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Preparation and chromatographic characteristics of a chiral-recognizing perphenylated cyclodextrin column

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Abstract

A novel bifunctional chiral recognizing column (PhCD column) was prepared by immobilizing, on silica gel, β-cyclodextrin (CD) which was perphenylated through the reaction of phenyl isocyanate. Using several enantiomeric compounds, chiral recognition by CD was determined to be maintained in a PhCD column. The chromatographic characteristics of a PhCD column were compared with those of a column (CD column) bound with underivatized CD, and the following properties were found. For polyaromatic compounds and alkylbenzene derivatives, hydrophobic interaction is predominant, and for phenylalkyl alcohols and amines whose enantiomers are hard to recognize on a CD column, specific enantioselectivity was exhibited under the effects of the chemical structure around the chiral centre of the solutes. This added function is possibly an induced fit caused by the phenyl cluster on the CD openings. Using these properties, various enantiomeric drugs, in particular β-blockers (amino alcohols), which are hardly separated on conventional chiral separation columns, could be separated.

1. Introduction

In the last decade, chirality-related chemistry has become extremely important. For instance, asymmetric synthesis has increased its importance in drug preparation because one member of an enantiomeric pair frequently shows high toxicity while the other is an effective drug. To detect how such drugs with chirality are converted *in vivo*, chiral recognition analysis has also become essential. In this field, HPLC has played an important role. HPLC separation enables us to conduct pharmacokinetics studies, metabolism analysis, detection of optical impurities, etc., and is making great contributions in the life sciences.

Many chiral stationary phases have been developed. Columns carrying various chiral recognizing linkers were produced first for indirect analyses in which organic solvents are adopted. Such columns have a limited range of separable compounds. In addition to the unambiguous analytical results, therefore, users were required to stock various types of columns, from which a suitable one is chosen according to the compounds sought. Columns bonded with proteins [1–5] have been developed for aqueous phase HPLC analysis. Although a single stationary phase can separate a wide variety of compounds, such protein-bonded columns also have problems such as low loadability of samples and instability

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of the phases. Also, the separation mechanism still remains to be solved, preventing further improvement of the columns.

Therefore, to over these shortcomings, it is worth investigating other stationary phases that can be used in aqueous mobile phases and separate a wide range of compounds. An example is cyclodextrin (CD) immobilized on supports; CD can recognize the chirality of some samples trapped in its cavity. Columns with the stationary phases α-, β- and γ-CDs, propylcarbamated CD and naphthylethylcarbamated CD have been developed [6–8] and are now commercially available. However, enantiomeric compounds are not always resolved satisfactorily on these CD columns and, therefore, further improvement is needed.

One idea for improvement is to modify CD stationary phases with linkers with potent hydrophilic or hydrophobic interactions so that this additional interaction can enhance the chiral recognition by CD. In this study, CD was linked with clusters of phenyl groups. The preparation was arranged so that phenyl groups are bound with all the hydroxyl groups on both the smaller and larger openings of the CD cone and the phenyl groups sticking out of the larger opening can interact with hydrophobic parts of the sample molecules in the outside of the cone. If this hydrophobic part of the solute is achiral, the hydrophobic interaction may regulate the fitness of the chiral part to the chiral recognizing centre in the cone. In this paper, we report the chromatographic characteristics of this bifunctional column.

2. Experimental

2.1. Chemicals and materials

β-Cyclodextrin (β-CD, referred to as CD hereafter) was purchased from Wako (Osaka, Japan). The silylating reagent 3-isocyanatopropyltriethoxysilane (95%) was obtained from Chisso Petrochemical (Tokyo, Japan) and was purified by distillation immediately prior to use. Phenyl isocyanate, the modifying reagent of CD, was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The silica gel used was Ultron SIL (a totally porous spherical silica gel with a mean particle diameter of 5 μm, a mean pore size of 120 Å and a specific surface area of 330 m^2/g), (Shinwa Chemical Industries, Kyoto, Japan). Other solvents used in the preparation of the stationary phase were of at least analytical-reagent grade and were carefully dried before use. Propranolol and its derivatives were kindly donated by Dr. J. Haginaka [9] of Mukogawa Women's University, and other samples were obtained from Wako.

