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Enantioselectivity of vesicle-forming chiral surfactants in capillary electrophoresis Role of the surfactant headgroup structure

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Abstract

Two vesicle-forming single-tailed amino acid derivatized surfactants sodium *N*-[4-*n*-dodecyloxybenzoyl]-L-leucinate (SDLL) and sodium *N*-[4-*n*-dodecyloxybenzoyl]-L-isoleucinate (SDLL) have been synthesized and used as pseudo-stationary phase in micellar electrokinetic chromatography to evaluate the role of steric factor of amino acid headgroup and hydrophobic/hydrophilic interactions for enantiomeric separations. The aggregation behavior of the surfactants has been studied in aqueous buffered solution using surface tension and fluorescence probe techniques. Results of these studies have suggested formation of vesicles in aqueous solutions. Microenvironment of the vesicle, which determines the depth of penetration of the analytes into vesicle was determined by fluorescence probe technique using pyrene, *N*-phenyl-1-naphthylamine (NPN), and 1,6-diphenyl-1,3,5-hexatriene (DPH) as probe molecules. Atropisomeric compounds (\pm) -1,1'-bi-2-naphthol (BOH), (\pm) -1,1'-binaphthyl-2,2'-diamine (BDA), (\pm) -1,1'-binaphthyl-2,2'-diylhydrogen phosphate (BNP) and Tröger's base (TB) and chiral compound benzoin (BZN) has been enantioseparated. The separations were optimized with respect to surfactant concentration, pH, and borate buffer concentration. SDLL was found to provide better resolution for BOH, BNP, and BZN. On the other hand, SDLIL offers better resolution for BDA. The chromatographic results have been discussed in the light of the aggregation behavior of the surfactants and the interaction of the solutes with the vesicles. © 2006 Elsevier B.V. All rights reserved.

Keywords: Capillary electrophoresis; Vesicle; Enantiomeric separation; Atropisomers

1. Introduction

Micellar electrokinetic chromatography (MEKC), introduced by Terabe et al. [1] has been proven to be a powerful tool for separation of a variety of analytes including pharmaceutical compounds, derivatized aminoacids, water and fat-soluble vitamins and herbicides [2–4]. However, due to electrophoretic mobility of the pseudo-stationary phase, MEKC is characterized by a limited migration time range. This creates a problem for separation of hydrophobic compounds as they have high partition coefficients and tend to have migration times close to the micellar migration time (t_{mc}) with high retention factors. In order to obtain optimum resolutions, the retention factors of the analytes have to be adjusted within the limited migration time range by changing the analytical parameters. For example,

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addition of organic solvents can alter the retention factors. However, it is not always possible to alter the retention factors by changing the analytical parameters due to the limitations in micelles themselves. This has led many researchers to seek alternative pseudo-stationary phases in MEKC. As discussed by Palmer [5], the pseudo-stationary phase in MEKC should have some characteristics to provide optimum resolution. They are: (i) it should be stable in the analytical conditions range to allow adjustment of retention factors, (ii) to minimize joule heating it should have low critical micellar concentration (cmc), (iii) it should have high electrophoretic mobility to provide a wide migration time range, (iv) should be monodisperse to provide high plate numbers, (v) should allow fast mass transfer for analytes between pseudo-stationary phase and aqueous phase, and (vi) phases with a wide variety of chemical structures should be available to provide chromatographic selectivity for a range of analytes. Clearly, due to the dynamic nature of the micelles, micellar phase cannot meet all the criteria. So, several alternative pseudo-stationary phases, such as cyclodextrin

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polymers [6], polyvinyl pyrrolidone [7], proteins [8], charged cyclodextrins [9], calixarenes [10], dendrimers [5,11] and polymerized micelle [5,12] have been employed to overcome the limitation of micellar phase. Recently, synthetic polymerized ionic surfactants with amino acid headgroups have been used as pseudo-stationary phase for enantiomeric separation of wide range of chiral compounds [13]. The use of polymeric surfactants overcome the limitation of dynamic nature of the micelles and provides reasonably wide migration range and better resolution [14,15]. More recently, vesicles have been used as an alternative pseudo-stationary phase in CE for achiral as well as chiral separations [16–24]. Vesicles are bilayer or multilayer aggregates bigger in size compared to the micelles and have higher surface charge density. Due to high electrophoretic mobility, vesicles provide wider migration window [16] and thus may have potential for separation of compounds with similar hydrophobicity. In the literature, there are only few reports of chiral separations using vesicles. We have recently reported the enantiomeric separation of atropisomeric binaphthyl derivatives and chiral compounds using spontaneously formed anionic vesicles of sodium N-[4-n-dodecyloxybenzoyl]-L-valinate (SDLV) as pseudo-stationary phase [20,21]. In another report, we have shown enantiomeric separation of some non-steroidal anti-inflammatory drugs using vesicles of a cationic surfactant (1R, 2S)-(-)-N-dodecyl-N-methylephedrinium bromide as pseudo-stationary phase [22].

