ELSEVIER

Journal of Chromatography B, 695 (1997) 131–145

JOURNAL OF CHROMATOGRAPHY B

Influence of the inorganic counterion on the chiral micellar electrokinetic separation of basic drugs using the surfactant N-dodecoxycarbonylvaline

Alicia G. Peterson, Joe P. Foley*

Department of Chemistry, Villanova University, Villanova, PA 19085-1699, U.S.A.

Received 23 October 1996; revised 3 April 1997; accepted 10 April 1997

Abstract

The chiral surfactant N-dodecoxycarbonylvaline (DDCV) has previously been used with a sodium counterion in micellar electrokinetic chromatography (MEKC) separations of β-blockers (pindolol, atenolol, metoprolol, acebutolol, alprenolol, oxprenolol and propranolol), β-agonists (N-methylpseudoephedrine, pseudoephedrine, ephedrine and norephedrine) and phenolic amino alcohols (norphenylephrine, synephrine, octopamine and salbutamol) in this laboratory. In the present study, we investigated the effects of three monovalent counterions – Li^+, Na^+ and K^+ – on the peak shape, efficiency, selectivity and retention of these 15 chiral pharmaceutical compounds with DDCV micelles. A much better (more symmetrical) peak shape was observed when Li^+ was employed, due to a better if still imperfect match of analyte and counterion mobilities; average asymmetry factors in LiDDCV, NaDDCV, and KDDCV buffers were 1.9, 3.7, and 4.2, respectively. An increase in efficiency of 50–100% for hydrophobic solutes and over 100% for many hydrophilic solutes was also observed in LiDDCV compared to NaDDCV and KDDCV, probably due to enhanced mass transfer. The influence of an organic modifier (acetonitrile) in the separation buffer was also studied for the LiDDCV, NaDDCV and KDDCV systems. Although a concentration of either 0 or 5% acetonitrile resulted in the best enantiomeric resolution for most analytes, the most hydrophobic solutes (alprenolol and propranolol) required the addition of 20–25% ACN. Due to differences in the conductance of Li^+, Na^+ and K^+, the advantage of using Li^+ as the counterion for anionic DDCV micelles (better peak shape, higher efficiency and resolution, with shorter analysis times), although apparent for comparisons at a given applied voltage, is more significant when considered in the more appropriate context of equivalent Joule heating.

Keywords: Enantiomer separations; Drugs; N-Dodecoxycarbonylvaline; Surfactants, chiral; Counterions

1. Introduction

A large number of drugs exist in enantiomeric forms. Frequently only one form is therapeutically active while the other is inactive and/or toxic. Separation methods for enantiomeric pairs have been extensively investigated in all areas of chromatography over the last 15 years. Capillary electrophoresis (CE) and micellar electrokinetic chromatography (MEKC) have generated much interest due to the high efficiencies of these separation modes.

Micellar electrokinetic chromatography was first introduced by Terabe in 1984 for the high resolution separation of neutral analytes [1]. Since then MEKC

*Corresponding author.

PII S0378-4347(97)00242-9

has been extended to include the separation of charged as well as chiral analytes. MEKC methods using the surfactant sodium dodecyl sulfate (SDS) in combination with chiral selectors, as cyclodextrin, maltose, or bile salts have been investigated [2,3]. Optically active amino acid-derivatized surfactants such as, sodium N-dodecanoyl-L-valinate, have been used successfully in chiral separations of both ionic and non-ionic enantiomers [4–6]. The commercially available, synthetic amino acid based chiral surfactant, N-dodecoxycarbonylvaline (DDCV) has been reported to have good selectivity for a wide variety of basic drugs such as β-blockers and β-agonists [7–9]. One of the features of DDCV is that it is available in optically pure forms (*R* or *S*), making reversal of elution order as easy as switching enantiomeric forms of the surfactant. This capability is important in determining the enantiomeric impurity of a drug.

The nature of the surfactant has a significant effect on the separation process in MEKC. Micelles are made up of individual surfactant monomers that will aggregate to form micelles at the critical micelle concentration (CMC). The surfactant monomers will orient themselves such that the hydrophobic tail is located in the interior and the hydrophilic head groups are on the exterior. Counterions bind non-stoichiometrically to the micellar surface and may control, or at least play an important role in determining, the surface chemistry of the micelle. The strength of the non-stoichiometric binding and the resulting surface chemistry should also have an effect on the separation process. Historically in MEKC, the surfactant is most often anionic and, moreover, has a sodium counterion.

Counterion effects in capillary zone electrophoresis (CZE) have been investigated extensively and are generally manifested in changes in the electroosmotic flow [10–14] or in the degree of electromigration dispersion of the analyte zones [15–19]. At present, however, only two studies have been published on the effects of the counterion on separations in MEKC. In 1993, Nielsen and Foley compared the chromatographic and electrokinetic properties of sodium dodecyl sulfate (SDS) micelles with magnesium dodecyl sulfate ($Mg(DS)_2$) micelles [20]. The Mg^{2+} counterion provided significantly larger methylene and functional group selectivities for the compounds studied compared to the Na^+ counterion. In 1995, Ahuja and Foley extensively compared MEKC separations obtained with SDS, lithium dodecyl sulfate (LiDS), and potassium dodecyl sulfate (KDS) [21]. In both studies, the identity of the counterion had a significant effect on the elution range, efficiency, peak shape, and the solvent strength of the organic modifier (acetonitrile).

In the present study we compare the effects of Li^+, Na^+ and K^+ counterions on the chiral separations of 15 basic pharmaceutical compounds using (*R*)- or (*S*)-DDCV micelles. Our objective was to examine the effects of these counterions on separation efficiency, peak shape, selectivity and elution range, the factors responsible for resolution in MEKC. Resolution is extremely important when determining the enantiomeric purity of a pharmaceutical drug. The basic drugs in this study, shown in Fig. 1, were (1) acebutolol, (2) alprenolol, (3) atenolol, (4) metoprolol, (5) oxprenolol, (6) pindolol, (7) propranolol, (8) ephedrine, (9) pseudoephedrine, (10) N-methylpseudoephedrine, (11) norephedrine, (12) norphenylephrine, (13) octopamine, (14) synephrine and (15) salbutamol.

2. Experimental

2.1. Instrumental

A Quanta 4000E CE system (Waters, Milford, MA, USA) equipped with temperature control and fixed wavelength UV detection at 214 nm was employed for all CZE separations performed in this study. All MEKC separations were performed in a 75-μm I.D. (363 μm O.D.) fused-silica capillary tube ranging in length from 52–55 cm (45–48 cm from inlet to detector) (Polymicro Technologies, Phoenix, AZ, USA). Sample introduction was performed hydrostatically for 2 s and the applied voltage for all separations was 12 kV. Data from the Quanta 4000E were collected and processed on a NEC Image 466es (Milford, MA, USA) using Millennium 2010 software (Millipore Corporation, Milford, MA, USA).

2.2. Materials

The zwitterionic buffer, CHES (2-[N-cyclohexylamine]ethanesulfonic acid) was purchased from Sigma (St. Louis, MO, USA). The chiral surfactant,

