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Review

Enantiomeric separations by use of polymeric surfactant electrokinetic chromatography

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Abstract

This review surveys the enantiomeric separation of drugs by electrokinetic chromatography using polymeric chiral surfactant pseudostationary phases. These phases have recently been shown to provide better mass transfer and increased rigidity and stability than regular micelles in micellar capillary electrophoresis. Characterization of the polymeric chiral surfactants is presented. Solution interactions of the pseudostationary phases via thermodynamics and fluorescence probe studies are evaluated. Also, case studies of enantiomeric separation of drugs using a single amino acid surfactant and the synergistic effect of the addition of γ-cyclodextrin to the buffer is discussed. The use of dipeptide surfactants for chiral drug separations is described as well.

Keywords: Reviews; Enantiomer separation; Electrokinetic chromatography; Pseudostationary phases; Thermodynamic parameters; Micellar electrokinetic chromatography; Surfactants

Contents

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1. Introduction

Electrokinetic chromatography (EKC) [1] is a mode of capillary electrophoresis (CE) based on electrophoretic migration. However, the solute to be separated is also partitioned between a mobile phase and a pseudostationary phase. When surfactants are used as pseudostationary phases, the technique is termed micellar electrokinetic capillary chromatography (MECC) [2] or more recently as micellar capillary electrophoresis (MCE) [3].

Introduced in 1984 by Terabe [4], MCE is a form of CE which has the ability to separate charged and neutral compounds simultaneously. Ionic micelles are the most widely used pseudostationary phases in MCE. The selectivity in MCE is achieved through differences in partitioning of the selectands in the micellar phase. The popularity of MCE is a result of the advantages it has over capillary zone electrophoresis (CZE) and high-performance liquid chromatography (HPLC) as an analytical method for pharmaceutical and biomedical applications [5–9]. The high efficiencies and resolution achieved with MCE make it useful for the separation of complex drug mixtures [10–14]. Conventional surfactants have a limited useful concentration range, with the lower range contingent on the CMC and the higher concentration dependent upon joule heating. Also, the concentration of surfactant depends on the CMC, which varies with temperature and salt concentrations. In particular, the effect of temperature on the micelle concentration is a significant problem for the reproducibility of migration times, because the migration time is proportional to the micelle concentration [15–24]. In order to compensate for some of the problems associated with the use of conventional micelles with CE, polymeric surfactants have been developed.

Polymeric surfactants have several distinct advantages over conventional micelles. Polymeric surfactants usually provide better mass transfer than conventional micelles. Also, the polymers are fixed in size and structure by covalent bonds, rather than the weak forces that result in self-assembly of surfactants in the micelle [25]. Other advantages of polymeric surfactants include enhanced stability, rigidity, and tolerance of organic solvents [26,27]. All of these advantages lead to an improvement in peak shape, resolution, and efficiency of the polymeric surfactants over conventional micelles.

In this review, we focus on the development of polymeric chiral surfactant pseudostationary phases in EKC for enantiomeric separation of chiral drugs. The theory, design, and synthesis of chiral polymeric surfactants have been reported in three reviews from the author's own laboratory [28–30]. Here, the characterization, the solution interactions with the analytes, and the applications of polymeric surfactants to chiral drug analysis are emphasized.

2. Polymeric chiral surfactants

The synthesis of a new, more rigid, highly ordered surfactant possessing universal separation abilities, with capabilities to improve reproducibility and increase elution range is of great interest in this area of EKC. In an effort to circumvent the problems of limited elution range and poor selectivity for hydrophobic analytes due to dynamic equilibrium, Larrabee and Sprague developed polymeric micelles [31]. They proposed that the elimination of dynamic equilibrium is due to the formation of covalent bonds between the surfactant aggregates. Based on this theory, Hara and Dobashi [20] and Warner and Wang [23] applied polymeric surfactants instead of regular micelles to EKC.

As mentioned above, polymeric surfactants have

certain distinct advantages over conventional surfactants for the enantiomeric separation of chiral compounds in CE [20,23]. According to Wang and Warner [23], polymeric surfactants, which were introduced in the 1970s [32], possess enhanced stability and controllable size, because the covalent bonds between these surfactant aggregates eliminate the normal dynamic equilibrium.

In order to illustrate the usefulness of this approach, Wang and Warner synthesized a polymeric chiral surfactant [poly(sodium undecanoyl-L-valinate) (Poly L-SUV) and its non-polymeric counterpart, sodium *N*-undecanoyl-L-valinate (L-SUV)]. A comparative study of their performance for the enantiomeric separation of chiral compounds in EKC was conducted. The enantiomeric separation of racemic 1,1′-binaphthyl-2,2′-diol (BOH) (Fig. 1) using Poly L-SUV and L-SUV micelles is shown in Fig. 2. The advantages of the polymer over the monomer are clearly demonstrated in this figure. As shown in Fig. 2A, the polymer was able to baseline resolve ($R_s >$ 1.5) the enantiomers of BOH at a concentration of 0.5% (w/v) (which is below the CMC of the monomer), while no separation was observed with the monomer of L-SUV at the same experimental conditions (Fig. 2B). Baseline resolution with the monomer was achieved at a concentration of 1% (w/v) (Fig. 2C). However, the peak efficiency was markedly lower (N=28 073) than with Poly L-SUV (N=102 240). The higher peak efficiency in the case of Poly L-SUV is believed to be a direct consequence of better mass transfer. The improved mass transfer is thought to be due to the fact that solutes do not penetrate as deeply into the polymeric micelles as compared to normal micelles [27,33].

The dynamics of conventional micelles also has a detrimental effect on peak efficiency. Micelles do not maintain a definite configuration but are in a dynamic association–dissociation equilibrium with monomeric surfactants in the bulk water phase. This association–dissociation equilibrium may affect the chiral recognition in micellar systems. In order to study this effect, the micelle-like polymer Poly L-SUV was synthesized and tested as a chiral selector in EKC. According to Dobashi et al., the hydrophobicity of this micelle-like polymer was lower than that observed for *N*-dodecanoyl-L-valinate (SDVal) micelles [24]. The authors considered this to be an indication of the greater water penetration into the interior region of this polymer, which still exhibits sufficient hydrophobicity to entrap enantiomeric solutes. This polymer gave separations for 3,5-dinitrobenzol amino acid isopropyl esters, but with severe peak tailing which disappeared in the presence of urea. Also, Poly L-SUV showed chromatographic resolution behavior similar to that of chiral micelles in EKC, indicating that chiral recognition is possible irrespective of the dynamic association equilibrium of ordinary surfactants in the bulk water phase. In addition, the authors observed less enantiomeric selectivity (Table 1) with the polymer as compared to the monomeric surfactant. They concluded that the micellar association–dissociation equilibrium does not affect the capacity for chiral separation, and the lower selectivity of a chiral polymer is partly due to spaces between surfactant monomers followed by water penetration to a greater extent into the interior core. It is interesting to note that the observation by Dobashi et al. [24] are in contradiction with the results of Wang and Warner [23]. The differences may be due to several factors including the analytes investigated by the researchers. More importantly, the authors polymerized their chiral polymers using very different techniques.

2.1. Synthesis

Wang and Warner [23] synthesized polymeric micelles according to a modified procedure by Lapidot et al. [34]. Undecylenic acid is reacted with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide to produce an ester and dicyclohexylurea. The ester, which is the common intermediate for all the compounds obtained, is then reacted with chiral single amino acids or dipeptides to generate the monomeric acylamino acid surfactant sodium *N*-undecylenyl-L-amino acids or L-dipeptides. These monomers are then polymerized (above the CMC) at 0.1 or 0.05 *M* aqueous solutions using γ radiation from a ^{60}Co source to provide the respective polymeric surfactants. Leydet et al. [35] have used an identical procedure to synthesize similar polymeric surfactants for AIDS testing.

In contrast to Wang and Warner, Dobashi et al. [24] prepared *N*-(10-undecenoyl)-L-valine (SUVAL) and *N*-(10-undecenoyl)-L-threonine (SDTHR) by

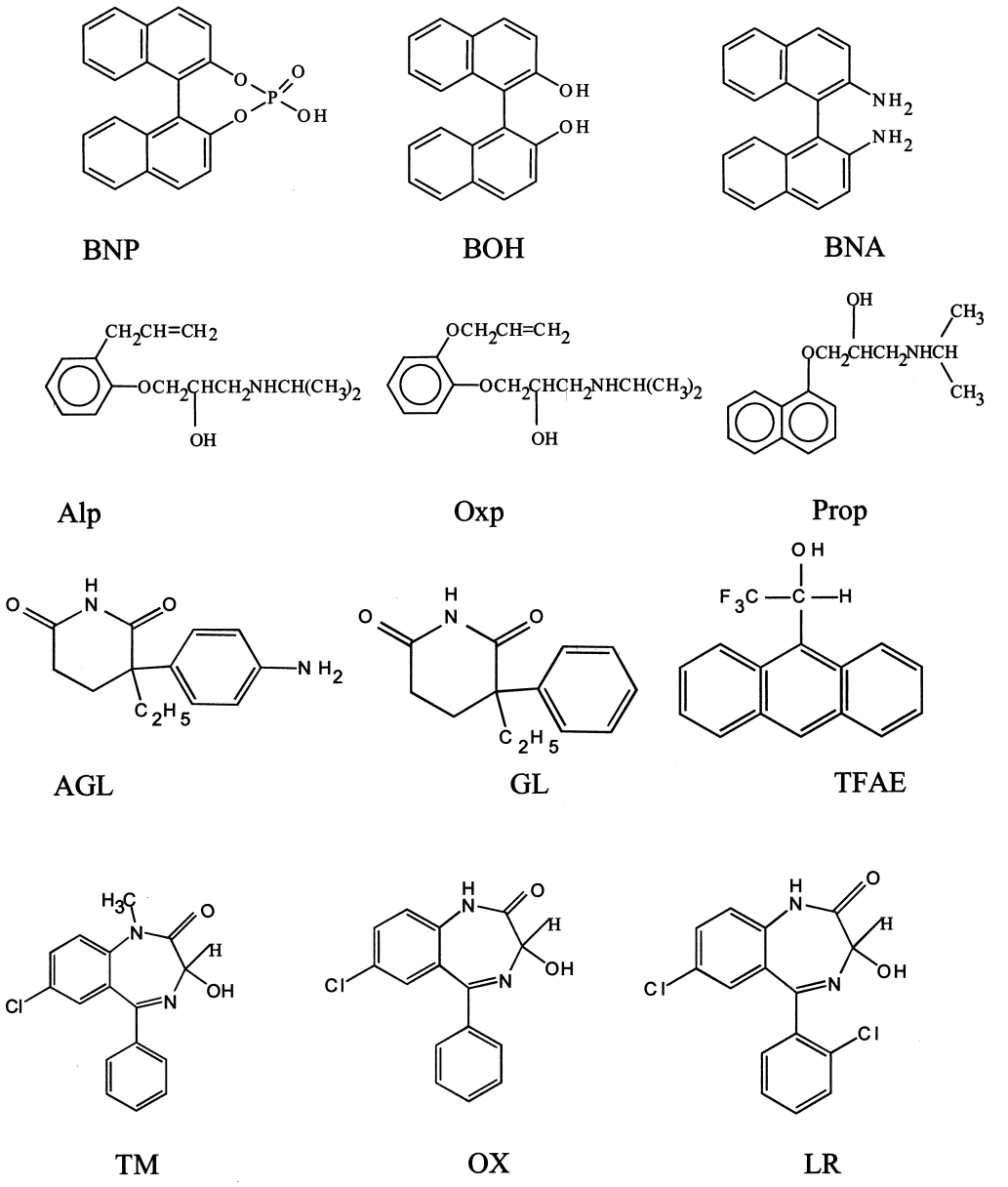

Fig. 1. Structure of chiral analytes.

reacting L-valine and L-threonine with 10-undecenoic acid, and *N*-hydroxysuccinimide ester in a procedure previously published by Guttman et al. [36]. The carboxylic acid is then converted to the corresponding salt. The monomeric surfactants (3.06 g) were dissolved in 100 ml of 0.3 *M* NaCl. The micellar solutions were irradiated with eight ultraviolet lamps under an argon atmosphere to obtain Poly L-SUV and Poly L-SUT.

