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Journal of Chromatography A, 1043 (2004) 291–302
JOURNAL OF CHROMATOGRAPHY A
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Chiral separations in microemulsion electrokinetic chromatography Use of micelle polymers and microemulsion polymers

Rashid Iqbal, Syed Asad Ali Rizvi, Cevdet Akbay, Shahab A. Shamsi*

Department of Chemistry, Center of Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303, USA

Received 23 December 2003; received in revised form 24 May 2004; accepted 28 May 2004

Abstract

In this study, microemulsions of the chiral surfactant polysodium *N*-undecenoyl-D-valinate (poly-D-SUV) was utilized for enantiomeric separation by investigating two approaches using polymeric chiral surfactant in microemulsion electrokinetic chromatography (MEEKC). In the first approach, poly-D-SUV was used as an emulsifier surfactant along with 1-butanol and *n*-heptane. Enantioseparation of anionic or partially anionic binaphthyl derivatives, anionic barbiturates, and cationic paveroline derivatives were achieved by varying the mass fraction of 1-butanol, *n*-heptane and poly-D-SUV. For anionic or partially anionic analytes, relatively lower mass fractions of *n*-heptane, and poly-D-SUV were found to give optimum chiral separations as compared to that for cationic solutes. In the second approach, the chiral microemulsion polymer was prepared by polymerizing mixtures of 3.50% (w/w) of sodium *N*-undecenoyl-D-valinate (D-SUV) and 0.82% (w/w) of *n*-heptane (core phase) at varying concentration of 1-butanol. After polymerization, the *n*-heptane and 1-butanol were removed to yield solvent free microemulsion polymers (MPs) which were then utilized for the separation of anionic binaphthyl derivatives and anionic barbiturates. When MPs of D-SUV were utilized for chiral separation, 1.00% (w/w) 1-butanol and 3.50% (w/w) 1-butanol was optimum for enantioseparation of (±)-BNP and (±)-BOH, respectively. On the other hand, for anionic (±)-barbiturates very low concentration of butanol (0.25%, w/w) provided optimum resolution. Compared with micellar electrokinetic chromatography (MEKC), the use of micelle polymers or microemulsion polymers in MEEKC showed dramatic enhancement for resolution of (±)-BNP, while this enhancement was less dramatic for other binaphthyls [(±)-BOH, (±)-BNA] as well as for (±)-barbiturates and (±)-paveroline derivatives. However, higher separation efficiency of the enantiomers was always observed with MEEKC than in MEKC.

Keywords: Enantiomer separation; Microemulsion electrokinetic chromatography; Micelle polymers; Atropisomers; Polysodium-*N*-undecenoyl-D-valinate; Barbiturates; Paverolines

1. Introduction

Capillary electrophoresis (CE) is a technique that offers rapid and high efficiency chiral separations, requiring low solvent and sample volume. These characteristics aided in the popularization of CE for chiral analysis [1,2]. Two CE techniques, which have been used for the separation of highly hydrophobic chiral and achiral compounds, are microemulsion electrokinetic chromatography (MEEKC) and micellar electrokinetic chromatography (MEKC). The separation mechanism in MEEKC is very similar to MEKC except the former technique utilizes buffered microemulsions (instead of micelles) for the separation of neutral and charged solutes based on their electrophoretic mobilities and hydrophobicities [3–6]. Microemulsions are typically transparent solutions and are thermodynamically stable dispersions of one liquid phase into another (usually, oil-in-water, o/w); stabilized by an interfacial film of a surfactant. Microemulsion systems are well known for their solubilizing capability and have been extensively used in enhanced oil recovery [7,8]. A typical microemulsion system for MEEKC consists of oil (usually *n*-heptane or octane) coated with a surfactant monolayer. The surfactant portion of the microemulsion is composed of a charged surfactant such as sodium dodecyl sulfate together with a co-surfactant (*n*-butanol or 1-propanol).

Although MEEKC has been shown to be a useful method for analysis of wide variety of compounds including

* Corresponding author. Tel.: +1 404 651 1297; fax: +1 404 651 2751.
E-mail address: chesas@panther.gsu.edu (S.A. Shamsi).

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doi:10.1016/j.chroma.2004.05.085

pharmaceutical [9–12], biomolecules [13,14], pesticides [15], and natural products [16,17], there are only three papers for separation of chiral molecules [18–20]. The first paper by Aiken and Huie [18] reported the resolution of ephedrine isomers employing (2*R*,3*R*)-di-*n*-butyl tartarate as a lipophilic oil constituent of the microemulsion. The second paper published by Pascoe and Foley [19] describes the use of a chiral surfactant, dodecoxycarboxyl valine (DDCV), to achieve separation of ephedrine and methyl ephedrine enantiomers. More recently, Mertzman and Foley [20] compared three low-interfacial tension oils (methyl formate, methyl acetate, methyl propionate) in combination with 1-butanol and DDCV for chiral separation of 14 cationic compounds. Therefore, it is of important to design new chiral selectors compatible for chiral separation of a wider range of compounds in MEEKC.

In the present work, we investigated the feasibility of using micelle polymers and microemulsion polymers (MPs) of sodium-*N*-undecenoyl-D-valinate (D-SUV) for the separation of some anionic, partially anionic and cationic enantiomers. Whilst the use of chiral micelle polymers has been recently reported using solvent-modified MEKC [21], in general the method described the chiral separations using hexanol and undecylenyl alcohol as a part of surfactant blend. Therefore, the aim of this work was to investigate the potential of micelle polymers and microemulsion polymers in MEEKC. Two different approaches were used to study the chiral separations. (I) The polymeric surfactant, poly-D-SUV, was used as an emulsifier surfactant to dynamically coat the oil-droplet. Using a four-component microemulsion system consisting of poly-D-SUV, 1-butanol, *n*-heptane and aqueous buffer chiral separation of (±)-binaphthyl derivatives, (±)-barbiturates and (±)-paveroline derivatives were examined. (II) The monomers of D-SUV were polymerized in the presence of varied concentration of 1-butanol (using a fixed concentration of *n*-heptane), followed by removal of residual 1-butanol and *n*-heptane via rotaevaporation and freeze-drying processes. The resulting MP was then used for chiral separation of (±)-binaphthyl derivatives and (±)-barbiturates. The separation parameters such as retention factor, resolution, separation factor and efficiency were evaluated and compared for the MEKC versus the two MEEKC approaches.

2. Experimental

2.1. Chemicals and reagents

The (±)-secobarbital and (±)-pentobarbital were obtained as racemic mixtures from Sigma (St. Louis, MO, USA). The binaphthyl derivatives [(±)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (BNP), (±)-1,1′-bi-2-naphthol (BOH), (±)-1,1′-binaphthyl-2,2′-diamine (BNA)], paveroline derivatives [(±)-laudanosoline and (±)-norlaudanosoline] were obtained as racemic mixtures from Aldrich (Milwaukee, WI, USA). Sodium hydroxide (98% assay) was obtained from Fisher Scientific (Fair Lawn, NJ, USA). The HPLC grade methanol, reagent grade sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), tris(hydroxymethyl)amino methane, 1-butanol and *n*-heptane were also obtained from Sigma. Chemicals used for the synthesis of surfactants including *N*,*N*-dicyclohexylcarbodimide (DCC), *N*-hydroxysuccinimide, D-valine and undecylenic acid were all obtained from Fluka (St. Louis, MO, USA). All standards and electrolytes were prepared with analytical-reagent grade chemicals and water used in all of the experiments was purified by a Barnstead Nanopure II water system (Barnstead International, Dubuque, IA, USA).

