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Journal of Chromatography A, 711 (1995) 297–304

JOURNAL OF CHROMATOGRAPHY A

Combined polymerized chiral micelle and γ-cyclodextrin for chiral separation in capillary electrophoresis

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First received 13 September 1994; revised manuscript received 27 April 1995; accepted 28 April 1995

Abstract

A combination of a polymerized chiral micelle, poly(sodium N-undecylenyl-D-valinate) [poly(D-SUV)] and γ-cyclodextrin (γ-CD) is used for the first time for chiral separation in capillary electrophoresis. A simple theory is presented to rationalize the synergistic effect of the enantioselectivity obtained by use of poly(D-SUV) and γ-CD in combination. A mixture of four enantiomeric pairs is successfully resolved by use of this combination. The resolutions of the enantiomers using this approach are far superior to those obtained by use of either poly(D-SUV) or γ-CD alone. In addition, the effects of the antipode (L-SUV), γ-CD concentration, buffer concentration, organic solvents, and urea concentration on the resolution are also examined.

1. Introduction

Chiral separation by use of capillary electrophoresis (CE) has become a research area of major focus in recent years [1–3]. This is largely due to the impact of CE on the pharmaceutical industry. Since a large fraction of therapeutic drugs are racemic compounds and many of them are either cationic or anionic, CE is an obvious tool to investigate the optical purity and separation of such drugs. Separation by use of CE offers greater separation efficiency within a shorter analysis time as compared to conventional chromatographic techniques. Direct chiral separation in CE is achieved either through the use of immobilized chiral phases or through the addition of chiral selectors as mobile phase additives. In the latter case, three types of chiral selectors are often employed: (1) host–guest chiral selectors [4–8], (2) chiral metal complexes [9,10], and (3) chiral surfactants [11–13]. The latter method has been termed micellar electrokinetic capillary chromatography (MECC).

Cyclodextrins (CDs) have been widely used as chiral selectors in chromatographic techniques and in CE [4–7,14] because of their ability to form highly selective molecular inclusion complexes with a variety of neutral or ionic organic species. The CDs are cyclic oligosaccharides consisting of α 1–4 linkage of D-(+)-glucopyranoside units. The unique torus shape of CDs and the fact that each glucose unit has five chiral centers are characteristics that have enabled these oligosaccharides to be effectively used in chiral separations. Since CDs are neutral compounds and migrate with the electroosmotic flow (EOF) in CE, their separation abilities are limited. Therefore, ionic derivatives of CDs and

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0021-9673/95/$09.50
SSDI 0021-9673(95)00515-3

CD–MECC have been used to achieve improved chiral separation [15–18]. The CD–MECC technique combines CDs and micellar systems into the same buffer. This approach was initially used to separate highly hydrophobic compounds [19], but is now widely used in chiral separations [16–18]. However, there is an inherent disadvantage with this approach. The available surfactant monomers in the buffer are partly associated in a complex with the CDs [20,21]. Thus, in many cases, complexation of surfactant monomers with CDs will interfere with interactions between the individual enantiomers and the CDs. This interference will directly affect the enantioselectivity of the system and may reduce the observed chiral resolution.

Chiral micelles have also been used for separation of enantiomers. Most polar organic solutes are able to interact with a micelle on the surface through polar–polar interactions as well as hydrophobic interactions with the core of the micelle. In addition, many chiral compounds will have chiral centers near a polar group. Thus, a surfactant having a chiral center near a polar head group should be suitable for enantiomeric separation by use of MECC. Different types of chiral surfactants have been used in CE chiral separation, including amino acid-derivatized synthetic surfactants, bile salts, and other natural surfactants. Furthermore, Okafo et al. [22] and Terabe et al. [23] have combined the use of bile salts and cyclodextrins for separation of enantiomers in a variety of compounds.

As discussed earlier, a normal micellar system combined with a CD will likely form an inclusion complex between the CD and the surfactant monomer. In many cases, this will reduce the enantioselectivity of CDs. In addition, the use of normal micelles in MECC separation has other limitations. For example, the concentration of the surfactant used in MECC has to be above the critical micellar concentration (CMC) in order to be effective. Thus, for a surfactant with a high CMC, the working concentration range may be very narrow, since at very high concentrations of a charged surfactant, excess heat will be generated in the capillary. This heat production will normally inhibit optimal separation.