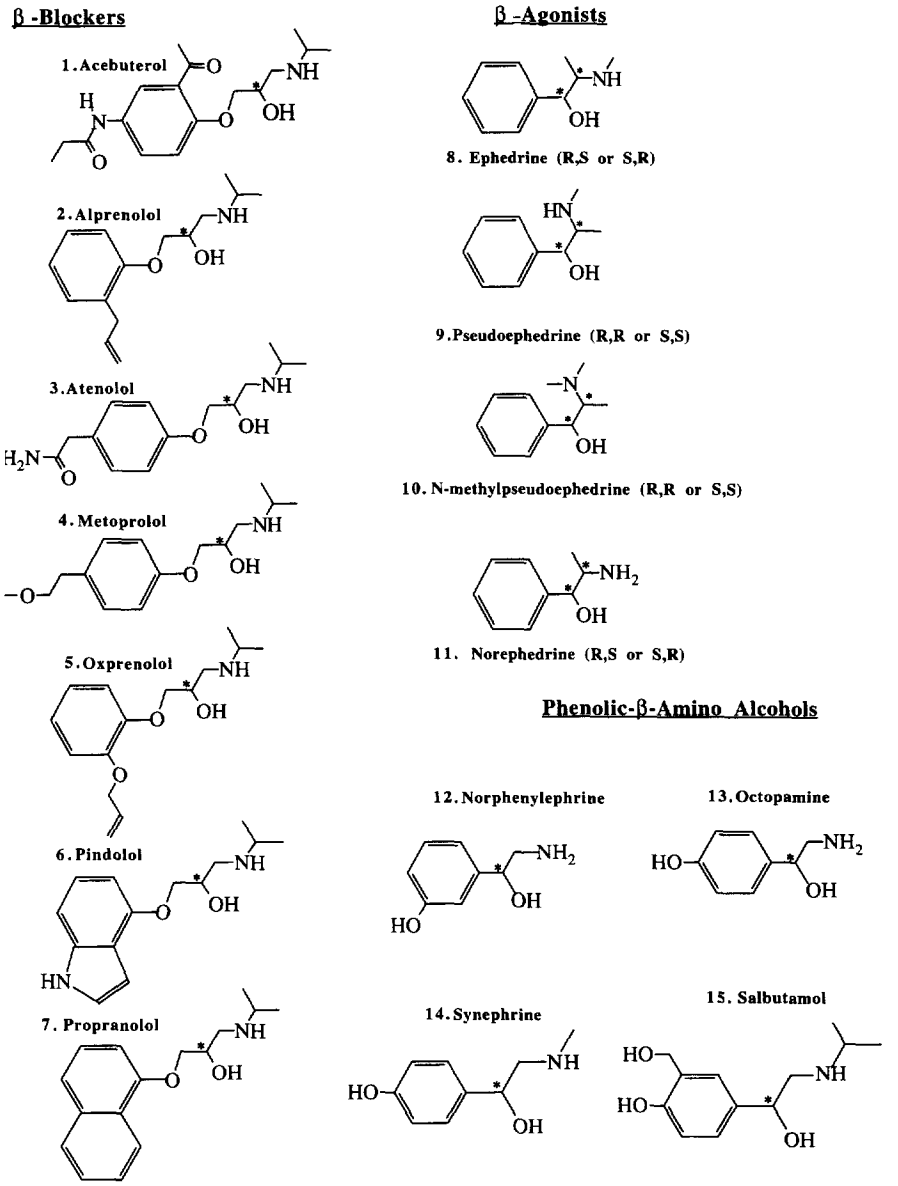

Fig. 1. Chemical structures of the chiral pharmaceutical analytes employed in this study.

(*R*)-N-dodecoxycarbonylvaline (Waters EnantioSelect (*S*)-and (*R*)-Val-1), was provided by Waters. Triethylamine (TEA) was purchased from J.T. Baker (Phillipsburg, NJ, USA). The following racemates or pure enantiomers were purchased from Sigma: racemic atenolol, (1*R*, 2*R*) (−)-N-methylpseudoephedrine, racemic metoprolol, racemic octopamine, racemic acebutolol, racemic alprenolol, racemic oxprenolol, (1*S*, 2*S*)-pseudoephedrine, (1*R*, 2*R*)-pseudoephedrine, racemic salbutamol and racemic synephrine. The following racemates or pure enantiomers were purchased from Aldrich (Milwaukee, WI, USA): (1*S*, 2*S*) (+)-N-methylpseudoephedrine, racemic pindolol, racemic propranolol, racemic norphenylephrine, racemic ephedrine and racemic norephedrine. Lithium hydroxide was purchased from

Sigma; in-house sodium hydroxide and potassium hydroxide were analytical reagent grade.

All studies were performed in MEKC buffer solutions containing 25 m*M* *S*-DDCV, 100 m*M* CHES and 10 m*M* triethylamine (TEA). MEKC electrolyte solutions were prepared by weighing out the appropriate chemicals and adding HPLC grade distilled water (J.T. Baker). The solutions were sonicated in a beaker and the pH monitored and adjusted with a 1.0 *M* solution of lithium hydroxide (LiOH), sodium hydroxide (NaOH) or potassium hydroxide (KOH), respectively, to keep the pH above 7. The contents of the beaker were transferred to a volumetric flask and enough TEA was added to make a final concentration of 10 m*M*. The appropriate amount of acetonitrile (v/v) was then added for studies involving organic modifier. The final pH was raised to 8.8 with 1.0 *M* of the appropriate hydroxide. CZE buffer solutions for measurement of $\mu_{ep,CZE}$ (see Eq. (2), Section 2.4) were made in the same manner as the MEKC separation buffer but with the DDCV omitted.

Given the 1:1 stoichiometry of the monoprotic surfactant DDCV (originally dissolved as the neutral acid) and the LiOH, NaOH or KOH used in the pH adjustment, the concentration of the counterion and the resulting anionic chiral surfactant is essentially the same; for brevity we will often refer to these MEKC buffers as LiDDCV, NaDDCV or KDDCV, respectively.

Stock sample solutions of the chiral analyte were prepared in methanol at concentrations of 2.00–4.00 mg/ml and diluted with separation buffer solution to a final concentration of 0.10–0.25 mg/ml (≤5% methanol). The final dilution also contained decanophenone (a t_{mc} marker) at a concentration of ~0.06 mg/ml.

2.3. Methods

The capillary was activated by first purging with 1.0 *M* LiOH, NaOH or KOH for 20 min and then 0.10 *M* of the respective base for an additional 20 min. The capillary was then rinsed and equilibrated with the separation buffer. The initial equilibration time was 3 h at the running voltage as described by Cohen and Grushka [22]. The capillary was used only with buffer solutions containing 10 m*M* TEA to maintain the conditioning of the capillary wall and enhance the repeatability of separations.

Methanol was used as the electroosmotic flow marker. Electroosmotic velocities were measured using a method previously published [23]. For each separation the elution time of the DDCV micelle (t_{mc}) was estimated using decanophenone. The t_{mc} values were confirmed with the iterative computation method developed by Bushey and Jorgenson [24]. Equations used in the calculations of retention factors, selectivity and efficiency are discussed in Section 2.4.

The separations were done in sets of five. Within each set of experiments, the capillaries were purged for 3 min with separation buffer. Between each set of experiments, the capillary was purged with the appropriate 0.1 *M* base. CZE separations, necessary to determine the charged analytes' electrophoretic mobilities (see Eq. (2)), were performed in sets of three. Previous experiments performed below the critical micelle concentration (0.5 m*M* DDCV) indicated that ion pairing was minimal [7], therefore the net mobility of these cationic analytes is equal to the sum of the coefficient of electroosmotic flow and their individual electrophoretic mobilities.

2.4. Calculations

The retention factors of the charged analytes were calculated via Eq. (1),

$$k = \frac{t_R(1 + \mu_r)t_0}{t_0(1 - t_R/t_{mc})} \quad (1)$$

where t_0 is the migration time of an unretained neutral compound (methanol in this study) and t_R and t_{mc} are the migration time of the charged analyte and micelle, respectively [25]. The method for t_{mc} measurement was described in Section 2.3. The remaining parameter in Eq. (1), $\mu_r = \mu_{ep}/\mu_{eo}$, is the analyte's relative electrophoretic mobility, i.e., the electrophoretic mobility of the analyte relative to the coefficient of electroosmotic flow. For charged solutes that swim downstream, $\mu_r > 0$ and vice-versa.

Ideally, μ_r is calculated after measuring both μ_{ep} and μ_{eo} under the same MEKC conditions used for the separation. Unfortunately, it is impossible to measure μ_{ep} under MEKC conditions, because of the

micelles' influence on analyte migration. Instead, μ_{ep} is measured with the same buffer system but with no surfactant ($C_{surf}=0$) or no micelles ($C_{surf}<$CMC), i.e., the mobilities of the charged analytes are measured under CZE-like conditions. One then assumes that $\mu_{ep,CZE} \approx \mu_{ep,MEKC}$, a reasonable assumption if the viscosity of the non-micellar and micellar separation buffers do not differ significantly. For these reasons, then, an accurate estimate of μ_r is obtained [25] using Eq. (2).

$$\mu_r = \frac{\mu_{ep,CZE}}{\mu_{eo,MEKC}} \quad (2)$$

The selectivities, α, were determined using Eq. (3):

$$\alpha = \frac{k_2}{k_1} \quad (3)$$

where k_1 and k_2 were the retention factors for the 1st and 2nd enantiomer, respectively.

