Vesicles as pseudostationary phase for enantiomer separation by capillary electrophoresis

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Abstract

A vesicle-forming single-tailed amino acid derivatized surfactant, sodium N-(4-n-dodecylxyloxybenzoyl)-L-valinate (SDLV) has been used as a chiral selector in micellar electrokinetic chromatography to study the molecular recognition of sterically hindered atropisomeric compounds (+)-(−)-binaphthol, (+)-(−)-binaphthyl diamine, (+)-(−)-binaphthol phosphate, Tröger’s base and the chiral compound benzoin (BZN). The aggregation behavior and microstructure of the surfactant were studied in separation buffer. The amphiphile was found to form bilayer vesicles in dilute aqueous solutions. The chromatographic separation of enantiomers by use of large liposome-like vesicles spontaneously formed by the amphiphile was explored. The separations were optimized with respect to voltage, pH, and surfactant and buffer concentrations. The resolutions obtained for the above mentioned racemates by use of SDLV vesicles as chiral selectors are higher compared to those reported for other chiral surfactant monomers. The results have been discussed in light of the aggregation behavior of the amphiphile in buffered aqueous solutions.

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Keywords: Capillary electrophoresis; Vesicles; Enantiomer separation; Atropisomers

1. Introduction

Capillary electrophoresis (CE) has recently emerged as a powerful technique for enantiomer separation. Many enantiomer separations have been reported by addition of “chiral selectors” to the buffer medium in capillary zone electrophoresis (CZE). The most commonly used chiral mobile phase additives include host-guest additives, such as cyclodextrins (CD) [1–3] and crown ethers [4-5]. Other background electrolyte (BGE) additives used are chiral ligand exchange reagents [6], macrocyclic antibiotics [7,8], heparin [9,10] and dextran sulfates [11]. A new class of compounds N-acylcalix-[4]-areneaminocacid derivatives has been also introduced by Pena et al. [12]. Successful enantiomer separations were also obtained by use of sodium dodecyl sulfate (SDS) in combination with chiral selectors e.g. cyclodextrin, maltose or bile salts [13,14]. For enantiomer separations by CE Terabe et al. [15] introduced a powerful mode often referred to as micellar electrokinetic chromatography (MEKC) that involved the use of a surfactant above its critical micellar concentration (cmc) in the BGE. Chiral surfactants such as glucopyranoside based phosphate and sulfate surfactants [16], digitonin [17], saponins [18] and amino acid derivatized surfactants sodium N-dodecanoyl-L-valinate [19–21] and sodium N-dodecoxycarbonylvalinate (DDCV) [22,23] demonstrated the utility of chiral MEKC approach. However, one of the disadvantages of MEKC using these surfactants is low migration range that affects the resolution. One approach to increase migration range is use of polymeric surfactants as pseudostationary phase in MEKC. In a review [24], C.P. Palmer has discussed the advantages of the use of polymeric surfactants in MEKC. More recently, polymeric dipeptide surfactants [25,26] and polymeric alkenoxy aminoacid surfactants [27,28] have been used for enantioseparations. An alternative of polymerized surfactants would be vesicles, which are bigger in size compared to that of normal micelles. Hong et al. [29] have used vesicles formed from

