ELSEVIER

Journal of Chromatography B, 657 (1994) 291–300

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Cyclodextrins as enantioselective mobile phase modifiers for chiral capillary electrophoresis Effects of pH and cyclodextrin concentration

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Abstract

When cyclodextrins (CDs) are used as enantioselective mobile phase modifiers for capillary electrophoresis, optimization of chiral resolution is usually carried out by varying operational parameters such as the choice of CD type, CD concentration and pH of the operating buffer. This work describes the effects of the above variables on the resolution of five chiral basic pharmaceuticals with different functional groups, using three different β-cyclodextrin (BCD) modifiers (BCD, 2-hydroxypropyl BCD and methyl BCD). In order to more clearly determine the specific role of pH, CD type and CD concentration on chiral resolution, the running buffers used in this work contained only the buffer salts and enantioselector. Very good resolution was achieved for chlorpheniramine and terbutaline; propranolol was able to be partially resolved. The degree of resolution achieved for these compounds is explained by examining the balance of specific and non-specific interactions between the solutes and the BCDs. Although the migration behavior of atropine and metoprolol indicates that they undergo marked interactions with the cyclodextrins in the running buffer, these interactions were not enantioselective, and these solutes were unable to be optically resolved under any of the conditions in our study.

1. Introduction

The use of cyclodextrins (CDs) for chiral separations in capillary electrophoresis (CE) has been growing in its number of applications, especially for pharmaceutical compounds [1–16]. However, the choice of CD type [4,5,9,10,14], CD concentration [1,4,5,7–10,15,16] and pH [1,9,15] of the operating buffer which is optimal for enantiomeric separation is not always intuitive. For example, Wren and Rowe [16] have developed a mathematical model to predict the optimum CD concentration for enantiomeric separations and have shown that as the affinity of the analyte for the CD becomes larger (*i.e.* their binding constant increases), *lower* CD concentrations are required for chiral resolution. Rawjee *et al.* [1,15] have derived an equilibrium model to describe the pH dependence of electrophoretic mobility and chiral selectivity for enantiomeric weak acids and bases and have found that the pH condition for optimum enantioselectivity is sometimes a non-intuitive choice. By individually studying the effects of pH, CD type and CD concentration on the resolution of chiral pharmaceuticals with different functional groups, better methods for choosing appropriate CE operating conditions can be developed for resolving many types of chiral compounds.

β-cyclodextrin (BCD) is a toroidal cyclic

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0378-4347/94/$07.00
SSDI 0378-4347(94)00139-Z

oligomer consisting of seven *d*-glucose units. The inner surface of the CD cavity is hydrophobic in nature and the outer surface hydrophilic [17,18]. In derivatized CDs, the outer rim hydroxyl groups in the 2, 3 and/or 6 positions have been chemically modified with various functional groups, and the overall hydrophobic character of the CD will therefore also depend on these functional groups. For inclusion complexation to occur between a guest molecule and the CD, some portion of the guest molecule must fit within the CD cavity. The driving forces for complex formation are reduction in the CD conformational strain energy and an increase in hydrogen bonding interactions between adjacent glucose units in the CD oligomer [17]. Therefore, complex formation is dependent on the CD conformation as well as the character of the guest molecule. Additionally, specific interactions between the glucose functional groups on the CD rim and the part of the solute molecule outside of the cavity must take place for chiral recognition to occur. These interactions include hydrophobic (Van der Waals), hydrogen bonding, dipole–dipole, and charge transfer interactions [17,18]. Our goal in utilizing native and derivatized CDs as CE running buffer additives and then monitoring the effects of CD type on CE chiral resolution is to ascertain the contributions of each type of interaction. In our studies discussed herein, the three types of CDs examined were BCD, 2-hydroxypropyl-β-cyclodextrin (MHPB), and randomly substituted methyl-β-cyclodextrin (MMB). Both MHPB and MMB not only have greater aqueous solubility than BCD but also vary in the types of specific interactions contributing to the inclusion process.