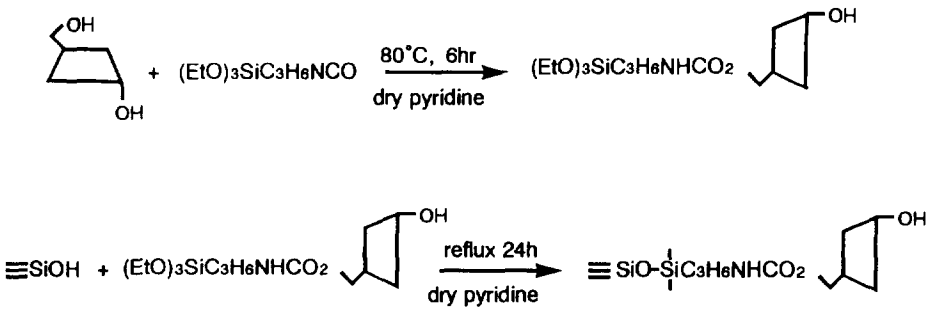
2.2. Preparation of chiral stationary phase

Two types of CD-bonded silica stationary phases were prepared. The bonding reactions used are shown in Fig. 1. All reactions were performed under anhydrous conditions.

2.3. Preparation of CD column

A 5-mmol amount of CD (ca. 5.7 g), dried at 80°C under vacuum for 8 h, was dissolved in 70 ml of dry pyridine with stirring, then 5 mmol of (3-isocyanatopropyl)triethoxysilane (ca. 1.50 g) was added to the solution under a nitrogen atmosphere. The solution was allowed to stand at ca. 80°C until both the disappearance of the adsorption at 2200–2300 cm^{-1} (N=C=O) and the appearance of carbonyl groups at 1700 cm^{-1} were detected. This modified CD solution (solution A) was also used for the preparation of a PhCD column. To this solution, vacuum-dried silica gel (6 g, 140°C, 8 h) was added with stirring. After cooling, the CD-bonded silica was filtered, washed successively with pyridine, acetone, methanol, water, tetrahydrofuran (THF) and dichloromethane and finally dried under vacuum at 60°C for about 8 h. The amount of CD immobilized on the silica material was measured as the mass difference of the dried particles before and after functionalization, i.e., 67.8 mmol/g.

CD-bonded Silca Gel



PhCD-bonded Silica Gel

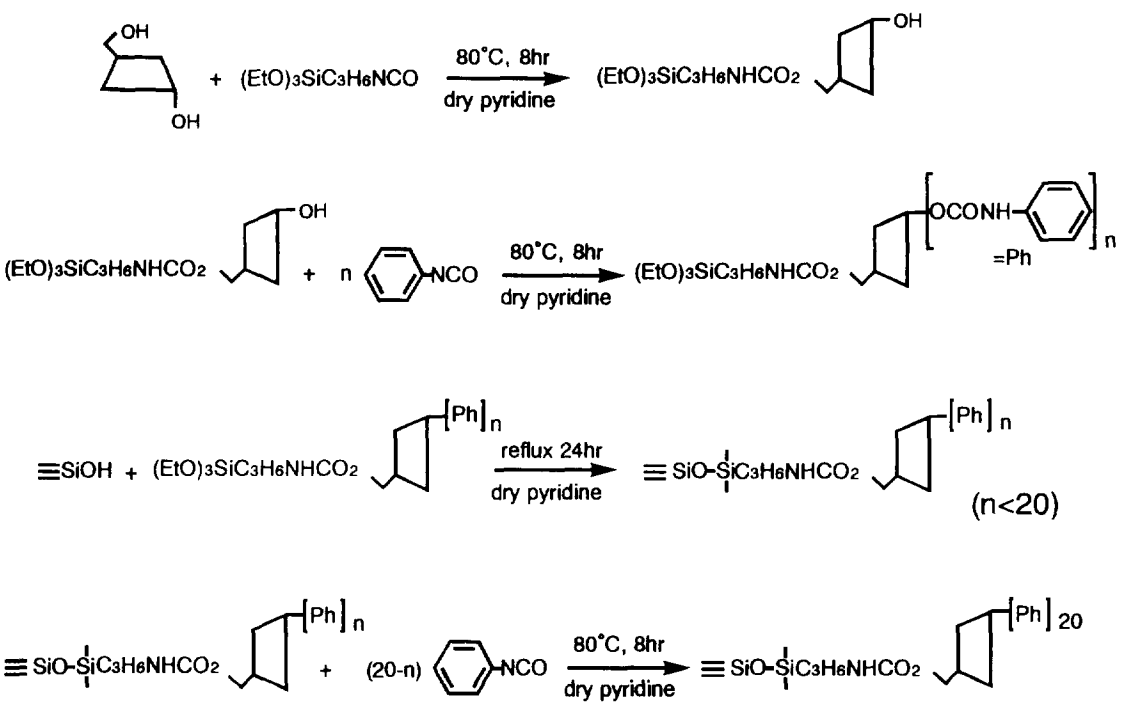


Fig. 1. Reaction schemes for the preparation of CD-bonded and PhCD-bonded silica gel.

