ELSEVIER

Journal of Chromatography A, 802 (1998) 95–105

JOURNAL OF CHROMATOGRAPHY A

Migration behavior and separation of tetracycline antibiotics by micellar electrokinetic chromatography

Yung-Chih Chen, Ching-Erh Lin*

Department of Chemistry, National Taiwan University, Taipei, Taiwan

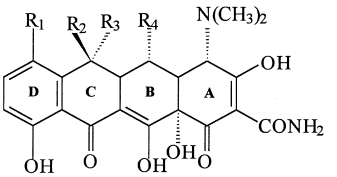
Abstract

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.

*Corresponding author.

PII S0021-9673(97)01181-3

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].

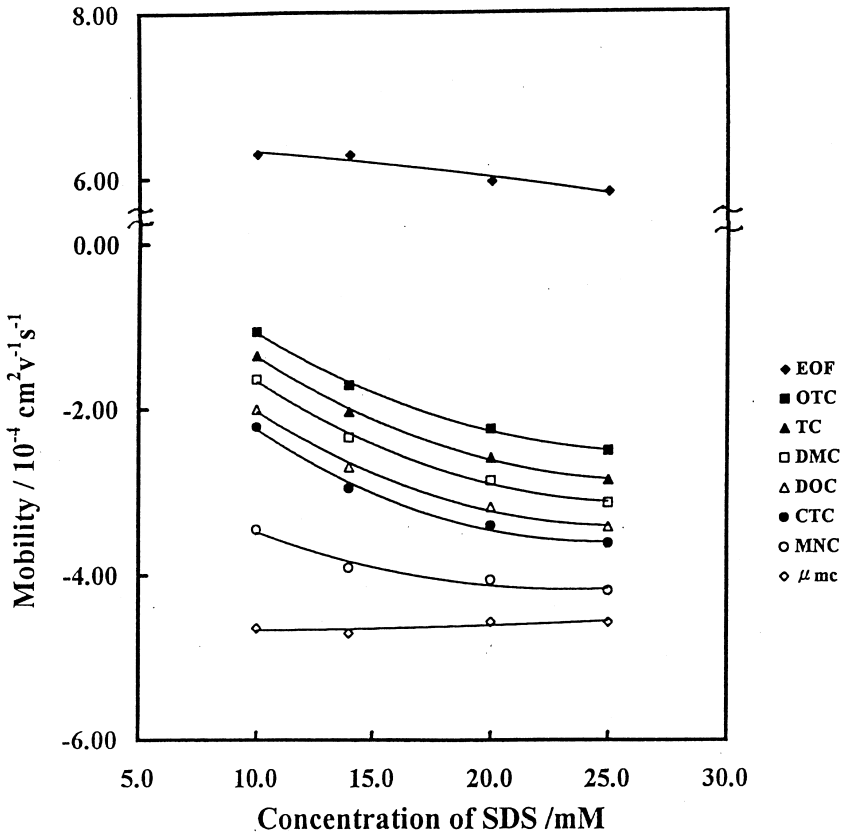


Fig. 2. Variation of the electrophoretic mobility of tetracyclines as a function of SDS concentration using 15 m*M* ammonium acetate buffer (pH 6.0). Capillary: 43 cm×50 μm, I.D., Other operating conditions: 15 kV, 25°C. Curve identification: (■) OTC, (▲) TC, (□) DMC, (△) DOC, (●) CTC, (○) MNC; the numbers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

