Novel chiral surfactant for the separation of enantiomers by micellar electrokinetic capillary chromatography

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Abstract

A novel chiral surfactant was prepared as both enantiomeric forms, (R)- and (S)-N-dodecoxycarbonylvaline, and employed for the separation of enantiomeric mixtures by micellar electrokinetic capillary chromatography (MECC). The enantioselectivities (α) obtained for twelve typical pharmaceutical amines using the (S)-surfactant were compared to those obtained with (S)-N-dodecanoylvaline, a chiral surfactant described in the literature. Higher enantioselectivities were seen for ten of the twelve compounds using (S)-N-dodecoxycarbonylvaline. Furthermore, (S)-N-dodecoxycarbonylvaline had significantly less background absorbance in the low UV. It is shown that *exact* enantiomer migration order reversal can be obtained by individually employing both enantiomeric forms of the surfactant. For ionizable compounds like the amines examined here, enantioselectivity can be optimized by changing the pH of the MECC buffer. Partitioning is optimized through surfactant concentration, organic additives and pH. The ability to achieve fast chiral separations is shown. A separation of ephedrine enantiomers in urine is shown, with the only sample preparation being filtration.

1. Introduction

An emerging trend in the pharmaceutical industry is the development of drugs as pure enantiomers (enantiopure drugs) rather than as racemic mixtures [1]. This change is due to the realization that the two enantiomers of a chiral compound can have widely different biological activities. The common analgesic, ibuprofen, now marketed by several firms as the racemic mixture, is a case in point. It has been determined that (S)-ibuprofen takes effect three times faster than (R)-ibuprofen. In other cases, the undesired enantiomer can lower the efficacy of the beneficial enantiomer, and in the worst case it can have adverse side effects. Regulatory

agencies worldwide have realized the potential benefits of enantiopure drugs and have published guidelines for the development of chiral drugs [2]. It is expected that many commercial racemic drugs will soon be available as the "pure" enantiomer. As a result, there exists a tremendous need for improved methods of determining optical purity. Since most "enantiopure" drugs have optical purity in excess of 95%, enantioselective chromatographic methods are required.

The use of LC chiral stationary phases is currently the predominant method for the separation of enantiomeric mixtures [3]. Pirkle-type, cyclodextrin, protein, cellulose and ligand-exchange chiral stationary phases are the most common. The 40–50 commercially available columns reflect the major problem associated with

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chiral LC. Specifically, because total plate numbers in chiral LC range from 1000–10 000, the minimum α value required to achieve baseline separation of an enantiomeric mixture is large. For example, with $N = 5000$ and $k = 1$, the minimum α required for baseline separation ($R_s = 1.5$) is 1.20. Since a given chiral phase only shows this α for a small number of compounds, it is typically necessary to screen several different types of chiral columns to achieve separation of a single enantiomeric mixture. Furthermore, simple changes in the mobile phase, such as concentration and type of modifiers, pH and ionic strength, often lead to unpredictable changes in enantioselectivity. Therefore, methods development in chiral LC is a tedious, empirical process.

Given a limit of 5000–10 000 plates, the goal in chiral LC has been to develop phases with higher α values. However, in view of the large number of chiral columns available, the goal of finding a single chiral phase which shows sufficient α for a broad range of compounds is likely to remain elusive. To achieve an improved situation whereby two to five chiral phases can separate the majority of chiral compounds, a separation format affording significantly higher efficiencies than LC is required.

Capillary electrophoresis (CE) is an attractive system for chiral separations because plate counts for small molecules are typically 100 000 or greater. In the example above, if $N = 100\,000$, with $k = 1$ and $R_s = 1.5$, the required α drops to 1.04. Therefore, a given chiral selectand will resolve a significantly higher percentage of enantiomeric mixtures in a CE versus an LC format. Furthermore, because the chiral selectand is dissolved in the CE buffer, several selectands and conditions can be automatically screened with a single CE instrument and capillary.

Many of the chiral separations by CE have employed natural products as the chiral selectand. These selectands include cyclodextrins [4–9] and proteins [10]. Cyclodextrin-containing electrolytes have successfully separated many different types of enantiomeric mixtures. However, both cyclodextrins and proteins are only available as the single enantiomer, making exact

enantiomer migration order reversal impossible. This ability is important to both improve quantitation of optical purity and to confirm if a resulting separation is a chiral one.

Micellar electrokinetic capillary chromatography (MECC), introduced by Terabe et al. [11] and recently reviewed [12], is a form of CE which has the ability to separate charged and uncharged compounds simultaneously. It is a very powerful separation method because high efficiencies are generally obtained (100 000 plates or more) and many parameters can be varied to optimize resolution.

Chiral CE separations have been achieved by MECC through the use of bile salts [13–15], which are naturally occurring surfactants. Only limited applications with bile salts have been reported so far. Like cyclodextrins and proteins, bile salts do not allow enantiomer migration order reversal. Furthermore, because of their low aggregation number, bile salts have only found use for hydrophobic analytes.

