Yu-San Huang[1]
Chung-Chen Tsai[1]
Ju-Tsung Liu[2]
Cheng-Huang Lin[1]

[1]Department of Chemistry, National Taiwan Normal University, Taipei, Taiwan
[2]Forensic Science Center, Military Police Command, Ministry of National Defense, Taipei, Taiwan

Comparison of the use of aqueous and nonaqueous buffers in association with cyclodextrin for the chiral separation of 3,4-methylenedioxymethamphetamine and related compounds

A comparison of the use of aqueous and nonaqueous buffers in association with β-CD for the chiral separation of (*R*)- and (*S*)-3,4-methylenedioxymethamphetamine and related compounds is described. The (*R*)- and (*S*)-isomers of 3,4-methylenedioxymethamphetamine (MDMA) and its major metabolite 3,4-methylenedioxyamphetamine (MDA) were prepared. Under aqueous and nonaqueous buffer conditions and based on the CZE and MEKC modes, the order of migration of (*R*)-MDA, (*S*)-MDA, (*R*)-MDMA, and the (*S*)-MDMA enantioisomers were determined. Several electrophoretic parameters, including the concentration of β-CD (aqueous, 25–60 mM; nonaqueous, 20–150 mM) used in the electrophoretic separation and the amount of organic solvents required for the separation, were optimized.

Keywords: Aqueous and nonaqueous buffers; Capillary electrophoresis; Enantioseparation; 3,4-Methylenedioxymethamphetamine DOI 10.1002/elps.200410374

1 Introduction

3,4-Methylenedioxymethamphetamine (MDMA) was first synthesized (1912) and patented (1914) by Merck Pharmaceuticals. The toxicity of MDMA was examined in ~1950 in animal tests. It has been a drug of abuse since ~1980 and was placed in Schedule I permanently in 1988 in the USA. In fact, Shulgin *et al.* in their publication *PiHKAL* (Phenethylamines I Have Known And Loved) have documented over 250 phenethylamine derivatives, including MDMA, 3,4-methylenedioxyamphetamine (MDA), *N,N*-dimethyl-3,4-methylenedioxyamphetamine (DMMDA), and many others in 1991; synthetic procedures were also reported in their monograph [1] MDMA and its major metabolite MDA all contain a chiral center and, as a result, can exist in (*R*)- and (*S*)-forms. Because of the difference in the pharmacological activity of these enantiomers [2, 3], it is necessary to determine the distribution of these enantiomers in clandestine tablets and in suspect urine samples. It is especially noteworthy that (−)-methamphetamine can also be extracted from a Vicks Inhaler [4] and that (−)-methamphetamine is used in certain prescription drugs [5]. To avoid errors in judgment, an enantiomeric analysis would be highly desirable. In a previous research project, the synthesis of the individual (*R*)-, (*S*)-MDA and (*R*)-, (*S*)-MDMA isomers was reported [6]. Using these standards, the quantitation and the distribution of the enantiomers (*R*)-, (*S*)-MDMA and (*R*)-, (*S*)-MDA (the pharmacologically active metabolite of MDMA) in clandestine tablets and suspect urine samples were investigated [6]. In this study, in a continuation of our investigation of the enantioseparation of these compounds, the use of both aqueous and nonaqueous buffers was examined, based on the CZE and MEKC modes. This is because nonaqueous CE (NACE) has rapidly grown in popularity and importance over the past few years [7–16]. Compared to aqueous CE, there are several advantages of NACE including a short analysis time, high separation efficiency, better solubility, and the superior stability of certain compounds in organic solvents compared to water, and its ease of interfacing with MS. This operation mode also offers the possibility of achieving a different selectivity, compared to aqueous CE, because the separation order can be altered by varying the ratio of the organic solvents used (for example, the ratio of methanol:ACN, *etc.*). Based on this concept, various aqueous and nonaqueous buffers were examined for the chiral separation of a racemic mixture. MDA, MDMA, DMMDA, 1-(1,3-benzodioxol)-5-yl-2-butylamine (BDB), and *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butylamine (MBDB) were used

