



ELSEVIER

Journal of Chromatography A, 923 (2001) 241–248

JOURNAL OF  
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Separation and selectivity of benzophenones in micellar electrokinetic chromatography using sodium dodecyl sulfate micelles or sodium cholate modified mixed micelles

Ching-Erh Lin\*, Mei-Ju Chen

*Department of Chemistry, National Taiwan University, 1 Roosevelt Road Section 4, Taipei 10764, Taiwan*

Received 9 January 2001; received in revised form 17 April 2001; accepted 17 May 2001

## Abstract

The separation and selectivity of nine benzophenones in micellar electrokinetic chromatography (MEKC) using sodium dodecyl sulfate (SDS) micelles or sodium cholate (SC) modified mixed micelles were investigated in the pH range 6.5–8.0. The results indicate that the combined effects of buffer pH and SC concentration can greatly affect the separation and selectivity of benzophenones, particularly for benzophenones possessing a hydroxyl substituent at the 4-position of the aromatic ring with respect to the carbonyl moiety when using SDS–SC mixed micelles. Better separability can be obtained with SDS–SC mixed micelles than with SDS micelles. Complete separation of nine benzophenones in MEKC can be achieved with an appropriate choice of buffer pH and the concentration of SDS micelles or SC modified mixed micelles. The dependence of the migration order of those benzophenones based on their structures and solute–micelle interactions is discussed. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Buffer composition; Benzophenones; Sodium dodecyl sulfate; Sodium cholate; Mixed micelles

## 1. Introduction

Micellar electrokinetic chromatography (MEKC) is a powerful analytical method which enables excellent separation of both charged and electrically neutral analytes [1–6]. The separation utilizing this technique is based on the difference in the partitioning of analytes between the micellar and aqueous phases. Thus, a successful separation can be achieved by the modification of the micellar phase or aqueous phase so that the partition coefficients of analytes or the interactions of analytes with the

micellar or aqueous phase can be differentiated from each other [7,8].

To modify the aqueous phase, organic modifiers [9,10], such as methanol, acetonitrile or tetrahydrofuran, etc., and/or electrolyte additives, such as cyclodextrins [11,12], urea [13], and hydrogen-bonding or complexing agents [14,15] are often added to the aqueous buffer electrolyte. On the other hand, modifications of the micellar phase can be made with the use of mixed micelles [16–21] or the addition of solubilized organic additives to the micelles [22–25] so that the separation and selectivity can be effectively affected. In addition to the types of micelles, buffer pH and micelle concentration (as well as the molar ratio in the case of mixed micelles) are often

\*Corresponding author. Tel.: +886-223-635-357; fax: +886-223-636-359.

considered to be the most important experimental parameters affecting the separation and selectivity in MEKC [24–27].

Benzophenones possess a characteristic of strong UV absorption and are widely used as sunscreen agents or photostabilizers in cosmetic products. For the past two decades, these compounds have been generally separated and determined by chromatographic methods [28–32]. In recent years, capillary electrophoresis has become an advantageous and powerful technique to separate diverse analytical samples [2–6]. This technique provides high resolution, great efficiency, rapid analysis, and small consumption of both sample and solvent in comparison with HPLC. However, to our knowledge, the application of this technique to the separation of benzophenones has not yet been reported.

In this study, the separation and selectivity of benzophenones in MEKC using sodium dodecyl sulfate (SDS) micelles or sodium cholate (SC) modified mixed micelles are investigated. Influences of buffer pH and the concentration of SDS micelles or SC modified mixed micelles on the separation and selectivity of nine benzophenones are examined. Here, we present the results of our investigation.

## 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. Uncoated fused-silica capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were used. The dimensions of the capillary are 57 cm×50 μm I.D. The effective length of the capillary is 50 cm from the injection end of the capillary. The CE system was interfaced with a microcomputer. 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

The nine benzophenones studied were obtained

from Sigma–Aldrich (St. Louis, MO, USA). Sodium cholate was purchased from Tokyo Kasei Kogyo (TCI, Tokyo, Japan). Sodium dodecyl sulfate was supplied from Merck (Darmstadt, Germany). These chemicals were used as received. All other chemicals were of analytical-grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of test solutes at a concentration of 10 μg/ml were prepared by dissolving analytes in an aqueous solution containing 5% methanol. The pH of a phosphate buffer was adjusted to a desired value by mixing various proportions of a certain concentration of sodium dihydrogenphosphate solution with the same concentration of disodium hydrogenphosphate solution. 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 5 s under a pressure of 0.5 p.s.i. (1 p.s.i.=6894.76 Pa). The measurements were run at least in triplicate to ensure reproducibility. A voltage of 20 kV was applied. The detection wavelength was set at 214 nm.

