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Research Article

Enantiomeric separations of binaphthyl derivatives by capillary electrophoresis using *N*-(2-hydroxydodecyl)-L-threonine as chiral selector: Effect of organic additives

The chiral selectivity of a novel amphiphile, *N*-(2-hydroxydodecyl)-L-threonine (2-HDT), was evaluated for enantiomeric resolution of three binaphthyl derivatives (±)−1,1′-bi-2-naphthol, (±)−1,1′-binaphthyl-2,2′-diamine, and (±)−1,1′-binaphthyl-2,2′-diylhdrogen phosphate (BNP) by micellar EKC. The effects of three organic modifiers, methanol, isopropanol, and acetonitrile, on the separations of enantiomers of these compounds were investigated. Separation of enantiomers could be achieved in relatively dilute solutions of the pure surfactant. However, best separations of enantiomers were obtained only in the presence of 10% v/v acetonitrile. Enantiomeric impurity in nonracemic mixtures of *R*- and *S*-forms of BNP was determined.

Keywords:
Binaphthyl derivatives / Enantioseparation / Fluorescence / Organic additives / Surface tension
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1 Introduction

In recent times, CE has emerged as an efficient analytical technique for the enantiomeric analysis in pharmaceutical science [1–5] and for various applications in bioanalytical sciences [6–8]. Enantioselective separation techniques in CE are not only used for the enantioseparation of drugs and pharmaceuticals, they have also been applied to analyze chiral pollutants [9], and foods and beverages [10, 11]. In fact, Cifuentes and co-workers [12] have used chiral electromigration methods for the analysis of chiral amino acids obtained from microalgae and genetically modified crops [13]. Since the enantiomeric resolution is based on stereoselective interactions between analyte and chiral selector, numerous chiral pseudostationary phases, *e.g.*, CDs [14–16], bile salts [17, 18], micelle/vesicle-forming synthetic chiral surfactant monomers [19–27] and their polymers [28–30], *etc.* have been employed in the past to achieve better selectivity, resolution, and efficiency. Many authors have reviewed the subject from time to time [9, 31–35]. The most popular mode in CE for the enantioseparation is micellar EKC (MEKC) that uses chiral surfactants and can be used to separate charged as well as neutral analytes. To obtain better enantiomeric resolution, the pseudostationary phase should have mainly two characteristics: (i) larger elution window and (ii) greater enantioselectivity. Our recent studies [24–26] have demonstrated that vesicle-forming surfactants offer a large elution window and hence better chiral resolution. Alternatively, separation scientists have employed organic solvents as additives to the buffer for greater selectivity [36–39]. Based on their interactions with the micelles organic modifiers have been classified into two classes, type I and II. Small polar molecules, such as MeOH, iPrOH, ACN, and urea, which are solubilized mainly in the bulk aqueous phase, are of the type II modifiers. On the other hand, polar molecules containing large hydrophobic part (*e.g.*, butanol, hexanol, octanol, *etc.*) and partitioned into the micellar phase are called type I modifiers. Type II modifiers being present in bulk help solubilization of hydrophobic analytes in water, and thus enhance their interactions with micellar phase. This is true when the modifier is present at higher concentrations. However, when present at low concentrations the modifier interacts directly with the micelles causing change in micellar properties [40]. For example, small concentrations of ethanol [41] or urea [42] reduce *cmc* value and increase mean aggregation number (N_{agg}) that is hydrodynamic size of the micelles. Such change in micellar properties can modify micelle–analyte interactions. In

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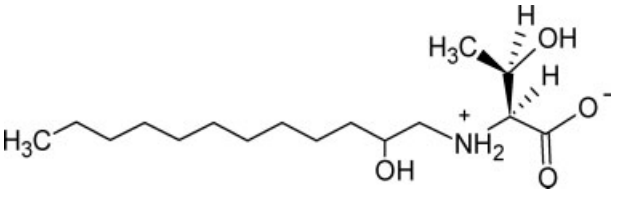
Abbreviations: BNA, (±)-1,1′-binaphthyl-2,2′-diamine; **BNP,** (±)-1,1′-binaphthyl-2,2′-diylhydrogen phosphate; **BOH,** (±)-1,1′-bi-2-naphthol; **2-HDT,** *N*-(2-hydroxydodecyl)-L-threonine

other words, addition of small amount of organic modifiers can modulate selectivity of the pseudostationary phase. Indeed, addition of these types of modifiers has resulted in a better selectivity and resolution of enantiomers [43].

