크로마토그래피 분리인자로서 β-Cyclodextrins을 담지시킨 유리구슬에 관한 연구

김만회
공군사관학교

A Study of Direct Loading of Beta-Cyclodextrins on Glass Beads as Chromatographic Separators

Manhoe Kim
Department of Chemistry, Air Force Academy, Cheong-ju, Chungbuk 363-849, Korea

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligopolysacharides containing from 6 to 13 glucose units bonded through 1,4-linkages. CDs are very promising molecules industrially because CDs have hydrophobic cavities surrounded by hydrophilic outer surfaces. Cyclodextrins forms complexes with many guest molecules. So cyclodextrins can be applied for a wide variety of possible uses such as enhancement of water solubility, stabilization of molecules, making or controlled release of hydrophobic substances, application in chromatography[Szejtli, 1998].

Various organic polymers containing β-CD were prepared by the casting and drying methods [Lee, 1981; Anzai et al., 1986; Miyata et al., 1994] and grafting methods of cyclodextrin-modified material to polymer [Sreenivasan, 1996; Sreenivasan, 1998].

The difficulty of the immobilization of large size CDs to inorganic surface is well known. The large size leads to decreased immobilization to the support [Zhao and He, 1994]. A number of cyclodextrin bonded inorganic phases were reported. A porous tubular ceramic membrane was impregnated with β-CD to obtain a chiral selective membrane with a linking spacer epichlorohydrin. More than half of the impregnated β-CD was washed from the membrane during permeation [Krieg et al., 2000]. Armstrong et al. attached CDs to silica by means of several silane linkages containing epoxy, double bond, alkyl halide group between cyclodextrins and silica [Armstrong and DeMond, 1984; Armstrong, 1985; Armstrong et al., 1985]. These silane linkages are so hydrolytically stable that CD bonded silica can be used to separate a wide variety of organic and inorganic substances with both normal and reverse mobile phases. The basis for the selectivity of the CD bonded phase is the ability of CDs to form inclusion complexes in liquid chromatographic separation. However, the effect of pore blocking and narrowing by the spacer groups in forming inclusion complexes was not studied well. Especially, large molecules such as naphthol isomers having double benzene ring will be very sensitive to the spacer groups.

In this study in order to provide more space to form inclusion complex for large molecules, a direct loading of CDs on glass beads was attempted as a novel method and the directly loaded inorganic supports were tested with a simple column chromatographic separation.

EXPERIMENTAL SECTION

Glass beads (particle size 50-100μm, pore size 31.6nm and surface area 97m^2/g) were used as supports. Beta-CD was attached to glass beads by a direct bonding method, using epoxy spacers, and cross-linking agent. 3-Glycidoxypropyltrimethoxysilane(Aldrich) was used as a spacer group. 1,8-Trichlorosilyloctane (Petrarch Systems) and epichlorohydrin (Aldrich) were used as cross-linking agents. Trimethylchlorosilane (Aldrich) was used for end-capping agent. HPLC grade of dimethylformamide (Aldrich), toluene (Aldrich), methanol (Aldrich), de-ionized water were used as solvents and washing solvents. A 10mL of burette (diameter 0.55cm, Pyrex Brand, Fischer) was used as a chromatography column. The length of packed column was 37cm and the free volume percentage of the packed β-CD bonded glass beads was 53%.

RESULTS AND DISCUSSION

Table 1 shows the weight losses of β-CD loaded glass beads in the TGA experiments. The loadings in Table 1 were defined the differences in sample weight percents between 150℃ and 600℃ at TGA experiments. The 'as synthesized' means the samples dried after washing with toluene and methyl alcohol by filtration. The 'washed in water' means the samples dried after washing with water.

Table 1. Amounts of loading on glass beads synthesized and washed in water

β-CD loaded samples	As synthesized (wt%)	Washed in water (wt%)
β-CD/3-glycidoxypropyltrimethoxysilane/glass beads	11.6	4.5
β-CD/glass beads	15.2	1.9
β-CD/glass beads/epichlrohydrin	22.4	2.2
β-CD/glass beads/1,8-trichlrosilyloctane(1.5g)	13.7	7.3
β-CD/glass beads/1,8-trichlrosilyloctane(8.0g)	20.2	10.4
β-CD/glass beads/trimethylchlorosilane	14.1	2.8
β-CD/glass beads/bromomethane	16.3	1.7

When Armstrongs method[Armstrong, 1985] was used, where 3-glycidoxypropyltrimethoxy-silane used as a spacer group, up to 11.6wt% of organic was loaded on glass beads. By comparison, typically 15.2 weight % of β-CD loading was obtained from the direct loading method. Direct loading method has merits of simple synthetic procedures, high cyclodextrin loading. However, the spacer group linked β-CD was more stable in polar mobile phases than the directly loaded one. The directly loaded sample lost most of the organic loaded when the sample was washed with water. The spacer group linked sample lost two thirds only when washed with water. This means that the sample synthesized contained organic not bonded to the glass beads.