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SDS and n-dodecyltrimethylammonium bromide (DTAB) as pseudostationary phase in electrokinetic chromatography to separate n-alkylphenones and found that the vesicular system provides about two times wider migration window, higher polar group selectivity, retention time and efficiency as compared to the SDS micellar system. Pascoe et al. [30] observed improvements in migration range and pH stability using CTAB/DDCV vesicles for hydrophobicity determination of some basic pharmaceutical drugs. To our knowledge there have been only a couple of reports on the successful use of vesicles as a pseudostationary phase in enantiomer separation by MEKC [31,32]. In a recent communication [31] we have demonstrated enantioseparations of two atropisomeric compounds using vesicle-forming single-tailed N-acylamino acid derivatized surfactant, sodium N-(4-n-dodecylxybenzoyl)-l-valinate (SDL V) as chiral selector in MEKC. We examine here in detail the chiral selectivity of SDL V for other atropisomers and enantiomers. SDL V differs from other N-acylamino acid surfactants in that it contains an aromatic ring and an ether linkage in addition to the amide bond and the hydrocarbon chain. It is believed that these types of functional groups are useful for providing the multiple interactions necessary for chiral discrimination. It will be shown that SDL V offers baseline separation of the atropisomeric compounds (+)-1,1′-bi-2-naphthol (BOH), (+)-1,1′-binaphthyl-2,2′-diamine (BDA), (+)-1,1′-binapthyl-2,2′-diyldihydrogenphosphate (BNP), and 2,8-dimethyl-6H,5,11-methanobenzol[b,f]j [1.5] diazozone (Tröger’s base, TB) as well as for the enantiomers of benzoin (BZN) at very low concentrations. A brief discussion on the aggregation properties and microstructures of the surfactant in aqueous buffer solutions has also been included.

2. Experimental

2.1. Chemicals and reagents

The racemates (+)-1,1′-bi-2-naphthol, (+)-1,1′-binaphthyl-2,2′-diamine, (+)-1,1′-binapthyl-2,2′-diyldihydrogenphosphate, Tröger’s base, benzoin, individual enantiomers and dodecanophene were purchased from sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA). The fluorescence probes, pyrene and 1,6-diphenyl-1,3,5-hexatriene (DPH) were obtained from Aldrich and recrystallized several times before use. Sodium tetraborate, sodium hydrogen phosphate and disodium hydrogen phosphate were purchased from SRL (Mumbai, India) and were used as received. Fused silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA).

The surfactant SDL V was synthesized as reported elsewhere [31]. Briefly, 4-dodecyl-oxybenzoic acid was first synthesized from 4-hydroxybenzoic acid and 1-bromododecane and purified according to the reported procedure [33]. The coupling of l-valine and 4-dodecyl-oxybenzoic acid was made via the formation of NHS ester in the presence of DCC [34]. The acid was recrystallized two to three times from ethanol–water mixture to eliminate DCU, a byproduct of the reaction. The sodium salt was prepared by stirring equimolar mixtures of sodium methoxide and N-(4-n-dodecylxybenzoyl)-l-valine in dry methanol for 6–8 h. The salt was obtained after evaporation of the solvent. It was recrystallized from ethanol–water until it was free from unreacted acid. The structure was confirmed by IR and 1H NMR spectra. The specific rotation of the surfactant was +26° (c = 1%, methanol). Molecular structure of SDL V is shown as an inset of Fig. 1.

2.2. Apparatus

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 an inbuilt temperature control system was employed for all separations performed in this study. Data was collected using a personal computer in conjunction with DAX 7.0 data acquisition and analysis software. The pH was measured by means of a digital pH meter model pH5652 (Electronics corporation of India limited, Calcutta, India) with a glass electrode. A Du Nuoy ring tensiometer (S.D. Hudson & Co., Kolkata) was used for surface tension measurements. The fluorescence measurements were performed on a Perkin Elmer LS-55 luminescence spectrometer equipped with a filter polarizer and thermostated cell holder. The temperature was controlled by use of circulating bath (Neslab, RTE 7).

2.3. Preparation of buffer solutions and samples

The borate and phosphate buffers were made by use of Milli-Q (18 MΩ resistivity) water. Run buffer solutions were prepared by dissolving the surfactant in desired concentra-
ions (20–60 mM) of borate buffer. The pH was then adjusted with either dilute sodium hydroxide or dilute HCl. The surfactant concentration ranges investigated were 0.5–6.0 mM. All the run buffers were filtered through a membrane filter of 0.22 µm pore size (Millipore, Bedford, MA, USA) and degassed in a Bandelin Sonorex (Model RK 100 H) ultrasonic bath for 5 min prior to use. Stock solutions of the racemic samples were prepared in methanol at a concentration of 2 mg/mL. The final sample solution for enantioseparation was prepared by diluting the stock solutions to 0.2 mg/mL with the buffer solution. The final sample contained 10% (v/v) methanol.

