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Separation of drug stereoisomers by capillary electrophoresis with cyclodextrins

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ABSTRACT

Using capillary electrophoresis, the enantiomers and isomers of several chiral drug molecules were resolved with cyclodextrins. Parameters affecting the resolution between (+)- and (−)-epinephrine, such as pH, cyclodextrin concentration, buffer concentration, and capillary dimensions were investigated. In addition to this, the effect of cyclodextrin type (β and several derivatized β-cyclodextrins) on resolution between stereoisomers of several chiral drug was also investigated. This study showed that the structural features of the molecule, the derivative groups on the cyclodextrin, the buffer composition and the capillary dimensions influence resolution. The chiral drugs used in this study were propranolol, atenolol, betaxolol, dipivefrin, AL03152 (an aldose reductase inhibitor), AL03363 (an oxidation product of AL03152) and the *cis*/*trans* isomers of pilocarpine.

INTRODUCTION

Regulatory agencies are now demanding more stringent investigations to evaluate the safety and efficacy of chiral drug products [I]. In order to ensure the purity of these chiral drugs and monitor stability studies, stereoselective analytical methods are needed.

Cyclodextrins have been used in HPLC [2–6] to resolve chiral compounds and they are now being used in capillary electrophoretic techniques [7–18] with very promising results. The properties of cyclodextrins which make them unique chiral selectors have been discussed in detail [19,20]. Only a brief review of their properties will be given here.

Cyclodextrins are chiral, neutral, cyclic polysaccharides composed of 6 to 8 d-glucose units having the shape of a hollow truncated cone. The interior of the cavity is hydrophobic and the rim of the cavity, lined with hydroxyl groups, is hydrophilic. Although there are still many fundamental questions on the chiral recognition process with cyclodextrins there are two generally accepted requirements for chiral recognition in aqueous solution [21]. First, the analyte must have a proper structural fit to the cyclodextrin. This fit is usually referred to as an inclusion complex and requires at least one aromatic ring structure in the molecule. Secondly, the groups on the rim of the cyclodextrin cavity must interact with a substituent group on or near the stereogenic center of the molecule. If this interaction is stronger for one of the two isomers they can be resolved from one another.

The stereoselectivity will change when some of the rim hydroxyl groups of β-cyclodextrin are replaced with other groups such as methyl, hydroxyethyl or hydroxypropyl. These derivatized cyclodextrins can provide unique selectivities for a separation.

In this work, cyclodextrins were added to the buffers used in capillary electrophoresis (CE) to resolve the stereoisomers of several chiral drug molecules. Parameters such as pH, buffer (Tris) concentration, cyclodextrin concentration, cyclodextrin type and

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capillary dimensions were varied to optimize these separations. These studies show that the structural features of the molecule, as well as the type of cyclodextrin, plays an important role in resolution.

EXPERIMENTAL

Chemicals

Orthophosphoric acid, sodium phosphate dibasic (Na_2HPO_4), hydrochloric acid and sodium hydroxide were obtained from J. T. Baker (Phillipsburg, NJ USA). Tris(hydroxymethyl)amino methane (Tris), (+, -)-epinephrine, (-)-epinephrine, and heptakis (2,6-di-O-methyl)β-cyclodextrin (Me-β-CD) were obtained from Sigma (St. Louis, MO, USA). β-Cyclodextrin (P-CD), y-cyclodextrin, (+)-atenolol, (−)-atenolol, (+,−)-propranolol, (+)-propranolol and pilcarpine were obtained from Aldrich (Milwaukee, WI, USA). A mixture of pilocarpine-isopilocarpine (50:50) was obtained from Inland Alkaloid (Pipton, IN, USA). Hydroxyethyl-β-cyclodextrin (HE-P-CD) and hydroxypropyl-β-cyclodextrin (HP-P-CD) were obtained from American Maize-Products Company (Hammond, IN, USA). Betaxolol was obtained from Laboratoires D'Etudes et de Recherches Synthelabo (Porcheville, France). Dipivefrin was obtained from Pharm-Eco (Simi Valley, CA, USA). AL03152 (2,7-difluoro-4-methoxyspiro(9H-fluorene-9,4′-imidazolidine)-2′,5′-dione) and AL03363 (2,7-difluoro-4-hydroxyspiro(9H-fluorene-9,4′-imidazolidine)-2′,5′dione) enantiomers were synthesized in-house. The AL03 152 enantiomers were available individually while the AL03363 enantiomers were only available as a racemic mixture.