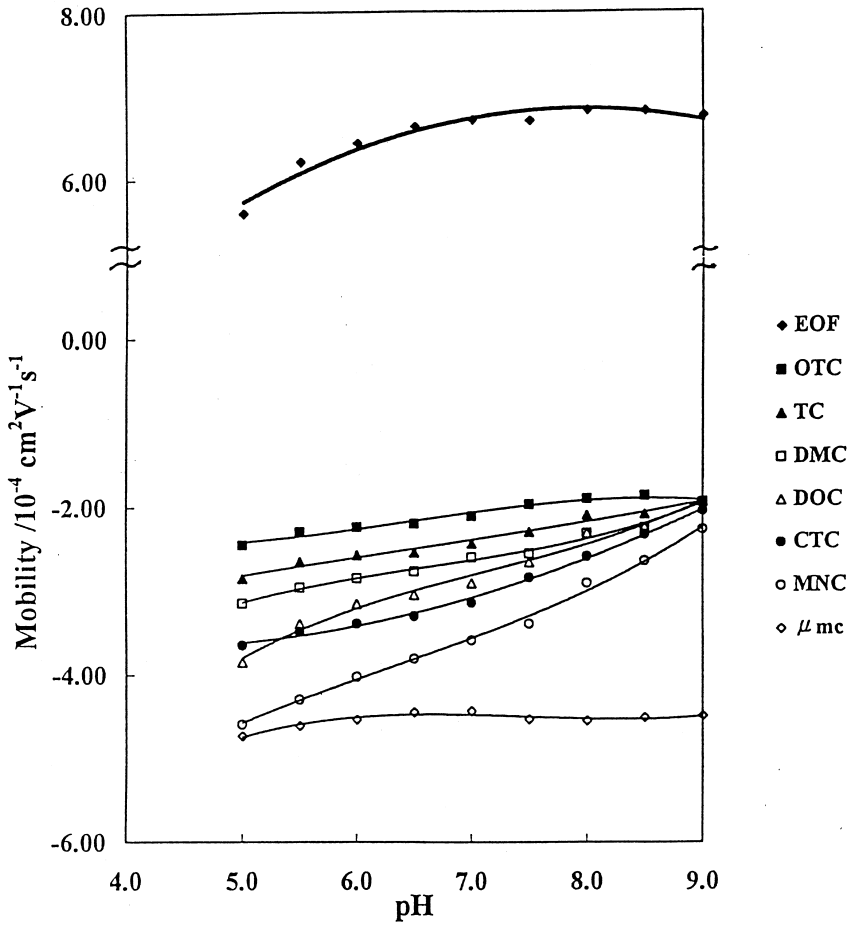


Fig. 3. Variation of the electrophoretic mobility of tetracyclines as a function of pH in the range 5.0–9.0 using 15 m*M* ammonium acetate buffer containing 20 m*M* SDS. Other operating conditions and peak identification are the same as for Fig. 2.

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.

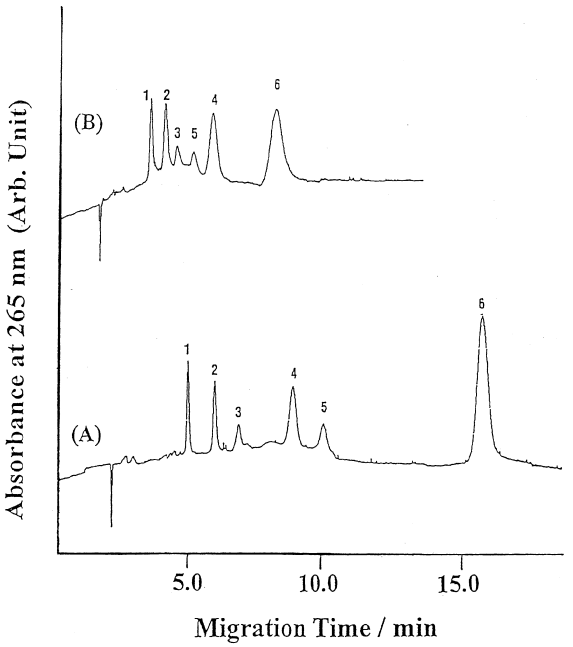


Fig. 4. Electropherograms of tetracyclines obtained with 15 m*M* ammonium acetate buffer containing 20 m*M* SDS at varied pH: (A) 5.0, (B) 6.5. Other operating conditions and peak identification as in Fig. 2.