Correspondence: Professor Cheng-Huang Lin, Department of Chemistry, National Taiwan Normal University, 88 Sec. 4, Tingchow Road, Taipei, Taiwan
E-mail: chenglin@cc.ntnu.edu.tw
Fax: +886-2-2932-4249

Abbreviations: **BDB**, 1-(1,3-benzodioxol)-5-yl-2-butylamine; **DMMDA**, *N,N*-dimethyl-3,4-methylenedioxyamphetamine; **MBDB**, *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butylamine; **MDA**, 3,4-methylenedioxyamphetamine; **MDMA**, 3,4-methylenedioxymethamphetamine; **NACE**, nonaqueous capillary electrophoresis; **SC**, sodium cholate

as model compounds. Several electrophoretic parameters, such as the concentration of β-CD used (aqueous, 25–60 mM; nonaqueous, 20–150 mM) in the electrophoretic separation, the amount of organic solvent required for the separation, were optimized and these data are reported herein.

2 Materials and methods

2.1 Chemicals

(*R*)-/(*S*)-MDMA, (*R*)-/(*S*)-MDA, BDB, DMMDA, and MBDB were synthesized and generously donated by the Forensic Science Center (Command of the Army Force of Military Police, Department of Defense, Taipei, Taiwan). SDS, CTAB, ACN, methanol (99.8%), and formamide (99.5%) were obtained from Acros (Geel, Belgium). β-CD and sodium phosphate were purchased from Sigma (St. Louis, MO, USA). Phosphate acid, ammonium acetate (CH_3COONH_4), and urea were obtained from J. T. Baker and Riedel-de Haën (Seelze, Germany).

2.2 Enantioselective synthesis of (*S*)-MDA and (*R*)-MDMA

The synthetic pathway for preparing these compounds has been described previously [6], and is abbreviated herein.

2.3 CE apparatus

The CE setup is similar to that described previously [6, 16], and is abbreviated herein.

3 Results and discussion

3.1 Aqueous buffers for enantioseparations

As it has been described previously [6], when the CZE mode was applied, the migration order observed is: MDA (MW 179.22) > DMMDA (MW 207.27) > MDMA (MW 193.24) > BDB (MW 193.24) > MBDB (MW 207.27). In principle, under the CZE mode, the analyte whose effective mobility is greater (often highly dependent on pH, that is, the pK_a of the solute) should move faster and, as a result, pass through the detection window quickly. DMMDA is a tertiary amine which has a much different molecular structure than the others (primary amine, MDA and BDB; secondary amine, MDMA and MBDB), making it difficult to predict and explain its migration order in the CZE mode. This is because amines are polar compounds and, except for tertiary amines, can form intermolecular hydrogen bonds. Thus, we suggest that the effective mobility of these amines could be in the order: $MDA^+ > DMMDA^+ > MDMA^+ > BDB^+ > MBDB^+$, indicated as numbers 1–5 in Table 1. This proposed order

Table 1. Effective mobility and migration orders (numbers, 1–10) for MDA (1°), MDMA (2°), BDB (1°), MBDB (2°), and DMMDA (3°) separated, using different aqueous and nonaqueous CE modes

Compound	MDA		MDMA		BDB	MBDB	DMMDA	Applied voltage
	(*R*)-	(*S*)-	(*R*)-	(*S*)-				
Molecular weight	179.22		193.24		193.24	207.27	207.27	
I. Aqueous								
Effective mobility	1		3		4	5	2	
CZE [6]	1		3		4	5	2	+
CZE + β-CD [6]	1	2	4	6	7, 8	9, 10	3, 5	+
MEKC (SDS)	1		3		–	4	2	–
MEKC (SDS) + β-CD	4		2		–	1	3	–
MEKC (CTAB)	3		2		–	1	2	–
MEKC (CTAB) + β-CD	2	1	6	4	–	7, 8	3, 5	–
II. Nonaqueous								
Effective mobility	1		2		–	3	4	
CZE	1		2		–	3	4	+
CZE + β-CD	1	2	3	4	–	5, 6	7, 8	+
MEKC (CTAB)	1		2		–	3	4	+
MEKC (SC) [13]	1		2		3	4	–	+

