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Journal of Chromatography A, 858 (1999) 219–227

JOURNAL OF CHROMATOGRAPHY A

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Chiral separations using polymeric dipeptide surfactants: effect of number of chiral centers and steric factors

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Received 9 February 1999; received in revised form 30 June 1999; accepted 16 July 1999

Abstract

Two polymeric dipeptide chiral surfactants (PDCSs), poly sodium *N*-undecanoyl isoleucyl-valinate (SUILV) with three chiral centers and poly sodium *N*-undecanoyl leucyl-valinate (SULV) with two chiral centers, have been evaluated and compared as chiral pseudo-stationary phases in electrokinetic capillary chromatography. The performance of these surfactants, in terms of enantioselectivity was examined using anionic, cationic and neutral analytes. Analyses of the data suggest that the enantiomeric resolutions of the analytes with these two PDCSs are dependent upon steric factors rather than number of stereogenic centers.

Keywords: Enantiomer separation; Pseudo-stationary phases; Surfactants; Peptides

1. Introduction

Micellar capillary electrophoresis (MCE) is one of the most common chiral separation modes in capillary electrophoresis (CE) [1–3]. Such separations depend on the addition of a chiral micelle as a pseudo-stationary phase in the background electrolyte (BGE). Several natural [4–8] and synthetic [9–25] pseudo-stationary phases have been reported for enantiomeric separation of chiral compounds. Recently, the use of polymeric surfactants as pseudo-stationary phases in MCE have attracted considerable interest [15–19]. This is because polymeric surfactants in MCE have some distinct advantages over conventional micelles, e.g., enhanced stability and rigidity, and absence of a critical micelle concentration. In addition, polymerization eliminates the dynamic equilibrium between surfactant monomers and micelles, resulting in faster mass transfer between analyte and pseudo-stationary phase [17,18]. In 1994, our laboratory reported the use of a polymeric amino acid-based surfactant, poly sodium *N*-undecanoyl L-valinate (L-SUV), for the separation of the optical isomers of (±)1,1′-bi-2-naphthol and laudonosine [17]. In subsequent papers, the use of poly L-SUV for the chiral separation of several other racemic compounds was also investigated [20,21].

In an effort to find chiral pseudo-stationary phases with even wider applicability, we embarked on a program based on gaining a mechanistic understanding of chiral interactions with polymeric dipeptide chiral surfactants (PDCSs). For example, the dipeptide surfactant poly sodium *N*-undecanoyl (L,L)-valyl-valinate with two chiral centers was recently compared to the single amino acid surfactant poly

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00810-9

L-SUV with only one chiral center [22]. The results of this study indicated a significant improvement in the chiral recognition for three out of four enantiomeric pairs using PDCS with two chiral centers as compared to single amino acid surfactants. In a subsequent paper, the effect of amino acid order of the PDCSs was examined [23]. In addition, diastereomeric surfactants of poly sodium *N*-undecanoyl leucyl-leucinate were used to determine the site of chiral recognition [24]. Based on fluorescence studies with respect to hydrophobicity, a structure of the PDCSs was proposed to explain its chiral interactions with some of the cationic, anionic and neutral analytes [25].

The present investigation evaluates how steric factors located near the chiral center of the N-terminal amino acid of PDCSs affects chiral recognition. Two PDCSs, poly sodium *N*-undecanoyl isoleucyl-valinate (SUILV) with three chiral centers and poly sodium *N*-undecanoyl leucyl-valinate (SULV) with two chiral centers are compared with respect to the enantiomeric separation of 12 chiral analytes in various forms (anionic, cationic and neutral).

