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Polymeric alkenoxy amino acid surfactants: II. Chiral separations of β -blockers with multiple stereogenic centers

Two amino acid-based (leucine and isoleucine) alkenoxy micelle polymers were employed in this study for the separation of multichiral center-bearing β -blockers, nadolol and labetalol. These polymers include polysodium *N*-undecenoxy carbonyl-L-leucinate (poly-L-SUCL) and polysodium *N*-undecenoxy carbonyl-L-isoleucinate (poly-L-SUCIL). Detailed synthesis and characterization were reported in our previous paper [26]. It was found that poly-L-SUCIL gives better chiral separation than poly-L-SUCL for both nadolol and labetalol isomers. The use of 50–100 mM poly-L-SUCIL as a single chiral selector provided separation of four and three isomers of labetalol and nadolol, respectively. Further optimization in separation of both enantiomeric pairs of nadolol and labetalol was achieved by evaluation of type and concentration of organic solvents, capillary temperature as well type and concentration of cyclodextrins. A synergistic approach, using a combination of poly-L-SUCIL and sulfated β -CD (S- β -CD) was evaluated and it showed dramatic separation for enantiomeric pairs of nadolol. On the other hand for labetalol enantiomers, separation was slightly decreased or remain unaffected using the dual chiral selector system. Finally, simultaneous separation of both nadolol and labetalol enantiomers was achieved in a single run using 25 mM poly-L-SUCIL and 5% w/v of S- β -CD in less than 35 min highlighting the importance of high-throughput chiral analysis.

Keywords: Cyclodextrin derivative / Enantiomer / Labetalol / Micellar electrokinetic chromatography / Micelle polymer / Nadolol / Organic modifier
DOI 10.1002/elps.200305762

1 Introduction

In the last decade, enantiomeric separation has become important for biomedical, environmental, agricultural and pharmaceutical research, because a large fraction of many thousand drugs in the market are chiral [1–4]. The annual sale of the world market for chiral drugs now exceed \$100 billion and is anticipated to increase at a good pace in this millennium [5]. Capillary electrophoresis (CE) has emerged as a versatile method for chiral analysis, due to high efficiency, high selectivity and low cost. Besides, the often-used neutral or charged cyclodextrins (CDs), the use of micelles as chiral selector in micellar electrokinetic chromatography (MEKC) has extended the

range of applicability of this technique for chiral analysis [6–9]. In particular, a significant number of studies in recent years have been reported regarding the use of chiral polymeric surfactants (also called molecular micelles or micelle polymers) for separation in chiral MEKC [10–23]. Several key advantages of polymeric surfactant over conventional micelles are noted: (i) due to zero critical micelle concentration (CMC) the chiral selector can be employed at very low molar concentration (e.g., much below the CMC of the monomer) [24]; (ii) the covalent linkage of hydrocarbon tail and concentration of chiral pseudophase is fixed and does not change with changes in pH, background electrolyte (BGE) and organic solvents; (iii) elution order of the enantiomers can be reversed on the fly (e.g., by simply changing the optical configuration of the polymeric surfactant). This last point is unique to polymeric surfactant compared to naturally occurring chiral selector employed in CE since the use of the former chiral selector conveniently reverse the migration order of two enantiomers to determine trace-level impurities and to confirm the identity of enantiomeric pair.

Molecular micelles of polyacylamino acid were first used by Wang and Warner [24] for chiral separation in MEKC. Very recently, the use of dipeptide molecular micelles has further

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Abbreviations: DM- β -CD, dimethyl- β -cyclodextrin; HP- β -CD, hydroxypropyl- β -cyclodextrin; HP- γ -CD, hydroxypropyl- γ -cyclodextrin; L-SUCIL, sodium *N*-undecenoxy carbonyl-L-isoleucinate; L-SUCL, sodium *N*-undecenoxy carbonyl-L-leucinate; S- β -CD, sulphated β -cyclodextrin; TEA, triethylamine; TM- β -CD, trimethyl- β -cyclodextrin

expanded the applicability of this class of surfactant for the separation of a large number of enantiomeric compounds [25]. Recently, our research group introduced a new class of molecular micelles based on alkenoxy amino acid or carbamate chemistry [26]. Two derivatives of polyalkenoxy amino acid or carbamate polymers (polysodium *N*-undecenoxy carbonyl-L-leucinate (poly-L-SUCL), polysodium *N*-undecenoxy carbonyl-L-isoleucinate (poly-L-SUCIL)) were synthesized (see Fig. 1), characterized and their performance was compared for simultaneous separation of eight β -blockers bearing a single chiral center.