The enantiomeric compounds that were examined in this study include atropine, chlorpheniramine, metoprolol, propranolol and terbutaline. In most of the previous literature describing optical resolution for these and other compounds via CE with CD additives, the addition of organic solvents, micelles and/or other compounds to the operating buffer was necessary to achieve good resolution. For example, terbutaline [4], propranolol [4,5,8,12,13,16] and chlorpheniramine [3] have been separated into their optical isomers via CE by utilizing at least one of the following additives in the operating buffer in various concentrations: acetonitrile [13], methanol [3,4,13], urea [3,4], and sodium dodecyl sulfate (SDS) [3]. Wren and Rowe [16], using a 50 m*M* lithium phosphate buffer at pH 2.5, and Peterson [5], using an operating buffer of Tris and sodium phosphate at pH 2.4, have shown that propranolol isomers can be separated using derivatized BCDs and no additives other than the buffer salts. Fanali [4] has also shown that by increasing the CD concentration, the enantiomers of terbutaline could be baseline resolved. However, the addition of urea as well as 30% methanol was necessary for partial resolution of propranolol. Rawjee *et al.* have recently reported chiral resolution of atropine using a running buffer containing hydroxypropyl-β-cyclodextrin and hydroxyethylcellulose [19]. Metoprolol thus far has not been able to be optically resolved successfully by CE, although Wren and Rowe have attempted to separate the latter solute via use of a methyl-CD buffer modifier [8].

The work detailed in this article demonstrates that complex mixtures of additives described in many previous literature reports are not always necessary for achieving good enantioselectivity of chiral drug compounds, and that optical separation can be accomplished in much simpler operating buffers by optimizing the CD concentration and type, as well as the pH of the operating buffer. All of the solutes examined in our study have functional groups surrounding the chiral center which are aromatic or basic. Therefore in addition to non-specific interactions between solute aromatic groups and the inner portion of the CD cavity, possible solute sites for specific hydrogen bonding interactions with the rim hydroxyls of the CD include–OH and–NH. For MMB, Van der Waals interactions between methyl groups on the CD rim and hydrophobic groups on the solute chiral center can also provide enantioselectivity. By varying the types of functional groups in the vicinity of the chiral center, various types of solute interactions with the CDs can be studied and used to aid in the determination of the optimum conditions for

enantioselectivity for specific groups of compounds.

2. Experimental

2.1. Reagents

Atropine (free base form), (+)-chlorpheniramine; (±)-chlorpheniramine maleate salt; (±)-metoprolol (+)-tartrate salt; (+)-propranolol hydrochloride; (±)-propranolol; (±)-terbutaline hydrogen chloride and β-cyclodextrin (BCD) were obtained from Sigma (St. Louis, MO, USA). The structural formula and pK_a for each of these compounds is shown in Fig. 1. Reagent grade sodium phosphate monobasic ($NaH_2PO_4 \cdot 2H_2O$), sodium phosphate dibasic ($Na_2HPO_4 \cdot 7H_2O$), phosphoric acid, sodium hydroxide and acetone were obtained from Fisher Scientific (Fair Lawn, NJ, USA); the latter reagent was used as a neutral marker in these studies. 2-Hydroxypropyl-β-cyclodextrin (MHPB) was obtained from American Maize Products (Hammond, IN, USA). The average degree of substitution in MHPB is one hydroxypropyl group per glucose unit. Randomly substituted methyl-β-cyclodextrin (MMB) was obtained from Pharmatec (Alachua, FL, USA). The average degree of substitution in MMB is 1.8 methyl groups per glucose unit.

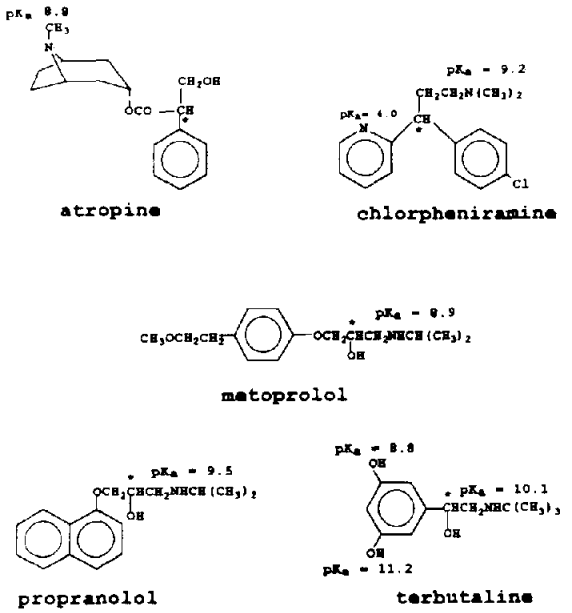

Fig. 1. Structures and pK_a values of the solutes examined in this study.