2.4. Electrophoretic technique

Electrophoretic separations were carried out with uncoated fused-silica capillaries having 50 µm internal diameter and 87 cm length (31.5 cm from inlet to detector). The untreated capillary was activated by first purging with 1 M NaOH for 30 min and then 0.1 M NaOH for additional 60 min. For MEKC separations, the capillary was treated successively with 0.1 M NaOH, water, BGE and run buffer for 5 min each before injection of a new sample. However, between two successive runs of the same sample, the capillary was rinsed with water, and run buffer only for 5 min each. The separations were carried out under constant applied voltage (15–25 kV). UV detection was performed at wavelength of 230 nm. The migration time of vesicle was calculated and was found to be 40.6 h. So the term (δ/ε0) is negligible and Eq. (2) reduces to

$$ k = (t_0 - t_1)/t_2$$

where $t_1$ and $t_0$ represents retention time of electroosmotic flow marker (methanol) and vesicle marker, respectively. The vesicle is negatively charged with higher charge density and hence migrates towards the anode at a velocity much larger than the micelles. The observed mobility of the vesicle was measured to be $1.25 \times 10^{-6} \text{cm}^2 \text{v}^{-1} \text{s}^{-1}$ and that of EOF was $4.0 \times 10^{-6} \text{cm}^2 \text{v}^{-1} \text{s}^{-1}$. From the observed mobility, the migration time of vesicle was calculated and was found to be 40.6 h. The cvc value in buffer is slightly lower than the reported value in water ($2.5 \times 10^{-6} \text{M}$) [31]. Since the solutions used for electrokinetic separations were made in buffers having different ionic strength than pure water, we have studied the aggregate behavior of SDBV in aqueous solution [37]. The cvc value in buffer is slightly lower than the reported value in water ($2.5 \times 10^{-6} \text{M}$) [31]. The formation of vesicles is suggested by the fluorescence probe studies using pyrene and DPH respectively, as polarity and membrane fluidity probe. The ratio $(I_1/I_3)$ of the first to the third vibronic bands in the fluorescence spectrum of pyrene was measured at surfactant concentration of 2 mM. The $I_1/I_3$ (1.06) value suggests that the dielectric constant ($\varepsilon$) of the microenvironment is about 5.62 [39]. The steady-
state fluorescence anisotropy (r) of DPH was also measured in the same solution at 30°C. The anisotropy value (0.112) is quite large compared to micelles. Similar values of r have also been reported for liposomes formed by phospholipids [40]. The values of I(3)/I(1) and r can be correlated to the microenvironment of pyrene and DPH probes, respectively. The larger value of r and the smaller value of I(3)/I(1) compared to the corresponding quantities in water indicate that the microenvironment of the probes is nonpolar as well as highly viscous than water suggesting formation of vesicular aggregates in buffer solutions. That the surfactant forms vesicles in buffer solution is further demonstrated by the transmission electron micrograph depicted in Fig. 1. The microscopic picture clearly shows the presence of closed spherical vesicles having internal diameter in the range 30–70 nm. It was observed that upon increasing the surfactant concentration large vesicles were formed. This was expected in view of the characteristics of the amphiphile. 

3.2. Enantioseparation

The low cvc value makes SDL V a very good candidate for use as a chiral selector in MEKC as it can be used at low concentration that makes the run buffer less viscous and less conducting. Further, the hydrophobic part of the vesicle bilayer provides hydrophobic–hydrophilic discrimination power. The vesicular solutions used for separation were 2–3 h old. However, no significant chromatographic differences were observed when solutions with different ageing period were used. Also no changes in chromatographic properties were observed when the surfactant solutions were filtered and reused after 1–2 days. It should be noted that the surfactant precipitated from buffer solution at pH <7.0 upon standing. Therefore, the separation was performed at pH ≥7.0. Borate buffer in the concentration range of 20–60 mM was used for the enantiomer separations as it had low conductivity and high buffer capacity around pH 9.2.