2.2. Characterization

The exact volume of a particle is a difficult quantity to measure. Therefore, one often uses partial

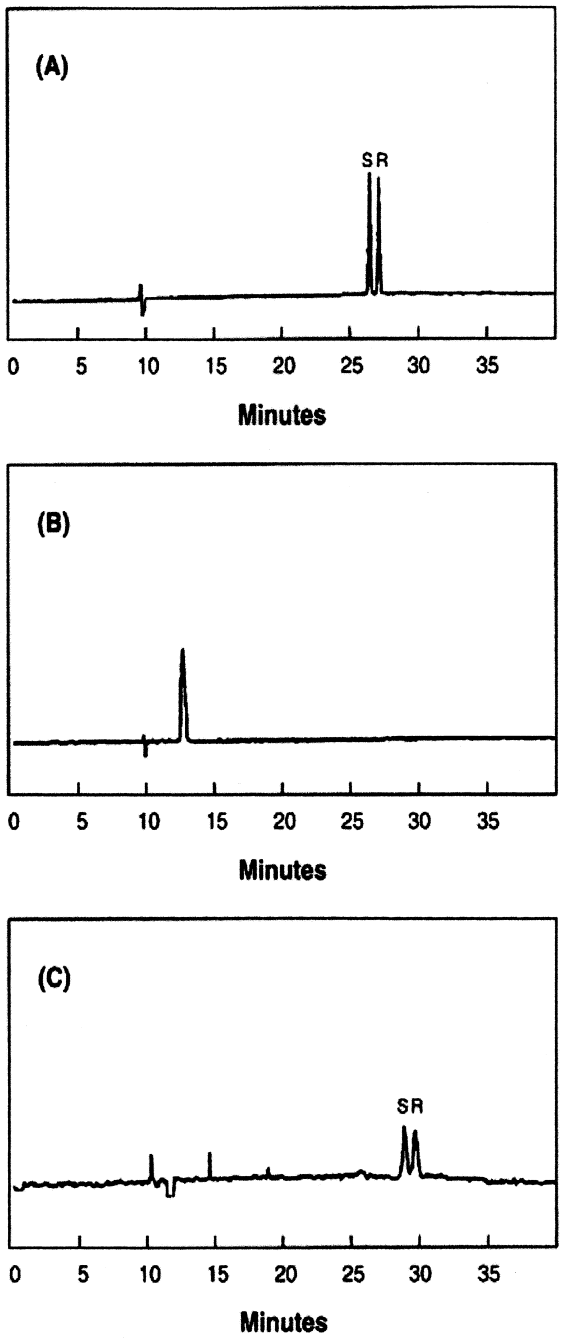

Fig. 2. Comparison between polymerized micelle and non-polymerized micelle for separation of (±)-1,1′-bi-2-naphthol. (A) 0.5% Poly L-SUV; (B) 0.5% L-SUV; (C) 1% L-SUV. Buffer, 25 m*M* borate buffer (pH=9.0); applied voltage, 12 kV; current, (A) 39, (B) 40, and (C) 51 mA; UV detection at 290 nm. From Ref. [29] with permission.

specific volume (V), which is defined as the increase in volume when 1 g of the dry solute is dissolved in a large volume of solvent. Yarabe et al. [37] determined the V values by the densitometry method.

The molecular masses and sedimentation coefficients of Poly L-SUV and Poly L-SUT were estimated by analytical ultracentrifugal measurements at different temperatures (Table 2) [37]. A straight line for ln A versus r was observed. This occurs only for highly monodispersed polymers [38–40]. A similar conclusion was drawn from a study of a Poly L-SUV with a pulsed-field gradient NMR technique (diffusion ordered spectroscopy, DOSY), by Harrell et al. for polymerization concentrations of 20–100 m*M* (Fig. 3). However, the authors observed polydispersity at polymerization concentration above 200 m*M* [41]. As shown in Table 2, the molecular masses and sedimentation coefficients of Poly L-SUV remained almost constant from 20 to 35°C. In contrast, the molecular masses and the sedimentation coefficient of Poly L-SUT increased roughly by a factor of two in the same temperature range. Perhaps, hydrogen bonding of the hydroxyl group of the threonine is the cause of this apparent dimerization. Both the molecular weights and the sedimentation coefficients of Poly L-SUV decrease between 35 and 40°C. This suggests that the polymers either undergo a conformational change or aggregate above 35°C.

The experimental molecular masses of various dipeptide terminated micelle polymers were determined using AUC by Haynes et al., Table 3 [42]. Poly (sodium undecanoyl L,L-valyl-valinate) (Poly L-SUVL) had the highest molecular mass (12 619±275) followed by poly (sodium undecanoyl L,L-threonyl-valinate) (Poly L,L-SUTL) (11 494±427), poly (sodium undecanoyl L,L-seryl-valinate) (Poly L,L-SUSL) (10 056±393) and poly (sodium undecanoyl L,L-alyl-valinate) (Poly L,L-SUAL) (10 056±185).

The experimental aggregation numbers (N) were obtained by dividing the molecular weight of the polymers by the molecular mass of the corresponding monomeric surfactant. The N values of the dipeptide surfactants followed the same trend as the molecular mass: Poly L,L-SUVL>Poly L,L-SUAL> Poly L,L-SUSL>Poly L,L-SUTL. The authors suggest that the differences in N from one micelle polymer to the next is probably related to the differential radical

Table 1
Optical resolution of racemic DNB-amino acid isopropyl esters by Poly SUVal and SDVal micellar solutions, determined by electrokinetic chromatography[a]

Amino acid	Poly SUVal				SDVal			
			SDS				SDS	
	k'_D[b]	α	k'_D[b]	α	k'_D[b]	α	k'_D[b]	α
Ala	0.28	1.18	0.64	1.14	0.48	1.23	1.02	1.17
Val	0.63	1.16	1.68	1.23	1.33	1.22	3.06	1.15
Leu	1.33	1.15	3.75	1.22	3.24	1.15	7.92	1.19
Phe	1.69	1.10	4.65	1.08	4.05	1.05	9.99	1.12

[a] Conditions as given in Table 1, except migrating solutions were used. Solutions were 0.76% Poly SUVal (equivalent to 0.025 *M* SUVal) in 0.025 *M* borate–0.05 *M* phosphate buffer (pH 7.0) containing 2 *M* urea and 0.76% Poly SUVal in 0.025 *M* borate–0.05 *M* phosphate buffer (pH 7.0) containing 2 *M* urea and 0.01 *M* SDS in the first and second columns, respectively. In the third and fourth columns, solutions were 0.025 *M* SDVal in the same buffer solution containing 2 *M* urea and 0.01 *M* SDS.
[b] D enantiomers eluted faster than L enantiomers. From Ref. [24] with permission.

termination rate of each polymer during radical polymerization or alternatively to the bulkiness of the dipeptide group.

No marked differences were observed for the CMC. However, Table 3 shows some differences in their specific rotations. The specific rotation for the micelle polymers followed the same trend as that of the monomers (unpolymerized micelle). The smaller experimental specific rotations for the polymers of L,L-SUAL and L,L-SUVL as compared to the monomers was attributed to the rigidity of micelle polymers over that of monomeric micelles. Regular micellar systems allow for less intermolecular interactions due to the presence of a dynamic equilibrium. This results in larger specific rotation values for traditional micelles.

Leydet et al. [35] reported that polymerization of similar ω-unsaturated surfactants lead to molecular weights in the range of 6000–10 000 by use of gel permeation chromatography (GPC). Dobashi et al. [24] estimated the weight-average molecular mass (M_w) and polydispersity (M_w/M_n) of Poly L-SUV where M_n is the number-average molecular mass by aqueous GPC analysis with pullulans as molecular mass calibration standards. They obtained 11 600 (1.06) without salt and 12 900 (1.05) with 0.1 *M* NaCl, and 13 600 (1.06) with 0.3 *M* NaCl. The M_w of the samples obtained from light-scattering data was 69 300 in 25 m*M* phosphate–borate buffer, corresponding to an aggregation number of 227. This aggregation number, and not 38 obtained by GPC, was likely more accurate according to the authors. However, the values obtained by GPC in our opinion are more representative of the true value since they are in close agreement with Leydet et al. as well as data obtained from our own laboratory [37,41,42].

Table 2
Molecular masses, sedimentation coefficients, and partial specific volumes[a]

T (°C)	Molecular mass (g/mol)		Sedimentation coefficients (10^{-13} svg)		Partial specific volume (ml/g)	
	PSUV	PSUT	PSUV	PSUT	PSUV	PSUT
20	9984±251	11252±415	0.67±0.03	1.03±0.03	0.804±0.005	0.772±0.002
25	9987±215	15049±306	0.82±0.05	1.58±0.04	0.806±0.003	0.776±0.002
30	9723±205	18320±384	0.78±0.04	1.69±0.03	0.814±0.002	0.779±0.004
35	10230±183	20403±329	0.89±0.02	1.83±0.05	0.813±0.005	0.781±0.002
40	9304±175	17554±498	1.02±0.02	1.54±0.04	0.817±0.005	0.786±0.004

[a] From Ref. [37] with permission.

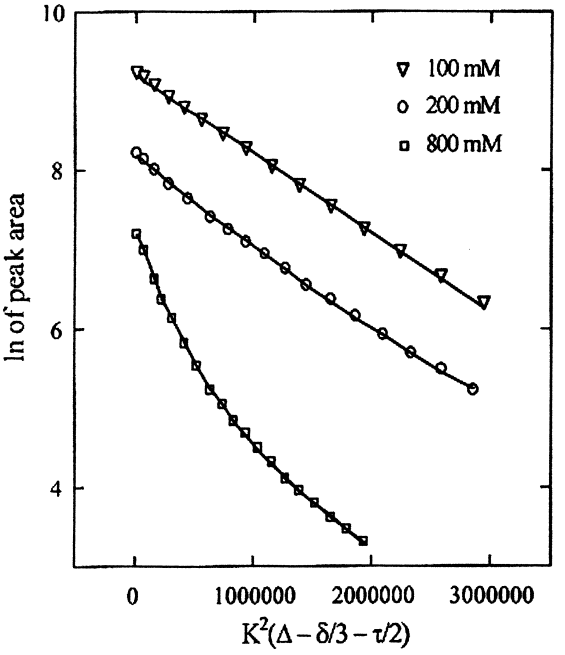

Fig. 3. Decay of NMR peak area with gradient strength for polymerization concentrations of 100, 200 and 800 m*M* samples indicating that those samples exhibit polydispersity. From Ref. [41] with permission.

3. Polymeric chiral surfactant–solute interactions

3.1. Thermodynamic studies of analyte solubilizations

The enthalpy (ΔH^{o}) and the entropy (ΔS^{o}) as the analyte moves from the aqueous phase into the polymeric surfactant phase have been evaluated [37]. The ΔH^{o} and ΔS^{o} as the analyte transfer from the aqueous phase to the micellar core of the polymerized surfactants were computed by using the slope ($-\Delta H^{o}/R$) and the *y*-intercept ($\Delta S^{o}/R+\ln \beta$) of the $\ln k$ versus $1/T$ plots (Fig. 4) of Van't Hoff equation. It is clear that the enthalpies, which are directly proportional to the slopes, do not differ significantly among the five PTH-amino acid analytes. However, considerable differences in retention factors were observed.

Table 4 provides a comparison of the thermodynamic results (ΔH^{o}, ΔS^{o}, ΔG^{o}) for the PTH-amino acids as they interact with Poly L-SUV and Poly L-SUT. It should be noted that Poly L-SUT is more polar than Poly L-SUV due to its hydroxyl group. Moreover, Poly L-SUT has a higher molecular mass

Table 3
Physicochemical properties of dipeptide terminated surfactant monomers

Characteristics	L-SUAL	L-SUVL	L-SUSL	L-SUTL
Molecular mass (g/mol)	363.5	396.5	384.5	398.5
Number of stereogenic centers	2	2	2	3
Critical micelle concentration (m*M*)[a]	~6	~7	~6	~6
Specific rotation[b]	−58.7° (±1.6)	−54.1° (±1.6)	−35.7° (±1.5)	−24.7° (±2.0)

Physicochemical properties of dipeptide terminated micelle polymers

Characteristics	Poly L-SUAL	Poly L-SUVL	Poly L-SUSL	Poly L-SUTL
Molecular mass (g/mol)	10 056±185	12 619±275	10 056±393	11 494±427
Experimental Aggregation number	~28	~32	~26	~29
Specific rotation[b]	−49.4° (±2.7)	−36.6° (±1.3)	−36.1° (±1.8)	−27.8° (±1.9)

[a] Determined in water using surface tension measurements.
[b] Determined in water $\lambda=589$ nm; $c=1$. From Ref. [42] with permission.