Among chiral drugs, β -blockers are one of the best-understood drugs known for their stereochemical impact on pharmacodynamics and pharmacokinetics. Most of the β -blockers bear a single chiral center. However, there are several multichiral-center β -blockers that are currently used in research as well as in clinical laboratory. Two such examples of β -blockers that possess several chiral centers are labetalol and nadolol (see Fig. 2 for structure) [27]. Labetalol, 2-hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl] benzamide is a type of therapeutic β -blocker with combined β - and α -receptor blocking properties. However, β -blocking activity is dominant. For labetalol, there are two asymmetric carbons resulting in four stereoisomers (*R,R*), (*S,S*), (*R,S*) and (*S,R*). Although the (*R,R*)-labetalol shows predominant β -adre-

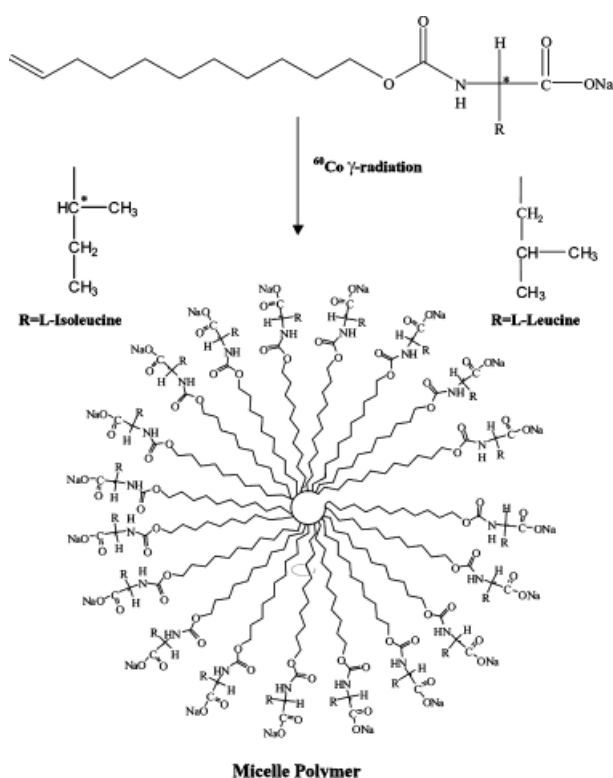


Figure 1. Structure of monomer and micelle polymer of alkenoxy surfactants.

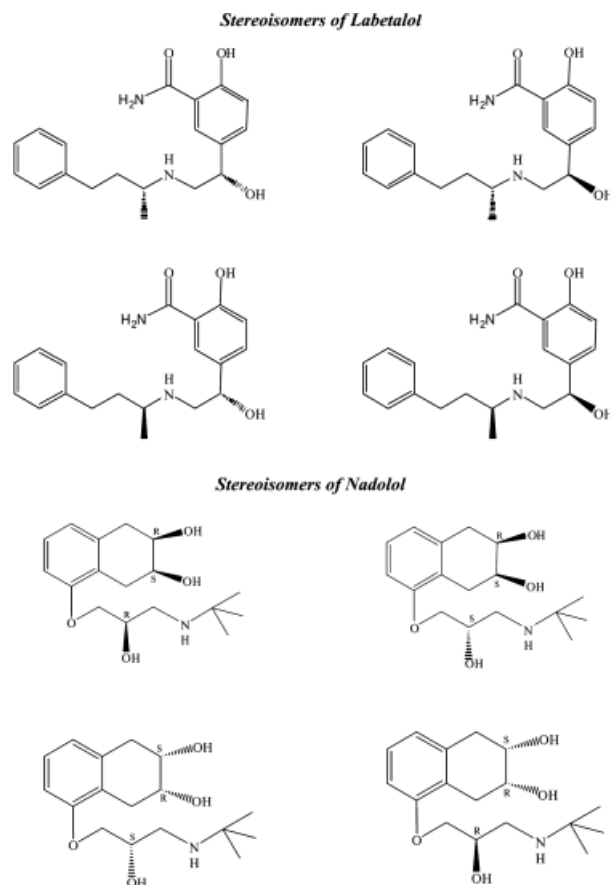


Figure 2. Structure of the stereoisomers of β -blockers, (\pm)-labetalol and (\pm)-nadolol.