3.2.1. Atropisomeric compounds

As previously reported by us [31], 2 mM of SDL V is required for baseline separation of the enantiomers of BOH and BNP. In this study, we have separated individual enantiomers of BOH with an optimized SDL V concentration of 2 mM and those of BNP with 5 mM SDL V in 50 mM borate buffer. The optimum separation of the enantiomers of BDA was obtained by use of the same condition as for BOH. The electropherograms for the optimized enantiomeric separation of the racemates are shown in Fig. 2A–C. The migration order was confirmed by spiking technique. The (R)+BOH and (S)+BDA enantiomer migrates slower. This suggests that the (R)+BOH, (R)+BDA and (S)+BNP has a higher affinity for the SDL V vesicles. This migration order is same as that obtained by Warner and co-workers [24,26] by use of polymeric l-valine surfactants. The resolution obtained for BOH (Ri = 5.23), BDA (Ri = 1.98), and BNP (Ri = 2.71) using SDL V vesicles is higher than the corresponding Ri values reported by other research groups using different micelle-forming surfactants including some polymeric systems [16,24,26]. However, Billiot et al. [25] and Rizvi and Shams [27] have reported better resolutions for the binaphthyl derivatives using poly(sodium undecanoyl-l-leucylalaninate) and poly(sodium N-undecenoxy carbonyl-l-leucinate), respectively, as chiral selectors. The examination of the electropherograms suggests that the migration time of the compounds is much longer with the vesicle solutions. Pascoe et al. [30] have also reported similar results in the case of CTAB/DDCV vesicles. Due to their bilayer structures, the vesicles have a higher charge density than that of typical micelles. Hence, SDL V vesicles have greater electrophoretic mobility in the opposite direction of EOF, which results in a wider migration window. The peak shapes were broader and the major part of this broadening was due to the lower migration velocity of the compounds across the detector window. The binaphthyl compounds are hydrophobic and therefore are retained longer resulting in enhanced Ri values.

To reduce the migration time of the analytes the separations were carried out with different voltages of 15, 18, 20, 22 and 25 kV. For BOH and BDA, 50 mM borate buffer pH 9.7 with 2 mM SDL V was used and for BNP, 50 mM borate buffer pH 10.3 with 5 mM of SDL V was used. The migra-

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**Fig. 2.** Optimized chiral MEKC separations of (A) BOH, (B) BDA and (C) BNP. Separation conditions: 50 mM borate buffer pH 9.7 containing 2 mM of SDL V (A and B) and 50 mM borate buffer pH 10.3 containing 5 mM SDL V (C); separation capillary: total length 87 cm, effective length 31.5 cm (50 µm I.D.); applied voltage 15 kV, detection wavelength 230 nm, temperature ~30°C.
Effect of surfactant and buffer concentrations, and pH on retention factor ($k_v$), selectivity ($\alpha$), resolution ($R_S$) and separation efficiency ($N_s$) of BOH and BNP

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<td>10.0</td>
<td>5.40</td>
<td>1.16</td>
</tr>
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NS: no separation; $N_s$: separation efficiency for the first enantiomer.

a Separation condition: 50 mM borate buffer pH 9.7 for BOH and pH 10.3 for BNP. Applied voltage 15 kV.
b Separation condition: 2 mM SDLV in pH 9.7 buffer for BOH and 7 mM SDLV in pH 10.3 buffer for BNP. Applied voltage 15 kV.
c Separation condition: 50 mM borate buffer with 2 mM SDLV for BOH and 50 mM borate buffer pH 10.3 with 5 mM SDLV for BNP. Applied voltage 15 kV.

