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Chiral separation of verapamil and related compounds using micellar electrokinetic capillary chromatography with mixed micelles of bile salt and polyoxyethylene ethers

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Abstract

Micellar electrokinetic capillary chromatography (MECC) using surfactant solutions containing sodium deoxycholate (NaDC), polyoxyethylene ethers, and methanol produced simultaneous baseline separation of the enantiomers of verapamil, norverapamil and gallopamil. Studies were conducted to determine the enantiomeric resolution obtained for verapamil and bi-2-naphthol with NaDC solutions, binary mixtures of NaDC with several polyoxyethylene ethers, and ternary mixtures of NaDC, polyoxyethylene ethers and methanol. Experiments were performed to determine the effect of three variables on chiral resolution: (1) the type of ether; (2) the mole fraction of ether in solutions with bile salt; and (3) the percentage of methanol in the mobile phase. The polyoxyethylene ethers studied included polyoxyethylene-8-decyl ether ($C_{10}E_8$), polyoxyethylene-6-dodecyl ether ($C_{12}E_6$), and polyoxyethylene-4-dodecyl ether ($C_{12}E_4$). Simultaneous baseline separation of the enantiomers of verapamil, norverapamil and gallopamil was obtained using a solution containing the ether $C_{12}E_4$. The mole fraction of ether was 0.30 and the total surfactant concentration was 50 m*M* in this solution. The solvent mixture used for this separation was 25% methanol by volume.

Keywords: Micellar electrokinetic chromatography; Enantiomer separation; Verapamil; Norverapamil; Gallopamil; Bi-2-naphthol

1. Introduction

The bile salts are naturally occurring chiral surfactants which have been used successfully as mobile-phase modifiers in micellar electrokinetic capillary chromatography (MECC) for enantiomeric separations [1–7]. Mixed micelles of bile salt and sodium dodecyl sulfate have also been used as mobile-phase modifiers in MECC [8]. The bile salts have been widely studied due to their physiological importance and unique chemical and physical properties [9]. The primary physiological role of these compounds is to solubilize fats during the digestion process. Many forms of the bile salts are found in nature. The class of compounds known as "bile salts" share a common sterol ring backbone but differ in the number, position and orientation of the hydroxyl groups. Naturally occurring bile salts are often

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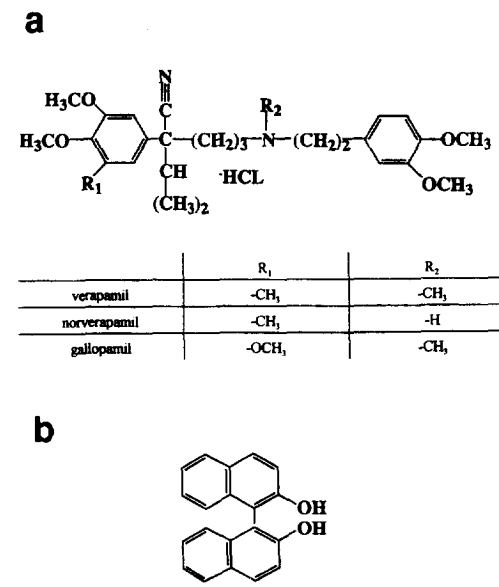
found conjugated through their acid functionality.

The aggregation behavior of the bile salts under various conditions has been studied using light scattering [9,10], nuclear magnetic resonance, electron spin resonance and X-ray techniques [11–14]. The literature values for the critical micelle concentrations (CMC) and aggregation numbers (AN) for the bile salts vary widely. A review by Kratohvil indicates that purification of the bile salts is of the utmost importance for obtaining reproducible data on the aggregation of these molecules [15]. The author gives the most reliable values for the CMC and AN of the unconjugated, dihydroxy bile salt, sodium deoxycholate (NaDC), as 2.4 m*M* and 7, respectively, in a solution containing 0.149 *M* NaCl. This aggregation number is still relatively small when compared to many other surfactants used as pseudophases in MECC. Sodium dodecyl sulfate, for example, has an AN of 64 under similar conditions [16].