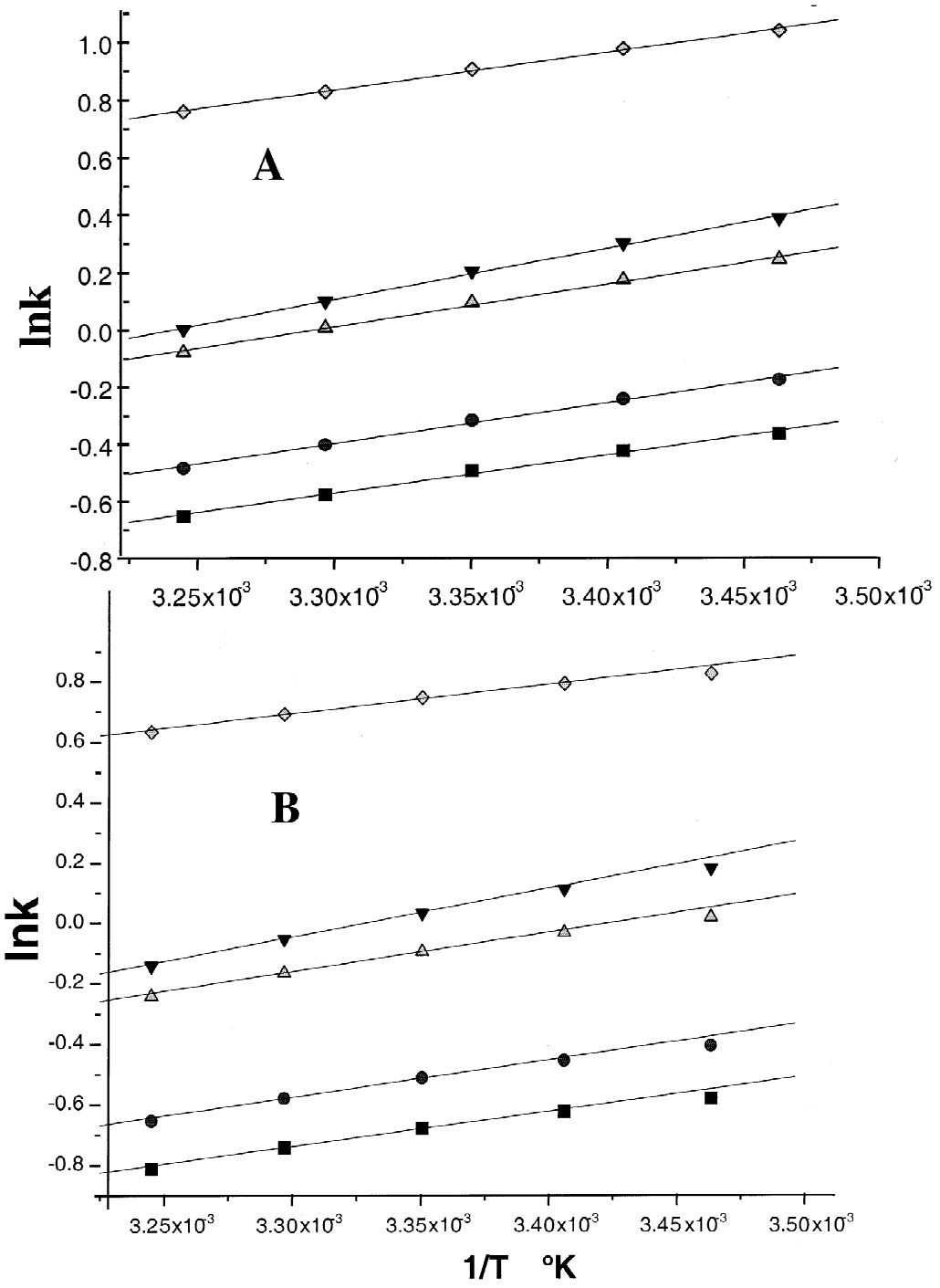

Fig. 4. The Van't Hoff plots of PTH-amino acids for poly (sodium undecanoyl-L-valinate) (A) and poly (sodium undecanoyl-L-threoninate) (B) for the second enantiomers of the PTH-amino acid standards. Conditions: 275 m*M* sodium phosphate dibasic; 20 m*M* boric acid, 10 m*M* triethylamine (pH 7.0), 25 kV applied voltage; 50 μA current; 25°C temperature. (A), 53 m*M* equivalent monomer concentration of PSUV is added to the buffer. (B), 53 m*M* equivalent monomer concentration of PSUT is added to the buffer. From Ref. [37] with permission.

Table 4
Enthalpies and entropies of transfer (micelle solubilization) for the PTH-amino acids[a]

Solute	Enantiomers	ΔH° (kJ/mol)		$\Delta(\Delta H)^\circ$ (kJ/mol)		ΔS° (J/mol K)		$\Delta(\Delta S)^\circ$ (J/mol K)	
		PSUV	PUST	PSUV	PSUT	PSUV	PSUT	PSUV	PSUT
PTH-valine	1	−10.56	−8.36			−4.06	2.05		
				−0.56	−0.48			−1.29	1.11
	2	−11.12	−8.84			−5.36	0.93		
PTH-norvaline	1	−11.05	−8.75			−4.24	2.10		
				−0.76	−0.66			−1.93	1.69
	2	−11.81	−9.42			−6.16	0.41		
PTH-phenylalanine	1	−11.85	−9.54			−3.37	3.10		
				−0.53	−0.40			−1.24	0.93
	2	−12.38	−9.94			−4.61	2.17		
PTH-α-aminocaprylic acid	1	−10.55	−7.26			7.88	17.75		
				−0.25	−0.14			−0.51	−0.20
	2	−10.78	−7.40			7.37	17.55		
PTH-tryptophan	1	−14.24	−11.93			−10.51	−3.94		
				−0.63	−0.38			−1.32	−0.77
	2	−14.84	−12.34			−11.82	−4.71		

[a] 2% (w/v) polymers, 275 m*M* boric acid, 20 m*M* sodium phosphate dibasic, 10 m*M* triethylamine, pH 7.0, 25 kV. From Ref. [37] with permission.

than Poly L-SUV. As a result, a stronger steric repulsion between the Poly L-SUT head group and the analytes minimizes the polar hydrogen-bonding interactions. Therefore, the authors suggest that the analytes reside primarily between the hydrophobic core and the polar head. Hence, they conclude that less efficient chiral recognition is observed when Poly L-SUT is used as a pseudostationary phase. The less efficient chiral recognition with Poly L-SUT may also result from the fact that the –OH group attached to the polar head is oriented towards the aqueous phase, away from the hydrophobic core.

Table 5 provides a summary of the distribution coefficients and the Gibbs free energies of the analytes at 25°C, the resolution, as well as selectivities (α) for various PTH-amino acid enantiomeric pairs. It is reasonable to assume that the aromatic rings of the solutes are included in the hydrophobic core of the polymeric micelle, while the amino acid groups interact with the polar groups of the polymeric micelle. Thus, selectivity will depend on how deep the analyte penetrates into the hydrophobic core of the polymeric surfactant. It was observed that a critical factor in selectivity was the degree of alkylation of the amino acid groups. The PTH-α-aminocaprylic acid, which has the highest degree of alkylation, gave the lowest selectivity and highest equilibrium constants. The higher partition coefficient obtained for PTH-α-aminocaprylic acid is believed to be due to the hydrophobicity of this amino acid. By consequence, it has a highest affinity for the polymeric micelle phases. It was interesting to note that the above analyte has the lowest $\Delta(\Delta G)$. This explains the observed low selectivity. Hydrogen bonding capability of the analyte plays an important role in selectivity as well. Tryptophan, which has an amide proton on its aromatic ring, has the highest $\Delta(\Delta G)$ and highest selectivity. Also, the $\Delta(\Delta G)$ are greater for Poly L-SUV than that of Poly L-SUT. This is in agreement with the better selectivity obtained when Poly L-SUV is used as the pseudostationary phase. As expected, the distribution coefficients were less for the less hydrophobic PTH-amino acid (valine) than for the more hydrophobic PTH-amino acid (α-aminocaprylic acid). Some of the differences in chiral selectivity observed with various dipeptide surfactants could be due to the differences in solution interactions with the analytes. Those implications are discussed in the next section.

3.2. Proposed structure of dipeptide surfactants

Billiot et al. [43] proposed that the lowest energy configuration of dipeptide surfactants in solution is

Table 5
Comparison of distribution coefficients, change in Gibbs free energy, resolution, and selectivities for the PTH-amino acids[a]

Solute	Enantiomers	K (25°C)		$\Delta G°$ (kJ/mol)		$\Delta(\Delta G°)$ (kJ/mol)		Resolution (25°C)		α (25°C)	
		PSUV	PSUT	PSUV	PSUT	PSUV	PSUT	PSUV	PSUT	PSUV	PSUT
PTH-valine	1	47	39	−9.31	−8.99						
						−0.16	−0.14	3.37	2.46	1.071	1.065
	2	50	42	−9.47	−9.13						
PTH-norvaline	1	56	47	−9.75	−9.40						
						−0.17	−0.15	3.47	3.17	1.075	1.070
	2	61	50	−9.91	−9.54						
PTH-phenylalanine	1	86	72	−10.81	−10.49						
						−0.15	−0.11	2.84	2.37	1.067	1.053
	2	92	76	−10.96	−10.61						
PTH-α-aminocaprylic acid	1	195	166	−12.97	−12.72						
						−0.09	−0.08	2.44	1.90	1.040	1.030
	2	204	171	−13.07	−12.80						
PTH-tryptophan	1	96	84	−10.98	−10.75						
						−0.22	−0.16	4.32	3.72	1.100	1.076
	2	106	90	−11.20	−10.89						

[a] 2% (w/v) polymers, 275 m*M* boric acid, 20 m*M* sodium phosphate dibasic, 10 m*M* triethylamine, pH 7.0, 25 kV. From Ref. [37] with permission.

when the larger of the two R-groups, i.e. the most hydrophobic, is directed towards the inner core of the polymeric surfactant, while the smaller, less hydrophobic R-group is twisted (due to steric constraints) more towards the bulk aqueous phase (Fig. 5). They illustrated the implications of the proposed structure by comparing the structure of poly (sodium *N*-undecanoyl-L,L-alanyl-leucinate) (Poly L,L-SUAL) and poly (sodium *N*-undecanoyl-L,L-leucyl-alaninate) (Poly L,L-SULA). Also shown in Fig. 5 is the

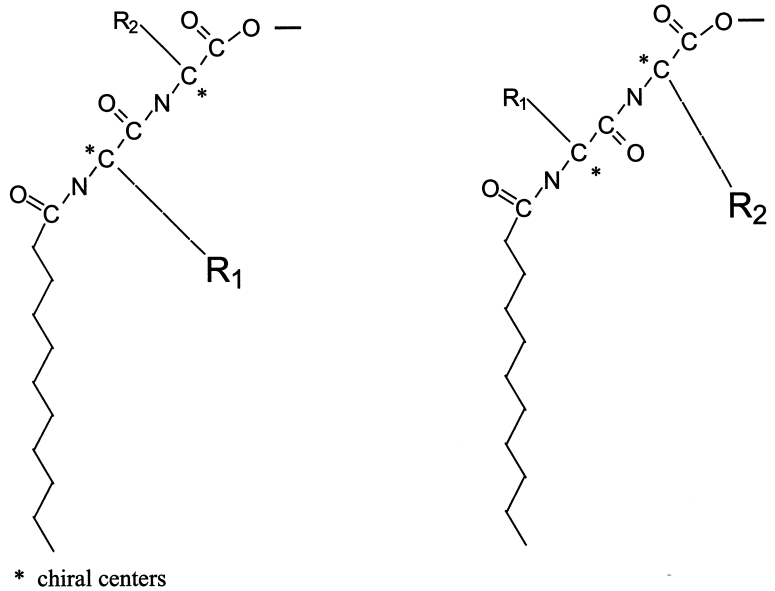

Fig. 5. Proposed structure of dipeptide surfactants. From Ref. [43] with permission.

proposed interaction of BOH with Poly L,L-SUAL and Poly L,L-SULA. When leucine, the larger of the two amino acids, is in the first (N-terminal) position (Fig. 6), they believe that the R-group of alanine is directed away from the hydrophobic core toward the aqueous phase. In this configuration, BOH can interact with all of the heteroatoms on Poly L,L-SULA, thus restricting the movement of BOH. The chiral selectivity of the surfactant is thus enhanced. If the larger amino acid (leucine) is in the second (C-terminal) position (Fig. 6), the chiral center attached to alanine is blocked resulting in reduced chiral selectivity of the surfactant. To better understand the role of the R-groups and the heteroatoms in the dipeptide surfactants, Billiot et al. [43] conducted fluorescent probe studies. The fluorescent probes used in their studies were prodan and pyrene. The two probes were used to compare the polarity/hydrophobicity of the microenviroment of the polymeric surfactant core. Fig. 7a–d depict the hydrophobicity trends for various polymeric surfactants. Fig. 9a shows that the core of poly (sodium undecanoyl-L-alaninate) (Poly L-SUA) is the least hydrophobic of the three single amino acid surfactants, followed by Poly L-SUV and poly (sodium undecanoyl-L-leucinate) (Poly L-SUL), as expected. The same trend was observed when glycine was held constant in the first position of the dipeptide surfactant (Fig. 7b). However, an unexpected increase in hydrophobicity was observed for the dipeptide surfactant with glycine in the first position when valine or leucine are held constant in the second position of the dipeptide and the amino acid in the first position is varied (Fig. 7c and d). These observations indicate that the amino acid order in polymeric dipeptide surfactants play an important role in the hydrophobicity of the surfactant core. It was also shown that the amino order has a significant effect on chiral selectivity. As shown in Fig. 8, BOH was always better resolved when the larger of the two amino acids is in the first position

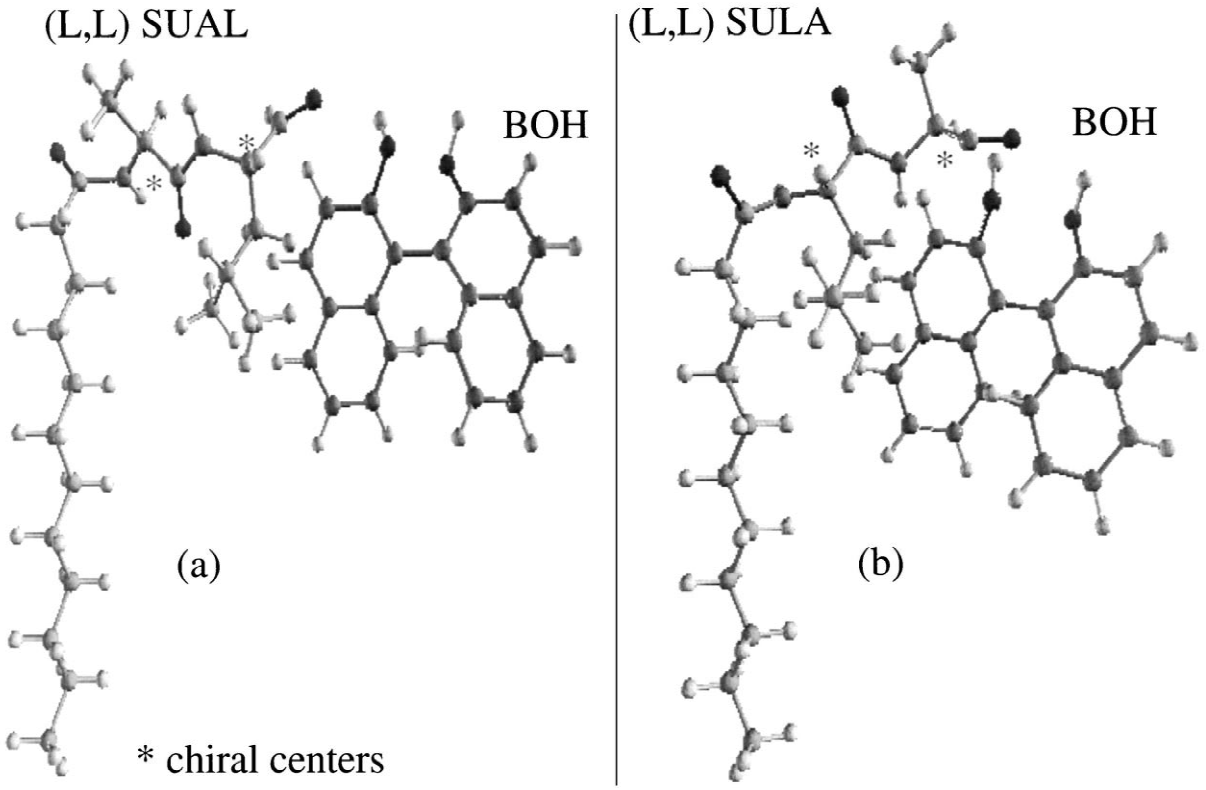

Fig. 6. Proposed interactions between BOH and Poly L-SUAL and Poly L-SULA. From Ref. [43] with permission.

