisfactory for most of the surfactant–analyte complexes, except DTAB–terbuthylazine, DTAB–propazine and TTAB–terbuthylazine complexes. Unfortunately, as indicated in Tables 2 and 3, the electrophoretic mobility of surfactant–analyte complexes, particularly, those of DTAB–analyte, are probably overevaluated. Thus, the binding constants of DTAB–analyte complexes calculated are too small, whereas the values evaluated for TTAB–analyte complexes are too large.

In order to improve the agreement between the predicted and experimental mobility curves of surfactant–analyte complexes and to obtain reasonable values of μ_{sc} of surfactant–analyte complexes, especially those of DTAB–analyte complexes, the correction of total surfactant concentration is necessary. This is done by subtracting the amount of surfactant monomers which are adsorbed onto the capillary surface in the process of electrophoretic separation from the total surfactant concentration. That is, the corrected total surfactant concentration is defined as the total concentration of surfactant monomers ($[S]_T$) minus the concentration of adsorbed surfactant monomers ($[S]_{ads}$).

The values of μ_{sc} of surfactant–analyte complexes decrease with increasing the concentration of adsorbed surfactant monomers. With $[S]_{ads}=0.1$ m*M* for TTAB and $[S]_{ads}=1.0$ m*M* for DTAB, the limiting electrophoretic mobility of TTAB–terbuthylazine complex is nearly equal to that of DTAB–terbuthylazine complex. As the electrophoretic mobility of TTAB–analyte complex should be smaller than that of the corresponding DTAB–analyte complex, the concentration of adsorbed surfactant monomers should not exceed 0.1 m*M* for TTAB–analyte complexes and should be less than 1.0 m*M* for DTAB–analyte complexes.

The concentration of adsorbed surfactant monomers is determined by assuming that the ratio of the electrophoretic mobility of DTAB–terbuthylazine complex to that of TTAB–terbuthylazine complex is equal to the ratio of the electrophoretic mobility of DTAB to that of TTAB, which is 1.08. In the present study, the adsorbed surfactant concentration ($[S]_{ads}$) is estimated to be about 0.085 m*M* for TTAB and about 0.85 m*M* for DTAB. The binding constants (K_{sc}) and the mobility (μ_{sc}) are then determined either by linear-plot method according to Eq. (4) or by curve-fitting method based on Eq. (3). The values of K_{sc} and μ_{sc} determined by linear-plot method are listed in Table 2 and these evaluated by curve-fitting method are given in Table 3. As shown in Fig. 2, the agreement between the predicted mobility curve (represented by dotted line) and the experimental mobility curve (indicated by data point) for each individual analyte, even for DTAB–terbuthylazine complex, is very good. The results also indicate that the strength of the interactions of these analytes with TTAB surfactant monomers is considerably strong, whereas that of the corresponding analyte with DTAB monomers is about 12- to 14-fold weaker. The binding constants of chloro-*s*-triazines to surfactant monomers increase in the order simazine$<$atrazine$<$propazine$<$terbuthylazine, as reflected from the extent of the variation in the effective electrophoretic mobility as a function of the concentration of surfactant monomers shown in Fig. 2.

3.3. Capillary zone electrophoretic separation of neutral species in the presence of cationic surfactant monomers

Two typical electropherograms of chloro-*s*-triazines obtained with a phosphate buffer (70 m*M*)

containing 1.0 m*M* TTAB and 8.0 m*M* DTAB, respectively, at pH 6.0 are shown in Fig. 3A and 3B. As demonstrated, complete separations of chloro-*s*-triazines in CZE were achieved in the presence of cationic surfactant monomers at a concentration below their critical micelle concentrations.

It is noteworthy that, as indicated by the magnitudes of the binding constants, the migration of these analytes follows the order simazine<atrazine<propazine<terbuthylazine. The binding constants of these analytes to TTAB and DTAB monomers evaluated with or without correction in total surfactant concentration are well correlated with log P_{ow}. The correlation coefficients (r^2) for the TTAB– and DTAB–analyte complexes with correction in total surfactant concentration are equal to 0.9970 and 0.99686, respectively. As the value of log P_{ow} is an index of the hydrophobicity of the solute, the results clearly indicate that the migration order of these chloro-*s*-triazines is primarily determined by their hydrophobicity.

3.4. Adsolubilization of chloro-s-triazine analytes

It is well known that the adsorption mechanism of cationic surfactants primarily involves two steps

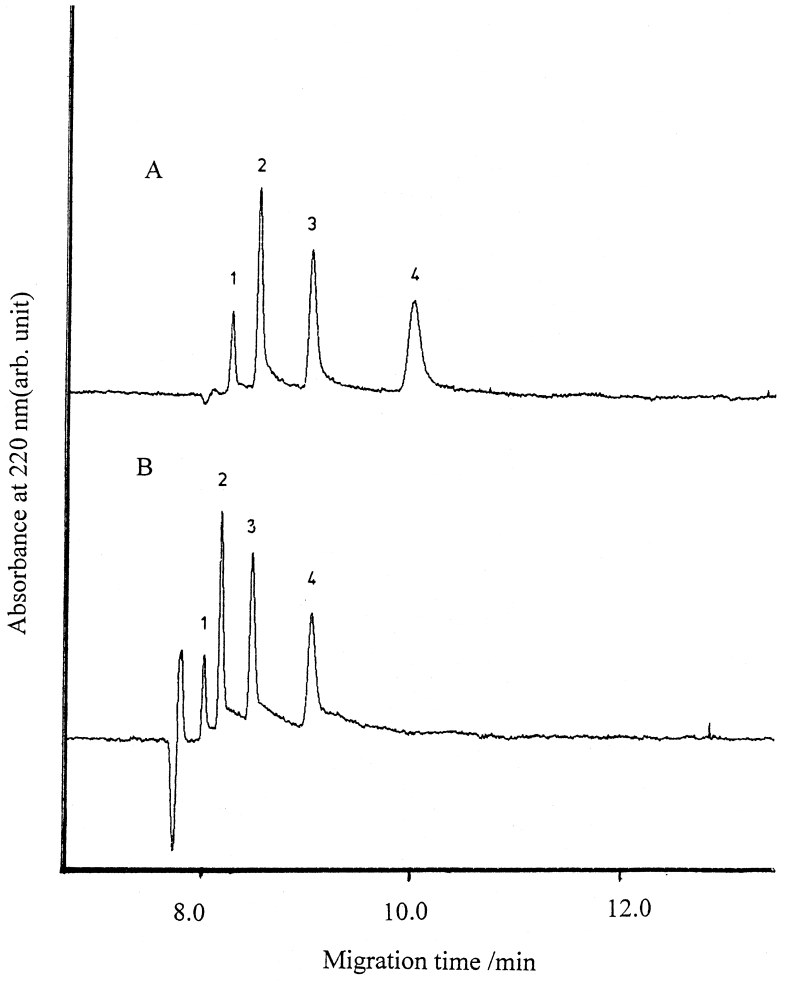


Fig. 3. Typical electropherograms of chloro-*s*-triazines obtained with addition of cationic surfactant monomers: (A) 1.0 m*M* TTAB; (B) 8.0 m*M* DTAB. Peak identification as in Fig. 2.

[7,8,40–42]. The first adsorption step corresponds to the adsorption of individual surfactant ions on the negative sites of the silica surface, driven by electrostatic interactions and the second adsorption step is driven by hydrophobic interactions between surfactant alkyl chains and is resulted in the formation of a bilayer of cationic surfactants or surface aggregates [7,8,40,43]. The adsorbed layer of cationic surfactants formed on the capillary surface provides hydrophobic circumstances which can incorporate hydrophobic compounds [7]. This phenomenon is refereed to as ''adsolubilization'' [7,38,41]. Moreover, surfactant adsorption on the surface is shown to continue even after the CMC is reached [8]. Thus after the adsorption of cationic surfactants on the capillary surface, a two-step process of adsorption–adsolubilization occurs for hydrophobic compounds and the analytes are partitioning between the adsolubilized surfactants and the aqueous phase containing surfactant monomers or micelles. For example, adsolubilization of 2-naphthol in the bilayer of the adsorbed dodecyltrimethylammonium chloride on the silica surface was reported [7]. The amount of adsolubilized analytes increases, reached a maximum, then decreases with increasing the concentration of cationic surfactants. Based on a similar argument, the adsolubilization of chloro-*s*-triazines is very likely to occur during the electrophoretic process. As shown in Fig. 3, the peak shape of chloro-*s*-triazines is somewhat skewed with TTAB surfactant monomers and is deteriorated to a greater extent with DTAB surfactant monomer at pH 6.0. The results suggest the occurrence of adsolubilization of chloro-*s*-triazines in the adsorbed bilayer of cationic surfactants on the capillary surface. The deterioration of peak shape can be improved by increasing the concentration of cationic surfactants or decreasing the content of methanol in the buffer solution so that the amount of adsolubilized analytes can be reduced. However, within the framework of CZE separation, the concentration of cationic surfactants is restricted to a value below the CMC. Thus the improvement of the peak shape of analytes is more or less limited and a symmetrical peak shape without tailing seems to be very difficult to obtain for analytes separated by CZE with cationic surfactants. Also, it should be noted that, as the analytes are in the neutral form, the separation of chloro-*s*-triazines is not effected by adsolubilization alone.

4. Conclusion

Complete separations of neutral species of chloro-*s*-triazines by CZE using cationic surfactant monomers as electrolyte modifier were achieved. The separation mechanism mainly based on a 1:1 interaction of analytes with cationic surfactant monomers is proposed. The migration order of these chloro-*s*-triazines is primarily determined by their hydrophobicity. Adsolubilization of these analytes is very likely to occur in the adsorbed bilayer of cationic surfactants. Adsolubilization can affect the asymmetry of the peak shape. However, adsolubilization alone is not effective enough to separate neutral species of chloro-*s*-triazines.

Acknowledgements

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On-line concentration of *s*-triazine herbicides in micellar electrokinetic chromatography using a cationic surfactant

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Abstract

On-line concentration of neutral species of *s*-triazine herbicides in micellar electrokinetic chromatography using tetradecylammonium bromide (TTAB) as a cationic surfactant was investigated. Factors affecting the stacking of analytes were examined. The results indicate that the stacking efficiency is markedly improved with addition of phosphate buffer in the sample matrix. It was found that, depending on the nature of the analytes, the most effective stacking of these analytes occurs when the ratio of the conductivity of buffer electrolyte to that of sample matrix is in the range 1.4–1.2, with sample matrix containing phosphate buffer. Micelle concentration in the separation buffer is also a crucial factor to enhance the stacking efficiency and detection sensitivity of analytes. Moreover, the stacking efficiency of each individual analyte depends on its binding constant to TTAB micelles. The concentration effect is primarily based on sweeping mechanism which is operated in a normal stacking mode with reversed electrode polarity in the presence of reversed electroosmotic flow. As a result of concentration enhancement, the detection limits of these herbicides can reach about 9–15 ng/ml with UV detection.

Keywords: Sample stacking; Sweeping; Triazines; Pesticides; Methylthio-*s*-triazines

1. Introduction

Capillary electrophoresis (CE) has been proven to be a powerful analytical tool for separating charged species of diverse samples, due to many of its advantageous features such as extremely high column efficiency, rapid analysis and small volumes of sample consumption in comparison with HPLC. For separating neutral analytes, micellar electrokinetic chromatography (MEKC) is the method of choice. However, the very limited optical path length, due to small inner diameter of the capillary (100–25 μm) and low sample volume injected (nL), make the detection of low-concentration samples with a UV detector difficult or even impossible without sample preconcentration.

To achieve more sensitive detection, fluorescence detection, particularly laser-induced fluorescence detection [1,2] may be used. Unfortunately, this type of detection is not generally applicable to environmental analysis because only a few compounds have native fluorescence and most analytes need to be derivatized with an appropriate fluorescent tag. In addition, only a limited number of laser sources are available.

Alternatively, on-line sample concentration by either field-amplified sample stacking [3–16] or isotachophoretic sample stacking [17–19] can be employed for enhancing detectability in capillary electrophoresis. For field-amplified sample stacking,

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upon the introduction of a long plug of low-conductivity sample solution into the capillary previously filled with a high-conductivity buffer electrolyte, sample stacking occurs at the concentration boundary between the low conductivity of sample zone and the high conductivity of separation zone when a high voltage is applied across the capillary. For neutral analytes, effective mobility necessary for stacking is provided by charged micelles in MEKC. Stacking with reversed migration micelles by large-volume sample injection may give enrichment factors of more than 500 [16–18]. Recently, head-column field-amplified sample stacking in binary system capillary electrophoresis has been demonstrated to provide over 1000-fold sensitivity enhancement [20,21]. Moreover, Terabe and co-workers [22–29] have reported that the stacking of neutral or ionic analytes in MEKC could be achieved based on sweeping technique. Depending on the nature of sample analytes, this technique can provide up to a 5000-fold concentration enhancement [22] or even approaching a million-fold sensitivity increase by applying cation-selective exhaustive injection and sweeping technique [28]. All these efforts make the detection of environmental analytical samples by UV absorption at trace concentration levels becoming possible.

s-Triazines are important selective pre- and post-emergence herbicides used widely for the control of broadleaf and grassy weeds [30]. These herbicides may contaminate drinking water sources. Six triazine herbicides, including prometryn and terbutryn are on the priority list in European Union drinking water guideline [31]. Simetryn and the two triazine herbicides aforementioned are on the priority list of pesticides in the USA national pesticide survey for a monitoring program on pesticides [31,32]. Because of their extensive use, relatively high persistence and toxicity in environmental matrices [31–33], *s*-triazines are of great environmental concern. Thus, the development of new analytical methods is desirable.

Several papers on the separation of *s*-triazines by MEKC have been reported [34–39]. The analysis of simazine and atrazine in samples of river water [35] and the determination of four chloro- and three methylthio-*s*-triazines in water [36] were conducted using sodium dodecyl sulfate (SDS) as an anionic surfactant. The separation of three chloro- and two methylthio-*s*-triazines was performed by partial filling micellar electrokinetic chromatography using SDS micelles [37]. The separation of prometon, prometryn and propazine was investigated using anionic octylglucoside-borate micelles at alkaline pH [34,38]. Recently, we reported the separation of thirteen *s*-triazines, including five chloro-, three methoxy- and five alkylthio-*s*-triazines, in MEKC using a cationic surfactant [39].

In this study, four methylthio-*s*-triazines are selected as test compounds. We demonstrate the influences of the concentration of both sample matrix and separation buffer on the stacking efficiency and detection sensitivity of these analytes by sweeping technique using a cationic surfactant. The concentration enhancement of neutral species of these *s*-triazines at pH 6.0 is studied and the limits of detection are determined.

2. Experimental

2.1. Chemicals and reagents

Four methylthio-*s*-triazines, including simetryn, ametryn, prometryn and terbutryn, were purchased from Supelco (Bellefonte, PA, USA). Tetradecyltrimethylammonium bromide (TTAB) was acquired from Tokyo Kasei Kogyo (TCI, Tokyo, Japan). All other chemicals were of analytical-reagent grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA)

Standard solutions of *s*-triazines were prepared at a concentration of 10 μg/ml in a solution containing 4% (v/v) acetonitrile. Further dilution of sample solution with deionized water down to 0.05 μg/ml was carried out in the determination of the limits of detection. The pH of the buffer and sample solutions was adjusted by mixing various proportions of a certain concentration of trisodium phosphate with the same concentration of phosphoric acid solutions to attain pH 6.0. All solutions were filtered through a membrane filter (0.22 μm) before use.

2.2. Apparatus

Electrophoretic experiments were performed using a Beckman Coulter (Fullerton, CA, USA) Model P/ACE MDQ capillary electrophoresis system,

equipped with a diode array UV–visible detector and an automatic injection system. The CE system with MDQ softwares was interfaced with a microcomputer and a Hewlett-Packard deskjet 670C printer. The fused-silica capillaries of 50 μm, I.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA). The total length of capillary is 60 cm and the position of UV detector is 10 cm from the anodic end. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of 0.01 pH unit. For conductivity measurements, a conductivity meter (Suntex SC-170, Taipei, Taiwan) calibrated with a 0.01 *M* KCl solution to a value of 1.413 mS/cm (at 25°C) was used.

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed 20 min with sodium hydroxide solution (1.0 *M*) at 25°C, followed by sodium hydroxide solution (0.1 *M*) for 20 min and then deionized and purified water for another 20 min at 25°C.

To ensure reproducibility, all experiments were performed at 25°C, and measurements were run at least in triplicate. The capillary was prewashed with running buffer for 5 min before each injection and postwashed for 5 min with deionized water to maintain proper reproducibility for run-to-run injections. Sample injections were made in pressure injection mode at a pressure of 68.9 mbar (1 p.s.i.) or indicated elsewhere. An applied voltage of −20 kV was selected in the electrophoretic separation. Detection was performed at 222 nm.

The stacking was performed by injecting sample solutions for a much longer time compared to the usual hydrodynamic injection. Sample solutions were introduced at the cathodic end of the capillary. Varied plug lengths of sample solution up to 18.1% occupancy of the capillary were assessed by introducing sample solution for a varied injection time up to 105 s.

3. Results and discussion

The addition of a cationic surfactant to the electrophoretic buffer may induce the reversal of the electroosmotic flow (EOF) in the electrophoretic separation. In fact, the EOF was reversed when the concentration of TTAB added in the phosphate buffer (70 m*M*) at pH 6.0 exceeded 0.2 m*M* [40]. In this study, on-line concentration of *s*-triazines was performed under the conditions of reversed EOF, as the concentrations of TTAB employed were much greater than the critical micelle concentration of TTAB which was determined to be 1.6±0.2 m*M* at pH 6.0 [41].