Some well-known factors that govern the chiral recognition ability of a chiral selector are hydrophobic/hydrophilic, hydrogen bonding and electrostatic interactions, steric factors, and number of stereogenic centers. Although the effect of hydrogen bonding, hydrophobic interactions, and steric factor on enantioselectivity has been extensively studied for micelleforming monomeric and polymeric surfactants [25–35], there is no such report in the literature for vesicle-forming surfactants. We report here the role of steric factor, hydrophobic interaction and the number of stereogenic center on chiral selectivity of vesicle-forming surfactants. In this work, two single-tailed N-acylamino acid surfactants, sodium N-[4n-dodecyloxybenzoyl]-L-leucinate (SDLL) and sodium N-[4*n*-dodecyloxybenzoyl]-L-isoleucinate (SDLIL) (see Fig. 1 for molecular structure) having increasing steric factors near the stereogenic center were synthesized. These surfactants differ from the previously reported valine analog SDLV in that SDLL has an isobutyl group instead of an isopropyl group at the stereogenic center of the surfactant. SDLIL on the other hand is isomeric to SDLL and has a sec-butyl group with two stereogenic centers near the polar head group. The difference in bulkiness of the amino acid side chain is expected to impart different steric effects, which can modulate the degree of analyte interaction with the polar groups around the stereogenic center. Studies on the aggregation behavior of the surfactants in aqueous solution have suggested that the surfactant molecules self-assemble to form vesicular structures. The enantioselectivity for each of the above vesicle-forming surfactants has been optimized and compared for atropisomeric compounds (\pm) -1,1'bi-2-naphthol (BOH), (\pm) -1,1'-binaphthyl-2,2'-diamine (BDA), (\pm) -1,1'-binaphthyl-2,2'-diylhydrogen phosphate (BNP), and



Fig. 1. Molecular structure of the surfactants and analytes.

Tröger's base (TB) and chiral compound benzoin (BZN) (see Fig. 1 for molecular structures).

2. Experimental

2.1. Materials

Fused silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). The racemates and pure isomers of (\pm) -1,1'-bi-2-naphthol, (\pm) -1,1'-binaphthyl-2,2'-diamine, (\pm) -1,1'-binaphthyl-2,2'-diylhydrogenphosphate, Tröger's base, benzoin and dodecanophenone were purchased from Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA). The fluorescence probes, pyrene, 1,6-diphenyl-1,3,5hexatriene (DPH), and *N*-phenyl-1-naphthylamine (NPN) were obtained from Aldrich and recrystallized several times from acetone–ethanol mixture before use. Sodium tetraborate, sodium hydrogenphosphate, disodium hydrogenphosphate were purchased from SRL (Mumbai, India) and were used as received. The surfactants SDLL and SDLIL were synthesized and purified following the procedure reported earlier by us [20,21,36].

2.2. Apparatus

The instrument used was a Prince CE system (Prince Technologies, The Netherlands) equipped with an autosampler, a Lambda 1010 variable wavelength UV-vis absorbance detector (Bischoff, Leonberg, Germany), and a temperature control system. An uncoated fused-silica capillary tube having 50 µm internal diameter and 87 cm total length (effective length of 31.5 cm from inlet to detector) was used for separations. The instrument control and data acquisition were performed using a personal computer in conjunction with Dax 7.0 data acquisition and analysis software. The pH measurements were done with a digital pH meter, Orion 710A+ (Thermo Orion, USA). A Du Nüoy ring tensiometer (S.D. Hudson & Co., Kolkata, India) was used for surface tension measurements. The measurement of specific rotation was performed with a Jasco P-1020 digital polarimeter. The steady-state fluorescence spectra were measured on a Spex Fluorolog model FL3-11 spectrofluorometer. Fluorescence anisotropy measurements were performed according to the procedure described in reference [36] on a Perkin Elmer LS-55 luminescence spectrometer equipped with polarizer and thermostated cell holder. Temperature was controlled by use of Thermo Neslab RTE-7 water circulating bath.

