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Effects of selected alcohols on chiral recognition via cyclodextrin inclusion complexation

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The effect of added alcohol on quinine and quinidine inclusion complexes is examined to determine the ability of cyclodextrin as a chiral discriminator via chromatographic and spectroscopic studies. The addition of a bulky alcohol to a chiral guest-cyclodextrin solution will discriminantly enhance fluorescence intensity. However, the added alcohol diminishes chiral recognition in a chromatographic system.

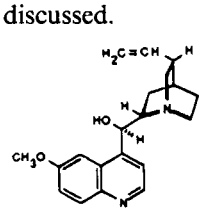
INTRODUCTION

Cyclodextrins have the ability to form inclusion complexes with a wide variety of molecules that fit within their cavity. The inclusion complexes formed between medicinal agents (guests) and the cyclodextrins (host) are of interest to the pharmaceutical industry because drug properties such as stability, solubility, and bioavailability can be enhanced and toxicity minimized.[1] Recently, the use of cyclodextrins as chiral discriminators has been studied with optically active molecules that form inclusion complexes.

The development of chiral chromatographic separations in which the cyclodextrin can be bonded either to the stationary phase or in the eluant has resulted in a variety of chiral analyses.[2,3] Mularz recommends the addition of an organic modifier, typically acetonitrile or methanol, to a cyclodextrin mobile phase for enhanced resolution.[2] Armstrong and co-workers have evaluated the effect of an organic modifier on retention and selectivity in reverse-phase liquid chromatographic separations on a cyclodextrin bonded phase.[4]

In addition, the use of alcohols has enhanced the spectroscopic properties of achiral cyclodextrin inclusion complexes. Matsui and Mochida reported larger binding constants as measured by absorption spectroscopy of azo dyes and beta-cyclodextrin with the addition of *n*-propanol, *n*-butanol and 2-butanol to a cyclodextrin solution.[5] Cruz discovered an enhancement of room-temperature phosphorescence with the addition of butyl alcohols to beta-cyclodextrin inclusion complexes of 1-bromonaphthalene.[6] Warner and co-workers have obtained a 20-fold enhancement of the formaton constant and a 2.5 fold enhancement in fluorescence lifetime with the addition of 10% tert-butyl alcohol to a pyrene/gamma-cyclodextrin inclusion complex.[7]

This paper will examine the effect of an organic modifier, such as selected alcohols, on the chromatographic and spectroscopic properties of chiral guests such as quinine, **1**, and quinidine, **2**, included within beta-cyclodextrin. The study will report the effect of the alcohols on the fluorescence intensity of the individual isomer and the differences between the isomers as a result of the formation of a cyclodextrin inclusion complex. The chromatographic separation resulting from the addition of the organic modifier to a beta-cyclodextrin mobile phase will also be evaluated. Finally, a comparison of the spectral and chromatographic results obtained with the addition of the alcohols to the cyclodextrin inclusion complexes will be discussed.



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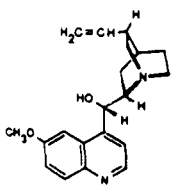

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EXPERIMENTAL

Instrumental

Fluorescence spectrophotometer. Fluorescence excitation and emission spectra were obtained with a Fluorolog 2 + 2 spectrofluorimeter (SPEX Industries, Metuchen, NJ), equipped with a double excitation and emission monochromators (spectral bandpass 2.0 nm mm^{-1}), a 450-W continuous xenon source and a Peltier-cooled Hamamatsu R928 photomultiplier tube. Conventional cuvettes were used. Data acquisition was done with a SPEX Datamate computer interfaced to the spectrofluorimeter. Hard copies of spectra were obtained with a Houston Instruments digital *x-y* plotter. All spectra were corrected for lamp intensity fluctuations.

