

An Attempt Directed toward Enhanced Shape Selectivity in Reversed-Phase Liquid Chromatography: Preparation of the Dodecylaminated β -Cyclodextrin-Bonded Phase

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With the aim of preparing a stationary phase with a high shape-recognition ability for liquid chromatography, a new bonded phase was synthesized by coupling multiply dodecylamino-substituted β -cyclodextrin (β -CD) to 3-glycidoxypropyl-derivatized silica gel. The stationary phase prepared in this way was expected to have increased shape selectivity compared with that of conventional reversed-phase materials, due to solute interactions with the alkyl chain piles built up on the β -CDs bonded to silica. The separation characteristics of the bonded phase were investigated using polycyclic aromatic hydrocarbons (PAHs) with different molecular shapes and compared with those of monomeric ODS and native β -cyclodextrin-bonded phases. The newly developed stationary phase was found to be highly selective for PAHs.

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Introduction

Solute shape selectivity in reversed-phase liquid chromatography (RPLC) has been a topic of much study over the last decade.^{1,2} Several classes of closely related compounds, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls, steroids, and carotenoids, can be separated in RPLC based on the molecular shape.³ Although the contribution of the reversed-phase material to shape selectivity is not completely understood, there have been a few trends observed in RPLC separations. Shape selectivity varies with carbon loading of the stationary phase, the length of the alkyl-chains bonded to silica gel and the properties of the silica substrate. Also, it is well known that shape selectivity is enhanced by reducing the column temperature or by increasing the organic-modifier content in the mobile phase. Furthermore, shape selectivity differences are strongly influenced by the kind of surface-modification chemistry used.⁴

It has been noted that polymeric octadecylsilane (ODS) silicas provide higher shape selectivity than monomeric counterparts in the separation of PAHs.⁵⁻⁷ This has been ascribed to increased chain ordering in polymeric phases, which results from their alkyl chain organization as a result of higher chain densities.^{4,6,8} Spectroscopic⁸⁻¹² and simulation¹³ studies have shown that chain ordering increases with increasing chain density.

The purpose of this paper is to report on the synthesis of a new RPLC bonded phase that is designed so as to have regularly spaced, ordered chains on a local level. The possible structure of the prepared stationary phase is depicted in Fig. 1. A number of PAHs were separated with this stationary phase,

and its selectivity was compared with those obtained with conventional stationary phases. The preliminary experiments revealed that the new stationary phase offers enhanced molecular shape-recognition performance.

It may be noted that, by far, a large number of modified β -CD stationary phases have been synthesized, most of which were directed toward chiral separations, but not toward RPLC separations. An exception to this trend has been presented by Ôi and Ishizuka.¹⁴ They synthesized dodecylamino- β -CD by the reaction of heptakis(6-bromo-6-deoxy)- β -CD with dodecylamine in dimethylformamide. ODS silicas were coated with the produced material and used as a stationary phase for the separation of structural isomers.

Experimental

Synthesis of the stationary phase

The preparation procedure of the stationary phase consisted of four separate stages (Fig. 2): (1) silylation of silica gel through reactions of 3-glycidoxypropyltrimethoxysilane with the surface silanol groups,¹⁵ which provides a site for subsequent bonding with dodecylaminated β -CD; (2) sulfonation of the primary hydroxyl groups of β -CD with mesitylsulfonyl chloride,¹⁶ (3) reaction of *n*-dodecylamine with mesitylenesulfonylated β -CD, yielding *n*-dodecylamino-derivatized β -CD (DA- β -CD) (the synthesis of heptakis(6-dodecylamino-6-deoxy)- β -CD has been already described by Takahashi *et al.*¹⁷); and (4) coupling of DA- β -CD with 3-glycidoxypropyl-derivatized silica.¹⁵

Reaction (2) can produce many positional isomers having different levels of substitution of the CD hydroxyl groups. The degree of *n*-dodecylamine substitution per CD was determined by proton NMR spectroscopy based on the peak-area ratio of the substituent groups to the β -CD in the spectra, and was found