In 1989, Dobashi et al. [16,17] described the synthesis of (S)-N-dodecanoylvaline and its use to separate neutral enantiomeric mixtures by CE. Subsequently, Terabe and co-workers [18–20] performed enantiomeric separations in MECC with the same surfactant. In both cases, the analytes were neutral amino acid derivatives. Otsuka and Terabe [19] found that adding methanol and/or urea to the MECC buffer improved the peak shape of the phenylthiohydantoin-amino acids investigated. A mixed micellar system with sodium dodecyl sulfate was employed to increase both the migration-time window and analyte capacity factors [20].

In principle, a chiral MECC system using synthetic chiral surfactants offers several advantages as a chiral separation system. These advantages include high efficiency, the ability to exactly reverse enantiomer migration order, tolerance of complex sample matrices, and simultaneous chiral and non-chiral separations. Because of the high efficiencies of MECC, we believe that only a few chiral surfactants will be needed to separate the majority of chiral compounds. To demonstrate these potential benefits, we have begun a program to synthesize novel chiral surfactants

and test their ability to separate enantiomeric mixtures in an MECC mode. Here, we report the results obtained using one of these chiral surfactants, (*S*)- and (*R*)-N-dodecoxycarbonylvaline.

2. Experimental

All CE separations were performed on either a Quanta 4000 or 4000E CE system (Waters, Milford, MA, USA). Buffers were prepared with surfactant and disodium phosphate and/or disodium tetraborate. The pH was then adjusted with either sodium hydroxide or phosphoric acid (J.T. Baker). Prior to use, AccuSep (Waters) capillaries, 60 cm (52.5 cm effective length) × 50 μm I.D., were rinsed for 5 min with 0.5 M NaOH. Between injections, the capillaries were rinsed with 0.1 M NaOH (3 min) and buffer (3 min). Unless indicated separations were performed with UV detection at 214 nm and a voltage of +12 kV. Hydrostatic injection times ranged from 2 to 20 s. All buffers and samples were filtered through a 0.45-μm filter (Millipore, Bedford, MA, USA). Separations were performed at ambient temperature. Samples were prepared as 10 mg/ml stock solutions in methanol and then diluted to 0.1 mg/ml in buffer. The micelle migration time was measured with the hydrophobic compound sulconazole. Racemates and the individual enantiomers (when possible) were purchased from Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA).

2.1. Synthesis of (S)-N-dodecanoylvaline

(*S*)-N-Dodecanoylvaline was prepared according to the procedure of Miyagishi and Nishida [21] by adding dodecanoyl chloride (Aldrich) to a 1 M sodium hydroxide solution containing (*S*)-valine (Aldrich). The structure was confirmed by ^{1}H NMR spectroscopy and HPLC analysis showed the product to be pure ($\geq$ 95%). ^{1}H NMR (C^{2}H$_3$O^{2}H, 300 mHz) δ 0.90 (t, J = 6.7 Hz, 3H), 0.965 (d, J = 6.9 Hz, 3H), 0.973 (d, J = 6.9 Hz, 3H), 1.29 (bs, 16 H), 1.62 (m, J =

7.1 Hz, 2H), 2.15 (m, J = 6.8 Hz, 1H), 2.28 (t, J = 7.5 Hz, 2H), 4.32 (d, J = 5.8 Hz, 1H).

2.2. Synthesis of dodecyl chloroformate

Dodecyl chloroformate was prepared according to the procedure of Eckert and Forster [22] by reacting 1-dodecanol with triphosgene. The product was used without further purification. The structure was confirmed by ^{1}H NMR (C^{2}HCl$_3$, 300 MHz) δ 0.88 (t, J = 6.6 Hz, 3H), 1.26 (bs, 18H), 1.72 (m, J = 7.0 Hz, 2H), 4.31 (t, J = 6.7 Hz, 2H).

2.3. Synthesis of (S)- and (R)-N-dodecoxycarbonylvaline

The (*S*)- and (*R*)-isomers of N-dodecoxycarbonylvaline were synthesized according to the procedure of Miyagishi and Nishida [21] by adding dodecyl chloroformate to individual 1 M sodium hydroxide solutions containing (*S*)- and (*R*)-valine (Aldrich), respectively. The structure was confirmed by ^{1}H NMR spectroscopy and HPLC analysis showed the product to be pure ($\geq$ 99.5%). ^{1}H NMR (C^{2}H$_3$O^{2}H, 300 mHz) δ 0.09 (t, J = 7.0 Hz, 3H), 0.94 (d, J = 6.9 Hz, 3H), 0.98 (d, J = 6.9 Hz, 3H), 1.29 (bs, 18 H), 1.63 (m, J = 6.85 Hz, 2H), 2.14 (m, J = 6.5 Hz, 1H), 4.04 (m, 3H).