HPLC system. A modular component liquid chromatographic system was used consisting of a Spectra Physics SP8700 extended range pump, a LDC UV monitor detector (254 nm) and a Rheodyne sample injector equipped with a 20-μL injection loop. The column was a Shandon (Sewickley, PA) C8 Hypersil WP300, 10μ (250 × 4.6 mm). A Model 5000 Fisher Recordall strip chart recorder was used to record the chromatograms.

Reagents and solvents

D-1-brompheniramine maleate was obtained from Schering-Plough Pharmaceuticals (Kenilworth, NJ). Quinine sulfate, quinidine sulfate, beta-cyclodetrin, *n*-butanol, *sec*-butanol, and *tert*-butanol were obtained from Aldrich Chemical Co. Methanol, ethanol, *n*-propanol and *iso*-propanol were obtained from Fisher Scientific Co. All reagents and solutes were used as received.

Procedure

Preparation of the spectrophotometric samples. Aqueous stock solutions of the quinine sulfate and quinidine sulfate were prepared at a concentration of 4.8×10^{-3} M. Aqueous beta-cyclodextrin stock solution was prepared at 15.0 mM. An aliquot of either isomer solution was mixed with distilled water and the beta-cyclodextrin solution to afford a 4.8×10^{-5} M analyte and either a 0 mM, 3.0 mM, 6.0 mM, 9.0 mM, or 12.0 mM cyclodextrin solution.

Spectrophotometric parameters. Excitation and emission slit widths were typically set at 2 mm for all spectra. Integration time of 1 second per sampling time was used with a 1 nanometer wavelength interval. Quinine and quinidine samples were excited at 300 nm and the emission spectra collected from 350 to 550 nm or 365 nm excitation with the emission spectra collected from 400 to 600 nm.

Preparation of the mobile phase. The beta-cyclodextrin mobile phase was prepared by dissolving the appropriate amount of cyclodextrin in a solution containing 8.0% of the desired alcohol (v/v) and 0.3% triethylamine (v/v) and the pH adjusted to 5.0 with acetic acid. The solution was filtered via vacuum through a 0.45-μm Nylon-66 membrane filter. The prepared mobile phase was degassed in the filtering flask employing vacuum and with constant stirring. Degassing conditions were maintained throughout the entire length of the experiments via a helium sparge.

Preparation of the chromatographic sample. Stock solutions of each of the two pairs of isomers were prepared in distilled water. The working concentration was 1.0 mg/mL.

Chromatographic parameters. A flow of 1.0 mL/min was used throughout the studies. Strip chart recorder speed was typically 0.25 cm/min. Retention times were measured manually by monitoring the time elapsed. The void volume, V_0, of the system was found to be 2.80 min which was used for all k' calculations.

RESULTS AND DISCUSSION

Fluorescence spectroscopy of quinine and quinidine inclusion complexes with beta-cyclodextrin

The selection of quinine and quinidine as guest analytes for this study was made on two factors: first, these isomers have been fully characterized as fluorescence standards;[8] second, their chromatographic separation via cyclodextrin chromatography has been demonstrated.[9] Therefore, all results forthcoming will be compared with those of published studies.

The effects of beta-cyclodextrin on the fluorescence spectra of quinine sulfate and quinidine sulfate are shown in Figs 1 and 2, respectively. A progressive increase in the cyclodextrin concentration, while the concentration of either quinine or quinidine is maintained constant, results in a corresponding intensity enhancement. A cyclodextrin concentration of 9.6 mM resulted in a 3.63 and a 4.32 intensity enhancement for quinine and quinidine, respectively. The enhancement is greater for quinidine as compared with quinine which indicates a greater inclusion complexation. These results are in agreement with the