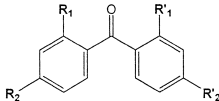
### 2.4. Mobility calculations

The electrophoretic mobility of analytes was calculated from the observed migration times as described previously [33,34].

## 3. Results and discussion

### 3.1. MEKC separation with SDS micelles

Fig. 1 shows the structures of the nine benzo-



No.	Benzophenones	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub> '	R <sub>2</sub> '
1	4,4'-Dihydroxy-benzophenone (4,4'-DHBP)	-H	-OH	-H	-OH
2	2,2',4,4'-Tetrahydroxy-benzophenone (2,2',4,4'-THBP)	-OH	-OH	-OH	-OH
3	2,4-Dihydroxy-benzophenone (2,4-DHBP)	-OH	-OH	-H	-H
4	2,2'-Dihydroxy-benzophenone (2,2'-DHBP)	-OH	-H	-OH	-H
5	2-Hydroxy-benzophenone (2-HBP)	-OH	-H	-H	-H
6	2,2'-Dihydroxy-4-methoxy-benzophenone (2,2'-DH-4-MBP)	-OH	-OCH <sub>3</sub>	-OH	-H
7	4,4'-Dimethoxy-benzophenone (4,4'-DMBP)	-H	-OCH <sub>3</sub>	-H	-OCH <sub>3</sub>
8	2-Hydroxy-4-methoxy-benzophenone (2-H-4-MBP)	-OH	-OCH <sub>3</sub>	-H	-H
9	2,2'-Dihydroxy-4,4'-dimethoxy-benzophenone (2,2'-DH-4,4'-DMBP)	-OH	-OCH <sub>3</sub>	-OH	-OCH <sub>3</sub>

Fig. 1. Structures of nine benzophenones studied.

phenones selected. Among them, the first three benzophenones which possess a hydroxyl group at the 4-position of the aromatic ring with respect to the carbonyl moiety of benzophenones are ionizable in the pH range studied. As the pH of the buffer determines the extent of the ionization of the analytes [26,27], manipulation of buffer pH becomes one of the key strategies to optimize the separation. In this study, combined effects of buffer pH and micelle concentration are taken into consideration to optimize the separation of the nine benzophenones selected.

### 3.1.1. Influence of buffer pH

Fig. 2 shows the variations in the effective electrophoretic mobility of nine benzophenones as a function of buffer pH in the range 6.5–8.0 using a phosphate buffer (20 mM) containing 20 mM SDS. As the  $pK_a$  values determined by capillary zone electrophoresis for 4,4'-DHBP (1), 2,2',4,4'-THBP (2) and 2,4-DHBP (3) are 7.60, 7.17 and 6.94, respectively [35], the effective electrophoretic mobility of these three analytes, migrating toward