2.2. Apparatus

The experiments were performed with an Isco 3850 CE system (Lincoln, NE, USA) equipped with a UV detector set at a wavelength of 210 nm. The electrodes at the injection and detector ends of the capillary were held at positive and ground potential, respectively. All experiments were performed at ambient temperature in constant-voltage mode at 10 kV. The observed current varied between 50 and 100 μA, depending on the CD used and the pH of the operating buffer.

An unmodified UV-transparent fused-silica capillary (60 cm × 0.075 mm I.D.; 35 cm from the buffer inlet reservoir to the detector; Polymicro Technologies, Phoenix, AZ, USA) was used for the separations. Samples with approximate concentrations of 1 mg/ml were injected by applying a vacuum of 0.5 p.s.i. (3447.38 Pa) for 2 s.

Electropherograms were recorded digitally with a PE Nelson intelligent interface (Cupertino, CA, USA) as well as in analog form with a Recordall 5000 strip chart recorder (Fisher Scientific).

2.3. Background electrolyte

A 50 m*M* sodium phosphate running buffer with HPLC-grade water (Nanopure; Sybron, Boston, MA, USA) as solvent was used for all experiments. Phosphoric acid or NaOH was used to adjust the appropriate buffer salt to the desired pH. For all of the compounds in the study, the pH values examined were 2.00, 4.00, 6.00 and 8.00. The following CD concentrations were also added to the above phosphate buffers and studied: 9.0 and 18.0 m*M* for all β-cyclodextrins, as well as 27.0 m*M* for both MHPB and MMB. Additionally, 1.0, 3.0 and 6.0 m*M* con-

centrations of all of the BCDs were utilized in the studies of atropine and metoprolol.

2.4. Procedure

The capillaries were pretreated overnight with 1.0 *M* NaOH. The capillary was then rinsed with water for 5 min, followed by equilibration with running buffer for 30 min. If the buffer solution was changed during the course of the day, it was rinsed for 5 min between buffers with 1.0 *M* NaOH and then with the appropriate buffer for an additional 5 min. Between solutes the entire system was flushed with fresh running buffer for 1–2 min.

Resolution between enantiomers was calculated via the equation:

$$R_s = \frac{2\Delta t_m}{w_1 + w_2}$$

where Δt_m (min) is the difference in migration times between the enantiomers and w_1 and w_2 are the widths of each peak at the base (min). All migration time and resolution data are reported as the average ± one standard deviation of three replicate analyses. For the buffers containing BCD and MHPB, the relative standard deviation (RSD) of the migration times ranged from 0.00 to 6.52%, with the vast majority of the RSD values ≤1.00%. In MMB-containing buffers, RSD values of the migration times ranged between 0.00 and 9.58%, with the vast majority in the range of 2.00 to 3.00%. The increased variance in migration times for the latter buffer is not surprising, since in the randomly substituted MMB the methyl substitution can occur at *any* of the rim hydroxyls of the BCD moiety (*i.e.* in the 2, 3 *or* 6 position) and this will lead to increased variance in the degree of solute–MMB interactions.

2.5. Molecular modeling studies

Molecular modeling studies of the minimum energy structures of metoprolol and atropine were performed using Insight II (version 2.1.0; Biosym Technologies, San Diego, CA, USA).

3. Results and discussion

In order to bring about chiral separation, a difference in electrophoretic mobility between the complexed and the free enantiomer is necessary. To see if this difference is present under the various experimental conditions in our study, the migration times (t_m) of each solute were compared between phosphate buffer alone at a specific pH and the same buffer with a specific concentration of CD added. Since the applied potential and capillary length were held constant for all of our experiments, the observed trends in migration time for our data are directly analogous to those for overall solute mobility. Of course, the contribution of electroosmotic flow (EOF) to overall mobility must also be taken into account, and the former parameter is pH dependent. Table 1 details the pH dependence of EOF for the sodium phosphate running buffer with no CD added; acetone was used as the neutral marker. It is clear from Table 1 that the very small magnitude of the measured electroosmotic mobility at pH ≤4.00 makes it a negligible contribution to overall solute mobility in this pH region. In accordance with this trend, Fig. 2a illustrates that the migration times of the solutes in phosphate buffer alone generally increase as the pH decreases, due to the pronounced decrease in EOF at pH ≤4.00. The exception to this trend is chlorpheniramine; its migration time at pH 2.00 is slightly *less* than that at pH 4.00 and is approximately half the magnitude of the migration times of the other

Table 1
Migration times (± 1 standard deviation) and electroosmotic flow (EOF) as a function of pH for the neutral marker (acetone) in the 50 m*M* phosphate running buffer

pH	Migration time (± SD) (min)	EOF ($cm^2/V \cdot s$)
2.00	171.325 (5.357)	$0.204 \cdot 10^{-4}$
3.10	97.233 (0.866)	$0.359 \cdot 10^{-4}$
4.00	53.377 (1.345)	$0.656 \cdot 10^{-4}$
6.00	8.411 (0.021)	$4.160 \cdot 10^{-4}$
8.00	6.871 (0.036)	$5.090 \cdot 10^{-4}$

Other experimental conditions are as stated in the text.

