ELSEVIER

Journal of Chromatography A 817 (1998) 121–128

JOURNAL OF CHROMATOGRAPHY A

Nonaqueous capillary electrophoresis chiral separations with quaternary ammonium β-cyclodextrin

Fang Wang[1], Morteza G. Khaledi*

Department of Chemistry, North Carolina State University, P.O. Box 8204, Raleigh, NC 27695-8204, USA

Abstract

Chiral separation of nonsteroidal anti-inflammatory drugs (profens), 1,1′-binaphthyl-2,2′-diyl-hydrogen phosphate, *N*-[1-(1-naphthyl)ethyl]phthalamic acid, and derivatized amino acids by a cationic cyclodextrin, quaternary ammonium β-cyclodextrin (QA-β-CD), was investigated by capillary electrophoresis (CE) in both aqueous and nonaqueous media. Several profens and amino acids could only be separated by QA-β-CD in pure formamide system. No chiral separation of the profens was achieved in the following solvents: *N*-methylformamide, methanol, dimethyl sulfoxide, and water; however, chiral separations of most of the amino acids were obtained in all of these solvents. The effects of other experimental parameters such as the CD concentration and apparent pH (pH*) were also investigated. The first application of nonaqueous CE chiral separation of ketoprofen in a commercially available sample, Actron, was also examined. In addition, the reversal of electroosmotic flow by QA-β-CD was observed in water, formamide, *N*-methylformamide, methanol, and dimethyl sulfoxide media. © 1998 Published by Elsevier Science B.V.

Keywords: Enantiomer separation; Buffer composition; Non-steroidal anti-inflammatory drugs; Amino acids; Profens; Binaphthyldiyl hydrogenphosphate; Naphthylethylphthalamic acid

1. Introduction

The applications of charged cyclodextrins (CDs) in aqueous CE chiral separation have received much attention recently. This is resulting from the fact that charged CDs have different separation selectivity from neutral CDs [1–6]. In principle, chiral resolution (R_S) equation could be expressed as [7,8]:

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{\Delta K[\mathrm{CD}]}{(1+K_1[\mathrm{CD}])(1+K_2[\mathrm{CD}])}\right)\times \left(\frac{\mu^f-\mu^c}{\mu_{avg}+\mu_{eo}}\right) \quad (1)$$

where N is the number of theoretical plates, K_1 and K_2 are the binding constants between the enantiomers and CD, μ^f and μ^c are the mobilities of enantiomers in free and in fully complexed forms, μ_{avg} is the average electrophoretic mobility of two enantiomers and μ_{eo} is the mobility of electroosmotic flow (EOF). For separation of charged enantiomers using oppositely charged CDs, higher separation selectivity ($\Delta\mu$) can be achieved due to a larger ''separation window'', i.e., the $\mu^f-\mu^c$ term in Eq. (1) [2]. Moreover, charged CDs can be used to establish the counter-EOF pattern to increase resolution [5]. Chiral separation of a large number of cationic analytes by the counter-EOF setup was achieved at a fixed concentration of a sulfated CD [5]. The disadvantage with the counter-EOF pattern formed by oppositely charged CDs is the band

*Corresponding author.

[1]Current address: Merck & Co., Inc., P.O. Box 200, RY80Y-115, Rahway, NJ 07065, USA.

0021-9673/98/$19.00
PII: S0021-9673(98)00484-1

broadening caused by diffusion due to long analysis times. Another factor that causes lower separation efficiency is the electrodispersion caused by the mobility mismatch between the charged CD–analyte complexes and the co-ions in the buffer [3].