nergic activity, the (*S,R*)-labetalol is most effective as a α -adrenergic blocker. In contrast, (*R,S*)- and (*S,S*)-labetalol possess only moderate pharmacological activity against α - and β -receptors. Similar to labetalol, nadolol, 5-[[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydro-*cis*-2,3-naphthalenediol] is a nonselective β -blocker, which also contains four stereoisomers (*RSR*, *SRS*, *RRS*, *SSR*). Nadolol is extensively employed in the treatment of hypertension and angina pectoris. A racemate mixture of (*RSR*)-nadolol and (*SRS*)-nadolol is considered more potent than the racemate mixture of (*SSR*)-nadolol and (*RRS*)-nadolol [28–30]. Since nadolol has three stereogenic centers it should result in eight possible stereoisomers. However, only four stereoisomers are possible because the two adjacent hydroxyl groups on the cyclohexane ring are “conformationally locked” in the *cis*-form due to the attachment with the flat benzene ring.

Multichiral-center drugs possess special challenge for separation, due to the complex structure of these analytes [31]. The separations of nadolol and labetalol have been attempted *via* high-performance liquid chromatog-

raphy (HPLC) using native CD or derivatized CD columns [29], or in supercritical fluid chromatography (SFC) using α_1 -acid glycoprotein column [30]. Although, the recent analytical applications of vancomycin column in HPLC for separation of these multichiral-center β -blockers seems promising [32]. In general, the separation of these analytes on the analytical scale is still a challenge. Compared to other separation techniques, CE is a more suitable technique for the analytical-scale separation of compounds with multiple stereogenic centers. For example, recent application of hepta-6-sulfate- β -CD as chiral selector in acidic and neutral BGE has demonstrated CE a viable technique for separation of four isomers of both labetalol [28, 29] and nadolol [28]. In this study, we report the chiral recognition of two alkenoxy amino acid micelle polymers, poly-L-SUCL and poly-L-SUCIL in MEKC for separation of multichiral-center β -blockers, labetalol and nadolol. In order to achieve the separation of all four stereoisomers of labetalol and nadolol, the influence of micelle polymer concentration, temperature, organic solvents, and types of CDs with and without micelle polymers have been studied. To the best of our knowledge, this is first report in which the application of polymeric surfactant with or without the use of CD has been explored for separation of multichiral-center compounds.

2 Materials and methods

2.1 Reagents and chemicals

The analytes nadolol and labetalol were obtained as mixture of four stereoisomers from Sigma (St. Louis, MO, USA). The BGE (2-(*N*-cyclohexylamino)ethanesulfonic acid; CHES) was of analytical reagent grade and was obtained from Fluka (Milwaukee, WI, USA). The β - and γ -CDs, sulfated β -CD (S- β -CD), hydroxypropyl- γ -CD (HP- γ -CD), hydroxypropyl- β -CD (HP- β -CD), heptakis(2,6-di-*O*-methyl)- β -CD (DM- β -CD), and heptakis(2,3,6-tri-*O*-methyl)- β -CD (TM- β -CD) were obtained from Sigma. Chemicals used for the synthesis of surfactants sodium *N*-undecenoxycarbonyl-L-leucinate (L-SUCL) and sodium *N*-undecenoxycarbonyl-L-isoleucinate (L-SUCIL) included: ω -undecylenyl alcohol, triphosgene, pyridine, dichloromethane, L-leucine, and L-isoleucine, all obtained from Aldrich (Milwaukee, WI, USA) and used as received. The complete synthesis, characterization and solution behavior studies of both L-SUCL and L-SUCIL and the corresponding polymeric surfactants (poly-L-SUCL and L-SUCIL) have been described elsewhere [26]. The lower limit of pH where both the monomers and polymers of L-SUCL and L-SUCIL were soluble in buffers was ca. 6. However, above pH 6 the surfactants were soluble up to any pH. The surfactants purity was confirmed by

¹H-NMR as mentioned in [26]. The LC-ESI-MS in scan mode of both acidic and sodium salt of L-UCL and L-UCIL provide molecular ion peaks at 327 *m/z* (acid form) and 349 *m/z* (salt form), respectively. Thus confirming the structure and identity of the synthesized surfactants.

