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Chiral separation of monoterpenes using mixtures of sulfated β-cyclodextrins and α-cyclodextrin as chiral additives in the reversed-polarity capillary electrophoresis mode

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Abstract

The use of mixtures of sulfated β-cyclodextrins and native α-cyclodextrin as chiral additives in capillary electrophoresis was evaluated for the chiral resolution of neutral, cyclic and bicyclic monoterpenes, including α-pinene, β-pinene, camphene and limonene. Binding properties of sulfated β-cyclodextrins towards these monoterpenes were studied. It was found that there was no enantioresolution of these terpenoids over the concentration range studied. However, the addition of α-cyclodextrin to the running electrolyte in addition to 6.5 m*M* sulfated β-cyclodextrins, imparted differences in the mobilities of the terpenoid enantiomers and resulted in remarkable enantiomeric separations of α-pinene (R_s=25), β-pinene (R_s=12), camphene (R_s=12) and limonene (R_s=4). The role of both α-cyclodextrin and sulfated β-cyclodextrins in these separations is discussed.

Keywords: Enantiomer separation; Cyclodextrin additives; Buffer composition; Monoterpenes; Terpenes; Cyclodextrins; Pinenes

1. Introduction

Cyclodextrins have been used extensively as chiral selectors for liquid chromatography and for capillary electrophoresis (CE) [1,2]. Because cyclodextrins are commonly available in three different sizes, a variety of racemic analytes may be separable with the judicious choice of the appropriate cyclodextrin. Nishi and Terabe [3] recently reviewed the applications of native and various derivatized cyclodextrins, such as methylated, hydroxypropylated and hydroxyethylated cyclodextrins, as chiral additives in CE to achieve enantioseparations. One fundamental limitation of neutral cyclodextrins for chiral separations in CE is that it is impossible to separate neutral analytes. This can be overcome by adding other charged components to the run buffer, such as ionic, micelle-forming surfactants [4].

Recently, anionic-substituted cyclodextrins, such as those with sulfobutyl [5,6], sulfoethyl [7], carboxymethyl [8,9] and sulfate [10,11] groups, have been effectively used as chiral selectors, both in chromatography and in CE. The use of charged chiral additives in the running buffer allows the separation of neutral analytes to take place and considerably widens the "separation window" [5]. Unfortunately, the longer migration times and the higher currents associated with charged additives often limited their

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use with weakly binding analytes. Recently, a successful alternative approach was reported in which commercially available mixtures of randomly substituted sulfated β-cyclodextrins were used with CE for the enantioseparation of approximately 80 compounds of pharmaceutical interest [12,13]. This technique uses the reversed-electrophoretic mode in which analytes are introduced at the cathodic end and detected at the anodic end of the capillary. Using this approach, higher concentrations of highly charged cyclodextrins not only shorten the analysis times but also enhance the enantioseparation of weakly binding analytes. This is because the weaker binding analytes generally need higher concentrations of the chiral selector in order to be enantioresolved [14]. In addition, the reversed-electrophoretic mode results in a reversal of the migration order for the enantiomers, compared to that obtained in the conventional electrophoretic mode.

Anigbogu et al. [15] reported a dual (neutral and charged) cyclodextrin mixture for the resolution of aminoglutethimide in CE. Despite an obvious complexation between carboxymethylated β-cyclodextrins and aminoglutethimide, no chiral resolution was observed. However, when neutral β-cyclodextrin was added to the running buffer containing carboxymethylated β-cyclodextrins, the enantioseparation of aminoglutethimide was possible. The neutral β-cyclodextrin provided the required difference in the binding of the enantiomers and the charged carboxymethylated β-cyclodextrins provided the appropriate mobility. The combination of these effects allowed enantioseparation to occur.

Monoterpenes such as α-pinene, β-pinene, camphene and limonene lack aromatic rings, hydroxyl groups and other strong dipolar groups. Consequently, solution-based enantioseparations of these compounds are not easy tasks. Also, the lack of functional groups limits the possibility of derivatizing these compounds. Most enantioresolutions of nonpolar hydrocarbons have been carried out by gas chromatographic techniques using various derivatized cyclodextrin stationary phases. Koscielski et al. [16] resolved α-pinene and β-pinene on packed GC columns where the supporting material was coated with a solution of α-cyclodextrin in formamide. Armstrong et al. [17] reported the resolution of bicyclic monoterpenes including α-pinene, β-pinene, limonene, camphene, borneol and isoborneol by using capillary GC columns coated with hydroxypropylated cyclodextrins. Recently, the same group (Armstrong and Zukowski, [18]) reported the direct enantiomeric resolution of α-pinene, β-pinene and camphene via reversed-phase HPLC with an α-cyclodextrin bonded stationary phase. It was this work that suggested that α-cyclodextrin could sterically discriminate between some hydrocarbon enantiomers in solution. Also, Botsi et al. [19] reported proton NMR resolution of racemic α-pinene using several different cyclodextrins, including α-cyclodextrin, methylated α- and β-cyclodextrins, as well as acetylated β-cyclodextrins, as chiral shift agents in water. Due to low solubility of the complex of α-pinene with either β-cyclodextrin or γ-cyclodextrin, no resolution of α-pinene had been reported.