2. Experimental

2.1. Chemicals

Dipeptides [(L,L) isoleucyl-valinate, (L,L) leucyl-valinate], undecylenic acid and *N*-hydroxysuccinimide were obtained from Sigma (St. Louis, MO, USA). The racemates (±)-1,1′-bi-2-naphthol (BOH), (±)-1,1′-binaphtyl-2,2-diamine (BNA), (±)-1,1′-binaphthyl-2,2′-dihydrogenphosphate (BNP), (DL)-aminoglutethimide (AGL), (DL)-glutethimide (GL), (±)-lorazepam (LR), (±)-2,2,2-trifluro-1-(9-anthryl)ethanol (TFAE), oxazepam (OX), temazepam (TM), propranolol (Prop), alprenolol (Alp) and oxprenolol (Oxp) were also purchased from Sigma. The structures of the chiral analytes under study are provided in Fig. 1.

2.2. Synthesis of polymeric dipeptide chiral surfactants

Surfactant monomers of undecenoyl (L,L)-isoleucyl-valinate and undecenoyl (L,L)-leucyl-valinate were synthesized from the *N*-hydroxysuccinimide ester of undecylenic acid according to the procedure reported by Wang and Warner [17]. A 100 m*M* sodium salt solution of each monomer was then polymerized by ^{60}Co-γ radiation. After polymerization, proton nuclear magnetic resonance (NMR) spectroscopy was used to confirm polymerization. Radiated polymers were dialyzed with a 2000 molecular mass cut-off and then lyophilized to obtain the dry product. All surfactants were found to be 99% pure or better as calculated from elemental analysis.

2.3. Capillary electrophoresis procedure

The MCE separations were performed on a Hewlett-Packard (HP) 3D CE Model G1600AX. The fused-silica capillary, 63.5 cm (effective length of 55 cm to detection window)×50 μm I.D., was purchased from Polymicro Technologies (Phoenix, AZ, USA) and mounted in an HP capillary cartridge. The cartridge temperature was maintained at 25°C for the separation of binaphthyl derivatives and 12°C for all other enantiomeric separations. The running BGEs were prepared in triply distilled water; surfactants were added and the pH adjusted by adding either HCl or NaOH to the BGE. All solutions were filtered through a 0.45-μm membrane filter before use.

A new capillary was conditioned for 30 min with 1 *M* NaOH at 60°C followed by 10 min with triply distilled water. The capillary was flushed with buffer for 2 min prior to injecting the sample. All analyte standard solutions were prepared in methanol–water (1:1) at 0.1–0.5 mg/ml. Samples were injected for 5 s at 10 mbar pressure. Separations were performed at +30 kV, with UV detection at 220 nm.

2.4. Optimized conditions

The MCE conditions, previously optimized [26] using amino acid based surfactants are as follows: (1) binaphthyl derivatives: BNP; 30 m*M* equivalent monomer concentration (EMC) of PDCS, BOH and BNA; 6 m*M* EMC of PDCS, 10 m*M* sodium borate, 100 m*M* Tris, pH 10.0 at 25°C, (2) β-blockers: (Prop, Alp, Oxp) 18 m*M* EMC of PDCS, 50 m*M* sodium borate, 300 m*M* 3-cyclohexylamino-1-propanesulfonic acid (CAPS), pH 8.5 at 12°C, (3) GL/