3.1. Effect of sample matrix on stacking efficiency

In a previous report [39], thirteen *s*-triazines, including the four chloro-*s*-triazines and four methylthio-*s*-triazines selected in this work, were completely separated by MEKC in a phosphate buffer (70 m*M*) containing TTAB (15 m*M*) as a cationic surfactant at pH 6.0. For a large volume of sample injection, however, reoptimization of separation parameters, such as phosphate concentration in the sample matrix and micelle concentration in the separation buffer, in particular, is necessary in order to achieve an effective and efficient stacking.

To examine the influences of sample matrix on the stacking efficiency and detection sensitivity of sample analytes, sample analytes were dissolved in an aqueous solution containing varied concentrations of either phosphate buffer in the range 10~70 m*M* and 4% acetonitrile as well. Fig. 1 shows some typical electropherograms of four methylthio-*s*-triazines obtained with sample solutions containing varied concentrations of phosphate buffer (0, 30, 50, 70 m*M*), while a separation buffer is composed of 40 m*M* phosphate buffer and 40 m*M* TTAB at pH 6.0. Sample analytes at a concentration of 10 μg/ml were injected for 30 s. As shown in Fig. 1A, the peaks of the four *s*-triazines obtained without addition of phosphate buffer in the sample matrix are rather broad and are poorly resolved. Apparently, the analytes are not in the efficient stacking conditions. The resolutions of peaks become even worse with a longer injection time. In contrast, as shown in Fig. 1B–D, with addition of phosphate buffer in the sample matrix, the peaks become sharpened and the peak height of these sample analytes increases with increasing phosphate concentration up to 50 m*M*. However, the peak height of these *s*-triazines de-

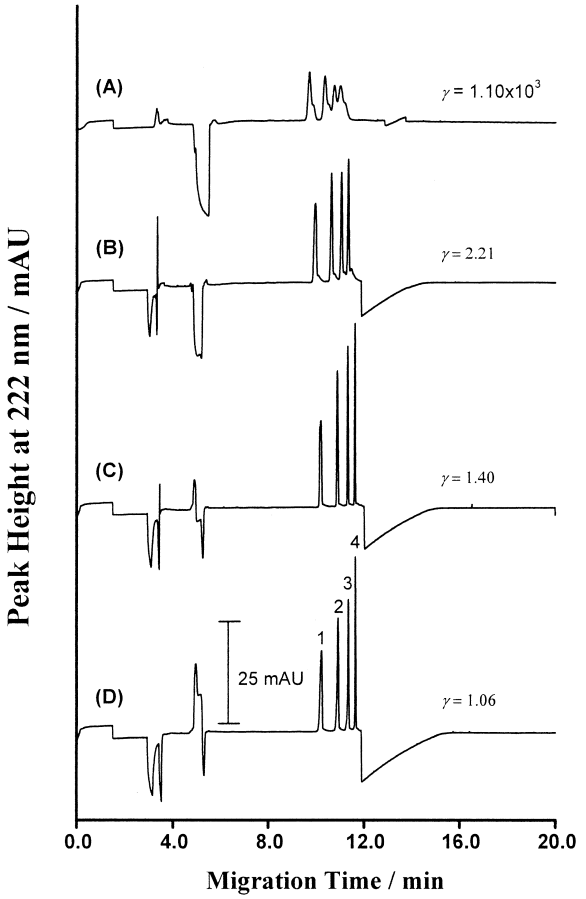

Fig. 1. Effect of sample matrix on the stacking efficiency and detection sensitivity of methylthio-*s*-triazines. Sample matrix: (A) water; (B) 30 m*M* phosphate buffer; (C) 50 m*M* phosphate buffer; (D) 70 m*M* phosphate buffer. Separation buffer, 40 m*M* TTAB in 40 m*M* phosphate buffer at pH 6.0; injection pressure, 1 p.s.i.; injection time, 30 s; capillary, 70 cm H 50 μm I.D; applied voltage, −20 kV; detection wavelength, 222 nm; temperature, 25°C; sample concentration, 10 μg/ml, sample dissolved in a sample matrix containing 4% acetonitrile solution. Peak identification, 1=simetryn, 2=ametryn, 3=prometryn, 4=terbutryn.

creases with further increasing the concentration of phosphate buffer in the sample matrix.

The conductivity values of sample matrices and those of buffer electrolytes at varied concentration were measured. The values of enhancement factor (γ) defined as the ratio of the conductivity of buffer electrolyte to that of sample matrix are indicated in Fig. 1. It should be noted that the γ value of the most effective stacking of analytes is in the range 1.4–1.2, instead of 1.0. Similar phenomena were observed for chloro-*s*-triazines as for methylthio-*s*-triazines and the γ value for most efficient stacking of these analytes was found to be 1.19 [42]. Evidently, the afore-mentioned results reveal that the stacking of analytes is primarily due to sweeping mechanism proposed by Quirino and Terabe [22], although, in this study, it is operated in a normal stacking mode with reversed electrode polarity in the presence of reversed electroosmotic flow. As the γ values of the most effective stacking are not closed to 1.0, the contribution of field-amplified sample stacking to the enhancement of detection sensitivity may not be completely ignored.

3.2. Stacking of analytes by sweeping

Fig. 2 depicts the schematic stacking mechanism of a neutral analyte dissolved in a sample matrix containing phosphate buffer with a separation buffer containing a cationic surfactant. As illustrated in Fig. 2A, the capillary column is initially filled with a micellar background electrolyte (BGE). A sample zone containing nonmicellar sample matrix or simply water is injected hydrodynamically with pressure for a period much longer than usual. By application of voltage at negative polarity (Fig. 2B), the electroosmotic flow is directed toward the anode (as cationic micelles is adsorbed on the capillary wall) and the micelles migrate toward the cathode. During

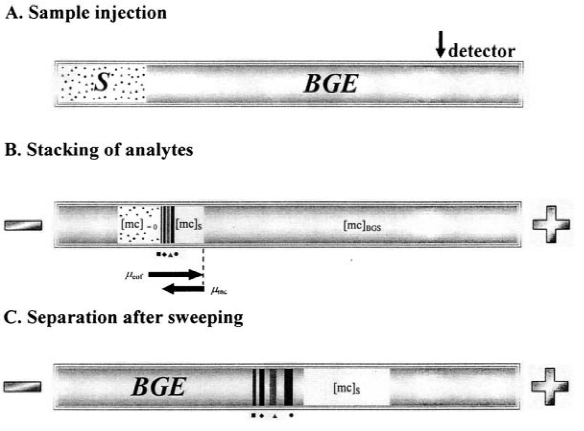

Fig. 2. Schematic diagram of a stacking mechanism by sweeping using a cationic surfactant (with sample matrix containing phosphate buffer).

sweeping, the analytes stacked at the concentration boundary between the region of $[mc]_s$ and that of [mc]=0 in the sample zone, where $[mc]_s$ denotes the concentration of micelles in the sample zone after sweeping and [mc]=0 indicates that no micelles are present in the original sample zone. The separation is then achieved via MEKC (Fig. 2C).

3.3. Effect of micelle concentration

The stacking efficiency and detection sensitivity of these test analytes are greatly affected by TTAB concentration. The peak height of each individual analyte increases with increasing micelle concentration until reaching the maximum, then it decreases with further increasing micelle concentration. Fig. 3 shows the variations of the peak height of methylthio-*s*-triazines as a function of TTAB concentration. As can be seen, the optimal TTAB concentrations determined for terbutryn, prometryn, ametryn and simetryn are about 30, 45, 50 and 70 m*M*, respectively, at the sample concentration of 10 μg/ml for a 30-s injection. In the present study, the optimal TTAB concentration for a simultaneous detection of methylthio-*s*-triazines is 40 m*M* for a 30-s injection and 60 m*M* for a 60-s or longer injection time. It should be noted that, with TTAB micelles at a concentration less than 20 m*M*, a satisfactory stacking of terbutryn for a 30-s injection is difficult.

3.4. Stacking efficiency versus sample plug length

The stacking efficiencies in terms of peak height (SE_{height}) for a neutral analyte is defined as the peak of the analyte for varied lengths of sample plug (H_{stack}) divided by the peak height of the corresponding analyte obtained for 1-s injection (H_{1s}). As a certain minimum injection time is required with Beckman A/PCE MDQ system for a particular injection pressure, H_{1s} is defined as the stacking efficient of a minimal injection time (H_{min}) divided by the minimal injection time. For example, with an injection pressure of 1 p.s.i., the minimum injection time is 3.5 s. Then H_{1s} is equivalent to $H_{3.5s}$ divided by 3.5.

For pressure injections, the length of sample plug in a capillary is directly proportional to the product of the injection pressure and injection time. Thus a 30-s injection of sample solution with a pressure of 1 p.s.i. corresponds to a sample plug length of 4.23 cm, which is 6.04% occupancy of the capillary.

Fig. 4 shows the effect of sample plug length on

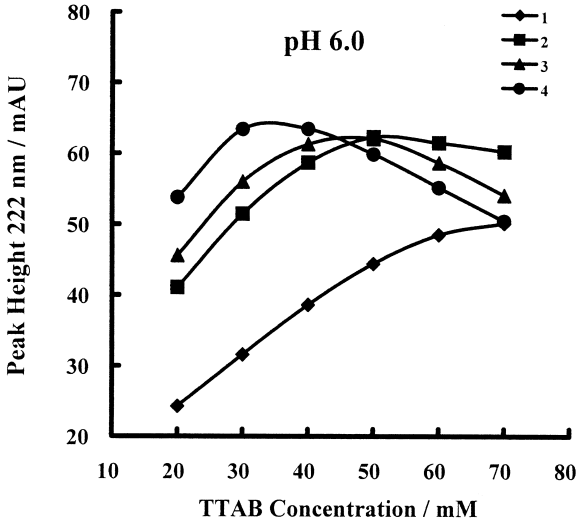

Fig. 3. The variation of peak heights of sample analytes as a function of TTAB concentration. The electrophoretic conditions are the same as for Fig. 1C.

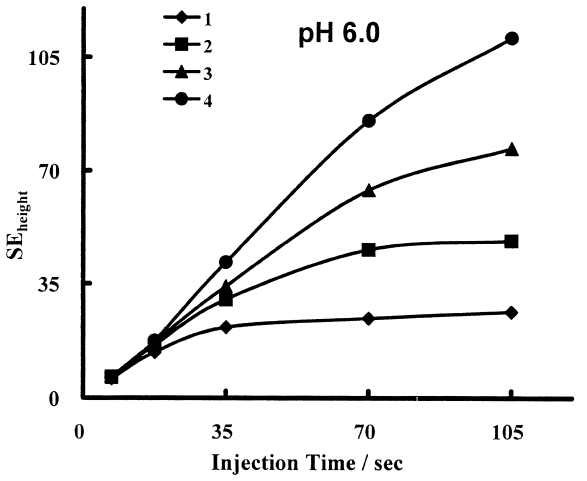

Fig. 4. Plots of SE_{height} versus injection time with a mixture of four methylthio-*s*-triazines at sample concentration of 1.0 μg/ml. Electrophoretic conditions are the same as for Fig. 1C, except sample concentration.

the SE_{height} using a mixture of four methylthio-*s*-triazines at a concentration of 1.0 μg/ml. As illustrated, the stacking efficiency of these test analytes increases linearly with increasing injection time up to about 70, 60, 35, and 20 s for terbutryn, prometryn, ametryn and simetryn, respectively, then increase gradually with further increasing injection time. Consequently, the maximum detection sensitivity of terbutryn, prometryn, ametryn, and simetryn are obtained with a duration of injection time of 70, 60, 35, and 20 s, respectively.

To demonstrate the stacking efficiency and the enhancement of detection sensitivity, Fig. 5 shows a typical electrophoreogram of the four methylthio-*s*-triazines obtained for a 30-s sample injection together with an electrophoreogram obtained for a 2.5-s injection under the optimal condition of usual injection time for comparison. Evidently, by the application of sweeping technique, the detection sensitivity of analytes can be greatly enhanced.

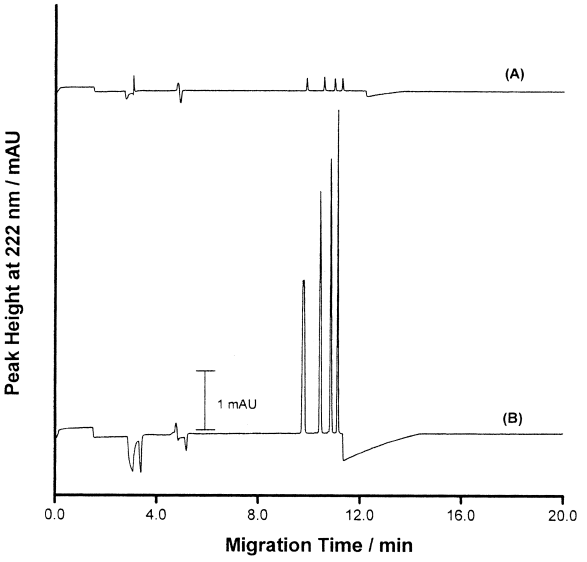

Fig. 5. Detection sensitivity of analytes measured under two different separation conditions: (A) without sample stacking (2.5-s injection with an injection pressure of 0.4 p.s.i.; sample concentration, 1.0 μg/ml); (B) with sweeping-stacking (30-s injection with an injection pressure of 1 p.s.i.; sample concentration, 1.0 μg/ml) Other operating conditions are the same as for Fig. 1C.

3.5. Peak width versus binding constant

It is of interest to note that, under the effective stacking conditions, the peak widths of these *s*-triazines increase in the order: simetryn>ametryn>prometryn>terbutryn. As the magnitudes of binding constants of methylthio-*s*-triazines to TTAB micelles also increase in the same order as for the peak width of the analytes [39], the dependence of the stacking of these analytes on their binding constants is evident. As a matter of fact, it is observed that the stronger the interaction between the analytes and the micelles, the narrower the peak width. As the binding constant of a sample analyte is linearly related to its retention factor, the result is qualitatively consistent with the finding obtained by Quirino and Terabe [22].

3.6. Detection limits and reproducibility

The limits of detection (LODs) at a signal-to-noise ratio (S/N) of 3, as well as the reproducibility of migration times and peak heights, for these *s*-triazines were determined. The migration times of these analytes were quite reproducible, with relative standard deviations (RSDs) varying in the range 0.8–1.0% ($n=8$). Variations of the peak height with RSDs less than 10.5% were obtained. The LODs determined for these methylthio-*s*-triazines with sample concentration in the range of 1000–50 ng/ml for a 30-s injection time are ranging from 9 ng/ml for simetryn to 15 ng/ml for terbutryn. Table 1 gives the data of analysis for these four analytes.

4. Conclusion

On-line concentration of neutral species of *s*-triazine herbicides in MEKC using a cationic surfactant is demonstrated. The stacking efficiency of analytes can be greatly enhanced by sweeping with addition of buffer electrolyte in the sample matrix and with an appropriate micelle concentration in the separation buffer. Reoptimization of separation parameters is necessary for a large-volume sample injection. For analytes with considerably different binding constants to the micelles, the optimal micelle concen-

Table 1
Limits of detection (LOD, $S/N=3$) and reproducibility of methylthio-*s*-triazines for 60-s injection time[a]

	Methylthio-*s*-triazines			
	Simetryn	Ametryn	Prometryn	Terbytryn
Equation of line	$y = 2.2324x - 0.0159$	$y = 2.3910x - 0.0232$	$y = 2.0220x - 0.0294$	$y = 1.9795x + 0.0488$
Coefficient of variation (R^2)	0.9998	0.9999	0.9991	0.9987
LOD (ng/ml)	13	9	14	15
RSD (%)				
Migration time	1.01	1.00	0.80	0.83
Peak height	8.7	9.1	9.4	10.5

[a] Sample matrix: 50 m*M* phosphate buffer containing less than 5% CH_3CN. Buffer electrolyte: 40 m*M* phosphate buffer containing 40 m*M* TTAB at pH 6.0.

tration for an efficient stacking may be different from one analyte to the other.

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Migration behavior and separation of tetracycline antibiotics by micellar electrokinetic chromatography

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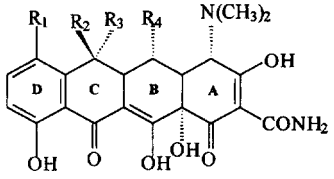
Abstract

The migration behavior and separation of six tetracyclines (TCs) were investigated by micellar electrokinetic chromatography (MEKC) in the pH range 5.0–9.0 using ammonium acetate buffer with the addition of sodium dodecyl sulfate (SDS). Mixed SDS–Brij 35, sodium cholate (SC) and tetradecyltrimethylammonium bromide (TTAB) were also used as surfactants. The influences of surfactant concentration and buffer pH on the separation of TCs were examined and the separations of TCs were optimized. Complete separation of six TCs was achieved within 8 min with 15 m*M* ammonium acetate buffer containing 20 m*M* SDS, with or without the addition of Brij 35 (0.135%, w/v), at pH 6.5 using a fused-silica capillary (42 cm×75 μm I.D.) at 15 kV. In general, good linear correlations of the logarithm of migration factor (log k') versus the logarithm of octanol–water partition coefficient (log P_{ow}) in these micellar systems, except for the TTAB–MEKC system, were obtained. The results indicate that the migration of TCs in MEKC is mainly based on hydrophobic interactions. However, hydrogen bonding interactions also play a significant role in influencing the chemical selectivity of TCs. In addition, the micelle–water partition coefficients (P_{mw}) of TCs, which are pH-dependent in the SDS–MEKC micellar system, are reported. © 1998 Elsevier Science B.V.