We recently synthesized a novel polymerized chiral micelle, poly(sodium N-undecylenyl-L-valinate) and successfully used it for chiral separation in MECC [24]. The polymerized micelle not only possesses normal micellar properties but also has several advantages over normal micelles, including enhanced stability because of covalent linkage of the surfactant monomers. This property can be used in CD–MECC to diminish the normal inclusion phenomena associated with surfactant monomers and CDs. There is also no CMC for polymerized micelles. This property allows use of the polymerized micelle in MECC over a wider working concentration range [25]. Finally, polymerized micelles are typically more rigid than normal micelles. Since the solute cannot penetrate deep into the rigid, polymerized micelles, interactions between the solute and the polymerized micelle must occur near the surface of the micelles. Thus, the mass transfer rate of the solute, between a polymerized micelle and the bulk solution, should be faster than that of a normal micellar system, which allows penetration of the solute via dynamic equilibrium.

In this manuscript, we report the first chiral separation which employs a combination of a polymerized chiral micelle and γ-CD. The polymerized chiral micelles used in this study are poly(sodium N-undecylenyl-L-valinate) [poly(L-SUV)] and its antipode poly(sodium N-undecylenyl-D-valinate) [poly(D-SUV)]. The separation of a mixture of four enantiomeric pairs by use of poly(D-SUV) and γ-CD is superior to the separation obtained by using either poly(D-SUV) or γ-CD, separately. The separation mechanism of this synergistic effect is presented. In addition, the effects of the antipode [poly (L-SUV)], γ-CD concentration, the buffer concentration, and organic solvents on the chiral separation are also evaluated.

2. Experimental

2.1. Materials

The procedure for synthesis of poly(L-SUV) and the characterization of poly(L-SUV) have been recently reported [24]. The same procedure

is used for synthesis of poly(D-SUV) ($[a]_D^{25} = +8.0^0$ ($c = 1.00$ in water)). It is found that poly(D-SUV) has the same physico-chemical properties as poly(L-SUV) in achiral environments. (±)-1,1′-Bi-2-naphthol (99%), (*R*)-(+)-1,1′-bi-2-naphthol (99%), *S*-(−)-1,1′-bi-2-naphthol (99%), (±)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate ($BNPO_4$) (99%), and (±)-verapamil (95%) were purchased from Aldrich (Milwaukee, WI, USA). D,L-Laudanosine (95%), L-valine (>99%), D-valine (99%), and undecylenic acid (>99%) were obtained from Sigma (St. Louis, MO, USA). The γ-cyclodextrin used in this study was a gift from American Maize Products (Hammond, IN, USA). These items were used as received.

2.2. Capillary electrophoresis

Micellar electrokinetic capillary chromatography experiments were conducted by use of a CES I capillary electrophoresis system (Dionex, Sunnyvale, CA, USA). Data were collected by use of an AI-450 chromatography workstation. An untreated fused-silica capillary (effective length 60 cm, 75 μm I.D.) was purchased from Polymicro Technologies (Phoenix, AZ, USA) and used as a separation column. The solution was buffered at pH 9 using borate buffer. The polymerized micelles and γ-CD were added directly to the buffer system. The buffer solutions were filtered through a 0.45-μm membrane filter prior to use. Separations were performed at 12 kV with UV detection at 280 nm. Samples were prepared in a methanol–water mixture in a concentration range from 0.02 to 0.1 mg/ml.