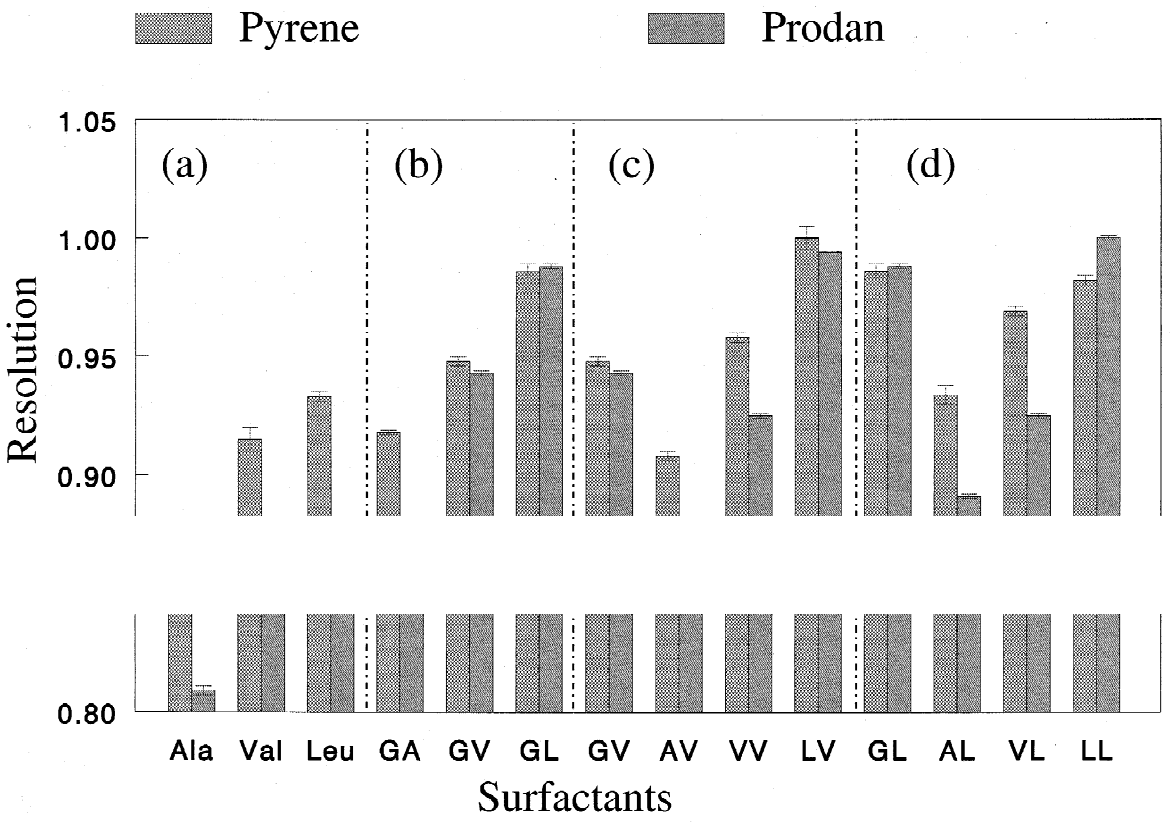

Fig. 7. Comparing the hydrophobicity of various polymeric dipeptide surfactants. From Ref. [43] with permission.

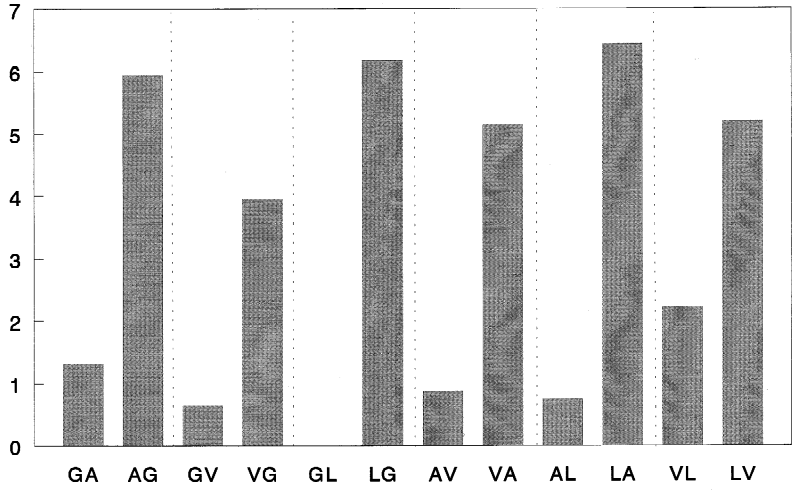

Fig. 8. Effect of amino acid order in dipeptide surfactants on the chiral resolution of BOH. EKC conditions: applied voltage +30 kV, buffer solution prepared with 100 m*M* Tris and 10 m*M* sodium borate at pH 10.0, column temperature 25°C, and 5 m*M* each of the polymeric surfactants at equivalent monomer concentrations. From Ref. [43] with permission.

of the dipeptide surfactant. NMR studies performed by Rugutt et al. also support the above observation [44]. The same trend was also observed for BNP (Fig. 9), except for Poly L-SUGA and Poly L-SUAG, which did not show any resolution. Another factor which can affect chiral selectivity with polymeric surfactants is the concentrations of surfactants used for polymerization. This issue is addressed in the following section.

3.3. Effect of polymerization concentration on chiral separation in electrokinetic chromatography

Harrell et al. [41] investigated how the concentration of the surfactant solution prior to polymerization affects the performance of the polymer in EKC. An EKC experiment that describes separation performance as a function of equivalent monomer concentration (EMC) was conducted. With polymerization concentrations of 20–200 m*M*, the optimum resolution for BOH and BNA was about 6 m*M* (Fig. 10a and b). Higher polymer concentrations were needed at higher polymerization concentrations (400–1000 m*M*) to achieve optimum resolution of BOH and BNA. The authors speculated that the above observation is because the polymers are monodispersed at lower polymerization concentrations and polydisperse at higher polymerization concentrations, as discussed in Section 2. The monodispersity seemed to enhance separation up to an optimum concentration.

The effect of polymerization concentration on EKC performance was also evaluated by running all of the polymers at 10 m*M*, which was the optimum EMC. As illustrated in Fig. 11a, the enantiomeric resolution of BOH, BNA and trifluoro-1-(9-anth-

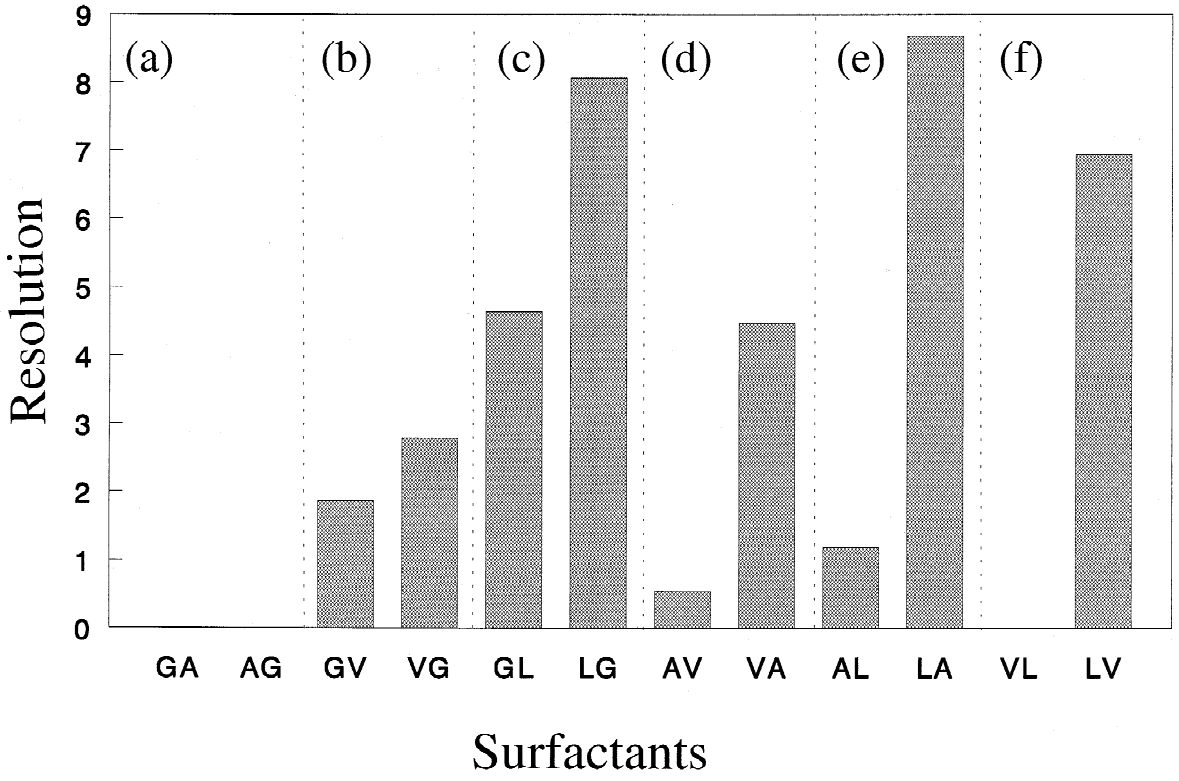

Fig. 9. Effect of amino acid order in dipeptide surfactants on the chiral resolution of BNP. EKC conditions: applied voltage +30 kV, buffer solution prepared with 100 m*M* Tris and 10 m*M* sodium borate at pH 10.0, column temperature 25°C, and 5 m*M* each of the polymeric surfactants at equivalent monomer concentrations. From Ref. [43] with permission.

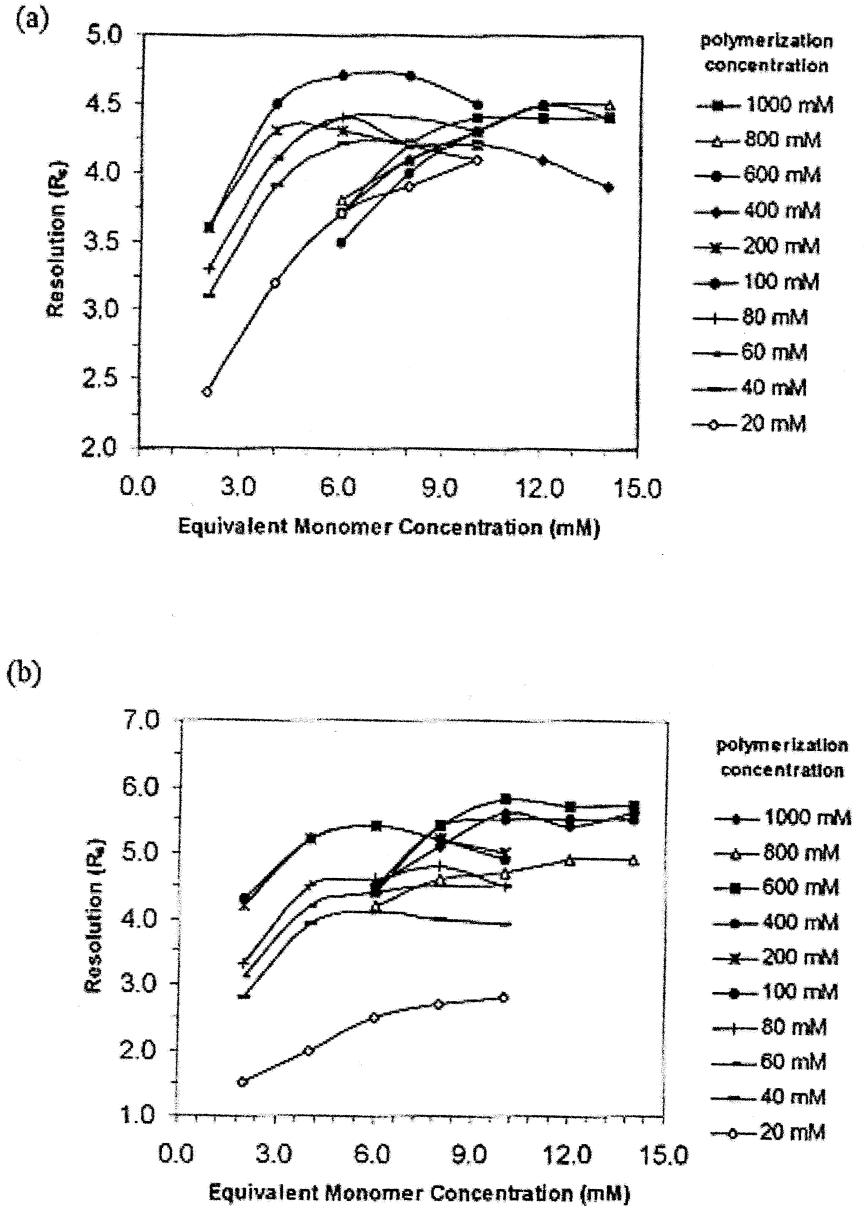

Fig. 10. Variation of resolution with equivalent monomer concentration (EMC) of each polymerization concentration of (a) 1,1′-bi-2-naphthol (BOH) and (b) binaphthyldiamine (BNA). From Ref. [43] with permission.