Keywords: Buffer composition; Tetracyclines; Antibiotics

1. Introduction

Tetracycline antibiotics are extensively used to control bacterial infections in both humans and animals. In addition to therapeutical use, these drugs are widely used as a feed supplement in animal husbandry. The structures of tetracyclines (TCs) studied are schematically shown in Fig. 1. These compounds possess multiple functional groups with acid–base properties: the first acid dissociation constant of tetracyclines, with a pK_a value of approximately 3.3, is attributed to the hydroxyl group of the tricarbonyl system in ring A; the second acid dissociation constant, with a pK_a value of approximate-



Tetracyclines	R_1	R_2	R_3	R_4
(1) Oxytetracycline (OTC)	H	OH	CH_3	OH
(2) Tetracycline (TC)	H	OH	CH_3	H
(3) Demeclocycline (DMC)	Cl	OH	H	H
(4) Chlortetracycline (CTC)	Cl	OH	CH_3	H
(5) Doxycycline (DOC)	H	H	CH_3	OH
(6) Minocycline (MNC)	$N(CH_3)_2$	H	H	H

Fig. 1. Structures of the tetracyclines studied.

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ly 7.6, is associated with the hydroxyl group of the dicarbonyl system between rings B and C; the third, with a pK_a value of approximately 9.7, is assigned to the amino group of the dimethylamino moiety in ring A; the fourth, with a pK_a value in the range 10.7–12.0, is associated with the phenolic hydroxyl group in ring D [1,2]. Accordingly, most TCs exhibit amphoteric character with an isoelectric point between 4 and 6. Thus, TCs exist in cationic form at more acidic pH values, in anionic form at more alkaline pH values, and in zwitterionic form at a pH near the isoelectric point, and depending on the pH of the buffer, can be separated as cations at low pH point as zwitterions near neutral pH, or as anions at an alkaline pH.

Tetracycline degrades to anhydrotetracycline (ATC) in acidic media at pH<2 [1]. TC and ATC epimerize to form 4-epitetracycline (ETC) and 4-epianhydrotetracycline (EATC), respectively, at pH 2–6 [1,3]. Likewise, other tetracyclines, such as oxytetracycline (OTC) and demeclocycline (DMC), also form degradation products [4]. Since commercially available TCs may contain some significant amounts of degradation products and EATC is shown to be renal toxic, the separation and monitoring of TCs and their impurities have drawn much attention.

High-performance liquid chromatography (HPLC) has been employed for separating and analyzing various TC mixtures over the past decades [5–11], despite the difficulties associated with peak tailing and low efficiency due to interaction with the residual silanol groups on silica-base packing material. In recent years, capillary electrophoresis (CE) has gained increased importance as a powerful analytical tool [12–17]. This is due to its many advantageous features such as extremely high efficiency, high resolution, short analysis time and small consumption of sample and solvent volume in comparison with HPLC.

TC and its degradation products were efficiently separated by capillary zone electrophoresis (CZE) using either a 20 m*M* phosphate buffer (pH 3.9) [18] or a basic 80 m*M* carbonate buffer (pH 9.0) [19], with the addition of 1–5 m*M* disodium ethylenediamine tetraacetate (EDTA), as a complexing agent. The analysis of DMC and its major impurities was investigated by adding Triton X-100 (0.35%, v/v) to a 50 m*M* phosphate running buffer containing 1 m*M* EDTA at pH 11.50 [4]. The electrophoretic behavior of seven tetracycline antibiotics, including TC, OTC, chlortetracycline (CTC), doxycyclines (DOC), DMC, methacycline (MC) and minocycline (MNC) has been characterized by CE using a phosphate buffer solution in the pH range 4–11 [20]. The optimum conditions for separating a mixture of TCs determined were: buffer pH, 7.5; buffer concentration, 4.3 m*M* and ionic strength, 18.2 m*M*. However, complete separation of these TCs was not achievable under these conditions [20]. Effective separation of various TCs, with the exception of DOC and MC, was readily achieved by CZE using a background electrolyte composed of 30 m*M* citric acid, 24.5 m*M* β-analine and methanol (40%, v/v) at pH 3.0 [21]. Moreover, the separations of impurities and degradation products of various TCs by CE and capillary electrochromatography (CEC) were compared [21]. The complete separation of four commercially available TCs (TC, OTC, DOC and CTC) by nonaqueous CE using methanol–acetonitrile (50:50, v/v) electrophoresis medium with the addition of different electrolytes was also demonstrated [22].

On the other hand, reports on the separation of tetracyclines by micellar electrokinetic capillary chromatography (MEKC) were few. Croubels et al. [23] analyzed TC, OTC, CTC and the degradation products of TC by adding nonionic surfactants such as Triton X-100 (0.05%) and Brij 35 (0.017%) to a phosphate buffer solution at pH 2.2 to improve the separation. However, complete separation of these tetracyclines was not achieved with the use of phosphate buffer as a background electrolyte. A method was developed for the determination of four tetracyclines, including TC, OTC, CTC and DOC, in bovine milk, serum and urine using a borate–phosphate buffer solution containing 10 m*M* sodium dodecyl sulfate (SDS) at pH 8.5 [24] with limits of detection down to a few ppb.

In this paper, we report the results of the separation of six tetracyclines by MEKC using an ammonium acetate buffer containing three different structural types of anionic surfactant and a cationic surfactant as micelles. The influences of buffer pH in the pH range 5.0–9.0 (for SDS only) and surfactant concentration, as well as the choice of background

electrolyte, on the separation of TCs are examined. The partition coefficients of tetracyclines to SDS micelles are evaluated and the migration order of tetracyclines was discussed based on quantitative structure-retention relationships (QSRRs).

2. Experimental

2.1. Chemicals and reagents

Six tetracyclines, including TC, OTC, CTC, DOC, MNC and DMC, SDS and sodium cholate and Sudan III were obtained from Sigma (St. Louis, MO, USA). Polyoxyethylene (23) dodecyl ether (Brij 35), tetradecyltrimethylammonium bromide (TTAB), ammonium acetate, and quinine hydrochloride were obtained from Aldrich (Milwaukee, WI, USA), TCI (Tokyo, Japan), Showa (Kyoto, Japan) and Kanto (Tokyo, Japan), respectively. All other chemicals were of analytical-reagent grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard stock solutions of six tetracyclines in aqueous solution at a concentration of 1000 μg/ml were prepared. When needed, various concentrations of sample solution ranging from 10–50 μg/ml were diluted from stock solution. Various proportions of 1 *M* acetic acid or 1 *M* trisodium phosphate were added to the ammonium acetate solution to reach a desired value in the pH range 5.0–9.0. All solutions were filtered through a membrane filter (0.22 μm) before use.

2.2. Apparatus

Separations were made with a CE system described previously [25]. The capillary dimensions were 43 cm×75 μm, I.D. The UV detection position was 7.0 cm from the cathodic end. Sample injection was done in a hydrodynamic mode for 1 s. The CE system was interfaced with a microcomputer and printer with software CE 1000 1.05 A. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of 0.01 pH unit.

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed using a standard sequence described previously [25]. To ensure reproducibility, all experiments were performed at 25°C and measurements were run at least in triplicate. The capillary was prewashed with running buffer for 2 min before each injection and postwashed with deionized water at 25°C for 5 min, followed with 0.1 *M* sodium hydroxide solution at 60°C for 5 min, and then with deionized water at 25°C for 5 min to maintain proper reproducibility for run-to-run injections. The detection wavelength was set at 265 nm.

2.4. Calculations

2.4.1. Electrophoretic mobility

The electrophoretic mobility of analytes was calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \quad (1)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an uncharged solute (dimethyl sulfoxide as neutral marker), L_t is the total length of capillary, L_d is the length of capillary between injection and detection, and V is the applied voltage.

2.4.2. Migration factor

The migration factor of analytes (k') was calculated from the observed migration times with the equation:

$$k' = \frac{t_m - t_0}{t_0\left(1 - \frac{t_m}{t_{mc}}\right)} \quad (2)$$

where t_o is the migration time in the absence of micelles, and t_{mc} is the migration time of micelles,

$$k' = \frac{(\mu_{ep} - \mu_0)}{(\mu_{mc} - \mu_{ep})} \quad (3)$$

where μ_{ep}, μ_o, and μ_{mc} are the electrophoretic

mobility of a solute, the electrophoretic mobility of a solute in the absence of micelles, and the electrophoretic mobility of micelle, respectively, calculated from the corresponding migration times.

In this work, quinine hydrochloride was used as a micelle marker for SDS and SDS–Brij 35, whereas Sudan III was used for SC and TTAB.

2.4.3. Partition coefficient

The migration factor (k') in MEKC is directly proportional to the micelle concentration through the following equation [26,27]:

$$k' = P_{mw}\nu([S] - CMC) \quad (4)$$

where P_{mw} is the partition coefficient of solutes between the aqueous and micellar phases, ν is the molar volume of the surfactant, [S] is the total surfactant concentration and CMC is the critical micellar concentration. With SDS as an anionic surfactant, ν is equal to 0.2483 l mol^{-1} [28].

3. Results and discussion

3.1. Choice of background electrolyte

As reported previously [23], complete separation of tetracyclines is difficult to achieve using a phosphate buffer containing different types of surfactants, such as SDS, cetyltrimethylammonium bromide (CTAB) and bile salt. In this work, for separating tetracyclines at a pH near their isoelectric points, ammonium acetate was selected as a running buffer. The optimum concentration of ammonium acetate buffer was determined to be 15 m*M*.

3.2. Influence of SDS concentration

Fig. 2 shows the variation of the electrophoretic mobility of tetracyclines as a function of SDS concentration at pH 6.0. The electrophoretic mobility of TCs (migrating toward anode) increases with SDS concentration. The electroosmotic flow decreases with increasing concentration of SDS, but the mobility of micelle is almost constant at varied concentration of SDS. Consequently, the migration windows become broader with increasing SDS concentration. The resolution of tetracyclines increases with increasing concentration of SDS, and the migration time also increases. So the optimum SDS concentration was determined to be about 15–20 m*M*.

3.3. Influence of buffer pH

Fig. 3 shows the variation of the electrophoretic mobility as a function of buffer pH in the range 5.0–9.0. The electrophoretic mobility of each individual tetracycline (migrating toward anode) decreased, but the electroosmotic flow increased, as the pH of the buffer increased. The variation in the electrophoretic mobility as a function of pH for tetracyclines having the hydroxyl group as the R_2 substituent, such as OTC, TC, DMC and CTC is considerably smaller than those of tetracyclines with a hydrogen atom as the R_2 substituent, such as DOC and MNC, thus leading the electrophoretic mobility curves of DOC and CTC to cross over at a pH of about 5.3. Fig. 4 shows the electropherograms of tetracyclines obtained in the SDS micellar system at pH 5.0 and 6.5. The reversal of the migration order of DOC and CTC was observed at these two pH values and a complete separation of six tetracyclines was achieved within 8 min at pH 6.5 with the migration following the order OTC<TC<DMC<DOC<CTC<MNC.

The marked decrease in the electrophoretic mobility of TCs with increasing buffer pH is believed partly due to the decrease in the mole fractions of positively charged species and neutral species and the increase in the mole fraction of negatively charged species, and partly due to the drastic decrease in the binding constants of TCs to micelles which will be described in Section 3.5.

3.4. Influence of concentration of other surfactants

Fig. 5 shows the variation of the electrophoretic mobility of TCs in a mixed micellar system containing 20 m*M* SDS and Brij 35 (up to 0.135%, w/v) at pH 6.5. The electrophoretic mobility of TCs (migrating toward anode) decreased with increasing concentration of Brij 35. This is expected owing to the decrease in the anionic charges of the SDS micelles on addition of Brij 35 [29,30].

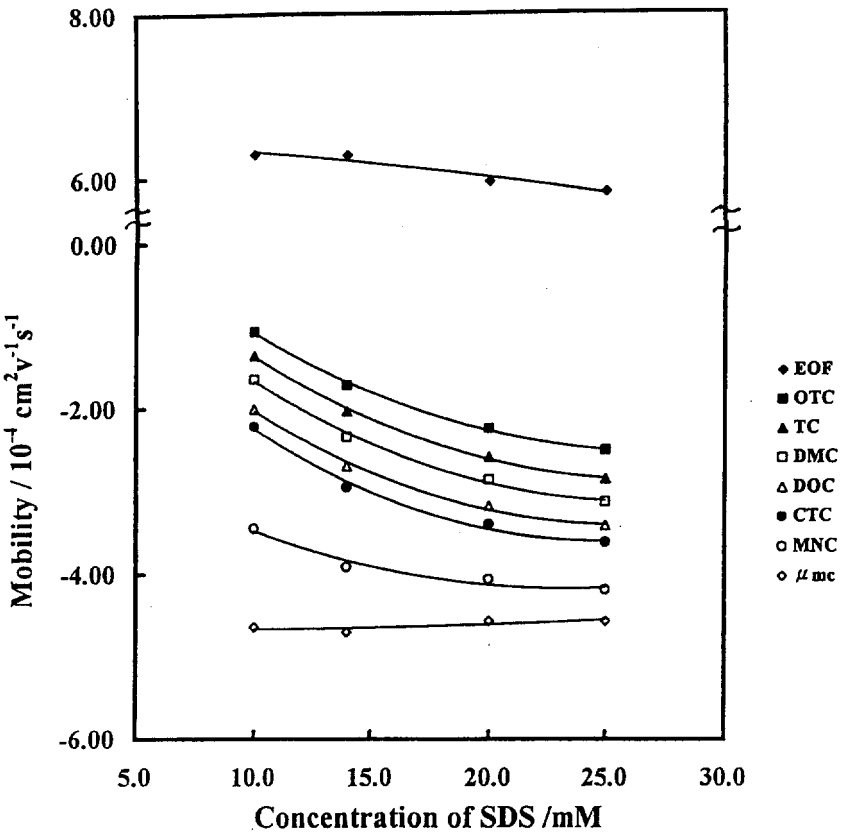

Fig. 2. Variation of the electrophoretic mobility of tetracyclines as a function of SDS concentration using 15 m*M* ammonium acetate buffer (pH 6.0). Capillary: 43 cm×50 μm, I.D., Other operating conditions: 15 kV, 25°C. Curve identification: (■) OTC, (▲) TC, (□) DMC, (△) DOC, (●) CTC, (○) MNC; the numbers of the analytes are shown in Fig. 1.

It is interesting that the variation in the electrophoretic mobility of TCs with the hydroxyl group as the R_2 substituent, such as OTC, TC, DMC and CTC, which are relatively less hydrophobic than DOC and MNC, is greater than those of TCs with a hydrogen atom as the R_2 substituent, such as the aforementioned DOC and MNC. Evidently, the addition of Brij 35 to the SDS system would decrease the electrostatic interactions between TCs and micelles, and the migration behavior of TCs in mixed micelles, as shown in Fig. 6, is understandable. The migration of TCs follows the order OTC< TC<DMC<CTC<DOC<MNC in the mixed SDS–Brij 35 micellar system.

Fig. 7 shows the variation of the electrophoretic mobility of TCs in the SC micellar system at pH 6.5. As in the case of the SDS system, the electrophoretic mobility of TCs (migrating toward anode) increased and the migration window became broader with increasing concentration of SC from 30 m*M* to 80 m*M*.

Complete separation of OTC and TC was achievable when the concentration of SC was raised above 70 m*M*. However, peaks of DMC and CTC are not resolved even with the concentration of SC at 80 m*M*. To avoid any complication caused by Joule heating, separation with a concentration of SC greater than 80 m*M* was not attempted. Fig. 8 shows the electropherogram of TCs obtained in the SC micellar system at pH 6.5.

Complete separation of TCs with ammonium acetate buffer in the TTAB micellar system is difficult. Peaks of CTC, DOC and MNC migrate together in the buffer system containing 10–30 m*M*

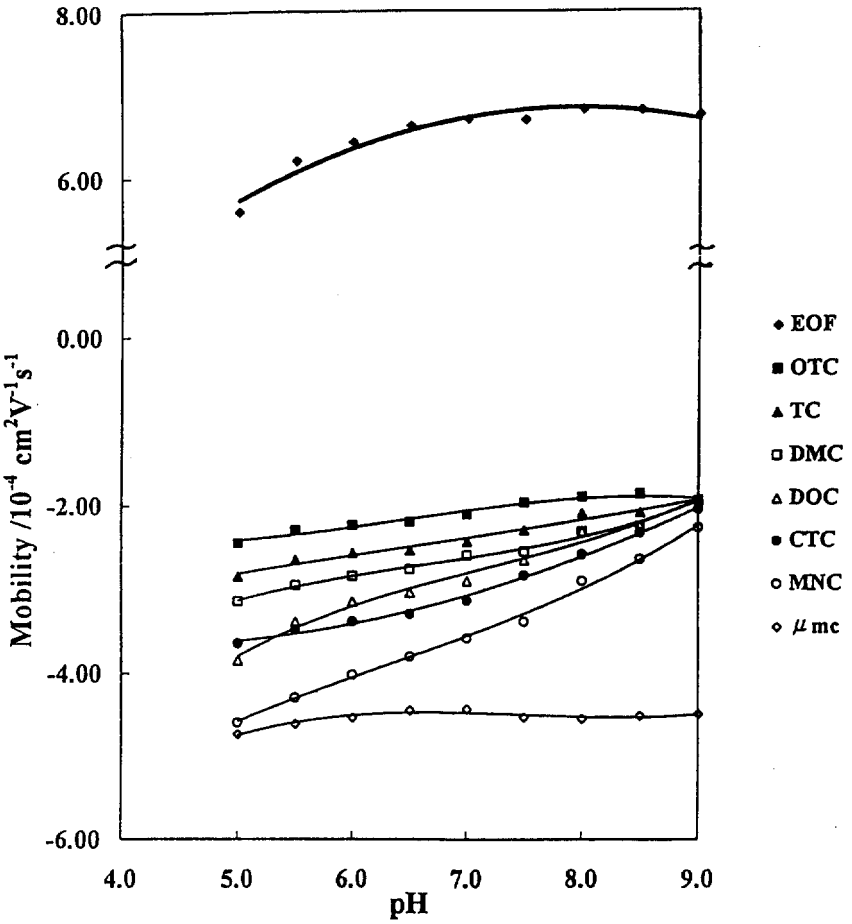

Fig. 3. Variation of the electrophoretic mobility of tetracyclines as a function of pH in the range 5.0–9.0 using 15 m*M* ammonium acetate buffer containing 20 m*M* SDS. Other operating conditions and peak identification are the same as for Fig. 2.