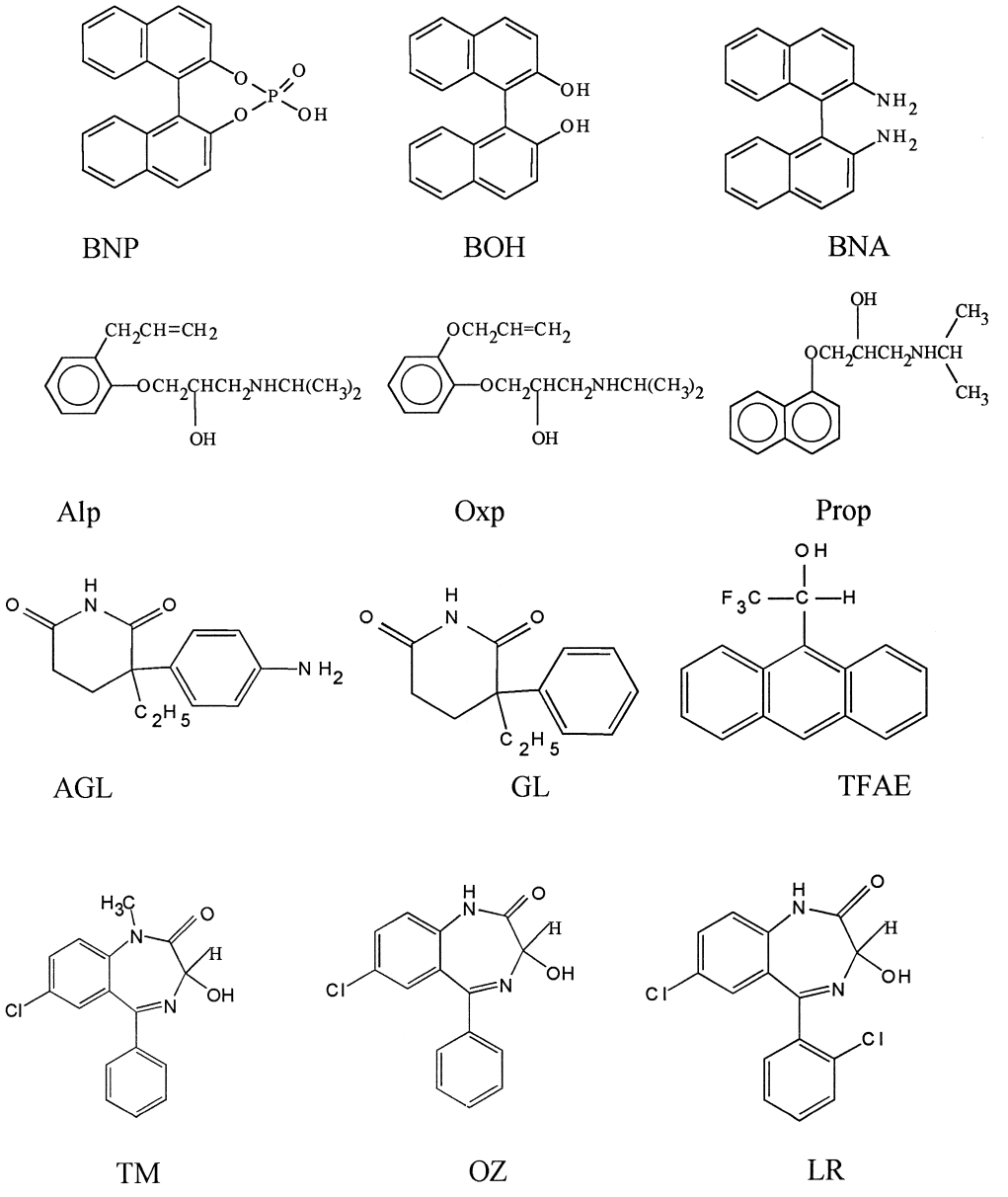

Fig. 1. Structures of the chiral analytes.

AGL: 80 m*M* EMC of PDCS, 50 m*M* Tris, pH 9.2 at 12°C, (4) benzodiazepines: TM; 20 m*M* EMC of PDCS, LR and OX; 12 m*M* EMC of PDCS, 25 m*M* Tris, 25 m*M* sodium borate, pH 8.5 at 12°C, (5) TFAE: 6 m*M* EMC of PDCS, 30 m*M* sodium borate, pH 10 at 12°C.