2.4. Preparation of PhCD column

To solution A (see above), phenyl isocyanate (ca. 7.5 g) was added with stirring and the reaction mixture was allowed to stand at ca. 80°C for 8 h. To this solution, vacuum-dried silica gel (6 g, 140°C, 8 h) was added with stirring and the mixture was refluxed for 24 h. To this dispersion were added 7.5 g of phenyl isocyanate. The purpose of this additional reaction was to introduce phenyl isocyanate to hydroxyl groups remaining unreacted in the first reaction. The mixture was allowed to stand for 8 h at 80°C and, after cooling, the phenylcarbamoylated CD-bonded silica was filtered, washed successively with pyridine, acetone, methanol, water, THF and dichloromethane and finally dried under vacuum at 60°C for ca. 8 h. The amount of phenylisocyanated CD layer on the silica material was measured as the mass difference of the dried particles before and after functionalization, i.e., 66.8 mmol/g.

2.5. Measurements

The chromatographic system used consisted of an LC-6A pumping system (Shimadzu, Kyoto, Japan), an SPD-6AV UV detector (Shimadzu) and a C-R6A integrator (Shimadzu). The chromatographic conditions are given later. IR spectra were obtained on an FT/IR-5300 spectrometer (JASCO, Tokyo, Japan).

3. Results and discussion

As mentioned under Experimental, we prepared a perphenylated CD column (PhCD column). One of the seven primary hydroxyl groups located at the smaller opening edge of the cone was used for linking CD to the silica support. The other six groups and fourteen secondary hydroxyl groups on the larger opening edge of the cone were all subjected to reaction with phenylisocyanate. This introduction of phenyl groups was intended to introduce a hydrophobic cluster above the CD larger opening of the cone. By forming this cluster, stationary phase will be bifunctional in chiral recognition: one is chiral recognition by the CD cone and the other is hydrophobic interaction by the phenyl cluster. If phenyl cluster traps the hydrophobic part of the solute, we may be able to control the fitness of some solute to the recognition centre in the CD cone, producing the induced fit of chiral molecules.

Our major aim in preparing this column was to separate enantiomeric drugs in aqueous media, particularly β-blocking drugs such as atenolol [10]. The structure of these drugs is commonly base on amino alcohols with chiral centres. Usually such drugs are difficult to separate into individual enantiomers with aqueous mobile phases.

We first investigated the chiral recognizing ability of the PhCD column. The samples used were commercially available drugs with chirality, ibuprofen ($pK_a = 4.4$), chlorpheniramine ($pK_a = 9.2$) and hexobarbital ($pK_a = 8.2$). The eluent used was 20 mM phosphate buffer containing 15% acetonitrile. Capacity factors (k') and separation factors (α) obtained at various pHs are summarized in Table 1 together with those obtained on the CD column for the same samples. At the pH values at which samples do not have charge, the chiral recognition ability [expressed as the separation factor (α)] of the PhCD column was almost equivalent with that of the CD column, implying that chiral recognition by the CD cone was maintained in the PhCD column. It was also determined that the k' values increased for the PhCD column. This is obviously due to the hydrophobic interaction by the phenyl cluster.