2.2 Apparatus

Chiral separations were performed using an Agilent CE system (Agilent Technologies, Palo Alto, CA, USA). The instrument is equipped with a 0–30 kV high-voltage power supply, a diode array detector for UV detection and Chemstation software for system control and data acquisition. The polyimide-coated fused-silica capillaries of 50 μ m ID and 150 μ m OD were obtained from Polymicro Technologies (Phoenix, AZ, USA). The capillary with 64.5 cm total length (56.0 cm from inlet to the detector) was prepared by burning about 3 mm polyimide to create a detection window. Since the best signal-to-noise ratio was obtained at 214 nm, this wavelength was used throughout the study.

2.3 Preparation of BGE and analyte solution

For all chiral CE experiments, the final BGE consisted of a 100 mM CHES buffered at pH 8.8 and 10 mM triethylamine (TEA) [33]. The desired pH value was obtained by using 1 M NaOH. The pH of BGE was adjusted before addition of any chiral selector or organic solvent. This BGE solution is finally filtered through a 0.45 μ m Nalgene syringe filter (Rochester, NY, USA). The running CE buffer solution was prepared by addition of each chiral selector (poly-L-SUCL, poly-L-SUCIL, native or derivitized CDs, S- β -CD, mixture of CD, and polymeric surfactants) with or without organic solvents (% w/v) to the BGE followed by ultrasonication for about 15–20 min. The diastereomeric solutions of nadolol and labetalol were prepared by dissolving in 50/50% v/v of methanol and water.

2.4 CE procedures

A new capillary was first conditioned for 1 h with 1 N NaOH at 50°C, followed by a 30 min rinse with triply deionized water. The capillary was preconditioned with the running buffer for 5 min before each run. Both nadolol and labetalol were injected for 1 s at 30 mbar pressure. All separations were performed at +20 kV and at 25°C unless otherwise mentioned. The chiral resolution (R_s) between four stereoisomers of labetalol and nadolol were calculated by the Agilent Chemstation software.

3 Results and discussion

Based on our previous results, 100 mM CHES buffered at pH 8.8 and 10 mM TEA was used for the separations [26, 33]. Optimization of the separation of each of the four stereoisomers of labetalol and nadolol was achieved by evaluating the parameters such as concentration of the polymeric surfactant (poly-L-SUCL and poly-L-SUCIL), organic solvents, temperature, types and concentrations of CD, as well as combination of CD and polymeric surfactants. The effects of all of these aforementioned parameters are discussed below.

3.1 Effect of the surfactant concentration

Table 1 shows the resolution of the stereoisomers of the studied β -blockers (nadolol (N1–N4) and labetalol (L1–L4)) at four different poly-L-SUCL and poly-L-SUCIL concentrations (25, 50, 75, and 100 mM) at optimum pH value of 8.8 [26, 33]. Several general trends are apparent from the data shown in Table 1. (i) At each equivalent molar concentration, poly-L-SUCIL always provided better resolution between each enantiomers and diastereomers of labetalol and nadolol. (ii) Resolution between the first pair of labetalol enantiomers (L1/L2) or the second pair of labetalol enantiomers (L3/L4) increased upon increasing poly-L-SUCIL concentration up to 50 mM, while the same pairs are unresolved at concentration > 25 mM poly-L-SUCL. (iii) The resolution between diastereomers of labetalol (L2/L3) continues to improve up to at least 75 mM and 50 mM poly-L-SUCIL and poly-L-SUCL, respectively. (iv) For nadolol the resolution between diastereomers (N2/N3) increases up to 50 mM poly-L-SUCIL and then at concentration > 50 mM it stays unchanged or decreased. However, using poly-L-SUCL the resolution between diastereomers of nadolol increases upon increasing concentration up to 100 mM. In addition, no resolution of either enantiomeric pair of nadolol (N1/N2 and N3/N4) was

observed at any concentration of poly-L-SUCL. On the other hand, using 100 mM poly-L-SUCIL the second enantiomeric pair of nadolol (N3/N4) showed slight resolution. This result shown in Table 1 is opposite to what we have observed previously for single chiral center bearing β -blockers, where poly-L-SUCL showed better resolution than poly-L-SUCIL for single chiral center β -blocker [26]. The improved resolution obtained for labetalol and nadolol using the latter polymeric surfactant can be attributed to the fact that these β -blockers bear multiple chiral centers, and thus can have multiple interactions with the two chiral centers of the poly-L-SUCIL.