Recently, chiral separation by nonaqueous CE (NACE) has received attention [9–15]. In this laboratory, chiral separation of several basic pharmaceutical racemates was achieved with both neutral and negatively charged CDs in purely organic solvents [10,11]. The change in solvents alters the binding constants between chiral selectors and enantiomers, and/or their separation selectivity. The benefit of reduction of binding constants in organic solvents is that it is easier to optimize separation selectivity for those compounds that strongly bind to chiral selectors in aqueous buffer. The ranges of CD concentration for chiral separation of these compounds in formamide were broader than those in the aqueous media. Especially, the application of oppositely charged CDs in NACE for separation of positively charged hydrophobic amines was better in the nonaqueous media [15]. Electrodispersion did not seem to pose a problem in the nonaqueous media. Stalcup and Gahm also reported chiral separations of acidic compounds and derivatives of amino acids using quinine as a chiral selector in methanol as the solvent [12]. The separation was based on the ion pairing and $\pi-\pi$ interactions. Valkó et al. used a neutral CD to separate dansyl amino acids in *N*-methylformamide media [13]. Bjornsdottir et al. reported chiral separation of some β-amino alcohols with (+)-*S*-camphorsulphonate in acetonitrile media [14]. The separation resulted from ion-pairing interactions between the positively charged enantiomers and the anionic chiral selector.

This report presents a continuation of the application of charged CDs in NACE chiral separation for acidic analytes and amino acids. The effect of a cationic quaternary ammonium-β-cyclodextrin (QA-β-CD) on EOF in both aqueous CE and NACE has been studied.

2. Experimental

2.1. Apparatus

All experiments were carried out on a laboratory-built unit. It consists of a ±30 kV high voltage power supply (Series EH, Glassman High Voltage, Whitehouse, NJ, USA) and an UV–visible detector (Model 200, Linear Instruments, Reno, NV, USA) operating at 254 nm in NACE and 214 nm in aqueous CE, respectively, and an electronic integrator (Hewlett–Packard, Avondale, PA, USA). Untreated fused-silica capillary tubes (Polymicro Technologies, Phoenix, AZ, USA) with 50 μm I.D.×363 μm O.D. were used. The total length of the capillary was 42 cm, and the length of the capillary to the detector was 31 cm. The capillary was jacketed in light mineral oil in order to maintain its temperature at 30°C using a constant-temperature circulator (Type K2-R, Lauda, Mainz, Germany). The injection was done by gravity injection.

2.2. Chemicals

QA-β-CD [empirical formula: $(C_6H_{10-n}O_5)_7 \cdot (C_6H_{15}ONCl)_n$, $n=3.8$] was a gift from American Maize-Products (Hammond, IN, USA). Since the CD is a mixture with different degrees of substitution, the CD concentration is expressed as mass/volume (w/v) %. Tris(hydroxymethyl)aminomethane (Tris), 1,1′-binaphthyl-2,2′-diylhydrogenphosphate (**10**), and *N*-[1-(1-naphthyl)-ethyl]phthalamic acid (**9**) were purchased from Aldrich (Milwaukee, WI, USA). The profens and derivatized amino acids were obtained from Sigma (St. Louis, MO, USA). Formamide and *N*-methylformamide were bought from Fluka (Buchs, Switzerland). *N,N*-Dimethylformamide, dimethyl sulfoxide, methanolammonium acetate, and acetic acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). The structures of the racemic compounds are shown in Fig. 1.

2.3. Safety

The safety precautions for formamide and its derivatives are the same as described previously [11]. Body, skin, and eye protection are also recommended by the manufacturer for using dimethyl sulfoxide.