One of the major disadvantages of the enantiomeric separations by MEKC is that it requires higher concentrations (two–ten times of CMC of the chiral surfactant making the method expensive. Therefore, it is important to use surfactants that have very low CMC values. In this work, we have employed an amino acid-derived amphiphile, *N*-(2-hydroxydodecyl)-L-threonine, 2-HDT (see Scheme 1 for molecular structure), which has two chiral centers and an OH group at the amino acid headgroup that through hydrogen bonding interaction can result in enhanced chiral selectivity. Also, the secondary amine and hydroxyl groups in the hydrocarbon chain can influence CMC and the self-assembly formation and hence enantiomeric resolution. There are some reports on the chiral separations using *N*-alkylamino acid derivatives by ligand-exchange CE [44–48]. However, to our knowledge, there is no report of the chiral separation using this type of amphiphile by MEKC. Several studies [25, 26, 49] have suggested that chiral resolution in MEKC depends on the nature and shape of the self-assemblies formed by the amphiphile in buffered medium. Our recent studies on the self-assembly properties and microstructure formation of this type of amphiphiles showed that they formed a rare type of bilayer tubular aggregates in alkaline solutions at a concentration above their CMC (Ghosh, A., Dey, J., submitted for publication). The tubules thus formed have multiple branches. We thought that such type of large tubular aggregates might have better selectivity compared to that of spherical micelles. The aim of this paper is to show the influences of (i) structure and chirality of 2-HDT and (ii) organic modifiers on the chiral recognition ability of the amphiphile with different types of compounds including binaphthyl dervatives. The chiral selectivity of 2-HDT was therefore evaluated for (±)-1,1′-bi-2-naphthol (BOH), (±)-1,1′-binaphthyl-2,2′-diamine (BNA), and (±)-1,1′-binaphthyl-2,2′-diylhydrogen phosphate (BNP), (±)-binaphthol-bis(trifluoromethane sulfonate), (±)-benzoin, (±)-benzoin ethyl ether, Tröger's base, (±)-warfarin, (±)-norephedrine, (±)-hesperitine, (±)-terbutaline, (±)-phenylalanine, and (±)-indoprofen. The effects of three organic additives methanol (MeOH), isopropanol (iPrOH), and ACN on the separations of enantiomers of these racemates were examined.



Scheme 1. Molecular structure of 2-HDT.

2 Materials and methods

2.1 Chemicals and reagents

The pure enantiomers and the racemic mixtures of BNP, BOH, and BNA, (±)-binaphthol-bis(trifluoromethane sulfonate), (±)-benzoin, (±)-benzoin ethyl ether, Tröger's base, (±)-warfarin, (±)-norephedrine, (±)-hesperitine, (±)-terbutaline, (±)-phenylalanine, (±)-indoprofen, and 1,2-epoxydodecane were purchased from Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA). The fluorescence probe, pyrene was obtained from Aldrich and recrystallized several times from acetone–ethanol mixture before use. Sodium tetraborate decahydrate, L-threonine, and triethylamine were purchased from SRL (Mumbai, India) and were used as received. Spectroscopic grade MeOH, acetonitrile, and iPrOH were obtained from SRL. The solvents were further dried and distilled before use.