Abbreviations: 1°, primary amine; 2°, secondary amine; 3°, tertiary amine; SC, sodium cholate ($C_{24}H_{39}O_5Na$)

was examined using various separation modes, as described below. These enantiomers can be completely separated when a longer capillary (total: 97 cm; effective length: 92 cm) was used and with β-CD added to the buffer [6] (CE buffer: 50 mM β-CD, 10 mM sodium cholate (SC), 50 mM phosphate buffer, 3 M urea in the same solution of methanol:ACN:water (M:A:W) = 14:4:82 v/v/v; pH 2.3). Thus, we propose that the effective mobility for these analytes in aqueous solution could be: (*R*)-MDA^+ > (*S*)-MDA^+ > (*R*)-$DMMDA^+$ > (*R*)-$MDMA^+$ > (*S*)-$DMMDA^+$ > (*S*)-$MDMA^+$ > (*R*)-BDB^+ > (*S*)-BDB^+ > (*R*)-$MBDB^+$ > (*S*)-$MBDB^+$, corresponding to peaks 1–10 in Table 1, respectively. Based on these proposals, we attempted to predict and explain their migration orders in subsequent CE separations.

Figure 1A shows typical electropherograms for a mixture of MDA, MDMA, MBDB, and DMMDA, using the normal aqueous MEKC mode; the inset shows the molecular structures of these compounds. In frame A, the CE buffer was a mixture of methanol–ACN–water (M:A:W = 26:6:68 v/v/v) which contained 50 mM phosphate buffer