Efficiencies, N, were calculated using the Foley–Dorsey Eq. (4):

$$N_{SYS} = \frac{41.7(t_R/W_{0.1})^2}{B/A + 1.25} \quad (4)$$

where t_R is the retention time of the enantiomer, $W_{0.1}$ is the width at 10% of the height and B/A is the asymmetry factor [26].

Resolution between enantiomers in MEKC [25] was calculated using Eq. (5),

$$R_S = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k_2}{1+k_{avg}}\right) \times \left(\frac{1+\mu_r-(t_0/t_{mc})}{1+\mu_r+(t_0/t_{mc})k_{avg}}\right) \quad (5)$$

where N is the average efficiency for the enantiomeric pair.

3. Results and discussion

3.1. Chromatographic consequences of counterion effects on micelles

DDCV monomers are composed of a C_{12} hydrophobic chain with an amino acid, valine, as a hydrophobic head group. At pH 8.8 they are negatively charged. Inorganic counterions such as Li^+, Na^+ or K^+ bind non-stoichiometrically to the micellar surface. The surface chemistry is influenced by the degree of hydration and the strength of binding of these counterions. The hydrated radii of the counterions Li^+, Na^+ and K^+ are, respectively, 0.34, 0.276 and 0.232 nm (Fig. 2). The larger the hydrated radius of the counterion, the greater the distance of closest approach between it and the anionic head group of the surfactant. From simple considerations of electrostatics ($F=q_1q_2/r^2$) and polarizability (which work in concert), one can surmise that the Li^+ counterion should bind less to the surface of a given anionic micelle than Na^+ or K^+ ion [21,27].

In general, changes in the degree of counterion binding to a given micelle will likely affect a number of important, interrelated characteristics of that micelle, including (i) the number of water molecules on the micelle surface (waters of hydration) [28]; (ii) the degree of electrostatic interaction between adjacent head groups; (iii) the degree of solvation of the

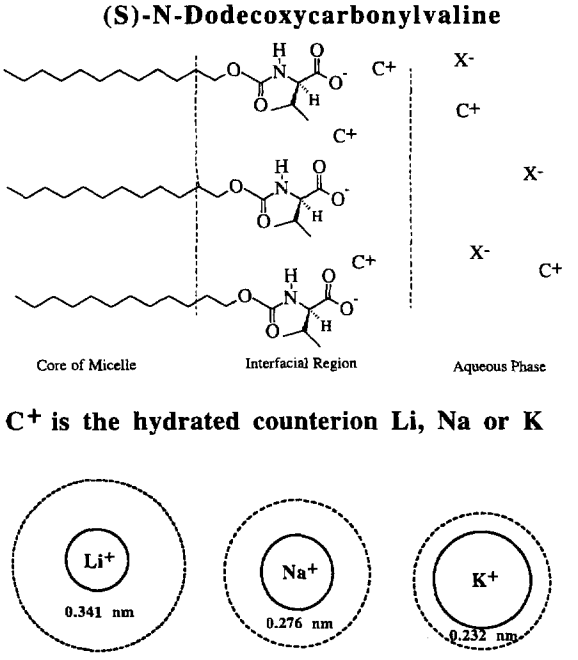

Fig. 2. Section of the interfacial region of a (*S*)-N-dodecoxycarbonylvaline (*S*-DDCV) micelle. The various regions and positions within that region where the hydrated counterion (C^+) may be present is shown. The hydrated radius (nm) is shown for Li^+, Na^+ and K^+.

micelle and thus its 'molecular weight' and hydrodynamic radius and (iv) the degree of penetration of the micelle by water, buffer species, organic solvent if present, or other compatible component of the buffered micellar solution. Moreover, changes in any or all of these 'secondary' micellar characteristics may significantly influence the overall organization of the micelle. For example, for 'pure', charged micelles, i.e., those made from a single surfactant of a single charge state, the greater repulsive forces between head groups due to less counterion binding is likely to result in a slight to moderate reorganization of the micelle that yields an increase in the average distance between head groups. Such reorganization could include a more staggered alignment of adjacent head groups, a solvent – or buffer – induced swelling of the micelle (an increase in its average size), or a modest reduction in the aggregation number.

The point of the above discussion is that the degree of counterion binding may play a significant role in determining the magnitude of one or more band-broadening phenomena associated with the micelle [25,29], as a consequence of changes in one or more micellar characteristics noted in the previous paragraph. Differences in zone broadening (efficiency) may, in some cases, be due to differences in the rate of mass transfer of the analyte between the aqueous mobile phase and the pseudostationary micellar phase. A higher mass transfer rate with a given counterion may be due to a decrease in the apparent 'viscosity' of the micelle in the interfacial and/or core regions, a reduction in electrical 'activation barriers' (charged solutes), or other, more complex combinations of numerous phenomena. Mass transfer differences have previously been hypothesized to be important in explaining the observed counterion effects on the efficiency of neutral solutes in MEKC with dodecylsulfate micelles [21]. Regardless of the reason(s), an increased rate of mass transfer should result in less band broadening and hence, better efficiency. As shown by Eq. (5) in Section 2.4, any enhancement in efficiency (N) or selectivity (α) will improve resolution, a parameter of utmost importance in any separation, but especially so for those involving difficult-to-separate enantiomeric solutes.

3.2. Joule heating

In the initial studies of NaDDCV, zwitterionic buffer systems were investigated because they offered lower conductivity when the buffer is adjusted close to its pK_a [8]. These buffer systems proved to have lower operating currents than inorganic buffer systems and thereby minimized the resultant Joule heating. Ohm's law plots were constructed with the present instrument and it was found that deviations from linearity did not occur until the power/length exceeded 1.0 W/m. When Ohm's law plots deviate from linearity, Joule heating can have an effect on peak shape and resolution [30].

In this study the Joule heating (in terms of power/length) at 12 kV for LiDDCV, NaDDCV and KDDCV without organic modifier was 0.40, 0.53 and 0.76 W/m, respectively. For similar separation buffers with 5% ACN, the Joule heating was 0.43 W/m, 0.63 W/m and 0.82 W/m. LiDDCV separation buffers containing 10, 15, 20 and 25% ACN were studied for the more hydrophobic β-blockers; the power levels with these LiDDCV buffers were always below 0.50 W/m.

Since the DDCV buffer systems (with CHES) are made by adjusting the pH of an aqueous solution of DDCV (the monoprotic acid) and CHES with LiOH, NaOH or KOH, the observed differences in current and the resultant Joule heating are due primarily to the differences in the mobilities of the counterions. In dilute aqueous buffer, the mobilities of Li^+, Na^+ and K^+ are 38.7, 50.5 and 73.5 ($\times 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$), respectively [29], which correlates well with 0.40, 0.53 and 0.76 W/m, the respective levels of Joule heating with equimolar LiDDCV, NaDDCV and KDDCV buffers without organic modifier. The disproportionate increase in current and Joule heating in going from 0 to 5% ACN for NaDDCV (+19%) compared to LiDDCV (+7.5%) and KDDCV (+7.9%) was inexplicable but reproducible.