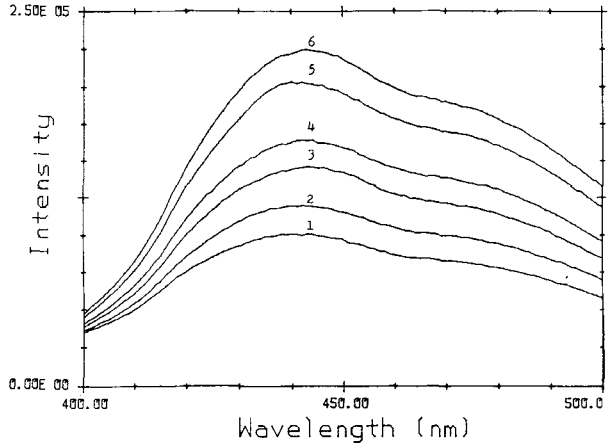

Figure 1 Fluorescence spectra of quinine sulfate with added cyclodextrin: 0 mM (1), 3 mM (2), 6 mM (3), 9 mM (4), 12 mM (5), and 15 mM (6).

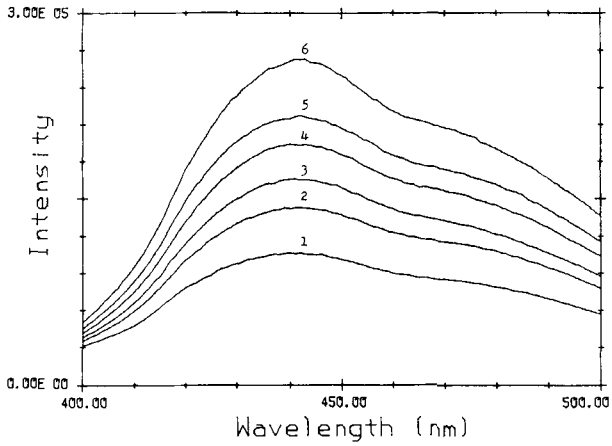

Figure 2 Fluorescence spectra of quinidine sulfate with added cyclodextrin: 0 mM (1), 3 mM (2), 6 mM (3), 9 mM (4), 12 mM (5), and 15 mM (6).

chromatographic separations achieved by Armstrong and co-workers,[9] in which a cyclodextrin bonded column eluted quinidine with a capacity factor of 2.16 versus 1.78 for quinine. This indicates greater binding of the quinidine diastereoisomer.

The fluorescence emission spectra of either isomer failed to detect an excimer band even in the beta-cyclodextrin solution. This observation rules out the possibility of a 2:1 or 2:2 guest/cyclodextrin complex. Thus, indirect evidence supports the 1:1 guest/cyclodextrin complex that is typical of beta-cyclodextrin complexes and similar guest molecules.

A progressive increase in the cyclodextrin concentration, while the analyte concentration is held constant, results in a corresponding intensity enhancement which is valid even with the addition of alcohols to the mobile phase. Table 1 provides the intensity enhancement ratio that is obtained with increasing cyclodextrin concentration with 5% methanol added to the cyclodextrin solutions. The quinidine isomer displays a greater intensity enhancement compared with the quinine isomer. Mularz has established that the greater the cyclodextrin concentration in the mobile phase, the greater the chiral recognition.[2] This concurs with the intensity enhancement reported above.

Table 1 Intensity enhancement obtained with increasing cyclodextrin concentration for quinidine and quinine

[beta-CD, mM]	*Quinidine* I/I_0	*Quinine* I/I_0
3.0	1.45	1.30
6.0	2.95	2.39
9.0	3.90	4.03
12.0	6.69	6.31

I_0 is the intensity of the isomer in a 5% methanol solution and I is the intensity of the isomer in a given cyclodextrin concentration containing 5% methanol by volume.

Table 2 Intensity enhancement obtained with a 12 mM cyclodextrin solution containing 5% alcohol

Alcohol	*Quinidine* I/I_0*	*Quinine* I/I_0	*Quinidine: Quinine ratio*
MeOH	6.59	6.00	1.10
EtOH	5.90	5.58	1.06
1-BuOH	6.80	6.80	1.00
sec-BuOH	10.37	7.17	1.45
tert-BuOH	11.55	7.29	1.58

* I_0 is the intensity of the isomer in a 5% alcohol solution and I is the intensity of the isomer in a 12 mM beta-cyclodextrin solution containing 5% of a given alcohol by volume.