2.2. Apparatus

Chiral separations were performed by use of a Beckman (Fullerton, CA, USA) P/ACE 5500 CE system equipped with Beckman P/ACE station version 1.2 for the instrumental control and data handling. The fused-silica capillary (50 μm i.d. × 320 μm o.d.) was obtained from Polymicro Technologies (Phoenix, AZ, USA). The total and effective (from inlet to detector) capillary length was 57.0 cm and 50.6 cm, respectively. A new capillary was first conditioned for 1 h with 1 M NaOH at 50 °C, followed by a 30 min rinse with triply deionized water. Before each run the capillary was preconditioned with the running buffer for 5 min between injections and 2 min with water. All separations were performed at +20 kV and at 25 °C unless otherwise mentioned. The separation parameters such as resolution factors (R_S), efficiency (N), and separation factors (α) were calculated using the common equations in chromatography.

In MEKC or MEEKC, the retention factor is expressed as:

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

where t_R, t_0 and t_{mc} are the retention times of one of the enantiomers, the bulk solution, and the micelle or microemulsion, respectively. However, in this study negative charge molecular micelle or microemulsion polymer (i.e., poly-D-SUV) migrated at a velocity much larger than the unpolymerized micelle or microemulsion towards the anodic (injection end). Thus, as t_{mc} approaches infinity, the term $(1 - t_R/t_{mc})$ in above equation is negligible and reduces to the following conventional chromatography equation [22,23]:

$$k' = \frac{t_r - t_0}{t_0}$$

2.3. Methods

2.3.1. Synthesis of polysodium N-undecanoyl-D-valinate

The complete synthesis and characterization of D-SUV and the corresponding polymeric surfactant (poly-D-SUV) has been described elsewhere [24].

2.3.2. Preparation of buffers and standard solutions

The first background electrolyte (BGE) at pH 7.0 was prepared by mixing 25 mM solutions of NaH_2PO_4 and Na_2HPO_4. The second BGE at pH 10.5 was a 100 mM Tris buffer solution. The desired pH values of the two aforementioned BGEs were adjusted by using either 1 M NaOH or 1 M HCl. The BGEs were filtered through a 0.45 μm membrane filter (Nalgene, Rochester, NY, USA) by creating a vacuum inside the syringe. This was followed by ultrasonication for 10 min to ensure properly degassed running buffers. The stock solutions of (±)-BNP, (±)-BOH, (±)-(BNA), (±)-secobarbital, (±)-pentobarbital, (±)-laudanosoline and (±)-nor-laudanosoline were dissolved either in 50:50 methanol:water or 80:20 methanol:water to give final concentration of 0.5 or 1.0 mg/mL of each analyte.

2.3.3. Preparation of the microemulsion using micelle polymer

All microemulsions were prepared on a (w/w) basis by mixing appropriate percentage (w/w) of 1-butanol, *n*-heptane, poly-D-SUV and BGE (phosphate buffer at pH 7.0 or Tris buffer at pH 10.0) in a 20 mL centrifuge tube. The mixture was placed in an ultrasonic bath for 90 min and the solution was then left to stand overnight at room temperature. With this procedure the transparent microemulsion solution was obtained within 6–8 h and was stable for several weeks.

2.3.4. Preparation of microemulsion polymers

Polymerized microemulsion or microemulsion polymers (MP) was prepared by mixing 3.00% (w/w) D-SUV, 0.82% (w/w) *n*-heptane, and varying amount of 1-butanol (0.0–7.50%, w/w). The mixture was ultrasonicated to obtain a clear solution, which was then polymerized for 30 h in ^{60}Co γ-irradiation (total dose = 240 MRad).

After polymerization, the solution was rotavaporized to remove residual organic solvents (1-butanol and *n*-heptane) not encapsulated in the MP, followed by freeze-drying to obtain a dry product. The microemulsions of MP were prepared by dissolving a given percentage amount of MP in buffer solution followed by ultrasonication for 10 min to ensure properly degassed microemulsion running buffers.

3. Results and discussion

3.1. MEEKC of binaphthyl derivatives

3.1.1. Effect of 1-butanol, n-heptane and poly-D-SUV concentration on the separation of (±)-BNP

The role of relatively polar water-miscible organic solvents (added as a co-surfactant) in MEEKC for separation mechanisms is reviewed recently by Klampfl [25]. It is hypothesized that, the use of 1-butanol forms the bridge between oil and water interphase and further reduces the surface tension of the microemulsion system to zero [25]. Previous studies have shown that varying 1-butanol concentration has a noticeable effect on the migration time and separation factor of the achiral analytes [26]. This has been attributed to the increase in BGE viscosity upon addition of 1-butanol. In this study, in the absence of 1-butanol no enantiomeric resolution (R_S) of (±)-BNP was observed. Upon addition of various mass fraction of 1-butanol ranging from 1.00 to 7.50% (w/w) the trend in k', t_0 and R_S and α were examined. Several points are worth mentioning. First, increasing the 1-butanol mass fraction in the range of 1.00–3.50% (w/w) was found to increase both k'_2 and R_S, as well as t_0 and α (Table 1). However, further increase in 1-butanol concentration above 3.50% (w/w) increased the t_0 but a decrease in k'_2, R_S and α values was observed. Second, an increase in

Table 1
Effect of varying the amount of 1-butanol, *n*-heptane and poly-D-SUV in the micelle polymer buffer on resolution (R_S), retention factor (k'_2), separation factor (α) of (±)-BNP and unretained time (t_0)

Microemulsion parameter	Concentration percentage (w/w)	R_S	k'_2	α	t_0 (min)
1-Butanol[a]	1.00	0.70	2.70	1.015	6.42
	3.50	2.15	5.00	1.044	7.77
	5.00	1.39	4.23	1.028	8.62
	6.49	0.75	3.18	1.022	9.09
	7.00	0.32	2.33	1.034	9.10
n-Heptane[b]	0.21	1.68	2.39	1.013	5.84
	0.42	1.78	2.41	1.021	5. 88
	0.82	2.15	5.00	1.042	5.81
	1.00	1.92	2.61	1.016	5.82
	1.60	1.88	2.75	1.026	5.81
poly-D-SUV[b]	0.25	1.85	3.38	1.030	8.33
	0.50	2.05	4.24	1.032	8.55
	0.76	2.15	5.26	1.046	8.59

[a] MEEKC conditions: 0.82% (w/w) *n*-heptane, 0.25% (w/w) poly-D-SUV, varied 1-butanol percentage (w/w), Na_2HPO_4/NaH_2PO_4 25 mM at pH 7.0, separation voltage +20 kV, capillary temperature 25 °C and UV detection wavelength at 220 nm.

[b] MEEKC conditions: 3.50% (w/w) 1-butanol, 0.25% (w/w) poly-D-SUV, varied *n*-heptane percentage (w/w). Other conditions are same as footnote 'a'.

t_0 increased k'_2 up to 3.50% (w/w) and further increase in t_0 resulted in reduced k'_2 values mainly due to drop in migration times (t_r) of (±)-BNP enantiomers. Previous studies have shown that higher 1-butanol content reduced migration time, but do not alter the k' values significantly [27]. Third, the trend of α is similar to that obtained for R_S except at 7.00% (w/w) 1-butanol concentration where R_S deteriorated significantly due to peak broadening, but slightly higher α was observed.

The first trend in R_S, k', and α can be explained by the fact that the chiral interactions (due to H-bonding and hydrophobic interaction) is enhanced between (±)-BNP and palisade layer of the poly-D-SUV microemulsion up to 3.50% (w/w) 1-butanol. However, at increased 1-butanol concentrations (3.50–7.00%, w/w); the hydrophobic as well as hydrogen bonding interactions interaction between (±)-BNP and the microemulsion is believed to be hindered by 1-butanol molecules, which resulted in a reduction of k', R_S and α, though t_0 continued to increase. We postulate that most of the poly-D-SUV micelles are occupied by 1-butanol molecules in the palisade layer and the micellar core at higher 1-butanol concentration. In the present study, higher 1-butanol concentrations (i.e., >3.50%, w/w) cause a gradual decrease in k'_2 but an increase in t_0 values.