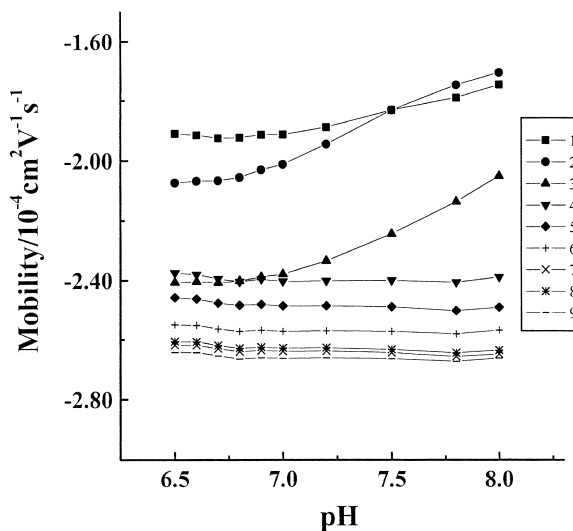


Fig. 2. Variations of the effective electrophoretic mobility of benzophenones as a function of buffer pH using 20 mM SDS–20 mM phosphate buffer system. Capillary: 57 cm×50  $\mu$ m, I.D.; sample concentration: 10  $\mu$ g/ml; detection wavelength: 214 nm; other operating conditions: 20 kV, 25°C. Curve identification, 1=4,4'-DHBP (■); 2=2,2',4,4'-THBP (●); 3=2,4-DHBP (▲); 4=2,2'-DHBP (▼); 5=2-HBP (◆); 6=2,2'-DH-4-MBP (+); 7=4,4'-DMBP (×); 8=2-H-4-MBP (\*); 9=2,2'-DH-4,4'-DMBP (–).

the anode, decreases markedly with increasing buffer pH from 6.8 to 8.0. This is probably due to the repulsive interaction between the anionic solute and anionic micelles, because the greater extent of the ionization of these three benzophenones, the less the extent of the interaction of solutes with micelles. As a result, a decrease in the electrophoretic mobility of the first three eluted benzophenones with increasing buffer pH is expected. However, the electrophoretic mobility of the rest of benzophenones increases slightly. Consequently, the selectivity of the first three pairs of eluted analytes may vary remarkably with buffer pH. For instance, the selectivity of peaks between 4,4'-DHBP (1) and 2,2',4,4'-THBP (2) decreases considerably with increasing buffer pH from 6.8 to 7.5, then increases slightly from pH 7.5 to 8.0; these two analytes comigrate at pH 7.5. Moreover, 2,4-DHBP (3) comigrates with 2,2'-DHBP (4) at pH 6.8. Consequently, the reversal of the migration order of these two pairs of analytes occurs when the buffer pH exceeds 7.5 and 6.8, respectively. It was observed that baseline separation of these nine analytes could be achieved with the

SDS micellar buffer system at about pH 6.5–6.7, 7.0–7.3 and 7.7–8.0. Fig. 3 shows such electropherograms of benzophenones obtained at pHs 6.6, 7.0 and 8.0.

It is noteworthy that, as shown in Fig. 3C, a fronting peak of 2,4-DHBP (3) was observed with a micellar buffer system containing 20 mM SDS at pH 8.0. This phenomenon, induced by the change in the pH of the background electrolyte, can be explained on the basis of the concept of peak shape diagram proposed by Gebauer, Bocek and coworkers [36,37]. Detailed analysis on the peak shape of benzophenones under various experimental conditions will be reported later.

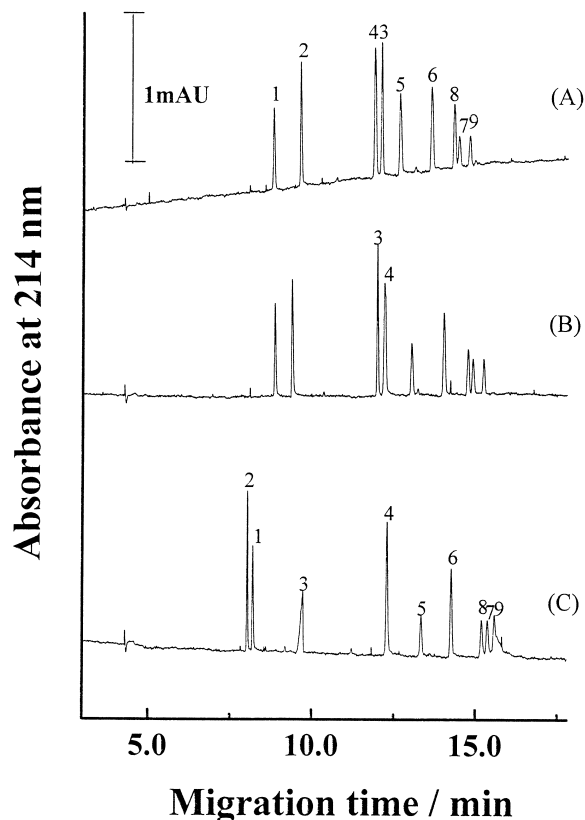


Fig. 3. Electropherograms of benzophenones obtained with a micellar phosphate buffer (20 mM) containing 20 mM SDS at varied buffer pH: (A) 6.6; (B) 7.0; (C) 8.0. Other operating conditions are the same as for Fig. 2. Peak identification and peak numbers are the same as for curve numbers in Fig. 2.