3.2.1. Effect of surfactant concentration. Concentration of chiral selector has significant effect on resolution. Different concentrations of SDLV in 50 mM borate buffer pH 9.7 were tested for separation of BOH and 50 mM borate, pH 10.3 for BNP. The variations of $k_1$, $\alpha$, $R_S$ and $N_s$ as surfactant concentration is increased are shown in Table 1. It can be observed that a minimum of 1 mM of SDLV was required for enantioreseparation of BNP. As surfactant concentration increases (0.5–6.0 mM) $k_1$ increases resulting in higher resolution. However, surfactant concentrations higher than 6 mM resulted in a higher resolution, but at the expense of longer run time. On the other hand, concentration as low as 0.5 mM of SDLV was required for enantiomer separation of BOH. The resolution obtained in the case of BOH is 2.71, which increased to 5.23 when 2 mM SDLV was used and the two peaks were well separated. Therefore, the subsequent work employed a surfactant concentration of 2 and 5 mM, respectively to maximize the resolution of BOH and BNP. From Table 1 it can be found that for BOH, selectivity and resolution as well as separation efficiency increases with the increase in surfactant concentration and then decreases passing through a maximum. At higher surfactant concentrations, the retention factor, $k_1$ for BOH decreased. This may be ascribed to the enhanced ionization of the analyte in the presence of high concentration of surfactant, which results in a weaker interaction with the vesicles and hence shorter retention time. The selectivity of the anionic form of BOH is expected to be less than the neutral form due to increased charge repulsions. As a result the $K_v$ value also decreased. In fact, change of $pK_v$ of organic acids and bases when solubilized in micelles have been reported by many authors [41,42]. In the absence of electrostatic interactions, the hydrophobic interaction dictates the degree of enantioselectivity. The role of hydrophobic interactions on the chiral selectivity and resolution is demonstrated by the low $\alpha$ (1.08) and $R_S$ (1.98) values and high $k_1$ (4.6) value for BDA using 2 mM SDLV. For BNP, however, both selectivity and resolution increases with increasing SDLV concentration, as it is less hydrophobic in comparison to BOH and BDA. The high retention time and resolutions obtained for BOH and BNP using the vesicular system compared to the unpolymerized micellar systems and some polymeric systems having the l-valine head group [16,43,44] can be attributed to the enhanced partitioning of the analytes with the hydrophobic layer of the vesicles.

3.2.1.2. Effect of buffer concentration. For separation of BOH and BDA, 2 mM SDLV in borate buffer of pH 9.7 and for BNP, 5 mM SDLV in borate buffer of pH 10.3 was employed. The migration time of BOH increased from 17.2 to 27.4 min from 51.1 min upon application of 25 kV and that of BNP decreased to 20.7 min from 38.2 min upon application of 22 kV. However, in all the cases, the resolution progressively decreased with the application of higher voltages due to lower retention factor. Since application of higher voltage often resulted in baseline distortion due to joule heating, all the further studies were carried out with 15 kV.
43.9 min as the buffer concentration was raised from 20 to 50 mM. No separation could be obtained below 20 mM of sodium tetraborate. The variations of retention factor, selectivity, resolution and separation efficiency for BOH and BNP with the buffer concentration are shown in Table 1. The retention factor as well as selectivity can be found to increase with buffer concentration. However, above 50 mM the resolution value dropped for both BOH and BNP. For both analytes, the change in separation efficiency is consistent with the change in resolution. It is well known that buffer concentration influences the magnitude of the EOF. Normally, a higher buffer concentration gives a lower EOF and vice versa [45]. Thus higher buffer concentration produces higher retention times. The increase of selectivity with buffer concentration may be due to the increase of ionic strength of the BGE solution. It is well established that increase of ionic strength (i) decreases the cmc of micelles and hence increase the micelle concentration in solution, and (ii) induces growth of the surfactant aggregates to form rod-like micelles as often indicated by the increase in viscosity of the solution [46]. These effects result in an increase of the partition coefficient of the analyte between the aqueous and micellar phase [47]. Taking into account the run time and resolution for BOH and BNP 50 mM borate was selected as the optimum concentration of buffer. The same optimized buffer concentration was also employed for BDA.