In general, a water-immiscible organic solvent (e.g., *n*-heptane) is needed to facilitate the formation of an actual oil phase of the microemulsion. Unlike 1-butanol, *n*-heptane penetrates completely into the hydrophobic micellar core of the micelle due to its lack of a hydrophilic moiety (e.g., absence of OH group). Thus, the hydrophobicity of the micellar phase increase as *n*-heptane concentration is increased from 0.21 to 0.82% (w/w) which in turn increased k'_2, R_S and α (Table 1). Hence, the optimum values of k'_2, R_S and α were found to be 0.82% (w/w). Further increase in *n*-heptane concentration decrease the k'_2 values, but R_S and α decrease only slightly. However, the concentration of *n*-heptane did not cause much change in t_0 and hence electroosmotic flow (EOF). Thus, it appears that the steady increase in hydrophobicity of the poly-D-SUV micelle caused by increased concentration of *n*-heptane from 0.21 to 0.82% (w/w) resulted in relatively stronger hydrophobic interaction between the micellar phase and the (±)-BNP that consequently increases the retention factors. However, as *n*-heptane concentration exceeds 0.82% (w/w) interaction between the micellar phase and the solute decreased. This is probably due to the fact that micellar phase is completely occupied by *n*-heptane molecules and no cavity remained for (±)-BNP solute to penetrate into the micellar core. Moreover, it is also important to note that at higher *n*-heptane concentration poor mass transfer of (±)-BNP between aqueous phase and microemulsion phase cause peak broadening hence a decrease in efficiency and R_S.

Next, the influence of poly-D-SUV concentration on k'_2 and R_S was studied. Table 1 shows that even a slight increase in t_0 will increase k'_2. Note, that the α values are initially almost constants compared to R_S values up to at least 0.50% (w/w) poly-D-SUV concentration, which may be due to EOF decrease that in turn influences R_S. In addition, it is clear that an increase in poly-D-SUV concentration over the range of 0.50–0.76% (w/w) resulted in an increase in both k'_2 and α values (Table 1). Since both N and α has opposing effect no significant increase in R_S of (±)-BNP was observed over the same range.

3.1.2. Comparison of MEEKC and MEKC on chiral separation of (±)-BNP

The electropherograms in Fig. 1 show the comparison of MEEKC, solvent-modified MEKC (using 1-butanol or *n*-heptane) and MEKC using poly-D-SUV surfactant. In this comparison, three parameters (e.g., R_S, α, and N) of (±)-BNP were investigated using the same buffer and same concentration of poly-D-SUV under optimum organic component (1-butanol and/or *n*-heptane) in the microemulsion. The MEEKC separation of (±)-BNP was performed using a microemulsion system consisting of 0.82% (w/w) *n*-heptane, 3.50% (w/w) 1-butanol, and 0.76% (w/w) poly-D-SUV (Fig. 1a). The high content of organic components in MEEKC led to highest R_S, α and N, but at the expense of longer migration time (ca. 70 min). Solvent-modified MEEKC separation with 1-butanol, but without *n*-heptane shows minor reductions in R_S, α and N as well as migration time decrease to ca. 65 min (Fig. 1b). Thus, the chiral separation of (±)-BNP is almost unaffected in the absence of *n*-heptane. This observation was further confirmed by performing two additional experiments. In the first experiment, solvent-modified MEEKC separation was performed without 1-butanol. As shown in Fig. 1c, no chiral R_S and chiral α of (±)-BNP enantiomers were obtained with more than half reduction in N and t_r values. In the second experiment, only pure micellar system with the same buffer and same concentration of poly-D-SUV, but with no *n*-heptane or 1-butanol was evaluated (Fig. 1d). Again, no chiral separation was observed. This suggests that 1-butanol was the principal component controlling the chiral separation of the MEEKC system. We hypothesized that the hydroxyl groups of 1-butanol are a major source of hydrogen bonding between (±)-BNP and the pseudostationary phase. Thus, reduction in R_S, N, and t_r values can be attributed to the significant decrease in hydrogen bonding due to the absence of 1-butanol.

3.1.3. Effect of 1-butanol on separation of (±)-BOH and (±)-BNA

As explained in the previous two sections, unlike *n*-heptane, the use of 1-butanol improves R_S, α and N values of (±)-BNP significantly. In this section, the effect of varying the amount of 1-butanol concentration on separation of two other binaphthyl derivatives [i.e. (±)-BOH and (±)-BNA] were investigated. As seen in Table 2, a gradual increase in k'_2 values of both (±)-BOH and (±)-BNA were observed upon an increase in 1-butanol mass fraction up to at least 2.00% (w/w). Further increase in percentage (w/w)

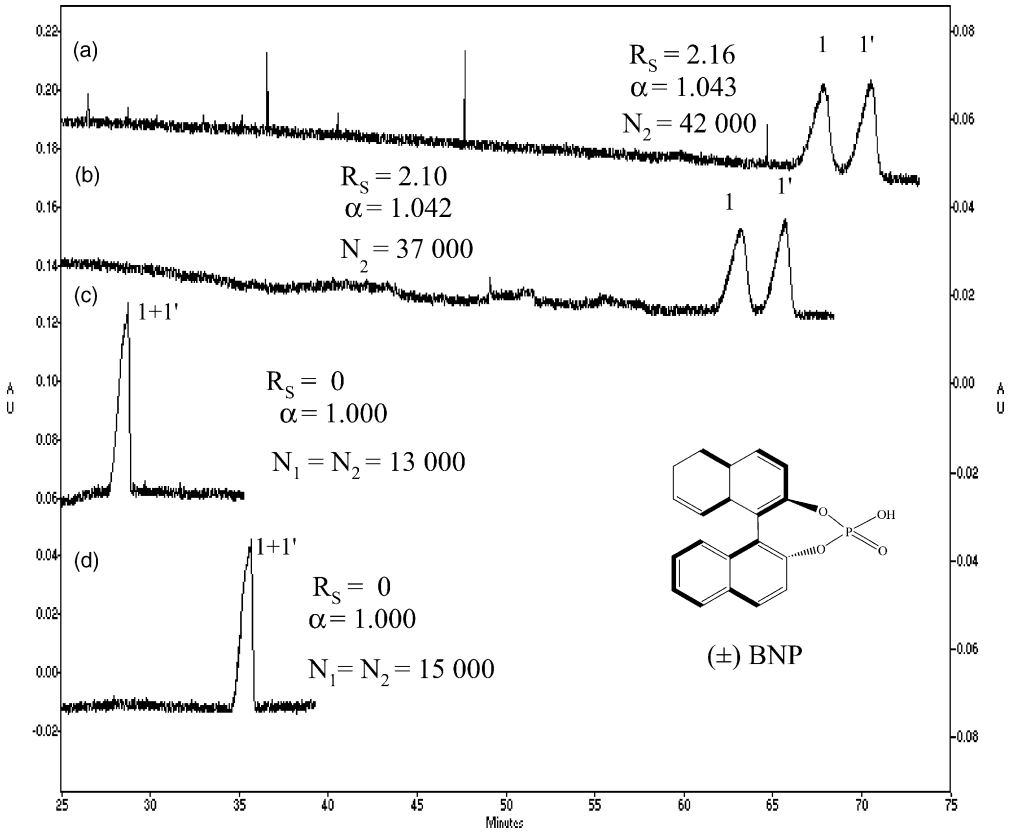

Fig. 1. Comparison of MEEKC and MEKC for chiral separation of (±)-BNP. Both MEEKC and MEKC contain poly-D-SUV 0.76% (w/w), Na_2HPO_4/NaH_2PO_4 25 mM at pH 7.0. Enantioseparations are using (a) MEEKC with 3.50% (w/w) 1-butanol and 0.82% (w/w) *n*-heptane (b) solvent-modified MEKC with 3.50% (w/w) 1-butanol (c) solvent-modified MEKC with 0.82% (w/w) *n*-heptane and (d) MEKC without 1-butanol and *n*-heptane. Other conditions as given in Table 1.