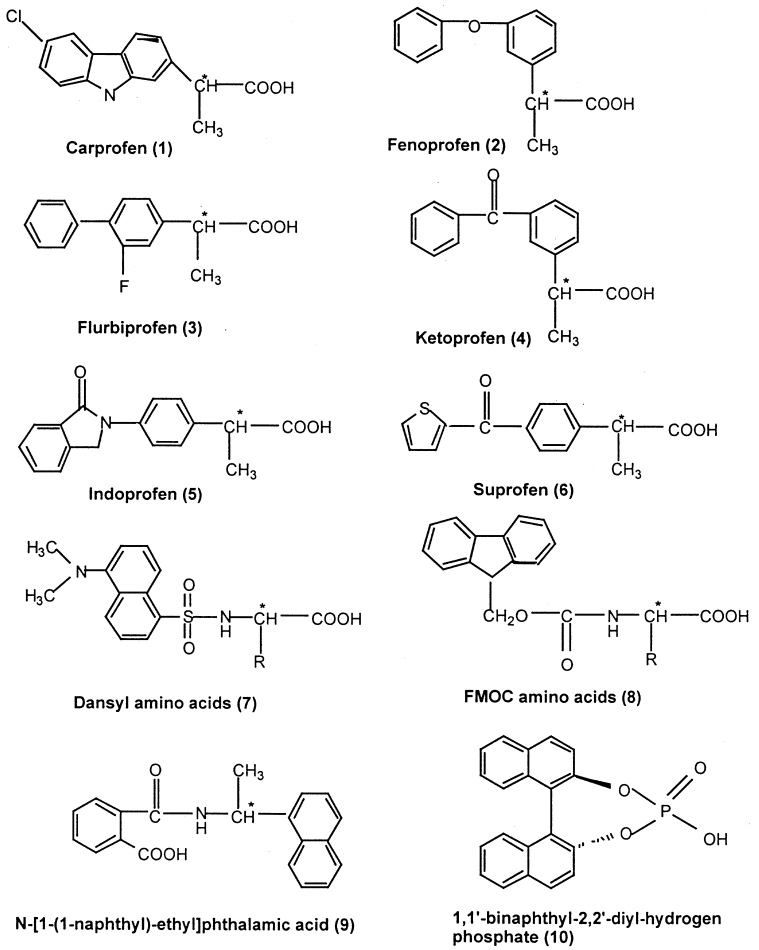

Fig. 1. Structure of chiral compounds.

3. Results and discussion

3.1. Effect of CD on EOF

Fig. 2 shows the effect of the concentration of QA-β-CD on EOF in both aqueous and formamide media. In the Tris–acetate aqueous buffer (pH= 8.06), EOF was $3.48 \cdot 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$ when $1.71 \cdot 10^{-3}$% (w/v) (~10 μ*M*) of QA-β-CD was added. The positive mobility indicates the migration direction from anode to cathode, vice versa; the negative mobility shows a reversal in migration direction due to the change in surface charges. The direction of EOF was reversed at $8.55 \cdot 10^{-3}$% (w/v) (~50 μ*M*) and EOF mobility was $-9.73 \cdot 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$ (Fig. 2). The EOF mobility was $-22.0 \cdot 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$ at 0.171% (w/v) (~1 m*M*), where it levelled off and increased only slightly at the higher CD concentrations (Fig. 2). In formamide, the direction of EOF was reversed upon addition of $1.71 \cdot 10^{-2}$% (w/v) (~100 μ*M*) of the cationic CD ($\mu_{eo} = -1.92 \cdot 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$). The EOF in formamide levelled off $-3.57 \cdot 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$, where the CD concentration was 1.71% (w/v) (~10 m*M*). Further increase in the CD concentration increased EOF slightly as it was in water (Fig. 2). The dependence of EOF on the concentration of the positively charged CD has the same trend in both aqueous and nonaqueous media. By comparing the EOF data in the aqueous buffer with that in form-

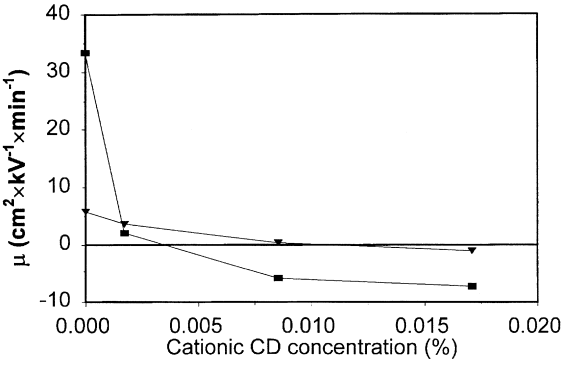

Fig. 2. Effect of QA-β-CD on EOF. Buffer electrolytes: (A) 50 m*M* Tris–25 m*M* acetic acid (pH = 8.06) in aqueous media (filled square), (B) 100 m*M* Tris–100 m*M* acetic acid (pH* = 7.5) in formamide (filled triangle), (C) variations of EOF at low CD concentration range. Field strength: ±476 V cm^{-1}. Positive voltage is applied when EOF is positive while negative voltage is used when EOF is negative.

amide media, it was found that the magnitude of EOF in water was larger than that in formamide, when the CD concentration was zero. In general, mobility in water is greater than in formamide due to a larger ratio of dielectric constant to viscosity (note that $\mu \propto \varepsilon/\eta$). A smaller amount of the CD was needed to reduce or reverse EOF in water than that in formamide. Apparently, the positively charged CD coats the capillary wall more effectively in water than in formamide. This might be because formamide is a basic solvent and interacts with the silanol groups on the wall; thus competes with the adsorption of the cationic CD. Similar results were observed in a previous study of tetraalkylammonium (TAA^+) ions [11].