3.3. Retention, enantioselectivity and achiral selectivity

Reported in Tables 1 and 2 are the retention factors of the first enantiomer of each solute to elute

Table 1
Effect of counterion and acetonitrile on retention and enantioselectivity of β-blockers

Counterion	ACN (%)	(*R*)-Atenolol	Acebutolol	Pindolol	Metroprolol	Oxprenolol	Alprenolol	Propranolol
Li	0	2.43 (1.06)	10.7 (1.03)	11.8 (1.07)	12.9 (1.06)	26.3 (1.04)	70.0 (1.00)	163 (1.00)
	5	0.94 (1.05)	5.11 (1.04)	9.11 (1.07)	10.3 (1.07)	18.4 (1.07)	68.0 (1.00)	80.0 (1.00)
	10	0.75 (1.05)	3.72 (1.04)	6.76 (1.06)	7.35 (1.07)	13.8 (1.07)	51.1 (1.00)	56.0 (1.00)
	15	0.80 (1.04)	2.21 (1.04)	4.03 (1.07)	4.70 (1.06)	8.31 (1.06)	32.0 (1.00)	39.0 (1.00)
	20	0.21 (1.00)	1.26 (1.04)	2.44 (1.06)	2.91 (1.05)	4.98 (1.05)	17.0 (1.06)	19.0 (1.05)
	25	0.12 (1.00)	1.12 (1.02)	1.36 (1.05)	1.63 (1.05)	2.57 (1.05)	7.02 (1.06)	7.67 (1.06)
Na	0	3.20 (1.05)	17.6 (1.00)	19.1 (1.07)	15.8 (1.07)	44.0 (1.00)	530 (1.00)	5400 (1.00)
	5	1.76 (1.05)	7.06 (1.04)	8.23 (1.07)	8.68 (1.07)	17.4(1.07)	65.9 (1.00)	94.0 (1.00)
K	0	1.56 (1.05)	8.80 (1.00)	12.8 (1.07)	12.8 (1.07)	21.9 (1.00)	86.0 (1.00)	5400 (1.00)
	5	1.13 (1.05)	6.49 (1.04)	7.60 (1.07)	7.30 (1.07)	16.2 (1.07)	60.0 (1.00)	88.0 (1.00)

in LiDDCV, NaDDCV and KDDCV buffer systems, along with enantioselectivities. When individual solutes were available in optically pure form (β-agonists and atenolol), the absolute configuration of the first enantiomer is provided. In all cases the absolute configuration of the first enantiomer was invariant among the three buffers. Except for atenolol, an anomalously hydrophilic β-blocker, the general order of elution was the phenolic amino alcohols, the β-agonists and then the β-blockers. Although this order correlated fairly well with overall hydrophobicity, some interesting exceptions were observed within the β-agonists, where the compound with a higher degree of methylation on the nitrogen always eluted before the less methylated compound of the same absolute configuration (ephedrine vs. norephedrine, N-methylpseudoephedrine vs. pseudoephedrine).

For the β-blockers in Table 1, there is a wide range of hydrophobicity and thus retention for a given set of buffer conditions. Although most enantiomers could be separated under purely aqueous

Table 2
Effect of counterion and acetonitrile on retention and enantioselectivity of β-agonists and phenolic amino alcohols

Counterion	ACN (%)	β-Agonists			
		(1*S*, 2*R*)-Norephedrine	(*S*,*S*)-N-methyl-Pseudoephedrine	(*S*,*S*)-Pseudoephedrine	(1*S*, 2*R*)-Ephedrine
Li	0	5.70 (1.10)	6.17 (1.31)	7.43 (1.23)	8.25 (1.11)
	5	3.81 (1.09)	3.71 (1.30)	6.70 (1.24)	6.57 (1.14)
Na	0	7.38 (1.10)	5.66 (1.30)	8.35 (1.22)	9.45 (1.11)
	5	4.10 (1.09)	3.49 (1.29)	4.90 (1.22)	5.04 (1.09)
K	0	6.35 (1.09)	5.40 (1.29)	7.38 (1.20)	7.00 (1.09)
	5	3.73 (1.09)	2.86 (1.27)	4.16 (1.21)	4.37 (1.09)
		Phenolic amino alcohols			
		Octopamine	Synephrine	Norphenylephrine	Salbutamol
Li	0	1.44 (1.05)	1.54 (1.07)	2.10 (1.09)	2.48 (1.05)
	5	1.16 (1.05)	1.08 (1.06)	1.46 (1.08)	1.94 (1.05)
Na	0	2.04 (1.07)	2.40 (1.07)	3.29 (1.09)	3.59 (1.05)
	5	1.12 (1.06)	1.21 (1.06)	1.76 (1.08)	2.01 (1.05)
K	0	1.72 (1.05)	1.56 (1.06)	2.60 (1.09)	3.14 (1.04)
	5	0.95 (1.05)	1.11 (1.06)	1.45 (1.08)	1.74 (1.05)

conditions (albeit with a higher than optimal degree of retention [25], the enantiomers of the two most hydrophobic β-blockers, alprenolol and propranolol, required a minimum of 20% ACN to achieve enantiomeric separation. The NaDDCV and KDDCV buffer systems could not be studied above 5% ACN because of insufficient solubility in the presence of the zwitterionic buffer CHES. When lithium was employed as the counterion, similar limitations in DDCV solubility with CHES at ACN concentrations as high as 25% were not observed. Note that in previous studies with NaDDCV, a sodium borate buffer was used at high ACN concentrations ($\leq$25%) to separate acebutolol, oxprenolol, alprenolol and propranolol [8].

With no ACN in the buffer, the trend in retention for all the β-blockers except propranolol was $k_{LiDDCV} \approx k_{KDDCV} \ll k_{NaDDCV}$. For propranolol, the trend was $k_{LiDDCV} \ll k_{NaDDCV} \approx k_{KDDCV}$, although the uncertainties in k are relatively high due to propranolol's nearness to t_{mc}. With the addition of 5% ACN, the observed trends in retention appeared to depend on the degree of retention: For the three most retained (hydrophobic) β-blockers – oxprenolol, alprenolol and propranolol – the retention factor of each solute was similar in all three buffer systems. For the two least retained (hydrophilic) β-blockers – atenolol and acebutolol – the trend in retention was $k_{LiDDCV} \ll k_{KDDCV} < k_{NaDDCV}$. Finally, for the two remaining, moderately retained β-blockers – pindolol and metoprolol – the trend in retention was $k_{KDDCV} < k_{NaDDCV} \ll k_{LiDDCV}$.

Table 2 gives the retention factors of the first enantiomer of each β-agonist and phenolic amino alcohol to elute and the enantioselectivities (in parentheses). With no ACN in the buffer, the general trend in retention for each of the β-agonists was $k_{KDDCV} < k_{LiDDCV} < k_{NaDDCV}$, with minor exceptions for norephedrine ($k_{LiDDCV} < k_{KDDCV}$) and N-methylpseudoephedrine ($k_{NaDDCV} < k_{LiDDCV}$). With the addition of 5% ACN, the trend was $k_{KDDCV} < k_{NaDDCV} < k_{LiDDCV}$, with only a minor exception for norephedrine ($k_{LiDDCV} < k_{NaDDCV}$). For each of the phenolic amino alcohols, the trend in retention with no ACN in the buffer was always $k_{LiDDCV} < k_{KDDCV} < k_{NaDDCV}$. With 5% ACN, the trend was $k_{KDDCV} < k_{LiDDCV} < k_{NaDDCV}$, with minor exceptions for octopamine ($k_{NaDDCV} < k_{LiDDCV}$) and synephrine ($k_{LiDDCV} < k_{KDDCV}$).

Whereas solute retention depended significantly on the counterion, enantioselectivity remained essentially constant for all three counterion systems. This is not surprising, since the chiral interactions depend primarily on the structures and absolute configurations of the analytes and the chiral surfactant (DDCV), neither of which is influenced by the micelle's counterion.

In contrast to the virtually constant enantioselectivity, numerous significant changes in achiral selectivity were observed as the counterion was varied, including several changes in the elution order (Table 3). In aqueous DDCV buffer, atenolol moved from fourth to second (almost tied for first) as the counterion was varied from Li^+ to K^+. Among the more hydrophobic compounds, metoprolol eluted after acebutolol and pindolol with Li^+ and K^+ but eluted before them with Na^+ as a counterion. In DDCV buffer with 5% ACN, atenolol moved from first to fourth to third as the counterion was varied from Li^+ to Na^+ to K^+; the order of synephrine and octopamine reversed when the counterion was switched from Li^+ to Na^+ or K^+. Among the more hydrophobic compounds, the elution order of acebutolol and pseudoephedrine reversed (with ephedrine in

Table 3
Effect of counterion and acetonitrile on elution order[a]

ACN (%)	Counterion	Elution order														
0	Li	13	14	12	3	15	11	9	10	8	1	6	4	5	2	7
	Na	13	14	3	12	15	10	9	11	8	4	1	6	5	2	7
	K	14	3	13	12	15	10	8	11	9	1	6	4	5	2	7
5	Li	3	14	13	12	15	10	1	11	8	9	6	4	5	2	7
	Na	13	14	12	3	15	10	9	11	8	1	6	4	5	2	7
	K	13	14	3	12	15	10	9	11	8	1	4	6	5	2	7

[a] Separation buffer as in Fig. 3, numbering scheme as in Fig. 1.

between) when the counterion was switched from Li^+ to Na^+ or K^+; the order of pindolol and metoprolol reversed when the counterion was switched from Li^+ or Na^+ to K^+.