3.2.1.3. Effect of pH. In MEKC, the electrophoretic mobility is pH-dependent. For charged compounds, variations in the buffer pH may lead to changes in dissociation of the compounds thus affecting their charge and thereby solute-micelle interactions and electrophoretic mobilities. The micelle-induced pK<sub>m</sub> shift has shown to affect the selectivity of ionic compounds significantly [48]. The partitioning of the solute between free solutions and pseudo-stationary phase is also found to be pH-dependent. As a result, in MEKC, large variations in selectivity are observed with the change in pH of the BGE. For the separation of BOH and BDA, the pH was varied between 8.5 and 10.3 using 50 mM borate containing 2 mM SDLV. Baseline separation of BOH and BDA was achieved in the pH range 8.5–10.3. For BNP, 50 mM borate buffer with 5 mM SDLV was employed. The variations of retention factor, selectivity, resolution and separation efficiency of BOH and BNP with pH are indicated in Table 1. In the pH range 8.5–10.3, BDA is neutral but BOH is partially ionized. The ionization of BNP results in a sharp change in ionization behavior of the analyte. Since the EOF induced by the borate ions is less dependent on the pH value, the change in migration time must be due either to the change in state of aggregation or to the increase in ionic strength of the BGE. However, in the pH range studied, no change in the state of aggregation is expected for SDLV surfactant. Therefore, the increase in migration time must be a result of the ionic strength of the buffer. The adjustment of pH results in a change in ionic strength the variation of which leads to the changes in the partition coefficients of the compounds between the aqueous and vesicle phase and thus affects retention factor. The data in Table 1 show that at the highest pH value, the retention factor for BOH is greater than that of BNP. This is expected because at pH 10.3 BNP is completely ionized whereas BOH is only partially ionized as the pK<sub>a</sub> of BOH is ~9.5 [44,49]. This suggests that partitioning of BOH into the vesicles is more compared to that of BNP. Among the atropisomeric compounds BDA is retained longer as it is uncharged in the working pH range. Also BOH was retained longer than BNP and hence chiral recognition was enhanced. At pH 10.3 the selectivity for BOH is more but the resolution is less. This is due to the broadening of the peaks. On the other hand, for BNP, the selectivity and resolution increases with pH up to 10.3. The slight inconsistency in the S values with the respective R<sub>S</sub> values in the case of BNP may be due the differences in ionic strength of the buffers. It was not possible to study at pH greater than 10.3 as the baseline became unstable.