Table 2
Effect of varying the amount of 1-butanol in the micelle polymer buffer on resolution (R_S), retention factor (k'_2), separation factor (α), efficiency (N_2) of (±)-BOH, (±)-BNA and unretained time (t_0)

Concentration 1-butanol percentage (w/w)	R_S	k'_2	α	N_2	t_0 (min)
Analyte (±)-BOH					
0.00	2.12	1.23	1.041	144 000	3.89
0.25	1.94	1.33	1.031	140 000	4.12
0.50	1.57	1.42	1.040	63 000	4.14
1.00	2.01	1.45	1.040	117 000	4.69
2.00	1.82	1.55	1.041	84 000	5.41
3.50	2.18	1.28	1.040	174 000	5.52
Analyte (±)-BNA					
0.00	0.83	1.26	1.027	58 000	
0.25	0.99	1.36	1.026	72 000	
0.50	1.24	1.39	1.027	108 000	
1.00	0.32	1.66	1.032	33 000	
2.00	0.66	2.16	1.040	26 000	
3.50	0.43	1.50	1.030	18 000	

MEEKC conditions: 0.25% (w/w) poly-D-SUV, 0.82% (w/w) *n*-heptane, 100 mM Tris buffer at pH 10.5. Other operating conditions as given in footnote of Table 1.

of 1-butanol led to a surprising drop in k'_2 values of both binaphthyl derivatives. However, it was noted that an increase in percentage (w/w) of 1-butanol caused a contiuous increase in t_0. Moreover, it was observed that R_S, N and α of (±)-BOH and (±)-BNA follows different trend when varying 1-butanol concentration. For example, an initial increase in 1-butanol up to 0.50% (w/w) increased both R_S and N of (±)-BNA. In contrast, an opposite affect was observed for (±)-BOH, though the α values remains fairly constant for both enantiomers. Further increases in 1-butanol concentration caused a significant deterioration in R_S and N of (±)-BNA, whereas the R_S and N of (±)-BOH was unaffected. Thus, the addition of 1-butanol as a co-surfactant to improve chiral resolution appears to be analyte dependent and a case should be made for its use.

3.2. MEEKC separation of barbiturate derivatives

3.2.1. Effect of 1-butanol, n-heptane and poly-D-SUV concentration on chiral separation of (±)-secobarbital and (±)-pentobarbital

The MEEKC of racemic barbiturates [(±)-secobarbital (*S*), (±)-pentobarbital (*P*)] was also studied by varying the 1-butanol, *n*-heptane and poly-D-SUV concentrations. The

Table 3
Effect of varying the amount of 1-butanol, *n*-heptane and poly-D-SUV in the micelle polymer buffer on resolution (R_S), retention factor (k'_2), separation factor (α) of (±) pentobarbital (*P*), secobarbital (*S*) and unretained time (t_0)

Microemulsion parameter	Concentration percentage (w/w)	R_S		k'_2		α	
		(±)-*P*	(±)-*S*	(±)-*P*	(±)-*S*	(±)-*P*	(±)-*S*
1-Butanol[a]	1.00	1.16	1.14	0.75	0.89	1.030	1.028
	3.50	1.96	1.92	0.83	0.93	1.039	1.033
	5.00	0.97	0.99	0.62	0.71	1.016	1.021
	6.49	0.43	0.45	0.57	0.66	1.023	1.029
n-Heptane[b]	0.21	1.14	1.18	0.56	0.65	1.025	1.018
	0.42	0.95	1.05	0.58	0.67	1.020	1.017
	0.82	1.89	1.93	0.80	0.96	1.042	1.035
	1.00	1.75	1.78	0.83	1.03	1.041	1.033
	1.60	1.43	1.46	0.88	1.06	1.037	1.031
poly-D-SUV[c]	0.25	0.54	0.57	0.30	0.40	1.007	1.006
	0.50	1.31	1.33	0.56	0.69	1.019	1.020
	0.76	1.92	1.94	0.80	0.96	1.027	1.021
	1.00	0.69	0.71	0.42	0.69	1.023	1.021

[a] MEEKC conditions: 0.82% (w/w) *n*-heptane, 0.76 % (w/w) poly-D-SUV, varied 1-butanol percentage (w/w). Na_2HPO_4/NaH_2PO_4 25 mM at pH 7.0, separation voltage +20 kV, capillary temperature 25 °C and UV detection wavelength at 220 nm.
[b] MEEKC conditions: 3.50% (w/w) 1-butanol, 0.25% (w/w) poly-D-SUV, varied *n*-heptane percentage (w/w). Other conditions are same as footnote 'a'.
[c] MEEKC conditions: 3.50% (w/w) 1-butanol, 0.82% (w/w) *n*-heptane, varied poly-D-SUV percentage (w/w). Other conditions are are same as footnote 'a'.

barbiturates whose pK_a values are in the range of 7.6–8.0, are supposed to be acidic solutes and can move as partially anionic species under MEEKC experiment performed at pH 7.0 [28]. Similar to anionic (±)-BNP, increasing 1-butanol mass fraction up to 3.50% (w/w) increased R_S and k'_2 values of both (±)-(*P*) and (±)-(*S*) (Table 3). Conversely, as 1-butanol concentration exceeded 3.50% (w/w), a steady decrease in both k'_2 and R_S of the two barbiturate derivatives was observed. Moreover, a major drop in α was observed at ≥3.50% (w/w) 1-butanol.

Highest resolution of the enantiomers of racemic barbiturates was also achieved at 0.82% (w/w) of *n*-heptane (Table 3). However, unlike (±)-BNP the affinity (i.e. k'_2) of the barbiturates enantiomers for the microemulsion phase remains fairly constant once 0.82% (w/w) of the *n*-heptane is exceeded. In addition, highest α of (±)-(*P*) and (±)-(*S*) was observed at highest *n*-heptane concentration (i.e.1.60%, w/w), but R_S decreased in comparison to 0.82% (w/w) *n*-heptane. The effect of poly-D-SUV surfactant on R_S, α and k'_2 of barbiturates was also optimized as shown in Table 3. As expected, a gradual increase in k'_2 values was obtained as poly-D-SUV surfactant concentration was increased from 0.25 to 1.00% (w/w). On the other hand, 0.76% (w/w) poly-D-SUV provided the optimum R_S. Further increase in mass fraction of poly-D-SUV to 1.00% (w/w) led to a remarkable decrease in R_S of barbiturates due to increase in peak widths, but α remains essentially the same. It should be noted that under the experimental conditions used, (±)-barbiturates are partially anionic but they are less hydrophobic than (±)-BNP. Therefore, k'_2 and R_S of barbiturates at equivalent poly-D-SUV concentration are much smaller for (±)-barbiturates than for (±)-BNP (Table 1 versus Table 3).