Cross-linking agents such as 1,8-trichlorosilyloctane and epichlorohydrin were tried to stabilize the directly loaded β-CD glass beads. The sample cross-linked with epichlorohydrin had 22.4wt% loading. The column packed with β-CD glass beads cross-linked by epichlorohydrin showed a high back pressure in column chromatography experiment. This result means that epichlorohydrin reacted with hydroxyl groups of β-CD and the flexible epichlorohydrin attached positioned at the outer surface of particle and decreased the portion

of macro passages between particles packed. However, most of the loadings cross-linked with epichlorohydrin were washed off by water. With chains as short as three carbon atoms in epichlorohydrin, cross-linking between CDs is difficult to accomplish because of the relative size of CD [Armstrong, 1985].

Comparing to the cross-linked phase by epichlorohydrin the phase cross-linked by 1,8-trichlorosilyloctane was much more stable in water. It had 7.3 weight percent of loading after washing with water. With a chain of ten atoms in 1,8-trichlorosilyloctane, the couplings between cyclodextrins can be efficiently formed. Amounts of loading were proportional to cross-linking agents used.

The selectivity of the β-CD bonded phase come from the difference of capability to form inclusion complexes in their hydrophobic cavity. Hydrophobic part of 2-naphthol is easy to be a guest molecule to the host β-CD because of the low steric hinderance of hydroxyl group attached at the tail section of the molecule. However, 1-naphthol is difficult to make an inclusion complex because of the high steric hindrance of hydroxyl group at the wing section of molecule.

When the column packed with β-CD bonded glass beads was applied for separation of naphthol isomers with a mobile phase of methyl alcohol, the column could differentiate two naphthol isomers. The composition ratios, 1-naphthol/2-naphthol, of the elution collected from the columns packed with different phases are shown in Table 2. Generally, 1-naphthol eluted earlier than 2-naphthol because of the difference in the capability to form inclusion complex. If the column material has a selectivity to naphthol isomers, the initial composition ratio of 1-naphthol/2-naphthol of the elution was large and decreased with proceeding elution. The column packed with β-CD directly bonded glass beads gave a distinct separation of naphthol isomers as shown in Table 2. However, a dramatically decreased selectivity between naphthol isomers was observed from the spacer group linked β-CD glass beads, although its loading was 11.6wt%. This result means that formation of inclusion complexes of 2-naphthol were limited by pore narrowing or pore blocking.

Table 2. 1-Naphthol/2-naphthol ratios of elute from the column packed with the differently bonded phases.

Bonding methods used	Sample number eluted sequentially from the column				
	1	2	3	4	5
Directly bonded phase	7.0	6.7	2.2	0.8	0.1
Spacer group linked phase (3-glycidoxypropyltrimethoxysilane)	1.40	1.15	1.05	0.88	0.7
Cross-linked phase (1,8-trichlorosilyloctane)	1.0	0.96	0.98	0.98	1.05
End-capped phase (trimethylchlorosilane)	1.0	0.98	1.02	0.99	1.02

The similar trend was observed from the column packed with cross-linked phase. The cross-linked β-CD directly bonded glass beads by 1,8-trichlorosilyloctane did not show any selectivity. The reason that the cross-linking agent does not show any separation of naphthol isomers come from the difference of the location of spacer group and cross-linking agent.

Basically spacer groups are located between glass beads and β-CD, in contrast with, cross-linking agents are on the outer surface of particles. As a result, the cross-linking agents have more chances to block or to narrow the β-CD pore than spacer groups.

Generally it is known that end-capping produces a more stable silica gel since silanols on the surface of the silica are sites most susceptible to dissolution in aqueous solution [Armstrong, 1985]. In addition, end-capping decrease the peak tailing and peak broadening phenomena when base samples such as amine compounds are analyzed because silanol groups act as a counter acid to the base compounds [Forgacs and Cserhati, 1997]. End-capped directly loaded β-CD glass beads with trimethylchlorosilane didnt show any separation selectivity between naphthol isomers. It is conformed that the end-capping agent, trimethylchlorosilane, not only make a change in the pore opening of β-CD because trimethylchlorosilane is attached on the rim of β-CD but also reduce the remaining silanol groups.

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