Mularz and co-workers achieved maximum chiral recognition when the organic modifier was present at a concentration of 6% or less by volume in a cyclodextrin mobile phase in the separation of *d,l*-pseudoephedrine.[10] The data in Table 2 provides the intensity enhancement obtained with the addition of 5% selected alcohols to a 12 mM beta-cyclodextrin concentration. The addition of 5% methanol, ethanol or 1-butanol to the cyclodextrin solution results in approximately equal intensity enhancement of about 6-fold, with the quinidine isomer displaying a slightly greater enhancement. It is apparent that increasing the chain length of a linear alcohol reduces the chiral recognition effectiveness of the cyclodextrin. Mularz finds essentially no difference in the chiral resolution when adding either methanol or ethanol to a cyclodextrin mobile phase.[2] However, the intensity enhancement is greatly increased when a bulky alcohol is utilized versus a linear alcohol. The enhancement

nearly doubles when *tert*-butanol is used instead of 1-butanol. Warner and co-workers report similar findings in which the fluorescence lifetime of the complex increases with increasing bulkiness of the added alcohol. They attribute the enhanced fluorescence to the following factors: protection from bimolecular quenchers, increased shielding from the bulk aqueous environment, increased rigidity of the included guest, and a decrease of other deactivation pathways with respect to fluorescence.[7] The difference in the intensity enhancement of quinidine versus quinine is greater as one increases the bulkiness of the alcohol. An intensity increase of about 10% using linear alcohols to 50% or better with increased bulkiness is evident with *tert*-butanol. Thus, the addition of a bulky alcohol increases the effectiveness of beta-cyclodextrin as a chiral discriminator as evident in the enhanced fluorescence intensity of quinidine as compared with quinine.

Chromatographic studies of quinine and quinidine inclusion complexes with beta-cyclodextrin

The use of organic modifiers in cyclodextrin chromatography is well documented. Cline Love and Arunyanart added 10% methanol to a cyclodextrin mobile phase to obtain enhanced formation constants for the complex formation of the guest with the cyclodextrin.[11] Armstrong and co-workers employed either methanol or acetonitrile typically from 10% to 40% in the mobile phase where a bonded cyclodextrin column was utilized in the separation of drug stereoisomers.[9] More recently, Mularz recommends acetonitrile in the separation of *d,l*-chlorpheniramine versus methanol and ethanol when added to a cyclodextrin mobile phase.[2] In addition, Warner and co-workers conclude that the presence of *tert*-butyl alcohol increases the strength of a cyclodextrin-pyrene complex resulting in a decrease in retention.[12] In an effort to determine whether the above findings obtained with spectroscopic studies are applicable to chromatographic resolution, selected alcohols will be added to cyclodextrin mobile phases. Calculations of resolution, selectivity and formation constants will determine the effectiveness of the selected alcohols as mobile phase modifiers that enhance the ability of cyclodextrins as chiral discriminators.

The resolution, *Rs*, of two adjacent peaks is defined as the distance between the two peak centers divided by the average peak width, as given by Equation 1.

$$Rs = (t_2 - t_1)/[1/2(t_{w1} + t_{w2})] \qquad (1)$$

The quantities t_1 and t_2 refer to the retention time of peaks 1 and 2, and t_{w1} and t_{w2} are their peak width values. As a reference point, when $Rs = 1$, the two peaks are well separated with only 2% of their area overlapping.[13] The resolution equation can also be given in terms of its three parameters:

$$Rs = 1/4(\alpha - 1)(N)^{1/2}(k'/(1 + k')) \qquad (2)$$

where α is the selectivity factor, N is the column efficiency, and k' is the capacity factor. The selectivity factor is defined as the ratio of k'_2 and k'_1, which is associated with the composition of the mobile and/or the stationary phase. The capacity factor, k', is defined as the moles of solute in the stationary phase divided by the moles of solute in the mobile phase. Thus, k' is a measurement of solvent strength and can be diminished by increasing the strength of the mobile phase.