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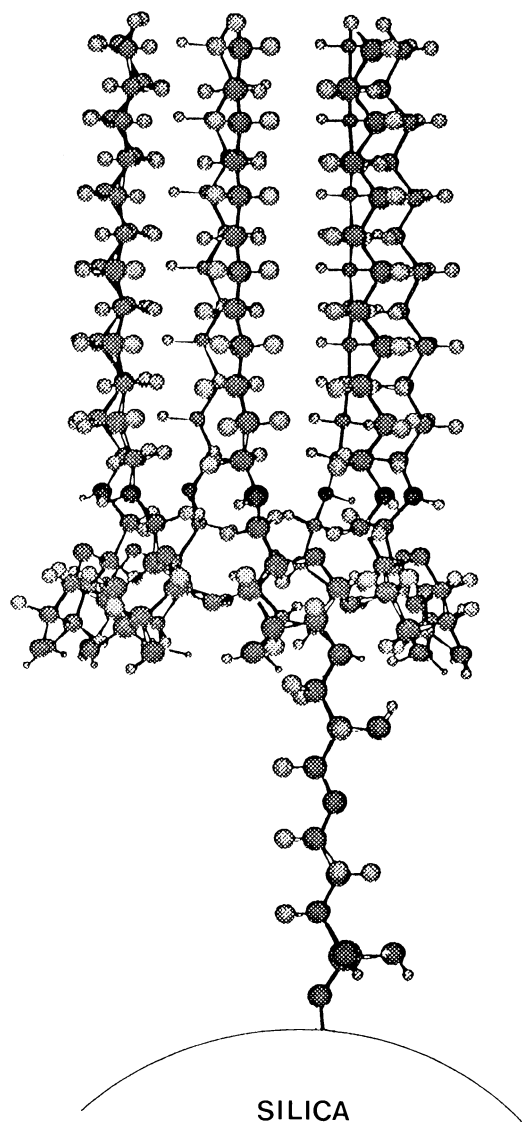


Fig. 1 Structure of the DA- β -CD bonded to silica.

to be 7.1–7.8. It is likely that the seven primary, more reactive hydroxyl groups were used up during derivatization, together with a small portion of the secondary hydroxyl groups. Both the 3-glycidoxypropyl-derivatized silica and the DA- β -CD bonded phase were submitted for carbon analysis. The density of DA- β -CD attached to the silica surface, a_{CD} (mol/m²), was calculated according to the following equation:¹⁸

$$\%C = \frac{1200n_{LNK}a_{LNK}S + 1200n_{CD}a_{CD}S}{1 + M_{LNK}a_{LNK}S + M_{CD}a_{CD}S}, \quad (1)$$

where %C is the carbon content of the finally obtained silica, S is the specific surface area (m²/g), and n_{LNK} and n_{CD} are the number of carbons of the linkage chain and DA- β -CD, respectively. M_{LNK} and M_{CD} are the formula weights of the linkage chain and DA- β -CD, respectively, and a_{LNK} is the surface coverage of the 3-glycidoxypropyl-derivatized silica (1.90 μ mol/m²), which was calculated from the equation derived by Berendsen and Galan.¹⁹ The DA- β -CD surface coverage was calculated to be 0.37 μ mol/m².

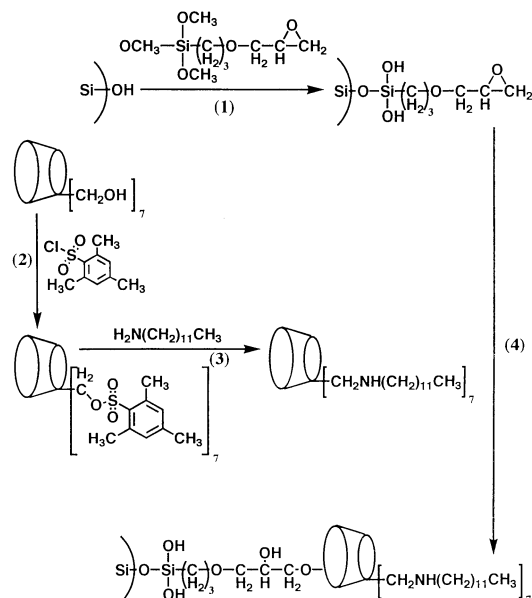


Fig. 2 Reaction scheme for preparing the DA- β -CD stationary phase.