Apparatus

The experiments were performed with a Dionex CES system (Sunnyvale, CA, USA) equipped with a UV detector. Separations were performed in unmodified fused-silica capillaries of varying lengths and internal diameters (Polymicro Technologies, Phoenix, AZ, USA). Samples were injected by gravity for 10 s at 50 mm. The injection end was the anode (+). The applied voltage was 10 or 15 kV, depending on the sample and the observed current was always less' than 50 μA. Separations were achieved with cyclodextrin buffers of varying concentrations and pH values. Electropherograms were recorded with a Spectra-Physics Chrom-Jet integrator (San Jose, CA, USA).

Procedure

At the beginning of each day, and whenever the buffer solution was changed, the capillary was pressure-rinsed two times for 180 s with 0.1 M H_3PO_4 and two times with 0.5 A4 NaOH. Then the entire system (capillary, source and destination vials) was rinsed four times with purified water and four times with the buffer solution. These rinse cycles were performed automatically by the instrument. Once this procedure was complete, it was only necessary to rinse the entire system one time with buffer after each injection. This one-time rinse cycle was performed automatically by the instrument.

Solutions

The basic compounds, epinephrine, atenolol, propranolol, betaxolol, dipivefrin and pilocarpine, were dissolved in 0.01 A4 HCl. The acidic compounds, AL03152 and AL03363, were dissolved in 0.01 A4 NaOH. High- and low-pH buffers were prepared containing varying amounts of the four cyclodextrins (β, Me-β-CD, HE-B-CD and HP-β-CD). The pH 11 buffers were composed of Tris and Na_2HPO_4 with the pH adjusted to 11 with NaOH. The pH 2.4-9 buffers were composed of Tris and the pH was adjusted with phosphoric acid and sodium hydroxide. All buffers were filtered through a 0.45-μm filter prior to use.

Calculations

The resolution (R) values were calculated by the following equation:

$$\text{Resolution (R)} = 2(d_2 - d_1)/(w_1 + w_2)$$

where d_1 and d_2 are the migration times, in cm, and w_1 and w_2 are the widths at the base, in cm, of the first and second peaks, respectively.

RESULTS AND DISCUSSIONS

Cyclodextrins have been used successfully in capillary isotachophoresis [7–9] and CE [10–17] to resolve chiral compounds. These reports have shown that pH [7], temperature [11], organic solvents [11], cellulose derivatives [16], cyclodextrin amount [7,8,11,12,15], and cyclodextrin type [12,15] can

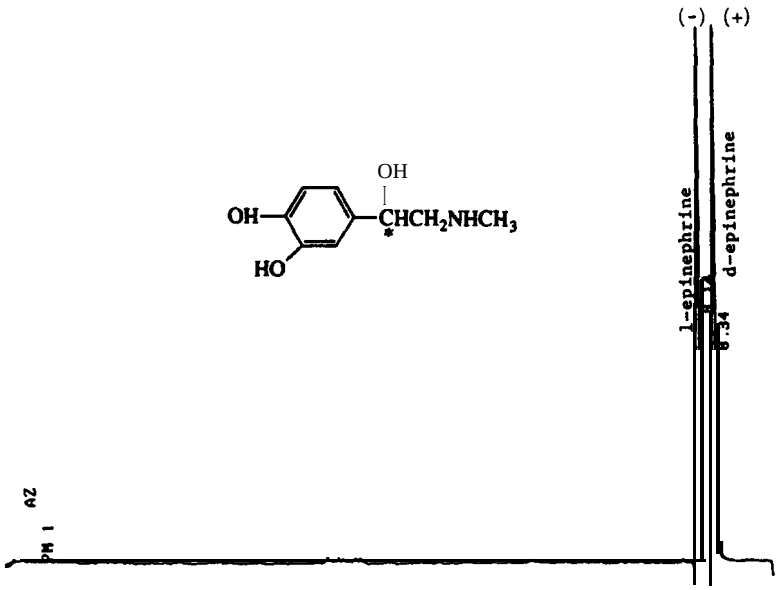

Fig. 1. Resolution of (+)- and (-)-epinephrine (25 ppm each isomer). Conditions: 20 m*M* Tri-H,PO,-9 m*M* Me-*β*-CD; pH 2.4, fused-silica capillary, 50 cm × 0.050 mm I.D. (45 cm to detector), 15 kV, 206 mm (0.1 AUF'S), 10 s 50 mm gravity inject.

influence resolution between isomers. Thin-layer chromatography separations with cyclodextrin mobile phases have also shown similar effects [22–24].