3. Results and discussion

The enantiomeric surfactants (*R*)- and (*S*)-N-dodecoxycarbonylvaline were readily soluble in typical MECC buffers (i.e. phosphate–borate and phosphate). At pH < 6.5, the surfactants precipitated from solution. Consequently, they were only used at pH $\geq$ 7.0 in this work. Tests showed the surfactants to be soluble at 200 mM. Since joule heating effects became severe (even at low voltages) above this concentration, an upper solubility limit was not determined.

MECC is a true chromatographic technique. Thus, chromatographic figures of merit such as capacity factor (k) and selectivity (α) can be used

to describe analyte interactions with the micelles. Enantioselectivity (α) is an important parameter which allows comparison of different chiral surfactants. Thus, accurate α measurements must be obtained.

In MECC, k is defined as [23]:

$$k = \frac{t_r - t_{aq}}{t_{aq}\left(1 - \dfrac{t_r}{t_{mc}}\right)} \qquad (1)$$

where t_r is the observed migration time of the solute, t_{mc} is the migration time of a solute which is completely partitioned into the micelle ($k = \infty$) and t_{aq} is the migration time of the solute if it does not interact with the micelle. We have substituted t_{aq}, or the time in the aqueous phase, for the more commonly used t_o, or electroosmotic flow time, to account for the fact that charged compounds will not migrate with the electroosmotic flow when in the aqueous phase.

For a neutral solute, t_{aq} is equal to the electroosmotic flow time (t_o) (usually obtained by injecting methanol or other organic solvent). In the case of charged solutes, t_{aq} is more difficult to measure. Khaledi and co-workers [24,25] have developed equations to determine the capacity factors of both anionic and cationic compounds in MECC. The important factor is that in capillary zone electrophoresis, cationic compounds will migrate before the electroosmotic flow marker, with an apparent mobility equal to the sum of the electroosmotic mobility and electrophoretic mobility:

$$\mu_{apparent} = \mu_{osmosis} + \mu_{electrophoretic} \qquad (2)$$

For a given buffer, the electroosmotic flow in the presence vs. the absence of micelles will be different. This difference is due to differences in viscosity and ionic strength. Thus, the strategy employed to calculate t_{aq} of the amines in this study involved two experiments. First, the *electrophoretic* mobilities of the compounds were measured in the same buffer (without the micelles) as used in MECC. This value was calculated by subtracting the electroosmotic mobility from the apparent mobility. Then, the electroosmotic mobility obtained in the MECC sepa-

ration of the compound was added to its electrophoretic mobility to obtain an apparent mobility in MECC as if there were no interaction with the micelles. The apparent mobility can be converted into a migration time to obtain t_{aq}. Since the two enantiomers of any compound will have the same electrophoretic mobility in the absence of a chiral selectand, they will have the same t_{aq} value as well. The major assumption of this approach is that the cationic compounds will not ion-pair with free surfactant molecules [24,25]. Experiments performed below the critical micelle concentration (CMC) of the surfactants indicated that ion-pairing was minimal.

The solute migration time, t_r, is obtained from the resulting electropherogram, while t_{mc} is obtained by injecting a very hydrophobic marker, such as Sudan III, and measuring the migration time [23]. Initially, Sudan III was used as the micelle marker. However, we found that the chiral compound sulconazole migrated at the same time as Sudan III at all surfactant concentrations examined. Because it was more soluble than Sudan III, sulconazole was used in most cases.

The separation of pseudoephedrine enantiomers using 50 mM (S)-N-dodecoxycarbonylvaline is shown in Fig. 1. The baseline disturbance at 8.650 min is the electroosmotic flow marker (methanol), and the peak at 46.550 min is the micelle marker (sulconazole). Pseudoephedrine is positively charged at this pH, and in
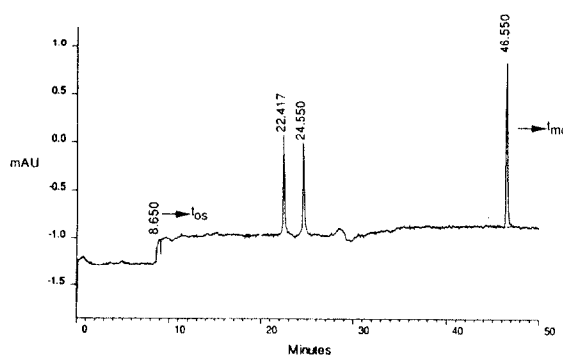

Fig. 1. Separation of pseudoephedrine enantiomers. Sample: 100 μg/ml racemic pseudoephedrine and 50 μg/ml sulconazole dissolved in buffer. Buffer: 50 mM (S)-N-dodecoxycarbonylvaline, 25 mM Na$_2$HPO$_4$–25 mM Na$_2$B$_4$O$_7$, pH 8.8.

free solution migrates before the electroosmotic flow marker. Thus, using the calculation described above, t_{aq} was assumed to be 6.389 min, its migration time in the absence of micelles. From this data and the migration time of the enantiomers, the capacity factor of the first enantiomer (k_1) was 4.84, and the capacity factor of the second enantiomer (k_2) was 6.01. The value of α (k_2/k_1) was 1.24. The average plate count for the two peaks was 104 000, and the resolution was 5.12.