The bile salts and polyoxyethylene ethers have been shown to form mixed micelles. The properties of these mixed micelles have been investigated [17–21]. The addition of polyoxyethylene-8-decyl ether ($C_{10}E_8$) in various mole ratios to NaDC has been shown to alter the CMC, the AN of the micelles, and the ability of the micelles to solubilize cholesterol. For example, a mixed solution of the ether $C_{10}E_8$ and NaDC containing an ether mole fraction of 0.25, was observed to have a CMC of 1.63 m*M* and an AN of 25. A solution containing NaDC was observed to have a CMC of 3.16 m*M* and an AN of 18 under the same experimental conditions [21]. Asano and coworkers indicate that the hydrophobicity of the interior of the mixed micelle decreases with increased mole fraction of ether. For example, increasing the mole fraction of ether from 0.00 to 0.43 in mixed solutions of NaDC and $C_{10}E_8$ results in the formation of micelles which decreased in polarity. A steady increase in the solubilization of cholesterol was observed for mixed solutions of NaDC and $C_{10}E_8$ when the mole fraction of ether was increased from 0.00 to 0.40. The results of fluorescence studies reported here confirm the increase in aggregation number of micelles in mixed solutions of NaDC and a similar ether, polyoxyethylene-4-dodecyl ether ($C_{12}E_4$, commonly known as Brij 30, ICI Americas, Wilmington DE, USA).

It was our goal to determine the ability of mixed micelles of bile salt and polyoxyethylene ethers to act as a pseudophase in MECC for chiral separations. These studies allowed us to gain an understanding of separations using mixed micelles and further expand the applications of bile salt MECC. The chiral separations of verapamil, norverapamil, gallopamil and bi-2-naphthol were chosen for the study (the structures for these compounds are given in Fig. 1). The separation for the enantiomers of bi-2-naphthol using bile salts with methanol as a mobile-phase modifier in MECC is well documented [22]. The separation of the enantiomers of bi-2-naphthol using mixed micellar solutions and mixed micellar solutions with methanol provided additional reference points for comparison to solutions of bile salt and bile salt with methanol.

Enantiomeric separation of the widely administered calcium ion channel blocking drug,



	R_1	R_2
verapamil	$-CH_3$	$-CH_3$
norverapamil	$-CH_3$	-H
gallopamil	$-OCH_3$	$-CH_3$

Fig. 1. Structures of solutes investigated. (a) Verapamil and related compounds; (b) bi-2-naphthol.

verapamil, its major metabolite, norverapamil, and a methoxy derivative, gallopamil, has been an area of active research. Chiral resolution of the verapamil enantiomers has been achieved with HPLC using several chiral stationary phases, including a α_1-acid glycoprotein [23,24], amylose Tris-3,5-dimethylphenylcarbamate [24], cyclodextrin [25], ovomucoid [26], and cellulose-based [27,28] columns. Chiral resolution for verapamil has been demonstrated with MECC using cyclodextrins with anionic surfactants [29]. Enantiomeric resolution has also been observed using some of the chiral stationary phases for norverapamil [23–25] and gallopamil [24]. The simultaneous separation and enantiomeric determination of verapamil and norverapamil has been reported using two separation schemes. The first involves coupled column achiral–chiral HPLC [23,25]. The second method involves derivatization of the norverapamil followed by chiral HPLC [24].

Experiments have been performed to determine the conditions under which mixed micelles of NaDC and each of the three ethers $C_{12}E_4$, $C_{10}E_8$, and polyoxyethylene-6-dodecyl ether ($C_{12}E_6$) enhance the chiral separations of verapamil and bi-2-naphthol. The results of these investigations have provided the conditions which allow simultaneous baseline separation of the enantiomers of verapamil, norverapamil, and gallopamil using MECC.

2. Experimental

2.1. Apparatus for CE

Studies were conducted using both a laboratory-assembled capillary electrophoresis instrument and a HPCE-3D (Hewlett-Packard, Wilmington, DE, USA) system. The laboratory-assembled instrument consisted of a 30 kV power supply (Model MJ30P400, Glassman High Voltage, Whitehouse Station, NJ, USA) and a variable-wavelength UV–Vis absorbance detector (Model AD-200, SpectroVision, Chelmsford, MA, USA). Experiments were performed using 50 μm I.D. bare fused-silica capillaries (Supelco, Bellefonte, PA, USA). The absorbance at 210 nm was recorded using both a strip chart recorder (Kipp and Zonen, Netherlands) and a Model 3390A integrator (Hewlett-Packard). Additional experiments for verapamil and bi-2-naphthol were performed using the HPCE-3D system with a HP bare fused-silica capillary to confirm the results obtained with the laboratory-assembled instrument.