### 3.1.2. Influence of SDS concentration

It was observed that the selectivity of benzophenones, except that of the peaks between 4,4'-DMBP (7) and 2-H-4-MBP (8), was not significantly affected when the micelle concentration of SDS was greater than 20 mM at pH 6.8. The peaks of benzophenones, except the first three analytes, became broadened with SDS at 15 mM and the peak shape of these benzophenones was even severely deteriorated with a micellar buffer system containing SDS at a concentration less than 12 mM at pH 6.8. This phenomenon of the peak deformation was also observed in the separation of corticosteroids with a micellar buffer electrolyte containing 6 mM SDS at pH 8.7 [16].

### 3.2. MEKC separation with SDS–SC mixed micelles

It has been reported that the selectivity of analytes in MEKC can be improved and the elution range in MEKC can be extended with the use of mixed micelles [33,38–42]. In this study, a less hydrophobic sodium cholate (SC) is chosen to form SDS–SC mixed micelles so that the separation window can be extended.

#### 3.2.1. Influence of buffer pH

Fig. 4 shows the variations of effective electrophoretic mobility of nine benzophenones as a function of buffer pH in the range 6.5–8.0 with a phosphate buffer (20 mM) containing 20 mM SDS and 10 mM SC. In comparison with the results shown in Fig. 2, the separation and selectivity of benzophenones, especially the first four analytes, are greatly affected with the use of the SC modified SDS micellar phase. Depending on the pH of the buffer, the electrophoretic mobility of 4,4'-DHBP (1) decreases greatly by 5–13%, with a greater extent of the variation at a pH in the lower buffer pH region; the electrophoretic mobility of 2,2'-DHBP (4), 4,4'-DMBP (7) and 2,4-DHBP (3) decreases also considerably. So does the electrophoretic mobility of the rest of benzophenones, except 2,2',4,4'-THBP (2), but to a less extent. On the contrary, the electrophoretic mobility of 2,2',4,4'-THBP (2) increases significantly by 3–6%. As a result, the selectivity and migration order of benzophenones in the pres-

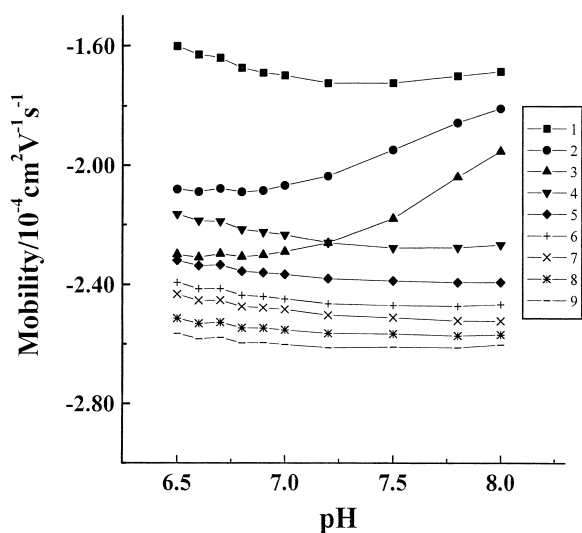


Fig. 4. Variations of the effective electrophoretic mobility of benzophenones as a function of buffer pH using a micellar phosphate buffer (20 mM) containing 20 mM SDS and 10 mM SC. Other operating conditions and curve identification are the same as for Fig. 2.

ence of SC differ considerably from those without the presence of SC.

It is noteworthy that, as a consequence of a greater extent of the decrease in the electrophoretic mobility of 2,2'-DHBP (4) than that of 2,4-DHBP (3) with the SC modified SDS micellar phase, the reversal of the migration order of these two benzophenones does not occur until the pH of the buffer electrolyte exceeds 7.2. Thus, 2,2'-DHBP (4) elutes before 2,4-DHBP (3) at pH 7.0. This is opposite to the migration order observed in Fig. 3B. Moreover, 2-H-4-MBP (8) migrates faster than 4,4'-DMBP (7) toward the anode in the presence of SC. Thus 4,4'-DMBP (7) elutes before 2-H-4-MBP (8). Furthermore, as a result of an increase in the electrophoretic mobility of 2,2',4,4'-THBP (2) with the presence of SC owing to the enhancement of hydrogen bonding interaction, 4,4'-DHBP (1) elutes before 2,2',4,4'-THBP (2) even at pH values in the range 7.5–8.0.