Polyaromatic compounds (benzene, naphthalene, anthracene and pyrene) were also tested to see the effect of the phenyl cluster. When the CD column was used, the k' values increased as the number of benzene rings increased, except for anthracene, as shown in Fig. 2. This result suggests that these polyaromatic samples were retained in an aqueous acetonitrile mobile phase by hydrophobic interaction by CD and that for anthracene, some unidentified exclusive forces were combined. On the PhCD column, all four compounds had larger k' values than those on

Table 1
Capacity factors (k') and separation factors (α) obtained for ibuprofen, chlorpheniramine and hexobarbital on Ultron ES-PhCD and Ultron ES-CD columns at various buffer pH values

Column	Compound	pH 3.0		pH 4.0		pH 5.0		pH 6.0		pH 7.0	
		k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
ES-PhCD	Ibuprofen	–[a]	–	–	–	24.87	1.06	7.31	1.05	2.19	1.05
(PhCD column)	Chlorpheniramine	4.31	1.00	9.28	1.00	18.31	1.00	47.50	1.00	–	–
	Hexobarbital	9.99	1.11	9.97	1.11	9.95	1.07	9.87	1.11	9.13	1.11
ES-CD	Ibuprofen	74.08	–	64.78	–	52.14	1.05	31.93	1.07	23.47	1.07
(CD column)	Chlorpheniramine	2.80	1.09	4.59	1.09	6.61	1.09	11.30	1.08	28.14	1.06
	Hexobarbital	6.35	1.17	6.47	1.17	6.25	1.17	6.37	1.17	6.18	1.16

Eluent: 20 m*M* phosphate buffer–acetonitrile (85:15, v/v).
[a] Compound was not retained.

the CD column and the k' values increased with increase in the ring number. These observations imply that on the PhCD column, hydrophobic interactions strengthened by the introduction of phenyl clusters dominate the retention of hydrophobic polyaromatic solutes. Such an enlarged retention due to the phenyl cluster was also obtained for alkylbenzenes. As represented in Fig. 3, on the PhCD column, the k' values increased with increasing carbon number of the alkyl groups and were much larger than those on the CD column. Consequently, on the PhCD column, the phenyl cluster has a substantial hydrophobic interaction with hydrophobic compounds.

For chiral compounds, CD and PhCD columns behaved differently, as shown in Fig. 4. The samples used were an amino ester, propranolol and its derivatives, which have a hydrophobic naphthalene ring and a chiral carbon several ångstroms distant from the ring. On the PhCD column, much larger k' values were produced for all the compounds tested compared with those

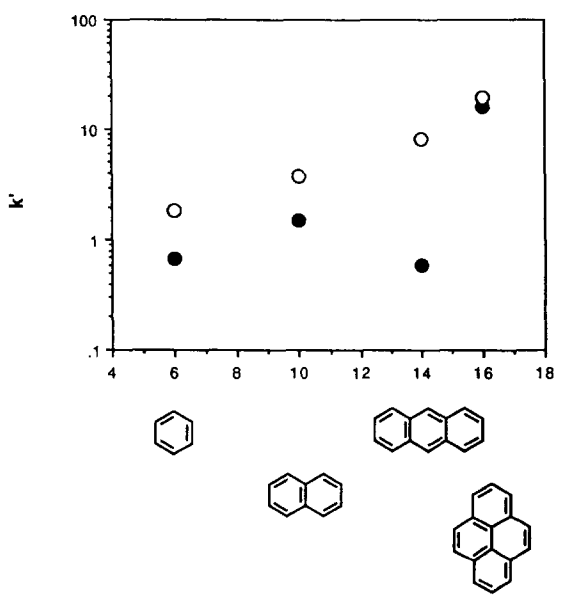


Fig. 2. Capacity factors obtained for polyaromatic compounds on (●) CD and (○) PhCD columns. HPLC conditions: columns, Ultron ES-CD (CD column, 150 × 6.0 mm I.D.) and Ultron ES-PhCD (PhCD column, 150 × 6.0 mm I.D.); eluent, water–acetonitrile (60:50, v/v); flow-rate, 1.2 ml/min; column temperature, 25°C; detection, UV at 254 nm.

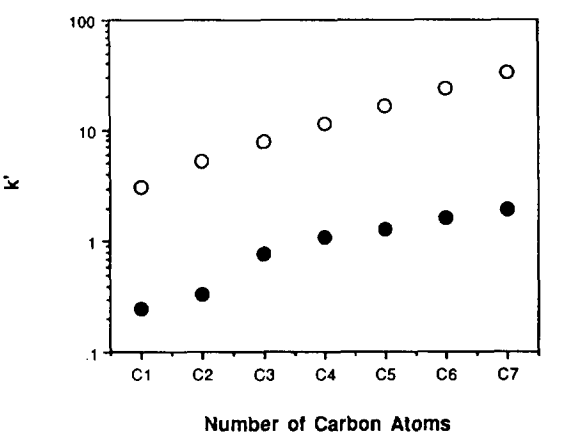


Fig. 3. Capacity factors obtained for alkylbenzenes on (●) CD and (○) PhCD columns. HPLC conditions as in Fig. 2.

