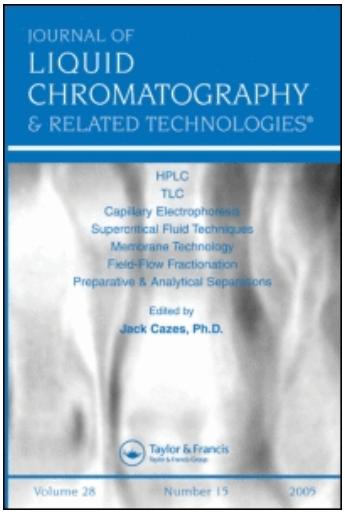

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Separation of Cyclodextrins Using Cyclodextrin Bonded Phases

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SEPARATION OF CYCLODEXTRINS USING CYCLODEXTRIN BONDED PHASES

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ABSTRACT

Two different cyclodextrin bonded phases (α and β) were used for the separation of α-, β- and γ- cyclodextrins. The β-cyclodextrin phase was found to be, in general, more effective at resolving the cyclodextrins than the α-cyclodextrin bonded phase. Acetonitrile/water mixtures were used as mobile phases. The effect of mobile phase composition on retention and resolution is examined. The elution order was found to be size dependent. The results are discussed in terms of the overall retention mechanism.

INTRODUCTION

Cyclodextrins (cyclic glucopyranosides bonded through α−1,4 linkages) have been used extensively as additives in the food, cosmetics, pharmaceutical products, and in a number of analytical methodologies. Cyclodextrins (CD) are formed through an enzymatic process which

produces mixtures of cyclodextrin homologues, each having a different number of glucose rings. Analysis of the reaction mixture is necessary for quality control and the optimization of reaction conditions resulting in the preferential production of a particular homologue. The enhanced bioavailability of some drugs in the presence of cyclodextrins has been reported [1]. For these applications, the determination of cyclodextrins in complex biological samples is essential.

Several chromatographic stationary phases have been proposed for the separation of CD's, including amine [2,3,4], cation exchange [5,6], phenyl [7], C_8 or C_{18} [3,4], as well as silica [3]. The amine columns, although effective at resolving the CD's, are susceptible to self-hydrolysis. The cation exchange methods yield broad peaks and long analysis times. The reversed-phase columns also give rise to broad peaks.

Detection of CD's is limited because of their lack of UV absorption. Other methods that have been applied include negative colorimetric [7], radioactive labeling [3] or derivatization [3]. The most widely used and easiest method of detection is refractive index. Recent investigations [8] have shown that for CD's, refractive index is actually more sensitive than low wavelength UV.

In the work presented here, cyclodextrin bonded phases were used to separate α-, β- and γ-CD's. The elution order was found to be related to solute size.

EXPERIMENTAL

The chromatographic measurements were made on a Shimadzu LC-6A Liquid Chromatograph interfaced with a C-R3A Chromatopac Data System. Detection was accomplished using a Waters Model R401 Differential Refractometer.

The chromatographic columns (received from Advanced Separations Technology, Inc., Whippany, NJ) were 250 mm x 4.6 mm i.d. stainless steel packed with 5μm Cyclobond I (β-CD) or Cyclobond III (α-CD).

The CD's (α-, β-, and γ-) were obtained from Ensuiko Sugar Refining Co., Ltd (Japan). HPLC grade acetonitrile (MeCN) and water were obtained from Fisher Scientific (St. Louis, MO) and used without further purification.

RESULTS AND DISCUSSION

The effect of mobile phase composition on solute retention is illustrated in Figure 1. As can be seen from the figure, increasing the amount of acetonitrile modifier increases the solute capacity factor and the column selectivity for the solutes. This effect is more dramatic on the α-CD phase. For a given mobile phase composition, solute capacity factors are larger on the α-CD column than on the β-CD column. For a large number of solutes that are successfully resolved on CD phases, the retention mechanism is related to the formation of an inclusion complex between the solute and the host CD [9]. However, inclusion complex formation is not believed to play a role in this particular separation. It is not likely that one CD can form an inclusion complex with another because of size, geometric and polarity restrictions. Furthermore, the mobile phases used in these CD separations contain high percentages of organic modifier, which preferentially resides in the CD apolar cavity. Retention is most likely the result of hydrogen bonding and dipolar interactions between the CD bonded ligand and the dissolved homologue. Also, it appears that the cyclodextrins are retained on the basis of molecular size (Figure 1 and Table I). The elution order obtained on the CD phase (α-, β-, and γ-) differ from the results obtained on a reversed-phase column by Koizumi, et. al. (γ-, α-, and β-) [4], which were correlated with the solute's solubility in water.