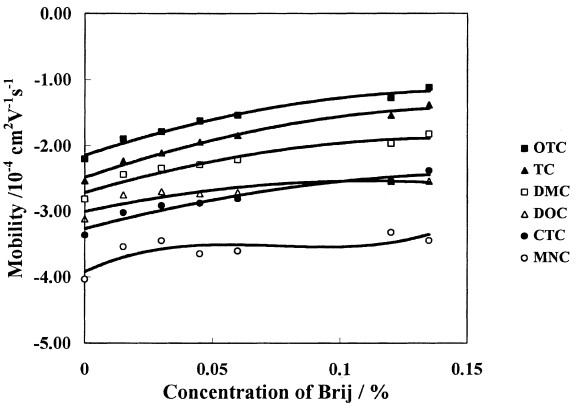


Fig. 5. Variation of the electrophoretic mobility of tetracyclines in a mixed micellar system containing 20 m*M* SDS and Brij 35 (up to 0.135%, w/v) with 15 m*M* ammonium acetate buffer (pH 6.5). Other operating conditions and peak identification as in Fig. 2.

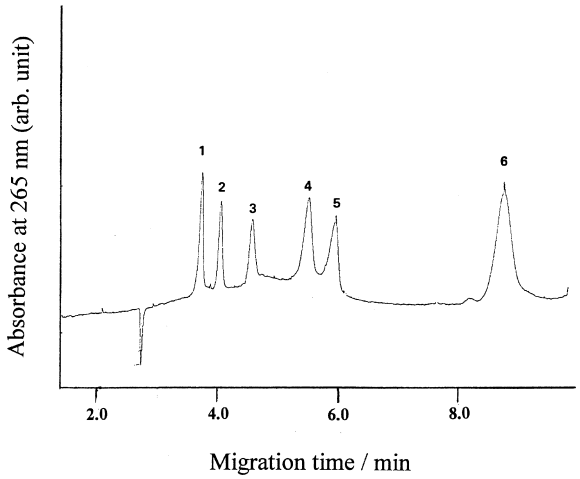


Fig. 6. Electropherogram of tetracyclines obtained with 15 m*M* ammonium acetate buffer containing 20 m*M* SDS and Brij 35 (0.135%, w/v) at pH 6.5. Other operating conditions and peak identification as in Fig. 2.

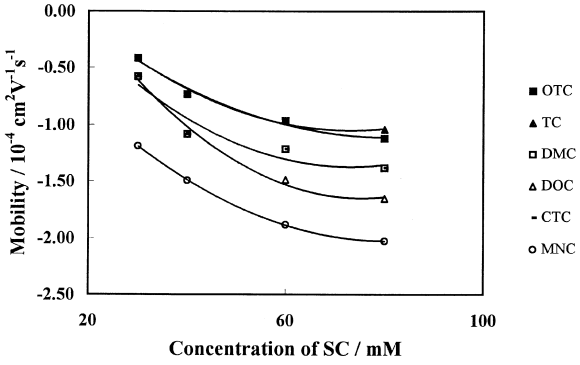


Fig. 7. Variation of the electrophoretic mobility of tetracyclines as a function of SC concentration using 15 m*M* ammonium acetate buffer (pH 6.5). Applied voltage: 12 kV. Other operating conditions and peak identification as in Fig. 2.

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

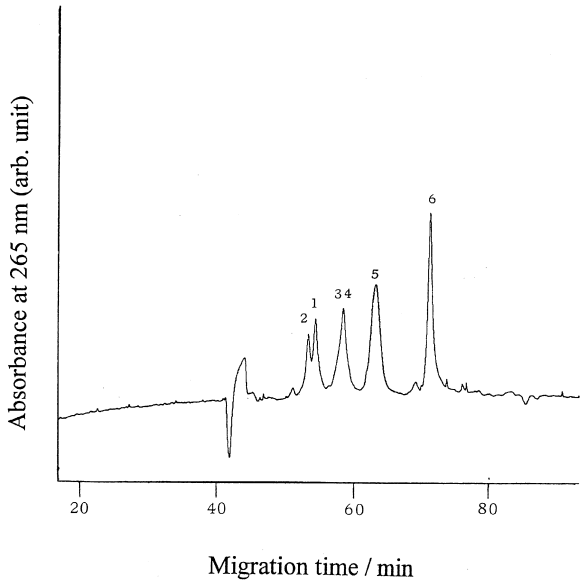


Fig. 8. Electropherogram of tetracyclines obtained with 15 m*M* ammonium acetate buffer containing 80 m*M* SC (pH 6.5). Other operating conditions and peak identification as in Fig. 7.