The separation in Fig. 1 was obtained using a simple phosphate–borate buffer, with no urea or methanol required to obtain symmetrical peaks. Symmetrical peaks were also obtained using (S)-N-dodecanoylvaline in the same buffer system. We found that adding urea and/or methanol to the buffer did not improve peak shapes for the amines investigated. The migration-time window for a neutral solute in Fig. 1 (t_{aq}/t_{mc}) was 0.19. This result compares favorably with the value of 0.16 reported by Terabe [19] for a buffer containing (S)-N-dodecanoylvaline, sodium dodecyl sulfate, urea and methanol. In summary, we found that a simple MECC system led to excellent peak shape and resolution for the analytes tested.

One of the initial motivations for synthesizing (S)-N-dodecoxycarbonylvaline was to obtain a chiral surfactant which had lower UV absorbance than (S)-N-dodecanoylvaline. A 25 mM solution of each surfactant was prepared in 25 mM disodium phosphate–25 mM disodium tetraborate buffer. The pH was adjusted to 8.8 with either sodium hydroxide or phosphoric acid. These solutions were drawn into a 50 μm I.D. capillary, and the absorbance at 214 nm measured. The absorbance of (S)-N-dodecanoylvaline was 30 mAU while that of (S)-N-dodecoxycarbonylvaline was 12 mAU. Since the phosphate–borate buffer also had an absorbance of 12 mAU, it was concluded that (S)-N-dodecoxycarbonylvaline led to no additional absorbance at 214 nm. This property is very important at high surfactant concentrations, i.e. >100 mM, where high backgrounds decrease the effective linear dynamic range of the detector.

The difference in structure between (S)-N-

dodecanoylvaline and (S)-N-dodecoxycarbonylvaline is the replacement of an amide group in (S)-N-dodecanoylvaline with a carbamate group in (S)-N-dodecoxycarbonylvaline (see Fig. 2). Since the two groups are adjacent to the chiral center in the surfactant, we tested the differences in enantioselectivity (α) between the two surfactants using twelve pharmaceutical amines as test probes. The data are summarized in Table 1. Unexpectedly, there was a large difference in enantioselectivity between the two surfactants. Specifically, (S)-N-dodecoxycarbonylvaline afforded higher α values for ten compounds. A separation of norephedrine enantiomers using the two surfactants is shown in Fig. 3. Other structural variations also impact enantioselectivity, and these results will be reported shortly.

To determine the influence of pH on partitioning, experiments were conducted at pH 7.0 and 8.8 using 25 mM (S)-N-dodecoxycarbonylvaline. All the amines investigated showed higher partitioning at pH 7.0. For instance ketamine, pK_a 7.5, had a capacity factor of 12.5 at pH 7.0 versus 2.2 at pH 8.8. The twelve test compounds in Table 1 all contain one or more amine groups, most of which have pK_a values between 7 and 10. Thus at pH 7.0 they were more positively
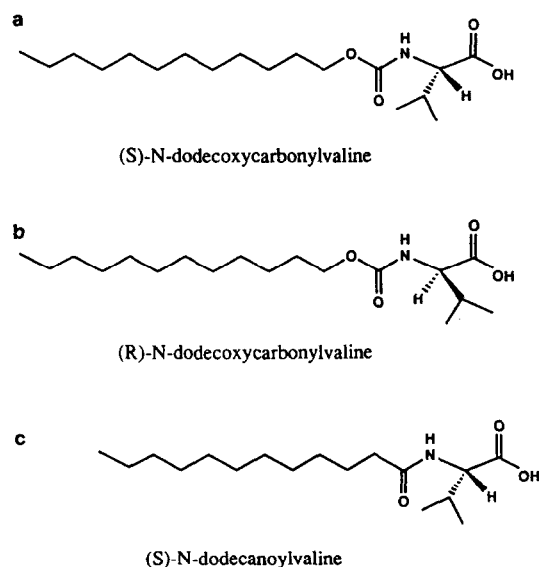
(S)-N-dodecoxycarbonylvaline

(R)-N-dodecoxycarbonylvaline

(S)-N-dodecanoylvaline

Fig. 2. Structures of the chiral surfactants.