Chemicals

3-Glycidoxypropyltrimethoxysilane, 2-mesitylsulfonyl chloride, and dodecylamine were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). β -CD was obtained from Kishida Chemical (Osaka, Japan). Phenanthro[3,4-c]phenanthrene (PhPh) was a gift from J. C. Fezer of Chevron Research (Richmond, CA, USA). The base silica material used for preparing the DA- β -CD phase was Develosil 300-5 (particle diameter, 5 μ m; a mean pore diameter, 26.9 nm; specific surface area, 156 m²/g) from Nomura Chemical (Seto, Japan).

Chromatographic measurements and molecular modeling

The stationary phases were slurry-packed into fused-silica capillaries of 0.53 mm i.d. (GL Sciences, Tokyo, Japan). Develosil ODS-5 (a monomeric phase; 3.0 μ mol ODS/m²; endcapped; 5 μ m particle diameter, Nomura Chemical) and Cyclobond-I (a β -CD bonded stationary phase; 20 μ m particle diameter; Advanced Separation Technologies, Whippany, NJ, USA) were used for comparison studies. The liquid-chromatographic system was composed of a Model MF-2 microfeeder (Azuma Denki Kogyo, Tokyo, Japan), a Uvidec 100-III ultraviolet detector (Jasco, Tokyo, Japan), and a Model 7520 injector with a 0.5 μ L loop (Rheodyne, Cotati, CA, USA). The flow rate was set at 2 μ L/min. The retention factors and selectivity coefficients⁶ were calculated as follows:

$$\text{retention factor } (k) = (t_R - t_0)/t_0, \quad (2)$$

$$\text{selectivity coefficient } (\alpha) = k \text{ ratio of planar/nonplanar or linear/nonlinear solutes}, \quad (3)$$

where t_0 is the mobile-phase holdup time and t_R is the retention time of the solute. Molecular-modeling studies were performed using Chem 3D molecular-modeling software developed by Cambridge Scientific Computing (Cambridge, MA, USA).