3. Results and discussion

The structures of the two PDCSs (SUILV and SULV) used in this study are shown in Fig. 2. As shown, the difference between these two polymers is in the N-terminal position of the dipeptide for each

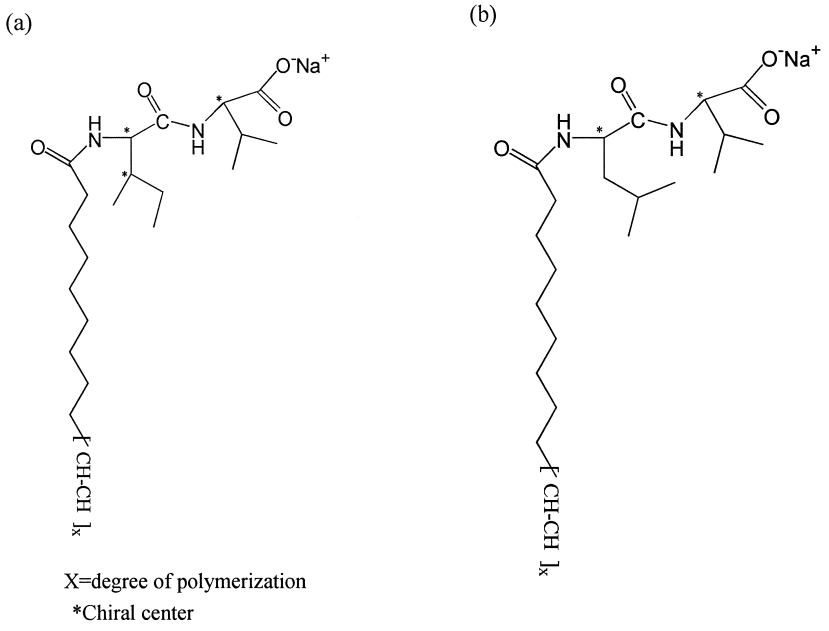

Fig. 2. Structure of the surfactants. (a) SUILV, (b) SULV.

surfactant chain. The C-terminal amino acids of both polymeric dipeptide surfactants are valine. Therefore, it is reasonable to assign any differences in observed enantioseparation of these two dipeptide surfactants to the change in the N-terminal amino acid or its impact on the structure of the PDCSs. Furthermore, the two amino acids in the N-terminal position have a couple of significant differences which should be taken into account when exploring differences in chiral resolution with these two surfactants. The most obvious difference is the fact that SUILV has three chiral centers while SULV has two chiral centers (Fig. 2). Another factor, which must be considered, is steric hindrance. The α-chiral carbon of isoleucine in SUILV is attached to a *sec*.-butyl group, whereas the α-chiral carbon of leucine in SULV is attached to an isobutyl group. Thus, the N-terminal α-chiral center on the SUILV is more sterically hindered as compared to the N-terminal α-chiral center on SULV.

3.1. Enantioseparation of binaphthyl derivatives

The initial set of compounds examined in this study is the binaphthyl derivatives, BNP, BOH and BNA. These compounds are atropisomers and therefore, do not have an asymmetric carbon but rather a chiral plane (C2 symmetry). The three binaphthyl derivatives examined in this study have varying degrees of hydrophobicity and charge states under the experimental conditions used. For example, BNP is anionic, BOH partially anionic, and BNA is neutral at the optimized pH of 10 used for these studies.

No significant difference in enantiomeric resolution was observed with the three-chiral-center dipeptide surfactant SUILV compared to the two-chiral-center surfactant SULV for the enantiomeric separation of BOH and BNA (Table 1). Both SUILV and SULV resolved the enantiomers of BNA with a resolution of ~5.1. Similarly, SUILV and SULV provided respective resolution values of 5.1 and 4.9 for the enantiomers of BOH. In contrast, the three-chiral-center dipeptide surfactant SUILV separated the enantiomers of BNP with a resolution of 3.5, while SULV with two chiral centers was able to resolve BNP with an enantiomeric resolution of 7.8 (Table 1). However, a slight decrease in enantioselectivity was observed using poly L-SULV, Fig. 3. From the chromatographic data shown in Table 1, it