Table 1
Comparison of enantioselectivities (α) obtained at pH 8.8 with 25 mM (S)-N-dodecoxycarbonylvaline vs. 25 mM (S)-N-dodecanoylvaline

Analyte	α	
	(S)-N-Dodecanoylvaline	(S)-N-Dodecoxycarbonylvaline
Atenolol	1	1.04
Bupivacaine	1.06	1.05
Ephedrine	1.05	1.10
Homatropine	1.02	1.03
Ketamine	1.05	1.01
Metoprolol	1.01	1.06
N-Methylpseudoephedrine	1.05	1.32
Norephedrine	1.04	1.10
Norphenylephrine	1.03	1.09
Octopamine	1	1.05
Pindolol	1.02	1.06
Terbutaline	1	1.01

Conditions: UV detection at 214 nm; 2-s hydrostatic injection; 60 cm × 50 μm capillary; + 12 kV; pH 8.8 buffer: 25 mM Na$_2$HPO$_4$–25 mM Na$_2$B$_4$O$_7$.

charged (as reflected in the difference in free solution mobilities between the two pH values). Since the micelles are anionic, it was not surprising that increased positive charge led to increased partitioning. Values of α were also measured for the twelve test compounds using (S)-N-

dodecoxycarbonylvaline at pH 7.0 and pH 8.8. The α values for nine compounds were higher at pH 7.0 (Table 2). For some compounds, such as bupivacaine, metoprolol and ketamine, enantioselectivity was significantly higher at pH 7.0. These results point to the importance of electro-
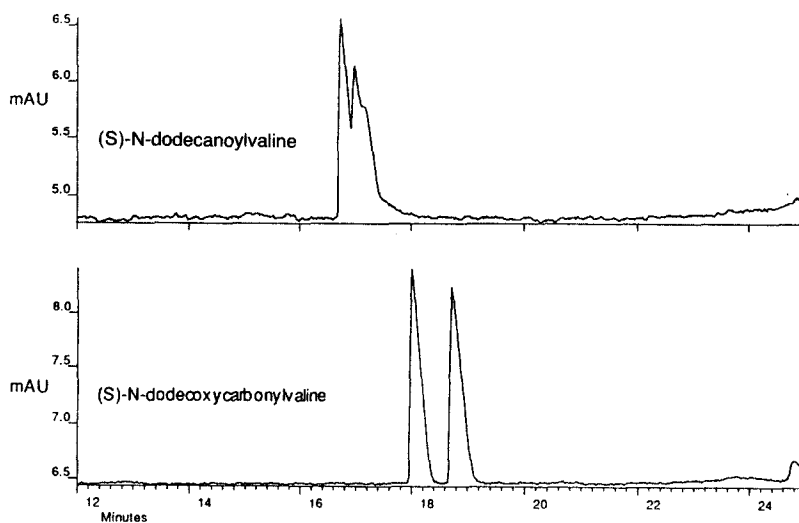

Fig. 3. Comparison of the separation of norephedrine enantiomers with (S)-N-dodecoxycarbonylvaline versus (S)-N-dodecanoylvaline. Sample: 100 μg/ml racemic norephedrine dissolved in buffer. Buffer: 25 mM (S)-N-dodecoxycarbonylvaline or (S)-N-dodecanoylvaline, 25 mM Na$_2$HPO$_4$–25 mM Na$_2$B$_4$O$_7$, pH 8.8.

Table 2
Comparison of enantioselectivities (α) obtained at pH 7.0 vs.
pH 8.8 with 25 mM (S)-N-dodecoxycarbonylvaline

Analyte	α	
	pH 7.0	pH 8.8
Atenolol	1.05	1.04
Bupivacaine	1.26	1.05
Ephedrine	1.14	1.10
Homatropine	1.03	1.03
Ketamine	1.06	1.01
Metoprolol	1.19	1.06
N-Methylpseudoephedrine	1.38	1.32
Norephedrine	1.12	1.10
Norphenylephrine	1.09	1.09
Octopamine	1.05	1.05
Pindolol	1.09	1.06
Terbutaline	1.02	1.01

Conditions: UV detection at 214 nm; 2-s hydrostatic injection; 60 cm × 50 μm capillary; + 12 kV; pH 7.0 buffer: 50 mM Na_2HPO_4; pH 8.8 buffer: 25 mM Na_2HPO_4–25 mM $Na_2B_4O_7$.

static interactions in determining enantioselectivity of the system.

Generally, we have found that under typical MECC operating conditions, (i.e. uncoated capillaries at pH ⩾ 7,) an α of 1.02, with sufficient optimization of k (see below), will allow baseline resolution. At pH 7, there was sufficient α on all but one of the compounds to allow baseline resolution, as opposed to three unresolved compounds at pH 8.8.