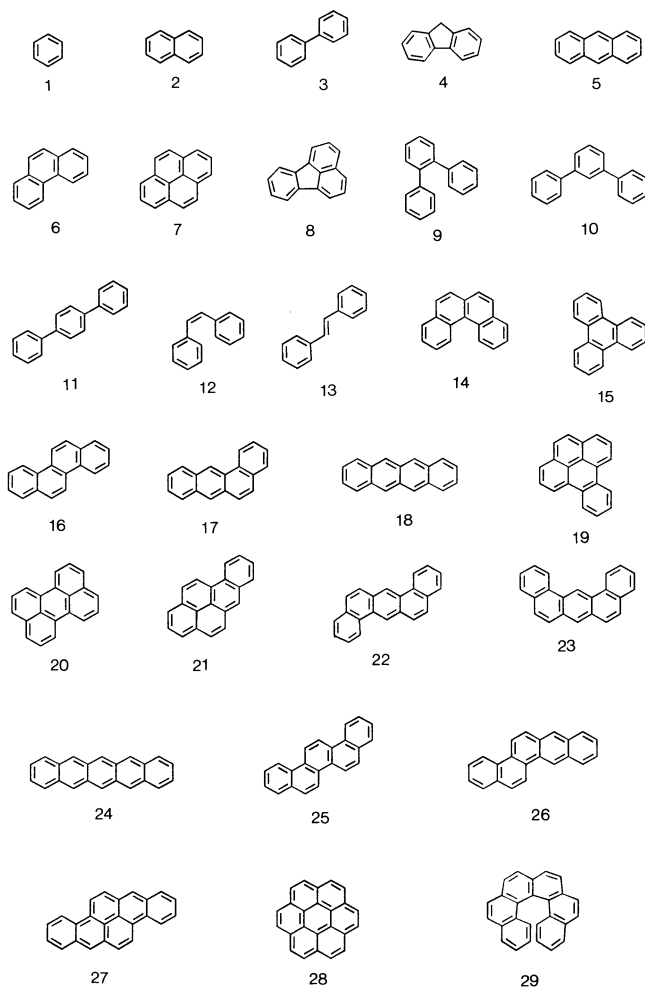


Fig. 3 Structures of the 29 PAHs used in these studies: (1) benzene; (2) naphthalene; (3) diphenyl; (4) fluorene; (5) anthracene; (6) phenanthrene; (7) pyrene; (8) fluoranthene; (9) *o*-terphenyl; (10) *m*-terphenyl; (11) *p*-terphenyl; (12) *cis*-stilbene; (13) *trans*-stilbene; (14) benzo[*c*]phenanthrene; (15) triphenylene; (16) chrysene; (17) benzo[*a*]anthracene; (18) naphthacene; (19) benzo[*e*]pyrene; (20) perylene; (21) benzo[*a*]pyrene; (22) dibenz[*a,h*]anthracene; (23) dibenz[*a,j*]anthracene; (24) pentacene; (25) picene; (26) benzo[*b*]chrysene; (27) dibenzo[*b,def*]chrysene; (28) coronene; (29) PhPh.

Results and Discussion

It is improbable that, by the reaction of silica gel with monofunctional silanes, the silica surface became jammed with alkyl ligands. A close spacing of the alkyl ligands is usually precluded by steric-hindrance effects and silica surface heterogeneity.⁸ The density of the silanol groups at the silica surface is ~ 8 mmol/m², and nearly one half of these groups can be substituted with long alkyl chains. On the other hand, for silylation with multifunctional silanes, the heterogeneity due to the silanol distributions is reduced, and higher surface coverages (up to ~ 6.4 μ mol/m² for silane polymerization in solution) can be achieved.^{4,20} Nevertheless, surface heterogeneity arises from deposition and bonding of silane polymers onto the silica surface during synthesis; the silica surface may be covered with clusters of high ligand density, which would induce chain ordering.⁸

We initially expected that the locally increased chain density

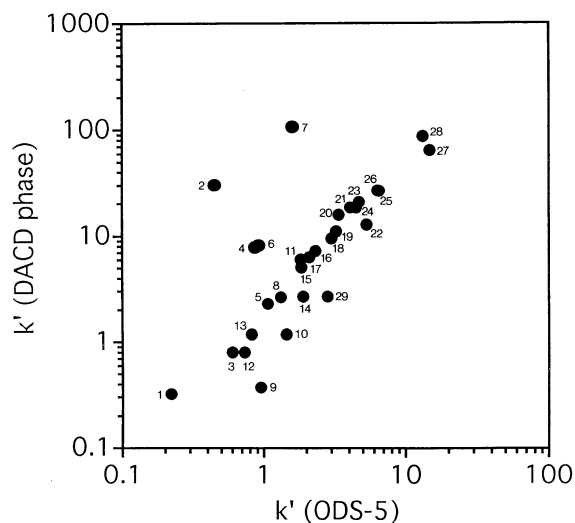


Fig. 4 Plot of capacity factors for PAHs in the DA- β -CD phase vs. the ODS-5 phase. Mobile phase: methanol/water (90:10, v/v). The numbers are as in Fig. 3.

of the DA- β -CD-bonded phase, compared to monomeric RPLC phases, would allow the chains to be more ordered, and therefore, to show enhanced solute shape selectivity. Namely, when all of the primary hydroxyl groups situated on one of the edges of the CD ring with an outer periphery diameter of 1.54 nm²¹ or 1.53 nm²² are replaced by dodecylamino groups, the alkyl chain density of the DA- β -CD phase is calculated to be ~ 6.2 μ mol/m² (3.7 chains/nm²) per CD.