As shown in Fig. 4, complete separation of the nine selected benzophenones can be achieved using SDS–SC mixed micelles at almost any pH in the range 6.5–8.0, except at about 7.2 at which the crossing of the mobility curves of 2,4-DHBP (3) and

2,2'-DHBP (4) occurs. Fig. 5 shows the three representative electropherograms of benzophenones obtained with 20 mM SDS–10 mM SC mixed micellar phase at pH 6.8, 7.0 and 7.5.

### 3.2.2. Influence of SC concentration at a chosen pH

Fig. 6A shows the variations of electrophoretic mobility of benzophenones as a function of SC concentration in the range 0–30 mM using a phosphate buffer (20 mM) containing 20 mM SDS and varied concentrations of SC at pH 7.0. As demonstrated, the reversal of the migration order of 2,4-DHBP (3) and 2,2'-DHBP (4) and that of 4,4'-DMBP (7) and 2-H-4-MBP (8) occurs with the

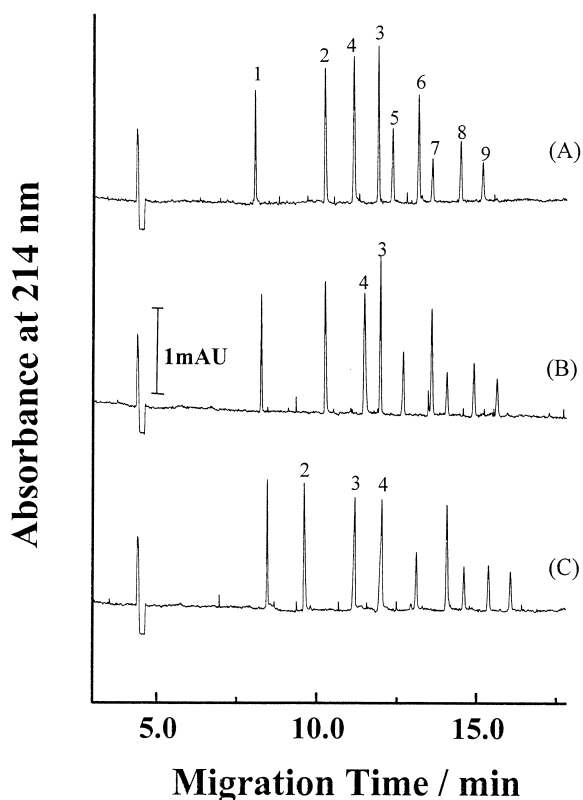


Fig. 5. Electropherograms of benzophenones obtained with a micellar phosphate buffer (20 mM) containing 20 mM SDS and 10 mM SC at varied buffer pH: (A) 6.8; (B) 7.0; (C) 7.5. Other operating conditions are the same as for Fig. 2. Peak identification and peak numbers are the same as for curve numbers in Fig. 2.