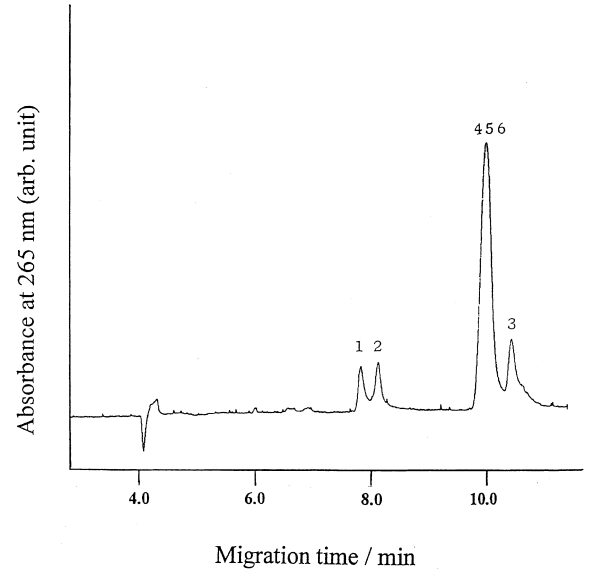


Fig. 9. Electropherogram of tetracyclines obtained with 30 m*M* ammonium acetate buffer containing 30 m*M* TTAB (pH 6.5). Applied voltage: −10 kV. Other operating conditions and peak identification as in Fig. 2.

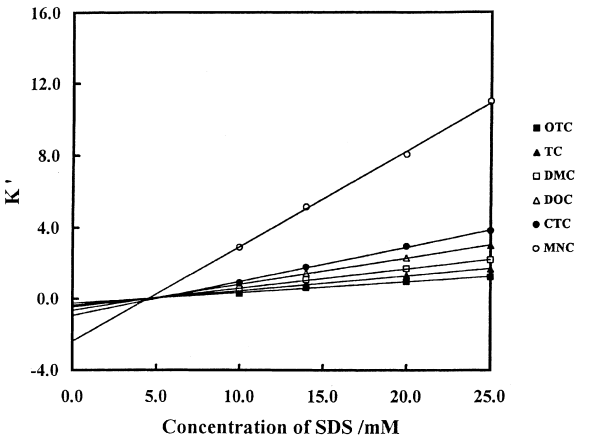


Fig. 10. Plots of migration factor (k') of tetracyclines as a function of SDS concentration at pH 6.0.