2.2 Synthesis of the chiral selector

The amphiphile 2-HDT was synthesized and purified following the procedure reported earlier [50]. Briefly, L-threonine (5.0 g, 42.0 mM) was dissolved in 65% v/v ethanol–water mixture using equivalent amount (42.0 mM) of triethylamine. An equimolar amount of 1,2-epoxydodecane (9.2 mL) was added to the mixture and stirred for 8 h at 60°C. The solvent was evaporated and the crude product was recrystallized from absolute ethanol. The structure was confirmed by ^{1}H, ^{13}C NMR, and FT-IR spectra, and elemental analysis. Optical activity was determined by measurement of specific rotation ($[\alpha]_D^{25} = -16.64$, c 0.2 MeOH). IR (KBr): 3400 (–OH), 2930 (alkyl), 1600 ($-CO_2^-$), and 1580 cm^{-1} ($-NH_2^+$); ^{1}H NMR (200 MHz, D_2O): $\delta = 0.75$ (3H, t, CH_3CH_2), 1.07 (3H, d, CH_3CH), 1.16 (16H, m, alkyl chain), 1.32 (2 H, m, CH_2CHOH), 2.35 (2 H, m, $CHOH$), 2.56 (2H, t, CH_2N), 3.55 (1H, s, CH_2CHOH), 3.74 (1H, $CHCOO$); ^{13}C NMR: δ(ppm) 13.7 (CH_3CH_2), 19.1 (CH_3CHOH), 22.5 (CH_3CH_2), 30.0 [$(CH_2)_8$], 50.4 (CH_2N), 54.0 ($CHCOO$), 70.0 ($CHOH$), 179.5 (COO); elemental analysis ($C_{16}H_{33}NO_4$): calculated 63.33, 10.96, 4.62, found 63.05, 11.14, 4.54.

2.3 Instrumental

The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker SEM 200 instrument using TMS or ACN as external standard. Melting point of compound was determined with an Instind (Kolkata) melting point apparatus in open capillaries. Optical activity of the compound was measured with a Jasco P-1020 digital polarimeter. The pH measurements were carried out with a digital pH meter Model pH 5652 (EC India, Kolkata) using a glass electrode. A Shimadzu UV-1601 spectrophotometer was used to record absorption spectra. FT-IR spectrum was recorded with a Thermo Nicolet Nexus 870 spectrometer. FT-IR spectra of the solid sample were taken in KBr pellet.

2.4 Physical characterization

2.4.1 Surface tension measurements

The CMC of the surfactant was determined by surface tension measurements by a torsion balance using the Du Nuöy ring detachment method. The platinum–iridium ring was regularly cleaned with ethanol–HCl solution. The instrument was calibrated and accuracy was checked by measuring the surface tension of distilled water. A stock solution of the surfactant was made in Milli-Q water (18.2 MΩ). An aliquot of this solution was transferred to a beaker containing known volume of water. The solution was gently stirred magnetically and allowed to stand for about 5 min at room temperature (~30°C) and then surface tension was measured. For each measurement at least three readings were taken and the mean γ value was recorded. The CMC value of the surfactant was obtained from the breakpoint of the plot of surface tension (γ mN/m) *versus* logarithm of [surfactant].

2.4.2 Fluorescence spectra

The steady-state fluorescence spectra of pyrene were recorded on a SPEX Fluorolog FL-3 spectrofluorometer. Saturated solutions of pyrene were used for sample preparation. The solutions were excited at a wavelength equal to 335 nm. The spectra were recorded in the wavelength range of 350–550 nm using ratio mode.

2.5 CE

The MEKC experiments were conducted by use of a Hewlett-Packard^{3D} CE system (Waldbronn, Germany). The instrument was equipped with a DAD for UV detection. In this study, the DAD was set at 230 and 254 nm for the detection of analytes. The instrument also consisted of a 0–30 kV high-voltage built-in power supply. Hewlett-Packard CE ChemStation software was used for control and data acquisition. Fused-silica capillaries employed in all experiments (50 μm id, 365 μm od) were purchased from Polymicro Technologies (Phoenix, AZ). The effective length of the capillaries was 32.5 cm, while the total length was 42.5 cm. Capillary was initially conditioned for 2 h with 1 M NaOH and then 30 min with 0.1 M NaOH at 30°C. The capillary was finally rinsed for 10 min with deionized water and 10 min with the run buffer prior to use. The desired temperature of the capillary was maintained using the instrument's thermostating system.