Fig. 3 shows the chiral separation of fenoprofen (**2**) with QA-β-CD in pure formamide (pH* = 7.5). Chiral separation of fenoprofen (**2**) was achieved in the QA-β-CD concentration range from 0.855 to 4.28% (w/v) (~5–25 m*M*). Further increase in the CD concentration caused a dramatic increase in current and decay in separation efficiency due to Joule heating. Baseline separation was achieved with plate counts around 200 000 in 2.57% (w/v) (~15 m*M*) of QA-β-CD. Chiral separations of other profens such as ketoprofen (**4**), suprofen (**6**), carprofen (**1**), and flurbiprofen (**3**), and dansyl amino acids (**7**) were also achieved in formamide. Naproxen and ibuprofen were not resolved with this CD in formamide.

Interestingly, no separation of the profens was observed in the aqueous media at pH = 8.06 in the CD concentration range from $1.71 \cdot 10^{-3}$ to 1.71% (w/v) (10 μ*M*–10 m*M*). No chiral separation was obtained for the profens in the counter-EOF setup [1.71% (w/v) QA-β-CD]. Server peak broadening of the profens and amino acids under the counter-EOF setup was observed in water due to electrodispersion, which was caused by the mobility mismatch between the analyte–cationic CD complexes and the co-ions in the supporting electrolytes. Chiral separations of 1,1′-binaphthyl-2,2′-diylhydrogen phosphate (**10**), *N*-[1-(1-naphthyl)-ethyl]phthalamic acid (**9**), and some amino acid derivatives were achieved in the aqueous media. On the other hand, no chiral separation of *N*-[1-(1-naphthyl)-ethyl]phthalamic acid (**9**) was observed in any of the organic solvents.

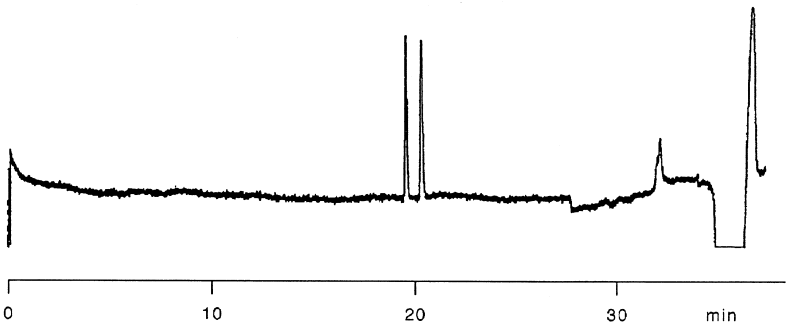

Fig. 3. Chiral separation of fenoprofen (**2**) in formamide. Buffer electrolyte: 2.57% w/v (~15 m*M*) QA-β-CD in 100 m*M* Tris–100 m*M* acetic acid in formamide (pH* = 7.5). Field strength: −476 V cm^{-1}.

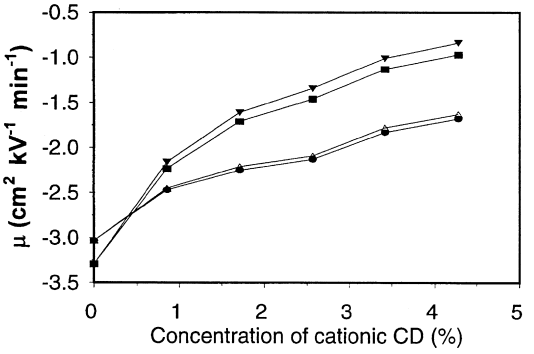

Fig. 4. Effect of the concentration of QA-β-CD on mobility in formamide. Buffer electrolyte: 100 m*M* Tris–100 m*M* acetic acid in formamide (pH*=7.5). Field strength: −476 V cm^{-1} except 476 V cm^{-1} at [QA-β-CD]=0. The first (filled square) and second (filled triangle) peaks of fenoprofen enantiomers (**2**), and the first (filled oval) and second (empty triangle) eluted peaks of ketoprofen optical isomers (**4**).