The separation of (+)- and (-)-epinephrine by CE with Me-/?-CD was shown by Fanali [12] with a coated capillary. In this investigation, the enantiomers of epinephrine were resolved with an unmodified fused-silica capillary. A typical electropherogram is shown in Fig. 1. The effect of pH, buffer concentration, cyclodextrin concentration and capillary dimensions on the resolution between these enantiomers is illustrated in Table I and II.

Table I shows that resolution between the enantiomers of epinephrine improves as pH decreases and buffer (Tris) concentration and cyclodextrin (Me-*β*-CD) concentration increases. At the

TABLE I

EFFECT OF BUFFER pH, TRIS CONCENTRATION AND CYCLODEXTRIN CONCENTRATION ON THE RESOLUTION OF (+)- AND (−)-EPINEPHRINE

Conditions: fused-silica capillary, 50 cm × 0.075 mm I.D. (45 cm to detector), 15 kV, 206 nm (0.1 AUFS), 10 s 50 mm gravity inject.

Buffer A: *20 mM Tris–H_3PO_4–9 mM Me-β-CD; pH (variable)*					
pH	*2.4*	*5*	*7*	*9*	11
Resolution	1.60	1.47	0.95	0	0
Buffer B: (variable) mM Tris–H_3PO_4–9 mM Me-B-CD; pH 2.4					
Tris (m*M*)	1	10	20	40	60
Resolution	0.88	1.6	1.6	1.6	1.1
Buffer C: *20 mM Tris–H_3PO_4–(variable) mM Me-β-CD; pH 2.4*					
Me-*β*-CD (m*M*)	4	9	18	28	
Resolution	1.25	1.60	2.38	2.53	

TABLE II

EFFECT OF CHANGING CAPILLARY LENGTH AND INTERNAL DIAMETER (I.D.) ON THE RESOLUTION OF (+)- AND (−)-EPINEPHRINE

Conditions: buffer, 20 m*M* Tris–H_3PO_4–9 m*M* Me-/?-CD; pH 2.4; 15 kV; 206 nm (0.1 AUFS), 10 s 50 mm gravity inject.

Capillary length (cm) × I.D. (mm) (to detector)	Resolution	Migration time (min)	
		(+)	(−)
45 × 0.025	3.2	12.54	12.95
45 × 0.050	2.5	11.38	11.69
45 × 0.075	1.6	9.23	9.41
55 × 0.075	2.5	15.58	15.98
65 × 0.075	2.8	21.88	22.45

TABLE III

EFFECT OF CYCLODEXTRIN TYPE ON CHIRAL RESOLUTION

See Figs. 2-5 for electropherograms and conditions.
Note: the basic enantiomers and isomers (propranolol, atenolol, pilocarpine) were only resolved with cyclodextrin buffers of pH 2.4 not pH 11. The acidic enantiomers (AL03152, AL03363) were only resolved with cyclodextrin buffers of pH 11 not pH 2.4.

Compounds	β-CD	Me-β-CD	HE-β-CD	HP-B-CD
Propranolol	×[a]	×	yes	yes
Atenolol	×	Partial	×	×
AL03152	yes"	yes	yes	yes
AL03363	yes	yes	yes	yes
Pilocarpine	yes	yes	×	×
Betaxolol	×	×	×	×
Dipivefrin	×	×	×	

[a] × = Isomers not resolved; yes = isomers resolved

lower pH values epinephrine is positively charged and less likely to degrade. Increasing the amount of cyclodextrin improves resolution significantly while only minor improvements in resolution were obtained when the Tris concentration was increased. A point is eventually reached where increasing the Tris and cyclodextrin concentration does not improve resolution further.