ryl)ethanol (TFAE) increased with polymerization concentration. Lower resolutions were observed at polymerization concentrations above 200 m*M*. The authors suggested that the polymers formed at lower polymerization concentrations are monodispersed and also more tightly packed as compared to the ones formed at higher polymerization concentrations. This results in a decreased head group surface area and hydrocarbon volume. Therefore, at the lower polymerization concentrations (20–80 m*M*), the low

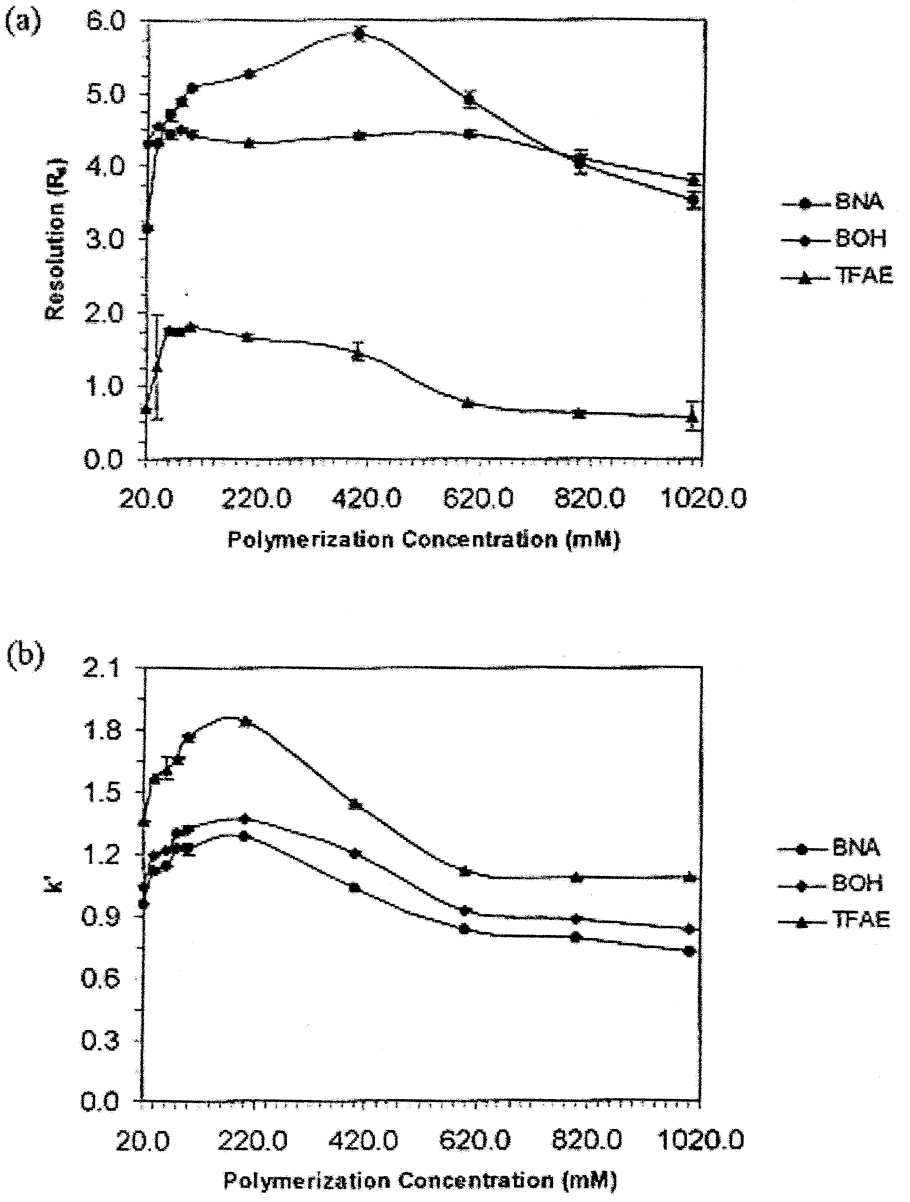

Fig. 11. (a) Variation of resolution with polymerization concentration using an equivalent monomer concentration (EMC) of 10 m*M* for (+) BNA, (O) BOH, and (▼) TFAE and (b) change of retention factor (k') with polymerization concentration of (+) BNA, (O) BOH, and (▼) TFAE. From Ref. [41] with permission.

hydrophobic character of the polymers do not allow penetration of the analyte into the hydrophobic core. On the other hand, the polymers produced at higher concentration (>200 m*M*) showed a decrease in resolution. According to the authors, this is probably due to shape changes and/or steric factors which inhibit the partitioning of the analyte into the polymer. It was further concluded that variations in

resolution and k' (Fig. 11b) observed as a function of concentrations are most likely a result of differences in the micelle polymer shape.

4. Application to chiral drug analysis

The separation of enantiomeric mixtures into individual optical isomers is very important to the pharmaceutical industry. For example, it is now well established that the pharmacokinetic characteristics of individual enantiomers of drugs are different [45]. Serious physiological problems may result from such differences. Therefore, various chromatographic methods for chiral separation and purification have been developed [46,47]. The following is a discussion of the use of polymeric chiral surfactants for enantioseparation of chiral drugs using MCE. A comprehensive discussion of structural changes of polymeric amino acid-based surfactants on enantioseparation, that will be beneficial to the reader, has been recently accepted for publication [48].

4.1. Single amino acid polymeric surfactants

4.1.1. Enantioseparation of paveroline analogs

The enantiomers of D,L-laudanosine, a cationic biosynthetic precursor of morphine, were separated using Poly L-SUV, a single amino acid chiral surfactant, as a pseudostationary phase [23]. The effect of pH on the enantiomeric separation of laudanosine is illustrated in Fig. 12. As observed, the enantiomers are only slightly resolved at pH 9. However, a nearly baseline resolution (R_s=1.2) is obtained at pH 10. The effect of pH on polymeric surfactants have been previously investigated by Chu and Thomas [49]. They demonstrated that at lower pH, the anionic polymeric surfactants examined in their study had a compact conformation. In contrast, the highly negatively charged polymeric surfactant may have a looser conformation due to electrostatic repulsion at higher pH. The authors speculated that the looser conformation of the micelle at higher pH may provide better interactions with the laudanosine enantiomers. However, recent work performed in Warner's laboratory shows that the various amino acid based polymeric surfactants examined in our studies are not affected by increases in pH. This is believed to be due to relatively strong hydrogen bonding occurring between the amino acid head groups [50].

Agnew et al. [51] also used Poly L-SUV to separate paveroline analogs (laudanosine, laudanosoline and norlaudanosoline). No baseline separation was observed under neutral or alkaline conditions for all three cationic paveroline analogs.

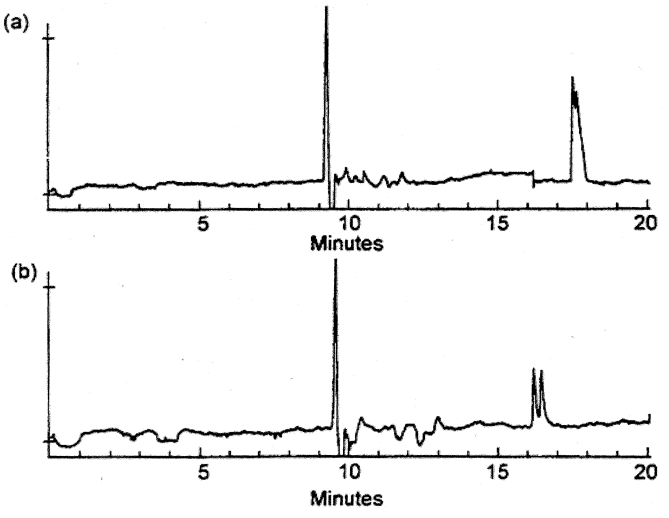

Fig. 12. Influence of pH on separation of D,L-laudanosine. (a) pH 9.0, (b) pH 10.0. Buffer, 0.5% Poly L-SUV in 25 m*M* borate buffer; UV detection at 254 nm, applied voltage, 12 kV. From Ref. [29] with permission.

The effect of coating the capillary with polyvinyl alcohol was investigated at pH 5.6 and 6.0 (Fig. 13a and b). The selectivity was enhanced using a coated capillary for norlaudanosoline. In contrast, enantioseparation could not be obtained with the coated capillary for laudanosine. Note, however, that laudanosine was reported to be almost baseline-resolved at pH 10.0 [23]. The order of elution was laudanosoline>norlaudanosoline>laudanosine. For the three analytes studied, the longer retention did not result in better selectivity and resolution.

4.1.2. Enantioseparation of warfarin/coumachlor

Warfarin, a coumarinic anticoagulant drug used for the treatment of thromboembolic diseases, was separated by Agnew et al. [51] using Poly L-SUV as well. It is well known that the (*S*)-enantiomer of the drug is more pharmacologically beneficial than the (*R*)-form. Coumachlor, an analog of warfarin, was also examined. Qualitative and quantitative experiments using both drugs have been documented by use of HPLC and GC [52,53].

The optimization of the chiral resolution of coumachlor and warfarin was performed in four different buffers in the acidic pH range from 5.5 to 6.5 with Poly L-SUV. The optimum mass fraction was 0.5% (w/v). The effect of pH on the chiral resolution of the two drugs is shown in Fig. 16. The two drugs were better resolved at pH 5.6 than at pH 6.5. The optimum pH was 5.9 for both drugs (figure not shown). Under these conditions (pH 5.9 and mass fraction of 0.5%, w/v), coumachlor was still better resolved than warfarin. Coumachlor has a lower electrophoretic mobility toward the cathode and a larger overall electronegative charge due to the chlorine group (Fig. 14). According to the authors,

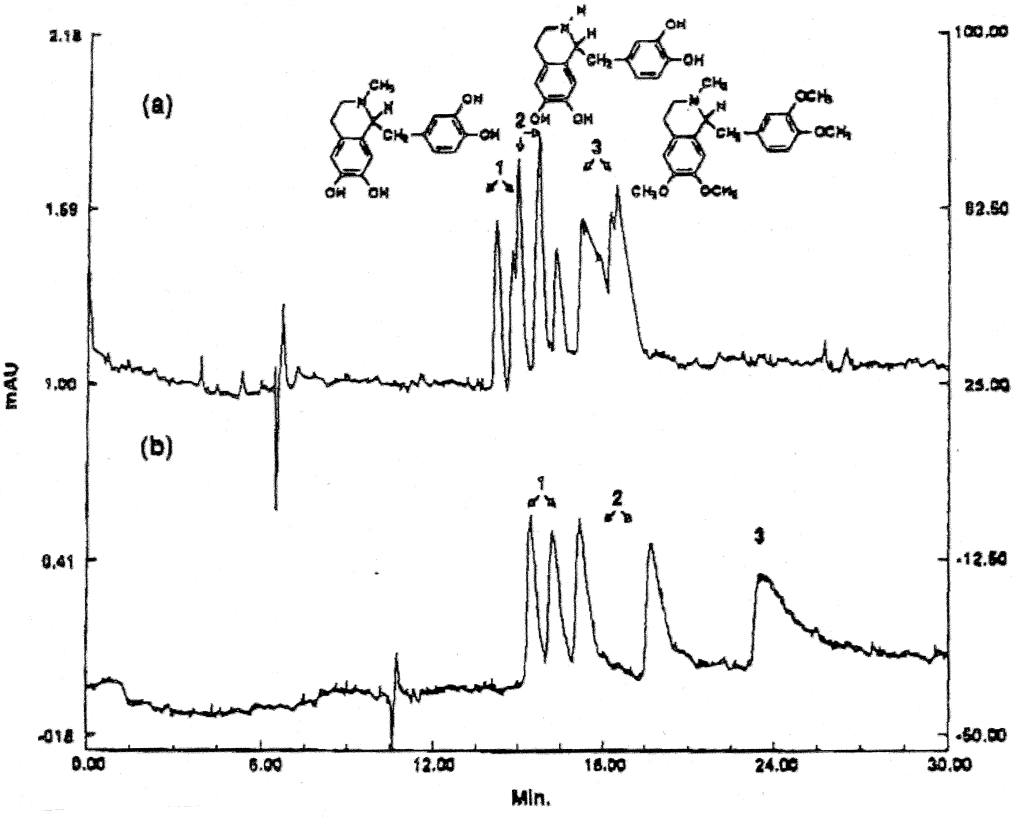

Fig. 13. Enantioseparation of paveroline derivatives using (a) uncoated silica capillary at pH 6 and (b) PVA coated capillary at pH 5.6. Peaks: (1) laudanosoline, (2) norlaudanosoline, (3) laudanosine. CE conditions: 55 cm (50.5 cm effective length)×50 μm, 0.25% Poly L-SUV with 25 m*M* dibasic phosphate. From Ref. [29] with permission.