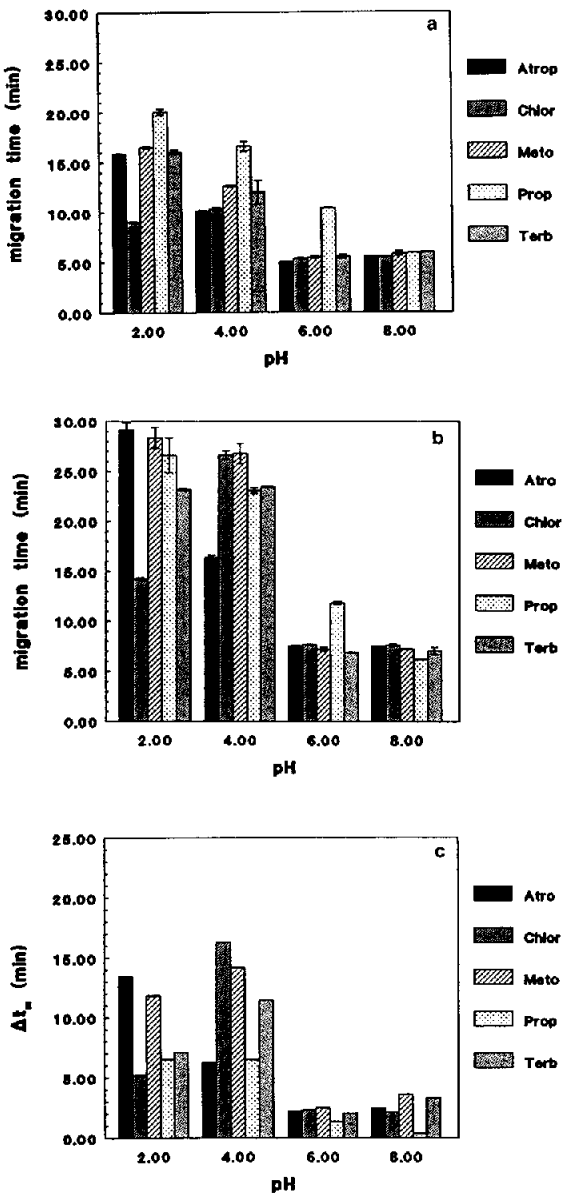

Fig. 2. The effect of the addition of cyclodextrin to the running buffer on migration time. (a) Plot of migration time (t_m) *vs.* pH for each compound examined in 50 m*M* phosphate buffer. (b) Same conditions as (a) except 9.0 m*M* BCD has been added to the running buffer. (c) Plot of the difference in migration time (Δt_m) *vs.* pH between conditions in (b) (with BCD) and conditions in (a) (phosphate alone).

compounds at pH 2.00. The reason for this difference is that the CE migration time for any compound is determined primarily by that compound's charge and size. Chlorpheniramine is approximately the same size as most of the other compounds, therefore its increased mobility at pH 2.00 can be attributed to its charge. Chlorpheniramine has two amine groups; the one located on the pyridine ring has a pK_a of 4.00 and the one on the amine side chain has a pK_a of 9.00. Therefore both amine groups will be fully protonated at pH 2.00. The amine groups of the other compounds will be fully protonated at the pH 2.00, 4.00 and 6.00 conditions. However the other compounds have only a single amine group, therefore, the +2 charge of chlorpheniramine at pH 2.00 as opposed to the +1 charge of the other solutes at this condition results in chlorpheniramine migrating approximately twice as fast.

Fig. 2a also exhibits that propranolol has a larger migration time than the other compounds studied at pH 6.00, 4.00 and 2.00. In the case of propranolol, the increased migration time is due to size considerations. As shown in Fig. 1, propranolol contains a naphthalene group. This rigid functional group has a greater cross-sectional area than the analogous groups on the other compounds studied and this results in propranolol exhibiting reduced mobility.