TTAB. Peaks of CTC and DOC are not separable even with the addition of acetonitrile (10%, v/v) or methanol (30%, v/v). Fig. 9 shows the electropherogram of TCs obtained in the TTAB micellar system at pH 6.5.

3.5. Partition coefficient

Fig. 10 shows the plots of capacity factor (k') calculated from Eq. (2) for various TCs versus SDS concentration. The partition coefficients of tetracyclines between aqueous and micellar phases (P_{mw}) were determined according to Eq. (4). Table 1 lists the partition coefficients (P_{mw}), capacity factors (k') and CMC values obtained for individual tetracycline in the SDS micellar system at pH 6.0. The average CMC value is 4.5±0.2 m*M*. As expected, this is smaller than the literature value of 8.2 m*M* owing to the presence of buffer electrolyte.

Table 2 gives the partition coefficients (P_{mw}) evaluated at five different pH values. The partition coefficient (P_{mw}) of individual tetracycline, which is pH-dependent, decreases quite drastically with increasing pH of the buffer. This is expected because the charge density of tetracyclines becomes more negative as the pH of the buffer increases from 6.0 to 8.0.

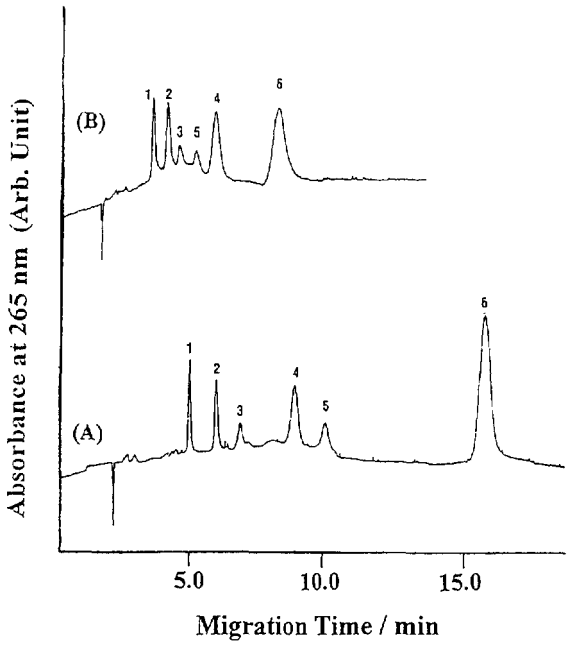

Fig. 4. Electropherograms of tetracyclines obtained with 15 m*M* ammonium acetate buffer containing 20 m*M* SDS at varied pH: (A) 5.0, (B) 6.5. Other operating conditions and peak identification as in Fig. 2.

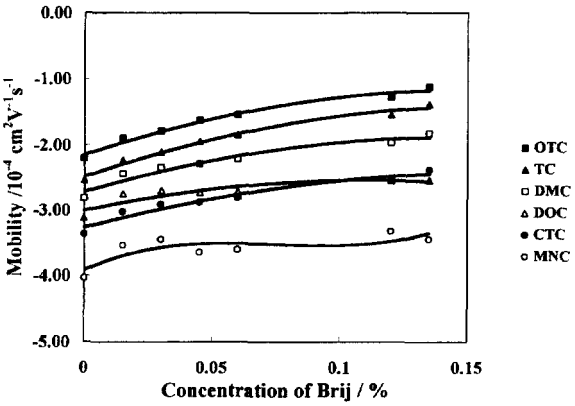

Fig. 5. Variation of the electrophoretic mobility of tetracyclines in a mixed micellar system containing 20 m*M* SDS and Brij 35 (up to 0.135%, w/v) with 15 m*M* ammonium acetate buffer (pH 6.5). Other operating conditions and peak identification as in Fig. 2.

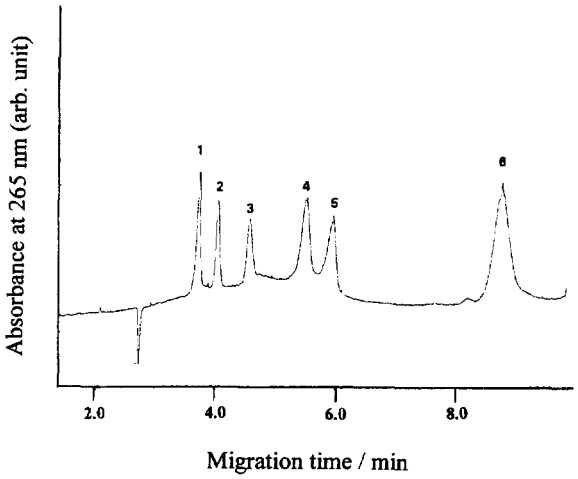

Fig. 6. Electropherogram of tetracyclines obtained with 15 m*M* ammonium acetate buffer containing 20 m*M* SDS and Brij 35 (0.135%, w/v) at pH 6.5. Other operating conditions and peak identification as in Fig. 2.

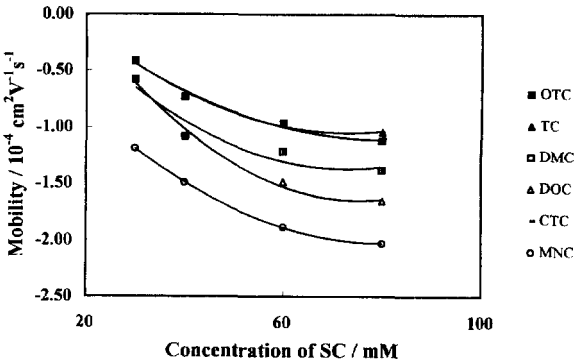

Fig. 7. Variation of the electrophoretic mobility of tetracyclines as a function of SC concentration using 15 m*M* ammonium acetate buffer (pH 6.5). Applied voltage: 12 kV. Other operating conditions and peak identification as in Fig. 2.

3.6. Migration order and the correlation of migration factor versus octanol–water partition coefficient

It has been generally accepted that separation of neutral and charged solutes in MEKC is based on the differential partitioning of solutes between aqueous phase and micellar phase in which hydrophobic

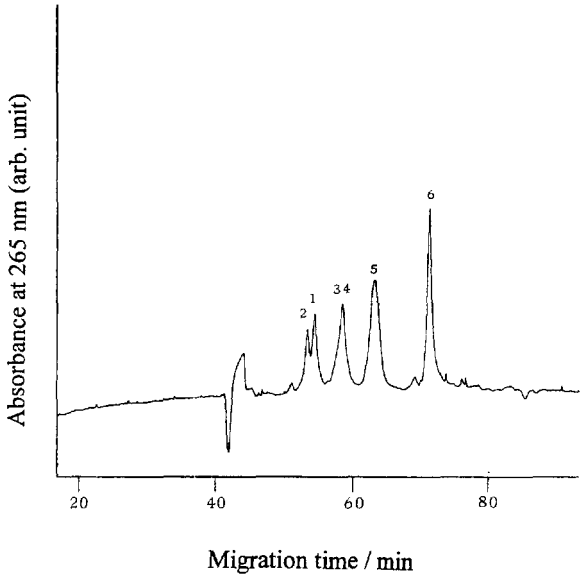

Fig. 8. Electropherogram of tetracyclines obtained with 15 m*M* ammonium acetate buffer containing 80 m*M* SC (pH 6.5). Other operating conditions and peak identification as in Fig. 7.

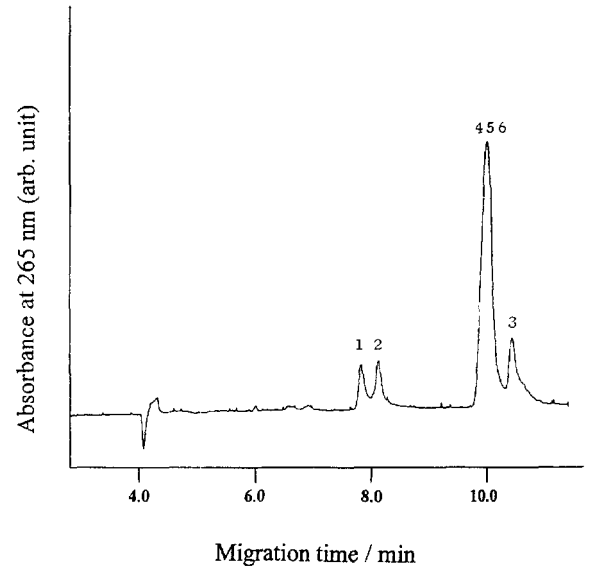

Fig. 9. Electropherogram of tetracyclines obtained with 30 m*M* ammonium acetate buffer containing 30 m*M* TTAB (pH 6.5). Applied voltage: −10 kV. Other operating conditions and peak identification as in Fig. 2.

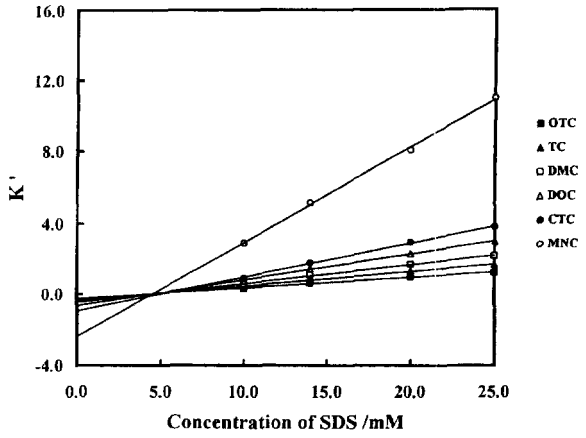

Fig. 10. Plots of migration factor (k') of tetracyclines as a function of SDS concentration at pH 6.0.

Table 1
Partition coefficients, capacity factor and CMC values of tetracyclines in SDS micellar system at pH 6.0

Tetracyclines	P_{mw}	k'	CMC (m*M*)	P_{ow}
OTC	240	0.96	4.32	0.08[a]
TC	330	1.31	4.58	0.09[b]
DMC	429	1.61	4.56	0.25[b]
DOC	587	2.11	4.55	0.95[a]
CTC	766	2.76	4.94	0.41[a]
MNC	3110	8.06	3.07	1.12[a]

[a] [37].
[b] [38].

interaction is the sole underlying force that influences the migration behavior of hydrophobic solutes. However, large differences in the migration behavior of solutes in MEKC with different types of surfactants suggest that this general belief is not accurate for all MEKC systems [31,32]. Depending on the chemical nature of both solutes and micelles, various chemical interactions other than hydrophobic interactions, such as dipolar or hydrogen bonding interactions, may occur between them in the partitioning process [31–33]. These interactions influence the migration behavior of solutes with various functional groups to a different extent, thus resulting in the differentiation of the selectivity and the alteration of the migration order of solutes.

In order to shed light on the migration order of tetracyclines, quantitative structure–retention relationship (QSRR) that describe the correlations be-

Table 2
Partition coefficients of tetracyclines measured with ammonium acetate–SDS buffer system at varied pH values

Tetracyclines	P_{mw}				
	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0
OTC	240	244	201	117	62
TC	330	335	282	181	78
DMC	429	394	292	242	66
DOC	587	541	423	270	89
CTC	766	729	540	276	133
MNC	3110	1258	889	367	300

tween migration factor and octanol–water partition coefficient, a hydrophobic parameter, were examined in various micellar systems to better understand the underlying chemical interactions that influence the migration and selectivity of tetracyclines. In this study, we selected the SDS, SDS–Brij 35, SC and TTAB micellar systems.

For a micellar system in MEKC 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 capacity factor ($\log k'$) and the logarithm of octanol–water partition coefficient ($\log P_{ow}$) as found in [34–36]:

$$\log k' = a \log P_{ow} + b \quad (5)$$

It has been generally accepted that the migration of solutes in MEKC correlates best with P_{ow} if the micellar system used has a similar hydrogen bonding affinity to 1-octanol [31,32]. Based on the values of P_{ow} of tetracyclines reported in the literature [37,38] and the capacity factor (k') of TCs calculated in this work, the plots of $\log k'$ vs. $\log P_{ow}$ in four different micellar system at pH 6.5, as shown in Fig. 11, were obtained. The correlation coefficient (r^2) for the SDS, SDS–Brij 35 and SC micellar systems are high. However, a poor correlation was observed for the TTAB system. Table 3 lists the results of a $\log k'$ vs. $\log P_{ow}$ linear regression.

As the TTAB micelles have the most hydrogen bond acceptor characteristics among the four micellar systems used in this study [31,32], it is not surprising that the overall trend of the migration behavior of TCs in MEKC with TTAB micelles does not correlate well with the values of $\log P_{ow}$, because tetracyclines also possess hydrogen bond acceptor characteristics. The reasons for the abnormally large electrophoretic mobility of DMC in the TTAB-MEKC system are not clear. As indicated in Table 3, the correlation is greatly improved when DMC is eliminated from sample solutes.

The hydrogen bond acceptor characteristics of SC are weaker than those of TTAB, but stronger than for 1-octanol. The existence of higher correlation for the SC system is attributed to a similar hydrogen bonding pattern between SC micelles and 1-octanol. This result is consistent with the findings obtained by Yang et al. [32,39].

The correlation of $\log k'$ vs. $\log P_{ow}$ in the SDS–MEKC system is not as good as for the SC system. This is attributed to the lower similarity in the hydrogen bonding pattern between SDS micelles and 1-octanol compared with that between SC micelles and 1-octanol. Moreover, the existence of congenerity problems in the SDS–MEKC system may cause the situation to be even worse [31,32]. However, as SDS micelles are stronger hydrogen bond donors than 1-octanol, they exhibit more selective interactions towards tetracyclines which have hydrogen bond acceptor characteristics.

The addition of Brij 35 to the SDS micellar system resulted in higher correlation. This is expected because the anionic charges located at the surface of the SDS micelles are shielded on addition of Brij 35, which has a long polyoxyethylene chain [40]. Therefore, excellent correlation of $\log k'$ vs. $\log P_{ow}$ in the mixed SDS–Brij 35 micellar system (with $r^2 > 0.942$) can be obtained.

As large differences in the migration behavior and selectivity for TCs in MEKC with different types of surfactants were observed and good linear correlations of $\log k'$ vs. $\log P_{ow}$ were obtained, the results reveal that the migration of TCs depends primarily

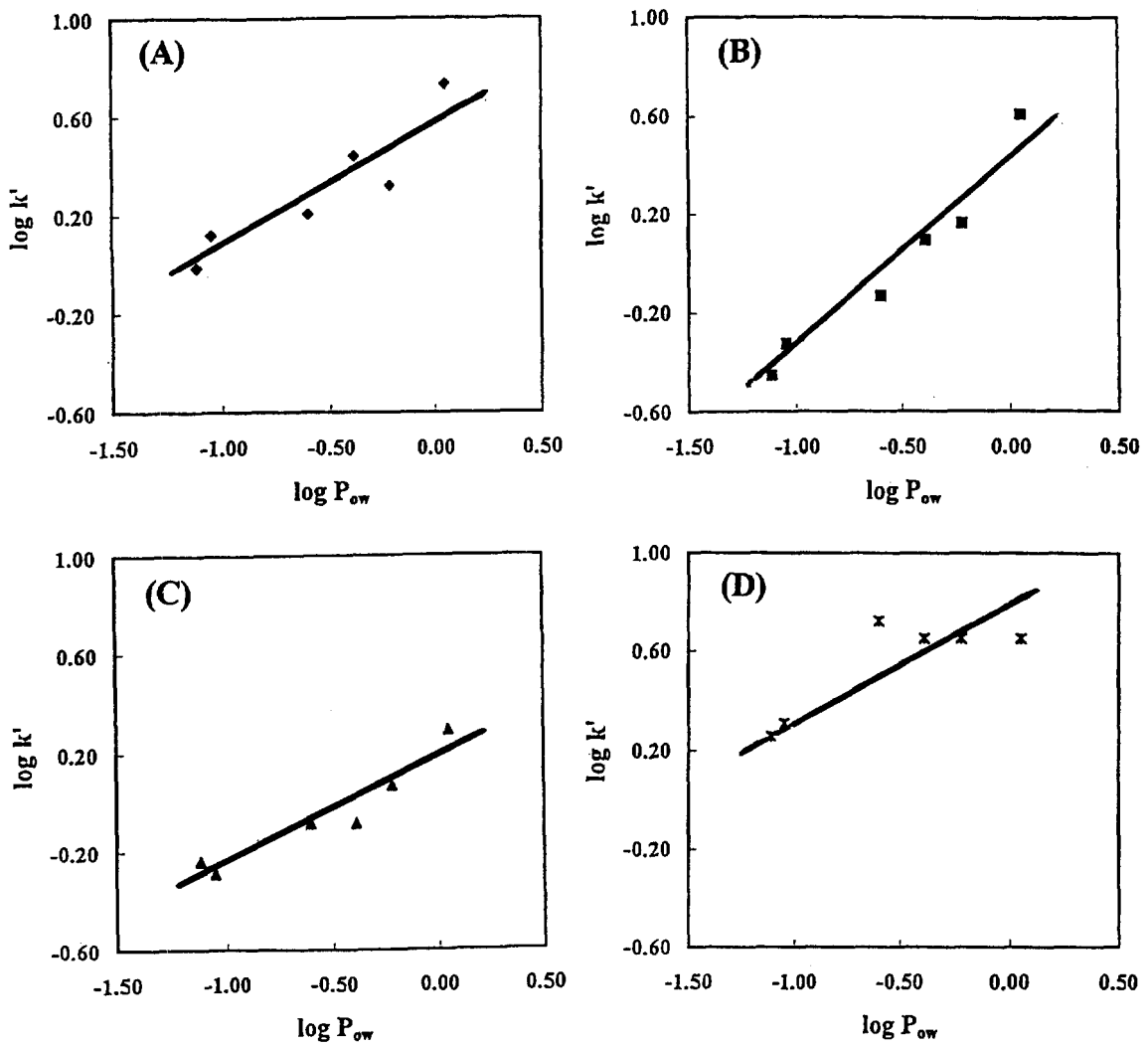

Fig. 11. Correlations of log k' versus log P_{ow} for tetracyclines in four different micellar systems at pH 6.5: (A), 20 m*M* SDS; (B), 20 m*M* SDS–Brij (0.135%, w/v); (C), 80 m*M* SC; (D), 30 m*M* TTAB.

on the extent of micellar solubilization based on hydrophobic interactions; however, hydrogen bonding interactions may play an important role in these micellar system.