3.2.2. Comparison of MEEKC and MEKC on chiral separation of (±)-barbiturates

Fig. 2 shows the comparison of MEKC and MEEKC of barbiturates. Lower R_S and *N* values of barbiturates were obtained using MEKC (Fig. 2a). Since (±)-(*P*) is slightly less hydrophobic (log *P* = 2.10) than (±)-(*S*) (log *P* = 2.33), therefore, the former elutes faster than the latter in all of the four separation systems. The solvent-modified MEKC with 0.82% (w/w) *n*-heptane provided only a slight increase in R_S and t_r of the both barbiturates (Fig. 2b). This can be attributed to the slight increase of hydrophobic character of the micelle polymer in the presence of *n*-heptane. However, *N* and α values for both solutes remained almost unchanged in both MEKC (Fig. 2a) and solvent-modified MEKC with *n*-heptane (Fig. 2b). As shown in Fig. 2c, the solvent-modified MEKC with 3.50% (w/w) 1-butanol increased both R_S and *N* values by at least two-fold with an expense of only 1-min increase in retention time. In addition, it is worth noting that the presence of hydroxyl groups in barbiturates generates hydrogen bonding between the analytes and 1-butanol, which is probably a vital source of the increased in retention values. Finally, MEEKC was applied to the separation of the barbiturates. As seen in the electropherogram in Fig. 2d, both R_S and *N* values were remarkably increased as compared to MEKC, but only slightly than the solvent-modified MEKC with 1-butanol. However, it is of interest to note that virtually equivalent α values were obtained for all four separation system. Furthermore, a minor decrease is observed in retention of the barbiturates (Fig. 2d) as compared to the solvent modified-MEKC with no *n*-heptane (Fig. 2c). This phenomenon may be attributed to a disrupt in the hydrogen bonding caused by presence of *n*-heptane.

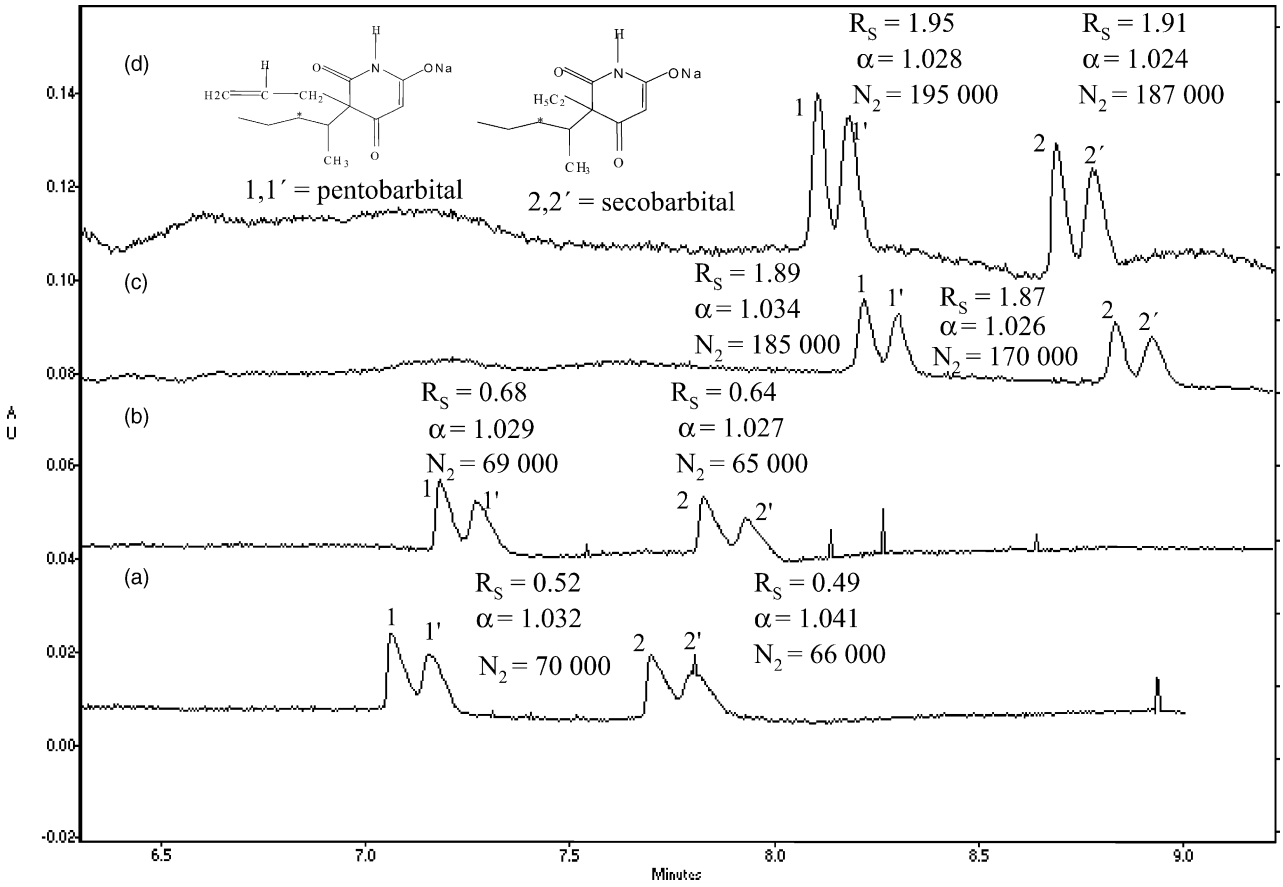

Fig. 2. Comparison of MEEKC and MEKC for simultaneous separation and enantioseparation of 1,1′ = (±) pentobarbital (*P*) and 2,2′ = (±) secobarbital (*S*). Both MEEKC and MEKC contain 0.76% (w/w) poly-D-SUV, Na_2HPO_4/NaH_2PO_4 25 mM at pH 7.0. Enantioseparation are using (a) MEKC without 1-butanol and *n*-heptane (b) solvent-modified MEKC with 0.82% (w/w) *n*-heptane (c) solvent-modified MEKC with 3.50% (w/w) 1-butanol and (d) MEEKC 3.50% (w/w) with 1-butanol and 0.82% (w/w) *n*-heptane. Other operating conditions as given in footnote of Table 1.

3.3. MEEKC separation of paveroline derivatives

3.3.1. Effect of 1-butanol, n-heptane and poly-D-SUV concentration on chiral separation of (±)-laudanosoline and (±)-norlaudanosoline

Two paveroline derivatives [(±)-laudanosoline (*L*) and (±)-norlaudanosoline (*NL*)] were studied. These two solutes are cationic biosynthetic precursors of morphine [28]. The effect of 1-butanol content ranging from 1.00 to 7.00% (w/w) on R_S, α and k'_2 of paveroline derivatives are summarized in Table 4. At lowest content of 1-butanol (i.e., 1.00%, w/w) the highest R_S, α and k'_2 were obtained. As 1-butanol content was increased, the R_S and k'_2 values decreased gradually. Since paveroline derivatives are positively charge they have their own electrophoretic mobilities that drag them faster towards the cathode (detection end) in the absence of 1-butanol. However, as more 1-butanol is added the analytes are less retained in the microemulsion droplet and migrate faster. Thus, it should be noted that in contrary to the negatively charged barbiturates and (±)-BNP, the drop in k'_2 of paveroline derivatives was observed at much lower concentration of 1-butanol [ca. 1.00% (w/w) 1-butanol]. A small but gradual increase in R_S and k'_2 is obtained as *n*-heptane concentration increased steadily (Table 4). An increase in hydrophobic character of micelle polymer upon addition of *n*-heptane may be responsible for the observed trend. Taking a closer look at the R_S values in Table 4 reveals almost no changes in chiral selectivity of paveroline derivatives, except for a very minor increase around 0.82% (w/w) *n*-heptane. It is clear from Table 4 that with increasing amount of poly-D-SUV, k'_2 was almost doubled. However, R_S increased only slightly. Again, no significant change in α of (±)-paveroline derivatives was observed over the entire concentration range of poly-D-SUV.