3.2 Effect of type of organic solvents

In general, organic solvents when used as a mixture with water, influence the electroosmotic flow (EOF) and effective mobility of the analyte due to the change in polarity, and the viscosity of the bulk electrolyte. In addition, organic solvents may increase the solubility and improve the peak shape because of stacking of the analytes and decrease partitioning between the solutes and the pseudostationary phase. Hence, organic solvents are favorably applied to optimize the separations in MEKC [34]. An increase in migration time for stereoisomers of labetalol was observed (data not shown) upon addition of different types of organic solvent in MEKC buffer. It was observed that addition of an alcohol to the MEKC buffer is not an effective way to increase the chiral R_s between the first pair of labetalol enantiomers (L1/L2). However, at least the use of methanol and butanol slightly improve the separation of the second pair of labetalol enantiomers (L3/L4). In addition, the R_s between the diastereomers (L2/L3) improved in all cases. The same approach was adopted for nadolol, but no improvement in separation of its stereoisomers was observed (data not shown).

Table 1. Effect of poly-L-SUCL and poly-L-SUCIL concentration on the chiral resolution of labetalol and nadolol^{a)}

Chiral analytes	Enantiomers	Poly-SUCIL concentration (mM)				Poly-SUCL concentration (mM)			
		25	50	75	100	25	50	75	100
Labetalol	L1/L2	0.36	0.37 ^{b)}	–	–	0.29	–	–	–
	L2/L3	1.16	1.27	1.38	1.16	0.52	1.05	1.05	1.10
	L3/L4	1.13	1.24	0.84	0.55	0.39	–	–	–
Nadolol	N1/N2	–	–	–	–	–	–	–	–
	N2/N3	0.57	2.08	2.01	1.54	0.34	1.57	1.85	2.01
	N3/N4	–	–	–	0.29 ^{c)}	–	–	–	–

a) Applied voltage, +20 kV; 25°C; sample concentration, 0.5 mg/mL; sample injection, pressure 50 mbar, 2 s; UV detection at 214 nm

b) Optimum resolution of both of the enantiomers observed and this leads to select this concentration of the polymeric surfactant for the further separation condition optimization

c) Very slight resolution was observed for the second enantiomer (N3/N4) of nadolol.

– No resolution was observed.

3.3 Effect of temperature

In chiral micellar electrokinetic chromatography (CMEKC) temperature is often regarded as a parameter that has an effect on resolution, selectivity and efficiency. In every instance, temperature increase will yield faster separation but decrease resolution. However, there may exist an isoenantioselective temperature (T_{isoenant}), depending on both the type of chiral analyte and chiral surfactant. For example, Billiot and Warner [35] recently showed that the presence of T_{isoenant} for (\pm)-1,1'-bainaphthyl phosphate using a chiral micelle polymer as a chiral selector. In our studies, the effect of temperature on separation was investigated in the temperature range of 25°C–11.6°C. However, this temperature optimization could only culminate for enantiomeric pairs of labetalol (Fig. 3), which showed maximum resolution for all four stereoisomers at 11.6°C. In contrast, resolution of the first enantiomeric pair of nadolol (N1/N2) remains unaffected, while only slight increase in R_s of the second enantiomeric pair of nadolol (N3/N4) was observed at 15°C (data not shown). Furthermore, it has been observed that lowering the temperature deteriorated the separation between the diastereomers of nadolol (N2 and N3).