4. Conclusion

Complete separation of six tetracyclines was achieved using ammonium acetate buffer with the addition of SDS or mixed SDS–Brij35 at pH 6.5 with an applied voltage of 15 kV. The results of present studies indicate that the migration of TCs in MEKC is primarily based on hydrophobic interaction. However, electrostatic interaction, which is primarily due to hydrogen bonding interaction, may play a significant role in influencing the selectivity of TCs, thus leading to the alteration of the migration order of TCs in MEKC with different structural types of surfactants.

Table 3
Quantitative relationships between hydrophobicity and migration factors of tetracyclines in MEKC

Surfactant	n	$\log k' = a \log P_{o/w} + b$		
		a	b	r^2
SDS	6	0.53	0.60	0.855
	5[a]	0.60	0.67	0.949
SDS–Brij 35	6	0.80	0.44	0.942
	5[b]	0.65	0.31	0.972
SC	6	0.43	0.18	0.901
	5[c]	0.45	0.21	0.957
TTAB	6	0.37	0.74	0.696
	5[d]	0.38	0.71	0.906

[a] Sample without DOC.
[b] Sample without MNC.
[c] Sample without CTC.
[d] Sample without DMC.

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A capillary electrophoresis study on the influence of β-cyclodextrin on the critical micelle concentration of sodium dodecyl sulfate

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Abstract

The influence of β-cyclodextrin (β-CD) on the critical micelle concentration (CMC) of sodium dodecyl sulfate (SDS) was investigated by capillary electrophoresis using anionic chlorophenols as probe molecules at pH 7.0. The variations of the electrophoretic mobility of probe molecules as a function of surfactant concentration in both premicellar and micellar regions in the absence and presence of β-CD was analyzed. The results indicate that, as a consequence of a strong inclusion complexation between β-CD and SDS, the encapsulation of β-CD with probe molecules is greatly diminished, or even vanished, in the presence of SDS. The complexes formed between β-CD and SDS monomers exist predominantly in the form of a 1:1 stoichiometry, while the complexes with a 2:1 stoichiometry reported previously in the literature as a minor component may exist by less than 10%. The elevation of the CMC value of SDS depends not only on the concentration of β-CD in the buffer electrolyte but also on methanol content in the sample solution. The binding constants of probe molecules to β-CD, to surfactant molecules, and to the complexes formed between β-CD and SDS are reported.

Keywords: Critical micellar concentration; Binding constants; Cyclodextrins; Sodium dodecylsulfate; Chlorophenols

1. Introduction

In capillary electrophoresis (CE), surfactants are added either as an electrolyte modifier in the buffer electrolyte in capillary zone electrophoresis (CZE) or used as a pseudo-stationary phase in the separation media in micellar electrokinetic chromatography (MEKC). Thus, a good knowledge of the critical micelle concentration (CMC) of a surfactant is desirable in order to optimize the separation. Various methods, including conductivity [1–4], surface tension [5], light scattering [6,7], spectrophotometry [3,4,8], cyclic voltammetry [9], NMR [10], speed of sound [11], and CE [12–18], were used to determine the CMC values of surfactants. Among them, CE has proven to be a convenient and useful technique for such a measurement. As the CMC value of a surfactant depends on the nature and operating conditions of the buffer electrolyte, e.g. ionic strength, temperature, buffer pH, additive of buffer electrolytes, etc., CE can be conveniently applied to a buffer system containing any electrolyte modifiers.

Three different approaches based on the CE technique have been proposed. The method based on the linear

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relationship of retention factor with micelle concentration was first introduced to yield the CMC value of a surfactant in MEKC [12,13]. However, the CMC values determined by this method were found to be unreliable in some occasions [12,13]. The second approach based on the variation of the effective electrophoretic mobility of a solute as a function of surfactant concentration in the premicellar and micellar regions was proposed [14,15]. Recently, the third method based on the measurements of the electric current of an electrolyte system as a function of surfactant concentration using CE instrumentation at a given voltage was suggested [17]. However, the drawback of this method is that the slopes of the straight lines corresponding to the premicellar and micellar states of surfactant in the concentration range studied need to be different so that the CMC value can be unambiguously determined.

Cyclodextrins (CDs) form inclusion complexes with a variety of hydrophobic and hydrophilic species [19,20], including surfactant molecules. Inclusion complexes with a 1:1 stoichiometry are usually assumed, although inclusion complexes with a 2:1 stoichiometry may occasionally be reported [21].

Cyclodextrins form inclusion complexes with sodium dodecyl sulfate (SDS) [11,22–27]. The addition of β-CD to the premicellar solution system appears to encapsulate surfactant molecules and to shift the equilibrium in favor of the formation of inclusion complexes between surfactant monomers and β-CD, thus, influencing considerably the micellization of surfactant molecules [28]. As a consequence, the CMC value of a surfactant increases with increasing concentration of β-CD [17,22,29–36].

Despite the fact that the CMC value of SDS was determined to be 14.8 m*M* by CE in the presence of 10 m*M* β-CD [17], the interaction of β-CD with SDS and the influence of β-CD on the micellization of SDS is not clearly understood. In this work, pentachlorophenol and 2,3,4,6-tetrachlorophenol are selected as probe molecules. The variations of the effective electrophoretic mobility of these two probe molecules as a function of surfactant concentration in the premicellar and micellar regions in the absence and in the presence of β-CD are analyzed and the binding constants of probe molecules to β-CD and to surfactant molecules are evaluated. The influence of β-CD on the CMC value of SDS and the interaction of β-CD with probe molecules and SDS can thus, be better understood. Moreover, as probe molecules are dissolved in an aqueous solution containing methanol, the influence of methanol content in the sample solution on the CMC value of SDS in the presence of β-CD is also examined.

2. Experimental

2.1. Apparatus

All CE experiments were performed on a Beckman P/ACE System MDQ with a photodiode array detector (Beckman Coulter, Fullerton, CA, USA). Uncoated fused-silica capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were used. The dimensions of the capillary are 50.2 cm×50 μm I.D. The effective length of the capillary is 40 cm from the injection end of the capillary. The CE system was interfaced with a microcomputer and a laser printer. System MDQ software (version 1.0.019) of Beckman was used for data acquisition. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of ±0.01 pH unit.

2.2. Chemicals and reagents

Pentachlorophenol (PCP) and 2,3,4,6-tetrachlorophenol (TTCP) were purchased from Tokyo Kasei Kogyo (TCI, Tokyo, Japan). β-CD and SDS were obtained from Merck (Germany). Sudam III, used as a micelle marker, was supplied by Janssen (Belgium). Methanol, used as a neutral marker, is of HPLC grade purchased from Mallinckrodt (USA). All other chemicals were of analytical grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Sample solutions were prepared by dissolving chlorophenols at a concentration of 10 μg/ml in an aqueous solution containing 5–30% methanol. The pH of a phosphate buffer was adjusted to the desired pH value (pH 7.0) by mixing various proportions of a certain concentration of sodium dihydrogenphosphate solution with the same concentration of disodium hydrogenphosphate solution. All buffer solutions, which contained various concentrations of SDS and β-CD and were freshly prepared weekly and stored in a refrigerator before use, were filtered through a membrane filter (0.22 μm).

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed 30 min with 1.0 *M* NaOH solution, followed by 30 min with deionized water at 25. Before each injection, the capillary was prewashed for 8 min with running buffer and postwashed for 5 min with deionized water, 5 min with 1.0 *M* NaOH, 5 min with 0.1 *M* NaOH, and 5 min with deionized water to maintain proper reproducibility of run-to-run injections. Sample injections were done in a hydrodynamic mode over 3.5 s under a pressure of 1.0 p.s.i. (1 p.s.i.=6894.76 Pa). The measurements were run at least in triplicate to ensure reproducibility. An applied voltage of 20 kV was selected to keep the total current less than 100 μA in order to avoid experimental complications resulting from Joule heating. The detection wavelength was set at 215 nm. The relative standard deviation of migration time is less than 0.6% (n=5).

2.4. Mobility calculations

The electrophoretic mobility of analytes was calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V} \cdot \left(\frac{1}{t_m} - \frac{1}{t_{eo}} \right)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an unchanged solute, L_t is the total length of capillary, L_d is the length of capillary between injection and detection, and V is the applied voltage.

2.5. Evaluation of binding constants

Theoretical equations for expressing the effective electrophoretic mobility of probe molecules as a function of either β-CD concentration and/or SDS concentration are derived and described in Section 3. The binding constants of probe molecules to β-CD, to SDS monomers, to SDS micelles and to the complexes formed between β-CD and SDS monomers were then evaluated by curve-fitting the predicted mobility data with the experimental mobility data through the utilization of Excel software.

3. Theoretical considerations on electrophoretic mobility

3.1. Influence of cyclodextrins

In an electrophoretic buffer system containing β-CD, the effective electrophoretic mobility of a fully dissociated species of a probe molecule can be phenomenologically expressed as:

$$\mu_{eff} = \alpha_{A^-} \mu_{A^-} + \alpha_{A^- \cdot CD} \mu_{A^- \cdot CD} \quad (1)$$

where α and μ denote the mole fraction and electrophoretic mobility of the ionic species involved. Thus, μ_{A^-} and $\mu_{A^-\cdot CD}$ represent the limiting electrophoretic mobility of the anionic species of a probe molecule (A^-) and the inclusion complex ($A^-\cdot CD$) formed between A^- and CD, respectively. The mole fractions α_{A^-} and $\alpha_{A^-\cdot CD}$, respectively, can be more specifically expressed in terms of the concentration of free CD molecules ([CD]), or the total concentration of CD molecules ($[CD]_t$), and the binding constants of A^- to CD ($K_{A^-\cdot CD}$) as [37,38]

$$\alpha_{A^-} = \frac{1}{1 + K_{A^-\cdot CD}[CD]}$$

and

$$\alpha_{A^-\cdot CD} = \frac{K_{A^-\cdot CD}[CD]}{1 + K_{A^-\cdot CD}[CD]}$$

where $[CD] = [CD]_t/(1 + K_{A^-\cdot CD}[A^-])$. Hence, Eq. (1) can be expressed as: [37,38]

$$\mu_{eff} = \frac{\mu_{A^-} + K_{A^-\cdot CD}[CD]\mu_{A-\cdot CD}}{1 + K_{A^-\cdot CD}[CD]} \quad (2)$$

3.2. Influence of surfactant molecules

In a similar fashion, in the presence of anionic surfactant monomers (S^-) without the addition of cyclodextrins, the effective electrophoretic mobility of an anionic probe molecule can be expressed as:

$$\mu_{eff} = \alpha_{A^-}\mu_{A^-} + \alpha_{A^-\cdot S^-}\mu_{A^-\cdot S^-} \quad (3)$$

where $A^-\cdot S^-$ represents the complex formed between A^- and surfactant monomers (S^-). Likewise, Eq. (3) can be specifically given by the following equation

$$\mu_{eff} = \frac{\mu_{A^-} + K_{A^-\cdot S^-}[S^-]\mu_{A^-\cdot S^-}}{1 + K_{A^-\cdot S^-}[S^-]} \quad (4)$$

where $[S^-]$ and $K_{A^-\cdot S^-}$ represent the concentration of surfactant monomers and the binding constant of the complex formed between A^- and S^-, respectively. For anionic surfactant monomers, the magnitude of $K_{A^-\cdot S^-}$ is expected to be small because of the repulsive interactions between the anionic probe molecule and anionic surfactant monomers.

With surfactant molecules at concentrations greater than the CMC, the effective electrophoretic mobility of an anionic probe molecule is expressed as:

$$\mu_{eff} = \alpha_{A^-}\mu_{A^-} + \alpha_{A^-\cdot S^-}\mu_{A^-\cdot S^-} + \alpha_{A^-\cdot M}\mu_{A^-\cdot M} \quad (5)$$

where $A^-\cdot M$ represents the complex formed between A^- and the anionic micelles. Likewise, Eq. (5) can be expressed as [16]:

$$\mu_{eff} = \frac{\mu_{A^-} + K_{A^-\cdot S^-}[CMC]\mu_{A^-\cdot S^-} + K_{A^-\cdot M}[M]\mu_M}{1 + K_{A^-\cdot S^-}[CMC] + K_{A^-\cdot M}[M]} \quad (6)$$

where $[S^-]$ is equal to the CMC of a surfactant, [M] is the concentration of surfactant micelles, $K_{A^-\cdot M}$ is the binding constant of A^- to the micelles and μ_M is the electrophoretic mobility of anionic micelles.

3.3. Influence of both cyclodextrins and surfactant molecules

In an electrophoretic buffer system containing both cyclodextrins and anionic surfactant monomers,

depending on their concentrations, the interactions of probe molecules with electrolyte modifiers become quite complicated because the following equilibria may be involved:

$$A^- + CD \overset{K_{A^-\cdot CD}}{\rightleftharpoons} A^- \cdot CD$$

$$A^- + S^- \overset{K_{A^-\cdot S^-}}{\rightleftharpoons} A^- \cdot S^-$$

$$S^- + CD \overset{K_1}{\rightleftharpoons} S^- \cdot CD$$

$$S^- \cdot CD + CD \overset{K_2}{\rightleftharpoons} S^- \cdot (CD)_2$$

$$S^- \cdot CD + A^- \overset{K_{S^-\cdot CD\cdot A^-}}{\rightleftharpoons} S^- \cdot CD \cdot A^-$$

$$S^- \cdot (CD)_2 + A^- \overset{K_{S^-\cdot (CD)_2\cdot A^-}}{\rightleftharpoons} S^- \cdot (CD)_2 \cdot A$$

where $K_{S^-\cdot CD\cdot A^-}$ and $K_{S^-\cdot (CD)2\cdot A^-}$ are the binding constant of the complexes formed between A^- and $S^-\cdot CD$ and that of the complexes formed between A^- and $S^-\cdot (CD)_2$, respectively; K_1 and K_2 are the binding constants of the complexes formed between SDS and β-CD and that of the complexes formed between $S^-\cdot CD$ and CD, respectively.

In the absence of surfactant micelles, the effective electrophoretic mobility of an anionic probe molecule may be composed of the following terms:

$$\mu_{eff} = \alpha_{A^-}\mu_{A^-} + \alpha_{A^-\cdot S^-}\mu_{A^-\cdot S^-} + \alpha_{A^-\cdot CD}\mu_{A^-\cdot CD} + \alpha_{S^-\cdot CD\cdot A^-}\mu_{S^-\cdot CD\cdot A^-} + \alpha_{S^-\cdot (CD)_2A^-}\mu_{S^-\cdot (CD)_2A^-} \quad (7)$$

It is noted that the analytical concentration of an anionic probe molecule ($[A^-]_T$), which is equal to $[A^-]+[A^-\cdot S^-]+[A^-\cdot CD]+[S^-\cdot CD\cdot A^-]+[S^-\cdot (CD)_2\cdot A^-]$, can be expressed in terms of $[S^-]$, [CD], and various binding constants as:

$$[A^-]_T = [A^-](1 + K_{A^-\cdot S^-}[S^-] + K_{A^-\cdot CD}[CD] + K_{S^-\cdot CD\cdot A^-}K_1[S^-][CD] + K_{S^-\cdot (CD)_2\cdot A^-}K_1K_2[S^-][CD]^2)$$

Similarly, the total concentration of β-CD ($[CD]_T$), which is equal to $[CD]+[S^-\cdot CD]+[A^-\cdot CD]+[S^-\cdot CD\cdot A^-]+2[S^-\cdot (CD)_2]+2[S^-\cdot (CD)_2\cdot A^-]$, can be expressed in terms of $[S^-]$, [CD], and various binding constants as:

$$\begin{aligned}[CD]_T &= [CD] + K_1[S^-][CD] + K_{A^-\cdot CD}[A^-][CD] \\ &\quad + K_1K_{S\cdot CD\cdot A^-}[A^-][S^-][CD] + 2K_1K_2[S^-][CD]^2 \\ &\quad + 2K_1K_2K_{S\cdot (CD)_2\cdot A^-}[A^-][S^-][CD]^2 \\ &= [CD](1 + K_1[S^-] + K_{A^-\cdot CD}[A^-] + K_1K_{S\cdot CD\cdot A^-}[A^-][S^-] \\ &\quad + 2K_1K_2[S^-][CD] \\ &\quad + 2K_1K_2K_{S\cdot CD_2\cdot A^-}[A^-][S^-][CD])\end{aligned}$$

Likewise, the total concentration of surfactant monomers ($[S^-]_T$), which is equal to $[S^-]+[A^-\cdot S^-]+[S^-\cdot CD]+[S^-\cdot (CD)_2]+[S^-\cdot CD\cdot A^-]+[S^-\cdot (CD)_2\cdot A^-]$, can be expressed as:

$$\begin{aligned}[S^-]_T &= [S^-](1 + K_{A^-\cdot S^-}[A^-] \\ &\quad + K_1[CD] + K_1K_2[CD]^2 + K_1K_{S^-\cdot CD\cdot A^-}[A^-][CD] \\ &\quad + K_1K_2K_{S^-\cdot (CD)_2\cdot A^-}[A^-][CD]^2)\end{aligned}$$