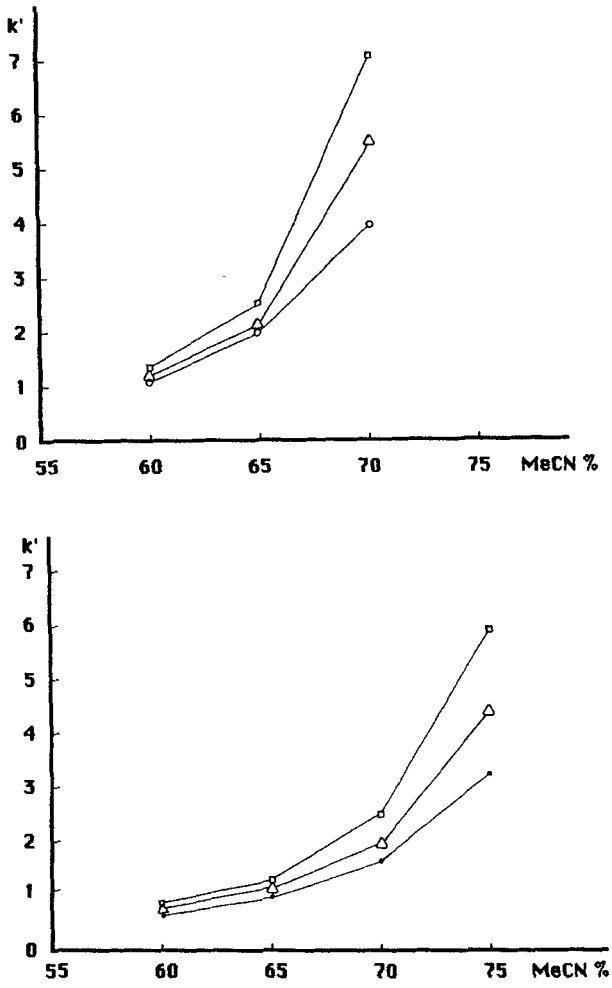

Figure 1. Plots of k' vs % MeCN in water for α-CD (O), β-CD (△) and γ-CD (□) on the α-CD column (a) and the β-CD (b) column.

TABLE I. Experimental Chromatographic Parameters for α-, β- and γ-CD on the α- and β-CD columns.

Column	α-CD			β-CD		
Mobile Phase	65% MeCN/H_2O[a]			70% MeCN/H_2O[a]		
Solute	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD
tr(min)[b]	8.65	9.62	10.63	8.09	9.55	11.17
k'[c]	2.09	2.45	2.80	1.97	2.50	3.10
N/250mm[d]	7800	6600	5000	8600	8400	6800
Symmetry Index[e]	0.83	0.85	0.71	0.89	0.91	0.78
α[f]	1.17	1.14		1.27	1.24	
R_S[g]	2.19	2.12		3.60	3.80	

[a]flow rate, 1 ml/min.; [b]solute retention time; [c]solute capacity factor; [d]number of theoretical plates; [e]calculated at 10% of the peak height; [f]separation factor; [g]resolution

Some of the principle differences between the α-CD and β-CD columns can be seen from the data in Table I. The compositions were adjusted to obtain comparable retention times on both columns. Using the usual parameters which characterize a chromatographic separation (i.e., α, R_S) it can be seen clearly that the β-CD column is more selective and efficient for these solutes than the α-CD column. Also, it should be noted that the number of theoretical plates decreases and the peak asymmetry increases with increasing solute molecular weight.

The effect of mobile phase flow rate on column efficiency for each solute and on both columns was also examined to provide insight into the source of column differences and to the separation mechanism. Van Deemter plots for the α-CD and β-CD columns are presented in Figure 2. From the figures, it can be seen that the differences in column efficiencies on the α-CD column between solutes is larger than on the β-CD column and the overall efficiency of the α-CD column is less than the β-CD column for these solutes.