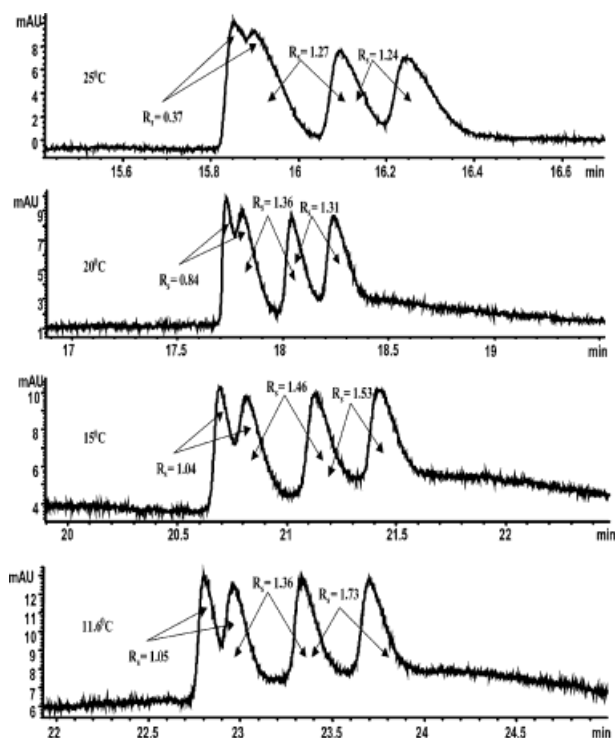


Figure 3. Effect of temperature on resolution of the enantiomeric pairs of labetalol. MEKC condition: 50 mM poly-L-SUCIL, 100 mM CHES/10 mM TEA, pH 8.8. Pressure injection, 30 mbar for 1 s; +20 kV applied for separations; UV detection at 214 nm.

3.4 Effect of concentration and type of native and derivatized CDs

The addition of CD into the micellar solution alters the partitioning of the solute between the micellar phase and the CD phase. It is well known that a variety of neutral and charged organic and inorganic molecules form highly selective molecular inclusion complexes with CDs. Since the first pair of enantiomers of nadolol (N1/N2) did not show any chiral resolution after the temperature variation studies, a combination of polymeric surfactant and different concentrations of CD as well as type of CD was explored. Several different concentrations of β -CD were employed in combination with 100 mM poly-L-SUCIL. Figure 4 shows the effect of concentration of β -CD on separation of nadolol stereoisomers. When only β -CD is used, no resolution was observed for any of the enantiomeric pairs of nadolol. However, upon addition of 100 mM poly-L-SUCIL to 0.1% β -CD, three stereoisomers of nadolol were partially resolved. Furthermore, the resolution increases gradually for each pairs N1/N3 and N3/N4 in the electropherogram, whereas this is not the case for peak pair N1/N2, which remains unresolved at all concentrations of β -CD. Several attempts were made to improve the separation of the enantiomeric pair (N1/N2) of nadolol using different types of CD (DM- β -CD, TM- β -CD, HP- β -CD, HP- γ -CD, β -CD and γ -CD) in combination with

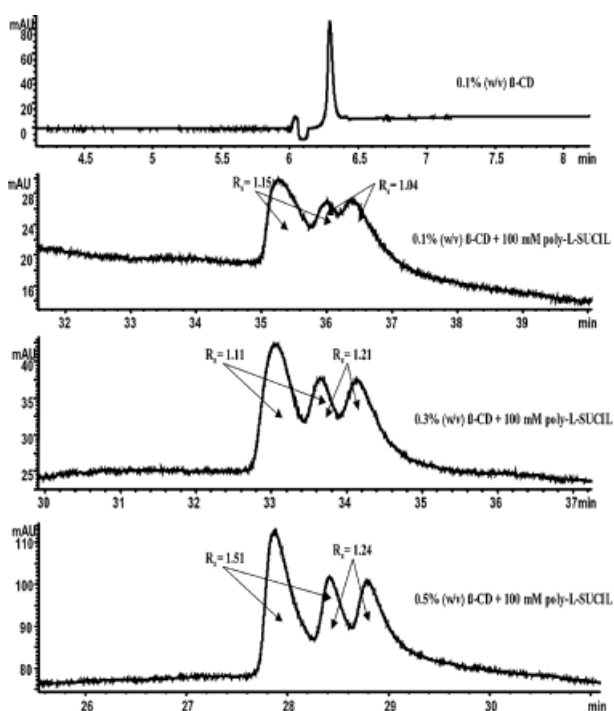


Figure 4. Effect of β -CD concentration (% w/v) in combination with 100 mM poly-L-SUCIL on the resolution of enantiomeric pairs of nadolol. MEKC conditions are same as Fig. 3, except 100 mM poly-SUCIL.

100 mM poly-L-SUCIL (data not shown). Only γ -CD showed any significant improvement in resolution of the second pair of nadolol enantiomers (N3/N4), while none of the native or derivitized CDs employed could resolve the first enantiomeric pair of nadolol (N1/N2).