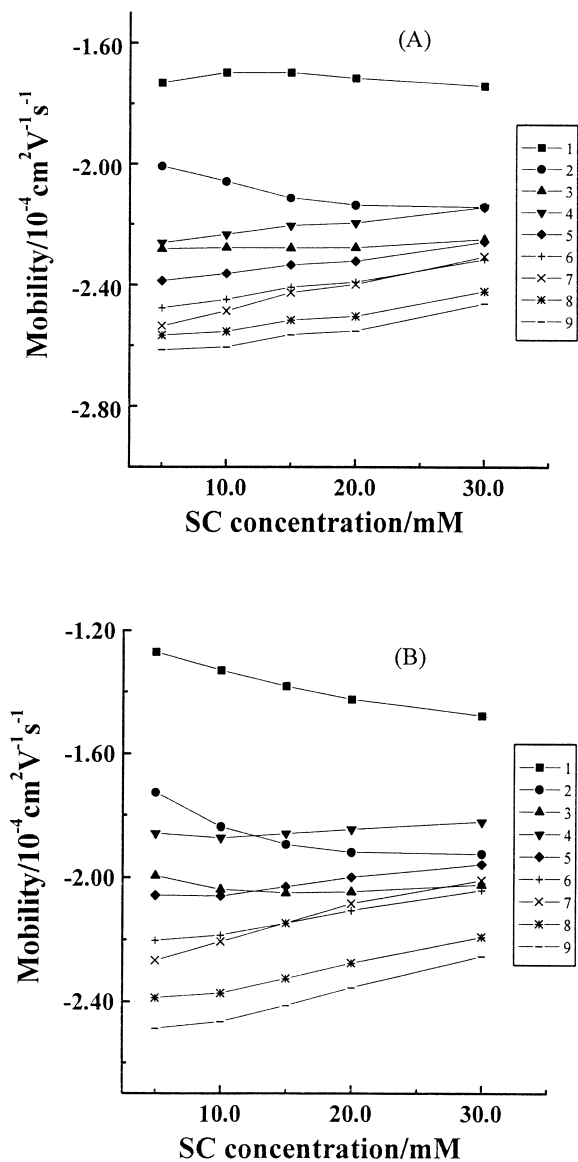


Fig. 6. Variations of the effective electrophoretic mobility of benzophenones as a function of SC concentration using a SC modified SDS micellar phosphate buffer (20 mM) at pH 7.0: (A) 20 mM SDS and (B) 10 mM SDS. Other operating conditions and curve identification are the same as for Fig. 2.

addition of 5 mM SC in the SDS micellar phase; 4,4'-DMBP (7) migrates closely with 2,2'-DH-4-MBP (6) when the SC concentration exceeds 15 mM. Therefore, in order to achieve baseline separation of those benzophenones, SC should be restricted at a concentration in the range 5–15 mM.

The trends of the variations of electrophoretic mobility of benzophenones as a function of SC concentration with a phosphate buffer containing 30 mM SDS are similar to those obtained with a mixed micellar phosphate buffer as for Fig. 6A. However, the separation window is considerably smaller than that observed in Fig. 6A and the influence of SC concentration on the separation and selectivity of benzophenones at pH 7.0 is comparatively smaller than those shown in Fig. 6A. As a matter of fact, complete separation of the nine selected benzophenones can only be achieved in a small SC concentration range around 17–22 mM.

The variations in the electrophoretic mobilities of benzophenones as a function of SC concentration with a phosphate buffer containing 10 mM SDS are shown in Fig. 6B. As the separation window is extended, in comparison with the one shown in Fig. 6A, the influence of SC concentration on the separation and selectivity of these nine benzophenones at pH 7.0 is comparatively greater than that observed in Fig. 6A. The reversal of the migration order of 2,2',4,4'-THBP (2) and 2,2'-DHBP (4), of 2,4-DHBP (3) and 2-HBP (5), and of 2,2'-DH-4-MBP (6) and 4,4'-DMBP (7) occurs with SC concentration exceeding 12, 12 and 15 mM, respectively. Thus, baseline separation of the nine selected benzophenones can be achieved with the SC concentration in the range of 5–10 mM, but may find difficult to achieve with SC in the concentration range of 11–17 mM. Nevertheless, these nine benzophenones could be effectively separated again with the addition of SC at a concentration in the range 17–23 mM.

### 3.3. Migration order versus structure of solutes and solute–micelles interaction

In MEKC separation, the electrophoretic mobility of a neutral solute depends solely on the extent of solute–micelle interaction, but for an ionizable solute, the electrophoretic mobility depends not only on the extent of solute–micelle interactions but also on the degree of ionization and its mass. As the interior core of SDS micelles is highly hydrophobic, the separation and selectivity of solutes in the SDS micellar phase is predominantly governed by hydrophobic interactions. The results shown in Fig. 2

indicate that, among the first five eluted benzophenones, the hydrophobicity of the analytes increases with decreasing the number of hydroxyl substituents at the 4-position. However, the electrophoretic mobility of benzophenones in MEKC increases with increasing the number of the hydroxyl groups at the 2-position. The hydroxyl group(s) at the 2-position of the aromatic ring can form intramolecular hydrogen bonds with nearby carbonyl group(s) of benzophenones [43]. Evidently, these results reveal that the interaction between solutes and SDS micelles is not solely determined by the hydrophobic interaction [25,34,44]. In view of the hydrogen bond donating characteristics of SDS micelles, hydrogen bonding interactions may play a significant role in the interaction between benzophenones and SDS micelles, especially for benzophenones possessing strong hydrogen bond accepting characteristics, such as 2,2',4,4'-THBP (2).