The same relative migration trends are observed upon addition of CD to the operating buffer (Fig. 2b). However, under the latter conditions, the migration time of each solute (except chlorpheniramine) at pH 2.00 and 4.00 increases by a factor of *ca.* 1.5. Of course, a small part of this increase in migration time is due to increased viscosity of the running buffer with the addition of CD. However, the effect of increased viscosity on migration time is small, as illustrated at pH 8.00 and 6.00 where in general the increase in the migration times upon addition of CD is about three minutes or less (Fig. 2c). The migration time of the neutral marker, acetone, followed similar trends to the latter case over the entire pH range. Therefore, at pH 2.00 and 4.00, the difference in solute migration time in the presence of CD (Δt_m) can be attributed to interactions between the solute and the CD moieties. At pH 6.00 and 8.00, the small magnitude of Δt_m implies that a much lesser degree

of interaction between the solute and CD is occurring.

If the difference in solute migration time (Δt_m) between that of the solute in the buffer alone and that in the same buffer with a specific concentration of CD added is compared, the magnitude of the analyte's binding constant with the CD can be approximated, since it will be proportional to Δt_m. Therefore the larger the Δt_m under a particular set of buffer conditions, the greater the relative degree of binding. As shown in Fig. 2c, Δt_m increases with decreasing pH, with pH 2.00 exhibiting the largest Δt_m values. As shown in Table 1, at pH 2.00 the EOF is almost negligible and is considerably less than at higher pH. This provides the analytes with additional time for interaction with the CD moieties as they migrate through the capillary. If the solutes are in contact with the CDs for an increased amount of time, a larger number of total solute exchanges between free solution and binding in a CD–solute complex will occur. However, increased migration times also lead to a greater degree of diffusional band broadening, and this is deleterious to overall resolution, particularly for closely spaced solute bands. In many cases the opportunity for an increased number of interactions between the solutes and CD moieties ultimately improves enantiomeric separation. However in those cases wherein the effects of band broadening at longer migration times negate those from increased CD–solute interactions, it should be possible to minimize the former effects by decreasing the capillary diameter and increasing the applied potential. The overall utility of this approach is currently under investigation in our laboratory.

The purpose of adding CDs to the running buffer is to achieve enantiomeric separation. The most important figure of merit for evaluating the success of this approach is measurement of the resolution value (R_s) between enantiomeric pairs. Table 2 is a summary of the resolution values measured for chlorpheniramine, propranolol and terbutaline at all conditions studied. Atropine and metoprolol were unable to be optically resolved under any of the conditions examined thus far in our laboratory. Resolution of enantiomers is dependent on both the strength and selectivity of the interaction between the CDs and the solute enantiomers. For the latter two compounds, the degree of binding is large, as is demonstrated by the fact that at most of the pH conditions evaluated in this study, particularly pH 2.00, their Δt_m values are either substantially greater than or are equal to those of the other three compounds (Fig. 2c). Therefore, atropine and metoprolol interact strongly with

Table 2
Effect of pH and cyclodextrin concentration on resolution

	Chlorpheniramine			Terbutaline			Propranolol		
	pH 6.00	pH 4.00	pH 2.00	pH 6.00	pH 4.00	pH 2.00	pH 6.00	pH 4.00	pH 2.00
9 m*M* BCD	X[a]	0.88 (0.00)	X	0.36 (0.00)	0.93 (0.00)	0.77 (0.01)	X	X	X
18 m*M* BCD	X	0.82 (0.03)	X	0.69 (0.00)	1.00(0.08)	0.98 (0.02)	X	X	X
9 m*M* MHPB	X	0.93 (0.05)	X	0.46 (0.00)	1.47 (0.15)	1.45 (0.06)	X	0.78 (0.06)	0.75 (0.04)
18 m*M* MHPB	X	1.40 (0.28)	X	0.65 (0.05)	2.46 (0.32)	2.60 (0.10)	X	0.67 (0.02)	0.81 (0.05)
27 m*M* MMPB	X	1.02 (0.04)	0.50 (0.02)	0.55 (0.01)	1.96 (0.07)	2.56 (0.14)	X	0.67 (0.04)	0.74 (0.01)
9 m*M* MMB	X	X	X	0.66 (0.04)	1.47 (0.12)	1.45 (0.02)	X	0.53 (0.03)	0.59 (0.01)
18 m*M* MMB	X	X	X	0.51 (0.04)	1.78 (0.11)	2.55 (0.42)	X	S[b]	0.57 (0.03)
27 m*M* MMB	X	X	X	0.67 (0.04)	1.50 (0.04)	1.76 (0.24)	X	S	S

A summary of the resolution achieved for chlorpheniramine, terbutaline and propranolol as a function of pH and cyclodextrin type and concentration. Resolution is calculated as stated in the text; the confidence interval (± 1 standard deviation) follows each resolution value.