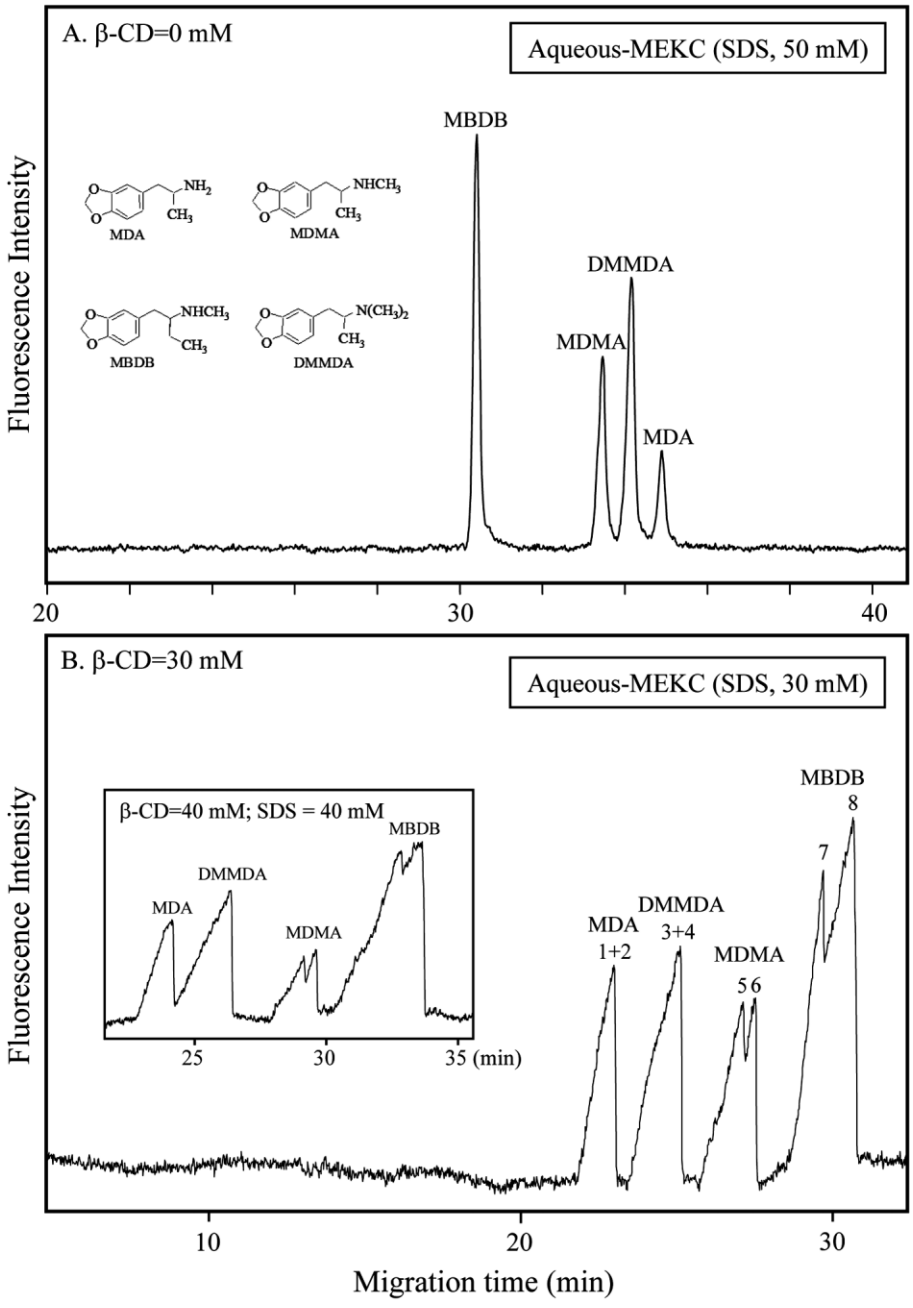

Figure 1. Typical fluorescence aqueous CE electropherograms of a mixture of MDA, DMMDA, MDMA, and MBDB using the normal aqueous-MEKC mode. (A) The CE buffer was a mixture of methanol–ACN–water (M:A:W = 26:6:68 v/v/v) which contained 50 mM phosphate buffer (pH 2.2) and 50 mM SDS. A capillary 92 cm in length (effective length: 87 cm) was used; the applied voltage was −15 kV (current, ~−20 μA). (B) CE buffer: by adjusting SDS to 30 mM and β-CD to 30 mM and additional urea (0.9 M) was added to the same solution described above. Inset shows the result obtained for a solution containing 40 mM β-CD and 40 mM SDS.

(pH 2.2) and 50 mM SDS. A 92 cm long capillary (effective length: 87 cm) was used; the applied voltage was −15 kV (current, ∼−20 μA). In order to optimize the separation condition, various ratios of methanol–ACN–water were investigated. When the ratio was altered to 30:7:63 (M:A:W v/v/v), a similar result was obtained but the migration time became longer. In either case, the optimized separation was obtained when the volume of methanol was adjusted to three- to fourfolds of ACN and the volume of water was adjusted to two- to threefolds of total organic solvents. Since the pH value was lower (very acidic conditions) and a negative charge was applied, the EOF should be extremely small; if it exists at all, the EOF should migrate in the reverse direction (in the direction of the inlet). Thus, the separation efficiency is completely determined by the interaction force between the analytes and the SDS micelles, as well as their effective mobility in such a solution. The migration order should be the opposite of the order of the effective mobility of themselves. This is consistent with the observed migration order: MBDB > MDMA > DMMDA > MDA (as shown in Fig. 1A). In an attempt to separate the enantiomers, β-CD (30 mM) was added to the same solution. Figure 1B shows the result of the separation. The inset shows the result obtained from a solution containing 40 mM β-CD and 40 mM SDS. In both cases, the separation is incomplete. It is known that, when the CD-modified MEKC mode is applied, the migration order can be altered since the CD modifier would disturb the nature of the interaction between the SDS micelles and the analytes [17]. As a result, the migration order and migration time would be changed when the ratio of the SDS and β-CD concentration is changed. The migration order now follows the order of effective mobility. However, the enantioseparation is incomplete. Peaks 5 and 6 could correspond to (*R*)- and (*S*)-MDMA, but it is difficult to distinguish them by spiking with an authentic (*R*)-MDMA standard. Instead of SDS, a cationic surfactant (CTAB, 0–80 mM; β-CD, 0 mM) was used and the results are shown in Fig. 2A. The CE buffer used was an aqueous solution that contained 20 mM of CH_3COONH_4 and 50 mM of CTAB, the pH of which was 6.5. The applied voltage was −20 kV (current, ∼ −40 μA). Since the concentration of CTAB was sufficiently high to cause a reversal in the EOF (moves in the direction of the outlet), as a result, the $CTAB^+$-$analyte^+$ micelles migrate toward the outlet along with the EOF. Since the EOF is the major source, the migration time should be shorter, compared to the case of SDS. Furthermore, the migration order should be the opposite of the order of effective mobility because a negative charge was applied in this case. This is also in agreement with the observed result for the electropherogram shown in Fig. 2A. However, in this case, the peak of MDMA and DMMDA cannot be separated; neither CTAB concentrations nor organic solvent ratios were changed. Similarly, in an attempt to separate the enantiomers, β-CD, was added to the same solution and the result is shown in Fig. 2B. Electropherograms a and b show the β-CD-modified MEKC (surfactant) electropherogram of the mixture before (electropherogram a) and after spiking with (*R*)-MDMA (electropherogram b), respectively. The optimized CE buffer was a 1% aqueous solution of ACN containing 20 mM CH_3COONH_4, 80 mM CTAB, and 60 mM β-CD; the pH of which was 6.5. Herein, various concentration ratios of CTAB and β-CD were examined, the separation efficiency was poorer. Furthermore, the 1% ACN in water is