3. Results and discussion

3.1. Theory

Since this CD-modified polymerized chiral micellar system is similar to a normal CD-MECC system, we can use normal CD-MECC theory [3] with minor modifications where appropriate. In our system, the polymerized chiral micelle behaves as a pseudo-stationary phase. It is assumed that the neutral γ-CD is part of the aqueous phase. In addition, it is assumed that the enantiomers interact independently with the polymerized micelle and the γ-CD. Thus, the capacity factor, k', can be defined as:

$$k' = \frac{n_{mp}}{n_{aq}} \tag{1}$$

where n_{mp} and n_{aq} are the moles of solute molecules associated with the polymerized micelle and the moles associated with the aqueous phase (including CD), respectively. In addition,

$$n_{aq} = n_f + n_{CD} \tag{2}$$

since the moles of solute in the aqueous phase include not only the moles of free solute, n_f, but also the solute molecules incorporated in CD, n_{CD}.

Based on a partitioning mechanism in this CD–chiral MECC system, there are two important partitions for an enantiomer A:

$$[A]_f \rightleftarrows [A]_{mp} \tag{3}$$

$$[A]_f \rightleftarrows [A]_{CD} \tag{4}$$

where $[A]_f$, $[A]_{mp}$, and $[A]_{CD}$ are the concentration of the enantiomer, A, in the aqueous phase, micelle polymer, and cyclodextrin, respectively. Thus, we can obtain

$$\frac{n_{mp,A}}{n_{f,A}} = K_{mp,A}\frac{V_{mp}}{V_f} \tag{5}$$

$$\frac{n_{CD,A}}{n_{f,A}} = K_{CD,A}\frac{V_{CD}}{V_f} \tag{6}$$

where K_{mp} and K_{CD} are partition coefficients between the polymerized micelle and the aqueous phase and between the CD and the aqueous phase, respectively, and V_f, V_{mp}, and V_{CD} are the volume of the aqueous phase, the micellar phase, and the cyclodextrin phase, respectively. We can combine Eqs. 1, 2, 5, and 6 to obtain

$$k' = \frac{K_{mp}V_{mp}}{V_f + V_{CD}K_{CD}} \tag{7}$$

Selectivity for an enantiomeric pair can be defined as

$$\alpha = \frac{k'_{app,B}}{k'_{app,A}} \quad (8)$$

where $k'_{app,A}$ and $k'_{app,B}$ are apparent capacity factors for the enantiomeric pair, A and B, respectively.

From Eqs. 7 and 8, we can derive:

$$\alpha = \frac{1 + \phi_{CD} K_{CD,A} K_{mp,B}}{1 + \phi_{CD} K_{CD,B} K_{mp,A}} \quad (9)$$

where ϕ_{CD} is the phase ratio of the volume of CD (V_{CD}) to that of the aqueous phase (V_f). Selectivity is directly related to resolution (R_s). For a given plate number N and apparent capacity factor k', the greater the value of α, the higher is the resolution (R_s). From Eq. 9, since α must be ≥ 1, it can be shown that there are only three combinations of these parameters: (1) if $K_{CD,A} > K_{CD,B}$ and $K_{mp,B} > K_{mp,A}$, chiral resolution will be superior to that obtained using either CD or the polymerized chiral micelle alone; (2) if $K_{CD,A} > K_{CD,B}$ and $K_{mp,A} > K_{mp,B}$, chiral resolution will be poorer than by use of γ-CD alone; (3) if $K_{CD,A} < K_{CD,B}$ and $K_{mp,B} > K_{mp,A}$, then resolution will be poorer than by use of the polymerized chiral micelle alone. Fig. 1 provides a demonstration of the enantiomeric separation obtained through a combination of γ-CD and poly(D-SUV) and also validates Eq. 9. When using only γ-CD as a chiral selector, *R*-1,1′-bi-2-naphthol has a high affinity for γ-CD and will migrate faster than the *S*-form (Fig. 1a). When using only poly(D-SUV) as a chiral selector, the *S*-1,1′-bi-2-naphthol interacts stronger with the polymer than the *R*-form and *R*-1,1′-bi-2-naphthol will migrate through the system faster than the *S*-form (Fig. 1b). If we combine these two chiral selectors, it is found that $K_{CD,R} > K_{CD,S}$ and $K_{mp,S} > K_{mp,R}$. Therefore, chiral resolution will be greater than by use of either chiral selector alone. This synergistic effect of γ-CD and poly(D-SUV) on the separation of *R,S*-1,1′-bi-2-naphthol is demonstrated in Fig. 1c. Furthermore, as shown in Fig. 1d, while combining γ-CD and poly(L-SUV), the chiral resolution is diminished, which corresponds to the condition where $K_{CD,R} > K_{CD,S}$ and $K_{mp,R} > K_{mp,S}$.