2.6 Preparation of background electrolyte and analyte solutions

The BGE for the HP^{3D}CE experiments consisted of different concentrations (40–60 mM) of $Na_2B_4O_7 \cdot 10H_2O$ at pH 10.3 containing various organic modifier with different percentage. The pH of these BGE was adjusted using 1 M sodium hydroxide. The surfactant concentration ranges investigated were 2–6 mM in pure buffer and 6–20 mM in the presence of additives. Fresh surfactant solutions were prepared daily, filtered through a 0.45 μm polypropylene filter, and degassed for at least 5 min prior to each experiment. The BGE was used to rinse the capillary for 5 min between runs. Stock solutions of the racemic samples were prepared in MeOH at a concentration of 1–2 mg/mL. Analyte solution was dissolved in 50:50 v/v MeOH/buffer in order to give a final concentration of 0.1–0.2 mg/mL. Each sample solution was degassed for 5 min prior to use.

2.7 Calculations

Chiral resolution (R_S) was calculated using the peak width at half-height method [51].

$$R_S = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_2 + 1}\right)\left(\frac{1 - {t_0}/{t_{mc}}}{1 - \left({t_0}/{t_{mc}}\right)k_1}\right) \quad (1)$$

where N is number of theoretical plates and t_{r1}, t_{r2}, t_0, and t_{mc} represent retention times of the first and second enantiomer, EOF marker (MeOH), and micelle marker (cetylpyridinium chloride), respectively. The values of N, k, and α were calculated using the following equations [52]:

$$N = 5.54\left(\frac{t_r}{w_{0.5}}\right)^2 \quad (2)$$

$$k = \frac{t_r - t_0}{t_0\left(1 - {t_r}/{t_{mc}}\right)} \quad (3)$$

$$\alpha = \frac{k_2}{k_1} \quad (4)$$

The t_{mc} value in 60 mM borate buffer (pH 10.3) containing 6 mM 2-HDT was found to be 48.2 min, which increased to 50.6 min upon addition of 6% v/v iPrOH. On the other hand, the t_{mc} value in 60 mM borate buffer (pH 10.3) containing 12 mM 2-HDT and 10% v/v MeOH was observed to be 218 min.

3 Results and discussion

3.1 Physicochemical properties of 2-HDT

First of all, it should be noted that 2-HDT exists as inner salt and thus it is insoluble in water at neutral pH. However, in dilute solutions of higher pH, the amphiphile becomes soluble as it ionizes to the anionic form. Therefore, the aggregation behavior of the amphiphile was studied in pure 50 mM borate buffer (pH 10.3) solutions and in the presence of 10% v/v MeOH, iPrOH, and ACN as additives. The CMC values of the amphiphile determined under different conditions are listed in Table 1. As observed the CMC value of 2-HDT increases upon addition of organic modifiers. This is indicative of reduction of dielectric constant of borate buffer

Table 1. Physicochemical properties of 2-HDT at 30°C

Properties	Borate buffer	50 mM Borate buffer + 10% v/v modifier		
		MeOH	iPrOH	ACN
cmc (mM)	0.55	0.93	0.96	0.90
γ_{cmc} (mN/m)	35.50	29.50	31.70	29.50
I_1/I_3	1.18 (1.74)	1.18 (1.52)	1.19 (1.47)	1.44 (1.54)

Quantities within parentheses are the corresponding values in the absence of 2-HDT.

in the presence of organic additives. It is important to note that in the presence of organic modifiers the surface tension value at the CMC (γ_{cmc}) is less compared to that in pure buffer. This might be beneficial to solute partitioning between the bulk and micellar phase.

To further substantiate this we have measured steady-state fluorescence spectra of pyrene to shed light on the polarity of the interfacial region of the micelle. It is well known that the ratio (I_1/I_3) of the intensities of the first and third vibronic transitions is sensitive to solvent polarity [53]. The I_1/I_3 ratio was therefore measured in the presence and absence of 2-HDT. The data are listed in Table 1. It is observed that upon addition of organic solvent the I_1/I_3 ratio decreased in going from pure aqueous buffer (1.74) to aqueous-organic buffers indicating decrease in the solvent polarity. In the presence of 2-HDT surfactant the I_1/I_3 ratio decreased further suggesting aggregate formation. Since pyrene molecule gets solubilized in the micelle–water interface, the lower I_1/I_3 ratio compared to that of surfactant free solutions (see Table 1) indicates that the micelle interface is much less polar than bulk solvent. The micropolarity of the aggregates remains almost unchanged in the presence of MeOH and iPrOH, but addition of ACN increases micropolarity of the aggregates.