3.2. CD Concentration effect

Fig. 4 shows the effect of the concentration of the CD on the electrophoretic mobilities of enantiomers of the profens in formamide. The analytes were negatively charged in the absence of the cationic CD (i.e. [QA-β-CD]=0). With the increase in the concentration of the positively charged CD, the mobilities of the test solutes decreased.

Fig. 5 shows the effect of the concentration of QA-β-CD on separation selectivity. With the increase in the concentration of the CD, the mobility difference increases and reaches a plateau. No maximum selectivity was observed in the CD concentration range. Among the analytes, dansyl–valine (**7**–**11**) (Table 1) has the highest separation selectivity, and dansyl–tryptophan (**7**–**10**) (Table 1) has the second highest. Fenoprofen (**2**) shows the highest $\Delta\mu$ among the profens.

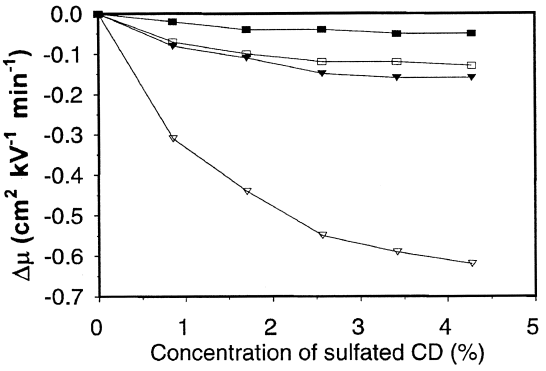

Fig. 5. Effect of the concentration of QA-β-CD on the mobility difference of some acidic solutes in formamide. Conditions as in Fig. 4. Test solutes: dansyl–valine (**7**–**11**) (empty triangle), dansyl–tryptophan (**7**–**10**) (filled triangle), fenoprofen (**2**) (empty square), and ketoprofen (**4**) (filled square).

3.3. Effect of pH*

Previously, Rawjee et al. studied the effect of pH on chiral selectivity and resolution [16]. It was found that the binding constants of enantiomers and/or their difference (ΔK) depend on their charges. pH also affects EOF. The influence of apparent pH (or pH*) on the mobilities of analytes in formamide is shown in Fig. 6. All the test analytes were negatively charged in the pH* range. With the increase in pH* from 6.5–7.5, the effective charges on the CD–enantiomer complexes became more negative. After pH*=7.5, the net mobilities remained relatively constant. This resulted from the fact that the analytes were totally dissociated after pH*=7.5. No obvious change in separation selectivity was observed for all the racemates from pH*=6.5–10.4.

3.4. Effect of solvents

The effect of solvents on chiral separation is listed on Table 1. The solvents studied were formamide, *N*-methylformamide, *N,N*-dimethylformamide, methanol, dimethyl sulfoxide, acetonitrile, and propylene carbonate. It was found that QA-β-CD is almost insoluble in acetonitrile and propylene carbonate due to the fact that both solvents belong to protophobic dipolar aprotic solvents [17]. It was also observed that the cationic CD is slightly soluble in *N,N*-dimethylformamide <0.171%, w/v). However, it can be dissolved very well in dimethyl sulfoxide (4.28%, w/v, or ~25 m*M*). Therefore, only formamide, *N*-methylformamide, methanol, and dimethyl sulfoxide were chosen to study the effect of solvents on chiral separation. The profens could only be resolved by the cationic CD in formamide. No chiral separation of these profens was found in the other solvents. Most of the dansyl amino acids (**7**) were separated in all the solvents. Chiral separation of two

Table 1
Effect of organic solvents for NACE chiral separation [a]