3.2.2. Enantiomer separation of benzoin

Benzoin and its derivatives have been employed in the development of antiseptic, astringent and anti-inflammatory drugs such as Tin-Ben. Unlike the atropisomeric compounds it has a chiral carbon atom in the molecule. In this study, baseline separation of benzoin was achieved by use of SDLV vesicles in a wide range of pH between 7.0 and 10.3 with phosphate and borate buffers. For separations at pH 7.0, 7.4 and 8.0, phosphate buffer (30 mM) was used as BGE. Benzoin could be separated at pH 7.0 using 4 mM SDLV but with low resolution (R<sub>S</sub> = 1.2). The optimum resolution (R<sub>S</sub> = 1.61) was obtained at pH 10.3 using 60 mM borate buffer containing 4 mM SDLV. No separation of the pair of enantiomers was obtained when buffer concentration was less than 30 mM. The electropherogram for chiral separation of benzoin is shown in Fig. 3A. The migration time of benzoin (15 min) is very less in comparison to BOH and BNP. Since benzoin is less hydrophobic than the binaphthyl compounds, the partitioning with the vesicles is less favorable resulting in a lower migration time. The enantiomeric separation of benzoin was optimized with respect to the SDLV concentration. SDLV in the concentration range 1-6 mM was used in 60 mM borate buffer of pH 8.5. The variations of k<sub>1</sub>, k<sub>2</sub>, R<sub>S</sub> and N values are summarized in Table 2. The data in Table suggest that no separation at concentrations below 1 mM of SDLV could be achieved. However, the separation improved with increasing SDLV concentration to 4 mM. The corresponding efficiency also increased with the vesicle concentration. Use of surfactant concentrations above 4 mM did not improve resolution significantly and the efficiency decreased. This may be a result of unsymmetrical peak shapes. The variation in pH has no effect on selectivity but the R<sub>S</sub> and N values inconsistent...
Fig. 3. Optimized chiral MEKC separations of (A) benzoin and (B) Tröger’s base. Separation conditions: (A) 60 mM borate buffer pH 10.3 containing 4 mM of SDL V; (B) 50 mM borate buffer pH 10.3 containing 2 mM SDL V and 2% methanol; all other conditions are same as in Fig. 2.

with each other. This is perhaps due to the variations in ionic strength of the BGE that causes variations in the migration range.

3.2.3. Enantiomer separation of Tröger’s base

The chirality of TB is due to the pyramidal nitrogens. The molecule has a slow rate of configurational inter-conversion as to allow resolution of the enantiomeric components [50].

Because of their very pronounced asymmetric character and the presence of rigid chiral groove, TB and its derivatives have been used as molecular receptors [51], chiral solvating agents [52] and as chiral modifiers in enantioselective reactions [53].

We have separated the enantiomers of TB using 2 mM SDL V in 50 mM borate buffer of pH 10.3. However, good peak shapes could be obtained only when 2% (v/v) methanol was used in the background electrolyte. At methanol concentrations lower or higher than 2%, the resolution decreased. The electropherogram for enantiomer separation of TB is shown in Fig. 3B. The optimization of enantiomer separation of TB was attempted in four different buffers in the alkaline pH range from 8.5 to 10.3 with 2 mM of SDL V. The optimum resolution ($R_s = 1.06, \alpha = 1.04$) was obtained with 50 mM borate buffer, pH 10.3 with 2 mM of SDL V. At lower pH the peak shapes deteriorated and separation could not obtained below pH 8.5.

Table 2

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</table>

$^a$ Separation condition: 60 mM borate buffer pH 9.7, applied voltage 15 kV.

$^b$ Separation condition: 40 mM borate buffer, 4 mM SDBV, applied voltage 15 kV.

$^c$ 30 mM phosphate buffer was used.

4. Conclusion

Vesicles prepared from a single-tailed amino acid derivative amphiphile, sodium N-(4-n-dodecylxoybenzoyl)-l-valinate have been used as a substitute of micelles in MEKC for enantiomer separations. Three rigid atropisomeric compounds ($\pm$)BOH, ($\pm$)BDA and ($\pm$)BNP, Tröger’s base, and benzoin having different hydrophobicity and ionic character have been enantiomerically resolved. The resolutions obtained for the racemates by use of SDL V vesicles as chiral selectors are higher compared to those reported for other monomeric chiral surfactant systems. The improved enantiomer separation may be due to the aromatic ring near the surfactant head group that facilitates formation of vesicles in solution. The major advantages of this surfactant are (i) low cvc and (ii) high hydrophobicity of the vesicular aggregates that facilitates partitioning of the analytes between aqueous and vesicle phase. Also, SDL V can be readily synthesized. The vesicular system provides larger migration window in comparison to normal micellar systems. Thus it can be a better chiral selector for hydrophobic chiral analytes. Active work is going on in our laboratory to separate enantiomers of other class of chiral drug molecules and to synthesize other amino acid derivatized chiral surfactants of this series.

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References