In MECC, k is related to surfactant concentration by the following equation [23]:

$$k = \frac{K\nu_s([\text{surf}] - \text{CMC})}{1 - \nu_s([\text{surf}] - \text{CMC})} \quad (3)$$

where K is the thermodynamic partition coefficient of the solute, ν_s is the partial molar volume of the surfactant, and CMC is the critical micelle concentration of the surfactant. As previously discussed [23], there is an optimum k value in MECC, which is determined by the ratio of t_{mc}/t_{aq} according to the following equation [26]:

$$k_{\text{optimum}} = \left(\frac{t_{mc}}{t_{aq}} \right)^{1/2} \quad (4)$$

where t_{mc} is the migration time of a solute completely partitioned into the micelle and t_{aq} is the migration time of the solute if there is no partitioning. (Note again that we have substituted t_{aq} for t_0.) Thus k, and hence resolution, can be optimized by changing the surfactant concentration.

Generally, 25 mM surfactant was first tested for separating an analyte. Then, if partitioning was too low, the surfactant concentration was increased. Fig. 4 shows the separation of atenolol enantiomers at surfactant concentrations of 25 and 100 mM. At 25 mM, k was 0.6 and resolution 0.8. Optimum k for these conditions was calculated to be 2.8. Thus, the surfactant concentration was increased to 100 mM; k increased to 2.5, thereby affording a resolution of 2.7.

An important aspect of the separations shown in Fig. 4 is that in both cases, α was 1.04 (while partitioning was significantly different). This result illustrates a large advantage of MECC over LC in methods development. The ability to optimize partitioning *independent of* α makes methods development much more straightforward in MECC. For a given column in LC, if partitioning is not optimum, the strength of the mobile phase must be altered. However, selectivity may change as well.

If partitioning is too high at 25 mM, lower surfactant concentrations may be employed.
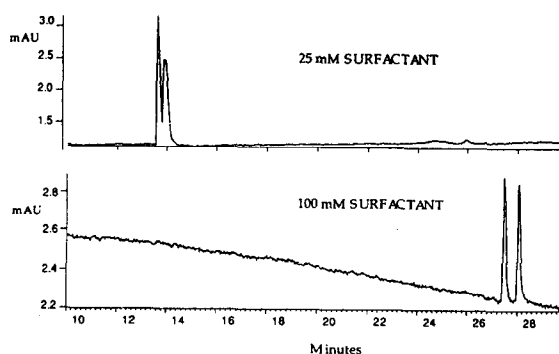

Fig. 4. Influence of surfactant concentration on the separation of atenolol enantiomers. Sample: 100 μg/ml racemic atenolol dissolved in buffer. Buffer: 25 or 100 mM (S)-N-dodecoxycarbonylvaline, 25 mM Na_2HPO_4–25 mM $Na_2B_4O_7$, pH 8.8.

However, the practical lower limit of surfactant concentration was 15 mM. Below this level, symmetrical peaks could not be obtained. In cases where k is still too large at 15 mM, organic solvent may be employed in order to decrease the analyte's thermodynamic partition coefficient. Fig. 5A and B show the separation of propranolol enantiomers at surfactant concentrations of 25 and 10 mM. At 25 mM, k was 50, and no resolution was apparent. At 10 mM, k decreased to 17, resulting in resolution. However, peak tailing and low plates were evident. At 25 mM surfactant with 30% acetonitrile, close to baseline resolution was obtained (Fig. 5C). Note that altering the analyte's thermodynamic partition coefficient by adding organic solvent, unlike changing the surfactant concentration, may change selectivity.

The ability to perform fast separations is one of the attractive features of CE. Analysis time is reduced by increasing the applied electric field.

However, there is a limit to how much the field can be increased before excessive Joule heating leads to band broadening and decreased resolution. To minimize the conductivity of the MECC buffer, the phosphate–borate buffer system at pH 8.8 was replaced with the zwitterionic buffer, 2-(N-cyclohexylamino)ethanesulfonic acid (CHES). This buffer permits the separation of N-methylpseudoephedrine enantiomers in less than 90 s using an electric field of 860 V/cm and a 30 cm capillary (Fig. 6).