Table II shows that decreasing the internal diameter (I.D.) of the capillary and increasing the length of the capillary will also improve resolution. Resolution improves because decreasing the column radius and increasing the column length enhances the capillaries ability to dissipate heat and minimizes thermally induced zone broadening [17]. These changes do not come without drawbacks. Increasing the length of the capillary will increase analysis time and decreasing the capillary I.D. will decrease the sensitivity. The sensitivity of the method decreases because detection is performed on-line and the absorbance is directly proportional to the pathlength of the cell which in this case is the capillary. Improving resolution by manipulating capillary dimensions may be a less expensive alternative to increasing the amount of cyclodextrin if the cyclodextrin is expensive. However, capillaries less than or equal to 25 μm I.D. tend to clog easily and can be troublesome to use.

Studies with propranolol, atenolol, pilocarpine, AL03 152, AL03363, betaxolol and dipivefrin show how resolution is influenced by the type of cyclodextrin (Table III). Table III shows that the enantiomers of propranolol, atenolol, AL03 152, AL03363 and the *cis/trans* isomers of pilocarpine could be resolved with at least one of the cyclodextrin buffers used in this study. The enantiomers of betaxolol and dipivefrin, however, were not resolved with any of these buffers.

Four cyclodextrins were used in this study; β-cyclodextrin, Me-β-CD, HE-B-CD and HP-β-CD. The cyclodextrins were dissolved in pH 2.4 (20 or 40 m*M* Tris) and pH 11 (20 m*M* Tris–10 m*M* Na_2HPO_4) buffers. The basic enantiomers (propranolo1 and atenolol) and the basic *cis/trans* isomers (pilocarpine/isopilocarpine) were only resolved with cyclodextrin buffers of pH 2.4 not pH11. The acidic enantiomers (AL03152 and AL03363) were only resolved with cyclodextrin buffers of pH 11 not pH 2.4. Apparently resolution only occurs when the molecules are charged, These separations, illustrated in Figs. 2-5 were optimized by adjusting the buffer composition and capillary dimensions.

Fig. 2 shows that the enantiomers of propranolol were equally resolved with a pH 2.4 HE-P-CD and HP-β-CD buffer. Racemic atenolol, however, was only partially resolved with a pH 2.4 Me-β-CD buffer (Fig. 3). Increasing the amount of Me-/?-CD from 28 m*M* to 35 m*M* did not improve resolution further.

The *cis/trans* isomers, pilocarpine and isopilocarpine, were resolved with equal concentrations of β- and Me-β-CD in a pH 2.4 buffer (Fig. 4). The resolution with 9 m*M* Me-P-CD (Fig. 4B) is clearly superior to that with 9 m*M* β-cyclodextrin (Fig. 4A). The separation with β-cyclodextrin can not be improved by increasing the cyclodextrin concentration since β-cyclodextrin is not soluble above 9 m*M* in this electrolyte system. However, the resolution can be improved by decreasing the capillary I.D. from 75 to 50 μm (Fig. 4C). Resolution with the 50 μm I.D. capillary using a β-cyclodextrin buffer is now sufficient for quantitation. This is important to note because beta cyclodextrin is less expensive than Me-B-CD. Fig. 4D shows that decreasing the capillary I.D. also improves resolution with the Me-P-CD buffer.

The enantiomers of AL03 152 and AL03363 were resolved with equal concentrations of all four cyclo-