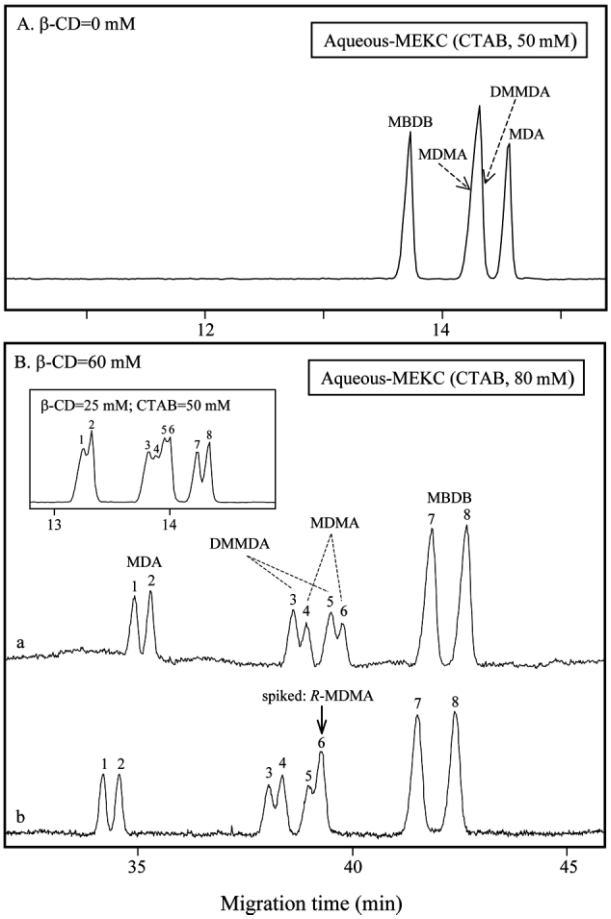

Figure 2. (A) CE buffer: an aqueous solution containing 20 mM CH_3COONH_4 and 50 mM CTAB, the pH of which was 6.5. Applied voltage was −20 kV (current, ∼−40 μA). (B) The electropherograms a and b show the β-CD-modified MEKC (surfactant) electropherogram of the mixture before (electropherogram a) and after spiking with (*R*)-MDMA (electropherogram b), respectively. CE buffer: an aqueous solution (containing 1% ACN) containing 20 mM CH_3COONH_4, 80 mM CTAB, and 60 mM β-CD, the pH of which was 6.5. Inset, the result obtained when the solution was changed to a solution containing 25 mM β-CD and 50 mM CTAB.