2.3. Methods

The untreated capillary was activated by first purging with 1 M NaOH for 60 min and then 0.1 M NaOH for additional 60 min. Running buffer solutions were prepared by dissolving the surfactant in desired concentrations (30-70 mM) of borate buffer. The pH was then adjusted with either dilute sodium hydroxide or dilute HCl. The surfactant concentration range investigated was 0.5–6 mM. All the running buffers were filtered through a membrane filter of 0.22-µm pore size (Millipore, Bedfold, MA, USA) and degassed in a Bandelin Sonorex, model RK 100 H ultrasonic bath (Bandelin Electronic, Berlin, Germany) for 5 min prior to use. For MEKC separations, the capillary was treated successively with 0.1 M NaOH, water and running buffer for 5 min each before sample injection. Between two successive runs the capillary was rinsed with water and buffer for 5 min each. UV detection was performed at wavelength of 230 nm. The surfactants have absorption maximum at 255 nm. At 230 nm, the molar absorptivity is relatively low $(4500 \text{ Lmol}^{-1} \text{ cm}^{-1})$ in comparison to the samples $(1,05,000 \text{ Lmol}^{-1} \text{ cm}^{-1} \text{ for BOH})$. So no detection problem was observed at 230 nm while using up to 7 mM of surfactants. Injection was performed by pressure method (20 mbar, 0.02 min). Stock solutions of the racemic samples were prepared in methanol at a concentration of 2 mg/ml.

The final sample solution for enantiomeric separation was prepared by diluting the stock solutions to 0.2 mg/ml with borate buffer. The final sample also contained 10% (v/v) methanol for BOH, BNP and 40% for BDA, BZN and TB. The vesicle mobility was measured using the procedure reported by Williams and Vigh [37] with slight modifications. The observed vesicle mobility ($\mu_{vs,obs}$) can be measured using the reported procedure [37] without measuring μ_{eo} (mobility of electroosmotic flow (EOF)) in the same run. The procedure was slightly modified to measure $\mu_{vs,eff}$ (effective vesicle mobility) and μ_{eo} in a single run. This was necessary because, it was found that the values of $\mu_{\rm vs,eff}$ and $\mu_{\rm eo}$ are very close. A slight variation in μ_{eo} (which is possible between two successive runs) would affect the observed mobility of the vesicles. The detail procedure is described under "supplementary materials". Two different hydrophobic compounds dodecanophenone and 2-ndodecyloxynaphthalene were used as the vesicle markers to confirm the vesicle migration time. The separations were carried out at ambient temperature ($\sim 25 \,^{\circ}$ C).

2.4. Calculations

Chiral resolution (R_s) was calculated using the method involving peak width at half-height [21,34,38].

$$R_{\rm s} = \frac{(2.35/2)(t_{\rm r2} - t_{\rm r1})}{W_{50(1)} + W_{50(2)}} \tag{1}$$

where t_{r1} and t_{r2} are the migration time and $W_{50(1)}$ and $W_{50(2)}$ are the peak widths at 50% height of the 1st and 2nd isomer, respectively. The retention factor, *k* for charged and neutral analytes was calculated using the equation [39]:

$$k = \frac{t_{\rm r}(1+\mu_{\rm r})-t_{\rm o}}{t_{\rm o}(1-t_{\rm r}/t_{\rm vs})}$$
(2)