3.5 Effect of charged CDs and synergism

Up to this point, using single-chiral selectors (polymeric surfactant, native or derivitized CD) or dual-chiral selector (combination of polymeric surfactant with native or derivitized CDs) could not show any remarkable result for nadolol isomers. Encouraged by the previous reports on the successful use of S- β -CD for the separation of nadolol and labetalol stereoisomers [28–30], we employed S- β -CD in combination with poly-L-SUCIL. In Fig. 5, the resolution of both of the enantiomeric pairs of nadolol is compared using either poly-L-SUCIL (Fig. 5a) or S- β -CD (Fig. 5b) or a combination of these two (Fig. 5c). The application of 25 mM poly-L-SUCIL alone does not show any separation of the critical enantiomeric pair (N1/N2) of nadolol, while the use of 5% w/v S- β -CD (under optimum condition) showed enhanced resolution for the second pair of enantiomers. A similar synergistic approach on

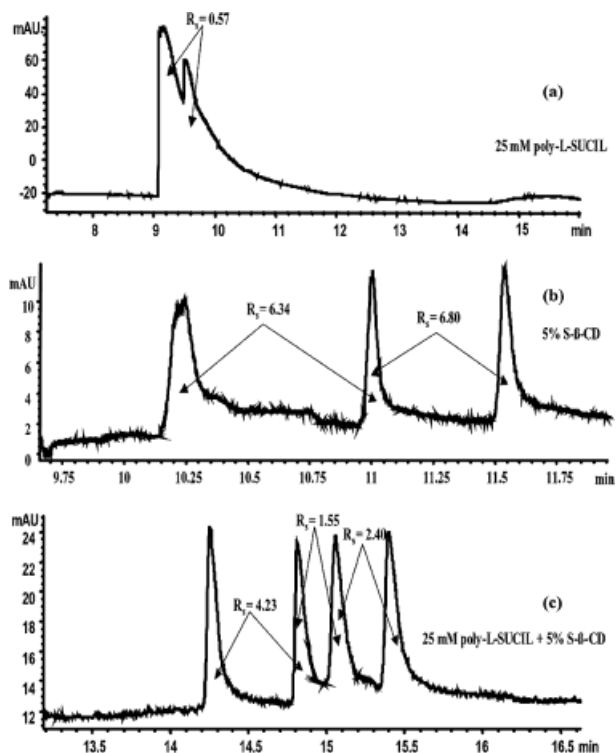


Figure 5. Effect of 5% w/v S- β -CD in combination with poly-SUCIL on the diastereomeric resolution of the enantiomeric pairs of nadolol. MEKC conditions are same as Fig. 3, except 25 mM poly-SUCIL and injection for 30 min bar for 2 s.

the use of polymeric surfactant and S- β -CD was also explored for the resolution of four isomers of labetalol (Fig. 6). As expected, the combination of two high-mobility chiral selectors (poly-L-SUCIL and S- β -CD) increases the migration time of all four isomers of labetalol, but resolution of the first enantiomeric pair (L1/L2) declined significantly, while only slight decrease in R_s was observed for the second enantiomeric pair (L3/L4). In general, the use of anionic CD in combination with anionic surfactant should not enhance the separation owing to unidirectional mobility of the two anionic chiral selectors. Apparently, the combined use of two chiral selectors results in increase partitioning of analytes (nadolol and labetalol) between S- β -CD and poly-L-SUCIL, consequently the retention time in CD-modified MEKC (CD-MEKC) is higher than either only CD or MEKC approach for both nadolol and labetalol. However, this increase in retention does not improve the R_s of labetalol as it did for nadolol. This is probably due to the fact that the value of migration time for nadolol is in the range of optimum capacity factor [36]. The simultaneous and enantioseparation of both nadolol and labetalol isomers could be conveniently achieved in high-throughput fashion. Therefore, using a combination of 25 mM poly-L-SUCIL and 5% w/v S- β -CD simultaneously, an enantioseparation of eight β -blockers was obtained with high resolutions within 35 min (Fig. 7).