3.3.2. Comparison of MEEKC and MEKC on chiral separation of paveroline derivatives

Comparison of MEEKC, MEKC, and solvent-modified MEKC of paveroline derivatives is shown in Fig. 3. The paveroline derivatives were well enantioseparated using MEKC (Fig. 3a). The electropherogram in Fig. 3b demonstrates that addition of *n*-heptane has no significant effect on R_S, α and t_r whereas a minor increase in efficiency was observed with solvent-modified MEKC using 0.82% (w/w) *n*-heptane. A slight decrease in retention time was observed when *n*-heptane was replaced by 3.50% (w/w)

Table 4
Effect of varying the amount of 1-butanol, *n*-heptane and poly-D-SUV in the micelle polymer buffer on resolution (R_S), retention factor (k'_2), separation factor (α) of (±) laudanosoline (*L*), (±) norlaudanosoline (*NL*) and unretained time (t_0)

Microemulsion parameter	Concentration percentage (w/w)	R_S		k'_2		α	
		(±)-*L*	(±)-*NL*	(±)-*L*	(±)-*NL*	(±)-*L*	(±)-*NL*
1-Butanol[a]	1.00	0.97	1.97	1.24	1.44	1.056	1.104
	3.50	0.82	1.76	0.41	0.49	1.057	1.095
	5.00	0.72	1.61	0.26	0.33	1.050	1.094
	6.49	0.45	0.69	0.13	0.16	1.031	1.088
	7.00	0.37	0.54	0.15	0.17	1.043	1.063
n-Heptane[b]	0.21	0.87	1.39	0.92	1.07	1.044	1.088
	0.42	0.91	1.47	0.96	1.11	1.047	1.095
	0.82	0.95	1.87	1.24	1.44	1.056	1.099
	1.00	1.23	2.05	1.28	1.47	1.047	1.104
	1.60	1.32	2.13	1.32	1.52	1.046	1.089
poly-D-SUV[c]	0.25	1.03	1.88	0.48	0.99	1.075	1.160
	0.50	1.15	2.17	0.81	1.24	1.278	1.112
	1.00	1.23	2.32	0.98	1.56	1.054	1.054

[a] MEEKC conditions: 0.82% (w/w) *n*-heptane, 0.76% (w/w) poly-D-SUV, varied 1-butanol percentage (w/w). Na_2HPO_4/NaH_2PO_4 25 mM at pH 7.0, separation voltage +20 kV, capillary temperature 25 °C and UV detection wavelength at 220 nm.
[b] MEEKC conditions: 1.00% (w/w) 1-butanol, 0.76% (w/w) poly-D-SUV, varied *n*-heptane percentage (w/w). Other conditions are same as footnote 'a'.
[c] MEEKC conditions: 1.00% (w/w) 1-butanol, 1.60% (w/w) *n*-heptane and varied poly-D-SUV percentage (w/w). Other conditions are same as footnote 'a'.

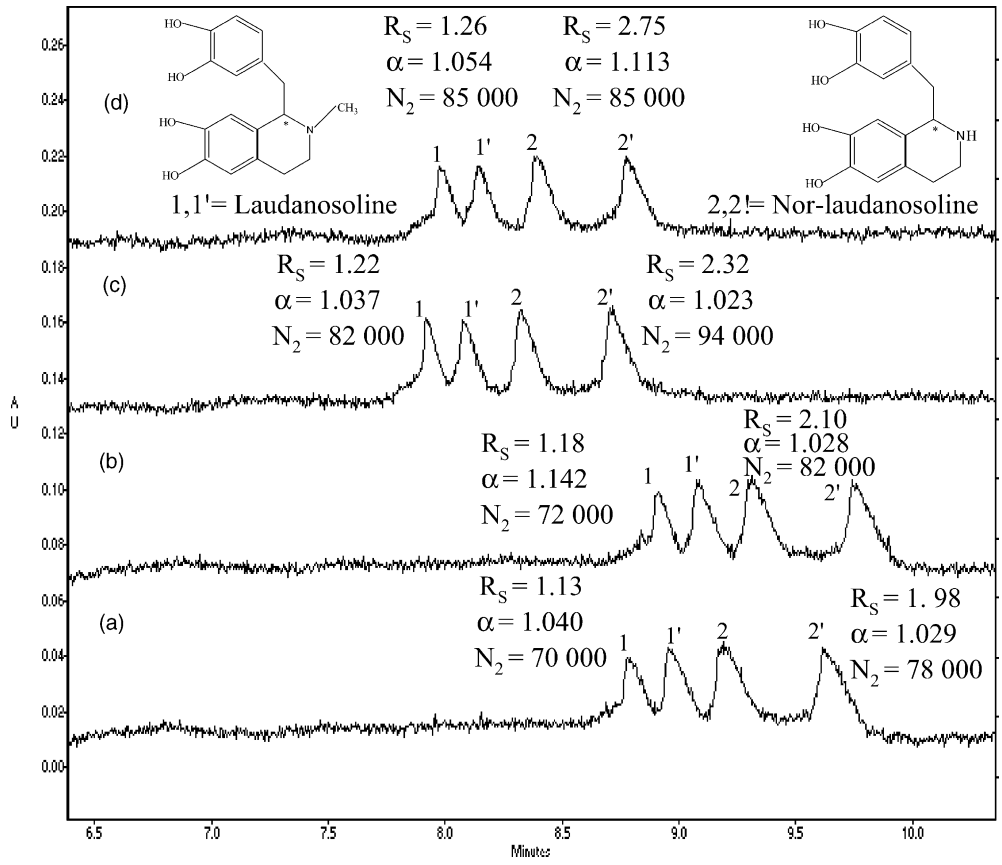

Fig. 3. Comparison of MEEKC and MEKC for simultaneous separation and enantioseparation of 1,1′ = (±)-laudanosoline (*L*) and 2,2′ = (±)-norlaudanosoline (*NL*). Both MEEKC and MEKC contain 1.00% (w/w) poly-D-SUV, Na_2HPO_4/NaH_2PO_4 25 mM at pH 7.0. Enantioseparations are using (a) MEKC without 1-butanol and *n*-heptane (b) solvent-modified MEKC with 1.60% (w/w) *n*-heptane (c) solvent-modified MEKC with 1.00% (w/w) 1-butanol and (d) MEEKC with 1.00% (w/w) 1-butanol and 1.60% (w/w) *n*-heptane.

1-butanol (Fig. 3c). There was also no significant effect on α and t_r of both (±)-(*L*) and (±)-(*NL*) in MEEKC system containing both *n*-heptane and 1-butanol, but increase in R_S of (±)-(*L*) is apparent (Fig. 3d). Nevertheless, comparison of MEKC (Fig. 3a) versus MEEKC (Fig. 3d) clearly shows that better R_S and *N* of paveroline derivatives can be achieved using the latter technique. With respect to the elution order, (±)-(*L*) and always elute before (±)-(*NL*) in all separation systems despite the fact that former has relatively higher hydrophobicity ($\log P = 1.19$) than the latter ($\log P = 0.81$). This demonstrates that the elution order of (±)-(*L*) and (±)-(*NL*) is not controlled by hydrophobic interaction alone, but hydrogen bonding plays an important role as well. The chemical structures of both solutes show that they are almost identical, except methyl group attached to the ring nitrogen in (±)-(*L*) and is replaced by hydrogen atom in (±)-(*NL*). The methyl group provides relatively higher hydrophobic character of (±)-(*L*) while the hydrogen atom increases the hydrogen bonding capability of (±)-(*NL*). Thus, (±)-(*NL*)forms relatively stronger hydrogen bonding with the poly-D-SUV through this extra hydrogen-bonding site. Consequently, longer retention was observed for (±)-(*NL*) as compared to (±)-(*L*).