3.2 Enantiomeric resolution of binaphthyl derivatives

As discussed in the preceding section, 2-HDT has a relatively low CMC value which makes it a good candidate for use as a pseudostationary phase in MEKC. However, the limitation of this surfactant is that being insoluble at neutral pH it can be employed only for the separations at higher pH. Accordingly, we performed the enantiomeric separations of BOH, BNA, BNP, (±)-binaphthol-bis(trifluoromethane sulfonate), (±)-benzoin, (±)-benzoin ethyl ether, Tröger's base, (±)-warfarin, (±)-norephedrine, (±)-hesperitine, (±)-terbutaline, (±)-phenylalanine, and (±)-indoprofen in borate buffer of pH 10.3. The maximum solubility of 2-HDT in borate buffer was observed to be ~6 mM. Therefore, we employed 2-HDT in the concentration range of 2–6 mM for the enantioseparations of all the aforesaid racemates. The separations were optimized with respect to surfactant and buffer concentration, and applied voltage. The pH variation was not considered for this work because of poor solubility of the amphiphile at pH<10.3. Although 2-HDT was highly soluble at pH>10.3, separations at higher pHs were given up because of noisy baseline. However, none of the racemates, except BOH, BNA, and BNP could be enantioseparated. Therefore, the enantioseparations of only three binaphthyl derivatives are discussed below.

In an attempt to optimize enantioseparations of the binaphthyl derivatives the effect of applied voltage in the range of 12.5–20 kV and borate buffer concentration in the range of 40–70 mM were studied. It was found that higher borate concentration and lower applied voltage resulted better enantioseparation, but at the cost of longer retention times. As a compromise, 60 mM borate buffer and 15 kV separation voltage were selected for the enantioseparations. At lowest applied voltage the enantiomers of all the three binaphthyl derivatives were well resolved, but retention times were too large. With the increase in voltage the retention time decreased with concomitant increase in resolution, which reached maximum at about 15 kV and then decreased with further increase in voltage.

The chiral selector concentration is considered to be an important factor that controls enantioselectivity. The enantioseparations of the binaphthyl derivaties were performed at 15 kV with 60 mM (BNP) and 50 mM (BOH and BNA) borate buffer containing 2–6 mM 2-HDT. In all the cases, resolution increased concomitantly with 2-HDT. Considering the peak shapes and resolution an optimum concentration of 6 mM 2-HDT was selected. The optimized electropherograms are shown in Fig. 1. It can be observed that best separations could be obtained within a relatively short time in 60 mM (BNP) and 50 mM (BOH and BNA) borate buffer (pH 10.3) at an applied voltage of 15 kV. The corre-

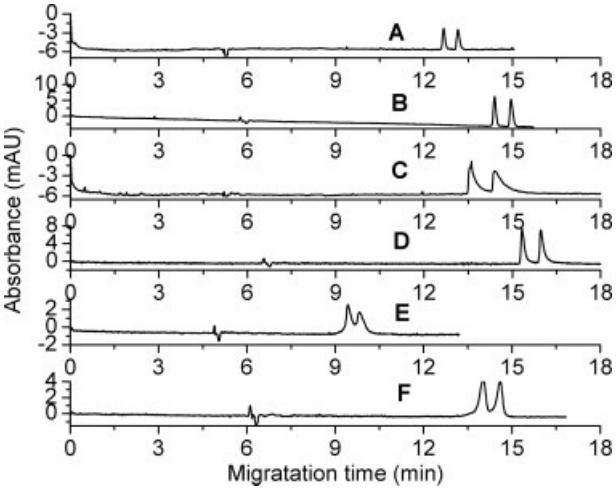


Figure 1. Electropherograms showing the enantiomeric separations of BNP (A, B), BOH (C, D), and BNA (E, F). Separation conditions: 6 mM 2-HDT, 60 mM (A, B), or 50 mM (C, D, E, F) borate buffer (pH 10.3), 2% (B), 6% (D), or 4% (F) v/v iPrOH, injection pressure 20 mb, injection time 2 s, applied voltage 15 kV, λ = 230 nm.