Type of solvents / Compounds	Formamide			*N*-Methylformamide			Methanol			Dimethyl sulfoxide		
	t_r	$\Delta\mu$ [b]	R_s	t_r	$\Delta\mu$ [b]	R_s	t_r	$\Delta\mu$ [b]	R_s	t_r	$\Delta\mu$ [b]	R_s
Carprofen (**1**)	13.91	−0.67	1.60	6.75	0	N.S.	7.07	0	N.S.	23.58	0	N.S.
	14.11											
Fenoprofen (**2**)	14.39	−1.50	3.22	6.11	0	N.S.	6.42	0	N.S.	22.92	0	N.S.
	14.81											
Flurbiprofen (**3**)	14.63	−0.33	0.76	5.97	0	N.S.	6.22	0	N.S.	21.70	0	N.S.
	14.73											
Ketoprofen (**4**)	11.55	−0.67	1.06	5.99	0	N.S.	6.13	0	N.S.	22.17	0	N.S.
	11.66											
Indoprofen (**5**)	13.61	−0.17	0.49	6.95	0	N.S.	5.38	0	N.S.	27.37	0	N.S.
	13.67											
Suprofen (**6**)	12.76	−0.33	0.79	6.12	0	N.S.	6.49	0	N.S.	22.41	0	N.S.
	12.85											
Dns–Aminobutyric acid (**7**–**1**)	9.87	−7.83	6.31	5.06	−1.67	0.62	5.87	−1.67	0.76	14.58	−0.33	0.49
	11.05			5.12			5.93			14.67		
Dns–Glutamic acid (**7**–**2**)	9.65	−7.33	5.72	5.16	−1.33	0.76	6.74	−1.17	0.70	15.69	0	N.S.
	10.69			5.21			6.80					
Dns–Leucine (**7**–**3**)	10.62	−6.33	5.41	5.37	−2.00	1.20	5.66	−0.83	0.51	16.89	−0.50	0.98
	11.70			5.43			5.75			17.10		
Dns–Methionine (**7**-−**4**)	9.98	−5.67	4.52	5.17	−1.00	0.61	5.63	0	N.S.	14.90	−0.33	0.63
	10.82			5.21						15.02		
Dns–Norleucine (**7**–**5**)	9.97	−5.67	4.52	5.25	−1.33	0.75	5.71	−1.00	0.55	15.07	−0.33	0.68
	10.81			5.30			5.75			15.20		
Dns–Norvaline (**7**–**6**)	9.92	−7.00	5.62	5.28	−1.33	0.75	5.40	−1.50	0.73	14.82	−0.50	0.80
	10.97			5.33			5.45			14.97		
Dns–Phenylalanine (**7**–**7**)	11.76	−2.33	2.24	5.37	−0.50	0.29	6.94	0	N.S.	15.60	0	N.S.
	12.24			5.39								
Dns−-Serine (**7**–**8**)	9.66	−6.00	4.66	5.06	−1.17	0.62	5.87	−1.50	0.80	12.39	0	N.S.
	10.50			5.10			5.93					
Dns–Threonine (**7**–**9**)	9.86	−7.83	6.36	5.10	−1.33	0.77	5.53	−2.80	1.42	11.13	0	N.S.
	11.05			5.15			5.63					
Dns–Tryptophan (**7**–**10**)	10.04	−5.50	4.44	5.74	−0.50	0.27	No			17.60	−0.33	0.63
	10.87			5.76			data			17.74		
Dns–Valine (**7**–**11**)	10.27	−11.17	9.60	5.25	−2.00	1.20	5.66	−2.33	1.25	15.43	−0.67	1.12
	12.20			5.33			5.75			15.65		
FMOC–Tryptophan (**8**–**1**)	10.72	−0.50	0.47	6.10	0	N.S.	6.97	0	N.S.	21.33	0	N.S.
	10.81											
FMOC–Valine (**8**–**2**)	9.90	−1.50	1.12	5.73	0	N.S.	5.45	0	N.S.	19.15	0	N.S.
	10.10											
1,1′-Binaphthyl-2,2′-diyl-hydrogen phosphate (**10**)	13.91	−0.67	1.60	6.75	0	N.S.	7.07	0	N.S.	13.44	−4.17	6.27
	14.11									14.55		