where t_0 and t_{vs} are the migration time of EOF marker (methanol) and vesicle marker, respectively, and $\mu_r = \mu_{ep}/\mu_{eo}$ is the relative electrophoretic mobility, i.e., the electrophoretic mobility (μ_{ep}) of the analyte (positive, zero, or negative) relative to the coefficient of electroosmotic flow (μ_{eo}). For the neutral analytes, $\mu_r = 0$ and for negatively charged analytes, $\mu_r < 0$. The μ_{ep} for the charged analytes was measured in the separation buffer system under CZE conditions (buffer with no vesicles). The vesicles have high electrophoretic mobility in the direction of anode and therefore have very high t_{vs} values. Hence, the t_{vs} values were measured using the method described by Williams and Vigh [37]. The mobility of EOF and effective vesicle mobility ($\mu_{eff,vs}$) were measured in a single run. The μ_{eo} , $\mu_{eff,vs}$, and t_{vs} values thus obtained for SDLL and SDLIL in 50 mM borate buffer, pH 9.7 using dodecanophenone are tabulated in Table 1. In order to compare the results with micelle-forming surfactant systems, the mobility of EOF and effective mobility of micelles of anionic surfactant sodium dodecylsulfate (SDS) was also determined in the same buffer system. The results are included in Table 1. The data in Table 1 clearly show that the vesicles have very high electrophoretic mobility compared to the SDS micelles and hence the vesicular system provides wider migration window compared to the micellar system. Since the value of t_{vs} is very large compared

Table 1

Mobility of EOF (μ_{eo}), effective mobility of pseudo-stationary phase ($\mu_{eff,psp}$), and migration time of the pseudo-stationary phase (t_{psp}) for SDLL, SDLIL, and SDS surfactants

Aggregate	$ \mu_{eo} $ (10 ⁻⁴ cm ² V ⁻¹ s ⁻¹)	$ \mu_{\rm eff,psp} $ (10 ⁻⁴ cm ² V ⁻¹ s ⁻¹)	t _{psp} (h)	
SDLL vesicles	3.52	-3.50	25.3	
SDLIL vesicles	3.513	-3.49	22.0	
SDS micelles	3.37	-1.92	0.35	

to t_r , the term t_r/t_{vs} is negligible. The Eq. (2) for retention factor then becomes:

$$k = \frac{t_{\rm r}(1+\mu_{\rm r}) - t_{\rm o}}{t_{\rm o}}$$
(3)

The selectivity (α) was calculated from the ratio of retention factors.

3. Results and discussion

3.1. Aggregation behavior

In our previous reports [20,21], we have shown that SDLV surfactant produces vesicular structures in aqueous solution. Since SDLL and SDLIL are structurally similar, they are also expected to form bilayer vesicles in water. The aggregation behavior of SDLL and SDLIL were studied in 50 mM borate buffer, pH 9.7. The critical vesicle concentration (cvc) was determined by surface tension and fluorescence probe methods. In surface tension studies, the break point of the surface tension versus log (concentration) plot gives the cvc value. On the other hand, NPN was used as the extrinsic fluorescence probe molecule to determine the cvc by fluorescence probe method. The shift in emission maximum $(\Delta \lambda)$ of the fluorescence emission spectrum of NPN in presence of the surfactants with respect to that in water was plotted against the surfactant concentration. The inflection point of the plot gave cvc value. The cvc values along with other physicochemical properties of the surfactants are tabulated in Table 2. The corresponding values for SDLV are also included in the table for comparison purposes. The cvc values obtained by surface tension and fluorescence probe methods are closely similar to each other. The cvc values of the surfactants are almost two orders of magnitude lower than other N-acylamino acid surfactants reported so far [40]. It is important to note that the cvc values of SDLL and

Table 2

Critical vesicle concentration (cvc), surface area per surfactant molecule (A_m) , shift of emission maximum $(\Delta\lambda)$ of NPN, polarity ratio (I_1/I_3) , and fluorescence anisotropy (r) of DPH of SDLL, SDLIL, and SDLV vesicles in 50 mM borate buffer, pH 9.7

Surfactant	$\operatorname{cvc} \times 10^5 (\mathrm{M})$	$A_{\rm m}$ (nm ² /molecule)	$\Delta\lambda$ (nm)	I_1/I_3	r
SDLL	0.92 (1.5)	0.75	36.0	1.01	0.115
SDLIL	1.0 (1.7)	0.58	36.0	1.03	0.093
SDLV	2.0 (2.7)	0.62	32.0	1.06	0.112

Values within the bracket are obtained by fluorescence probe method.