3.4. MEEKC of binaphthyl and barbiturates derivatives using microemulsion polymers

As mentioned earlier in Sections 3.1–3.3, no significant change in separation factor of enantiomers was induced by use of different concentrations of oil phase (e.g., *n*-heptane in microemulsion of poly-D-SUV). Therefore, surfactant of D-SUV was polymerized in the microemulsion form only in the presence of varied percentage (w/w) of 1-butanol at a fixed 0.82% (w/w) concentration of *n*-heptane. As discussed in Section 2.3.4, after polymerization the residuals of 1-butanol and *n*-heptane were evaporated from the mi-

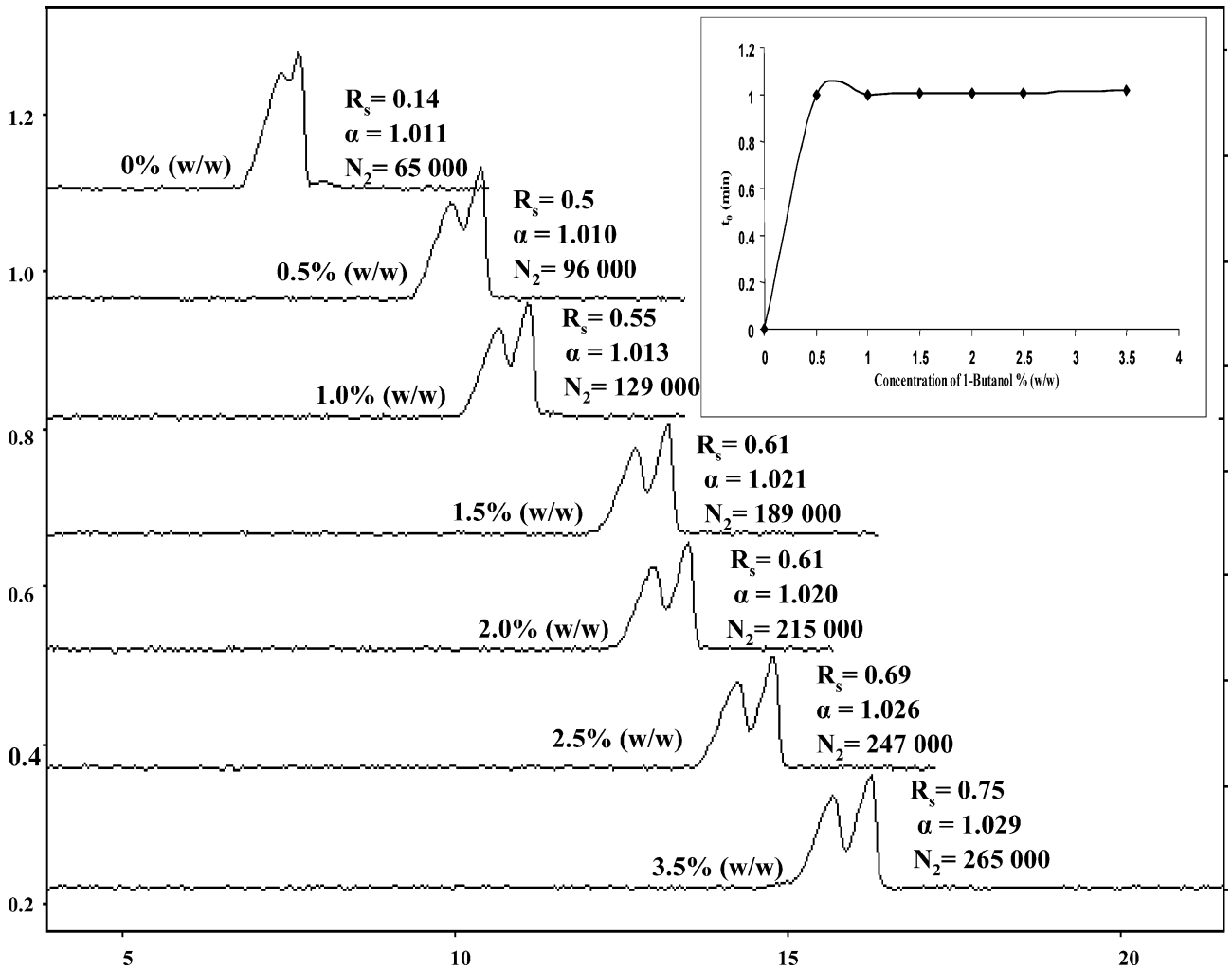

Fig. 4. Effect of variation of the amount of 1-butanol in microemulsion polymers (MP) on the resolution of (±) BNP. MEEKC conditions: 0.76% (w/v) microemulsion polymer (MP) of D-SUV 25 °C, pH 7.0 25 mM phosphate buffer. Pressure injection: 50 mbar, 2 s, +30 kV applied for separations. UV detection at 220 nm. Microemulsion were prepared by dissolving 100 mM D-SUV, *n*-heptane 0.82% (w/w), with various concentrations of 1-butanol polymerized with ^{60}Co γ-radiation. Inset shows the variation of unretained (t_0) time with the variation of 1-butanol.

croemulsion polymer (MP) followed by a freeze-drying process. In order to test the utility of MP as a pseudostationary phase, four racemic analytes [(±)-BNP, (±)-BOH, (±)-(S) and (±)-(P)] were examined. The separation parameters such as R_S, α, k' and t_0 were then evaluated and compared as briefly discussed in the following sections.

3.4.1. Effect of 1-butanol concentration on the separation of (±)-BNP, (±)-BOH, (±)-(S) and (±)-(P)

A comparison of the chiral separation of the (±)-BNP with MP of poly-D-SUV versus the unmodified poly-D-SUV reveals that the later provided high R_S and N (Fig. 4). For example, (±)-BNP was only partially resolved by the use of the unmodified poly-D-SUV. However, both R_S and N of (±)-BNP increased when D-SUV was polymerized in the presence of 0.50–3.50% (w/w) of 1-butanol. In particular, when D-SUV was polymerized with 3.50% (w/w) 1-butanol and 0.82% (w/w) *n*-heptane, highest R_S and N of (±)-BNP was observed with only a slight increase in α. In addition, a flat trend in t_0 value (Fig. 4 inset) between 0.50 and 3.50% (w/w) 1-butanol illustrates very stable EOF over this range. However, further increase in 1-butanol concentration from 3.50 to 7.50% (w/w) resulted in loss of R_S with concomitant decrease in t_r and N as was observed in MEEKC using micelle polymer (data not shown).

Upon comparing the R_S of (±)-BNP shown in Fig. 4 versus Table 1, it is evident that MEEKC using micelle polymer provides higher R_S as compared to using microemulsion polymer, but the migration times are always shorter using the latter. These observations can be explained by understanding the differences in the separation system compositions for the two approaches. It is well known that micellar core volume and the interactions between the analyte and the micellar head group has profound effect on the association of the analyte with the micellar core [28–30]. In MEEKC using MP, the excess of 1-butanol and *n*-heptane have been evaporated after polymerization. Thus, only a small amount of these two organic solvents is encapsulated inside the micellar core. The size-to-charge ratio of the micelles is increased in presence of 1-butanol, which is known