Table 1
Resolution, selectivity[a], and capacity[a] factors of enantiomers

	SUILV	SULV
BNP		
R_s	3.5±0.1	7.8±0.3
k'	1.14	1.22
α	1.06	1.08
BOH		
R_s	5.1±0.1	4.9±0.1
k'	1.12	0.98
α	1.10	1.06
BNA		
R_s	5.1±0.2	5.1±0.3
k'	1.16	0.94
α	1.10	1.04
Alp		
R_s	0.74±0.44	1.4±0.2
k'	0.36	0.38
α	1.04	1.04
Oxp		
R_s	0.91±0.23	1.20±0.46
k'	1.12	1.15
α	1.02	1.02
Prop		
R_s	1.40±0.31	1.78±0.10
k'	1.72	1.77
α	1.02	1.03
AGL		
R_s	6.02±0.48	6.53±0.06
k'	0.68	0.68
α	1.08	1.09
GL		
R_s	1.50±0.01	1.41±0.01
k'	1.11	1.12
α	1.01	1.02
TM		
R_s	2.01±0.06	4.02±0.07
k'	1.43	1.24
α	1.04	1.02
LR		
R_s	3.49±0.04	2.68±0.05
k'	1.40	1.13
α	1.04	1.03
OX		
R_s	5.43±0.06	1.61±0.03
k'	1.13	1.31
α	1.06	1.02
TFAE		
R_s	1.5±0.03	0.74±0.03
k'	1.91	2.04
α	1.02	1.78

[a] ±0.01 average standard deviation of three consecutive CE runs.

can be concluded that even though poly L-SUILV interact stronger with enantiomers of BNA and BOH, than the SULV, enantiomeric resolution of these analytes does not change. Furthermore, it is interesting to note that the more sterically hindered, more polar analyte (BNP) showed a significant difference in enantiomeric selectivity using SUILV as compared to SULV, though no drastic increase in enantioselectivity was observed with the latter PDCS.

3.2. Enantioseparation of β-blockers

The β-blockers (Oxp, Alp and Prop) are a family of compounds that are used for the treatment of hypertension [27]. In most cases, the (*S*)-enantiomer of these drugs is more potent than the (*R*)-enantiomer. The structures of these positively charged compounds are similar. They all possess an alkanolamine side chain attached to one or two aromatic rings (Fig. 1).

As with BOH and BNA, no significant differences in enantiomeric resolution or enantioselectivity of the β-blockers was observed with poly L-SUILV as compared to SULV. PolySUILV provides enantiomeric resolution values of 1.2, 1.4 and 1.8 for Oxp, Alp and Prop, respectively. In contrast, poly L-SULV resolves these enantiomers with resolutions of 0.9, 0.7 and 1.4, respectively (Table 1). It should be mentioned that relatively high experimental errors in resolution values of β-blockers is possibly due to the adsorption of the positively charged analyte to the capillary wall. Previous studies in our research group have shown that electrostatic interaction between the positively charged β-blockers and the negatively charged dipeptide surfactants appears to be the primary factor in binding of this class of compounds to the polar head of the micelle polymers [24]. Therefore, it is mainly the C-terminal or outside amino acid (valine) which is involved in enantiomeric recognition of these relatively hydrophilic, cationic (i.e., Prop, Alp, Oxp) analytes. In other words, the N-terminal amino acids, i.e., leucine of poly L-SULV and isoleucine of SUILV, do not contribute significantly to the enantiomeric recognition of the β-blockers. This is consistent with very similar capacity factors and selectivity factors ob-