very important in terms of achieving a complete chiral separation. If the percentage of ACN in water was increased to 5%, the separation was incomplete. Instead of ACN, 1–3% methanol was used, but the result was not satisfactory. When additional modified surfactants (including octyltrimethylammonium bromide, OTAB, and tetradecyltrimethylammoniumbromide, TTAB; 0–10 mM, respectively) were also added to the running buffer, the separation efficiency was not further improved. The inset in Fig. 2B shows the result obtained when the solution was changed to a solution containing 25 mM β-CD and 50 mM CTAB. As a result, the separation is incomplete. It appears that (*R*)- and (*S*)-isomers are present in equal amounts naturally. Thus, in electropherogram a, peaks 3 and 5 are assigned to DMMDA, peaks 4 and 6 to MDMA. After spiking, it was found that peak 6 corresponds to (*R*)-MDMA. In other words, peak 4 corresponds to (*S*)-MDMA. Thus, we conclude that these enantiomers can be completely separated when a β-CD-modified MEKC (cationic surfactant, CTAB) is used. The separation time is shorter than that for the CZE mode. We conclude that in a normal aqueous phase the effective mobility of these analytes would be: (*R*)-MDA$^+$ > (*S*)-MDA$^+$ > (*R*)-DMMDA$^+$ > (*R*)-MDMA$^+$ > (*S*)-DMMDA$^+$ > (*S*)-MDMA$^+$ > (*R*)-BDB$^+$ > (*S*)-BDB$^+$ > (*R*)-MBDB$^+$ > (*S*)-MBDB$^+$. The migration order does not follow the mass *per* charge well.

3.2 Nonaqueous buffers for enantioseparations

Amines are all capable of forming hydrogen bonds with water. However, in a nonaqueous phase, they may have a different effective mobility compared to that in an aqueous phase. Figure 3A shows typical electropherograms for the mixture using the nonaqueous CZE mode (frame A, β-CD = 0 mM; frame B, β-CD = 150 mM). In frame A, the CE buffer was a methanol solution which contained only 50 mM CH_3COONH_4. A 50 μm ID capillary 96 cm in length (effective length: 92 cm) was used; the applied voltage was +25 kV (current, ~+8 μA). The concentration of MDMA used was 10 ppm. The migration order was: MDA > MDMA > MBDB > DMMDA; basically the compounds migrated in the order of mass *per* charge. This is because, in a nonaqueous solution, where methanol is an amphiprotic solvent, only the concentration of hydronium ions is very low. As a result, in such a condition, the effective mobility should basically agree with the mass *per* charge. We suggest that the effective mobility order of these amines could be: MDA$^+$ > MDMA$^+$ > (BDB$^+$>) MBDB$^+$ > DMMDA$^+$. Figure 3B shows the β-CD-modified NACE electropherogram of the mixture before (electropherogram in frame B) and after spiking with (*R*)-MDMA (electropherogram in the inset), respectively. The