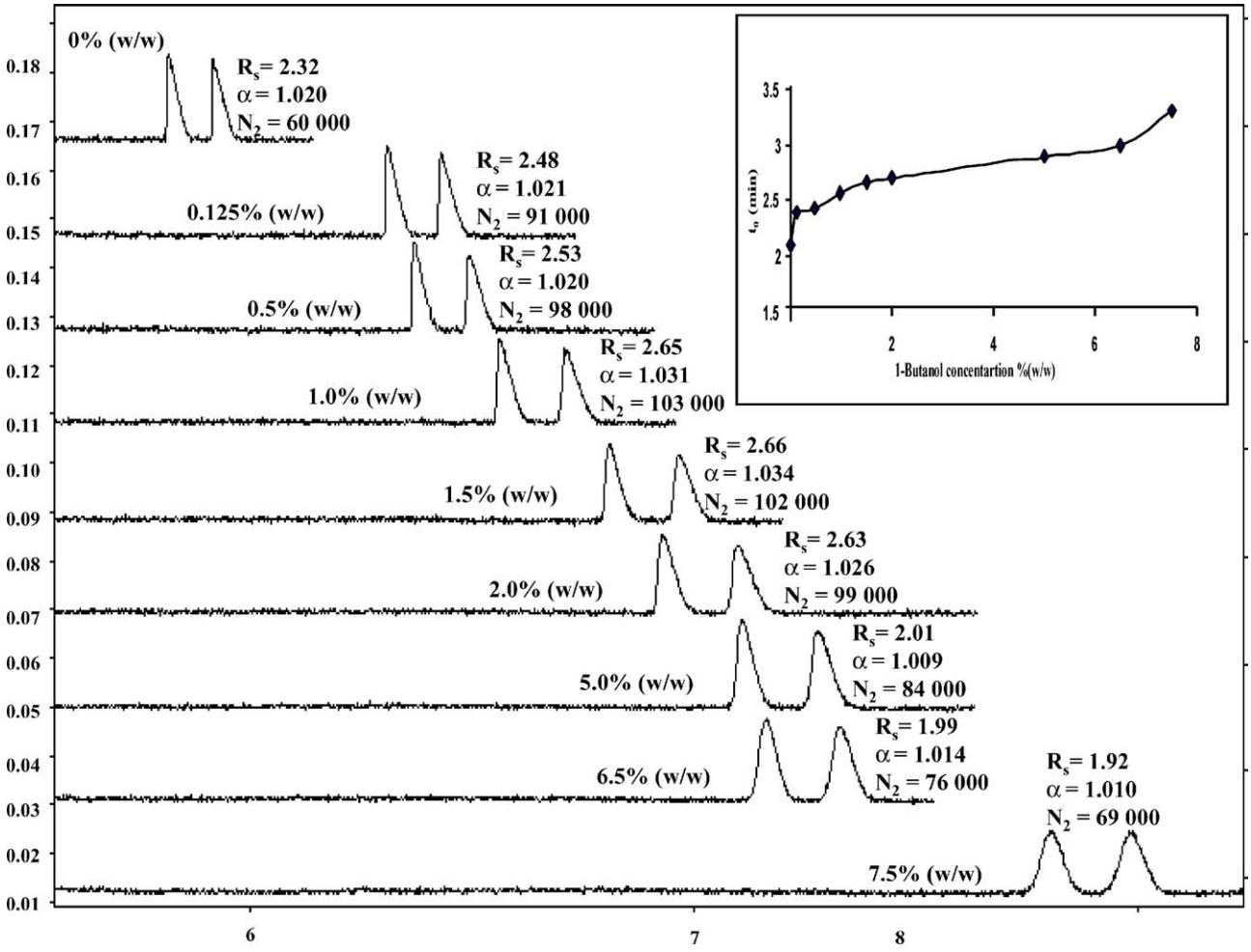

Fig. 5. Effect of variation of the amount of 1-butanol in microemulsion polymers (MP) on the resolution of (±)-BOH. MEEKC conditions: 0.25% (w/v) MP of D-SUV 25 °C. Other MEEKC conditions and microemulsion preparation are same as Fig. 4 except the 25 mM sodium borate at pH 9.0 was used. Inset shows the variation of unretained (t_0) time with the variation of 1-butanol.

to reduce the charge density and to promote formation of micelles with a more open structure [31–33]. As a result, up to 3.50% (w/w) 1-butanol in the surfactant, still promote favorable chiral interaction of (±)-BNP with MP. However, further addition of 1-butanol (>3.50%, w/w) saturates most of the palisade region. Hence, (±)-BNP cannot interact sufficiently with the MP of D-SUV. Therefore, chiral R_S and t_r decrease (data not shown) (Fig. 4).

The enantiomers of (±)-BOH are partially anionic in pH 9.0 buffer though still have overwhelming hydrophobic character. The chiral R_S of (±)-BOH only increased slightly up to 1.50% (w/w) of 1-butanol and further increment in 1-butanol resulted in loss of chiral R_S due to a gradual drop in N and α, however t_r continued to increase (Fig. 5). One of the explanation is that (±)-BOH is hydrophobic enough to penetrate deeper in the micellar core and when 1-butanol concentration is increased in the palisade layer the (±)-BOH interacts strongly with the 1-butanol through hydrogen bonding and reduces chiral resolution due to its poor access to the chiral head group of the MP of D-SUV. This resulted in an increase in t_r, peak broadening and loss of chiral resolution. In addition, similar approach was also adopted for enantioseparation of electrically neutral (±)-BNA. However, MP did not show any improved chiral resolution of (±)-BNA (data not shown).

The effect of 1-butanol concentration on the R_S, α, N_2 and t_0 for the simultaneous enantioseparation of (±)-(*P*) and (±)-(*S*) was also examined (data not shown). The R_S for both (±)-(*P*) and (±)-(*S*) increased slightly up to 0.25% (w/w) 1-butanol, then R_S deteriorated at highest 1-butanol concentration (i.e., 7.50% w/w), but with only minor drop in t_r and t_0 values. Furthermore, the efficiency of the enantiomers and α values remained comparable (data not shown).

4. Conclusions

MEEKC using either micelle polymers or microemulsion polymers was found to be useful separation systems for chiral separation of binaphthyls, barbiturates, and paveroline derivatives. In the first separation system using micelle polymers, the effect of organic solvents (1-butanol, *n*-heptane) as constituents of microemulsion on R_S, k', α, and N were investigated for all three classes of chiral solutes. Several trends are noticeable. First, for anionic [(±)-BNP, (±)-(*P*) and (±)-(*S*)], partially anionic [(±)-BOH] and neutral [(±)-BNA] solutes, it seems obvious that k'_2 increases with an increase in mass fraction of 1-butanol upto atleast 3.50% (w/w). However, further increase in 1-butanol concentration resulted in a drop in k'_2 that in turn deteriorated the resolution of the aforementioned enantiomers. In general, low-to-intermediate mass fraction of 1-butanol provided higher R_S and constant t_0 values of anionic and neutral solutes. Second, the increase in 1-butanol concentration leads to a decrease in k'_2 of cationic [(±)-(*L*) and (±)-(*NL*)] solutes, which is in contrast to the trends observed for anionic or neutral analytes. Third, variation in the composition of the oil phase (*n*-heptane) indicates that the maximum k'_2 is typically seen around 0.82% (w/w). In fact, at concentration >0.82% (w/w) *n*-heptane the k'_2 values of all solutes were reduced. In general, the use of *n*-heptane was shown to have only slight influence on R_S of chiral solutes. For anionic and neutral solutes optimum chiral R_S was found to be 0.82% (w/w) whereas for cationic solutes slightly higher [∼1.00% (w/w) *n*-heptane] provided maximum chiral R_S. However, it should be noted that variation in 1-butanol or *n*-heptane concentration provided no significant change in separation factor of any neutral or charge enantiomers.

The other separation system that utilizes the microemulsion polymer of D-SUV showed significant improvement in R_S and N of (±)-BNP as compared to MEKC. On the other hand, for (±)-BOH as well as for (±)-(*S*) and (±)-(*P*) both MEKC and MEEKC provided similar R_S and N. Finally, we believe that the use of microemulsion polymers may still provide higher enantioselectivity for other classes of chiral solutes. Therefore, further investigation is warranted by varying the microemulsion constituents during polymerization process to fully exploit the potential of this novel technique in CE.

Acknowledgements

This work was supported by a grant from the National Institute of Health (Grant No. 62314-02).

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