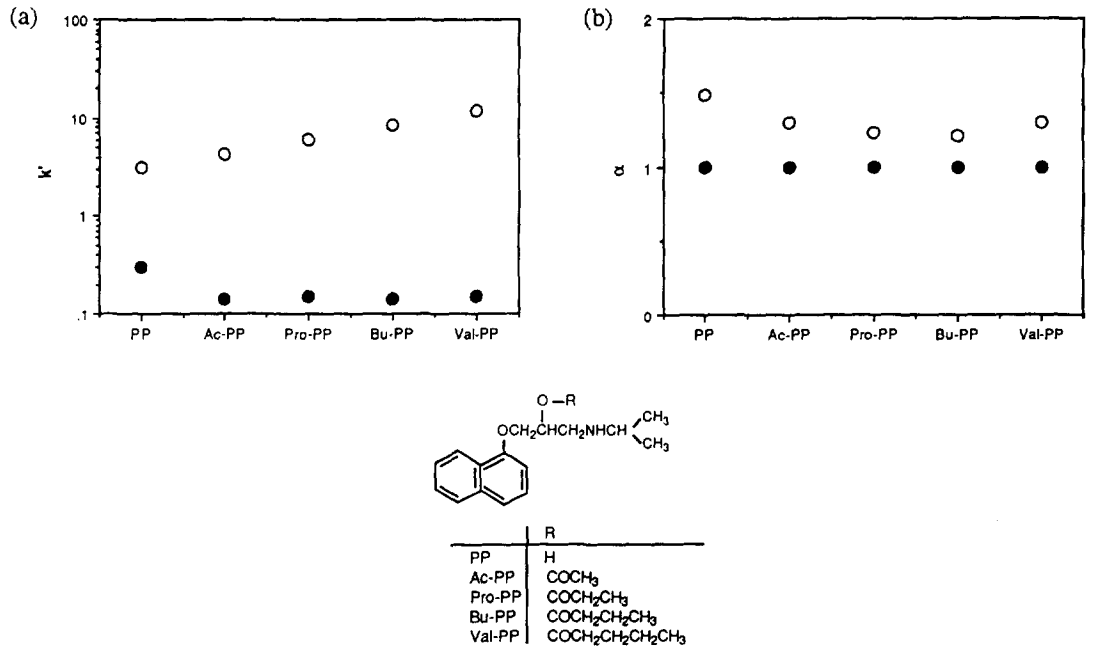


Fig. 4. Capacity factors and separation factors obtained for propranolol and its ester derivatives on (●) CD and (○) PhCD columns. HPLC conditions: columns, as in Fig. 2; eluent, 20 m*M* phosphate buffer (pH 4.6)–acetonitrile (65:35, v/v); flow-rate, 1.0 ml/min; column temperature, 25°C; detection, UV at 220 nm.

suggests that both the naphthalene ring and alkyl chain had hydrophobic interactions with the hydrophobic phenyl cluster of the PhCD column. When the length of the alkyl chain of the ester branch was increased, the k' values increased on the PhCD column whereas they remained almost

Table 2
Separation of enantiometeric pairs on Ultron ES-PhCD

Substance	R_s	Substance	R_s
Acetylpheneturide	4.48	Eperisone	1.94
Alprenolol	2.70	Flavanone	3.01
Arotinolol	1.71	Ibuprofen	0.42
Atenolol	2.03	Oxprenolol	0.69
Benzoin	0.92	Phenylethyl alcohol	1.35
1,1'-Bi-2-naphthol	4.50	Phenylethylamine	1.36
Biperiden	0.73	Pindolol	1.78
Bunitrolol	3.26	Proglumide	0.49
Bupivacaine	1.34	Propranolol	2.55
Chlormezanone	1.76	*trans*-Stilbene oxide	3.33
Chlorphenesin	2.31	Trihexyphenidyl	0.89
DBD-APys[a]	2.82	DNB-MBA[b]	2.11

[a] 4-(N,N-Dimethylaminosulphonyl)-7-(3-aminopyrrolidine)-2,1,3-benzoxadiazole.
[b] N-3,5-Dinitrobenzoyl-α-methylbenzylamine.

constant on the CD column. This trend also supports hydrophobic interactions. To see if this hydrophobic interaction controlled the fitting of the chiral centre of the solutes in the CD cavity, separation factors (α) were also measured. As shown in Fig. 4b, the test samples had α values larger than 1 on the PhCD column whereas the values were always 1 for the separation on the CD column. The results indicate that chiral recognition by the CD cavity was enhanced by the introduction of phenyl clusters. As no recognition was observed when the CD column was used, such recognition by the PhCD column was probably produced by the cooperative action of both functions, hydrophobic interaction and inclusion.