Table 2. Separation parameters for the enantiomeric separations of BNP, BOH, and BNA using 6 mM 2-HDT in 60 mM (for BNP) or 50 mM (for BOH, and BNA) borate buffer (pH 10.3) containing different percentage of MeOH, iPrOH, and ACN

Analyte	t_0	t_2	k_1	α	R_S	N
BNP						
No additive	5.23	13.15	1.93	1.08	2.70	85 300
2% MeOH	5.57	14.16	1.63	1.06	1.52	39 852
2% iPrOH	5.85	14.95	1.63	1.07	2.43	109 211
2% ACN	5.61	13.67	1.54	1.05	1.42	58 525
BOH						
No additive	5.10	11.94	1.64	1.08	0.89	10 911
6% MeOH	5.90	13.09	1.28	1.06	0.80	14 908
6% iPrOH	6.56	15.97	1.53	1.07	2.11	78 191
6% ACN	5.78	13.27	1.44	1.08	1.51	52 928
BNA						
No additive	5.46	10.00	0.86	1.10	0.78	8 677
4% MeOH	5.54	12.11	1.22	1.08	1.14	19 937
4% iPrOH	6.22	14.60	1.41	1.07	1.23	25 680
4% ACN	5.53	13.13	1.42	1.09	0.98	18 250

sponding separation parameters have been compiled in Table 2. The data suggest that although the chiral selectivity in the case of BNA is higher than that of BNP or BOH, the R_S value decreases in the order BNP>BOH>BNA. On the other hand, both BNP and BOH have equal α values, but R_S value of the former is greater than that of the latter. The observed difference is perhaps due to (i) the number of theoretical plates (N), which decrease in the order BNP>BOH>BNA and (ii) weaker interactions with the micelles as indicated by the lower values of retention factors.

3.2.1 Influence of organic modifiers

Since the atropisomers of BOH and BNA were poorly resolved with buffers containing only 2-HDT, organic modifiers such as MeOH, iPrOH, and ACN were evaluated in this study, as they are known to improve enantioselectivity and resolution in MEKC systems [54–56]. Usually a high content of an organic modifier is required for the separation of highly hydrophobic compounds in MEKC. Since organic cosolvents change dielectric constant, viscosity, and electrical conductivity of the BGE, they are expected to influence CMC, shape and size of the aggregates formed by the amphiphile in water. The pK_a values of compounds can also change upon addition of organic compounds. It has been observed that the influence of organic cosolvents on the pK_a values is not significant even in the presence of 30% v/v modifier in aqueous BGE [57, 58]. However, at higher concentrations, a considerable shift of the pK_a values is observed and thus can affect the selectivity of the separation. Therefore, we attempted to perform the separations first in the presence of a small volume percentage of the organic additives. Consequently, the effects of cosolvents were optimized for BNP, BOH, and BNA. It should be noted that 2-HDT is poorly soluble in MeOH, ACN, and iPrOH. It was observed that decrease of pH below 10.3 caused precipitation of 2-HDT from solutions containing higher percentages of organic solvents. Only at pH>10.3, the solubility of 2-HDT increased significantly. However, poor baseline of the electropherograms at pH>10.3 prevented us from studying the effect of pH on the separations of atropisomers of binaphthyl derivatives in the presence of organic additives. The separation buffers employed were 60 mM (BNP) and 50 mM (BOH and BNA) borate (pH 10.3) containing 6 mM 2-HDT and 2–10% v/v of the organic additives. Systematic studies suggested that although addition of MeOH and ACN improved separations, atropisomers of BOH and BNA were best resolved when 6 and 4% v/v of iPrOH, respectively, was added to the separation buffer. However, a decrease in the R_S value was observed in the case of BNP even in the presence of only 2% iPrOH. In fact, addition of equal volume percentage of MeOH and ACN decreased resolution to a greater extent. The electropherograms showing optimum resolutions of the atropisomers of BNP, BOH, and BNA thus obtained are included in Fig. 1. The relevant separation parameters are listed in Table 2. As seen in Table 2 there is a significant improvement in the enantiomer resolution of BOH and BNA upon addition of small amount (2–6%) MeOH, iPrOH, or ACN in borate buffer (pH 10.3) containing 6 mM 2-HDT. The chiral selectivity, however, did not change. The result is consistent with the increase in the separation efficiency (N). The decrease in the resolution in the case of BNP upon addition of a small amount of organic additive may be attributed to the decrease in the polarity of micelle interface that in turn reduces the micelle–solute interaction as BNP is completely ionized at the separation pH. On the other hand, in the separation buffer, BNA and BOH ($pK_a = 9.5$) [59] being present in the neutral and partially ionized form, respectively, the micelle–solute interaction becomes stronger causing increase in the R_S value.