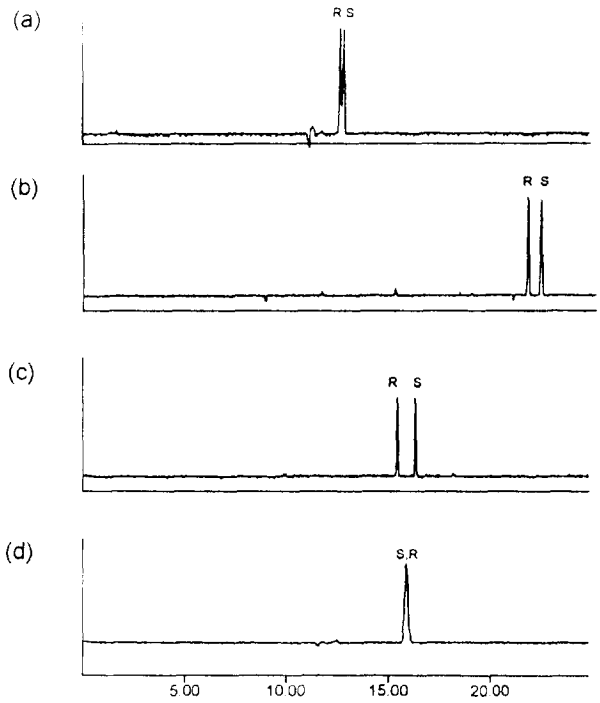

Fig. 1. Chiral separation of (±)-1,1′-binaphthol by use of poly(D-SUV) and γ-CD. CE conditions: 25 m*M* borate buffer (pH 9); applied voltage, 12 kV; UV detection, 280 nm. (a) 10 m*M* γ-CD, (b) 0.5% poly(D-SUV), (c) 10 m*M* γ-CD and 0.5% poly(D-SUV), (d) 10 m*M* γ-CD and 0.5% poly(L-SUV).

3.2. Separation of mixtures of chiral compounds by use of γ-CD and poly(D-SUV)

A combination of γ-CD and poly(D-SUV) not only enhances chiral selectivity but also extends the migration window of CD-modified CE since CDs are neutral compounds and migrate with the EOF. Fig. 2 shows the separation of a mixture of four enantiomeric compounds. Using either γ-CD or poly(D-SUV) alone at the concentrations examined, no satisfactory resolution is obtained (Fig. 2a,b). However, use of both γ-CD and poly(D-SUV) at the same concentrations resolves three enantiomeric pairs (Fig. 2c). With further optimization, all four compounds are resolved (Fig. 2d).

Since there are a number of parameters which affect chiral separations, the separation mechanism of polymerized chiral micelles is not always