In this study, substantial enantioresolution of the neutral bicyclic monoterpenoids, such as α-pinene, β-pinene, camphene and limonene, by a CE technique are reported. The chiral separations were achieved using only mixtures of α-cyclodextrin and sulfated β-cyclodextrins as chiral additives in CE.

2. Experimental

2.1. Materials

Sulfated β-cyclodextrins (average degree of substitution, four) were obtained from American Maize Products (Hammond, IN, USA). α-Cyclodextrin was obtained from ASTEC (Whippany, NJ, USA). Racemic solutes as well as pure enantiomers were obtained from Aldrich (Milwaukee, WI, USA) or Fluka (Buchs, Switzerland). NaH_2PO_4 and H_3PO_4 were obtained from Aldrich.

2.2. Methods

A Waters Quanta 4000 CE system was used. The length of the capillary to the detector was 50 cm and was 57.6 cm end-to-end. The inner diameter of the capillary was 75 μm and UV detection was accomplished at 214 nm. The buffer was prepared using a 10 m*M* NaH_2PO_4 solution, adjusted to pH 3.3 by addition of H_3PO_4. Deionized water was used to prepare the buffer solution. All samples were dis-

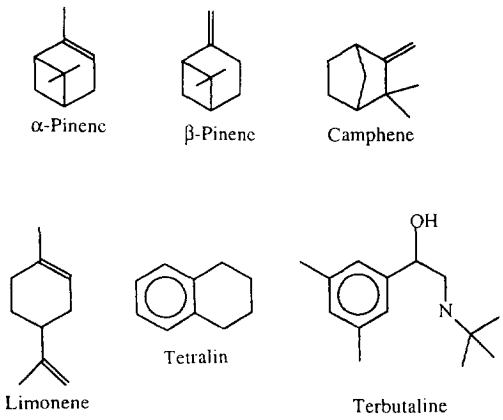

Fig. 1. Structures of monoterpenes, tetralin and terbutaline.

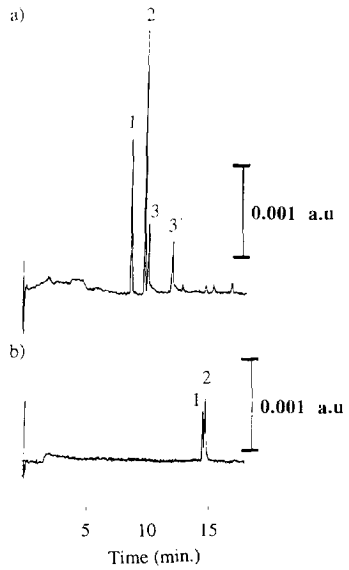

Fig. 2. (a) Electropherogram of the separation of α-pinene (1), tetralin (2) and the terbutaline enantiomers (3, 3'). The running electrolyte consisted of 6.5 m*M* sulfated β-cyclodextrins in 10 m*M* phosphate buffer (pH 3.3). (b) Electropherogram of α-pinene (1) and β-pinene (2). The electrolyte consisted of 1.3 m*M* sulfated β-cyclodextrins in 10 m*M* phosphate buffer, pH 3.3.

solved in methanol at about 1 mg/ml. The samples were hydrostatically (2 or 4 s) introduced into the cathodic end of the capillary. The analysis was carried out at 21±2°C. A voltage of −20 kV was used for all electrophoretic analysis.

3. Results and discussion

Structures of the monoterpenes examined in this study, together with terbutaline and tetralin are shown in Fig. 1. Binding characteristics of monoterpenes with sulfated β-cyclodextrins were studied. The electrophoretic data obtained, at two different sulfated β-cyclodextrin concentrations, in the reversed-electrophoretic mode, are listed in Table 1.