SDLIL surfactants are lower than that of SDLV. This is due to the bulky headgroup of the former surfactants, which reduces ionic repulsion and thereby facilitates aggregate formation. The minimum surface area per surfactant head group (A_m) , calculated from the slope of the linear part of the surface tension versus log (concentration) plot by use of Gibbs adsorption equation [41] are less than $1.0 \text{ nm}^2/\text{molecule}$. This means that the hydrophobic chains of the surfactant molecules are tightly packed in the bilayer self-assembly. The lower values of cvc and A_m are indicative of vesicle formation in buffered solution. It should be noted that the A_m values of the surfactants are in the order SDLL > SDLIL. This may suggest that the amino acid side chain of the headgroup of SDLIL surfactant being longer folds back into the hydrocarbon core of the bilayer assembly. This is substantiated by the results of fluorescence probe studies.

The fluorescence probe studies were performed in order to understand the nature of the microenvironment of the aggregates. We measured the fluorescence spectra of pyrene and NPN in presence of the surfactants at concentrations above their respective cvc values to determine micropolarity. The intensity ratio of the first and third vibronic bands (I_1/I_3) of pyrene fluorescence is strongly dependent on the polarity of the environment in which it is solubilized [42]. Low value of I_1/I_3 relative to that in water (1.71) indicates less polar environment. The polarity ratio, I_1/I_3 of pyrene was therefore measured in 2 mM buffered aqueous solutions of SDLL and SDLIL. The data are included in Table 2. It can be observed that the polarity of the solubilization site of pyrene is slightly more in SDLV compared to that of SDLL and SDLIL surfactant self-assemblies. This might be due to the difference in hydrophobicity of the amino acid side chain of the surfactant molecules. We have also used NPN as probe molecule to determine micropolarity since they are known to be soluble in the interfacial region of the aggregates. NPN has been used as polarity probe by many researchers because it is poorly soluble in water and its fluorescence emission spectrum exhibits a large blue shift with a concomitant rise in intensity in going from a polar to nonpolar environment [43]. We have measured the fluorescence spectra of NPN in presence of all the three surfactants SDLV, SDLL, and SDLIL. The spectral shifts $(\Delta \lambda)$ relative to that in pure water are included in Table 2. It can be seen that $\Delta\lambda$ values are in the order SDLIL \approx SDLL > SDLV. This means that the local polarity of the vesicle-water interface increases in the order SDLIL \approx SDLL < SDLV. This supports our conclusion that the amino acid side chain of the surfactant headgroup in SDLL and SDLIL folds back into the hydrocarbon core, which results in a tighter packing of the hydrocarbon chains through enhanced hydrophobic attractions. Consequently, the degree of water penetration is decreased and thus polarity at the interface is reduced. The steady-state fluorescence anisotropy (r) values (Table 2) of the DPH probe in the self-assemblies of the surfactants also support this. The relatively large values of r indicate that the microenvironment of DPH is more viscous compared to bulk water. In fact, the values of r are comparable to that of liposomes formed by lecithin (0.098) [44]. Such highly non-polar and viscous microenvironment can therefore be attributed to the formation of bilayer membrane structures by these surfactants. In fact, in our earlier publications [20,21] we have shown that the microscopic images of aqueous SDLV solution exhibit spherical vesicles.