[a] X = No separation ($R_s = 0$).

[b] S = $0 < R_s < 0.15$.

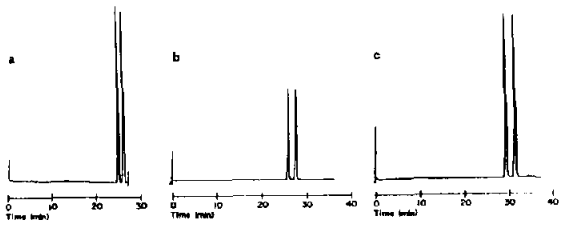

Fig. 3. Separation of terbutaline enantiomers as a function of MHPB concentration at pH 4.00. (a) 9.0 m*M*, (b) 18.0 m*M*, (c) 27.0 m*M* MHPB.

the CDs. According to Wren and Rowe's predictions, the stronger the binding of the solute–CD complex, the lower the CD concentration needed to achieve optimal resolution [16]. However in our study, even when low concentrations of CDs were examined (1.0, 3.0 and 6.0 m*M*), enantiomeric separations for atropine and metoprolol could not be achieved and only one broad peak was obtained for each compound. One possible explanation for these observed results is that the difference in CD binding between enantiomers may be so slight that CE operating conditions which would bring about optimal resolution (*i.e.* larger applied potential and smaller capillary diameter) are critical for achieving any degree of enantiomeric separation. Another explanation is that even though there are strong interactions between the solute and the CDs, they are not enantioselective. In such a case, both enantiomers would exhibit virtually identical electrophoretic mobilities, and this would also result in the appearance of only a single broad peak. In order to further explore the latter possibility, molecular modeling studies of the structures of these solutes were carried out under pH conditions comparable to those used in this study. The minimum energy configurations of atropine at pH 2.00 and 4.00 result in orientations wherein the aromatic and hydroxyl portions of the molecule are perpendicular at such an angle as to hinder the aromatic ring from inclusion into the CD cavity to any significant extent. Additionally, the width of this end of the molecule is *ca.* 7.8 Å. This is equivalent to the diameter of the β-cyclodextrin cavity [18]. At higher pH the solute width increases to *ca.* 8.2 Å, and this deleterious effect on CD inclusion complexation is corroborated experimentally by the smaller Δt_m measured for atropine at pH ≥ 4.00 (Fig. 2c). It is possible that inclusion complexation of atropine might therefore be facilitated by addition of a small amount of an organic modifier to the pH 2.00 BCD running buffer, in order to decrease the rigidity of the CD cavity [17]. For metoprolol, the width of the molecule in two of the three minimum energy conformations is slightly less (*ca.* 7.3 Å) than the diameter of the BCD cavity. However, the length of the molecule in these conformations (*ca.* 13.2–13.4 Å) serves to hinder the specific interactions at the BCD rim which are essential for enantioselectivity, since the height of the BCD cavity is only about 7.9 Å [18].

Enantiomeric resolution results for the other three compounds are summarized in Table 2. For both propranolol and chlorpheniramine, the (−) isomer exhibited the shorter migration time. Since only the racemic terbutaline was examined in our studies, the migration order for its enantiomers could not be determined. Overall, the best enantiomeric separations achieved under the operating conditions investigated in this study were for terbutaline. Terbutaline is also the only compound examined wherein its enantiomers were resolved to some extent with all three CDs. This is likely due to the hydroxyl groups present on the aromatic ring, which are available for hydrogen bonding with both the primary *and* secondary hydroxyl groups on MHPB and BCD. Better enantioselectivity is achieved with MHPB than with BCD because the hydroxyl on the MHPB propyl group is motionally less restricted than the rim hydroxyls in BCD. In the former case, this allows much freer access to the amine nitrogen adjacent to the chiral center on terbutaline. The bulky groups on the solute's amine side chain serve to limit that chain's conformational modes. The increase in specific interactions with the MHPB result in improved enantioselectivity over BCD. In BCD, the 2-propyl group at the rim is not present and the steric hindrance of the bulky *tert.*-butyl group on the solute's amine nitrogen impairs secondary hydrogen bonding interactions

with the more static BCD rim hydroxyls, even though inclusion of the aromatic portion of terbutaline is doubtless occurring within the BCD cavity.