2.2. Materials

Sodium deoxycholate (NaDC) was purchased from Aldrich (Milwaukee, WI, USA). The manufacturer stated that purity of NaDC was greater than 98%. The bile salt was recrystallized from ethanol prior to use [15]. Polyoxyethylene ethers, bi-2-naphthol enantiomers, 1-dodecylpyridinium chloride hydrate, and pyrene were purchased from Aldrich and used as received. The *R*- and *S*-enantiomers of verapamil hydrochloride and methoxyverapamil hydrochloride (gallopamil hydrochloride) were purchased from Research Biochemicals (Natick, MA, USA). The *R*- and *S*-enantiomers of norverapamil were kindly provided by Dr. L. Miller (Searle Chemical Sciences Department, Skokie, IL, USA). Reagent-grade sodium hydroxide and sodium chloride and HPLC-grade methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). Sudan III, a widely used micelle marker, was purchased from Central Scientific (New York, NY, USA). Deionized and distilled water was used for the preparation of all solutions.

2.3. Experimental technique

Prior to performing an experiment, the capillary was rinsed with a 0.1 *M* sodium hydroxide solution for 2 min. The surfactant solution used for the experiment was rinsed through the capillary for an additional 2 min by applying pressure to a vial containing the solution on the cathode side of the capillary. Injection of analytes was performed hydrodynamically when using the laboratory constructed CE instrument. The injection was performed by raising the cathode end of the column to a height of 15 cm above the anode

for a specified time interval from 1 to 6 s. Injections, when using the HPCE system, were performed hydrodynamically by applying pressure to the vial containing the analyte solution.

The capillary used for experiments with the laboratory constructed instrument had an overall length of 75 cm with a length of 65 cm to detection. The capillary used for experiments with the HPCE instrument had an extended light path. The capillary internal diameter of 50 μm was increased to 100 μm for the detection window. The capillary used had an overall length of 64.5 cm with a length of 56 cm to detection. The applied voltage was held constant at 20 kV for both instruments. Typical experimental runs involved rinsing the column with the NaOH solution, followed by the surfactant solution, injecting the solution containing the solute(s) of interest and then simultaneously switching the voltage on and starting the data collection.

The resolution reported is based on baseline width measurements from the electropherograms. This facilitated comparison of the data collected both on the laboratory-assembled CE and the HPCE instruments. The HPCE software contained resolution calculation algorithms which resulted in higher resolution values than the manual calculation results reported here.

2.4. Solution preparation

All experiments were performed using solutions with a total surfactant concentration of 50 m*M*. Surfactant solutions were prepared by mixing appropriate volumes of stock solutions containing each type of surfactant. The solutions were prepared containing methanol in the indicated percentages by volume. Polyoxyethylene ether stock solutions were prepared by dissolving the appropriate quantity of the surfactant in an aqueous solution containing 16 m*M* NaCl to achieve a final surfactant concentration of 50 m*M*. NaCl was added to provide a near-constant current in all capillary electrophoresis experiments. A 100 m*M* NaDC stock solution was prepared by dissolving the appropriate quantity of the bile salt in water. Mixed micellar solutions were prepared by combining appropriate volumes of the NaDC stock solution, the polyoxyethylene ether stock solution, methanol and water. The pH of the resultant solutions was between 8.1 and 8.3. Solutions of verapamil, norverapamil, gallopamil and bi-2-naphthol in methanol were prepared from the individual enantiomers. The injected concentration of each enantiomer was 0.25 mg/ml. A fat soluble dye, Sudan III, was added to provide an indication of the micellar migration time.

2.5. Experimental conditions investigated

The mole fractions of ether and volume percentages of methanol for the solutions studied are given in Table 1.