Fig. 2a is an electropherogram showing the migration of α-pinene together with terbutaline and achiral tetralin using 6.5 m*M* sulfated β-cyclodextrins in the running buffer. Achiral tetralin and terbutaline are chosen for the purpose of comparison. α-Pinene migrated faster than tetralin, indicating that α-pinene binds more strongly to the sulfated cyclodextrins than tetralin. The earlier migration of a neutral analyte in the reversed-electrophoretic polarity mode indicated a stronger interaction with sulfated cyclodextrins [13]. This shows the importance of hydrophobic-driven complexation between α-pinene and sulfated β-cyclodextrins. Terbutaline is positively charged under these experimental conditions (pH 3.3). The fact that terbutaline migrates towards the anode and has a large separation factor demonstrates both the highly negatively charged nature of the complex, as well as the ability of the sulfated β-cyclodextrins to discrimate on the basis of chirality.

Table 1
Migration times of monoterpenes[a]

Concentration of sulfated β-cyclodextrins (m*M*)	Current (μA)	Migration time (min)			
		α-Pinene	β-Pinene	Camphene	Limonene
1.3	24	14.32	14.53	14.53	16.33
6.5	52	9.18	9.18	9.18	9.40

[a] Electrophoretic conditions: electrolytes consisted of 1.3 or 6.5 m*M* sulfated β-cyclodextrins in 10 m*M* phosphate buffer, pH 3.3. −20 kV were applied.

However, there is no enantioseparation of α-pinene and β-pinene with sulfated β-cyclodextrins in the running electrolyte (Table 1). This indicates that there is no difference in the binding constants between sulfated β-cyclodextrins and the pinene enantiomers. No enantioresolution of camphene and limonene was obtained using these experimental conditions. β-Pinene and camphene migrated together and limonene migrated more slowly than any other monoterpene used in this study. This indicates that limonene had the weakest interaction with the sulfated β-cyclodextrins.

Wren and Rowe [14] indicated that higher concentrations of a chiral selector can sometimes result in poorer enantioseparations. To check on the possibility that the lack of a chiral separation may have been due to the high concentration of the chiral selector, the experiment was repeated with lower levels (1.3 m*M*) of sulfated β-cyclodextrins. No chiral separation of monoterpenes was observed using the 1.3 m*M* solution of sulfated β-cyclodextrins. Apparently, the sulfated β-cyclodextrins do not have significant differences in affinity for the monoterpene enantiomers. However, by reducing the concentration of sulfated β-cyclodextrins, it was possible to distinguish the relative strength of interaction between α- and β-pinene. The earlier migration of α- and β-pinene, as shown in Fig. 2b, indicates that α-pinene binds more tightly to sulfated β-cyclodextrins than does β-pinene. The lack of a difference in the migration times between camphene and β-pinene indicates that they have similar strength interactions with the sulfated β-cyclodextrins under these experimental conditions. Therefore, the order of relative binding strength of monoterpenes to the sulfated cyclodextrins is α-pinene>β-pinene≈camphene>limonene.

As mentioned previously, it is possible to impart differences in mobilities of enantiomers by adding a second chiral selector [15]. α-Cyclodextrin is the logical choice for these analytes, considering previous LC reports on its enantiodiscrimination of monoterpenes [18]. Indeed, the combination of α-cyclodextrin and sulfated β-cyclodextrins provided the desired enantioseparations. The resultant electrophoretic data, including migration times and resolutions, are shown in Figs. 3 and 4, respectively. Generally, addition of α-cyclodextrin to running buffer containing 6.5 m*M* sulfated β-cyclodextrins results in longer migration times and increased resolution.