MMB in the running buffer also serves to bring about separation of the enantiomers of terbutaline, with a resolution greater than 1.00 (Table 2). Enantioselectivity with MMB is likely due to hydrophobic interactions between the methyl groups on MMB and the *tert.*-butyl group on the amine side chain of the solute. However, hydrophobic interactions (Van der Waals attractions) are weaker and less directed than the more specific hydrogen bonding interactions between terbutaline and MHPB, ultimately resulting in less enantioselectivity when MMB is used as the chiral modifier. Resolution for the terbutaline enantiomers therefore decreases in the order MHPB > MMB > BCD.

Not only does the type of CD have a strong effect on enantioselectivity; the concentration of the CD in the running buffer is also very important. As shown in Table 2 and the electropherograms in Fig. 3, terbutaline enantioselectivity is optimal for the intermediate concentration range in our study. The optimum concentration under these buffer conditions is 18.0 m*M*, and if lower or higher concentrations are utilized, the resolution decreases. NMR studies ongoing in our laboratory [20] indicate that the latter case may be due to self-association of BCD monomers under completely aqueous conditions, leading to decreased enantioselectivity.

Propranolol is structurally the most hydrophobic compound examined in this study (log $P_{o/w}$ equal to 3.65; [21]). Therefore one might predict that the best resolution should occur with the most hydrophobic CD (MMB) because of hydrophobic interactions with the methyl groups located on the rim of the CD. However, the best separation occurs with the addition of MHPB to the running buffer (Table 2). Resolution of propranolol decreases in the order MHPB > MMB ≫ BCD, with no separation occurring with BCD under the conditions examined. Closer examination of the structure of propanolol helps to explain this trend. Because of the size of the rigid naphthalene ring with respect to the diameter of the BCD cavity, propranolol would fit snugly inside of the CD cavity. The remainder of the binding interaction and enantioselectivity is caused by secondary interactions with the groups located on the CD rim. Because BCD can only accommodate two fused aromatic rings into its inner cavity, particularly under completely aqueous conditions, it is likely that the bulkier propranolol molecule does not complex as deeply within the BCD cavity as terbutaline. Other workers [4,8,12,13] have approached this problem by adding organic modifiers to the running buffer. These organic modifiers decrease the rigidity of the cavity, because the solvation of BCD modifies the BCD conformation from a lower entropy (aqueous) state to a higher entropy (organic modifier) state [17]. This facilitates the inclusion of the naphthalene group within the BCD cavity.

With the addition of a propyl spacer to the rim of BCD (MHPB), not only does the rigidity of the cavity decrease, which will allow improved inclusion of the naphthalene group, but secondary interactions between the hydroxyl on the propyl spacer and the hydroxyl and amine groups off of the chiral center of propranolol are enhanced. Because the naphthalene group limits the motional freedom of the solute side chain, the propyl spacer on MHPB is necessary for enantiomeric separation, because it imparts greater motional freedom to the rim hydroxyl group. Also, the propyl spacer on the MHPB hydroxyl is of appropriate length for interaction with the side chain hydroxyl and amine groups on propranolol. The addition of methyl groups on the rim of MMB also reduces the rigidity of the CD cavity. However, its secondary interactions with the isopropyl group on the propranolol side chain are more hydrophobic in nature, as opposed to the hydrogen bonding interactions taking place with MHPB. The former interactions are weaker and thereby result in less enantioselectivity, just as was seen for terbutaline.

For propranolol, there is very little difference in resolution at pH 4.00 and 2.00 and it also appears to be independent of CD concentration over the range in this study (Table 2 and Fig. 4).

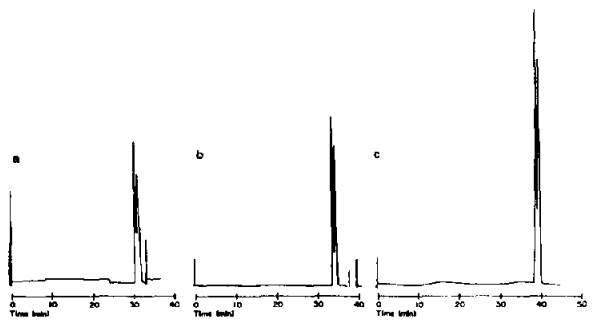

Fig. 4. Separation of propranolol enantiomers as a function of MHPB concentration at pH 2.00. (a) 9.0 m*M*, (b) 18.0 m*M*, (c) 27.0 m*M* MHPB.