2.6. Fluorescence studies

Pyrene was used as a probe to determine the AN of the micelles using a method reported for mixtures of bile salts and polyoxyethylene ethers [17–21]. The fluorescence emission spectrum of pyrene in the micellar solutions was measured from 350 nm to 500 nm using an excitation wavelength of 333 nm. The instrument used for

Table 1
The ethers investigated at each mole fraction and percentage of methanol in the surfactant solution

MeOH conc. (%)	Mole fractions			
	0.1	0.2	0.3	0.4
0	$C_{10}E_8$	$C_{10}E_8^a$ $C_{12}E_6$ $C_{12}E_4^a$	$C_{10}E_8$ $C_{12}E_6^a$ $C_{12}E_4^a$	 $C_{12}E_6$ $C_{12}E_4^a$
5	$C_{10}E_8$	$C_{10}E_8^a$ $C_{12}E_6$ $C_{12}E_4$	$C_{10}E_8$ $C_{12}E_6$ $C_{12}E_4^a$	 $C_{12}E_6$ $C_{12}E_4$
15	$C_{10}E_8^a$	$C_{10}E_8^a$ $C_{12}E_6^a$ $C_{12}E_4^a$	$C_{10}E_8^a$ $C_{12}E_6^a$ $C_{12}E_4^a$	 $C_{12}E_6$ $C_{12}E_4^a$
25	$C_{10}E_8$	$C_{10}E_8^a$ $C_{12}E_6$ $C_{12}E_4$	$C_{10}E_8$ $C_{12}E_6$ $C_{12}E_4^a$	 $C_{12}E_6$ $C_{12}E_4$

[a] Experiments which were duplicated using the HPCE.

these experiments was an SLM Aminco Bowman Series 2 Luminescence Spectrometer (Rochester, NY, USA). The emission bandpass and scan rate were set to 0.5 nm and 0.5 nm/s, respectively. The fluorescence quenching studies, which allowed the AN to be calculated, utilized 1-dodecylpyridinium chloride as a fluorescence quencher for pyrene. These studies were conducted for solutions of NaDC and mixed surfactant solutions of NaDC and $C_{12}E_4$.

3. Results

3.1. Capillary electrophoresis

MECC studies were conducted using solutions containing NaDC alone, in binary mixtures with ether or methanol, and in ternary mixtures with ether and methanol. The studies included solvent solutions having 5, 15 and 25% methanol by volume at four ether mole fractions as detailed in Table 1. Plots of the resolution obtained for the enantiomers of verapamil versus percent methanol for each mole fraction of $C_{12}E_4$, $C_{12}E_6$, and $C_{10}E_8$ are given in Figs. 2, 3 and 4, respectively. A plot of the resolution obtained for the bi-2-naphthol enantiomers versus percent methanol for NaDC alone, and with each of the three ethers investigated containing 0.2 mole fraction ether, is given in Fig. 5. An electropherogram

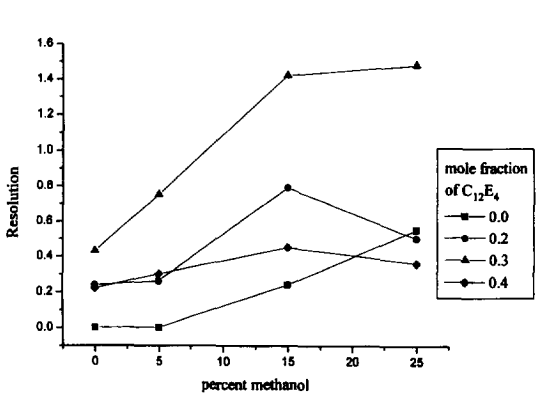


Fig. 2. Observed enantiomeric resolution of verapamil at four mole fractions of $C_{12}E_4$ with increasing methanol percentage in a total surfactant concentration of 50 m*M* for the NaDC/$C_{12}E_4$ mixed system.

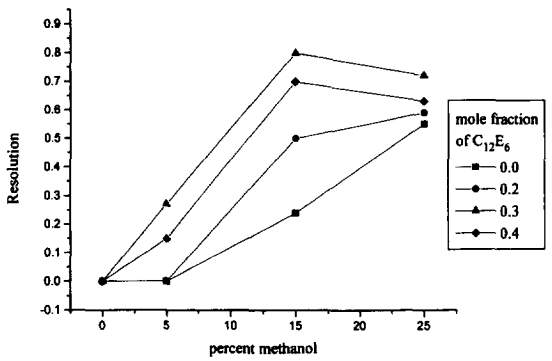


Fig. 3. Observed enantiomeric resolution of verapamil at four mole fractions of $C_{12}E_6$ with increasing methanol percentage in a total surfactant concentration of 50 m*M* for the NaDC/$C_{12}E_6$ mixed system.