[a] Buffer: 3.42% w/v (~20 m*M*) QA-β-CD in 20 m*M* ammonium acetate +1% acetic acid.
[b] (10^{-6} cm^2 V^{-1} s^{-1}). pH*: (A) 7.1 in formamide, (B) 7.2 in *N*-methylformamide, (C) 6.0 in methanol, and (D) 10.1 in dimethyl sulfoxide. Field strength: −714 V cm^{-1}. t_r = Retention time in min; Dns = dansyl = 5-dimethylaminonaphthalene-1-sulfonyl; N.S. = no separation.

g-fluorenylmethoxycarbonyl (FMOC) amino acids (**8**–**1**,**2**) was only observed in formamide. For most of the test solutes, better chiral separation was obtained in formamide than in other solvents. Chiral resolution for these analytes was almost the same in *N*-methylformamide, methanol, and dimethyl sulfoxide. Previously, it was shown that the binding constants of basic compounds with the neutral CD in formamide are almost five times larger than those in *N*-methylformamide [11]. Thus, the cationic CD concentration used in these three solvents can be far from the optimum concentration. The only exception

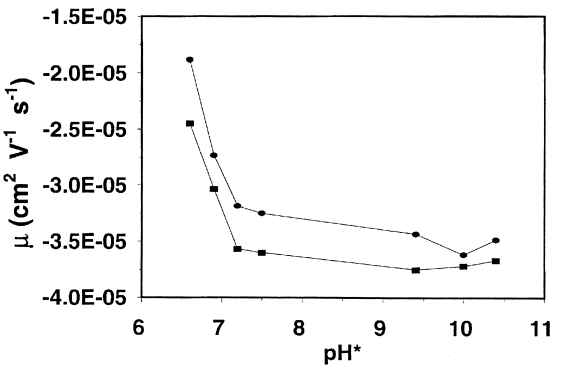

Fig. 6. Effect of pH* on the mobilities of acidic compounds in formamide. Buffer electrolyte: 1.71% w/v (~10 m*M*) QA-β-CD in Tris–acetate buffer in formamide. The total concentration of Tris plus acetic acid was kept at 200 m*M*. Field strength: −714 V cm^{-1}. Solutes: suprofen (**6**) (filled triangle), and flurbiprofen (**3**) (empty oval).

was 1,1′-binaphthyl-2,2′-diylhydrogenphosphate (**10**). It has very high separation selectivity and resolution in dimethyl sulfoxide. The reason is not clear.

3.5. Sample analysis

Fig. 7 shows the chiral separation of ketoprofen (**4**) in Actron – a pain reliever/fever reducer medicine – after 40-fold dilution. According to the manufacturer, the active ingredient contains 12.5 mg ketoprofen (**4**). The inactive ingredients contain corn starch, croscarmellose sodium, hydroxypropylmethylcellulose, lactose, magnesium stearate, polyethylene glycol, and titanium dioxide. By comparing the electropherograms of the standard (Fig. 7A) and the real sample (Fig. 7B), one can find that the active ingredient is a racemic mixture. The inactive ingredients did not show any peak in the electropherogram due to the fact that most of them do not absorb UV