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

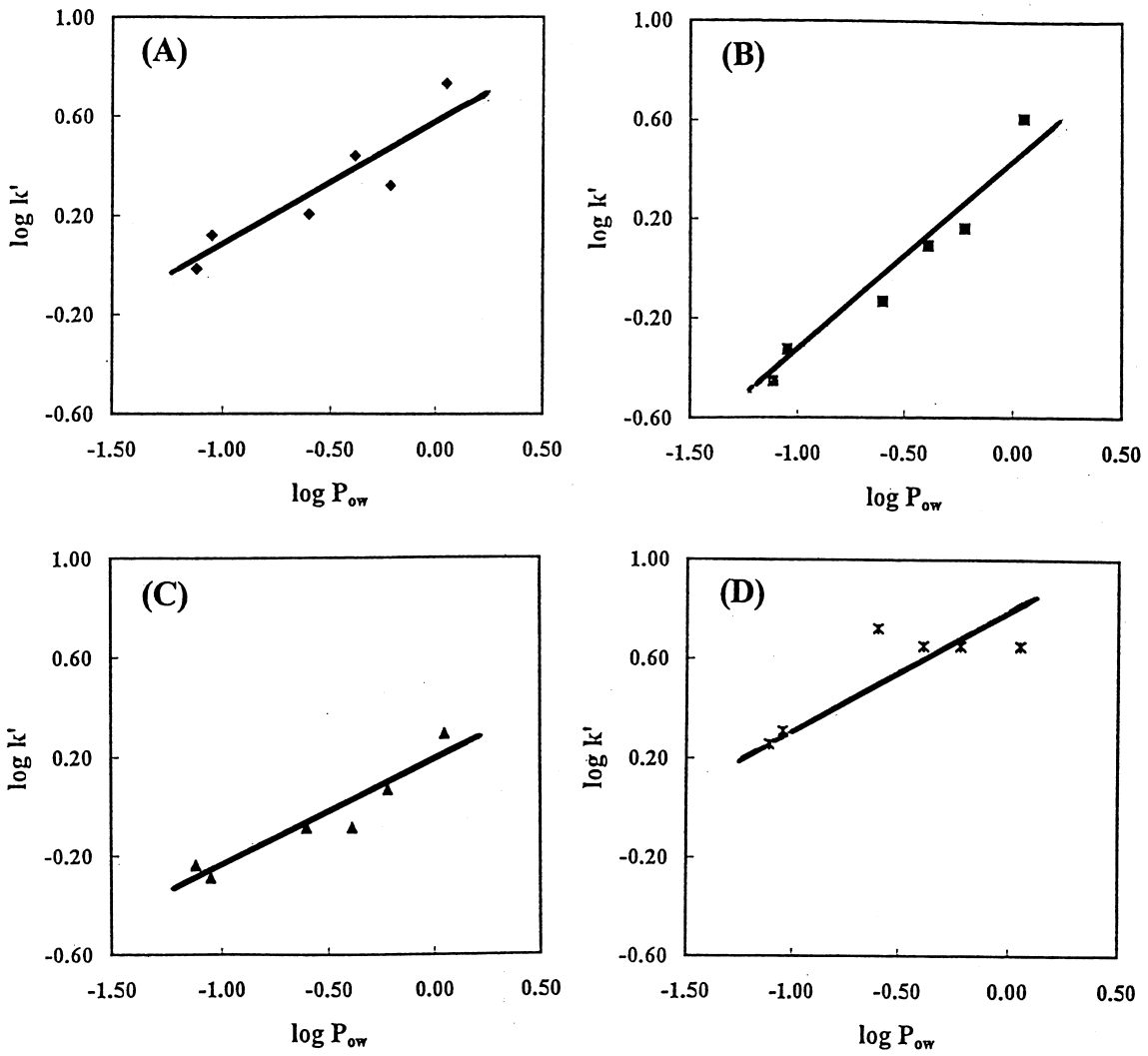


Fig. 11. Correlations of log k' versus log P_{ow} for tetracyclines in four different micellar systems at pH 6.5: (A), 20 m*M* SDS; (B), 20 m*M* SDS–Brij (0.135%, w/v); (C), 80 m*M* SC; (D), 30 m*M* TTAB.

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	$\log k' = a \log P_{ow} + b$		
		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.

Acknowledgements

We thank the National Science Council of Taiwan for financial support.