When the existence of the complexes with a 2:1 stoichiometry formed between CD and surfactant monomers is indicative and significant, the last term may not be eliminated. If this is the case, then the effective electrophoretic mobility of an anionic probe molecule in the premicellar concentration region can be specifically expressed as:

$$\mu_{\text{eff}} = \frac{\mu_{A^-} + K_{A^-\cdot S^-}[S^-]\mu_{A^-\cdot S^-} + K_{A^-\cdot CD}[CD]\mu_{A^-\cdot CD} + K_1K_{S^-\cdot CD\cdot A^-}[S^-][CD]\mu_{S^-\cdot CD\cdot A^-} + K_1K_2K_{S^-\cdot (CD)_2\cdot A^-}[S^-][CD]^2\mu_{S^-\cdot (CD)_2\cdot A^-}}{1 + K_{A^-\cdot S^-}[S^-] + K_{A^-\cdot CD}[CD] + K_1K_{S^-\cdot CD\cdot A^-}[S^-][CD] + K_1K_2K_{S^-\cdot (CD)_2\cdot A^-}[S^-][CD]^2} \quad (8)$$

where

$$[CD] = \frac{[CD]_T}{1 + K_1[S^-] + K_{A^-\cdot CD}[A^-] + K_1K_{S^-\cdot CD\cdot A^-}[A^-][S^-] + 2K_1K_2[S^-][CD] + 2K_1K_2K_{S^-\cdot (CD)_2\cdot A^-}[A^-][S^-][CD]}$$

$$[S^-] = \frac{[S^-]_T}{1 + K_{A^-\cdot S^-}[A^-] + K_1[CD] + K_1K_2[CD]^2 + K_1K_{S^-\cdot CD\cdot A^-}[A^-][CD] + K_1K_2K_{S^-\cdot (CD)_2\cdot A^-}[A^-][CD]^2}$$

and

$$[A^-] = \frac{[A^-]_T}{1 + K_{A^-\cdot S^-}[S^-] + K_{A^-\cdot CD}[CD] + K_1K_{S^-\cdot CD\cdot A^-}[S^-][CD] + K_1K_2K_{S^-\cdot (CD)_2\cdot A^-}[S^-][CD]^2}$$

Likewise, based on a similar consideration as described in Section 3.2 and the aforementioned paragraphs of this section, the effective electrophoretic mobility of an anionic probe molecule with surfactants in the micellar concentration region can be specifically expressed as:

$$\mu_{\text{eff}} = \frac{\mu_{A^-} + K_{A^-\cdot S^-}[CMC]\mu_{A^-\cdot S^-} + K_{A^-\cdot CD}[CD]\mu_{A^-\cdot CD} + K_1K_{S^-\cdot CD\cdot A^-}[CMC][CD]\mu_{S^-\cdot CD\cdot A^-}}{1 + K_{A^-\cdot S^-}[CMC] + K_{A^-\cdot CD}[CD] + K_1K_{S^-\cdot CD\cdot A^-}[CMC][CD] + K_1K_2K_{S^-\cdot (CD)_2\cdot A^-}[CMC][CD]^2 + K_{A^-\cdot M}[M]} + \frac{K_1K_2K_{S^-\cdot (CD)_2\cdot A^-}[CMC][CD]^2_{S\cdot CD_2\cdot A^-} + K_{A^-M}[M]_M}{1 + K_{A^-\cdot S^-}[CMC] + K_{A^-\cdot CD}[CD] + K_1K_{S^-\cdot CD\cdot A^-}[CMC][CD] + K_1K_2K_{S^-\cdot (CD)_2\cdot A^-}[CMC][CD]^2 + K_{A^-\cdot M}[M]} \quad (9)$$

Consequently, according to Eqs. (2), (4), (6), (8) and (9), the migration behavior of a probe molecule under various electrophoretic conditions can be predicted, provided that the binding constants and the necessary mobility data are available.

4. Result and discussion

4.1. Electrophoretic mobility as a function of β-CD concentration

Fig. 1 shows the variation of the electrophoretic mobility of the two chlorophenols studied as a function of β-CD concentration in the range 0–9.0 m*M* with chlorophenols dissolved in a 20% (v/v) methanolic solution. The experimental data are shown by the data points, while the predicted curves are represented by the solid lines. As can be seen, the electrophoretic mobility of TTCP decreases more rapidly than that of PCP with increasing β-CD concentration, because the binding constant of TTCP with β-CD is greater than that of PCP with β-CD. According to Eq. (2), the fitting of the predicted mobility curve to the experimental data of these two chlorophenols allows us to evaluate the binding constants of inclusion complexes formed between chlorophenols and β-CD. The binding constants of PCP- and TTCP- complexes evaluated are 280 and 2900

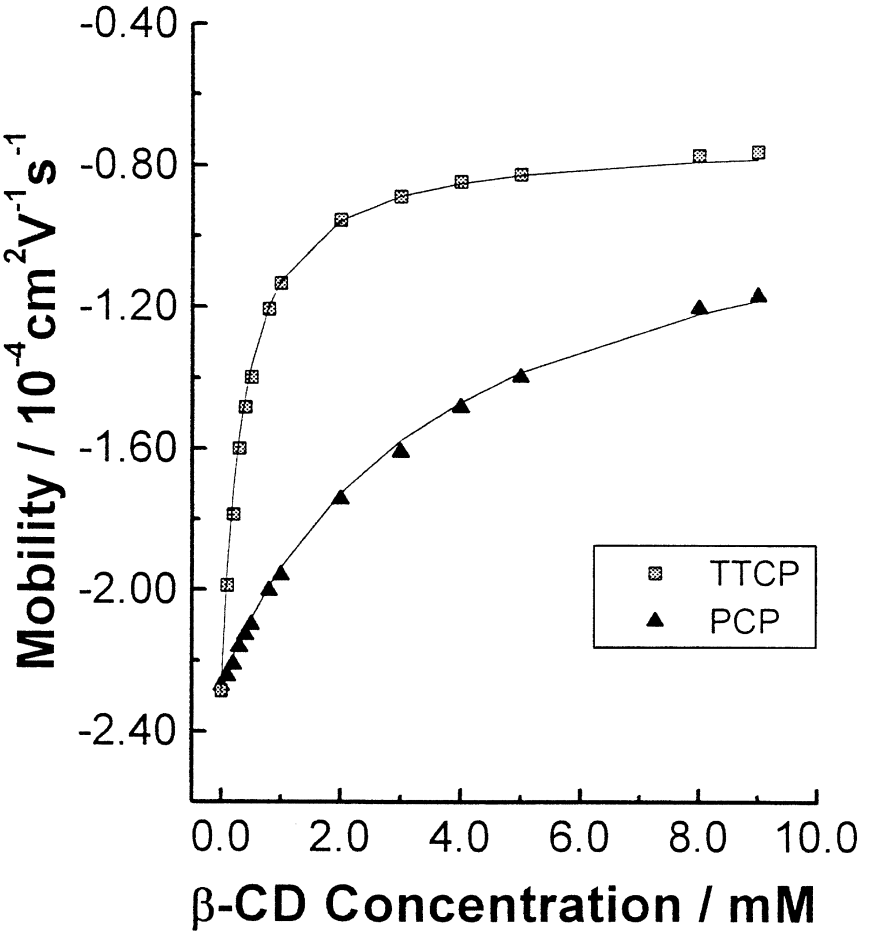

Fig. 1. The agreement between the predicted mobility curves (represented by solid lines) and experimental mobility data (shown by data points) of chlorophenols as a function of β-CD concentration: (▲), PCP; (▩), TTCP. Sample solution: chlorophenols (10 μg/ml) dissolved in a 20% methanolic aqueous solution. Buffer: phosphate buffer (70 m*M*) at pH 7.0. Capillary: 50.2 cm (effective length, 40 cm)×50 μm I.D. Detection wavelength: 215 nm. Other operating conditions: 20 kV, 25°C.

M^{-1}, respectively, with the latter being ten times larger than the former. The results clearly indicate that these two chlorophenols interact strongly with β-CD, as reflected from the variation of the electrophoretic mobility of chlorophenols shown in Fig. 1.

4.2. Electrophoretic mobility as a function of SDS concentration

4.2.1. In the absence of β-CD

Fig. 2 shows the variation of the electrophoretic mobility of chlorophenols as a function of SDS concentration in the premicellar region with chlorophenols dissolved in a 20% (v/v) methanolic solution. The observed electrophoretic mobilities are represented by the data points. The electrophoretic mobility of the two chlorophenols, migrating toward the anode, increases slightly with increasing monomeric SDS concentration from 0 to 4.5 m*M*. This is ascribed to the weak binding between the probe molecules and anionic SDS monomers. The binding constants of the two chlorophenols to anionic SDS monomers are evaluated by fitting

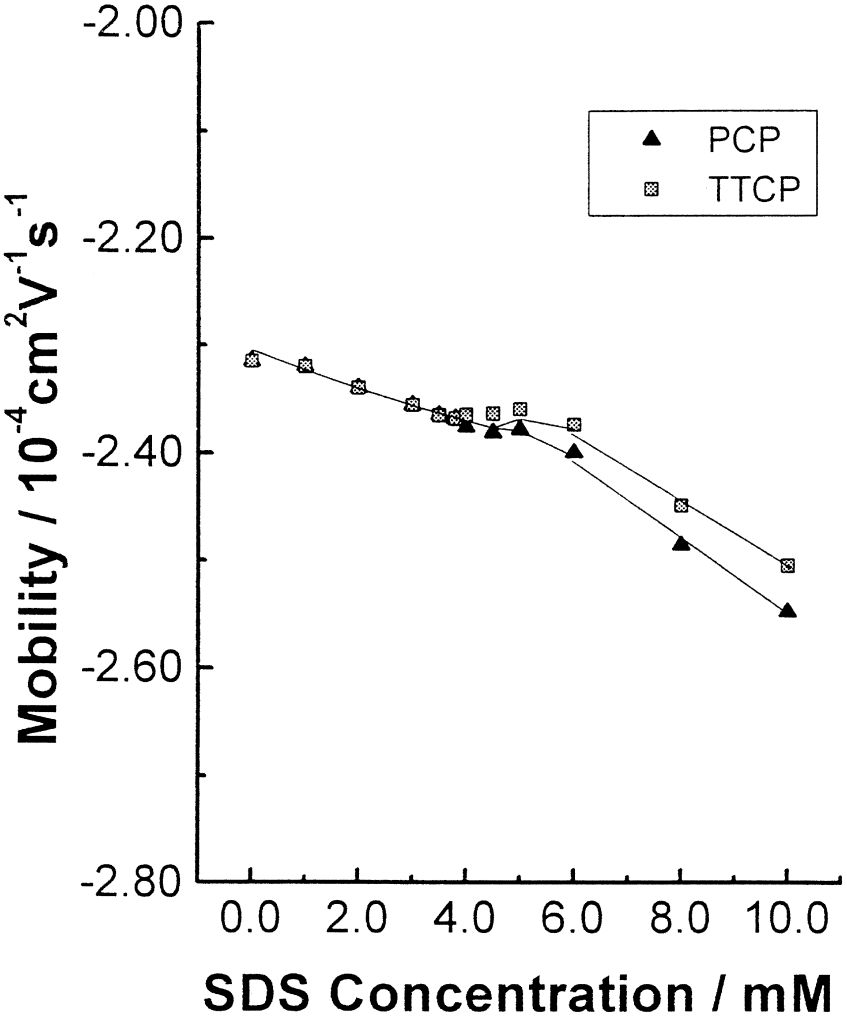

Fig. 2. The agreement between the predicted mobility curves (represented by solid lines) and experimental mobility data (shown by data points) of chlorophenols as a function of SDS concentration in the absence of β-CD: (▲), PCP; (▩), TTCP. Sample solution and operating conditions are the same as for Fig. 1.

the predicted electrophoretic mobility to the observed values using Eq. (4). The binding constants of TTCP and PCP obtained are 57 and 55 M^{-1}, respectively. These two values of binding constants are quite reasonable, judging from the variation of the electrophoretic mobility of chlorophenols shown in Fig. 2.

4.2.2. In the presence of β-CD

Fig. 3 shows the variation of the electrophoretic mobility of chlorophenols as a function of SDS concentration in the premicellar and micellar regions in the presence of β-CD at some fixed concentrations with chlorophenols dissolved in 20% (v/v) methanolic aqueous solution. As can be seen, the mobility curve of each probe molecule can be divided into two regions with two inflection points. The mobility increases (migration toward the anode) rapidly until the first inflection point is reached. Then the electrophoretic mobility of each probe molecule varies gradually with increasing the concentration of SDS monomers. This phenomenon, which is similar to the one

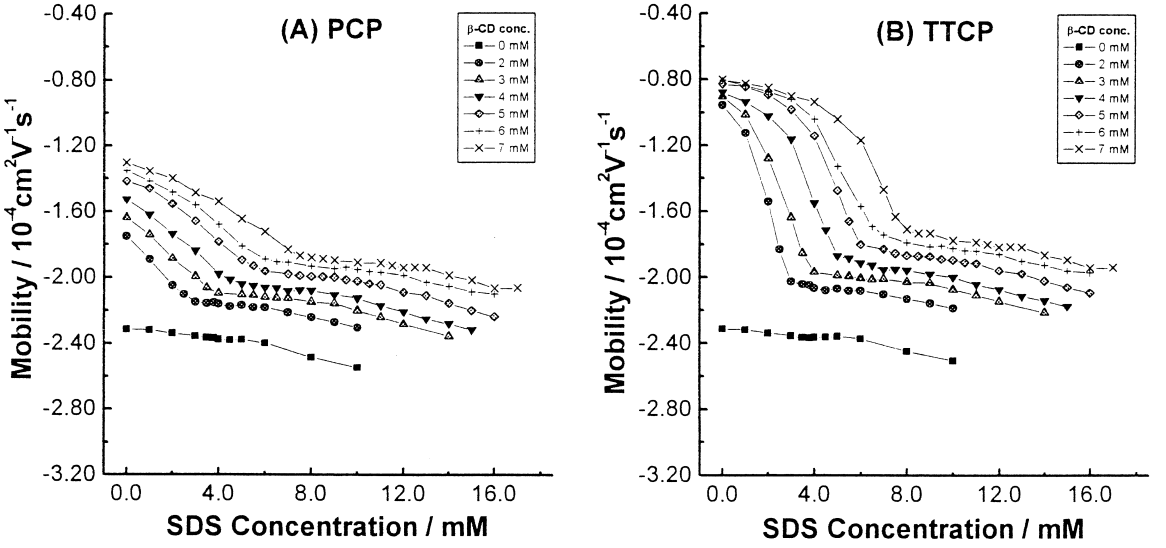

Fig. 3. Variation of electrophoretic mobility of chlorophenols as a function of SDS concentration with β-CD at some fixed concentrations in a SDS–phosphate buffer system: (A) PCP, and (B) TTCP. Operating conditions as for Fig. 1.

described in Section 4.2.1, reveals that the inclusion complexation between β-CD and anionic probe molecules becomes unfavorable in the presence of SDS, because the complexation between β-CD and SDS monomers is comparatively much stronger than that of β-CD with probe molecules. As the binding constant of β-CD to SDS reported by Wan Yunus et al. is 21 000 M^{-1} [24] and those of β-CD to TTCP and PCP evaluated in Section 4.1 are 2900 and 280 M^{-1}, respectively, β-CD molecules are expected to bind preferentially to SDS monomers, instead of binding to chlorophenols. In fact, the complexes formed between probe molecules and β-CD break up almost completely in the presence of SDS at concentrations above the first inflection point.

4.3. Prediction of electrophoretic mobility and evaluation of binding constants

For a better understanding on the stoichiometry of the complexes formed between β-CD and SDS monomers, the interactions of β-CD with probe molecules and SDS, and the influences of β-CD on the CMC value of SDS, the prediction of the effective electrophoretic mobility of probe molecules as a function of SDS concentration in the presence of β-CD is attempted. The binding constants of probe molecules to the complexes formed between β-CD and SDS, as well as that of β-CD with SDS, are evaluated. These values are determined by curve-fitting the predicted mobility data as a function of SDS concentration with the experimental mobility data through the utilization of Excel software.