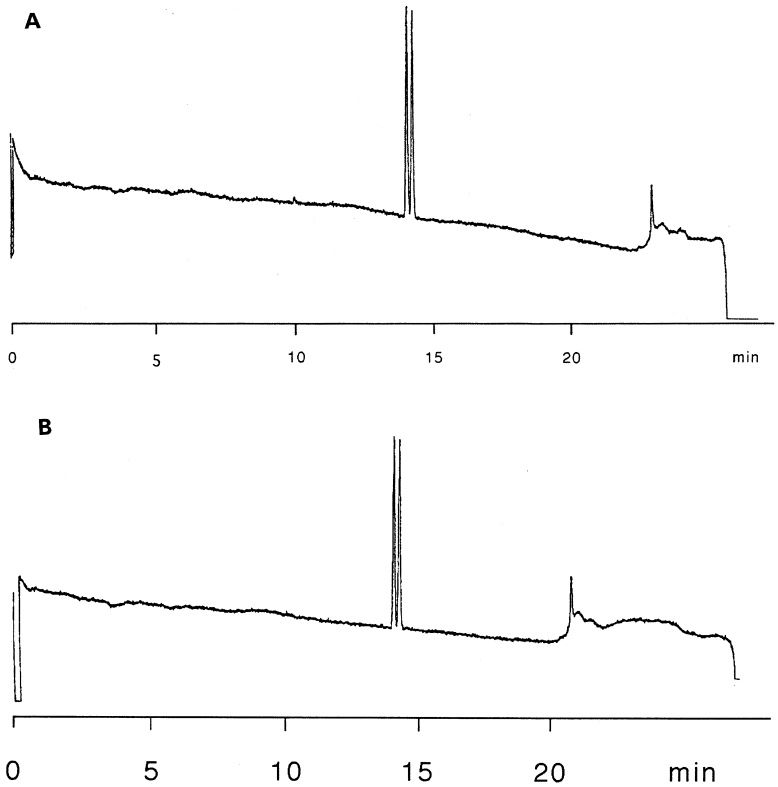

Fig. 7. Nonaqueous chiral separation of ketoprofen (**4**) in Actron. Buffer electrolytes: 4.28% w/v (~25 m*M*) QA-β-CD in 100 m*M* Tris–100 m*M* acetic acid in formamide (pH*=7.5). Field strength: −476 V cm^{-1}. Solutes: (A) ketoprofen (**4**) standard, (B) Actron sample.

light significantly at the operating wavelength. Because the enantiomers of ketoprofen (**4**) were nearly separated at baseline, the percentage of each enantiomer in the racemic mixture can be estimated from their peak area ratio. Due to the lack of the pure enantiomer standard, the identity of sample peaks could not be determined. They are defined by their elution order. In this sample, peak area ratio (peak 1/peak 2) is 44.4/45.2.

4. Conclusion

A cationic CD was used for chiral separation of acidic racemic compounds and derivatized amino acids in NACE. EOF was reversed in pure water, formamide, *N*-methylformamide, methanol, and dimethyl sulfoxide media with the addition of the cationic CD. No chiral separation of the profens was achieved in aqueous CE apparently due to a lack of separation selectivity. However, the profens were separated by QA-β-CD in formamide.

Acknowledgements

The authors gratefully acknowledge the funding by the National Institutes of Health (GM 38738). We also thank American Maize-Products Co. for providing the cationic cyclodextrin in this study.

References

[1] S. Terabe, Trends Anal. Chem. 8 (1989) 129.
[2] R.J. Tait, D.O. Thompson, V.J. Stella, J.F. Stobaugh, Anal. Chem. 66 (1994) 4013.
[3] I.S. Lurie, R.F.X. Klein, T.A. Dal Cason, M.J. LaBelle, R. Brenneisen, R.E. Weinberger, Anal. Chem. 66 (1994) 4019.
[4] T. Schmitt, H. Engelhardt, Chromatographia 37 (1993) 475.
[5] A.M. Stalcup, K.H. Gahm, Anal. Chem. 68 (1996) 1360.
[6] F. Wang, M.G. Khaledi, J. Microcolumn Sep., (1998) in press.
[7] J.C. Giddings, Sep. Sci. 4 (1969) 181.
[8] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.
[9] B. Ye, M.G. Khaledi, Presented at the 6th International Symposium on High Performance Capillary Electrophoresis, San Diego, CA, Abstract No. 133, 31 January–3 February 1994.
[10] M.G. Khaledi, F. Wang, C. Quang, Presented at the 1995 Pittsburgh Conference, New Orleans, LA, Abstract No. 51, 1995.
[11] F. Wang, M.G. Khaledi, Anal. Chem. 68 (1996) 3460.
[12] A.M. Stalcup, K.H. Gahm, J. Microcolumn Sep. 8 (1996) 145.
[13] I.E. Valko, H. Siren, M.-L. Riekkola, J. Chromatogr. A 737 (1996) 263.
[14] I. Bjoernsdottir, S. Terabe, S.H. Hansen, J. Chromatogr. A 745 (1996) 37.
[15] F. Wang, M.G. Khaledi, J. Chromatogr. B, in press.
[16] Y.Y. Rawjee, D. U Staerk, G. Vigh, J. Chromatogr. 635 (1988) 291.
[17] I.M. Kolthoff, Anal. Chem. 46 (1974) 1992.