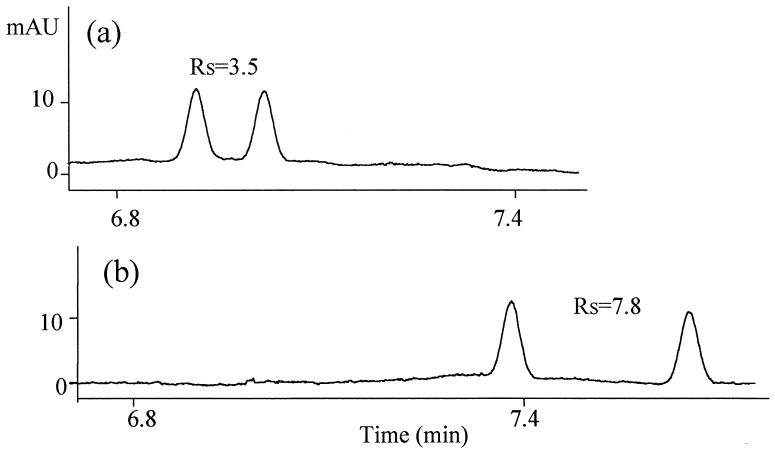

Fig. 3. Enantiomeric separation of BNP. (a) SUILV, (b) SULV, CE conditions: 30 m*M* EMC of PDCS, 10 m*M* sodium borate, 100 m*M* Tris, pH 10 at 25°C. UV detection at 220 nm.

tained for all three enantiomeric pairs of β-blockers using either poly L-SUILV or poly L-SULV.

3.3. Enantioseparation of glutethimide/aminoglutethimide

Glutethimide (GL) and aminoglutethimide (AGL) have been used extensively as anticonvulsant drugs [27]. As shown in Fig. 1, the difference in the structures of GL and AGL is that AGL has an amine moiety attached to its benzene ring as compared to GL with no functional group on the benzene ring. The structures of these two analytes suggest that GL is more hydrophobic than AGL. This is consistent with the elution order of AGL and GL. A comparison of the enantiomeric separation of AGL and GL using SUILV and SULV is shown in Fig. 4. The former PDCS provides a resolution of 6.0 for AGL, while the latter resolves the enantiomers of this analyte with a resolution of 6.5. The resolution values for the enantiomers of GL with SUILV and SULV are 1.5 and 1.4, respectively. Note that the enantiomeric resolution of AGL (containing an extra hydrogen bonding site) is always larger than GL using either SUILV or SULV. Furthermore, analyses of the data indicate that the third chiral center of

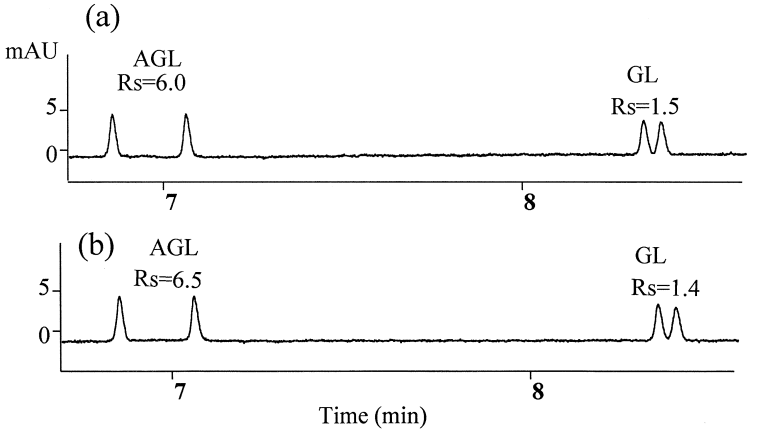

Fig. 4. Enantiomeric separation of AGL/GL. (a) SUILV, (b) SULV. CE conditions: 80 m*M* EMC of PDCS, 25 m*M* Tris, 25 m*M* sodium borate, pH 8.5 at 12°C. UV detection at 220 nm.

SUILV does not significantly improve the chiral resolution nor it has any significant impact on capacity factor and enantioselectivity of GL and AGL.

3.4. Enantioseparation of benzodiazepines

The effect of two chiral centers vs. three chiral centers was further investigated with three neutral benzodiazepines (TM, LR and OX). These compounds are used as hypnotics, tranquilizers and anticonvulants [28]. Although the benzodiazepine class of analytes possesses similar aromatic skeletons, the difference lies in the number and type of substituents attached to the aromatic ring. For example, note the methyl group located on the nitrogen in the seven-member-ring of TM and the chlorine in the *ortho* position of the lower benzene ring of LR (Fig. 1).