An analogous trend was observed for phenylalkyl alcohols (α-phenylethyl alcohol, 1-phenyl-1-propanol, 4-phenyl-2-butanol and 1-phenyl-1-pentanol). As depicted in Fig. 5a, on the PhCD column, the k' values increased with increase in the chain length of alkyl groups whereas on the CD column such trend was not observed. This result also shows that phenyl clusters interact with hydrophobic parts of the solutes dominating the retention. For this series of samples, the PhCD column also produced a highly specific chiral recognition, which is represented by the α values. As shown in Fig. 5b, 4-phenyl-2-butanol had an extremely large α value compared with the others; it should be noted that on the CD column, all the samples exhibited α values of nearly 1, showing no specific chiral recognition. Probably on the PhCD column, a hydrophobic part (probably $-CH_2CHPh$) interacted with the phenyl cluster so that the chiral centre of this solute [$CH_3^*CH(OH)CH_2-$] was located at the chiral recognizing region of the CD cone. This induced fit is a novel characteristic of this column. It seems that in the case of the other samples, the chiral centre [-*CH(OH)Ph] could not fit the recognition region because the hydrophobic interaction of the phenyl cluster and the benzene ring of the samples prevented the chiral centre entering the CD cone.

To see how effective the PhCD column is, enantiomeric drugs and chemicals were examined. Table 2 summarizes samples that showed good resolution. It is notable that β-blockers, which are hardly separated on conventional chiral columns, produced good resolution for their pairs of enantiomers on the PhCD column.

Consequently, by introducing a phenyl cluster on the large opening of CD and immobilizing this perphenylated CD on silica gel, a novel chiral recognizing column could be prepared.

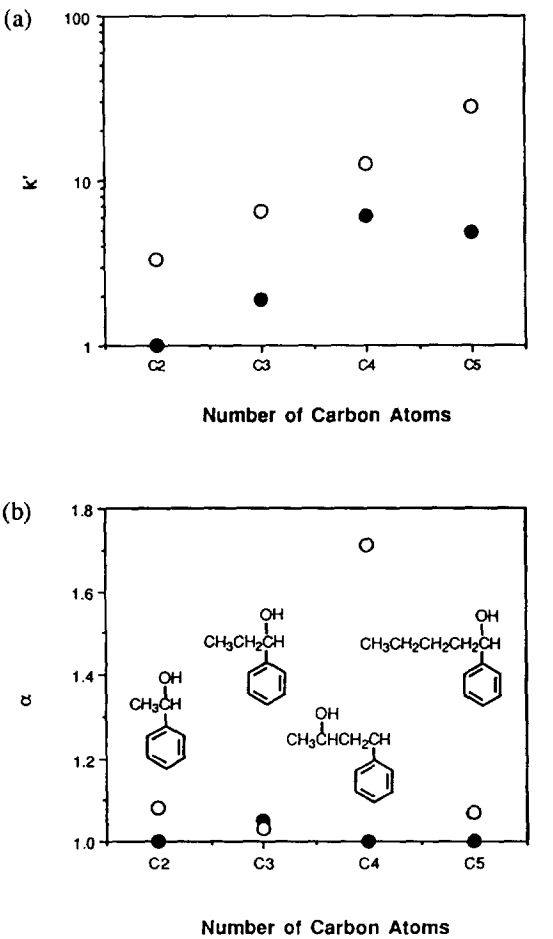


Fig. 5. Capacity factors and separation factors obtained for phenylalkyl alcohols on (●) CD and (○) PhCD columns. HPLC conditions: columns as in Fig. 2; eluent, 20 m*M* phosphate buffer (pH 4.6)–acetonitrile (80:20, v/v); flow-rate, 1.2 ml/min; column temperature, 25°C; detection, UV at 220 nm.

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