3.2. Enantiomeric separations

3.2.1. Optimization of separation parameters

Three analytical parameters, i.e., buffer pH, buffer concentration, and surfactant concentration were optimized for the enantiomeric separation of the test analytes. Based on our previous experience, borate buffer was found to be a suitable BGE for the optimizations. The systematic procedure for optimization of pH for the enantiomeric separation involved use of either 2 mM (for BOH, BDA and TB) or 4 mM (for BNP and BZN) of SDLL or SDLIL in 50 mM borate buffer in the alkaline pH range of 8.5-10.3. The applied voltage was 15 kV. From the pH optimization study, it was found that the enantiomers of BOH and BDA were best resolved at pH 9.7 and BNP, BZN, and TB were best resolved at pH 10.3. Further optimization experiments were carried out under these optimum pH conditions by varying the borate buffer concentration. The optimum borate buffer concentration for BOH, BNP and TB was 50 mM and for BZN it was 60 mM. For BDA though 50 mM borate buffer offered better resolution, the migration time was too long (55 min). Therefore, 30 mM borate buffer was used for further studies. These optimized conditions are same as found by us for these analytes using SDLV as chiral selector [21]. The above-optimized conditions of pH and buffer concentration were used to study the effect of surfactant concentration for the enantiomeric separation of each individual analyte. Surfactant concentrations in the range of 0.5-6.0 mM were used. The effect of SDLL concentration on resolution (R_s) for different analytes is shown in Fig. 2. Since there was no major change in the structure of the aggregates formed in the studied surfactant concentration range, the selectivity (α) practically remains unaltered. From the figure it can be found that for binaphthyl derivatives, resolution increases with SDLL concentration, reaches a maximum and then decreases. On the other hand, resolution increases with increase of surfactant concentration up to 4 mM and then remains almost constant for BZN. The optimum SDLL concentration for BOH and BDA is 2 mM whereas for BNP and BZN it is 4 mM. TB could not be baseline separated using SDLL. The best separation could be obtained using 4 mM SDLL and the resolution obtained was $0.96 (\alpha = 1.02)$. The applied voltage was 15 kV for all the experiments except for TB for which it was 20 kV. It is to be noted that application of higher voltage (25-30 kV) reduces the migration time of the analytes. However, this often resulted in distortion of the baseline due to the joule heating. Therefore, all the separations were carried out using applied voltage of 15-20 kV. The optimized electropherograms for different analytes using SDLL is shown in Fig. 3. Similar concentration optimization studies for SDLIL were also carried out for each individual analyte. The data are presented in Fig. 4. Unlike SDLL, 1 mM of SDLIL was needed for the optimum separation of BOH. Also in the working concentration range (0.5–7.0 mM) of SDLIL, the resolution for BNP and BZN increases continuously with increase of surfactant concentration. As in the case of SDLL, the selectivity values, within the experimental error limit, remain



Fig. 2. Effect of SDLL concentration on resolution of different chiral analytes. Separation conditions: 30 and 50 mM borate buffer pH 9.7 for BDA and BOH, 50 and 60 mM borate buffer pH 10.3 for BNP and BZN. Applied voltage 15 kV.

unchanged. It can be noted that the minimum SDLIL concentration required for enantiomeric separation of BNP and BZN are 2 and 3 mM, respectively. The optimum SDLIL concentration for BDA is same as that of SDLL. TB was better enantioseparated using SDLIL than SDLL. The resolution obtained for TB using 4 mM of SDLIL was 1.11 ($\alpha = 1.03$). The electropherograms for the optimized enantiomeric separation of the racemates using SDLIL are shown in Fig. 5. The optimum selectivity obtained for BOH (1.15 & 1.09), BDA (1.13 & 1.15) and BNP (1.09 &1.10) using these vesicle-forming surfactants SDLL and SDLIL, respectively, are higher than the reported values obtained by Rizvi et al. [34] and Thibodeaux et al. [35] using corresponding polymeric alkenoxy amino acid derivatized and polymeric N-undecenoyl-L-amino acid derivatized surfactants, respectively. Also, SDLL is a better chiral selector for BOH and BNP compared to the micelle-forming polymeric dipeptide surfactants, in which L-leucine is in the outer side (C-terminal position) of the dipeptide linkage [28,32,33]. However, Billiot et al. have reported higher resolution values for BDA using those polymeric dipeptide surfactants [33].

3.2.2. Role of surfactant headgroup structure on enantioselectivity

In an attempt to identify the factors which influence the chiral selectivity of the vesicle-forming surfactants, the α and R_s values obtained for different analytes using a particular chiral selector (SDLL or SDLIL) and the results obtained for a particular ana-



Fig. 3. Electropherograms for optimized enantiomeric separation of (A) BOH, (B) BDA, (C) BNP, (D) BZN and (E) TB using SDLL. MEKC condition: 30 and 50 mM borate buffer pH 9.7 with 2 mM SDLL for BDA and BOH, 50 mM borate buffer pH 10.3 with 4 mM SDLL for BNP and TB, and 60 mM borate buffer pH 10.3 with 4 mM SDLL for BZN. Applied voltage 15 kV for all except TB (20 kV). Separation capillary: total length 87 cm, effective length 31.5 cm (50 μ m ID); detection wavelength 230 nm, temperature ~25 °C.

lyte using different chiral selectors were compared. The results obtained for the analytes employed in this study are summarized in Table 3. The values (except those obtained with SDLV surfactant) presented in the table are the mean value of three



Fig. 4. Effect of SDLIL concentration on resolution of the chiral analytes. Separation conditions are same as in Fig. 2 except the applied voltage of 25 kV for BOH, 20 kV for BDA and BNP.