Several interesting differences in resolution and selectivity factors were observed for the benzodiazepams. Although TM interacts stronger with SUILV as compared to SULV, the enantiomers of TM are better resolved with the latter (Fig. 5). PolySUILV resolves the enantiomers of TM with a resolution of 2.0 and selectivity factor of 1.04 while SULV is able to separate the enantiomers of TM with a resolution of 4.0 and selectivity factor of 1.02 (Table 1). In contrast, the capacity factor for OX indicates that enantiomers of this analyte interact stronger with SUILV than SULV resulting in an improvement in enantioselectivity. Note that the capacity factors for OX are 1.06 for SUILV and 1.02 for SULV. Examination of the structures of TM and OX suggests that the latter analyte has more hydrogen bonding sites and it is less sterically hindered. The methyl group of TM may affect chiral selectivity in two ways. First, the methyl group blocks the hydrogen binding site of TM; second, it increases the steric hindrance. Fluorescence and NMR have been utilized in our laboratory to further understand the interaction of TM with polymeric surfactants.

Lorazepam is the third benzodiazepine compound investigated in this study. PolySUILV and polySULV were able to separate the enantiomers of LR with resolutions of 3.5 and 2.7, respectively (Fig. 6). Lorazepam and OX differ by a chlorine atom located on the ortho position of the free benzene ring of LR. The presence of the extra chlorine group may limit the movement of the benzene ring inside the micellar cavity resulting in a decline in enantioselectivity of the LR compared to OX with these two polymeric surfactants. More studies with a larger group of dipeptide surfactants, containing isoleucine (isoleucine–alanine, isoleucine–glycine and isoleucine–isoleucine) are planned to further investigate the factors responsible for chiral recognition of benzodiazepams.

3.5. Enantioseparation of (±)-2,2,2-trifluro-1-(9-anthryl)ethanol

The enantiomers of TFAE have been used in chiral NMR to resolve the hydrogen signals of

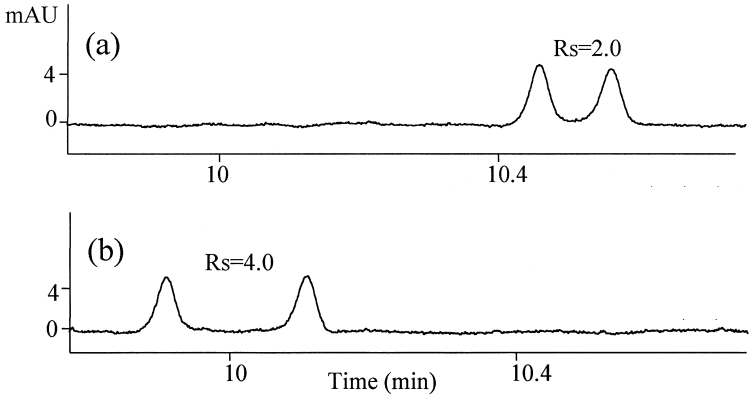

Fig. 5. Enantiomeric separation of TM. (a) SUILV, (b) SULV. CE conditions: 20 m*M* EMC of PDCS, 50 m*M* Tris, pH 9.2 at 12°C. UV detection at 220 nm.