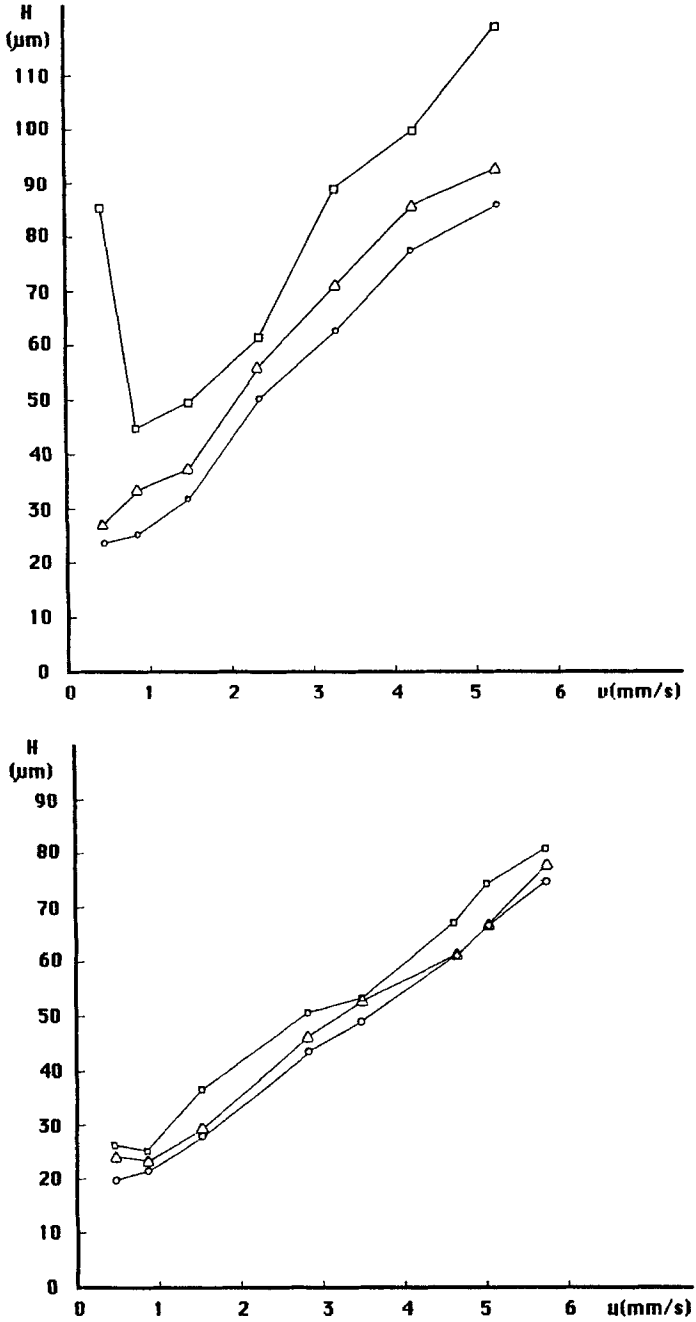

Figure 2. Plots of the theoretical plate height, H, vs linear velocity, μ, for α-CD (O), β-CD (△) and γ-CD (□) on the α-CD column (a; 65% MeCN/H_2O) and the β-CD column (b; 70% MeCN/H_2O).

The decrease in column efficiency with increasing flow rate is also more pronounced on the α-CD column than on the β-CD column. As noted in Table I, the column efficiencies for the solutes decrease with increasing solute size. One might explain these results in terms of a decrease in the solute diffusivity with increasing molecular weight. The molecular weight differences between these solutes is less than 30%; therefore, the differences in diffusivities for these solutes are expected to be relatively small and other factors should be considered. The fact that retention of these solutes is related to solute size suggests that the larger CD's, having more sites for hydrogen bonding with the CD bonded ligands, may undergo slower on-off kinetics at the stationary phase/mobile phase interface, resulting in greater mass transfer band broadening for the higher molecular weight solutes.

As indicated in Figure 1, the separation of CD's is sensitive to changes in mobile phase composition. Typical separations of the CD's on the β-CD column are presented in Figure 3. Similar results were obtained on both columns. The chromatograms illustrate the fact that the solutes are well resolved in less than ten minutes, using high aqueous mobile phase compositions. Increasing the organic content of the mobile phase increases solute retention and resolution without degrading peak shape. Pharmacological studies involving drug uptake in the presence of CD's may require quick analysis times with a minimum of sample clean-up. For complex biological samples, it may be necessary to use high organic mobile phase compositions to isolate the CD's from other sample components.