independent measurements. The corresponding reproducibility value has been indicated. The related data for the same analytes obtained using SDLV [21] surfactant have been also included in the table for comparison purposes. A close look at the R_s values (Table 3) of the racemates one can easily recognize the effect of surfactant headgroup structure on the enantiomeric separations. However, α is preferred over R_s for comparison because the latter parameter is affected by efficiency and retention factors in addition to selectivity. It is believed that chiral separation is a result of three major factors such as, hydrophobic, hydrogenbonding, and electrostatic interactions that dictate the degree of interaction of the analyte with the chiral selector. However,

Table 3

Retention factor (k	 selectivity 	(α) and reso	lution (R_c)	values for	various analy	vtes with SD	LL SDLIL	and SDLV	surfactant
recention factor (A	c), selectivity	(u) and 1030	fution (ns)	ranaes ioi	various anal	ytes with DD	LL, DLLL	, and DDL	Surfactant

Recention ratio (κ_i , selectivity (α' and resolution (κ_s) values for various analytes with SDEE, SDEE, and SDEV surfactants								
Surfactant		BDA	BOH	BNP	ТВ	BZN		
SDLL	k_1	4.55 ± 0.05	1.96 ± 0.13	0.55 ± 0.03	1.82 ± 0.15	0.53 ± 0.04		
	α	1.13 ± 0.015	1.15 ± 0.015	1.09 ± 0.005	1.02 ± 0.007	1.11 ± 0.01		
	R _s	2.23 ± 0.02	4.30 ± 0.66	1.56 ± 0.22	0.95 ± 0.07	2.30 ± 0.15		
SDLIL	k_1	5.63 ± 0.94	1.82 ± 0.10	0.43 ± 0.12	3.61 ± 0.52	0.43 ± 0.09		
	α	1.15 ± 0.008	1.09 ± 0.01	1.10 ± 0.005	1.03 ± 0.01	1.08 ± 0.01		
	R _s	2.96 ± 0.26	2.06 ± 0.05	1.40 ± 0.03	1.10 ± 0.08	1.12 ± 0.05		
SDLV ^a	k_1	4.64	2.97	0.67	2.02	0.65		
	α	1.09	1.11	1.14	1.04	1.07		
	$R_{\rm s}$	1.98	5.23	2.20	1.07	1.61		

Separation conditions: 50 mM borate buffer pH 9.7 with 2 mM surfactant for BDA and BOH; 50 and 60 mM borate buffer pH 10.3 with 4 mM surfactant for BNP and BZN; 50 mM borate buffer pH 10.3 with 4 mM surfactant for TB. Applied voltage +15 kV.

^a Taken from reference [21].

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Fig. 5. Electropherograms for enantiomeric separation of chiral analytes using SDLIL. MEKC condition: SDLIL concentration of 1 mM for BOH, 2 mM for BDA, 4 mM for BNP and TB, 7 mM for BZN. Applied voltage 15 kV. All other conditions are same as in Fig. 3.

the role of steric factor should not be ruled out. To evaluate the role of hydrophilic/hydrophobic interactions on enantioselectivity, the retention factors, k_1 , obtained for the binaphthyl derivatives using a particular surfactant system were considered. The binaphthyl derivatives were chosen for comparison because they are structurally similar but differ from each other in degree of ionization at a particular pH. Since BDA, BOH, and BNP have pK_a values close to that of 2-naphthylamine $(pK_a = 4.1)$ [45], 2-naphthol $(pK_a = 9.46)$ [45], and diphenylhydrogenphosphate ($pK_a = 1.12$) [46], respectively, at the working pH of enantiomeric separations (pH 9.7 for BDA, BOH and 10.3 for BNP), BDA is neutral, BOH is partially ionized and BNP is completely ionized [28,34]. So, BDA is expected to interact strongly with the hydrophobic bilayer of the vesicles. Due to negatively charged surface of the vesicles, BOH will interact less and the BNP will interact least owing to the electrostatic repulsions. Thus, the partition coefficients of the analytes are in the order BDA>BOH>BNP. This is reflected in the k_1 values for the three analytes using any of the chiral selectors (see Table 3). The same explanation also hold true for the chiral compound BZN. BZN being less hydrophobic compared to binaphthyl derivatives interact weakly with the vesicles and have lowest retention factor. An interesting observation made