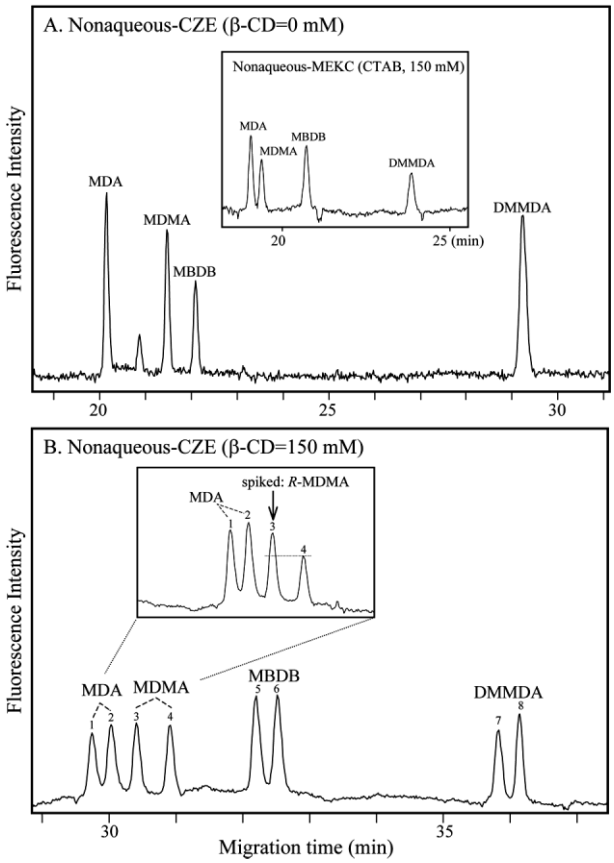

Figure 3. Typical electropherograms of a mixture of MDA, DMMDA, MDMA, and MBDB using the nonaqueous CZE mode (frame A, β-CD = 0 mM; frame B, β-CD = 150 mM). In (A), the CE buffer is a methanol solution which contained only 50 mM CH_3COONH_4; a 50 μm ID capillary 96 cm in length (effective length: 92 cm) was used; the applied voltage was +25 kV (current, ~+8 μA). Concentration of MDMA used was 10 ppm. Inset in (A), typical electropherogram for the mixture obtained using the NA-MEKC mode. CE buffer: a mixture of methanol–formamide (M:F = 70:30 v/v) which contained 20 mM CH_3COONH_4 and 150 mM CTAB; the applied voltage was +15 kV (current, ~+30 μA). (B) CE buffer: a formamide solution containing 30 mM of CH_3COONH_4 and 150 mM of β-CD. Inset in (B), the same conditions but additional (*R*)-MDMA was added.

optimized NA-CZE buffer was a formamide solution containing 30 mM of CH_3COONH_4 and 150 mM of β-CD. β-CD dissolves with difficulty in methanol and this is the reason why a formamide solution was used. After spiking, it was found that peak 3 corresponds to (*R*)-MDMA and peak 4 belongs to (*S*)-MDMA. Thus, we conclude that these enantiomers can be completely separated, much easily and faster than when aqueous CE is used, when the β-CD-modified NACE mode is used. Thus, we conclude that, in a nonaqueous phase, the effective mobility of

these analytes would be: (*R*)-MDA$^+$ > (*S*)-MDA$^+$ > (*R*)-MDMA$^+$ > (*S*)-MDMA$^+$ > ((*R*)-BDB$^+$ > (*S*)-BDB$^+$>) (*R*)-MBDB$^+$ > (*S*)-MBDB$^+$ > (*R*)-DMMDA$^+$ > (*S*)-DMMDA$^+$. In either the aqueous or nonaqueous case, the effective mobility of the (*R*)-isomer is greater than the (*S*)-isomer. These data are summarized in Table 1. The inset in Fig. 3A shows the result obtained when the NA-MEKC mode was used. The optimized buffer was a mixture of methanol–formamide (M:F = 70:30 v/v) which contained 20 mM of CH_3COONH_4 and 150 mM of CTAB; capillary 52 cm in length (effective length: 47 cm); the applied voltage was +15 kV (current, ~+30 μA). The migration order is also in agreement with the result obtained when the NA-CZE mode was used. Instead of CTAB, an SC ($C_{24}H_{39}O_5Na$) surfactant had been used previously in a similar separation experiment [13]. In that case, the CE buffer was a mixture solution of formamide–methanol–ethanol (30:20:50 v/v/v) and contained 20 mM CH_3COONH_4 and 150 mM SC; the applied voltage was +15 kV. The migration order was: MDA > 3,4-MDMA > (2,3-MDMA>) BDB > MBDB, which was basically in agreement with our proposed order in this study. However, it should be noted that the migration order would be changed if a nonpolar solvent, such as hexane, were to be used or mixed together. A methanol–hexane solution (7:3 v/v), containing 100 mM SC and 20 mM CH_3COONH_4 had previously been examined [13]. Under such conditions, the migration order was observed to be: MDA > MBDB > (2,3-MDMA>) BDB > 3,4-MDMA, *i.e.*, the migration order would be affected when a polar/nonpolar nonaqueous solution was used.