During our studies on the effect of organic additives we observed that in the presence of higher volume percentage the organic additives, a higher concentration of chiral selector was required for the enantioseparations. This is consistent with the increase in CMC of the chiral selector in the presence of organic modifiers. It is not surprising that retention time increases with the increase in the modifier concentration. As expected an enormous increase in the retention time was observed in the presence of organic modifier at a concentration >10% v/v in the BGE. Also the increase in modifier concentration above 10% did not alter the selectivity, but resolution decreased. Therefore, chiral selector concentration was optimized in the presence of 10% organic additives. Consequently, the separations were then carried out in borate buffers (pH 10.3) containing 2-HDT in the concentration range of 6–20 mM. It was found that best resolution for BNP, BOH, and BNA were obtained with borate buffer containing 10, 12, and 15 mM 2-HDT, respec-

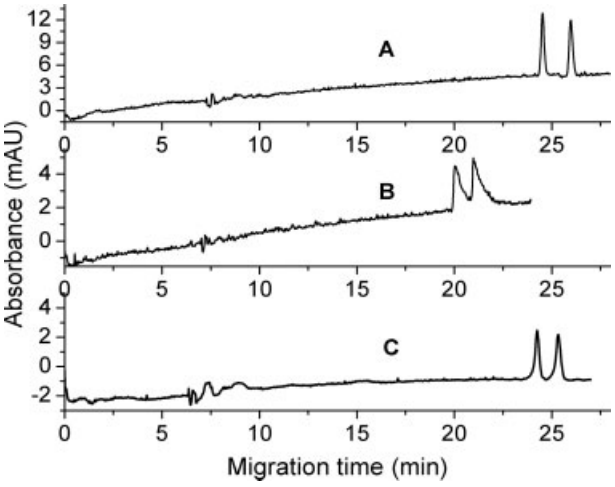


Figure 2. Electropherograms showing the enantiomeric separations of BNP (A), BOH (B), and BNA (C). Separation condition: (A) 10 mM 2-HDT, 60 mM borate buffer (pH 10.3), 10% v/v ACN, (B) 12 mM 2-HDT, 50 mM borate buffer (pH 10.3), 10% v/v ACN, and (C) 15 mM 2-HDT, 50 mM borate buffer (pH 10.3), 10% v/v ACN, injection pressure 20 mb, injection time 2 s, applied voltage 15 kV, λ = 230 nm.

Table 3. Separation parameters for enantiomeric resolutions of BNP with 10 mM 2-HDT in 60 mM borate buffer, and with 12 and 15 mM 2-HDT, respectively for BOH and BNA in 50 mM borate buffer (pH 10.3) containing 10% v/v MeOH, iPrOH, or ACN

Analyte	t_0	t_2	k_1	α	R_S	N
BNP						
10% MeOH	8.60	32.14	2.56	1.11	3.55	47 814
10% iPrOH	10.62	45.13	3.09	1.11	4.24	77 307
10% ACN	7.57	25.97	2.24	1.13	5.37	91 562
BOH						
10% MeOH	8.19	35.80	3.18	1.10	1.95	15 942
10% iPrOH	10.17	40.32	2.83	1.10	2.60	32 680
10% ACN	7.22	20.98	1.83	1.07	2.24	46 074
BNA						
10% MeOH	7.75	37.73	3.79	1.06	1.30	21 405
10% iPrOH	9.42	60.36	5.18	1.10	1.69	14 938
10% ACN	6.50	25.33	2.81	1.06	2.48	53 391

tively. The relevant separation parameters are included in Table 3. It is observed that BNP, BOH, and BNA are best separated in the presence of 10% ACN. Although the R_S value for BOH is slightly higher in the presence of 10% iPrOH, the corresponding retention time is very large compared to that in ACN and therefore, was not considered as optimum condition. The optimized electropherograms are depicted in Fig. 2. The resolutions of atropisomers of the analytes achieved in this work are better compared to those obtained by others using monomeric and polymerized chiral surfactants [51, 60–63].