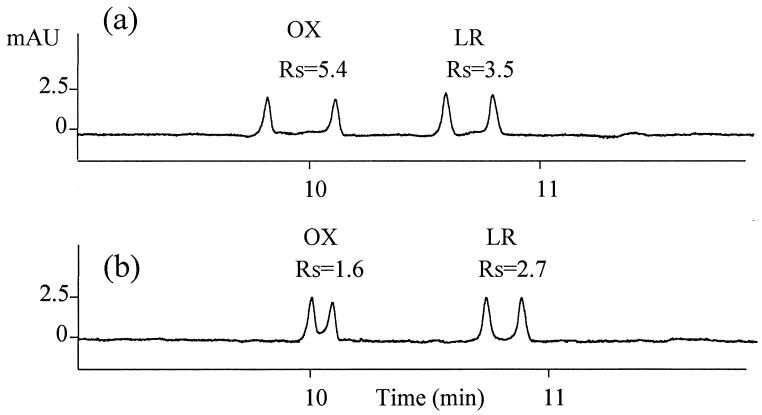

Fig. 6. Enantiomeric separation of LR/OX. (a) SUILV, (b) SULV. CE conditions: 12 m*M* EMC of PDCS. Other conditions as in Fig. 5.

various enantiomers [29]. Fig. 7 compares the separation of the TFAE enantiomers with the two polymeric surfactants, SULV and SUILV. Note the difference in enantiomeric resolution, i.e., an R_s of 1.5 with SUILV and an R_s of 0.7 with SULV. A comparison of k' and capacity factors shown in Table 1 indicates a weaker interaction and relatively smaller enantioselectivity of this analyte with SUILV than that of SULV. This suggests that steric matching has more influence on chiral recognition than the number of chiral centers for TFAE.

4. Conclusions

Of the 12 chiral analytes examined in this study, LR, OX and TFAE showed an improvement in chiral recognition with the three-chiral-center dipeptide surfactant SUILV compared to two-chiral-center dipeptide surfactant SULV. In contrast, the enantiomeric resolution of BNP and TM decreased with the former compared to the latter. In addition, no significant differences were observed when comparing the three-chiral-center surfactants versus the two-

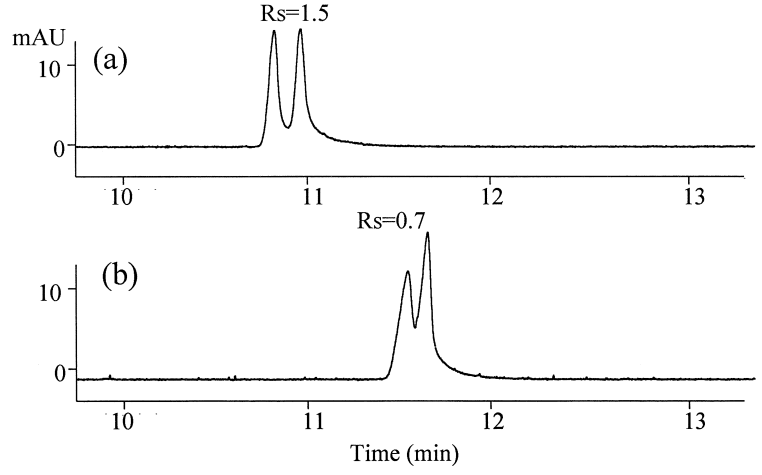

Fig. 7. Enantiomeric separation of TFAE. (a) SUILV, (b) SULV. CE conditions: 6 m*M* EMC of PDCS, 30 m*M* sodium borate, pH 10 at 12°C. UV detection at 220 nm.

chiral-center surfactants for BOH, BNA, Alp, Oxp, Prop, AGL and GL. The results suggest that in some cases the presence of *sec*.-butyl group of SUILV may limit access of the analytes to the second chiral center of this surfactant, resulting in a decline in chiral recognition of BNP and TM. However, with other analytes, it appears that steric repulsion by the methyl group of the *sec*.-butyl moiety may assist in stereoselectivity of the polymer toward the analytes, resulting in an improvement in the chiral separation of the OX, LR and TFAE.

It should be noted that the third chiral center of SUILV is adjacent to the second chiral center and it lacks a hydrogen bonding site. This is critical when hydrogen bonding is important in chiral recognition of a given analyte. Further studies are planned with a variety of analytes to understand the mechanism of chiral separations using amino acid based surfactants.

Acknowledgements

This work was supported through a grant from the National Institute of Health (GM 39844). I.M.W. also acknowledges the Phillip W. West endowment for partial support of this work.

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