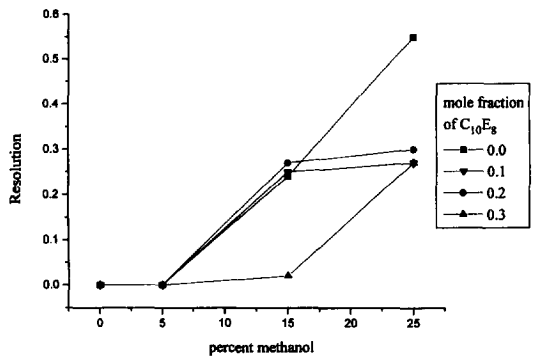


Fig. 4. Observed enantiomeric resolution of verapamil at four mole fractions of $C_{10}E_8$ with increasing methanol percentage in a total surfactant concentration of 50 m*M* for the NaDC/$C_{10}E_8$ mixed system.

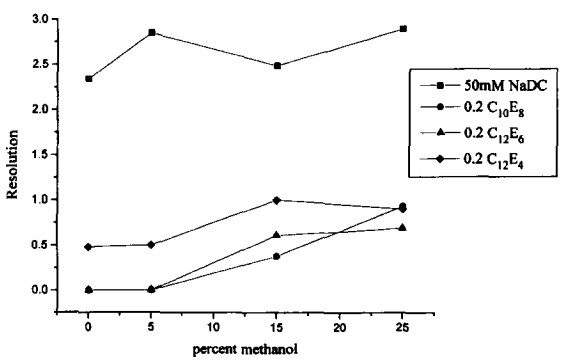


Fig. 5. Observed enantiomeric resolution of bi-2-naphthol for solutions of NaDC and mole fraction 0.2 of $C_{12}E_4$, $C_{12}E_6$ and $C_{10}E_8$ with increasing methanol percentage.

showing the separation of the enantiomers of verapamil, norverapamil and gallopamil under mixed micellar conditions is given in Fig. 6.

3.2. Fluorescence

Fluorescence quenching studies were conducted for solutions having a total surfactant concentration of 50 m*M* using a pyrene concentration of $1 \cdot 10^{-6}$ *M* and 1-dodecylpyridinium chloride concentrations from 25 m*M* to 75 m*M*. The aggregation numbers of micelles formed in solutions of NaDC and NaDC/$C_{12}E_4$ containing mole fraction 0.3 ether were calculated to be 10 and 39, respectively.

4. Discussion

4.1. Evaluation of mobile phases containing $C_{12}E_4$

Mixed surfactant solutions containing $C_{12}E_4$ and NaDC were shown to increase resolution for the verapamil enantiomers and decrease resolution for the bi-2-naphthol enantiomers compared to solutions containing NaDC alone. As shown in Figs. 1a and 1b, the structures of verapamil and bi-2-naphthol are quite different. The enantiomers of bi-2-naphthol do not have an asymmetric carbon. Verapamil has an asymmetric carbon which distinguishes the *R*- and *S*-enantiomers. As shown in Fig. 2, enantiomeric resolution was