3.2.2 Enatioseparation of nonracemic mixtures

In order to determine enantiomeric purity, we have performed separations using nonracemic mixtures of BNP. The determination of enantiomeric purity of compounds is very important in the synthesis of chiral substances. In synthetic organic chemistry, enantiomeric excess (ee) is often used as a measure of enantiomeric purity. In analytical chemistry, however, enantiomeric impurity (ei) is usually quantified. Assuming *R*-configuration is an impurity with *S*-configuration, ei can be defined as

$$\text{ei} = 100\frac{x_R}{(x_R + x_S)} = 100\frac{A_R}{(A_R + A_S)}(\%) \quad (5)$$

where x is mole or weight fraction and A is the area of the peak of enantiomer. The ei value thus obtained for *R*-configuration for various nonracemic mixtures have been used to construct a calibration curve (Fig. 3). It has been reported that there can be a change of migration order when analyzing enantiomeric purity. However, no such change in the migration order of the enantiomers was observed in the case of BNP. It is also important to note that an LOD of at least 1% w/w of *R*-configuration could be achieved in the case of BNP.

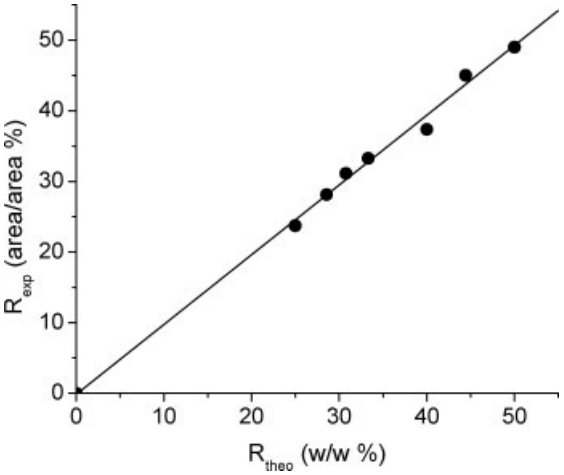


Figure 3. Calibration curve for the determination of enantiomeric impurity of BNP. Separation conditions: 10 mM 2-HDT, 60 mM borate buffer (pH 10.3), 10% MeOH, injection pressure 20 mb, injection time 2 s, applied voltage 15 kV, λ = 230 nm.

4 Concluding remarks

In conclusion, we have synthesized a novel chiral surfactant 2-HDT, which has a relatively lower CMC. The CMC value was observed to increase in the presence of 10% v/v MeOH, iPrOH, and ACN. Lower CMC value makes 2-HDT a good candidate for the chiral separations using MEKC. Different types of chiral compounds were employed for this study. However, only atropisomers of three binaphthyl derivatives

BOH, BNA, and BNP could be separated. Although reasonably good chiral resolution was achieved for BNP by use of pure surfactant solution, the addition of low percentage (2%) of organic modifiers such as MeOH, ACN, and iPrOH decreased resolution. On the other hand, poor resolution obtained for BOH and BNA in pure buffer significantly improved in the presence of small amount of cosolvent. When present in small amount, iPrOH is the most efficient modifier in increasing enantiomeric resolution than MeOH and ACN. Maximum resolution of BNP, BOH, and BNA, however, was obtained in the presence of 10% v/v ACN. The resolutions of atropisomers of the analytes achieved in this work are better compared to those obtained by others using monomeric as well as polymerized chiral amino acid-derived surfactants. Finally, we were able to determine enantiomeric impurity as low as 1% w/w of the *R*-isomer of BNP. Although insolubility in water poses a limitation for the use of 2-HDT in applications where neutral solutions are employed, it could be used as an efficient chiral selector for the separations at higher pH.

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