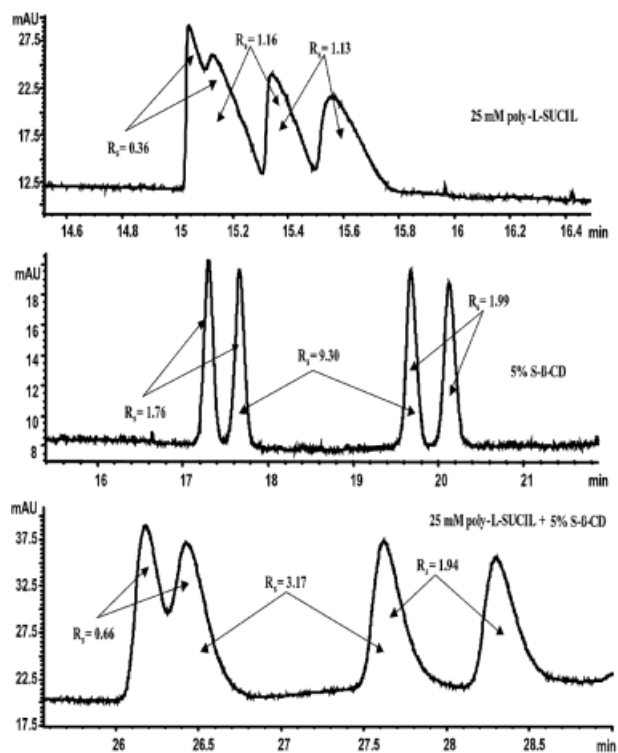


Figure 6. Effect of 5% w/v S- β -CD in combination with poly-SUCIL on the resolution of enantiomeric pairs of labetalol. MEKC conditions are same as Fig. 5.

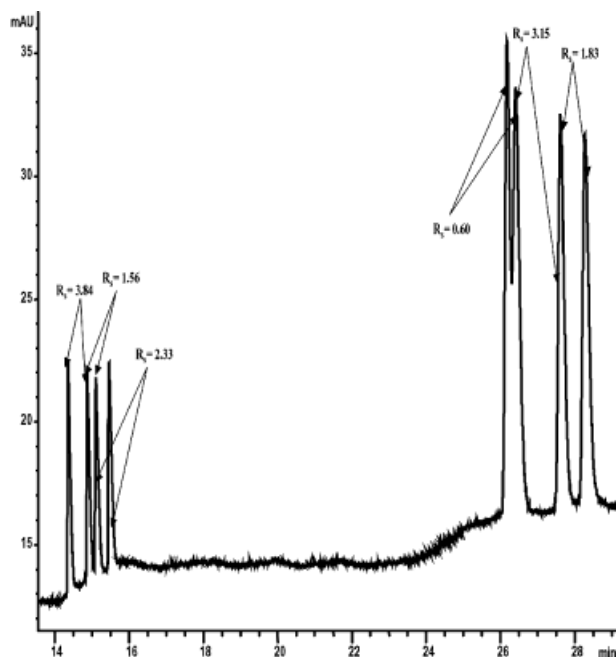


Figure 7. Simultaneous separation of four isomers of nadolol (N1, N2, N3, N4) and labetalol (L1, L2, L3, L4). MEKC conditions are same as Fig. 5.

4 Concluding remarks

Two derivatives of polymeric alkenoxy amino acid (poly-L-SUCL vs. poly-L-SUCIL) were introduced and compared for the separation of multichiral-center β -blockers. In contrast to our previous findings, two chiral centers-bearing polymeric surfactant, poly-L-SUCIL showed better chiral resolution than poly-L-SUCL with one chiral center. We hypothesize that β -blockers with multiple chiral centers have multiple interactions with the two chiral centers of poly-L-SUCIL, which causes an increase in chiral resolution. Hence, favorable steric interactions may play a significant role in this chiral recognition mechanism. The addition of organic solvent to the MEKC buffer increases retention, but cause a decrease in resolution of both labetalol and nadolol isomers. The result from the temperature studies indicates that chiral separation improved for labetalol while it was reduced for nadolol. The enantioselectivity was further optimized by addition of several neutral and charged CDs. Neutral (native or derivitized) CDs in combination with poly-L-SUCIL did not show any notable improvement in the separation of stereoisomers of labetalol and nadolol. The most useful type of charged CD that affected the separation was S- β -CD which when combined with poly-L-SUCIL caused a dramatic increase in resolution, resulting in baseline separation of all four stereoisomers of nadolol. On the other hand, using the same combination, resolution was slightly reduced for

the first enantiomeric pair (L1/L2) and remains unaffected for the second enantiomeric pair (L3/L4) of labetalol. The MEKC studies on several classes of structurally similar chiral analytes (e.g., binaphthyl derivatives, benzodiazepines) are underway in our laboratory to understand the relationships between the chemical structure of analytes and the type of alkenoxy polymeric surfactant. Such studies will provide useful insight in chiral recognition mechanism in MEKC.

The financial support of this research from the National Institute of Health (Grant No. GM 62314-02) is gratefully acknowledged.

Received August 18, 2003

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