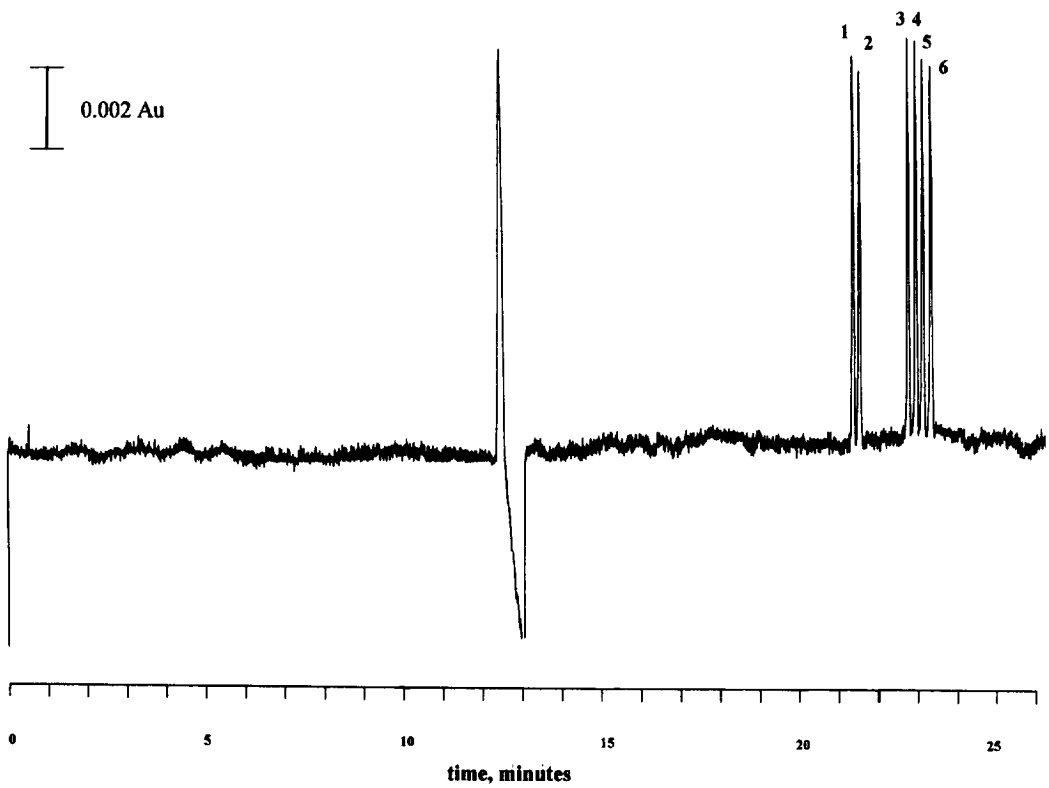


Fig. 6. Capillary electropherogram of racemic verapamil, norverapamil and gallopamil using a mixed surfactant solution of NaDC and $C_{12}E_4$. The solution contained $C_{12}E_4$ mole fraction of 0.3, 25% methanol, and a total surfactant concentration of 50 m*M*. The solution injected contained each enantiomer at a concentration of 0.25 mg/ml in methanol. Applied voltage 20 kV. Absorbance at 210 nm. Observed average current of 8.4 μA. Acquired using the laboratory-assembled CE instrument. Peaks: 1 = (−)-gallopamil, 2 = (+)-gallopamil, 3 = (−)-verapamil, 4 = (+)-verapamil, 5 = (−)-norverapamil, and 6 = (+)-norverapamil.

not observed for verapamil using a solution containing only NaDC at a concentration of 50 m*M*. Baseline resolution was achieved for the enantiomers of bi-2-naphthol, however using the same solution (Fig. 5). Several reports in the literature indicate that larger and less hydrophobic mixed micelles are formed in solutions containing both polyoxyethylene ether and bile salt [17–21]. Our experiments with NaDC show that the micellar AN increases with added ether. The AN increases from 10, with no ether, to 39 at a mole fraction of 0.3 for the ether $C_{12}E_4$. Four plots are given in Fig. 2 showing the observed enantiomeric resolution of verapamil versus methanol percentage for solutions of NaDC and three mixed micellar ratios with $C_{12}E_4$. The three levels investigated were mole fractions of 0.2, 0.3 and 0.4 $C_{12}E_4$. With no methanol in the solution, these data show an increase in resolution for the verapamil enantiomers using the mixed micelles. As the mole fraction of $C_{12}E_4$ increased, the enantiomers of verapamil separated with a resolution of 0.45 observed at a mole fraction of 0.30. The effect of methanol as a mobile-phase modifier on the resolution obtained for the enantiomers of verapamil for solutions of these surfactants is shown in Fig. 2. The resolution of the verapamil enantiomers increases as the percentage of methanol in the mobile phase increases in all four solutions up to 15% methanol. For a mole fraction of 0.30, the resolution increases to 25% methanol. A resolution of 0.55 was obtained for the verapamil enantiomers using a 50 m*M* NaDC solution containing 25% methanol. A resolution of 1.5 was obtained for the verapamil enantiomers, using a solution of NaDC and $C_{12}E_4$, consisting of a mole fraction of 0.30 $C_{12}E_4$ and 25% methanol.