The simulation of mobility curves as a function of SDS concentration in the premicellar region was carried out according to Eq. (8). The binding constants ($K_{A^-\cdot CD}$ and $K_{A^-\cdot S^-}$) and mobilities (μ_{A^-}, $\mu_{A^-\cdot CD}$ and $\mu_{A^-\cdot S^-}$) evaluated from Eqs. (2) and (4), the binding constants (K_1 and K_2) reported by Jobe et al. [36], and the mobilities of the complexes formed between probe molecules and β-CD–SDS complexes estimated according to Offord's equation [39] were all used as trial values, and the most suitable values of the binding constants and limiting mobilities of various complexes were obtained by varying these parameters and the binding constants ($K_{A^-\cdot S^-\cdot CD}$ and $K_{A^-\cdot S^-\cdot (CD)_2}$) until the predicted mobility curves were best fitted to the observed mobility curves. A typical predicted mobility curve of TTCP (dissolved in 20% methanolic aqueous solution) in the presence of 3 m*M* β-CD is shown in Fig. 4. As can be seen, the agreement between the predicted and observed mobility

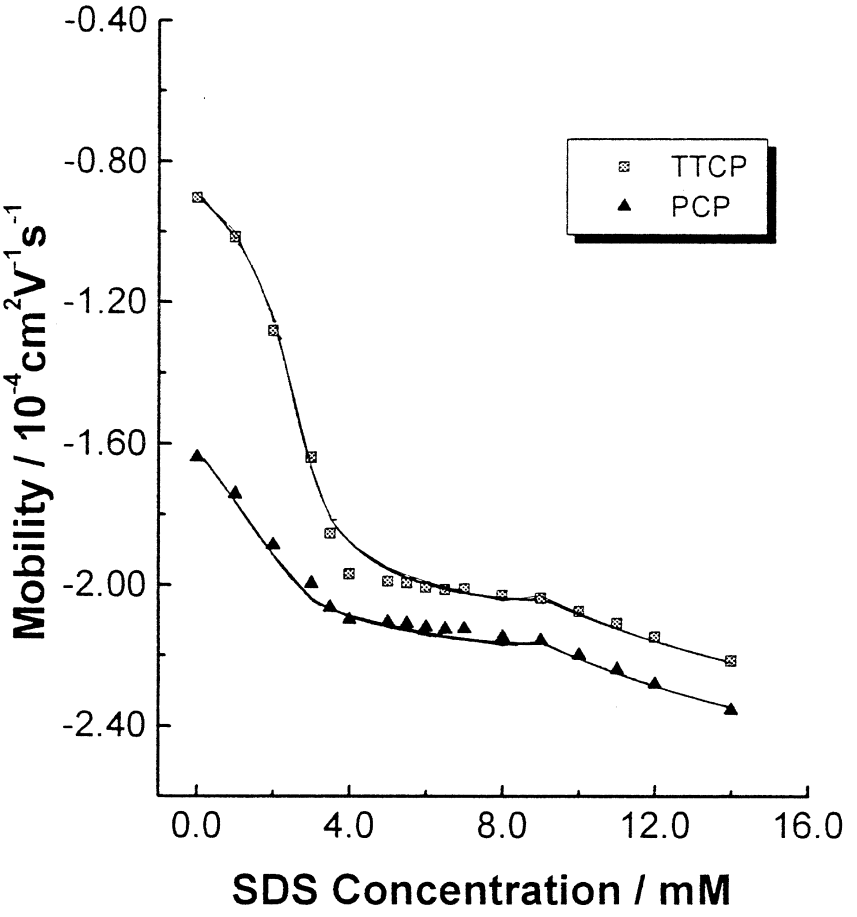

Fig. 4. The agreement between the predicted mobility curves (represented by solid lines) and experimental mobility data (denoted by data points) of TTCP as a function of SDS concentration in a SDS–phosphate buffer containing 3 m*M* β-CD. Sample solution and operating conditions as for Fig. 3.

curves is quite satisfactory. Table 1 lists the best fitted values of binding constants and mobilities of TTCP obtained. It should be pointed out here that better fitting of the mobility curve can be obtained with K_1 equal to about 48 000 M^{-1} than with K_1 equal to 21 000 M^{-1}.

A literature survey reveals that the values of binding constants for inclusion complexes involving SDS and β-CD can vary by several order of magnitude for the same system when different experimental methods are employed [26]. The K_1 values reported in the literature are: 210 [31], 356 [33], 500 [11], 1300–7230 [22], 3200–18 000 [23], 3630 [33], 8360 [25], 18 500 [40], 21 000 [24], and 25 600 [34]. The K_1 values of the complexes with a 1:1 stoichiometry reported in the literature ranging from 210 to 8360 M^{-1} are clearly unacceptable [26]. The K_1 values measured by fluorescence [34], UV and visible absorbance probes [24], and electromotive force (emf) methods involving the use of a SDS membrane selective electrode [40] give 25 600, 21 000 and 18 500 M^{-1}, respectively. These values evaluated by using three different experimental techniques are reasonably close. As the simulated mobility curves shown in Fig. 4 can yield better fitting when K_1 value greater than 21 000 M^{-1} is adapted as a parameter, the result reveals that the K_1 values ranging from 21 000 to 25 600 M^{-1} or even greater are acceptable.

Table 1
The mobility data of chlorophenols and binding constants of chlorophenols to β-CD, SDS or β-CD–SDS complexes in 70 m*M* phosphate buffer at pH 7.0

	Mobility ($\times 10^{-4}$ cm^2·V^{-1}·s^{-1})			Binding constant[a] (M^{-1})	
	TTCP	PCP		TTCP	PCP
μ_{A^-}	−2.32	−2.35	$K_{A^-\cdot CD}$	2900	280
$\mu_{A^-\cdot CD}$	−0.73	−0.75	$K_{A^-\cdot S^-}$	57	55
$\mu_{A^-\cdot S^-}$	−2.70	−2.74	$K_{S^-\cdot CD\cdot A^-}$	180	120
$\mu_{S^-\cdot CD\cdot A^-}$	−1.28	−1.30	$\frac{K_{A^-\cdot M}}{n}$	40	30
$\mu_{A^-\cdot M}$	−2.65	−2.65	$K_{S^-\cdot (CD)_2\cdot A^-}$	nil	nil

[a] $K_1 = 48\,000\ M^{-1}$; $K_2 = 210\ M^{-1}$; n, aggregation number of micelles.

4.4. Stoichiometry of β-CD–SDS complexes

As illustrated in Fig. 3, the first inflection point, which indicates the SDS concentration required to dissociate completely the complexes formed between chlorophenols and β-CD, occurs at higher SDS concentrations as β-CD concentration in the buffer solution increases. However, the mole ratios of the change in β-CD concentration (Δ[β-CD]) to the change in SDS concentration (Δ[SDS]) at these inflection points vary between 1:1.05 and 1:0.90. The result reveals that the complexes formed between β-CD and SDS exist predominantly in the form of a 1:1 stoichiometry, while the complexes with a 2:1 stoichiometry (β-CD:SDS) reported previously in the literature as a minor component may exist by less than 10%.

4.5. Influence of β-CD on CMC value of SDS

As shown in Fig. 3, the observation of a second inflection point for each mobility curve of probe molecules as a function of SDS concentration in the premicellar and micellar regions allows us to determine the CMC value of SDS. For these two probe molecules with β-CD at some fixed concentrations, the second inflection point occurs correspondingly at the higher SDS concentrations as β-CD concentration increases. The CMC values of SDS determined with β-CD at some fixed concentrations in the range 2–7 m*M* [with probe molecules dissolved in a 20% (v/v) methanolic aqueous solution] are given in Table 2. Thus the elevation of the CMC value of SDS depending on β-CD concentration in the buffer electrolyte is unambiguously evident. This is consistent with the results obtained by Junquera et al. [11] and Cifuentes et al. [17].

4.6. Influence of methanol content on the CMC of SDS

It has been demonstrated that organic solvents in the sample solution may affect the micellization of surfactant molecules [32]. As hydrophobic samples are often dissolved in a methanolic solution, it is of interest to determine the CMC values and to examine the influence of methanol content on the CMC values with such sample solutions.

Fig. 5A and B shows the variations of electrophoretic mobility of TTCP, dissolved in 5% and 30% (v/v) methanolic solutions, respectively, as a function of SDS concentration in the presence of β-CD at 3, 5, and 7 m*M*. For comparison, the variations of electrophoretic mobility of these two chlorophenols in the absence of β-CD are also included. As expected, the trends in the variation of electrophoretic mobility of chlorophenols are similar to those observed in Fig. 3B. The inflection points of the mobility curve at a given concentration of β-CD occur at relatively lower or higher concentrations of SDS than those of the corresponding mobility curve of TTCP as observed in Fig. 3B. Again, as indicated from the first inflection points, the complexes formed

Table 2
The CMC values of SDS determined in the presence of β-CD using TTCP (dissolved in 20% methanol solution) as a probe molecule and the change in mole ratio of β-CD to SDS

[β-CD]/m*M*	[SDS]/m*M* (at 1st inflection point)	Mole ratio (Δ[β-CD]/Δ[SDS])	CMC value/m*M*
2.0	3.00±0.05		7.7±0.2
		1: 0.90	
3.0	3.90±0.05		8.9±0.2
		1: 1.05	
4.0	4.95±0.05		9.9±0.2
		1: 0.90	
5.0	5.85±0.05		10.9±0.2
		1: 0.95	
6.0	6.80±0.05		11.9±0.2
		1: 0.95	
7.0	7.75±0.05		12.9±0.2

between β-CD and SDS have predominately a 1:1 stoichiometry. The CMC values of SDS determined from the second inflection points with β-CD at a concentration of 3, 5, and 7 m*M* are 7.0, 9.2 and 11.2 m*M*, respectively, for TTCP dissolved in a 5% methanolic aqueous solution and are 9.6, 11.5 and 13.5 m*M*, respectively, for TTCP dissolved in a 30% methanolic aqueous solution.

Fig. 6 shows the plots of the CMC values of SDS obtained at varied concentrations of β-CD with sample solutions containing 5%, 20% and 30% (v/v) methanol. As can be seen, three parallel straight lines are obtained and the increment of the CMC value at a particular β-CD concentration correlates well with methanol content in the sample solution. The results clearly indicate that not only β-CD concentration in the buffer electrolyte, but also methanol content in the sample solution, contributes to the elevation of the CMC value of SDS.

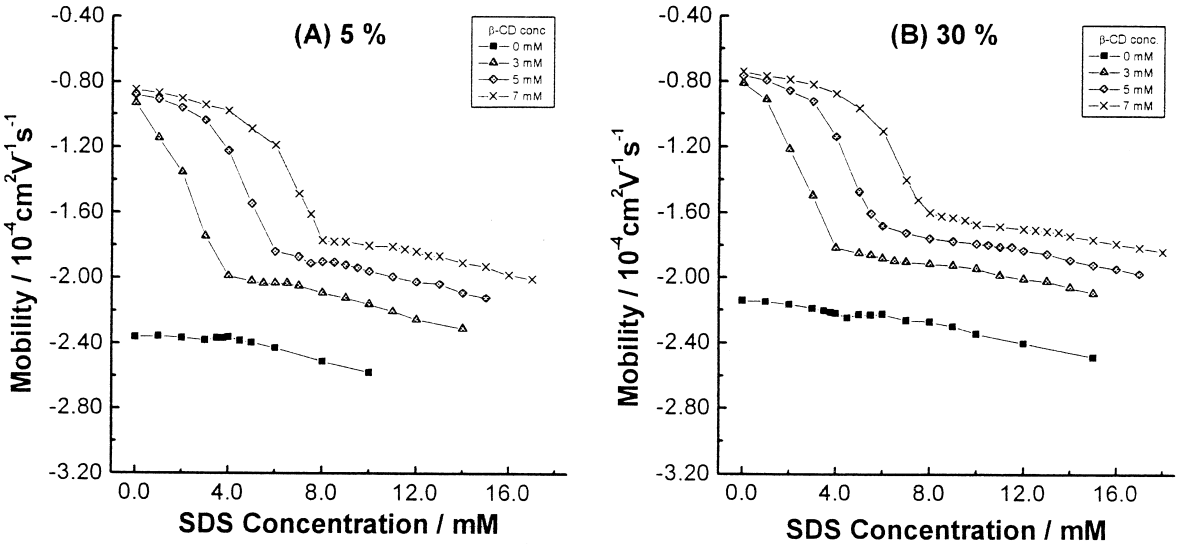

Fig. 5. Variation of electrophoretic mobility of TTCP as a function of SDS concentration with β-CD at varied concentrations in the range 0–7 m*M* obtained by dissolving probe molecules in a (A) 5% and (B) 30% methanolic aqueous solution. Operating conditions as for Fig. 3.

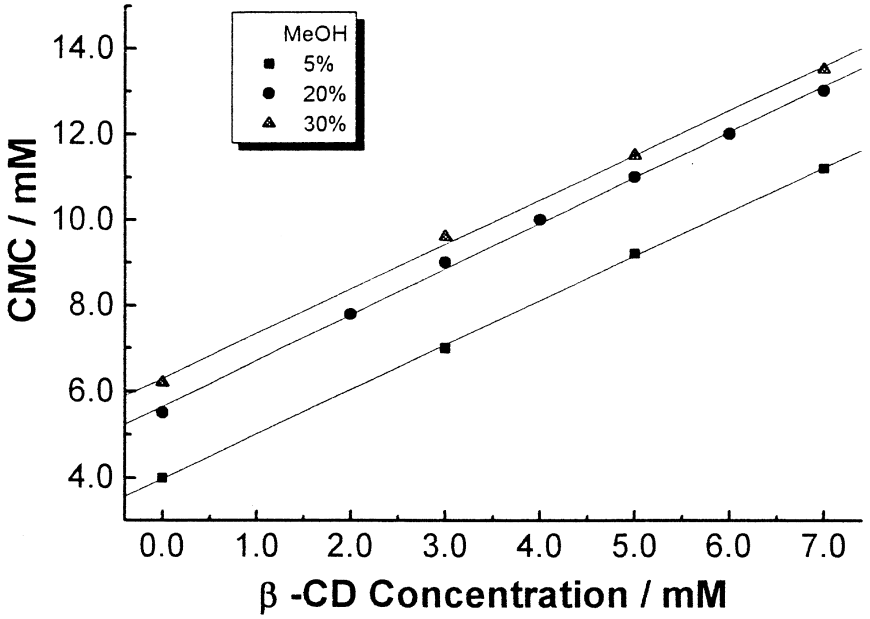

Fig. 6. Correlation of the CMC values of SDS with β-CD concentration for probe molecules dissolved in varied methanolic aqueous solutions: (■), 5%; (●), 20%; (▲), 30%.

5. Conclusion

Capillary electrophoresis is a convenient and useful technique to determine the CMC values of surfactants. With the proper choice of a probe molecule, the stoichiometry of the complexes formed between β-CD and surfactant molecules can be elucidated, and the interactions of β-CD with surfactant molecules and the influence of β-CD on the micellization of a surfactant can be better understood through the analysis of the variation of the electrophoretic mobility of a probe molecule as a function of surfactant concentration.

Acknowledgements

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出席國際學術會議報告書 (國科會補助)

89 年 10 月 12 日

報告人姓名	林敬二	服務機關名稱及職務	國立台灣大學 化學系教授
會議期間及地點	89 年 9 月 10-13 日 斯洛伐克, Bratislava 市	國科會 研究計劃	NSC 89-2113-M-002-055
會議名稱	(中文) 第 12 屆毛細管電分離技術國際研討會議 (英文) 12th International Symposium on Capillary Electroseparation Technique		
發表論文題目	(中文) 利用陽離子界面活性劑以微胞電動層析法對氯-三阱環之線上濃縮之研究 (英文) On-line concentration of chloro-s-triazines in micellar electrokinetic chromatography using a cationic surfactant (A-38)		

報告內容

一、參加會議經過：

第 12 屆毛細管電分離技術國際研討會於斯洛伐克(Slovak)的 Bratislava 市舉行。會期為 9 月 10-13 日，2000 年，由斯洛伐克的 Comenius 大學的 D. Kaniansky 教授籌辦。此次研討會主要由斯洛伐克的 Comenius 大學及奧地利的維也納大學為主辦單位。

此次研討會共有口頭論文及壁報論文共一百餘篇的發表。參加人數約有二百多人。由中歐及東歐來的學者特別多，學生很少。大會於 9 月 10 日下午揭幕。首先由荷蘭的 I. F. M. Everaerts 教授簡述毛細管電泳之新進發展，接著由瑞典的 S. Hjerten、日本的 S. Terabe、美國的 F. Foret 及 N. A. Guzman 等四位國際重量級的大師做專題演講，展開為期四天的學術活動。晚上舉行酒會招待。此次大會專題演講及學術研討分主題但未分場地進行研討，然而研究氣氛相當熱絡。此次研討會的主題除了等速電泳外，尚包括 chiral separation，CEC，bioseparation，pharmaceutical and biomedical 及 sample concentration 等。研討會所邀請及參加的國際知名學者不少，聆聽他們的研究結果，對毛細管電泳分離的現況及未來研究趨勢，會更能掌握。此研討會雖比 HPCE 國際研討會的規模小很多，但中東歐的學者參加人數顯得較多，但研討會內容仍具有其特色及價值。

在此研討會中，筆者提出一篇論文發表 – 論文題目為 ： On-line concentration of chloro-s-triazines in micellar electrokinetic chromatography using a cationic surfactant. **甚獲同一研究領域的學者所重視。此論文將發表刊登於** J. Chromatogr. A **專刊中。**

二、與會心得 ：

此次參加毛細管電分離技術國際研討會，除了能多了解此研究領域進展的現況，吸收新知識，交換研究心得之外，並能多認識一些國際知名學者。此次研討會所發表的論文有不少值得觀摩學習，並能啟發研究構想，實在獲益不少。台灣學者應該多多參加。

三、考察參觀活動 ：**除參觀儀器展外，大會未有安排。**

四、攜回資料 ：**論文摘要乙冊。**

五、建議 ：

參加國際學術研討會，不僅參加者能增廣見聞與學識，也可讓國際人士瞭解台灣學者的研究成果，應多多鼓勵參加。

出席國際學術會議報告書 (國科會補助)

90 年 11 月 20 日

報告人姓名	林敬二	服務機關名稱及職務	國立台灣大學 化學系教授
會議期間及地點	90 年 11 月 11-14 日 日本，京都市	國科會 研究計劃	NSC 90-2113-M-002-059
會議名稱	(中文) 高效能液相分離國際研討會議— HPLC Kyoto (英文) International Symposium on High Performance Liquid Phase Separations HPLC Kyoto		
發表論文題目	(中文) 利用環糊精為對掌選擇試劑以毛細管區帶電泳法進行 Phenothiazines 的對掌分離 (英文) Enantioseparation of phenothiazines in capillary zone electrophoresis using cyclodextrins as chiral selectors		