Because the enantioselectivities for most adjacent compounds were moderate and similar, the elution order for the second enantiomer of each compound is usually the same as that reported in Table 3 for the first enantiomer. The only potential differences in elution order are those separations in which the enantioselectivity is greater than the achiral selectivity (adjacent compound selectivity), i.e., a condition that frequently occurred for compounds that eluted adjacent to pseudoephedrine and/or N-methylpseudoephedrine.

From the perspective of resolution, one of the more important changes in achiral selectivity with micellar counterion was the separation of pindolol and metoprolol. Whereas they co-eluted in NaDDCV and KDDCV separation buffers, they were partially resolved with LiDDCV. As the percentage of ACN in the LiDDCV buffer was increased, baseline resolution between the later-eluting pindolol enantiomer and the earlier-eluting metoprolol enantiomer was achieved, due to the increasing achiral selectivity.

3.4. Peak shape, efficiency, elution range, resolution and analysis time

Shown in Fig. 3 are representative separations of the three types of pharmaceutical compounds we examined (β-blockers, β-agonists and phenolic amino alcohols (Fig. 1) using LiDDCV, NaDDCV and KDDCV buffer systems, respectively, without ACN. Importantly, peak width, tailing, overlap and analysis time all increase as the DDCV counterion is changed from Li^+ to Na^+ to K^+.

The effect of the DDCV counterion on the peak shape of the second enantiomer of each compound in the present study is shown in Table 4. As is evident from the data, the trend for each compound is $(B/A)_{Li^+} \ll (B/A)_{Na^+} < (B/A)_{K^+}$. When the individual asymmetry factors were averaged over each counterion, the results were 1.9, 3.7 and 4.2 for Li^+, Na^+ and K^+, respectively. Almost exactly the same results were obtained for the means when interior averaging was performed, i.e., when the highest and lowest asymmetry factors for a given counterion were omitted. Thus, despite a few apparent outliers, the most symmetric peaks were clearly obtained with LiDDCV and the least symmetric were obtained with KDDCV.

Although it is frequently difficult to deconvolute all potential sources of peak asymmetry, the data of Table 4 provide strong evidence that an important cause of peak asymmetry in this study is electromigration dispersion [15–17], i.e., the distortion of analyte zones as a result of significant differences in the electrophoretic mobilities of the analytes in the sample and like-charged ions present in the sample and/or separation buffer. As mentioned earlier in our discussion of Joule heating, the mobilities of Li^+, Na^+ and K^+ in dilute aqueous buffer are 38.7, 50.5 and 73.5 ($\times 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$) [29], whereas the mobilities of the 15 compounds of this study (measured in the absence of DDCV) ranged from 10–25×10^{-5} $cm^2\ V^{-1}\ s^{-1}$.

The above mobility data for the counterions and solutes explain, at least qualitatively, the trend in solute asymmetry factors noted above. First, since the mobilities of the analytes are lower than those of the counterions, the analyte peaks are tailed, in agreement with electromigration dispersion theory. Moreover, of the three monovalent counterions employed in this study, the best peak shapes were obtained when Li^+, the counterion whose mobility most closely matched the analytes was employed, also in agreement with the theory. Finally, although beyond the scope of the present study, even better (more symmetrical) peak shapes might be expected if a counterion less mobile than Li^+ was substituted in the separation buffer or if the analyte to counterion concentration ratio was lower.

A close inspection of Table 4 also reveals a moderate dependence of peak shape on the degree of retention. For a given counterion, the mean asymmetry factor of highly retained compounds (1, 4–6) is significantly lower than that for low to moderately retained compounds (3, 8–15) in both the aqueous MEKC buffer and the buffer with 5% ACN.

The lower asymmetry factors observed for the highly retained compounds are probably due to the reduced effect of electromigration dispersion on these compounds and not to the relatively small, random (w.r.t. retention) differences in mobilities

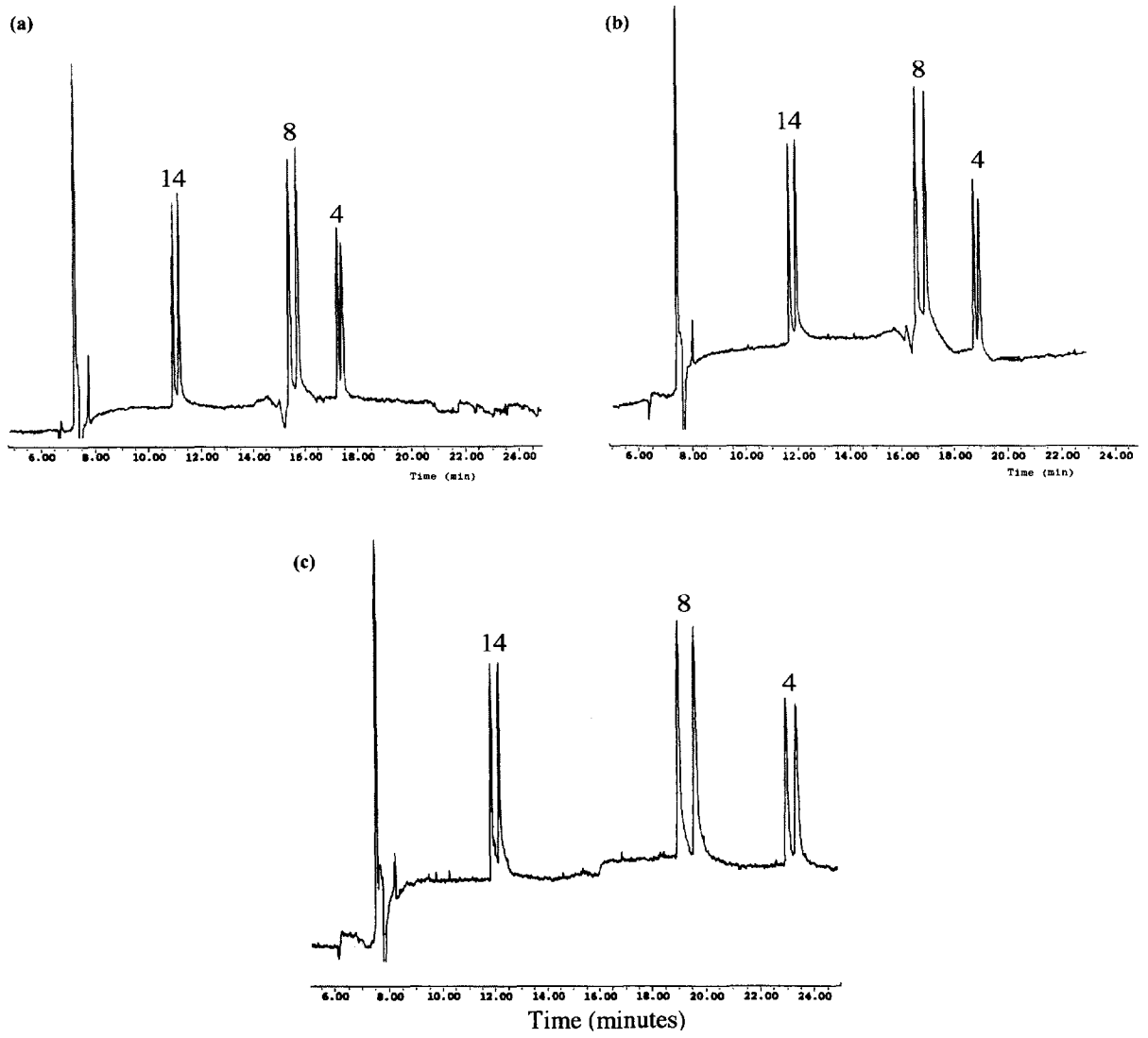

Fig. 3. Effect of counterion (Li^+, Na^+ or K^+) on micellar electrokinetic separation of synephrine (14), ephedrine (8) and metoprolol (4). Separation buffer: 25 m*M* (*S*)-DDCV, 100 m*M* CHES, and 10 m*M* TEA, adjusted to pH 8.8 with (A) LiOH; (B) NaOH or (C) KOH.

among all compounds of this study. Electromigration dispersion is a phenomenon that presumably occurs only (or mostly) in free solution; highly retained compounds, by definition, spend more time in micelles and therefore under finite elution range conditions spend less time in free solution. Thus, highly retained compounds undergo less electromigration dispersion in MEKC and their corresponding zones and peak profiles should therefore be more symmetrical.