As benzophenones with hydroxyl substituent(s) at the 4-position of the aromatic ring are comparatively less hydrophobic than those with a hydrogen atom which, in turn, are less hydrophobic than those with methoxy substituent(s), the first two benzophenones are the least hydrophobic compounds and the first five benzophenones are certainly less hydrophobic than the rest of the analytes which possess a methoxy group at the 4-position of the aromatic ring.

As illustrated in Figs. 2 and 4, there is a greater difference between the electrophoretic mobility between 2,2',4,4'-THBP (2) and 4,4'-DHBP (1) with SDS–SC mixed micelles than with SDS micelles, indicating that the hydrogen bonding interactions involved between 2,2',4,4'-THBP (2) and SDS–SC mixed micelles is stronger than that between 2,2',4,4'-THBP (2) and SDS micelles.

Similar arguments are applicable to the explanation of the migration order of the last four eluted analytes. In this case, the hydrophobic interaction between solutes and micelles increases with increasing the number of methoxy substituent(s) at the 4-position of the aromatic ring, whereas the hydrogen bonding interaction is influenced by the number of the hydroxyl group at the 2-position. As a result, 2,2'-DH-4,4'-DMBP (9) migrates after 4,4'-DMBP (7) and 2-H-4-MBP (8), whereas 4,4'-DMBP (7) migrates before 2-H-4-MBP (8) in the presence of SC.

#### 4. Conclusion

The separation and selectivity of the nine benzophenones selected can be mediated by manipulation of buffer pH and SDS micelle concentration or SC modified SDS micelle concentration. With the SC modified SDS micellar buffer system, the separation window is considerably extended. Complete separation of nine benzophenones in MEKC can be achieved with an appropriate choice of buffer pH and the concentration of SDS micelles or SC modified mixed micelles. Moreover, the migration order of those benzophenones can be explained on the basis of their structures and solute–micelle interactions. The results of the present investigation reveal that, in addition to predominant hydrophobic interactions, hydrogen bonding interactions may also play a significant role in the separation of these benzophenones.

#### Acknowledgements

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

#### References

- [1] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, *Anal. Chem.* 56 (1984) 111.
- [2] S.F.Y. Li, *Capillary Electrophoresis: Principles, Practice and Applications*, Elsevier, Amsterdam, 1993.
- [3] N.A. Guzman (Ed.), *Capillary Electrophoresis Technology*, Marcel Dekker, New York, 1993.
- [4] P. Camilleri (Ed.), *Capillary Electrophoresis*, CRC Press, Boca Raton, FL, 1993.
- [5] H. Shintani, J. Polonsky (Eds.), *Handbook of Capillary Electrophoresis Applications*, Blackie, London, 1997.
- [6] M.G. Khaledi (Ed.), *High Performance Capillary Electrophoresis: Theory, Techniques and Applications*, Wiley, New York, 1998.
- [7] S. Katsuta, K. Saitoh, *J. Chromatogr. A* 780 (1997) 165.
- [8] T. De Boer, R.A. de Zeeuw, G.J. de Jong, K. Ensing, *Electrophoresis* 20 (1999) 2989.
- [9] S. Takeda, S. Wakilda, M. Yamane, A. Kawahara, K. Higashi, *Anal. Chem.* 65 (1993) 2489.
- [10] K. Sarmini, E. Kennler, *J. Chromatogr. A* 792 (1997) 3.
- [11] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.G. Patterson, B.L. Karger, K. Hosoya, N. Tanaka, *J. Chromatogr.* 516 (1990) 23.