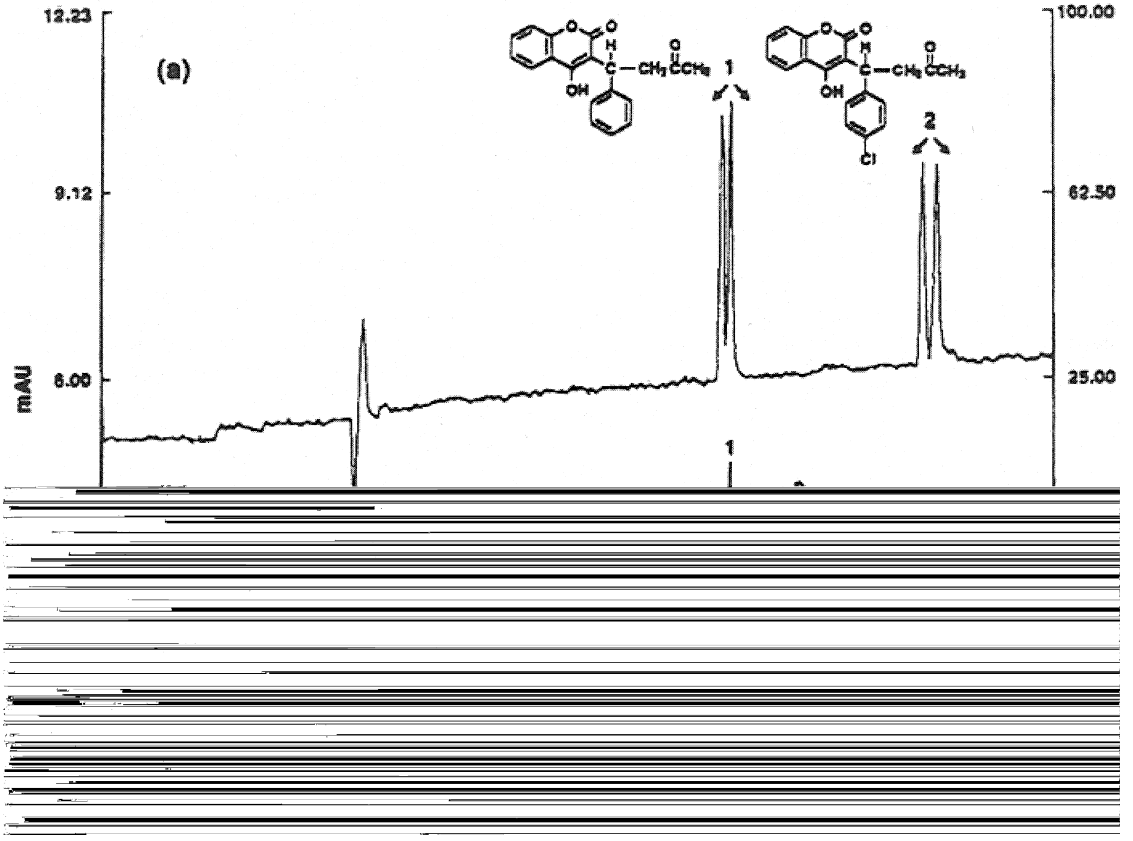

Fig. 14. Chiral separation of enantiomeric mixtures of warfarin (peak 1) and coumachlor (peak 2) at (a) pH 5.6 and (b) pH 6.5. CE conditions: capillary, 55 cm (50.5 cm effective length)×50 μm, 0.5% Poly L-SUV with 25 m*M* monobasic phosphate. From Ref. [29] with permission.

the dipole–dipole forces with the amide proton on the micelle seemed to be increased by the chlorine group. The negative charge on the racemate of coumachlor probably enhanced enantioseparation [54].

4.2. Synergistic effect of a chiral polymeric chiral surfactant and γ-cyclodextrins on the separation of laudanosine/verapamil

The ability of cyclodextrins (CDs) to form highly selective molecular inclusion complexes with a variety of neutral and ionic organic species has led to their popularity in chromatographic techniques, including CE [55–59]. However, the separations abilities of naturally occurring CDs are limited because CDs are neutral and migrate with the electroosmotic flow. As a result, ionic CDs and cyclodextrin–micellar capillary electrophoresis (CD–MCE) are often used for chiral separations [60–64]. However, there are disadvantages of using CD–MCE. Mainly, the surfactant monomers in the buffer partly complex with the CDs [65,66]. In order to alleviate this problem, Wang and Warner investigated the use of polymeric surfactants with CDs. A combination of a polymeric chiral surfactant, poly (sodium *N*-undecanoyl-D-valinate) (Poly D-SUV) and γ-CD was used by Wang and Warner [65] for the chiral separation of D,L-laudanosine and (±)-verapamil (Fig. 15). They demonstrated that the enantiomeric separation of the four enantiomers using Poly D-SUV and γ-CD is better than using either chiral selector alone. They also presented a modified EKC theory that explained the synergistic effect on enantioselec-

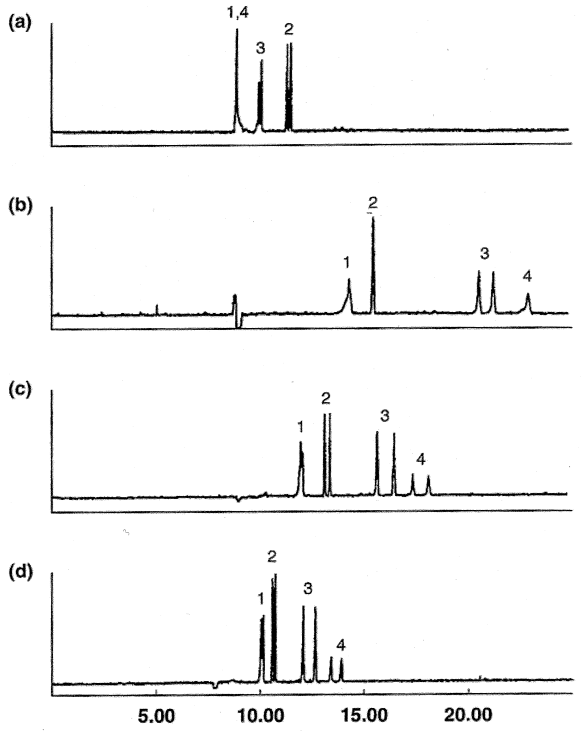

Fig. 15. Chiral separation of four enantiomeric pairs. Peaks: 1=D,L-laudanosine, 2=(±)-BNP, 3=(±)-BOH, 4=(±)-verapamil. CE conditions: (a) 10 m*M* γ-CD, (b) 0.5% Poly D-SUV, (c) 10 m*M* γ-CD and 0.5% Poly D-SUV, buffer for (a), (b) and (c) is 25 m*M* borate (pH 9), buffer for (d) is 10 m*M* γ-CD and 0.5% Poly D-SUV in 5 m*M* borate (pH 9). Applied voltage, 12 kV; UV detection, 280 nm. From Ref. [29] with permission.

tivity using both chiral selectors in the same buffer. No satisfactory resolution was observed (Fig. 15a and b) by using either γ-CD or Poly D-SUV alone within the concentration range of their study. Both of the chiral compounds were enantiomerically resolved when both γ-CD and Poly D-SUV were added to the buffer solution (Fig. 15c). Enantiomeric separation at optimized conditions is shown in Fig. 15d.

4.3. Comparison of single amino acid and dipeptide surfactants for enantiomeric separation of β-blockers

Encouraged by the initial success of the single amino acid based polymeric surfactants, Warner's research group expanded their studies to include the investigation of polymeric dipeptide surfactants. Shamsi et al. [67] compared the enantioselectivity of the polymeric dipeptide surfactant sodium *N*-undecanoyl-L,L-valyl-valinate (Poly L,L-SUVV) to the single amino acid based polymeric surfactant Poly L-SUV for the chiral separation of propanolol (PROP) and alprenol (ALP), which are two basic drugs called β-adrenergic blockers (β-blockers) (see Fig. 1 for structures of these analytes). These cationic drugs have been used for the treatment of hypertension, angina pectoris and arrhythmia. The (*S*)-enantiomer of these drugs is more potent than the (*R*)-enantiomer [68,69]. Both compounds have an alkanolamine side chain terminating in a secondary amine group and an aromatic group. The secondary amine of β-blockers has a pK_a of approximately 9.2–9.6. Therefore, the best pH (9.2) for chiral separation of PROP and ALP was found near their p*K*a. The authors also studied the effect of concentration of the pseudostationary phases on separation factors [capacity factors (k'), resolution (R_s) and efficiency (N)] for PROP enantiomers (Table 6). For PROP, k' increased as a function of the concentration of the polymer owing to an increase in hydrophobic and electrostatic interaction of anionic polymeric phase and cationic enantiomers. At the equivalent monomer concentration (EMC), the migration times were longer with Poly L-SUV than with Poly L,L-SUVV. Chiral recognition was significantly enhanced with Poly L,L-SUVV as compared to that with Poly L-SUV. Optimum separation was achieved for both PROP and ALP enantiomers at 0.5% (w/v) Poly L-SUVV. However, no resolution was observed at any concentration with Poly L-SUV. The electropherograms for the simultaneous separations of the enantiomers of ALP and PROP using optimized conditions with Poly L-SUV and Poly L-SUVV are shown in Fig. 16. It is interesting to note that the (*S*)-enantiomer of each drug always eluted first. This means that the (*R*)-enantiomer has a higher affinity for the polymeric surfactant than the (*S*)-enantiomer.

4.4. Diastereomeric dipeptide surfactants for enantiomeric separation of β-blockers

Four different optical configurations of Poly (sodium *N*-undecanoyl-L-leucyl-leucinate) [(Poly L,L-SULL), (Poly L,D-SULL), (Poly D,L-SULL), (Poly D,D-SULL)] were examined by Billiot et al. [70] as a

Table 6
Comparison of migration factors, resolution, and efficiency for PROP enantiomers, obtained using various concentrations of Poly L-SUV and Poly L,L-SUVV[a]

Concentration (%, w/v)	Equivalent monomer concentration (m*M*)	*k′*	R_s	*N*
0.075 Poly SUV		0.63	0.0	107 500
0.10 Poly SUVV	2.5	0.64	1.1	104 060
0.19 Poly SUV		0.63	0.0	113 500
0.25 Poly SUVV	6.2	0.64	1.1	186 500
0.38 Poly SUV		0.63	0.0	114 200
0.50 Poly SUVV	12.4	0.64	1.1	290 000
0.57 Poly SUV		0.63	0.0	116 600
0.75 Poly SUVV	18.6	0.64	1.1	248 600
0.76 Poly SUV		0.63	0.0	127 400
1.00 Poly SUVV	24.8	0.64	1.1	231 700

[a] Buffer 50 m*M* $Na_2B_4O_7$ at pH 9.2. Sample injected under pressure for 2 s (0.1 mg/ml). Applied voltage +20 kV; current 50–87 μA. UV detection at 214 nm. From Ref. [67] with permission.

diagnostic tool to investigate chiral molecular interactions via EKC. The single amino acid surfactants of opposite optical configuration (Poly L-SUL) and (Poly D-SUL) were used to determine the elution order of the enantiomers. The *R* form of both ALP and PROP eluted first in the case of Poly D-SUL

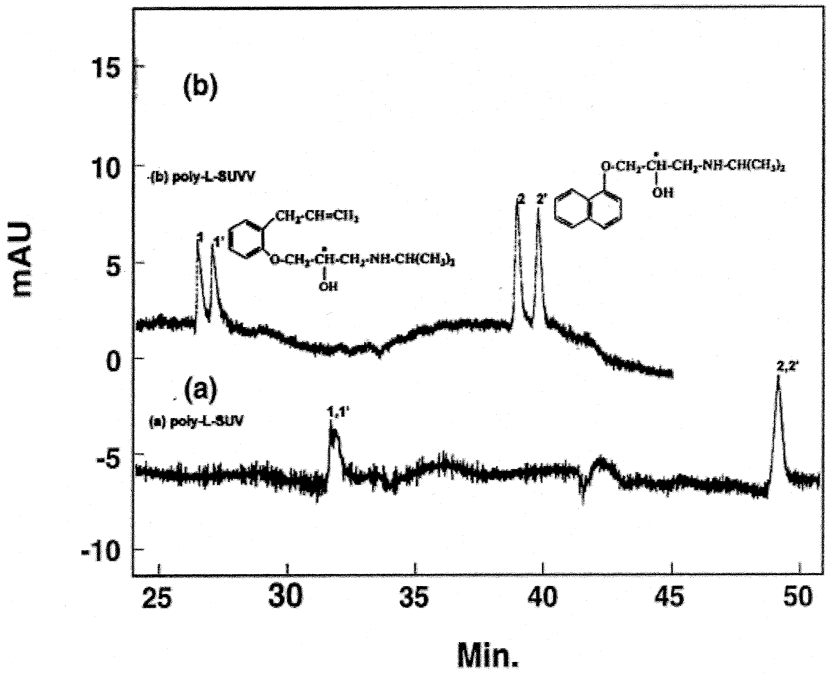

Fig. 16. Comparison of polymerized anionic surfactants for the separation of basic enantiomers. EKC conditions: 0.57 and 0.5% (w/v) of Poly L-SUV or Poly L-SUVV, respectively, 50 m*M* $Na_2B_4O_7$ buffered at pH 9.2. Peak identification: 0.2 mg/ml each of one (1) (*S*)-(−)-ALP, (1′) (*R*)-(+)-ALP; 0.1 mg/ml each of (2) (*S*)-(−)-PROP, (2′) (*R*)-(+)-PROP. Pressure injection for 2 s; +20 kV applied voltage for separation; Current, 85 mA for Poly L-SUV and 56 mA for Poly L-SUVV. UV detection was at 214 nm. From Ref. [67] with permission.