In addition to the dramatic effect of counterion on peak shape (asymmetry), a similar effect on efficiency was also observed. As shown in Fig. 4 (solutes 1, 3–6) and Fig. 5 (solutes 8–15) for the second enantiomer, the efficiency (calculated via Eq. (4)) is frequently 3–5 times higher using Li^+ as the counterion instead of Na^+ or K^+, with either 0 or 5% ACN in the separation buffer. With no ACN in the buffer, the average plate counts with Na^+ and Li^+ were 35 000 and 117 000, respectively. With 5%

Table 4
Effect of counterion on peak asymmetry

Compound	ACN (%)	Asymmetry factor (B/A)[a]		
		LiDDCV	NaDDCV	KDDCV
β-Blockers				
Acebutolol	0	1.3	1.8	1.8
	5	2.0	2.6	2.6
Alprenolol	0	NR[b]	NR	NR
	5	NR	NR	NR
Atenolol	0	2.2	3.4	3.1
	5	2.1	2.3	3.8
Metoprolol	0	2.4	2.2	2.1
	5	1.8	3.6	4.3
Oxprenolol	0	1.7	1.9	2.3
	5	1.5	3.0	3.9
Pindolol	0	1.1	1.9	3.0
	5	1.4	2.7	3.2
Propranolol	0	NR[b]	NR	NR
	5	NR	NR	NR
β-Agonists				
Ephedrine	0	2.3	4.4	3.0
	5	3.1	8.7	5.5
Pseudoephedrine	0	3.3	4.4	8.2
	5	2.9	7.2	8.7
N-Methylpseudoephedrine	0	3.1	7.2	7.3
	5	3.9	5.3	9.0
Norephedrine	0	1.4	5.3	3.0
	5	1.8	4.5	4.9
Phenolic amino alcohols				
Norphenylephrine	0	0.9	5.6	6.7
	5	1.3	4.2	5.6
Octopamine	0	2.5	5.8	5.8
	5	2.1	3.1	4.5
Synephrine	0	0.8	3.3	5.5
	5	1.9	2.0	5.0
Salbutamol	0	1.6	4.8	3.6
	5	1.7	3.7	3.8
Overall mean	0 & 5	2.0	4.0	4.6
Mean, compounds 3, 8–15[c]	0 & 5	2.2	4.7	5.4
Mean, compounds 1, 4–6	0 & 5	1.7	2.5	2.9
Mean	0	1.9	4.0	4.3
Mean, compounds 3, 8–15	0	2.0	4.9	5.1
Mean, compounds 1, 4–6	0	1.6	2.0	2.3
Mean	5	2.1	4.1	5.0
Mean, compounds 3, 8–15	5	2.3	4.6	5.6
Mean, compounds 1, 4–6	5	1.7	3.0	3.5

[a] Average of three measurements of B/A at 10%.
[b] Not reported due to co-elution of the enantiomers.
[c] Compound numbers refer to the numbering scheme of Fig. 1.

ACN, the average plate counts with Na^+ and Li^+ were 59 000 and 131 000.

Although a detailed comparison of the analytes' peak shapes to the exponentially modified Gaussian [31] or other asymmetric peak shape models was beyond our scope, based on the qualitative resemblance of the peaks in this study to the EMG model, it is reasonable to employ the latter to interpret the

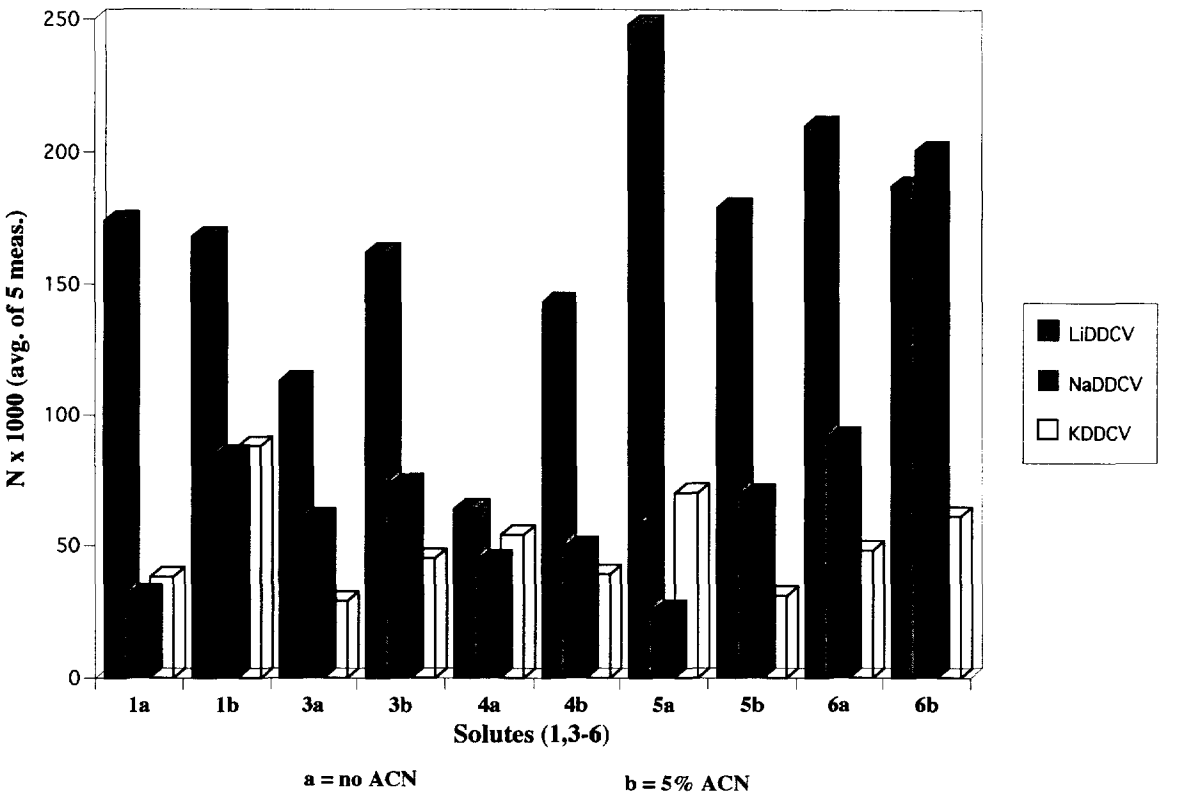

Fig. 4. Effect of counterion (Li^+, Na^+ or K^+) on separation efficiencies (N) of β-blockers (1, 3–6). Separation buffer as in Fig. 3, but with either 0 or 5% ACN (a or b, respectively).

efficiency results. Based on a universal EMG data set [32] in which the asymmetric band broadening component varies while the symmetric band broadening remains constant and assuming that electromigration dispersion is the only significant source of asymmetric band broadening, the observed trend in solute efficiency, $N_{\mathrm{LiDDCV}} \gg N_{\mathrm{NaDDCV}} \geq N_{\mathrm{KDDCV}}$, is largely, but not entirely, explained by the much more symmetric peak shapes obtained with LiDDCV.

In addition to counterion effects, a close inspection of Figs. 4 and 5 reveals a moderate-to-strong dependence of efficiency on the degree of retention. First, with either Na^+ or Li^+ as a counterion, the efficiency of highly retained compounds (1, 4–6) is nearly double (1.7 to 1.9 times) that of low to moderately retained compounds (3, 8–15) in the aqueous MEKC buffer. In the buffer with 5% ACN, the plate count was 1.5 to 2.5 times higher for the highly retained compounds. The greater efficiency for the highly retained compounds is due in part to the reduced electromigration dispersion they undergo; this was discussed earlier to explain the better (less asymmetric) peak shapes observed for highly retained compounds (Table 4).