The formation constant, K_f, of the guest/host complex is obtained according to the equation derived by Cline Love and Arunyanart[11] and expressed as:

$$k' = \phi[L_s]K_1/1 + K_f[CD] \qquad (3)$$

where k' is the capacity factor which is a function of chromatographic retention, ϕ is the phase ratio of the stationary volume to the mobile phase, $[L_s]$ is the number of stationary phase sites, K_1 is the solute-stationary phase equilibrium constant.

The use of alcohols to enhance the ability of cyclodextrins as chiral discriminators was initially tested on the diastereoisomers quinine and quinidine. The effectiveness of the alcohols was determined by evaluating the resolution as given by Equation 1. The differences in the formation constants of the isomers were obtained via Equation 3, where the cyclodextrin in the mobile phase was varied from 3.0 mM to 15.0 mM. Resolution and selectivity were calculated using the data corresponding to the 15.0 mM beta-cyclodextrin mobile phase. The data is summarized in Table 3. Figures 3 and 4 illustrate the changes in the K_f as a result of adding ethanol or isopropanol as the mobile phase additive as per Equation 3. The addition of *n*-propanol to the cyclodextrin mobile phase resulted in the loss of inclusion complexation, as indicated by the inability to calculate the K_f. Figure 4 demonstrates the diminished effectiveness of the cyclodextrin to form a complex with the isomers, as noted by the lesser slopes obtained compared to Figure 3.

Table 3 Effectiveness of alcohols as mobile phase modifiers in enhancing the ability of cyclodextrins as chiral discriminators of quinine and quinidine

Alcohol:	R_s	*Alpha*	K_{f1}	K_{f2}	$[K_{f1}-K_{f2}]$
8% EtOH	3.41	1.56	53	43	10
8% IPA	3.25	1.30	10	4	6
8% *n*-PrOH	1.80	1.35	—	—	—

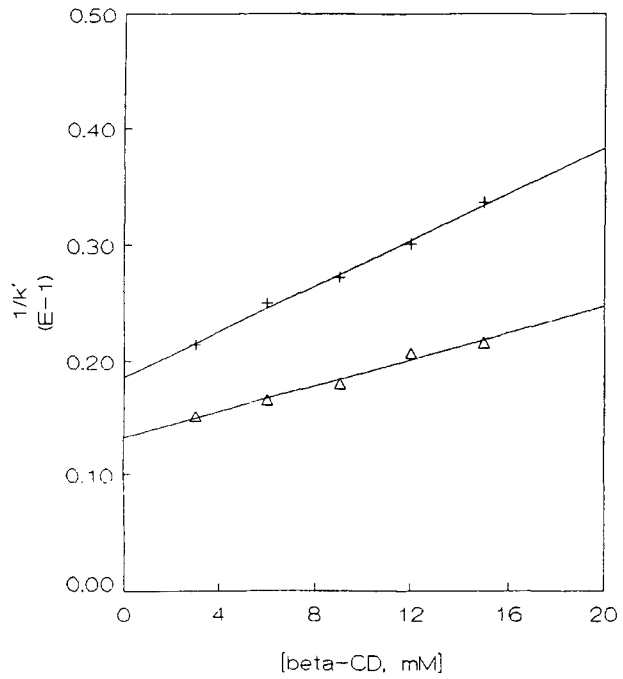

Figure 3 $1/k'$ vs [beta-CD]. Mobile phase contained cyclodextrin in 5% ethanol. Symbols are: quinidine (+) and quinine (△).