The effect of methanol on the enantiomeric separation of bi-2-naphthol was also investigated. The plots given in Fig. 5 show the resolution obtained for the enantiomers of bi-2-naphthol versus the percentage of methanol in the solutions. The results are presented for 50 m*M* solutions of NaDC and 50 m*M* solutions of NaDC in mixtures with each of the three ethers. A resolution of 2.9 was observed for the bi-2-naphthol enantiomers using a NaDC in a solution containing 25% methanol. The use of mixed micellar solutions with $C_{12}E_4$ and NaDC, with and without methanol, decreased the resolution observed for the bi-2-naphthol enantiomers relative to solutions of NaDC.

4.2. Evaluation of mobile phases containing $C_{12}E_6$ and $C_{10}E_8$

Experiments were performed to determine if other closely related ethers have similar effects on the chiral resolution of verapamil and related compounds. Two other ethers which have a longer ether chain component were evaluated. The commercially available polyoxyethylene ethers, $C_{10}E_8$ and $C_{12}E_6$, were chosen for these studies. The experiments performed for the $C_{12}E_4$ containing mobile phases were repeated using $C_{10}E_8$ and $C_{12}E_6$.

Four plots are given in Fig. 3 showing the observed enantiomeric resolution of verapamil versus methanol percentage for solutions of NaDC and three mixed micellar ratios with $C_{12}E_6$. The three levels investigated were mole fractions of 0.2, 0.3 and 0.4 with $C_{12}E_6$. No enhancement in the enantiomeric resolution of verapamil was obtained using solutions containing NaDC and $C_{12}E_6$ relative to solutions containing NaDC. Increased resolution is obtained with solutions containing NaDC, $C_{12}E_6$ and methanol compared to solutions containing NaDC and methanol. The highest resolution of 0.8 for the enantiomers of verapamil was observed, in the $C_{12}E_6$ study, using a mobile phase consisting of mole fraction $C_{12}E_6$ of 0.3 and 15% methanol. The same trend in results was observed using solutions containing $C_{12}E_4$.

Four plots are given in Fig. 4 showing the observed enantiomeric resolution of verapamil versus methanol percentage for solutions of NaDC and three mixed micellar ratios with $C_{10}E_8$. The three levels investigated were mole fractions of 0.1, 0.2 and 0.3 with $C_{10}E_8$. A slight enhancement in the resolution of the verapamil enantiomers is observed for solutions containing mole fractions $C_{10}E_8$ of 0.1 and 0.2 with 15% methanol, relative to solutions containing NaDC

with 15% methanol. The resolution obtained for the verapamil enantiomers using solutions containing $C_{10}E_8$, with and without added methanol, was consistently lower than the resolution obtained using solutions containing $C_{12}E_4$ or $C_{12}E_6$ under similar conditions.

The plots given in Fig. 5 show the resolution of the enantiomers of bi-2-naphthol versus the percentage of methanol in the solutions. The results are presented for 50 m*M* solutions of NaDC and 50 m*M* solutions of NaDC in mixtures with each of the three ethers. The resolution obtained for the bi-2-naphthol enantiomers using solutions containing $C_{10}E_8$ and $C_{12}E_6$, with and without added methanol, was consistently lower than the resolution obtained using solutions containing NaDC.

5. Conclusions

Mixed micelles have the potential to provide an infinitely variable pseudostationary phase in micellar electrokinetic capillary chromatography. Studies have shown that bile salt solutions are capable of providing chiral resolution for some enantiomeric pairs. It is known that binary solutions of polyoxyethylene ethers and bile salts result in mixed micelles with larger aggregation numbers and decreased micellar interior hydrophobicity relative to solutions of bile salt alone. Thus, one approach to varying the pseudostationary phase to enhance the separation of less hydrophobic compounds is to use mixtures of the bile salts and one of the commercially available polyoxyethylene ethers. The ability of solutions of sodium deoxycholate and polyoxyethylene ethers to provide the desired separations was carefully evaluated by varying mole fraction composition of each ether and percentage of methanol in the 50 m*M* surfactant solutions. These mixed micelles were found to increase the observed resolution for verapamil and related compounds and perform well with organic modifiers such as methanol. The solution which was found to provide the best separation for the verapamil enantiomers also provided chiral separations of closely related compounds, norverapamil and gallopamil. Thus, using a mobile phase containing NaDC, $C_{12}E_4$ and methanol, the simultaneous baseline separation of the enantiomers of verapamil, norverapamil and gallopamil was achieved.

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