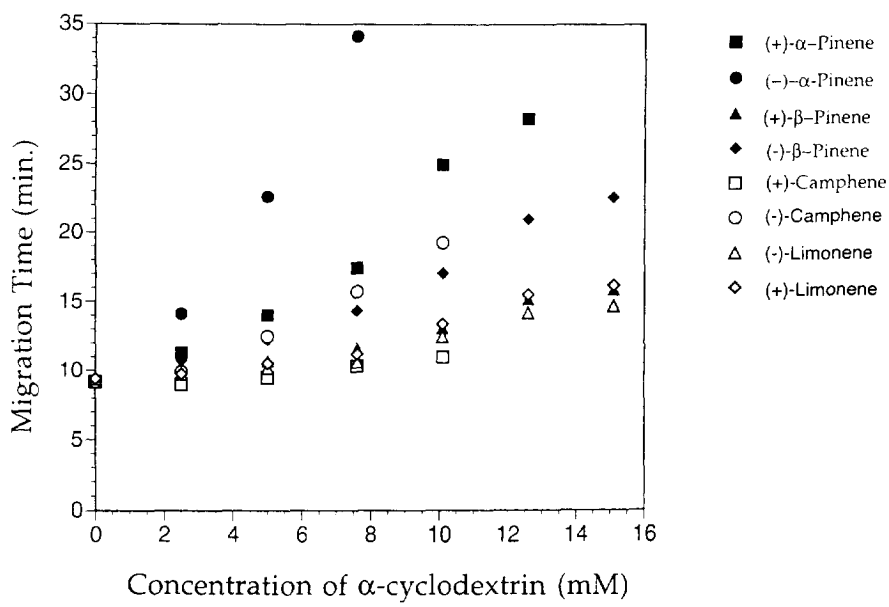

Fig. 3. Effect of the concentration of α-cyclodextrin on the migration time of terpenes. The electrolyte consisted of 6.5 m*M* sulfated β-cyclodextrins in 10 m*M* phosphate buffer, pH 3.3.

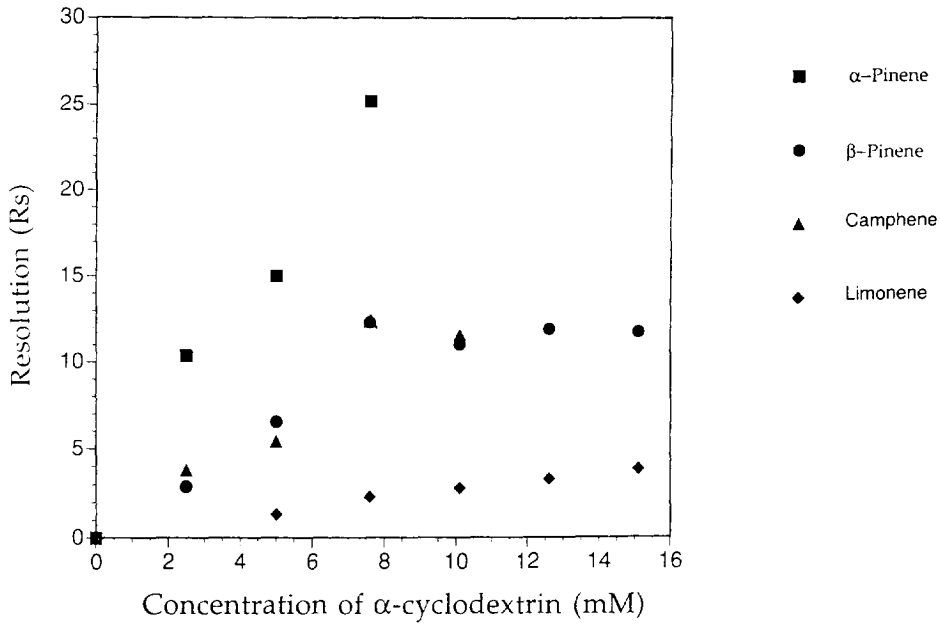

Fig. 4. Effect of the concentration of α-cyclodextrin on the resolution of terpenes.

Addition of 1.0 m*M* α-cyclodextrin results in almost baseline separation of α-pinene enantiomers, as shown in Fig. 5a. However, there was no resolution of β-pinene, camphene and limonene under these experimental conditions. Addition of 5.0 m*M* α-cyclodextrin results in greater than baseline resolution of β-pinene and a reversed migration order for both α- and β-pinene (Fig. 5b). This indicates that α-pinene binds more strongly to both α-cyclodextrin and sulfated β-cyclodextrins than does β-pinene. Further increases in the concentration of α-cyclodextrin in the running buffer resulted in better enantioseparation of α-pinene (Fig. 4). A remarkably large resolution (R_s=25) of α-pinene was achieved with 7.5 m*M* α-cyclodextrin. Resolution of β-pinene also was greatly enhanced (R_s= 12) using these experimental conditions. No further enhancement in the resolution of β-pinene was obtained at concentrations of α-cyclodextrin greater than 7.5 m*M*.

Enantioresolution of camphene also was achieved by the addition of α-cyclodextrin (above 2 m*M*) to the running buffer. *rac*-Camphene was prepared by combining an equal concentration of (+)-camphene (Aldrich C301) and (−)-camphene (Fluka 21290). It