The remaining differences in solute efficiencies observed in LiDDCV, NaDDCV and KDDCV buffers are most likely due to different rates of mass transfer as discussed in Section 3.1. A strong dependence in the efficiency of neutral solutes (which cannot undergo electromigration dispersion) on the surfactant counterion has previously been reported for dodecyl sulfate micelles [21]. In the present study of DDCV micelles, the variations in efficiency among solutes are probably due to differences in their polarity, locus of micellar solubilization, degree of hydration,

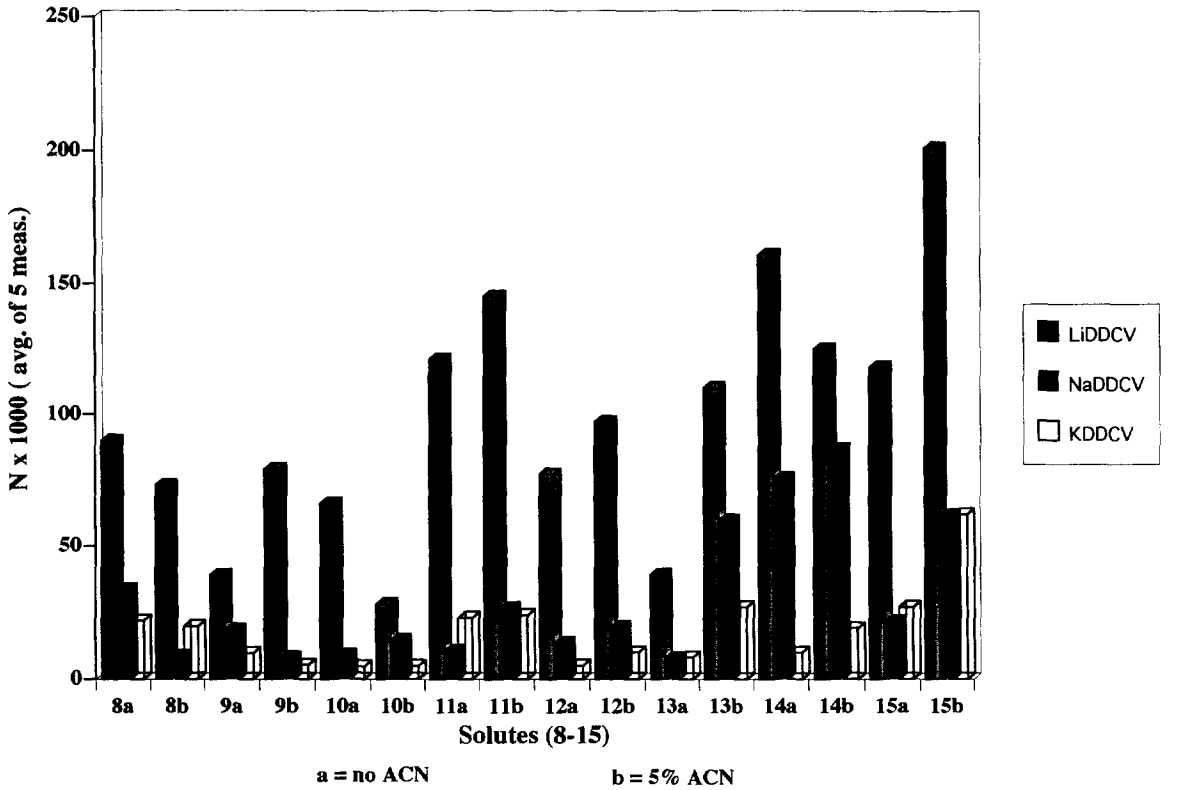

Fig. 5. Effect of counterion (Li^+, Na^+ or K^+) on separation efficiencies (N) of β-agonists (8–11) and phenolic amino alcohols (12–15). Separation buffer as in Fig. 4.

size and other solute characteristics, as well as a continuous variation in apparent intramicellar 'viscosity' from the interfacial to the core region of the micelle. For example, in contrast to non-polar (hydrophobic) solutes, many polar solutes drag water with them into the interfacial region of a micelle [33,34].

The elution ranges for the LiDDCV, NaDDCV and KDDCV separation buffers with no ACN were 2.5, 3.1 and 3.4, respectively. With 5% ACN, the elution ranges increased to 2.8, 3.8 and 4.8 respectively, representing a 10%, 18% and 30% change. For the LiDDCV buffer with 25% ACN, the elution range increased to 3.3, a 30% change from pure aqueous conditions.

Although a decrease in the elution range (t_{mc}/t_0) will result in a slight to moderate decrease in resolution if other chromatographic variables are constant, in the present study the much higher efficiencies typically observed with LiDDCV for both hydrophilic and hydrophobic analytes were more than sufficient to compensate for the somewhat lower elution range. The overall result with LiDDCV compared to NaDDCV or KDDCV at the same applied voltage was a slightly to moderately faster separation, with somewhat better resolution.

Finally, although the time required for the separations in Fig. 3 or similar ones sometimes differed only slightly between LiDDCV and NaDDCV, the results shown were obtained at the same applied voltage. When the separations were performed in the more appropriate context of equal Joule heating instead of equal applied voltage (not shown), the advantages of using Li^+ as the counterion for DDCV micelles (shorter analysis times, higher efficiency and resolution) were more pronounced.

3.5. Chiral separations with lithium as the optimal surfactant counterion

Based on the results presented, Li^+ ion is clearly

the best counterion among Li^+, Na^+ and K^+ for the chiral resolution of the pharmaceutical compounds of this study. Shown in Figs. 6–8 are additional separations obtained with LiDDCV buffer systems that were either impossible or not nearly as good with NaDDCV or KDDCV buffers. In Fig. 6, a typical MEKC separation of a range of hydrophilic and hydrophobic enantiomers is shown using a LiDDCV separation buffer without organic modifier. Figs. 7 and 8 illustrate the improvement in resolution of closely eluting diastereomers, ephedrine and pseudoephedrine and the β-blockers pindolol and metoprolol, respectively, with the addition of 5% and

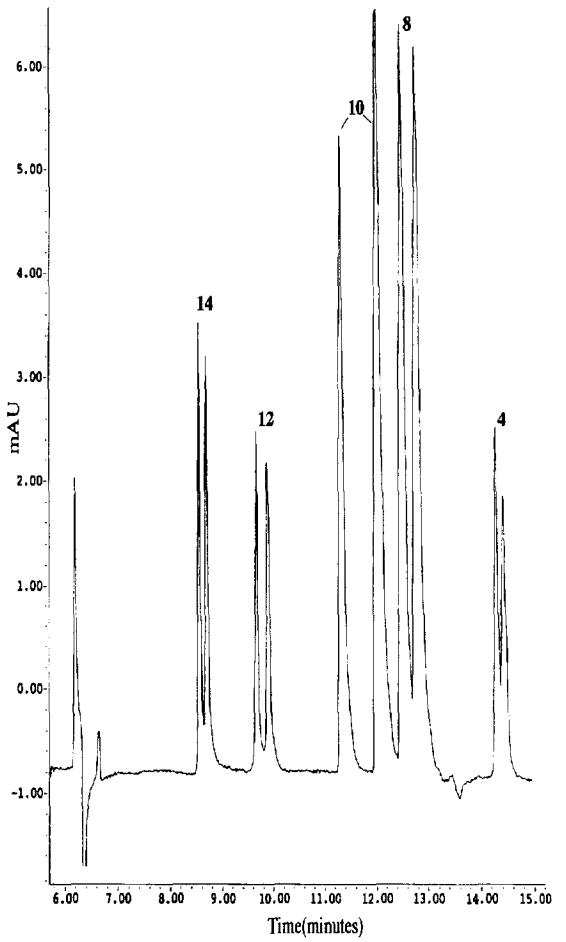

Fig. 6. Micellar electrokinetic chromatogram of synephrine (14), norphenylephrine (12), N-methylpseudoephedrine (10), ephedrine (8) and metoprolol (4). Separation buffer: 25 m*M* (*S*)-DDCV, 100 m*M* CHES, and 10 m*M* TEA, adjusted to pH 8.8 with LiOH.