4 Concluding remarks

The enantioisomers of MDMA and related compounds were identified using aqueous and nonaqueous buffers, based on the CZE and MEKC modes. In previous research [6], in which an aqueous-CZE mode was used, the effective mobility of these analytes would be: (*R*)-MDA$^+$ > (*S*)-MDA$^+$ > (*R*)-DMMDA$^+$ > (*R*)-MDMA$^+$ > (*S*)-DMMDA$^+$ > (*S*)-MDMA$^+$ > (*R*)-BDB$^+$ > (*S*)-BDB$^+$ > (*R*)-MBDB$^+$ > (*S*)-MBDB$^+$; the relationship between the migration orders and the *m/z* values of the compounds was not clear. However, by applying nonaqueous CZE and MEKC modes in this study, a reasonable relationship was found. In a nonaqueous phase, the effective mobility of these analytes would be: (*R*)-MDA$^+$ > (*S*)-MDA$^+$ > (*R*)-MDMA$^+$ > (*S*)-MDMA$^+$ > ((*R*)-BDB$^+$ > (*S*)-BDB$^+$>) (*R*)-MBDB$^+$ > (*S*)-MBDB$^+$ > (*R*)-DMMDA$^+$ > (*S*)-DMMDA$^+$. The migration order basically follows the mass *per* charge, unless a polar/nonpolar nonaqueous solution is used. In either case, the (*R*)-isomer migrates faster than the (*S*)-isomer.

This work was supported by a grant from the National Science Council of Taiwan under Contract No. NSC-92–2113-M-003–023. Permission was obtained from Pharmaceutical Affairs, Department of Health, Taiwan (License Number: ARR089000035). The authors wish to thank the Forensic Science Center (Taiwan Command of the Army Force of Military Police) for generously donating the standards.

Received December 6, 2004
Revised April 11, 2005
Accepted April 25, 2005

5 References

[1] Skinner, H. F., *Forensic Sci. Int.* 1990, *48*, 123–134.
[2] Varesio, E., Veuthey, J.-L., *J. Chromatogr. A* 1995, *717*, 219–228.
[3] Moore, K. A., Mozayani, A., Fierro, M. F., Poklis, A., *Forensic Sci. Int.* 1996, *83*, 111–119.
[4] Fitzgerald, R. L., Ramos, J. M., Bogema, S. C., Poklis, A., *J. Anal. Toxicol.* 1988, *12*, 255–259.
[5] Schachter, M., Marsden, C. D., Parkes, J. D., Jenner, P., Testa, B., *J. Neurol. Neurosurg. Psychiatry* 1980, *43*, 1016–1021.
[6] Huang, Y.-S., Liu, J.-T., Lin, L.-C., Fang, C., Lin, C.-H., *Electrophoresis* 2003, *24*, 1097–1104.
[7] Cherkaoui, S., Geiser, L., Veuthey, J.-L., *Chromatographia* 2000, *52*, 403–407.
[8] Miller, J. T., Shea, D., Khaledi, M. G., *J. Chromatogr. A* 2000, *888*, 251–266.
[9] Backofen, U., Matysik, F.-M., Hoffmann, W., Lunte, C. E., *Fresenius' J. Anal. Chem.* 2000, *367*, 359–363.
[10] Wang, F., Khaledi, M. G., *J. Chromatogr. A* 2000, *875*, 277–293.
[11] Cherkaoui, S., Veuthey, J. L., *J. Chromatogr. A* 2000, *874*, 121–129.
[12] Ward, V. L., Khaledi, M. G., *J. Chromatogr. A* 1999, *859*, 203–219.
[13] Chung, Y.-L., Liu, J.-T., Lin, C.-H., *J. Chromatogr. B* 2001, *759*, 219–226.
[14] Chen, Y.-H., Lin, C.-H., *Electrophoresis* 2001, *22*, 2574–2579.
[15] Chen, Y.-H., Chung, Y.-L., Lin, C.-H., *J. Chromatogr. A* 2002, *943*, 287–294.
[16] Tsai, C.-H., Fang, C., Liu, J.-T., Lin, C.-H., *Electrophoresis* 2004, *25*, 1601–1606.
[17] Sabbah, S., Scriba, G. K. E., *J. Chromatogr. A* 2000, *894*, 267–272.