- [12] T. De Boer, R. Bijima, K. Ensing, J. Cap. *Electrophoresis* 5 (1998) 65.
- [13] S. Terabe, Y. Ishihama, H. Nishi, T. Fukuyama, K. Otsuka, J. *Chromatogr.* 545 (1991) 359.
- [14] Y. Esaka, Y. Yamaguchi, K. Kano, M. Goto, *Anal. Chem.* 66 (1994) 2441.
- [15] Y. Esaka, M. Goto, H. Haraguchi, T. Ikeda, K. Kano, J. *Chromatogr. A* 711 (1995) 305.
- [16] E.L. Little, J.P. Foley, *J. Microcol. Sep.* 4 (1992) 145.
- [17] E.S. Ahuja, B.P. Preston, J.P. Foley, *J. Chromatogr. B* 657 (1994) 271.
- [18] S.K. Wiedmer, M.-L. Riekkola, *Anal. Chem.* 69 (1997) 1577.
- [19] C.P. Ong, C.L. Ng, H.K. Lee, S.F.Y. Li, *Electrophoresis* 15 (1994) 1273.
- [20] Y. Esaka, M. Kobayashi, T. Ikeda, K. Kano, *J. Chromatogr. A* 736 (1996) 273.
- [21] Y. Esaka, K. Tanaka, B. Uno, M. Goto, *Anal. Chem.* 69 (1997) 1332.
- [22] S. Katsuta, K. Saitoh, *Anal. Lett.* 27 (1994) 743.
- [23] S. Katsuta, T. Tsumura, K. Saitoh, N. Teramae, *J. Chromatogr. A* 705 (1995) 319.
- [24] S. Yang, M.G. Khaledi, *J. Chromatogr. A* 692 (1995) 301.
- [25] S. Yang, M.G. Khaledi, *Anal. Chem.* 67 (1995) 499.
- [26] S.C. Smith, M.G. Khaledi, *Anal. Chem.* 65 (1993) 193.
- [27] C.E. Lin, W.C. Lin, W.C. Chiou, *J. Chromatogr. A* 705 (1995) 325.
- [28] K. Ikeda, S. Suzuki, Y. Watanabe, *J. Chromatogr.* 513 (1990) 321.
- [29] D.K.L. Hodegeman, *J. Chromatogr.* 214 (1981) 237.
- [30] H.S.I. Tan, R. Sih, S.E. Moseley, J.L. Lichtin, *J. Chromatogr.* 587 (1991) 325.
- [31] K.W. Ro, J.B. Choi, M.H. Lee, J.W. Kim, *J. Chromatogr. A* 688 (1994) 375.
- [32] L.H. Wang, *Chromatographia* 50 (1999) 565.
- [33] C.E. Lin, C.C. Chen, H.W. Chen, H.C. Huang, C.H. Lin, Y.C. Liu, *J. Chromatogr. A* 910 (2001) 165.
- [34] C.E. Lin, M.J. Chen, H.C. Huang, H.W. Chen, *J. Chromatogr. A* (2001), in press.
- [35] M.J. Chen, C.E. Lin, unpublished results.
- [36] P. Gebauer, P. Bocek, *Anal. Chem.* 69 (1997) 1557.
- [37] P. Gebauer, P. Borecka, P. Bocek, *Anal. Chem.* 70 (1998) 3397.
- [38] H.T. Rasmussen, L.K. Goebel, H.M. Menair, *J. High Resolut. Chromatogr.* 14 (1991) 25.
- [39] Q. Wu, H.A. Claessens, C.A. Cramers, *Chromatographia* 34 (1992) 25.
- [40] E.L. Little, J.P. Foley, *J. Microcol. Sep.* 4 (1992) 145.
- [41] Y. Esaka, M. Kobayashi, T. Ikeda, K. Kano, *J. Chromatogr. A* 736 (1996) 273.
- [42] E.S. Ahuja, E.L. Little, K.R. Nielsen, J.P. Foley, *Anal. Chem.* 67 (1995) 26.
- [43] M.R. Clark, L.E. Garcia-Roura, C.R. Clark, *J. Liq. Chromatogr.* 11 (1988) 3213.
- [44] C.E. Lin, Y.C. Chen, C.C. Chang, D.Z. Wang, *J. Chromatogr. A* 775 (1997) 349.