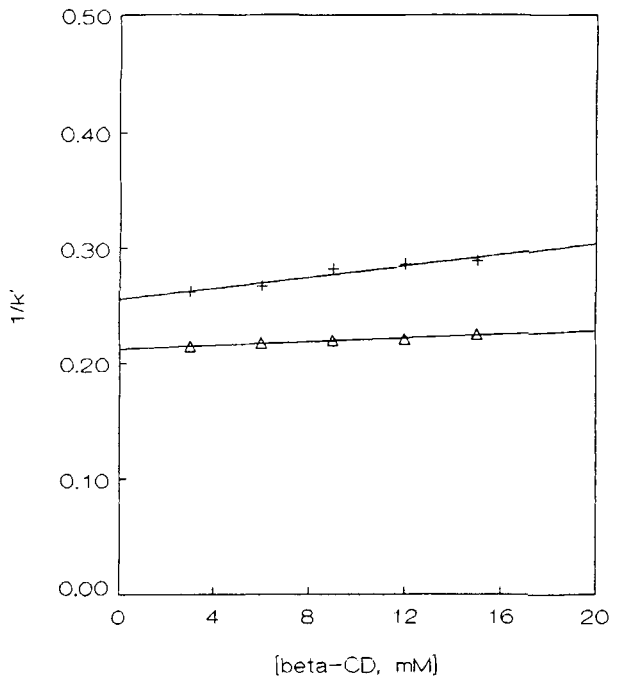

Figure 4 $1/k'$ vs [beta-CD]. Mobile phase contained cyclodextrin in 5% isopropanol. Symbols are: quinidine (+) and quinine (△).

The data indicates that the selectivity of the diastereoisomers is greater than 1 with all three alcohols. Yet, the K_f values are extremely small relative to typical values, which can range from 200 to 700, as obtained by Mularz.[2] Thus, the ability of the cyclodextrin to form an inclusion complex is diminished with the addition of propanol versus ethanol. The isomer discrimination is slightly diminished, as shown by the lower values obtained for the resolution and selectivity. The ability to resolve quinine and quinidine via reverse-phase chromatography without the aid of a chiral discriminator has been reported by Cline Love and Arunyanart utilizing a micellar mobile phase.[14] Therefore, the R_s of 1.76 obtained by Armstrong and co-workers[9] may not be a true indication of the ability of cyclodextrins as chiral discriminators.

Mularz has demonstrated the ability to resolve, *d,l*-brompheniramine, which contains one chiral center.[2] To further test the effect of alcohols on a cyclodextrin mobile phase, *d,l*-brompheniramine was separated with mobile phases ranging from 3.0 mM to 15.0 mM beta-cyclodextrin. The results are given in Table 4 for the four most common types of alcohols utilized in liquid chromatography. As described above, the K_f values are obtained by varying the cyclodextrin concentration from 3.0 to 15.0 mM and calculated via Equation 3. The R_s, alpha, k'_1 and k'_2 values are calculated from the chromotograms obtained with a 15.0 mM beta-cyclodextrin. Figure 5 illustrates the effect of methanol as a modifier on the K_f of a cyclodextrin mobile phase.

The data in Table 4 clearly indicates that the less polar alcohols in a cyclodextrin mobile phase will markedly reduce the ability of the cyclodextrin as a