from the data in Table 3 is that there is no direct relationship between the k_1 and α values. For example, the k_1 value for BDA is much higher than that of BOH using all the three chiral selectors, but the α value is slightly lower for BDA when SDLV and SDLL are the chiral selectors. Similarly, the k_1 values for BNP and BZN are very low compared to that of BOH and BDA, but the α values are comparable. This indicates that hydrophobic/hydrophilic interaction has major influence on the retention factors but seems to have less pronounced effect on chiral selectivity of the vesicle-forming surfactants. However, Bicchi et al. [47] have emphasized the importance of hydrophobic interactions on chiral recognition in their study involving enantiomeric separation of y-lactones and other compounds using derivatized cyclodextrins as chiral selectors. The other major force that could affect the enantioselectivity of the chiral surfactants under investigation is the steric interactions near the stereogenic center of the surfactant headgroup. To evaluate this, the α and R_s values obtained for the analytes using surfactants SDLV, SDLL, and SDLIL having increasing order of bulky groups near the stereogenic center were also compared. The data in Table 3 indicate that α for BDA decreases in the order SDLIL > SDLV and the order for BNP is SDVL > SDLL \approx SDLIL. The stereogenic center is less sterically hindered in SDLV and hence BDA penetrates deeper into the hydrocarbon core of the vesicle, which weakens interactions with the stereogenic center. This is reflected by the lowest α -value. Because of the steric hindrance of the sec-butyl group in SDLIL, BDA molecule is unable to penetrate deeper into the bilayer core and as a result its interaction with the stereogenic center is enhanced as manifested by the large α -value. In SDLL surfactant, BDA has intermediate selectivity. The effect of steric factor on chiral selectivity can also be observed for BNP. The α value for BNP is more in SDLV and less in SDLL and SDLIL. This means that the selectivity decreases due to increase of steric factors. Billiot and Warner [33] have, however, reported increase of chiral selectivity for BNP with increase of steric factors near the stereogenic center in their study using micelle-forming sodium undecenoyl-L-amino acidate as well as corresponding dipeptide surfactants. Rizvi et al. have also reported better resolution for BNP using L-leucine derived surfactant than corresponding L-isoleucine derived surfactant in their study using polymeric alkenoxy amino acid surfactant systems [34]. The contradictory behavior of the vesicular system for BNP can be attributed to the higher surface charge density of the vesicles in comparison to the micelles. Strong electrostatic repulsions compared to micelles force the BNP molecule to stay near the vesicle surface. The increase of steric factors further retards the penetration into vesicle bilayer that results in lower enantioselectivity. Among the three chiral selectors, SDLL provides highest α value for BOH and BZN. The slightly lower value of α for BOH and BZN in SDLIL is perhaps due to the presence of two stereogenic centers the relative orientation of which does not favor intercalation of the molecules in the vesicle. This is in contrast to that reported by Thibodeaux et al. [35] who have observed higher enantioselectivity for four out of five compounds including BDA and BNP using polymeric sodium N-undecenoyl-L-isoleucine surfactant in comparison to other amino acid based polymeric surfactants. This may again be ascribed to the difference in type of aggregate formed, i.e., micellar versus vesicular structures.

4. Concluding remarks

In summary, two vesicle-forming surfactants SDLL and SDLIL were used as chiral selectors for enantiomeric separation of atropisomeric compounds BOH, BNP, BDA, TB and chiral compound BZN. It has been shown that SDLL is a better chiral selector for BOH and BZN, where as SDLIL acts as a better chiral selector for BDA. The selectivity observed for BOH, BNP and BDA with these vesicle-forming surfactants are better than the reported values using different polymeric L-leucine and L-isoleucine derivatized surfactant systems. Comparison of the results of this study with our earlier published results for SDLV has helped to identify the factors responsible for chiral recognition of the vesicle forming surfactants. The structure of the surfactant headgroup has been shown to have significant effect on enantioselectivity of the chiral surfactants. The number of stereogenic centers seems to decrease enantioselectivity in the case of BOH and BZN. Although the chiral recognition mechanism for vesicular system appears to be the same as that of the polymeric micellar systems, the different trend in the values of resolutions observed for some analytes (for example BNP) is due to the difference in the nature of aggregate formed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2006.06.036.

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