References

[1] L.A. Mitscher, The Chemistry of the Tetracycline Antibiotics (Medicinal Research Series, Vol. 9), Marcel Dekker, New York, 1978.
[2] N.E. Rigler, S.P. Bag, D.E. Leyden, J.L. Sudmeier, C.N. Reilley, Anal. Chem. 37 (1965) 872.
[3] N.H. Khan, P. Wera, E. Roets, J. Hoogmartens, J. Liq. Chromatogr. 13 (1990) 1351.
[4] Y.M. Li, A.V. Schepdael, E. Roets, J. Hoogmartens, J. Chromatogr. A 740 (1996) 119.
[5] S.A. Barker, C.C. Walker, J. Chromatogr. 624 (1992) 195.
[6] R.W. Fedeniuk, S. Ramamurthi, A.R. McCurdy, J. Chromatogr. B. 677 (1996) 291.
[7] H. Oka, Y. Ikai, N. Kawamura, K. Uno, M. Yamada, J. Chromatogr. 393 (1987) 285.
[8] H. Oka, Y. Ikai, N. Kawamura, K. Uno, M. Yamada, J. Chromatogr. 400 (1987) 253.
[9] H. Oka, Y. Ikai, N. Kawamura, K. Uno, M. Yamada, J. Chromatogr. 411 (1987) 313.
[10] W. Nadong, J. Thuranira, K. Vermeulen, E. Roets, J. Hoogmartens, J. Liq. Chromatogr. 15 (1992) 2529.
[11] W. Nadong, K. Verresen, E. Roets, J. Hoogmartens, J. Chromatogr. 586 (1991) 61.
[12] S.F.Y. Li, Capillary Electrophoresis: Principles, Practice and Applications, Elsevier, Amsterdam, 1993.
[13] N.A. Guzman (Editor), Capillary Electrophoresis Technology, Marcel Dekker, New York, 1993.
[14] P. Camilleri (Editor), Capillary Electrophoresis: Theory and Practice, CRC Press, Boca Raton, FL, 1993.
[15] D. Coleman (Editor), Directory of Capillary Electrophoresis, Elsevier, Amsterdam, 1994.
[16] J.P. Landers (Editor), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1994.
[17] H. Shintani, J. Polonsky (Editors), Handbook of Capillary Electrophoresis Applications, Blackie, London, 1997.
[18] C.-X. Zhang, Z.-P. Sun, D.-K. Ling, Y.-J. Zhang, J. Chromatogr. 627 (1992) 281.
[19] A.V. Schepdael, J. Saevels, X. Lepoudre, R. Kibaya, N.Z. Gang, E. Roets, J. Hoogmartens, J. High Resolut. Chromatogr. 18 (1995) 695.
[20] M.F.M. Tavares, V.L. McGuffin, J. Chromatogr. A 686 (1994) 129.
[21] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 736 (1996) 313.
[22] J. Tjornelund, S.H. Hansen, J. Chromatogr. A 737 (1996) 291.
[23] S. Croubels, W. Baeyens, C. Dewaele, C.V. Peteghem, J. Chromatogr. A 673 (1994) 267.
[24] C.L. Chen, X. Gu, J. AOAC Int. 78 (1995) 1369.
[25] C.E. Lin, W.C. Lin, W.C. Chiou, J. Chromatogr. A 722 (1996) 333.
[26] S. Terabe, K. Otsuka, K. I chikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[27] M.G. Khaledi, S.C. Smith, J.K. Strasters, Anal. Chem. 63 (1991) 1820.
[28] D.W. Armstrong, G.Y. Stine, J. Am. Chem. Soc. 105 (1983) 2962.
[29] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[30] S. Terabe, H. Ozaki, Y. Ishihama, Bunseki Kagaku 42 (1993) 859.
[31] S. Yang, M.G. Khaledi, Anal. Chem. 67 (1995) 499.
[32] S. Yang, J.G. Bumgarner, L.F.R. Kruk, M.G. Khaledi, J. Chromatogr. A 721 (1996) 323.
[33] S. Yang, M.G. Khaledi, J. Chromatogr. A 692 (1995) 301.
[34] C. Hansch, A. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley-Interscience, New York, 1979.
[35] C. Hansch, in: E. Aries (Editor), Drug Design, Vol. 1, Academic Press, New York, 1979, p. 271.
[36] R. Kaliszan, Quantitative Structure—Chromatographic Retention Relationships, Wiley-Interscience, New York, 1987.
[37] J.L. Colaizzi, P.R. Klink, J. Pharm. Sci. 58 (1969) 1184.
[38] G. Miller, H. Smith, W. Rock, S. Hedberg, J. Pharm. Sci. 66 (1977) 88.
[39] S. Yang, J.G. Bumgarner, M.G. Khaledi, J. Chromatogr. A 738 (1996) 265.
[40] Y. Ishihama, Y. Oda, K. Uchikawa, N. Asakawa, Chem. Pharm. Bull. 42 (1994) 1525.