(Fig. 17a). However, a reversal of elution order was observed by replacing Poly D-SUL with Poly L-SUL (Fig. 17b). As expected, a reversal of enantiomeric order was also observed for Poly L,L-SULL as compared to Poly D,D-SULL (Fig. 17c–d). A comparison of the electropherograms showed that a similar elution order was observed for the enantiomers of ALP and PROP for Poly D-SUL, Poly D,D-SULL and Poly L,D-SULL. (i.e. the *S* form of both ALP and PROP always eluted first). In contrast, the *S* form elutes last in the case of Poly L-SUL, Poly L,L-SULL and Poly D,L-SULL. From this it can be inferred that the chiral recognition of ALP and PROP occur primarily at the C-terminal amino acid of the

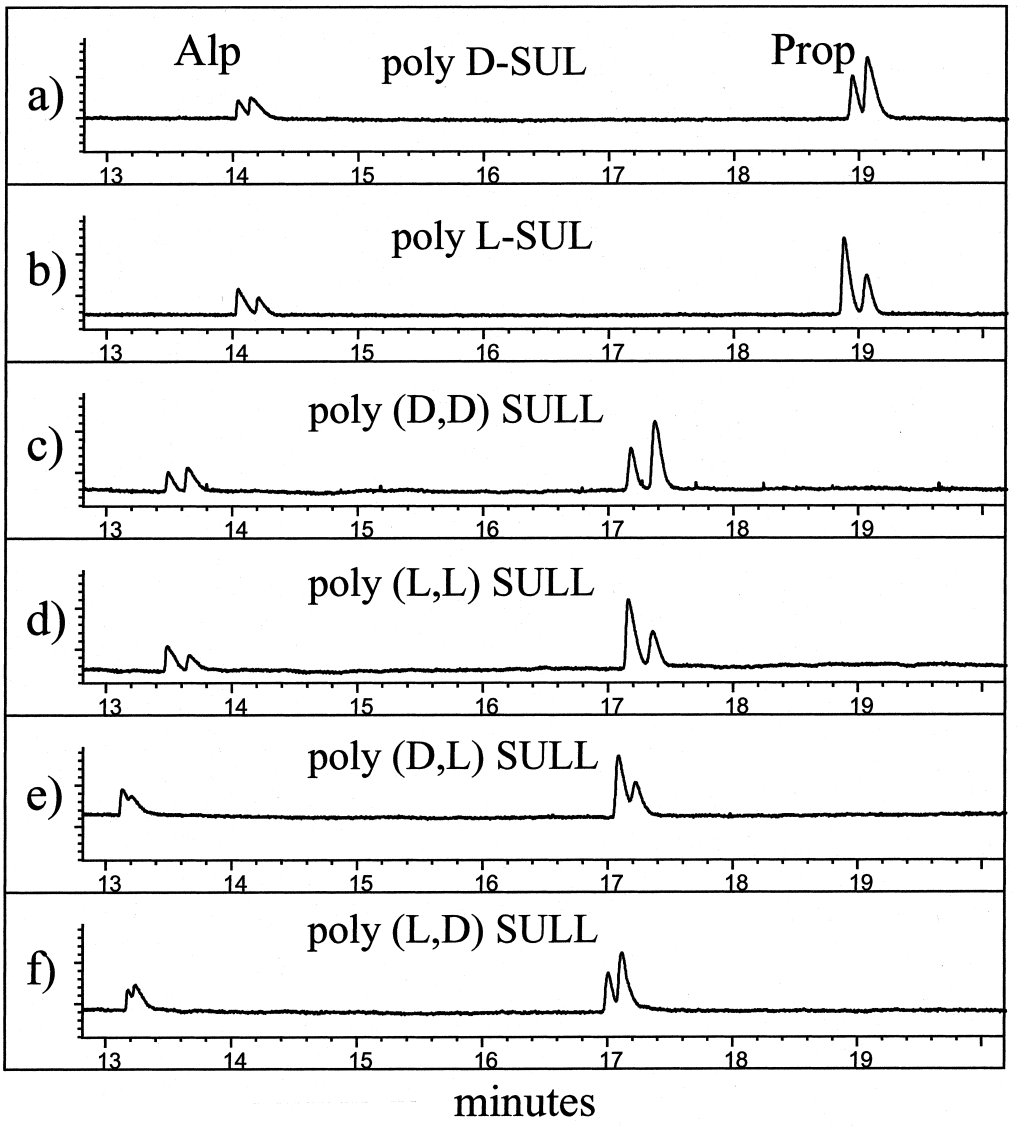

Fig. 17. Comparison of elution order and enantioseparation of alprenol (ALP) and propanolol (PROP) [*R*(+)/*S*(−) ratio = 2:1] with various polymeric surfactants; (a) Poly D-SUL, (b) Poly L-SUL, (c) Poly D,D-SULL, (d) Poly L,L-SULL, (e) Poly D,L-SUL, (f) Poly L,D-SULL. EKC conditions: applied voltage +30 kV, buffer solution prepared with 50 m*M* sodium borate and 300 m*M* CAPS at pH 8.5, column temperature 12°C, 18 m*M* each of the polymeric surfactants at equivalent monomer concentrations. From Ref. [69] with permission.

polymeric dipeptide surfactants, R_2 of Fig. 5. Comparison of the resolution of the enantiomers of ALP and PROP with dipeptide surfactants (Poly L,L-SULG and Poly L,L-SUGL) containing one chiral center supported the evidence of the preferential interaction of ALP and PROP with the outside (C-terminal) amino acid. As a result, the studies suggest that the cationic drugs (ALP and PROP) interact preferentially with the outermost amino acid of the dipeptide surfactant.

4.5. Comparison of two chiral center and three chiral center dipeptide polymeric surfactants

In order to further investigate the synergistic effect of additional chiral centers, Haddadian et al. [3], in Warner's group, compared the chiral separation ability of poly (sodium *N*-undecanoyl-L,L-isoleucyl-valinate) (Poly L,L-SUILV) (three chiral centers) and poly (sodium *N*-undecanoyl-L,L-leucyl-valinate) (Poly L,L-SULV) (two chiral centers). Both polymeric surfactants contain a valine as the C-terminal amino acid. The obvious difference is then in the N-terminal position. Leucine has only one chiral center while isoleucine has two. Therefore, any differences in chiral recognition generated by the two pseudostationary phases can be attributed to the N-terminal amino acids. In addition to possessing an extra chiral center, the α-chiral carbon of isoleucine in Poly L,L-SUILV is more sterically hindered. The α-chiral carbon of isoleucine is attached to a *sec*-butyl group, while the α-chiral carbon of leucine is attached to an isobutyl group. Thus, it can be inferred that the N-terminal α-chiral center on Poly L,L-SUILV is more sterically hindered than the N-terminal α-chiral center on Poly L,L-SULV.

4.5.1. Enantioseparation of β-blockers

The enantioseparation capability of three chiral centers was compared to that of two chiral center pseudostationary phases for the separation of positively charged enantiomers of β-blockers: ALP, oxprenolol (OXP) and PROP. Resolution values of 1.2, 1.4, and 1.8, respectively, were obtained for the three analytes with Poly L,L-SULV as the pseudostationary phases. In contrast, Poly L,L-SUILV provided resolution values of 0.9, 0.7, and 1.4, respectively, for the three analytes. No significant differences in the enantiomeric resolution between the polymeric pseudostationary phases were observed for the separation of the β-blockers because of the standard deviations (Table 7). The authors concluded that the above observation was due to very similar capacity factors and selectivity factors obtained for all of the three enantiomeric pairs of β-blockers. In addition, as discussed in the previous section (Section 4.4), the cationic β-blockers interact preferentially with the outside (C-terminal) amino acid. Therefore, since the C-terminal amino acid is the same for both surfactants, no significant difference between the two surfactants would be expected for the β-blockers.

4.5.2. Enantioseparation of glutethimide/aminoglutethimide

Glutethimide (GL) and aminoglutethimide (AGL) have been used extensively as anticonvulsant drugs [71]. GL and AGL differ in structure by an amine moiety attached to the benzene ring of AGL (Fig. 1). A more polar interaction and an extra H-bonding site for AGL is provided by the amine group. Enantioseparation of AGL and GL using Poly L,L-SUILV and Poly L,L-SULV is shown in (Fig. 18). As with the β-blockers, no significant difference was observed in the chiral separation ability of the two surfactants for AGL and GL. Resolution values of 1.5 and 1.4 were obtained for the enantiomers of GL with Poly L,L-SUILV and Poly L,L-SULV, respectively. In contrast,

Table 7
Enantioseparation of β-blockers[a]

Analyte	R_s	
	SUILV	SULV
BNP	3.5±0.1	7.8±0.3
BOH	5.1±0.1	4.9±0.1
BNA	5.1±0.2	5.1±0.3
ALP	0.74±0.44	1.4±0.2
OXP	0.91±0.23	1.20±0.46
PROP	1.40±0.31	1.78±0.10
AGL	6.02±0.48	6.53±0.06
GL	1.50±0.01	1.41±0.01
TM	2.01±0.06	4.02±0.07
LR	3.49±0.04	2.68±0.05
OX	5.43±0.06	1.61±0.03
TFAE	1.5±0.03	0.74±0.03

[a] Separation conditions for each analyte is described in the legends of Figs. 18–20. From Ref. [3] with permission.

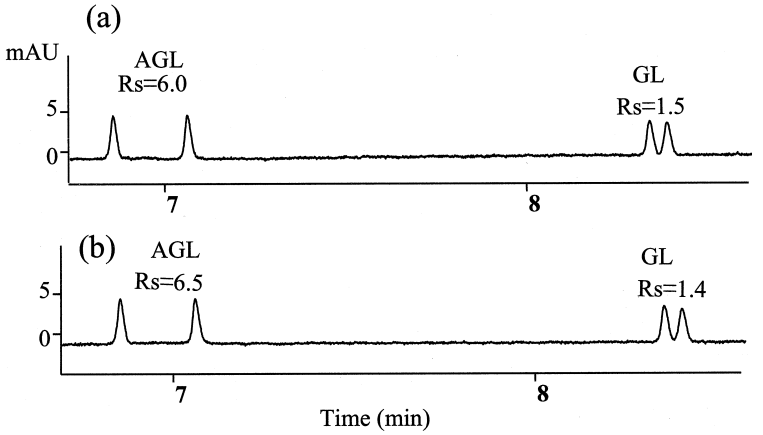

Fig. 18. Enantiomeric separation of AGL/GL; (a) SUILV, (b) SULV. CE conditions: 20 m*M* EMC of PDCS, 50 m*M* Tris, pH 9.2 at 12°C. UV detection at 220 nm. From Ref. [3] with permission.

the same polymeric surfactants provided much higher resolutions for AGL (R_s of 6.0 and 6.5, respectively). It is interesting to note that AGL, which contains an extra hydrogen bonding site, is resolved a lot better than that of GL using either Poly L,L-SUILV or Poly L,L-SULV.

4.5.3. Enantioseparation of benzodiazepines

The effect of the number of chiral centers on enantioselectivity was also investigated with three neutral benzodiazepines, temazepam (TM), lorazepam (LR) and oxazepam (OX). These drugs are used as hypnotics, tranquilizers, and anticonvulsants [71]. The difference in structure of these drugs reside in the type of functional group attached to the aromatic ring. A methyl group is located on the nitrogen in the seven ring of TM while a chlorine group is located at the ortho position of the lower benzene ring of LR (Fig. 1). The enantiomers of TM were resolved better with Poly L,L-SULV, as compared to Poly L,L-SUILV, in spite of the stronger interaction of the analyte with Poly L,L-SUILV (Fig. 19). The enantiomers of TM were separated with a resolution value of 2.0 with Poly L,L-SUILV, while Poly L,L-SULV provided a resolution of 4.0. In contrast, LR and OX showed an improvement in chiral recognition with Poly L,L-SUILV as compared to Poly L,L-SULV (Fig. 20).

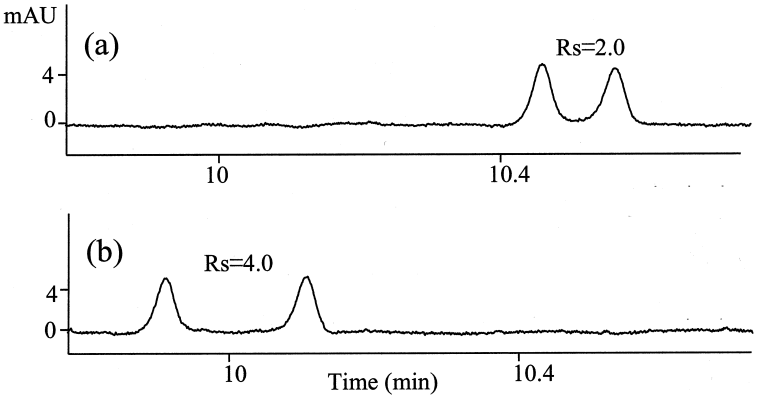

Fig. 19. Enantiomeric separation of TM; (a) SUILV, (b) SULV. CE conditions: 20 m*M* EMC of PDCS, 20 m*M* sodium borate, 50 m*M* Tris, pH 9.2 at 12°C. UV detection at 220 nm. From Ref. [68] with permission.