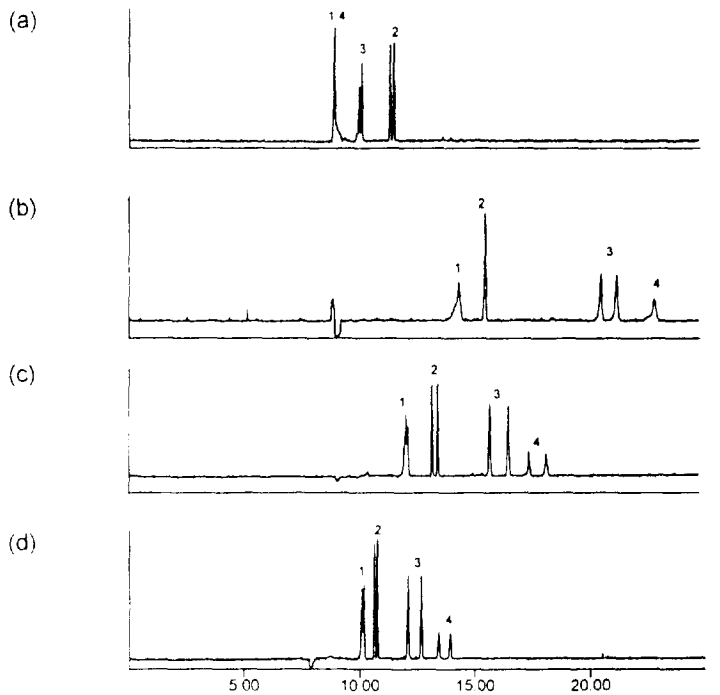

Fig. 2. Chiral separation of four enantiomeric pairs. Peaks: 1 = D,L-laudanosine, 2 = (±)-$BNPO_4$, 3 = (±)-1,1'-binaphthol, 4 = (±)-verapamil. CE conditions: (a) 10 m*M* γ-CD, (b) 0.5% poly(D-SUV), (c) 10 m*M* γ-CD and 0.5% poly(D-SUV), buffer for (a), (b) and (c) is 25 m*M* borate (pH 9). (d) 10 m*M* γ-CD and 0.5% poly(D-SUV), 5 m*M* borate (pH 9). Applied voltage, 12 kV; UV detection, 280 nm.

clear. In the present work, a series of experiments are conducted in order to optimize this CD–chiral MECC system.

3.3. Effect of γ-CD concentration on enantiomeric resolution

The effect of γ-CD concentration on the separation is investigated over the concentration range 5–20 m*M*. Fig. 3 shows the effect of CD concentration on the resolution of four enantiomeric compounds. Resolution generally increases with increasing cyclodextrin concentration up to a point where maximum separation is reached. Further increases in CD concentration result in a decrease in resolution. The optimum concentration of cyclodextrin is dependent on the enantiomeric pair being separated. These observations are consistent with data in the literature [7]. There are several models which can be used for quantitative optimization

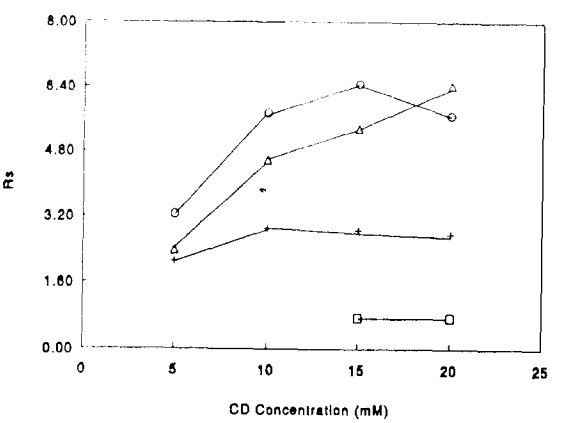

Fig. 3. Effect of γ-CD concentration on the resolution of the enantiomeric mixture. CE conditions: 0.5% poly(D-SUV), 25 m*M* borate (pH 9), 5–20 m*M* γ-CD; applied voltage, 12 kV; UV detection, 280 nm. (○) (±)-1,1'-binaphthol, (△) (±)-verapamil, (+) (±)-$BNPO_4$, (□) D,L-laudanosine.

[7,26,27]. However, we can qualitatively estimate the optimum CD concentration for (±)-$BNPO_4$ to be approximately 10 m*M*; for (±)-1,1′-bi-2-naphthol the optimum concentration is approximately 15 m*M*; for (±)-verapamil the optimum concentration is above 20 m*M*. In the case of D,L-laudanosine, an increase in γ-CD concentration initially improves the enantiomeric separation but further increase of the concentration does not significantly enhance the resolution. This observation suggests that the interactions between D,L-laudanosine enantiomers and γ-CD are weak and the resolution cannot be improved significantly by the addition of γ-CD alone.