報告內容

一、參加會議經過：

高效液相分離國際研討會於日本京都市舉行。會期為 9 月 11-14 日，2001 年，由日本京都纖維工藝大學(Kyoto Institute of Technology)的 N. Tanaka 教授及姬路工藝大學(Himeji Institute of Technology)的 S. Terabe 教授籌辦。此次研討會主要由日本的層析科學學會為主辦單位。

此次研討會共有口頭論文 66 篇及壁報論文 160 篇共二百餘篇的發表。參加人數約有二百多人。大會於 9 月 11 日下午揭幕。首先由美國東北大學的 B. L. Karger 教授講述利用 LC、CE、MS 等技術對高解析度蛋白基因體分離之新進發展，接著由日本東京大學的 Y. Sakaki 教授及美國 Oak Ridge 國家實驗室的 I. M. Ransey 博士等二位國際重量級的大師做專題演講，展開為期四天的學術活動。晚上舉行酒會招待。此次大會專題演講及學術研討分主題及二個場地進行研討，研討氣氛相當熱絡。此次研討會的主題包括蛋白質因體、藥物及生醫、新管柱技術、靜相示性、對掌分離、新靜相、微分離技術、微晶片、單細胞分析。研討會所邀請及參加的國際知名學者不少，聆聽他們的研究結果，對毛細管電泳分離的現況及未來研究趨勢，會更能掌握。此研討會雖比 HPLC 國際研討會的規模小一點，且受美國 911 事件影響，使得參加人數頓顯更少，但研討會內容仍具有其特色及價值。

在此研討會中，筆者受邀做專題報告— 論文題目為 : Enantioseparation of phenothiazines in capillary zone electrophoresis using cyclodextrins as chiral selectors，甚獲同一研究領域的學者所重視。此論文將發表刊登於 J. Chromatogr. A 中。此研討會另外也邀請暨南大學應化系主任楊重熙教授做一專題報告。筆者並受邀為 Session chairman 主持 Chiral separation 的 session。

二、與會心得 :

此次參加毛細管電分離技術國際研討會，除了能多了解此研究領域進展的現況，吸收新知識，交換研究心得之外，並能多認識一些國際知名學者。此次研討會所發表的論文有不少值得觀摩學習，並能啟發研究構想，實在獲益不少。台灣學者除了筆者與楊教授外，另外還有中國醫藥研究所的蔡東湖教授及暨南大學應化系的何佳安教授參加，但參加人數還是太少，應該多多參加。

三、考察參觀活動 : 除參觀儀器展外，大會另外安排京都市郊多處神社及廟宇。

四、攜回資料 : 論文摘要乙冊。

五、建議 :

參加國際學術研討會，不僅參加者能增廣見聞與學識，也可讓國際人士瞭解台灣學者的研究成果，應多多鼓勵參加。

出席國際學術會議報告書 (國科會補助)

91 年 5 月 20 日

報告人姓名	林敬二	服務機關名稱及職務	國立台灣大學 化學系教授
會議期間及地點	91 年 4 月 13-18 日 瑞典，斯德哥爾摩	國科會 研究計劃	NSC-90-2113-M-002-059
會議名稱	(中文) 第 15 屆微尺標分離及分析國際研討會-HPCE 2002 (英文) International Symposium on Micro Scale Separations and Analysis-HPCE 2002		
發表論文題目	(1) (中文) 啡噻吨在環糊精修飾微胞電動力層析法中的對掌分離 (英文) Enantioseparation of phenothiazines in Cyclodextrin-Modified Micellar Electrokinetic Chromatography (P176) (2) (中文) 啡噻吨在環糊精修飾區帶電泳法中的分離與遷移行為 (英文) Separation and Migration Behavior of phenothiazines in Cyclodextrin-Modified Capillary Zone Electrophoresis (P175)		

報告內容

一、參加會議經過：

第 15 屆微尺標分離及技術國際研討會於瑞典斯德哥爾摩市舉行。會期為 4 月 13-18 日，2002 年，由瑞典 Uppsala University 的 Westerlund 教授籌辦及瑞典化學會主辦。會場設於 Stockholm University。

此次研討會共有口頭論文 83 篇及壁報論文 258 篇共三百四十餘篇的發表。參加人數約有五百多人。大會於 4 月 14 日下午揭幕，由瑞士日內瓦大學的 D. Hochstrasser 教授講述醫學上蛋白基因體之新進發展，接著由美國 Advion BioSciences 公司的 J. Henion 博士及荷蘭 University Twente MESA 研究所的 Albert Berg 教授做專題演講，分別講述自動化奈電灑質譜法以及 Lab-on-a-chip 系統與微流體及奈流體裝置。隨後展開為期四天的學術活動。晚上舉行酒會招待。

此次大會專題演講及學術研討分幾個主題在二個場地同時進行研討，研討氣氛相當熱絡。此次研討會的主題著重於基因體學、蛋白質體學、藥物及生醫應用、微流體學及微晶片、對掌分離、新偵測方法、微分離技術、生化分析之濃縮技術、分離原理等。研討會所邀請及參加的國際知名學者不計其數，聆聽他們的研究結果，對毛細管電泳分離的現況及未來研究趨勢會更能掌握。此次研討會的許多主題及邀請演講中與我個人的研究最有關聯的是對掌分離與線上濃縮技術。因此瑞士 University of Bern 的

W. Thormann 教授的講題" Chiral capillary electrophoresis in drug bioanalysis "（KL3），德國 University of Munster 的 B. Chankvetadze 教授的講題 " Enantioseparations in capillary electromigration techniques "（KL8），及日本 Himeji Institute of Technology 的 S. Terabe 教授的講題 " On-line sample concentration techniques for high sensitivity capillary electrophoresis using sweeping and dynamic pH-junction "（KL17）最有助益也特別值得一提。至於壁報論文方面，也有不少是關於對掌分離與線上濃縮技術的論文。這些論文的瞭解對目前的研究工作與方向提供了不少值得思考的地方，例如雙環糊精系統應用在對掌異構物順序的辨別與指認以及遷移順序反轉原理的探討等等，而讓我有 " 不虛此行 " 的感覺。

在此研討會中，筆者提出二篇論文發表。論文題目為：（1）Enantioseparation of phenothiazines in Cyclodextrin-Modified Micellar Electrokinetic Chromatography ；（2） Separation and Migration Behavior of phenothiazines in Cyclodextrin-Modified Capillary Zone Electrophoresis，甚得同一研究領域的學者的興趣。此論文將可發表刊登於 J. Chromatogr. A 中。此次研討會另有本系張煥宗教授、交通大學應化系謝有容教授、成功大學化學系陳淑慧教授、靜宜大學應化系王書蘋教授的參與。台大藥學系孫紹文教授及本系張煥宗教授的學生亦參與此盛會。

二、與會心得：

此次參加微尺標分離及分析國際研討會，除了能多了解此研究領域進展的現況，吸收新知識，交換研究心得之外，並能多認識一些國際知名學者。此次研討會所發表的論文有不少值得觀摩學習，並能啟發研究構想，實在獲益不少，值得參加。國內學者參加國際會議之人數仍然不多，應多參與以提昇國際間之學術地位。

三、考察參觀活動：除參觀儀器展外，大會特別請求斯德哥爾摩市府安排接待餐會並參觀諾貝爾獎頒獎典禮之場所。

四、攜回資料：論文摘要乙冊。

五、建議：

參加國際學術研討會，不僅參加者能增廣見聞與學識，也可讓國際人士瞭解台灣學者的研究成果，應多多鼓勵參加。

出席國際學術會議報告書 (國科會補助)

91 年 12 月 20 日

報告人姓名	林敬二	服務機關名稱及職務	國立台灣大學 化學系教授
會議期間及地點	91 年 10 月 11-14 日 中國，上海	國科會 研究計劃	NSC-91-2113-M-002-054
會議名稱	(中文) 第 4 屆亞太微尺標分離及分析國際研討會-APCE 2002 (英文) 4th Asia-Pacific International Symposium on Microscale Separations and Analysis-APCE 2002		
發表論文題目	(1) (中文) 啡噻井在環糊精修飾區帶電泳法中的對掌分離與遷移反轉 (英文) Enantioseparation and Migration Reversal of Phenothiazines in Cyclodextrin-Modified Capillary Zone Electrophoresis (L052) (2) (中文) pH 對啡噻井電泳行為的影響及以毛細管區帶電泳法對 pKa 值之測定 (英文) Influence of pH on Electrophoretic Behavior of phenothiazines and Determination of pKa values by Capillary Zone Electrophoresis (P119) (3) (中文) 遷移行為的預期及以毛細管區帶電泳法對含六氫吡井取代基之奎寧酮(quinolones)的 pKa 值之測定 (英文) Prediction of Migration Behavior and Determination of pKa values of Quinolones with a Piperazine Substituent by Capillary Zone Electrophoresis (P118)		

報告內容

一、參加會議經過：

第 4 **屆亞太微尺標分離及技術國際研討會於中國大陸，上海市舉行。會期為** 10 **月** 11-14 **日，**2002 **年，由中國科學院大連化物所的林炳承教授籌辦及中國科學院大連化物所主辦。會場設於上海的中油日航大酒店。**

此次研討會共有口頭論文 66 **篇及壁報論文** 106 **篇共** 172 **篇論文的發表。參加人數約有三百多人，主要是大陸的學者及學生。大會於** 10 **月** 11 **日傍晚舉行接待酒會。**12 **日上午正式揭幕，由英國帝國學院的** A. Manz **教授講述以微晶片基底之分析系統之新近發展，接著由瑞典的** Stella Hjerten **教授做專題演講，講述微電泳新近發展的方法。美國華盛頓大學的** N. Dovichi **教授因事未克前來參加。隨後展開為期三天的學術活動。**

此次大會專題演講及學術研討分幾個主題在二個場地同時進行研討，研討氣氛還算熱絡。此次研討會的主題著重於基因學、蛋白質體學、藥物及生醫應用、微流體學及微晶片、對掌分離、新偵測方法、微分離技術、生化分析之濃縮技術、分離原理等。研討會所邀請及參加的國際知名學者不計其數，聆聽他們的研究結果，對毛細管電泳分離的現況及未來研究趨勢會更能掌握。此次研討會的許多主題及邀請演講中與我個人的研究最有關聯的是對掌分離與線上濃縮技術。因此美國 Caliper **公司的** R. L. Chien **博士的講題**" Sample stacking in microfluidic devices " **（**L066**），香港** Hong Kong Baptist University **的** Carmen W. Huei **教授的講題** " Acetonitrile stacking in capillary electrophoresis for the determination of positional and optical isomers " **（**L010**），及日本** Himeji Institute of Technology **的** S. Terabe **教授的講題** " Development of on-line sample preconcentration techniques by manipulating sample capillary matrices and background electrolytes in capillary electrophoresis " **（**L009**）最有助益也特別值得一提。至於壁報論文方面，也有一些是關於對掌分離與線上濃縮技術的論文。這些論文的瞭解對目前的研究工作與方向提供了不少值得思考的地方，收穫不少。**

在此研討會中，筆者提出三篇論文發表。一篇口頭論文，二篇壁報論文。論文題目為：（1） Enantioseparation and Migration Reversal of Phenothiazines in Cyclodextrin-Modified Capillary Zone Electrophoresis；（2） Influence of pH on Electrophoretic Behavior of phenothiazines and Determination of pKa values by Capillary Zone Electrophoresis；（3） Prediction of Migration Behavior and Determination of pKa values of Quinolones with a Piperazine Substituent by Capillary Zone Electrophoresis **甚得同一研究領域的學者的興趣。此論文希望可發表刊登於** Electrophoresis **期刊上。此次研討會另有交通大學應化系謝有容教授、成功大學化學系陳淑慧教授（由學生代表出席）、靜宜大學應化系王書蘋教授的參與。**

二、與會心得：

1. **此次參加亞太微尺標分離及分析國際研討會，除了能多了解亞太及國際上研究領域進展的現況，吸收新知識，交換研究心得之外，並能多認識一些國際知名學者。此次研討會所發表的論文雖然不算多，因有不少國際知名學者參與盛會，還是值得觀摩學習，也能啟發研究構想，值得參加。國內學者參加國際會議之人數仍然不多，應多參與以提昇亞太國際間之學術地位。**
2. **此次論文的發表主要以中國學者為主，論文數目雖然有些，但研究水準一般還是較低。**

三、考察參觀活動：**除參觀儀器展外，大會特別安排在東方明珠塔上參加研討會宴會，讓人感受上海的繁榮熱鬧。**

四、攜回資料：**論文摘要乙冊。**

五、建議：

參加國際學術研討會，不僅參加者能增廣見聞與學識，也可讓國際人士瞭解台灣學者的研究成果，應多多鼓勵參加。

出席國際學術會議報告書 (國科會補助)

92 年 7 月 12 日

報告人姓名	林敬二	服務機關名稱及職務	國立台灣大學 化學系教授
會議期間及地點	92 年 6 月 15-19 日 法國，尼斯	國科會 研究計劃	NSC-91-2113-M-002-054
會議名稱	(中文) 第 27 屆高效液相分離國際研討會-HPLC 2003 (英文) International Symposium on High Performance Liquid Phase Separation-HPLC 2003		
發表論文題目	(中文) 以雙環糊精在硼酸鹽錯合下利用區帶電泳法探討苯偶坐咽之對掌分離及羥基苯偶坐咽對掌異構物之遷移反轉 (英文) Enantioseparation of benzoins and enantiomers migration reversal of hydrobenzoin in capillary zone electrophoresis with dual cyclodextrins in the presence of borate complexation		

報告內容

一、參加會議經過：

第 27 屆高效液相分離國際研討會於法國尼斯舉行。會期為 6 月 15-19 日，2003 年，由法國的 A. M. Siouffi 教授籌辦及法國分離科學學會主辦。會場設於尼斯的國際會議中心(Acropolis)。

此次研討會共有口頭論文 98 篇及壁報論文 608 篇共 706 篇論文的發表。參加人數約有一千二百多人。大會於 6 月 16 日上午正式揭幕。此次大會的揭幕式與往年有所不同，主辦人顯然別出心裁，在大會主席簡單致詞後隨及由一團樂隊演奏一些輕鬆活潑的樂曲來助興，並放映所有參加國的國旗(共 54 國)，中華民國國旗與中華人民共和國的國旗可以同時並存。在中國的強權與長期"鴨霸"的作風下，實在難得見到這種場面，也因此令人振奮不已。筆者曾在酒會中特地詢問主辦人 Siouffi 教授他是否知道"台灣"有別於"中國"，他回答說"當然知道"。所以"台灣"應該走出去，才不會被"中國"壓得動彈不得。此次學術會議首先由法國法醫學研究所的 P. Kintz 博士介紹 LC/MS 與 LC/MS/MS 在法醫毒物學上的就近發展。他強調 LC/MS 的分析應用於法醫學及法醫毒物學上使得法醫學上的偵查與鑑定能有一大突破。而 LC/MS/MS 的應用更讓樣品的處理過程簡化而省時方便。接著由美國 Oak Ridge 國家實驗室的 J. M. Ramsey 博士講述奈米尺標管道中的分子傳送現象。管道尺標由 1 μm 減小至 100 nm 時分子的傳送現象與分離機制的瞭解將引發新的革命性的技術，隨後展開為期四天的學術活動。晚上舉行酒會招待。

此次大會專題演講及學術研討分幾個主題在二個場地同時進行研討，研討氣氛相當熱絡。此次研討會的主題著重於 Monolithic 管柱及靜相、蛋白質體學、毛細管電泳、對掌分離、新偵測方法、微小化及微分離技術、分離原理、SFC/高分子聚合物、儀器連線等。研討會所邀請及參加的國際知名學者不計其數，聆聽他們的研究結果，對層析及毛細管電泳分離的現況及未來研究趨勢將會更能掌握。此次研討會的許多主題及邀請演講中與我個人的研究最有關聯的是對掌分離，與毛細管電泳線上濃縮技

術。因此比利時 University of Liege 的 J. Crommen 教授的講題 "Modulation of selectivity for the enantiomeric separation of basic compounds in non-aqueous CE using anionic cyclodextrins in combination with ion-pairing reagents"，奧地利 University of Vienna 的 W. Lindner 教授的講題 "Development of novel chiral anion exchangers: reflections on mechanisms, pros and cons"，及日本 Himeji Institute of Technology 的 S. Terabe 教授的講題 "CE-MS with on-line sample preconcentration for high sensitivity analysis of proteins and peptides" 最有助益也特別值得一提。至於壁報論文方面，也有一些是關於對掌分離與線上濃縮技術的論文。這些論文的瞭解對目前的研究工作與方向提供了不少值得思考的地方。

在此研討會中，筆者提出一篇論文發表。論文題目為：Enantioseparation of benzoins and enantiomers migration reversal of hydrobenzoin in capillary zone electrophoresis with dual cyclodextrins in the presence of borate complexation。此論文將發表刊登於 J. Chromatogr. A 上。此次研討會另有清華大學化學系黃賢達教授、東吳大學化學系傅明仁教授、台大食品科技研究所孫璐西教授、靜宜大學應化系王書蘋教授、輔仁大學食品營養系的陳朝輝教授及中國醫藥研究所的蔡東湖教授的參與。

二、與會心得：

此次參加高效液相分離國際研討會，除了能多了解此研究領域進展的現況，吸收新知識，交換研究心得之外，並能多認識一些國際知名學者。此次研討會所發表的論文不少，更有一些是值得觀摩學習的論文。能啟發研究構想，實在獲益不少，值得參加。今年國內學者參加國際會議之人數較多一些，這是可喜的事。應多鼓勵參與以提昇國際間之學術地位。

三、考察參觀活動：除參觀儀器展外，大會特別選在 Moggin 安排接待餐會以瞭解法國南部地中海沿海地區的村落生活與民俗。

四、攜回資料：論文摘要乙冊。

五、建議：

參加國際學術研討會，不僅參加者能增廣見聞與學識，也可讓國際人士瞭解台灣學者的研究成果，應多多鼓勵參加。