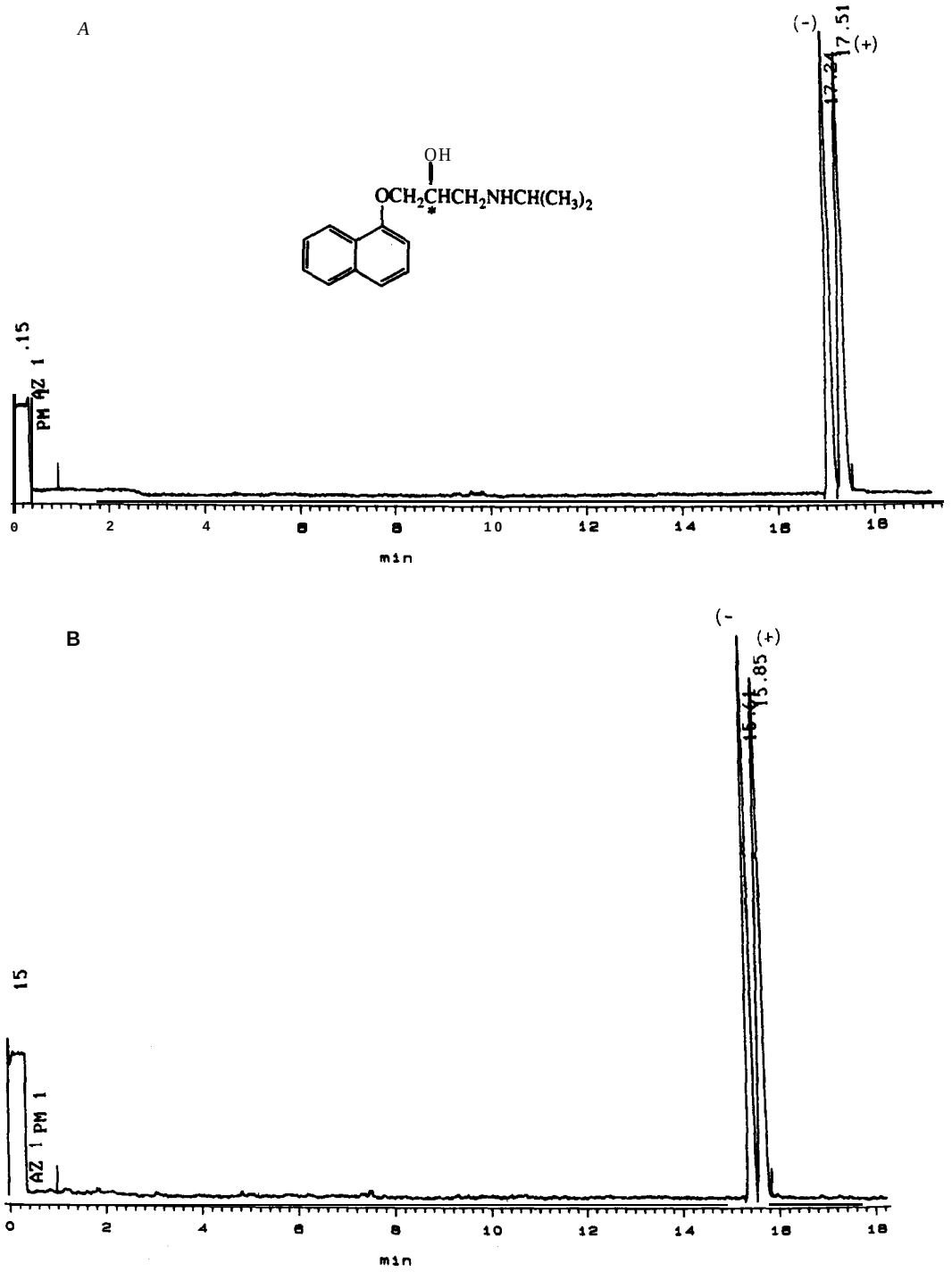

Fig. 2. Resolution of (+)- and (-)-propranolol(48 ppm each) with HE-β-CD and HP-B-CD. Conditions: fused-silica capillary, 50 cm × 0.050 mm I.D. (45 cm to detector), 15 kV, 206 mm (0.05 AUFS), 10 s 50 mm gravity inject. (A) 20 m*M* Tris–H_3PO_4–28 m*M* HE-/LCD; pH 2.4; (B) 20 m*M* Tris–H_3PO_4–28 m*M* HP-P-CD; pH 2.4.