3.4. Effect of buffer concentration on enantiomeric resolution

Generally, the buffer concentration controls the ionic strength of the electrolyte. Increasing the buffer concentration will reduce the EOF and also increase the viscosity of the electrolyte. This expands the migration time window for the CD–MECC system. However, this does not necessarily mean that resolution of the enantiomers will be increased. In our chiral separations, an increase in the borate buffer concentration from 5 to 45 m*M* lengthens the migration time for each compound. The resolutions of most enantiomeric pairs are enhanced, except for D,L-laudanosine where the resolution is decreased (Fig. 4). These observations are consistent with results obtained in our previous study [24]. The charged chiral polymer is more flexible at lower buffer concentration than at higher buffer concentration. This is because, at low buffer concentrations the polymerized micelle has fewer closely associated counterions than at high buffer concentrations. Thus, the micelle can extend more at low buffer concentrations than at high concentrations of buffer. This effect may induce a larger difference in affinities between the individual D- and L-enantiomeric forms at the lower buffer concentration and thus results in better resolution.

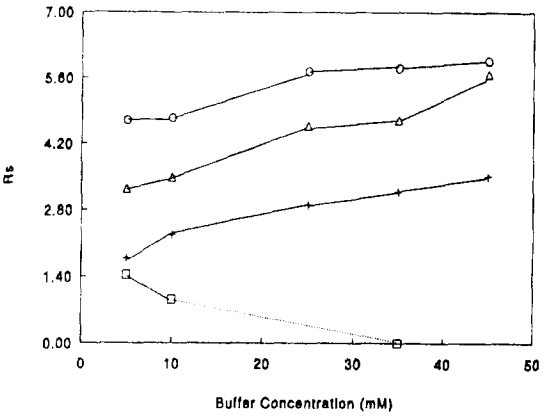

Fig. 4. Effect of buffer concentration on the resolution of the enantiomeric mixture. CE conditions: 0.5% poly(D-SUV), 5–45 m*M* borate (pH 9), 10 m*M* γ-CD; applied voltage, 12 kV; UV detection, 280 nm. (○) (±)-1,1′-binaphthol, (△) (±)-verapamil, (+) (±)-$BNPO_4$, (□) D,L-laudanosine.

3.5. Effect of organic solvents on enantiomeric resolution

The effects of organic modifiers on chiral resolution will not only depend on the type of chiral selectors used in the system but also on the properties of the enantiomers, e.g. their hydrophobic and hydrophilic properties. In general, the migration time of the samples increases with an increase in concentration of organic solvents. This is due to a decrease in the EOF. In normal MECC or CD–MECC, if the concentration of an organic solvent such as methanol is very high, the micelle will decompose into surfactant monomers and solute–micelle interaction is not possible. However, in our system, at very high concentrations of methanol (40%), the enantiomeric resolutions of the compounds examined are still very good (Fig. 5), except for D,L-laudanosine. Even in the presence of a small amount of methanol, separation of D,L-laudanosine is impaired. It is also very interesting in Fig. 5 that the remaining three enantiomeric pairs behave very differently. For example, the enantiomeric resolution of (±)-1,1′-bi-2-naphthol is improved by increasing methanol concentration. When only the poly(D-SUV) is used in the buffer, the resolution of (±)-1,1′-bi-

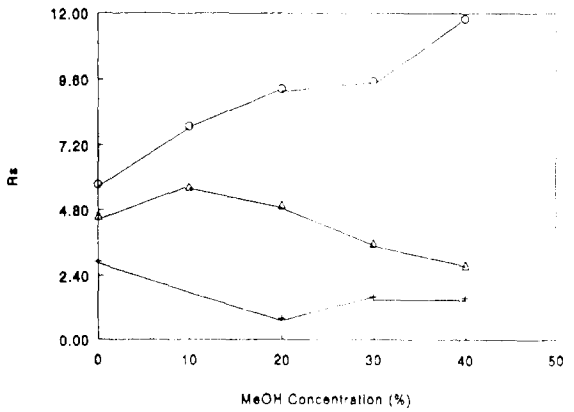

Fig. 5. Effect of methanol concentration on the resolution of the enantiomeric mixture. CE conditions: 0.5% poly(D-SUV), 25 m*M* borate (pH 9), 10 m*M* γ-CD, 0–40% MeOH; applied voltage, 12 kV; UV detection, 280 nm. (○) (±)-1,1′-binaphthol, (△) (±)-verapamil, (+) (±)-$BNPO_4$.