The ability to exactly invert the chirality of the system is an important feature of any chiral separation system. To this end, (S)- and (R)-N-dodecoxycarbonylvaline (Fig. 2a and b) were used to separate a 3:1 ratio of (S):(R) benzoin. As expected, the migration order of the two enantiomers was reversed (Fig. 7). When the (R)-surfactant was employed, the (R)-enantiomer of benzoin migrated first (top separation), while with the (S)-surfactant, the (S)-enantiomer
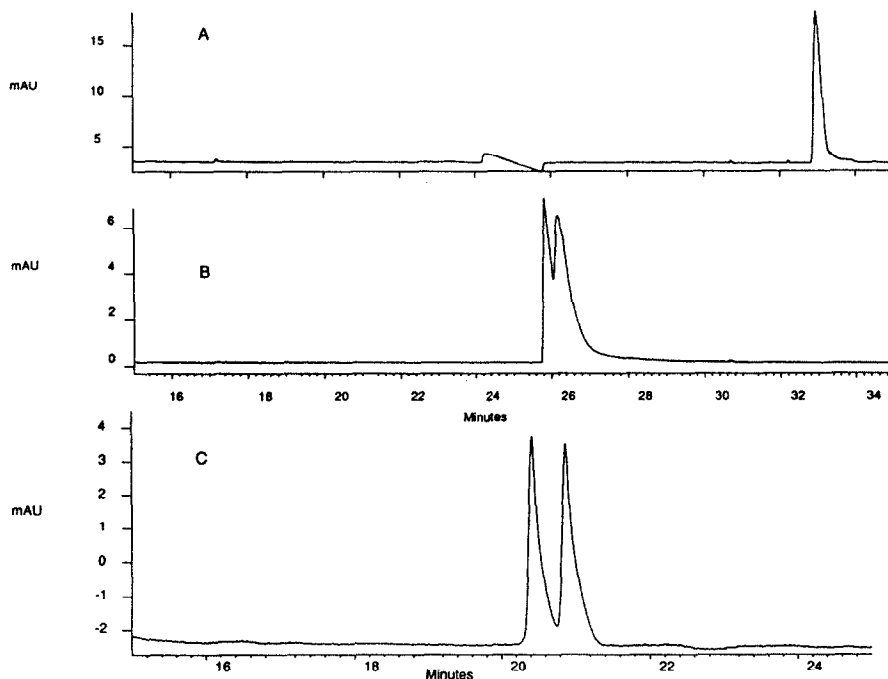

Fig. 5. Influence of surfactant concentration and acetonitrile on the separation of propranolol enantiomers. Sample: 100 μg/ml racemic propranolol dissolved in buffer. Buffers: (A) 25 mM (S)-N-dodecoxycarbonylvaline, 25 mM Na$_2$HPO$_4$–25 mM Na$_2$B$_4$O$_7$, pH 8.8; (B) 10 mM (S)-N-dodecoxycarbonylvaline, 25 mM Na$_2$HPO$_4$–25 mM Na$_2$B$_4$O$_7$, pH 8.8; (C) 25 mM (S)-N-dodecoxy-carbonylvaline, 25 mM Na$_2$HPO$_4$–25 mM Na$_2$B$_4$O$_7$, pH 8.8, 30% acetonitrile.
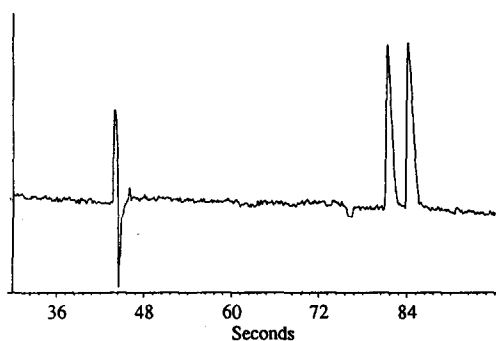
Fig. 6. Fast separation of N-methylpseudoepherine enantiomers. Sample: 100 μg/ml racemic N-methylpseudoephedrine dissolved in buffer. Buffer: 25 mM (S)-N-dodecoxycarbonylvaline, 50 mM CHES, pH 8.8. Capillary: 35 cm × 50 μm I.D. Voltage: + 30 kV.

of benzoin migrated first. These two separations were performed sequentially on the same capillary. The α value was 1.05 and efficiencies were greater than 100 000 for both peaks.

In Fig. 7, the migration times of the first and second peaks of each separation are the same (2% difference). Migration order inversion of this nature can only be realized when the two enantiomers of the chiral selectand are employed separately and all other parameters kept constant. The migration order of enantiomers can
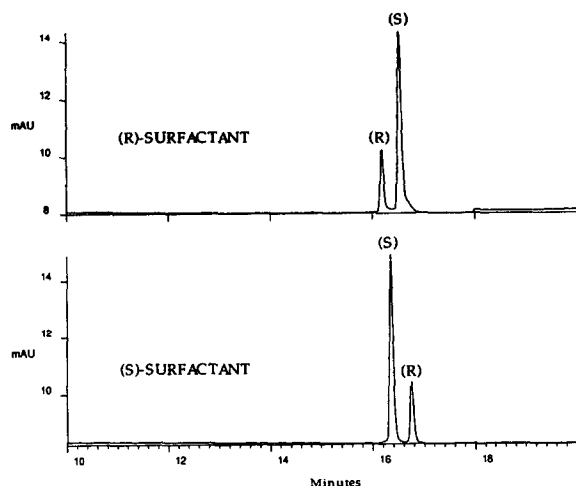
sometimes be reversed with cyclodextrins in CE, but it requires the development of a new set of separation conditions. Thus, the amount of methods development time is significantly increased without the guarantee of enantiomer migration order reversal.