We compared the retention properties of the DA- β -CD bonded phase for 28 PAHs and benzene, shown in Fig. 3, with those of Develosil-ODS and Cyclobond-I. The retention factors for these solutes separated on the DA- β -CD and Develosil ODS-5 phases are plotted against each other in Fig. 4. The plot indicates that the two phases have similar separation selectivity. It would seem that the conformation of the lipophilic chains of the DA- β -CD phase is similar to that of the monomeric ODS phase, which would explain their similar selectivity. For several solutes (*e.g.*, naphthalene, fluorene, phenanthrene, pyrene, and *o*-terphenyl), however, the selectivity is considerably different between those stationary phases. It seems reasonable to assume that the seven dodecyl-chains attached to the narrow end of β -CD are ordered to form a cavity-like structure, allowing hydrophobic interactions with solutes, possibly in the form of a host-guest complex. In that event, the selectivity differences could be explained by considering the geometric characteristics of the guest molecules and the cavity-like structure. When DA- β -CD has the conformation depicted in Fig. 1, the distances between the nearest-neighboring alkyl chains and between the alkyl chains separated by three D-glucopyranose units are ~ 0.43 and 0.88 nm, respectively; they were estimated based on the positions of the primary hydroxyl groups of β -CD. The widths of naphthalene, fluorene, phenanthrene, and pyrene range from 0.74 to 0.90 nm, which seem to be mandatory to fit into the cavity-like structure. On the other hand, the inclusion of *o*-terphenyl into the cavity-like structure may be prohibited due to its bulky shape and, consequently, it would be retained to a lesser extent than their planar analogues.

Figure 5 shows a similar plot for a comparison of the chromatographic selectivity of the DA- β -CD phase vs. that of the Cyclobond-I phase. The scattered plot indicates the poor

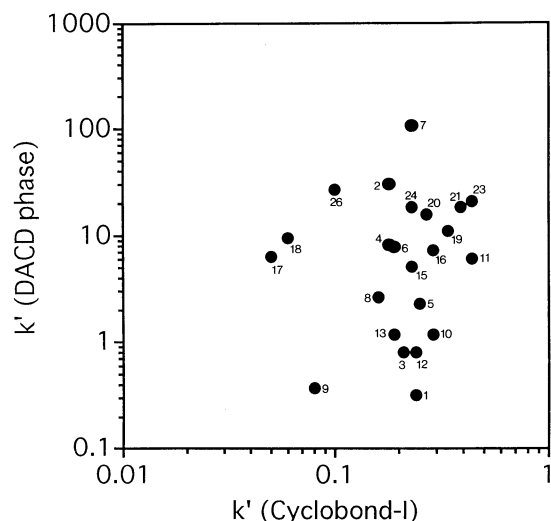


Fig. 5 Plot of the capacity factors for PAHs in the DA- β -CD phase vs. the Cyclobond-I phase. Mobile phase: methanol/water = 90:10, v/v (DA- β -CD phase); 70:30 (Cyclobond-I). The numbers are as in Fig. 3.

correlation between the selectivities of the two stationary phases and, therefore, the contribution of the β -CD moiety (*i.e.*, the effects due to the β -CD cavity) to the retention of PAHs on the DA- β -CD phase appears to be insignificant.

Table 1 lists the selectivity coefficients for pairs of PAH solutes with different degrees of molecular planarity and linearity. It can be seen that planar and linear molecules are selectively retained in the DA- β -CD phase. The α values in the DA- β -CD phase are remarkably larger than those in the monomeric ODS phase. It is noteworthy that the α values in the DA- β -CD phase are significantly large, even compared with the polymeric ODS phases,⁶ although a direct comparison is not appropriate because different mobile phases were used.

In conclusion, a dodecylaminated β -CD bonded phase was prepared aiming at the realization of highly shape-selective separation in RPLC. In the synthetic procedure, the distance between the neighboring alkyl chains is dictated by the locations of the primary hydroxyl groups of β -CD. The synthetic procedure presented here can be viewed as an approach for preparing monomeric phases having as many alkyl chains as possible in a small limited area. The enhanced molecular shape recognition ability of the DA- β -CD bonded phase is likely to in part be due to the high ligand density on a local level, and in part due to the inclusion complex formation of a solute with a cavity-like structure, although their contributions to the separation selectivity are indistinguishable.

Table 1 Selectivity coefficients of PAHs on three columns^a

Pair of solute	DACD	ODS-5	Cyclobond-I
<i>p/m</i> -Terphenyl	5.13	1.26	2.67
<i>p/o</i> -Terphenyl	14.0	1.93	2.67
Coronene/PhPh	32.4	4.66	—

a. Mobile phase: methanol/water (90:10).

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