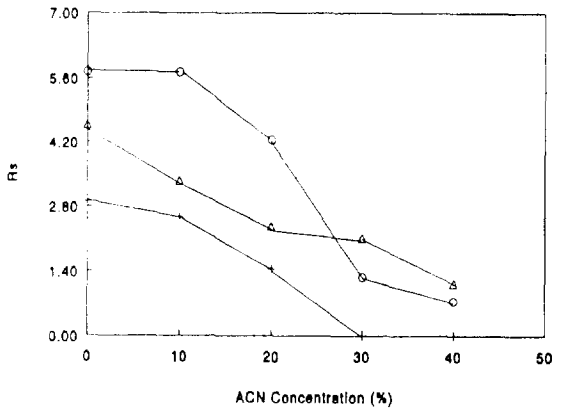

Fig. 6. Effect of ACN concentration on the resolution of the enantiomeric mixture. CE conditions: 0.5% poly(D-SUV), 25 m*M* borate (pH 9), 10 m*M* γ-CD, 0–40% ACN; applied voltage, 12 kV; UV detection, 280 nm. (○) (±)-1,1′-binaphthol, (△) (±)-verapamil, (+) (±)-$BNPO_4$.

2-naphthol is increased with an increase in methanol concentration. However, when only γ-CD is used, resolution is slightly decreased with an increase in methanol concentration. Thus, this combination shows a net increase of resolution upon addition of methanol. Enantiomeric resolution of (±)-verapamil increases with increasing concentration of methanol up to 10%. However, above 10% methanol, the resolution starts to decrease with increasing concentration of methanol. In contrast, the enantioselectivity of (±)-$BNPO_4$ is decreased with increasing methanol concentration up to 10%. Upon further addition of methanol, the resolution starts to increase, although the highest resolution is still obtained using the buffer without methanol. These observations show that the addition of methanol to the buffer produces different effects on the interactions between individual enantiomeric pairs with the chiral selectors [poly(D-SUV), γ-CD]. Since these effects are also solute-dependent, different resolution curves for different enantiomeric pairs are obtained.

Acetonitrile (ACN) is another frequently used organic solvent. As shown in Fig. 6, the addition of ACN decreases the resolution of the enantiomers. Since the pair of D,L-laudanosine has the weakest interaction with both the polymerized chiral micelle and the γ-CD, the presence of a small amount of ACN impairs the resolution of the enantiomers. It is known that ACN can displace solutes from cyclodextrin cavities, which would be expected to reduce the chiral recognition of the γ-CD.

4. Conclusions

As shown in these studies, chiral separation of the four enantiomeric pairs by use of poly(D-SUV) and γ-CD is superior to the use of either chiral selector alone. The modified theory we present here adequately explains the observed synergistic effect on the enantioselectivity by use of both chiral selectors. Since surfactant monomers do not exist in the covalently bonded polymerized micelle, the interference of surfactant monomers on the enantioselectivity of γ-CD is eliminated. Therefore, the use of a combination of poly(D-SUV) and γ-CD for chiral separation in CE proves to be a promising method for separation of mixtures of enantiomers. In addition, optimization of chiral separations in this binary system is more complicated than by use of only one chiral selector. This is because there are numerous factors which can affect the

enantioselectivity of this system. According to our experiments, some effects on the resolution of the enantiomers are very obvious, while others are ambiguous. It should be noted that each pair of enantiomers reaches an optimum resolution at a characteristic concentration of γ-CD. This is similar to what is observed in CD-modified CE. An increase in the buffer concentration causes the resolution of the enantiomers to increase, except for DL-laudanosine. The addition of ACN to the buffer reduces the enantioselectivity of the chiral system. However, the effect of the addition of methanol is more complicated and more solute-dependent.

Acknowledgements

This work was supported in part by a grant from the National Science Foundation (CHE-9224177). The authors also thank G.A. Reed of American Maize Products for kindly donating the CDs used in this study.

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