In conjunction with the aforementioned pharmacological studies, analysis of the CD's in biological samples requires minimal sample work-up and therefore, the method of detection needs to be sensitive and not require derivatization techniques. We found detection based on refractive index to be particularly useful and up to an order of magnitude more sensitive than

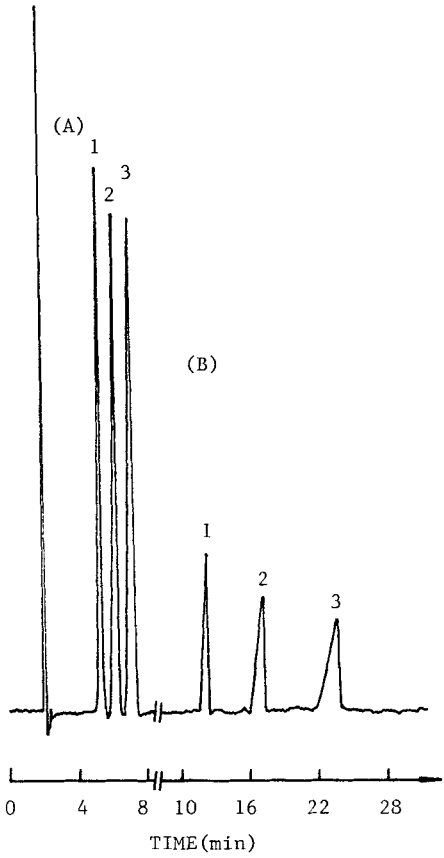

Figure 3. Chromatograms of α-, β-, and γ-CD on the β-CD on the β-CD column. Mobile phase (a) 75% MeCN/H_2O, (b) 80% MeCN/H_2O; flow rate, 1.5 mL/min.

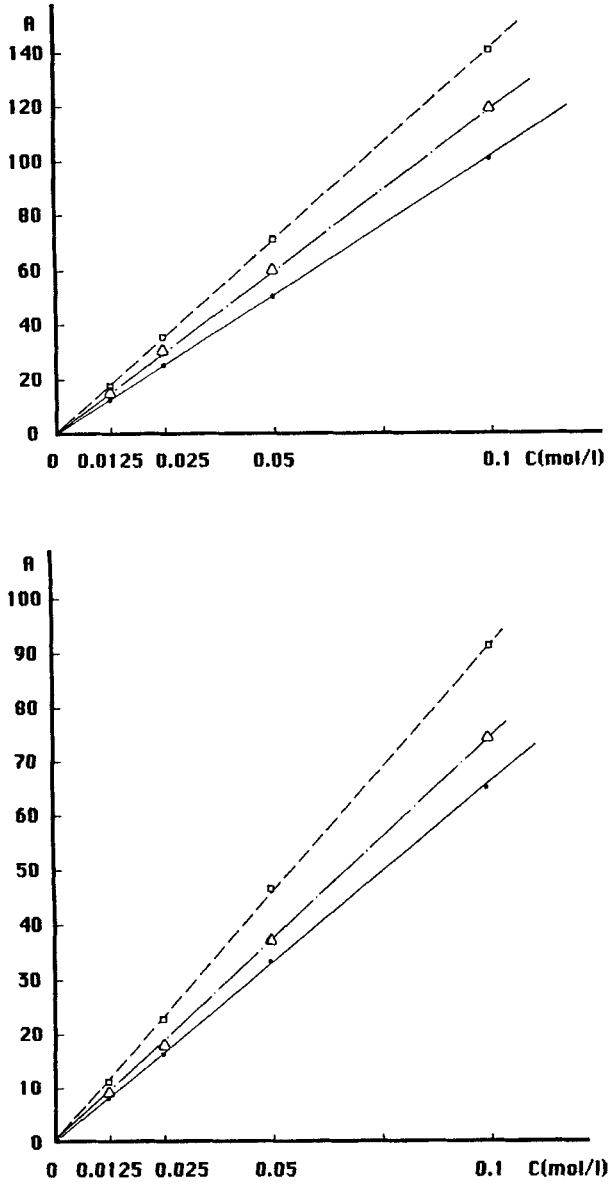

Figure 4. Plots of peak area vs solute concentration for α-CD (O), β-CD (△) and γ-CD (□) on the α-CD column (a; 65% MeCN/H_2O) and the β-CD column (b; 70% MeOH/H_2O).

low wavelength UV detection [8]. The relationship between solute concentration and peak area is plotted for all three solutes on the α-CD and on the β-CD columns (Figure 4). The results presented here compare favorably the results obtained using low wavelength UV detection [8]. As can be seen from the figures, excellent linearity was observed over an order of magnitude in solute concentration.

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