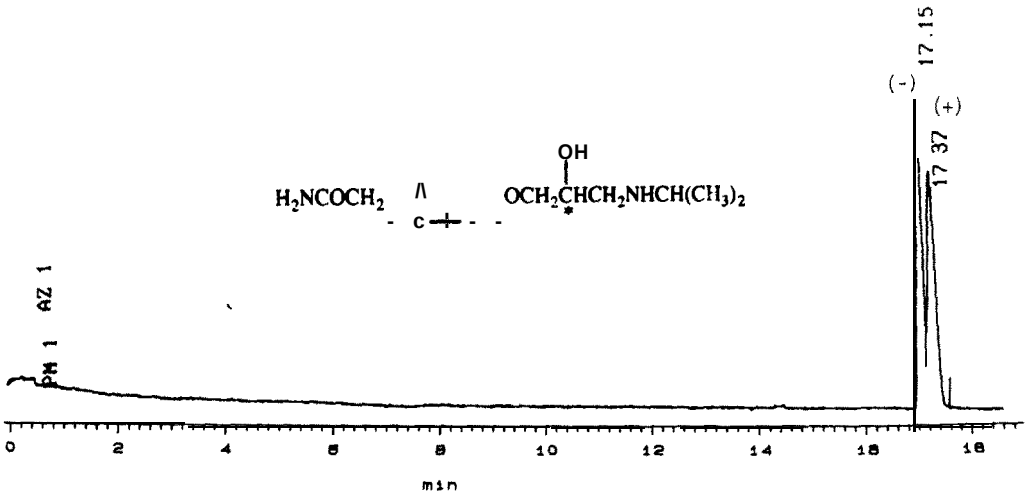

Fig. 3. Resolution of (+)- and (−)-atenolol (50 ppm each). Conditions: same as Fig. 5 except buffer, 20 m*M* Tris–H_3PO_4–28 m*M* Me-&CD; pH 2.4.

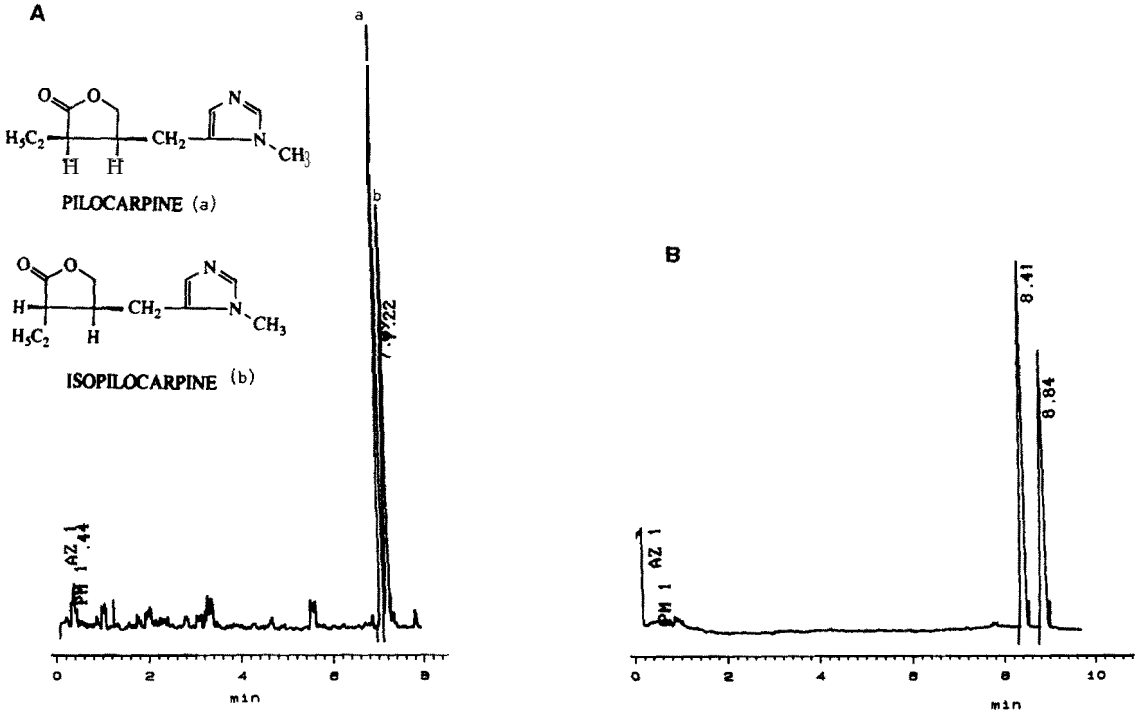

Fig. 4.