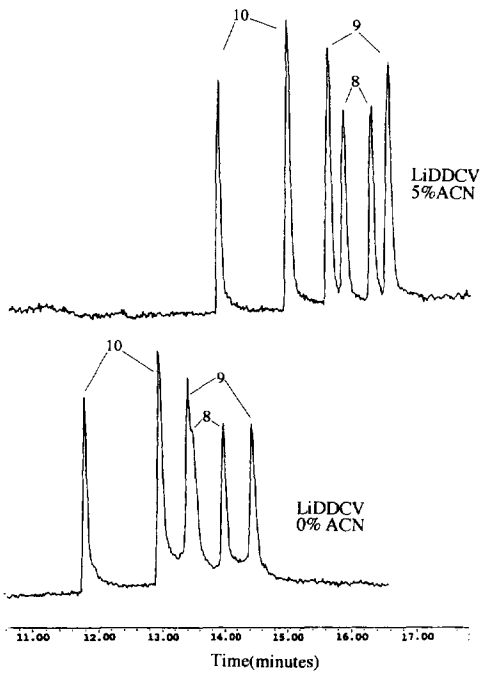

Fig. 7. Micellar electrokinetic chromatogram of β-agonists N-methylpseudoephedrine (10), pseudoephedrine (9) and ephedrine (8). Buffer composition as in Fig. 6, with either 0 or 5% ACN.

15% ACN. Such improvements were typically not observed with Na^+ or K^+ as counterions.

4. Conclusion

The nature of the counterion can significantly influence separations using N-dodecoxycarbonylvaline without loss of enantiomeric selectivity for the 15 chiral analytes in this study. The improvements in peak shape and the resultant rise in efficiencies are dramatic with the use of Li^+ as the counterion. The LiDDCV buffer system can also be used with up to 25% organic modifier without solubility problems, making it useful for a wide range of solutes with different hydrophobicity. Optimum separations of most analytes in this study, however, were obtained with either 0 or 5% ACN in the separation buffer. Other advantages of using LiDDCV over NaDDCV or KDDCV include shorter analysis times and lower operating currents at equal applied voltages, or much shorter analysis times at equal levels of Joule heating.

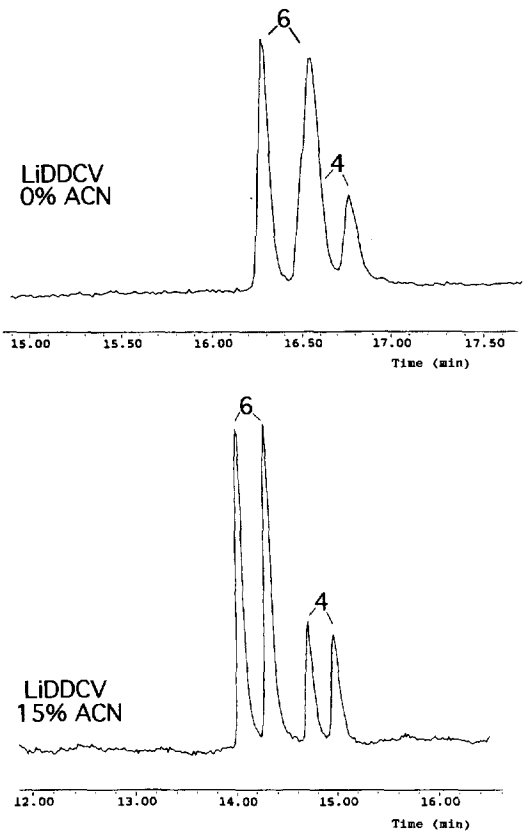

Fig. 8. Micellar electrokinetic chromatogram of β-blockers metoprolol (4) and pindolol (6). Buffer composition as in Fig. 6, with either 0 or 15% ACN.

Acknowledgments

The authors would like to thank Waters of Milford, MA, USA for the use of the Quanta 4000E capillary electrophoresis system and for providing the Waters EnantioSelect (*R*)- and (*S*)-Val-1 used for this research.

References

[1] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111–113.
[2] R.O. Cole, M.J. Sepaniak, W.L. Hinze, J. High Resol. Chromatogr. 13 (1990) 579–582.
[3] H. Nishi, T. Fukuyama, M. Matsao, S. Terabe, J. Microcol. Sep. 1 (1989) 234.
[4] K. Otsuka, J. Kawahara, K. Tatekawa, S. Terabe, J. Chromatogr. 559 (1991) 209–214.
[5] K. Otsuka, K. Karuhaka, M. Higashimori, S. Terabe, J. Chromatogr. A 680 (1994) 317–320.
[6] A. Dobashi, T. Ono, S. Hara, J. Yamaguchi, J. Chromatogr. 480 (1989) 413–420.
[7] J.R. Mazzeo, E.R. Grover, M.E. Swartz, J.S. Petersen, J. Chromatogr. A 680 (1994) 125–135.
[8] A.G. Peterson, E.S. Ahuja, J.P. Foley, J. Chromatogr. B 683 (1996) 15–28.
[9] M.E. Swartz, J.R. Mazzeo, E.R. Grover, P.R. Brown, H.Y. Aboulenein, J. Chromatogr. A 724 (1996) 307–316.
[10] I.Z. Atamna, C.J. Metral, G.M. Muschik, H.J. Issaq, J. Liq. Chromatogr. 13 (1990) 2517.
[11] J.E. Dickens, J. Gorse, J.A. Everhart, M. Ryan, J. Chromatogr. B 657 (1994) 401–407.
[12] H.J. Issaq, I.Z. Atamna, C.J. Metral, G.M. Muschik, J. Liq. Chromatogr. 13 (1990) 1247.
[13] H.J. Issaq, I.Z. Atamna, G.M. Muschik, G.M. Janini, Chromatographia 32 (1991) 155–161.
[14] H.J. Issaq, G.M. Janini, I.Z. Atamna, G.M. Muschik, J. Lukszo, J. Liq. Chromatogr. 15 (1992) 1129–1142.
[15] R. Virtanen, Acta Polytech. Scand. 123 (1974) 1–67.
[16] F.E.P. Mikkers, F.M. Everaerts, T.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 1–10.
[17] F. Foret, L. Krivánková and P. Bocek, Capillary Zone Electrophoresis; VCH Publishers, New York, 1993, pp. 27–33.
[18] Y.Y. Rawjee, R.L. Williams, G. Vigh, Anal Chem. 66 (1994) 3777–3781.
[19] R.L. Williams, G. Vigh, J. Chromatogr. A 730 (1996) 273–278.
[20] K.R. Nielson, J.P. Foley, J. Microcolumn. Sep. 5 (1993) 347–360.
[21] E.S. Ahuja, J.P. Foley, Anal. Chem 67 (1995) 2315–2324.
[22] N. Cohen, E. Grushka, J. Chromatogr. A. 678 (1994) 167–175.
[23] E.S. Ahuja, E.L. Little, J.P. Foley, J. Liq. Chromatogr. 15 (1992) 1099–1113.
[24] M.M. Bushey, J.W. Jorgenson, J. Microcol. Sep. 1 (1989) 125–130.
[25] J.P. Foley and E.S. Ahuja, in S.M. Lunte and D.M. Radzik (Editors), Pharmaceutical and Biomedical Applications of Capillary Electrophoresis, Vol. 2, Pergamon Press, Tarrytown, NY, 1996, pp. 81–178.
[26] J.P. Foley, J.G. Dorsey, Anal. Chem. 55 (1983) 730–737.
[27] M.J. Rosen, Surfactants and Interfacial Phenomena, John Wiley, 2nd ed., 1989, pp. 431.
[28] D. Myers, Surfactant Science and Technology, VCH Publishers, New York, 2nd ed., 1992, pp. 329.
[29] J. Vindevogel and P. Sandra, Introduction to Micellar Electrokinetic Chromatography, Huthig, Oxford, CT, 1992.
[30] H.J. Issaq, I.Z. Atamna, G.M. Muschik, G.M. Janini, Chromatographia 32 (1991) 155–161.
[31] M.S. Jeansonne, J.P. Foley, J. Chromatogr. Sci. 29 (1991) 258–266.
[32] J.P. Foley, J.G. Dorsey, J. Chromatogr. Sci. 22 (1984) 40–46.
[33] R.S. Tsai, W. Fan, N. Tatar, P. Carrupt, B. Testa, L. Kier, J. Am. Chem. Soc. 115 (1993) 9632–9639.
[34] S. Mall, G. Buckton, T. Gregori, D.A. Rawlins, J. Phys. Chem. 99 (1995) 8356–8361.