An experiment was performed in which the total concentration of surfactant was constant (25 mM) but different ratios of (R)-:(S)-N-dodecoxycarbonylvaline were used to separate an analyte with a large α value (N-methylpseudoephedrine, α = 1.30). As seen in Fig. 8, α changed linearly with the % (R)-N-dodecoxycarbonylvaline. At 100% (R)- and 0% (R)-surfactant, α was 1.30, although the migration order of the two enantiomers was reversed. At 50% (R)-surfactant, no enantioselectivity was seen. At intermediate percentages, the resulting α was linear with % (R)-surfactant. These data suggest that the chiral recognition process involves one surfactant molecule interacting with one analyte molecule. Otherwise the α data might not be linear with surfactant optical purity. We are investigating the mixing of other chiral surfactants to improve our understanding of the chiral recognition process.

MECC has been used to perform separations of complex sample matrices such as urine, because micelles can solubilize proteinaceous
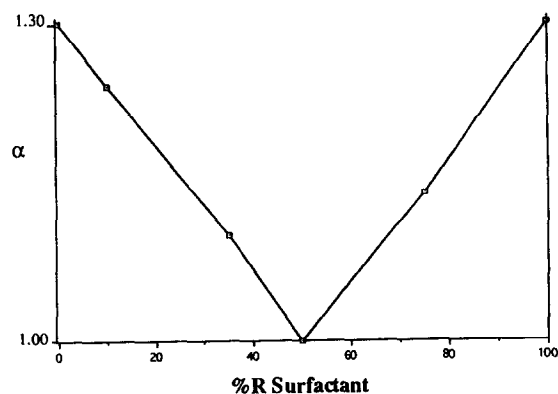

Fig. 7. Migration order reversal of benzoin enantiomers. Sample: 3:1 ratio of (S)- to (R)-benzoin dissolved in buffer. Buffer: 25 mM (R)- or (S)-N-dodecoxycarbonylvaline, 25 mM Na$_2$HPO$_4$–25 mM Na$_2$B$_4$O$_7$, pH 8.8.

Fig. 8. Graph of enantioselectivity vs. % (R)-N-dodecoxycarbonylvaline. Sample: 100 μg/ml racemic N-methylpseudoephedrine dissolved in buffer. Buffer: 25 mM N-dodecoxycarbonylvaline, different ratios of (R):(S), 25 mM Na$_2$HPO$_4$–25 mM Na$_2$B$_4$O$_7$, pH 8.8.
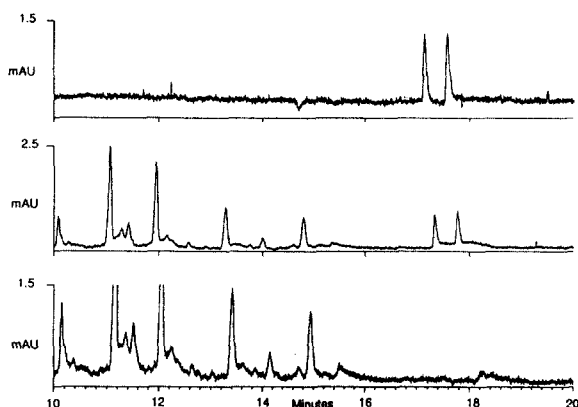
Fig. 9. Separation of ephedrine enantiomers spiked into urine. Samples: 100 μg/ml racemic ephedrine in buffer (top), 100 μg/ml racemic ephedrine in urine (middle), blank urine (bottom). Buffer: 50 mM (S)-N-dodecoxycarbonylvaline, 25 mM Na_2HPO_4–25 mM $Na_2B_4O_7$, pH 8.8.

material [27,28]. Fig. 9 shows the separation of 100 μg/ml racemic ephedrine spiked into urine using 50 mM (S)-N-dodecoxycarbonylvaline. At 25 mM surfactant, one of the ephedrine enantiomers co-migrated with a urine matrix peak. Increasing the surfactant concentration to 50 mM resolved the ephedrine enantiomer from the urine matrix peak. The only sample preparation in this case was a simple filtration step. This separation is an excellent example of the ability to perform non-chiral and chiral separations simultaneously in MECC with synthetic chiral surfactants.

4. Conclusions

MECC using synthetic chiral surfactants shows great promise as a solution to many chiral separation problems. The chiral surfactant described here can be prepared as both enantiomers, (S)- and (R)-N-dodecoxycarbonylvaline, allowing *exact* enantiomer migration order reversal. Enantioselectivity data obtained when different ratios of the (R)- and (S)-surfactants were mixed suggested that the chiral recognition process was based on one surfactant molecule recognizing one analyte molecule. For twelve pharmaceutical amines investigated, (S)-N-dodecoxycar-

bonylvaline was able to exceed a resolution objective of 1.5 for eleven. Resolution was maximized through optimization of surfactant concentration. For charged compounds, like the amines examined here, pH could be used to alter enantioselectivity and partitioning. Rapid chiral separations could be obtained by using zwitterionic buffers and high electric fields. Chiral separations in complex matrices could also be performed, with non-chiral and chiral separations occurring simultaneously. Future communications will report the performance of additional chiral surfactants as well as separation of additional compound classes.

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