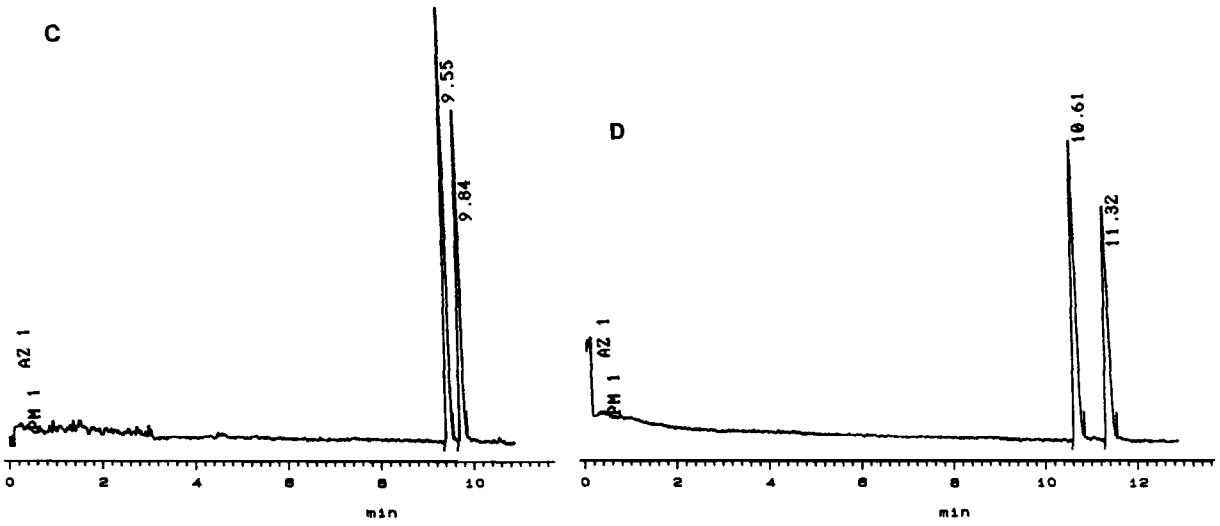

Fig. 4. (a) Separation of isopilocarpine-pilocarpine mixture (125 ppm each). Conditions: 40 m*M* Tris–H_3PO_4–9 m*M* β-CD; pH 2.4, fused-silica capillary, 50 cm × 0.075 mm I.D. (45 cm to detector), 15 kV, 206 nm (0.1 AUFS), 10 s 50 mm gravity inject; (B) same as A except buffer is 40 m*M* Tris–H_3PO_4–9 m*M* Me-/l-CD, pH 2.4; (C) same as A except 0.05 AUFS and fused-silica capillary is 0.050 mm I.D.; (D) same as B except 0.05 AUFS and fused-silica capillary is 0.050 mm I.D.

dextrins (β, Me-β-CD, HE-P-CD and HP-P-CD) in a pH 11 buffer (Table IV). Table IV shows that resolution between the enantiomers of these structurally similar compounds, with equal concentrations of the same cyclodextrin, can vary considerably (structures are show in Fig. 5). The AL03152 enantiomers were best resolved with Me-β-CD (Fig. 5A) while the AL03363 enantiomers were best resolved with β-cyclodextrin (Fig. 5B). The AL03 152 compound has an $-OCH_3$ group on the aromatic ring and AL03363 has a hydroxyl group in this position. Structurally, these compounds are very similar but resolution varies considerably with the same cyclodextrin as shown in Table IV. Clearly, the structural features of the molecule as well as the type of cyclodextrin play a role in resolution.

TABLE IV

RESOLUTION VALUES FOR THE AL03152 AND AL03363 ENANTIOMERS WITH CYCLODEXTRINS

Conditions: buffer, 20 m*M* Tris-10 m*M* Na_2HPO_4–9 m*M* cyclodextrin, pH 11, rest of conditions same as Fig. 5.

Cyclodextrin	AL03 152	AL03363
β-CD	1.1	3.4
Me-β-CD	5.7	1.2
HE-β-CD	3.6	0.73
HP-β-CD	3.5	2.4

Betaxolol and dipivefrin were not resolved with any of the cyclodextrin buffers used in this study. The structures of these molecules are shown in Fig. 6. y-Cyclodextrin, which has a larger cavity than β-cyclodextrin, gave no resolution either. The bulky groups opposite the chiral center attached to the aromatic ring probably prevent inclusion inside the cyclodextrin cavity. It is quite possible that if an inclusion complex does not form the enantiomers can not be resolved from one another in aqueous solution.