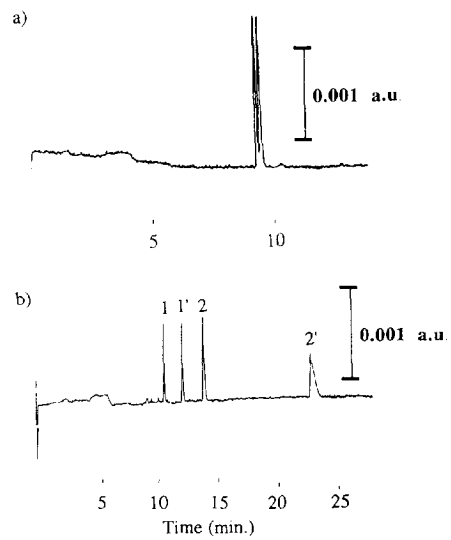

Fig. 5. Electropherograms of the chiral separation of α-pinene and β-pinene. (a) *rac*-α-Pinene, (b) (+)-β-pinene (1), (−)-β-pinene (1'), (+)-α-pinene (2) and (−)-α-pinene (2'). Electrolytes consisted of 1.0 m*M* α-cyclodextrin and 6.5 m*M* sulfated β-cyclodextrins (a) and 5.0 m*M* α-cyclodextrin and 6.5 m*M* sulfated β-cyclodextrins (b) in 10 m*M* phosphate buffer, pH 3.3.

was noted by Armstrong and Zukowski [18] that commercial "pure" chiral standards of camphene actually contain enantiomeric impurities. As shown in Fig. 6a, there appear to be three components in the *rac*-camphene. The first peak corresponds to tetralin that was added to the sample. Both enantiomeric standards of camphene were analyzed individually and it was found that both standards contained all three components, in different proportions. The relative peak area of the three components in (+)-camphene is 54:9:27% and in (−)-camphene is 19:34:47% for peaks 2, 3 and 4, respectively. On consideration of the previous HPLC analysis of the individual camphene enantiomers by Armstrong and Zukowski [18], the first peak (peak 2) was assigned to the (+)-enantiomer and the last peak (peak 4) to (−)-camphene. Peak 3 is currently regarded as an unidentified impurity.

Limonene, which is the weakest binder to sulfated β-cyclodextrins, seems to require the highest concentration of α-cyclodextrin to achieve the same extent of resolution obtained for other monoterpenes that were investigated. This again suggests that it has the weakest interaction with α-cyclodextrin.

(−)-Enantiomers of monoterpenes, with the exception of limonene, migrated more slowly than their positive counterparts, indicating that (−)-enantiomers bind strongly to α-cyclodextrin, which is consistent with the results obtained using HPLC [18]. The last eluting peak in HPLC indicates a stronger interaction and it corresponds to the slowly moving enantiomer in CE under the present experimental set-up. The (+)-enantiomer of limonene migrates more slowly than its negative counterpart, indicating that the (+)-enantiomer binds more strongly to α-cyclodextrin, as shown in Fig. 6b.

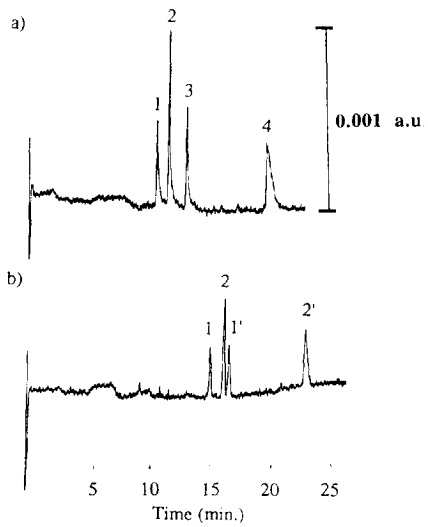

Fig. 6. (a) Electropherogram of the separation of tetraline (1), (+)-camphene (2) and (−)-camphene (4). Peak 3 is an unidentified impurity. The electrolyte consisted of 10 m*M* α-cyclodextrin and 6.5 m*M* sulfated β-cyclodextrins in 10 m*M* phosphate buffer, pH 3.3. (b) Electropherograms of the separation of (−)-limonene (1), (+)-β-pinene (2), (+)-limonene (1′) and (−)-β-pinene (2′). The electrolyte consisted of 15.1 m*M* α-cyclodextrin and 6.5 m*M* sulfated β-cyclodextrins in 10 m*M* phosphate buffer, pH 3.3.

4. Conclusions

The successful use of mixtures of α-cyclodextrin and sulfated β-cyclodextrins as chiral selectors in CE was reported for the enantioseparation of monoterpenes, including α-pinene, β-pinene, camphene and limonene. Negatively charged sulfated β-cyclodextrins, which failed to resolve monoterpenes, acted as non-specific carriers to the anodic end of the capillary. Addition of α-cyclodextrin to the electrolyte containing sulfated β-cyclodextrins induced differences in the mobility of enantiomers, resulting in remarkable chiral resolutions. α-Pinene binds the most strongly to both α-cyclodextrin and sulfated β-cyclodextrins and limonene was the least strongly bound of all the monoterpenes studied.

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