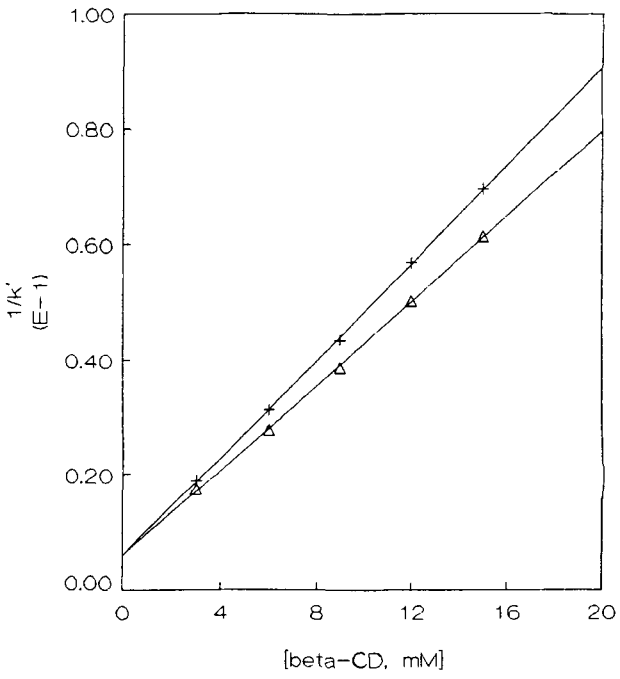

Figure 5 $1/k'$ vs [beta-CD] for (+)-bromopheniramine (+) and (−)-bromopheniramine (△). Cyclodextrin mobile phase contained 5% methanol (v/v).

Table 4 Effectiveness of alcohols as mobile phase modifiers in enhancing the ability of cyclodextrins as chiral discriminators of *d,l*-brompheniramine

Alcohol:	R_s	*Alpha*	k'_1/k'_2	K_{f1}	K_{f2}	$[K_{f1}-K_{f2}]$
0%	1.19	1.12	30.3/27.5	690	836	146
8% MeOH	1.07	1.13	16.3/14.4	614	702	88
8% EtOH	1.03	1.13	11.3/10.0	383	347	36
8% *n*-PrOH	0.35	1.07	6.6/6.1	113	94	19
8% IPA	0.23	1.05	8.5/8.1	128	108	20

Table 5 Dielectric constant of the solvents

Solvent:	*Dielectric constant (20 °C)*
Water	80
Methanol	32.7
Ethanol	24.6
n-Propanol	20.3
iso-Propanol	20.3

chiral discriminator. The R_s values decrease slightly from 1.19 with no alcohol to 1.03 with the addition of ethanol to the mobile phase. In the case of propyl alcohol, the R_s values fall significantly below 0.5, indicating that minimal resolution of the isomers is achieved. The selectivity factor is approaching 1 as the polarity of the added alcohol is decreased. The differences in the K_f value are also diminished with the addition of a nonpolar alcohol. The maximum resolution is obtained at higher cyclodextrin concentrations, which is in agreement with the findings of Mularz.[2]

Two possible explanations for the decreased chiral recognition with the addition of propanol to a cyclodextrin mobile phase are a decrease in the hydrophobicity of the bulk aqueous solution and a significant increase in the solvent strength of the mobile phase. The polarity of the alcohols serves as a useful guide[15] to solvent strength and is provided in Table 5. The dielectric constant of water is much greater than that of the alcohols. An 8% addition of an alcohol to a cyclodextrin solution would proportionately decrease the polarity of the bulk aqueous solution, which would reduce the binding force for the guest-host complex formation. Thus, the decrease in polarity with higher alcohols parallels the decrease in formation constants with the addition of an alcohol to the cyclodextrin solution. The increase in solvent strength of the mobile phase is indicated by reduced k' values. The k' values given in Table 4 show that the capacity factor of the brompheniramine isomers is significantly reduced with propanol versus ethanol versus methanol as the added organic modifier. Thus, the data supports the two explanations provided for the decrease in chiral recognition with the addition of higher alcohols to a cyclodextrin mobile phase.

CONCLUSIONS

The addition of selected alcohols to a cyclodextrin inclusion complex will not enhance chiral recognition as determined by chromatographic or fluorometric analyses to the same extent as previously expected. The addition of a bulky alcohol to a chiral guest-cyclodextrin solution will discriminantly enhance fluorescence intensity. In contrast, the addition of a higher alcohol to a cyclodextrin chromatographic system will diminish the effectiveness of the cyclodextrin as a chiral resolving agent. The role of the alcohol, in the two analytical techniques is markedly different. In the spectroscopic technique, the alcohols clearly enhance the pathways by which fluorescence is observed resulting in greater intensities. In the chromatographic technique, the alcohols reduce the selectivity and increase the strength of the bulk aqueous phase, resulting in diminished chiral recognition.

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