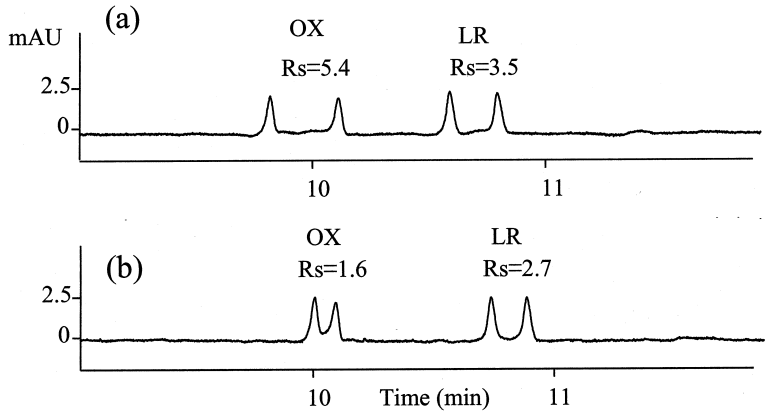

Fig. 20. Enantiomeric separation of LR/OX; (a) SUILV, (b) SULV. CE conditions: 30 m*M* EMC of PDCS, 6 m*M* EMC of PDCS, 30 m*M* sodium borate, pH 10 at 12°C. UV detection at 220 nm. From Ref. [68] with permission.

4.6. Effect of hydroxyl group attached to the polar head of polymeric surfactants on enantioseparation of benzodiazepines

In continuation of a program aimed at gaining a better understanding of chiral recognition with polymeric surfactants, Haynes et al. of Warner's group synthesized polymeric dipeptide surfactants with an –OH group on the polar head (i.e. Poly L,L-SUSL and Poly L,L-SUTL) [42]. The difference between Poly L,L-SUAL and Poly L,L-SUSL is the –OH group attached to the polar head of the latter polymeric pseudostationary phase (Fig. 21). Similarly, Poly L,L-SUVL and Poly L,L-SUTL differ by an –OH group attached to the polar head of Poly L,L-SUTL. In addition, Poly L,L-SUTL contains three chiral centers.

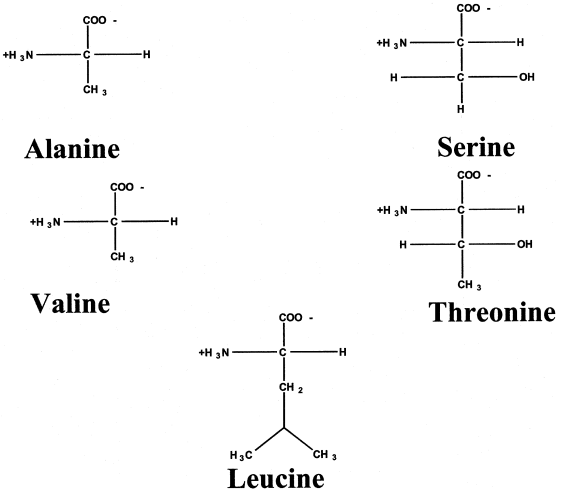

Fig. 21. Structure of amino acids.

The enantioselectivity of Poly L,L-SUAL and Poly L,L-SUSL, as well as Poly L,L-SUVL and Poly L,L-SUTL were compared for the enantiomeric separation of TM, LR and OX. The authors gave two possible explanations for the enhancement in resolution when Poly L,L-SUTL is used as pseudostationary phases as opposed to Poly L,L-SUSL (Fig. 22): (1) the presence of an extra heteroatom on the polar head of Poly L,L-SUTL increases H-bonding to the surfactant, and (2) the synergistic effect of three chiral centers increases the stereoselectivity of the surfactant. A maximum chiral resolution of 1.8 and 1.0 for OX was achieved at 5 m*M* EMC and at 20 m*M* EMC for Poly L,L-SUSL and Poly L,L-SUTL, respectively (Fig. 23). According to the authors, the –OH group at the β-carbon of Poly L,L-SUSL and Poly L,L-SUTL is an essential factor involved in the enantiomeric separation of OX. As shown in Fig. 24, the data collected for LZ was inconclusive as far as a mechanism for enantiomeric resolution is concerned. Neither the synergistic effect of the chiral centers nor the hydrogen bonding capability of the –OH group appeared to play a significant role in improving the chiral selectivity of LZ. The authors speculated that the advantage of having the –OH group may have been minimized by two factors: (1) the presence of an extra chloride atom on the lower aromatic ring of

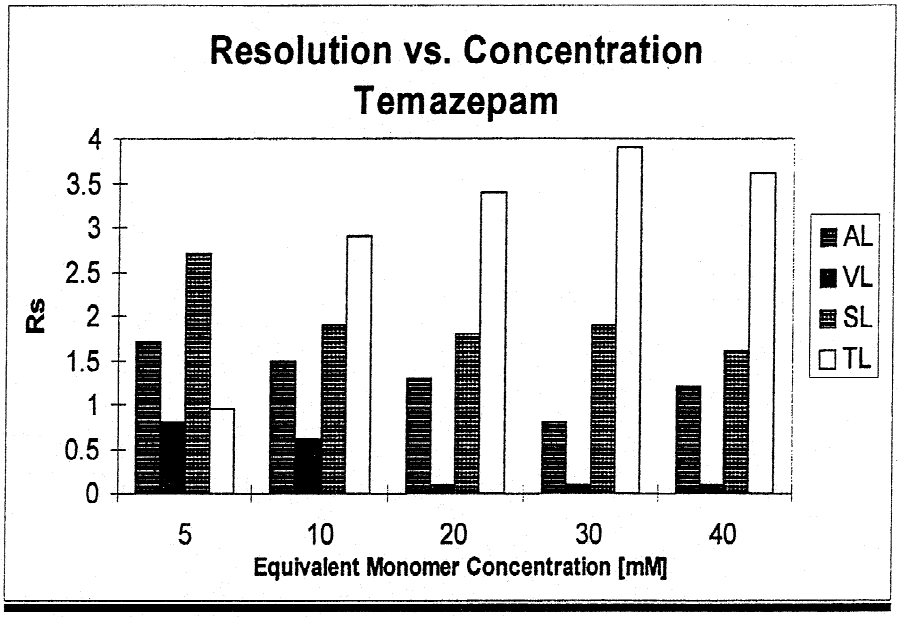

Fig. 22. Resolution of TM as a function of EMC. EKC conditions: 100 m*M* Tris–10 m*M* borate buffer (pH 10); 30 kV; 254 nm, and pressure injection of 5 s at 10 mbar. From Ref. [42] with permission.

LZ and (2) steric bulkiness of the dipeptide surfactants may have deteriorated the H-bonding interaction.

5. Conclusions

Polymeric chiral surfactants offer an efficient

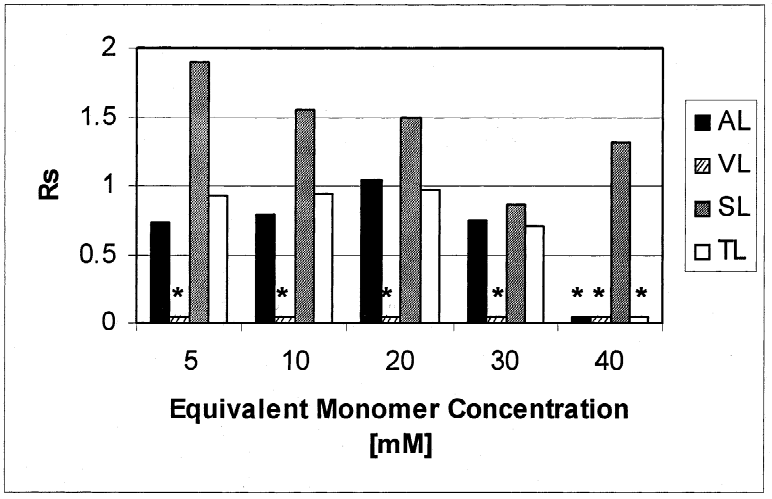

Fig. 23. Resolution of OX as a function of equivalent monomer concentration (EMC). EKC conditions: 100 m*M* Tris–10 m*M* borate buffer (pH 10); 30 kV; 254 nm, and pressure injection of 5 s at 10 mbar. From Ref. [42] with permission.

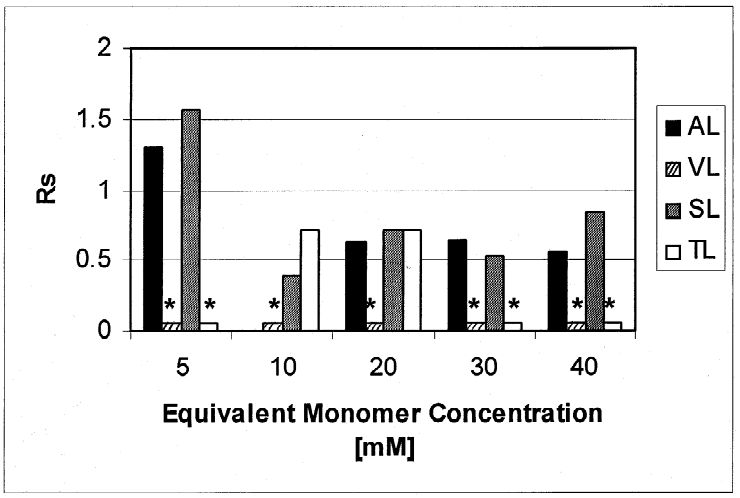

Fig. 24. Resolution of LZ as a function of equivalent monomer concentration (EMC). EKC conditions: 100 m*M* Tris–10 m*M* borate buffer (pH 10); 30 kV; 254 nm, and pressure injection of 5 s at 10 mbar. From Ref. [42] with permission.

alternative to regular micelles in EKC. This is because of its proven improvement in efficiency, resolution, and selectivity. In addition, the recent characterization of these polymeric pseudostationary phases have led to a better understanding of the solution behavior and probable mechanisms of interaction with chiral compounds.

6. Future directions

The separation of chiral drugs using polymeric surfactants in EKC are often superior to separations by monomeric ones. The main advantages of polymeric surfactants are chemical stability, zero CMC, tolerance of organic solvents in the 50 to 60% range and lower joule heating. More fundamental studies such as the ones presented in this review will facilitate the development and introduction of new polymeric pseudophases. In addition to both thermodynamics of interactions through Van't Hoff and fluorescence studies, more solution interactions using such techniques as GPC, light scattering, and nuclear magnetic spectroscopy should provide information regarding chemical interactions between solutes and polymer pseudo-phases.

7. Abbreviations

AGL	(±)-Aminoglutethimide
AIDS	Acquired immunodeficiency syndrome
ALP	(±)-Alprenol
BNP	(±)-1,1′-Binaphthyl-2,2′-diyl hydrogenphosphate
BOH	(±)-1,1′-Bi-2-naphthol
CAPS	3-Cyclohexylamino-1-propanesulfonic acid
CD	Cyclodextrin
CE	Capillary electrophoresis
CMC	Critical micellar concentration
CZE	Capillary zone electrophoresis
DNB	Dinitrobenzyl
DOSY	Diffusion ordered spectroscopy
EKC	Electrokinetic chromatography
EMC	Equivalent monomer concentration
GC	Gas chromatography
GL	(±)-Glutethimide
GPC	Gel permeation chromatography
HPLC	High-performance liquid chromatography
L-SUV	sodium *N*-undecanoyl-L-valinate

LR	(±)-Lorazepam
MCE	Micellar capillary electrophoresis
NMR	Nuclear magnetic resonance
OXP	(±)-Oxprenol
OX	(±)-Oxazepam
Poly L-SUV	Poly (sodium *N*-undecanoyl-L-valinate)
Poly L-SUVal	Poly (sodium 10-undecanoyl-L-valinate)
Poly L-SUT	Poly (sodium *N*-undecanoyl-L-threoninate)
Poly L-SUV	Poly (sodium *N*-undecanoyl-L-valinate)
Poly (L,L)-SUAL	Poly (sodium *N*-undecanoyl-L,L-alyl-leucinate)
Poly (L,L)-SULA	Poly (sodium *N*-undecanoyl-L,L-leucyl-alinate)
Poly L-SUA	Poly (sodium *N*-undecanoyl-L-alinate)
Poly L-SUL	Poly (sodium *N*-undecanoyl-L-leucinate)
Poly D-SUV	Poly (sodium *N*-undecanoyl-D-valinate)
Poly (L,L)-SUVV	Poly (sodium *N*-undecanoyl-L,L-valyl-valinate)
Poly (L,L)-SULV	Poly (sodium *N*-undecanoyl-L,L-leucyl-valinate)
Poly (L,L)-SUILV	Poly (sodium *N*-undecanoyl-L,L-Ileucyl-valinate)
Poly (L,L)-SULL	Poly (sodium *N*-undecanoyl-L,L-leucyl-leucinate)
Poly (L,D)-SULL	Poly (sodium *N*-undecanoyl-L,D-leucyl-leucinate)
Poly (D,L)-SULL	Poly (sodium *N*-undecanoyl-D,L-leucyl-leucinate)
Poly (D,D)-SULL	Poly (sodium *N*-undecanoyl-D,D-leucyl-leucinate)
Poly (L,L)-SUGL	Poly (sodium *N*-undecanoyl-L,L-glycyl-leucinate)
Poly (L,L)-SULG	Poly (sodium *N*-undecanoyl-L,L-leucyl-glycinate)
Poly (L,L)-SUSL	Poly (sodium *N*-undecanoyl-L,L-seryl-leucinate)
Poly (L,L)-SUTL	Poly (sodium *N*-undecanoyl-L,L-threonyl-glycinate)
PROP	(±)-Propanolol
PTH	Phenyl thiohydration
PVA	Poly(vinyl alcohol)
SDVal	*N*-Decanoyl-L-valinate
TM	(±)-Temazepam

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