The mechanism of these chiral separations is believed to be from inclusion complex formation between the analyte and the cyclodextrin. Some additional studies, however, are needed to confirm this. Binding studies between the drug and the cyclodextrin as well as additional experiments using the linear form of the cyclodextrin (if available) would be helpful.

CONCLUSIONS

These experiments show that resolution between

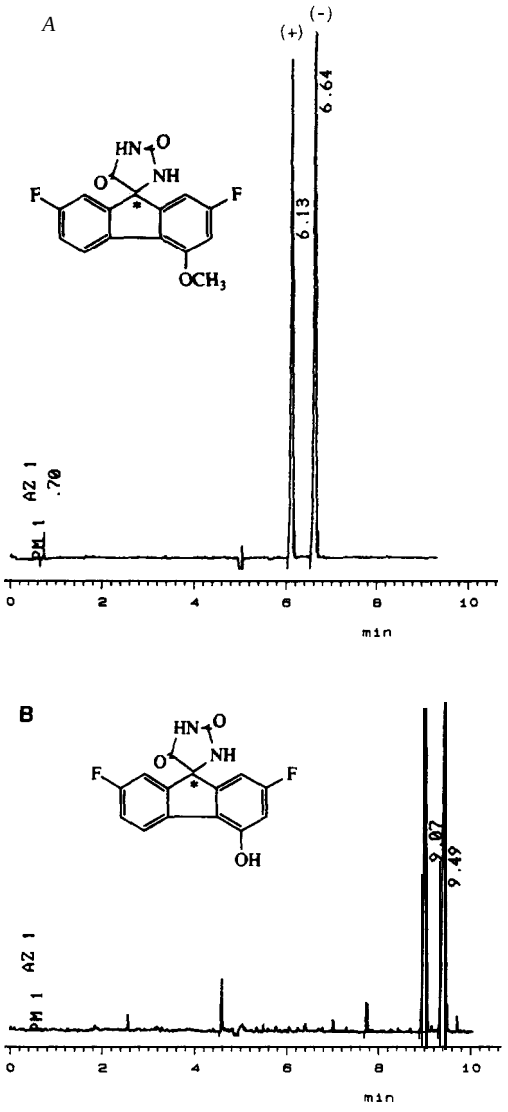

Fig. 5. (A) Resolution of (+)- and (−)-AL03152 (50 ppm each). Conditions: 20 m*M* Tris–10 m*M* Na_2HPO_4–9 m*M* Me-*β*-CD, pH 11, fused-silica, 50 cm × 0.075 mm I.D. (45 cm to detector), 10 kV, 225 nm (0.1 AUFS), 10 s 50 mm gravity inject; (B) AL03363 enantiomers (50 ppm each), same as A except buffer contains 9 m*M* *β*-CD.

stereoisomers of acidic and basic chiral drug molecules can be obtained rapidly with CE by adding to the buffer a chiral reagent such as cyclodextrin. The acidic enantiomers were best resolved in high pH buffers while the basic enantiomers and isomers

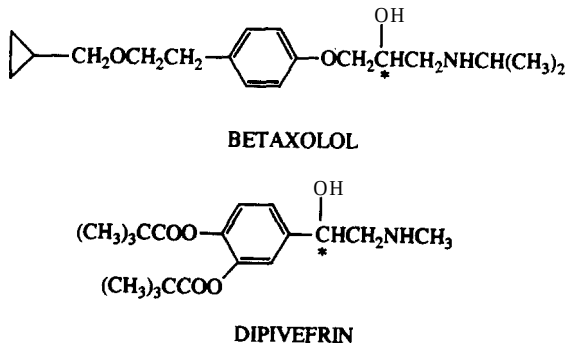

Fig. 6. Structures of betaxolol and dipivefrin

were best resolved in low pH buffers. In addition to pH, decreasing the capillary I.D. and increasing the cyclodextrin concentration can improve resolution. Minor improvements in resolution can be obtained by increasing capillary length or increasing buffer (Tris) concentration. The cyclodextrin type as well as the structural features of the molecule play an important role in resolution. Structural features such as bulky groups opposite the chiral center can affect inclusion complex formation while derivative groups on the rim of the cyclodextrin cavity can affect stereoselectivity.

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