It is likely that the ideal CD concentration for enantioselectivity has not yet been reached, and that by varying the CD concentration further the optimum CD concentration may be determined. Ongoing experiments in our laboratory are evaluating this possibility.

The separation of the enantiomers of chlorpheniramine proved to be extremely pH dependent. As shown in Fig. 5 and Table 2, enantiomeric separation of this compound can only be successfully accomplished at pH 4.00. Examination of the Δt_m values for chlorpheniramine demonstrates that there is little interaction between chlorpheniramine and any CD at pH 2.00. The reason for this is a pH dependent conformational change of chlorpheniramine which occurs upon full protonation of the pyridinyl nitrogen

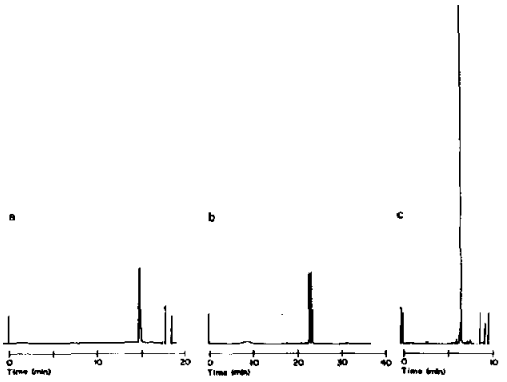

Fig. 5. Separation of chlorpheniramine enantiomers in 18.0 m*M* MHPB as a function of pH. (a) pH 2.00, (b) pH 4.00, (c) pH 6.00.

(pK_a of 4.00) and hinders both inclusion as well as specific interactions at the CD rim. This conformational change has been extensively studied in our lab via NMR and molecular modeling studies, and is to be further detailed elsewhere [20]. Table 2 illustrates that resolution between the chlorpheniramine enantiomers decreases in the order MHPB > BCD ≫ MMB. The overall best operating condition for enantiomeric separation is 18 m*M* MHPB at pH 4.00 (Fig. 5). Molecular modeling indicates that even at pH 4.00, there is restricted rotational motion of the amine side chain on the chiral center, which is caused by electrostatic repulsion between the protonated amine groups on the side chain and the partially protonated pyridinyl amine, as well as steric hindrance caused from $\pi-\pi$ interactions between the two aromatic ring groups. Since the hydroxyl group of MHPB is tethered to the propyl spacer, allowing more motional flexibility than the analogous hydroxyl groups on the rim of BCD, the probability of hydrogen bonding interactions with the side chain groups of chlorpheniramine would be enhanced for MHPB. Enantiomeric separation is not achieved with MMB, even though the migration time of chlorpheniramine is significantly larger in MMB than in phosphate buffer alone. It is likely that either secondary interactions between the MMB and chlorpheniramine are too weak for enantioselectivity or that the aromatic groups which typically include into the CD cavity are in this case primarily interacting with the methyl groups on the outside rim of MMB.

4. Conclusions

Our present studies illustrate that the common operational practice of assuming "the greater the concentration of cyclodextrin in the running buffer, the better the chiral resolution" is not always a good approach for method development when using CDs as enantioselective modifiers in CE. Moreover, the basic solutes in our present study were only resolved under acidic running buffer conditions, which parallels the behavior of the basic solutes in Peterson's work [5]. How-

ever, optimization of chiral resolution is a function not only of pH and CD type and concentration; the degree of binding between the CD and the solute also plays an important role in enantioselectivity. The relative contributions of each of these parameters is also determined by the types of functional groups adjacent to the chiral center of the solutes. We are currently measuring CD–solute binding constants via both CE and NMR methods, and will be presenting a more detailed treatment of the relationship between binding constants and chiral resolution in a future paper.

Acknowledgements

The authors thank Jay Amicangelo for assistance with molecular modeling, American Maize Products and Pharmatec Inc. for gifts of the MHPB and MMB respectively, and the University of Vermont Research Advisory Council for providing funding for the purchase of the capillary electropherograph. Partial financial support of this work from the American Cancer Society, Vermont Division is also gratefully acknowledged.

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