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

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Abstract−Beta-cyclodextrin (β-CD) was successfully loaded on the surface of glass beads without spacer group or cross-linking agent. The directly loaded β-CD was stable in toluene and methanol flows but critically hurt in more polar solvent such as water and dimethylformamide. Cross-linking could stabilize the directly bonded β-CD glass beads. Pore openings of cyclodextrin can be narrowed or blocked by end-capping agent, spacer groups, and cross-linking agents. The β-CD directly bonded glass beads have a capability to form inclusion complexes with larger host molecules because this method can avoid pore narrowing and blocking problems.

Key words: Beta-Cyclodextrin, Glass Beads, Cross-Linking, Chromatographic Separation

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 they have hydrophobic cavities surrounded by hydrophilic outer surfaces. Since cyclodextrins form complexes with many guest molecules they can be applied for a wide variety of possible uses such as enhancement of water solubility, stabilization of molecules, controlled release of hydrophobic substances, and application in chromatography [Szejtli, 1998]. The liquid chromatographic method can be applied for the separation of thermally sensitive molecules such as proteins [Janson, 2001] and large size molecules such as naphthol somers [Moon et al., 1989] and Naphthalene isomers [Kim et al., 2001].

Various organic polymers containing β-CD were prepared by the casting and drying method [Lee, 1981; Anzai et al., 1986; Miyata et al., 1994] and grafting method of cyclodextrin-modified material to polymer [Sreenivasan, 1996, 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 has not been 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 50-100 μm, pore size 31.6 nm and surface area 97 m^2/g) were used as supports. Beta-CD was attached to glass beads by a direct bonding method, using epoxy spacer, 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 10 mL burette (diameter 0.55 cm, Pyrex Brand, Fischer) was used as a chromatography column. The length of packed column was 37 cm and the free volume percentage of the packed β-CD bonded glass beads was 53%.

Epoxy spacer cyclodextrin-bonded glass beads were prepared according to the method described by Armstrong [Armstrong, 1985]. In order to synthesize directly bonded CDs on the solid surfaces, basically 350 mL of dimethylformamide (DMF) was heated to 153 °C and refluxed and 25 g of glass beads dried in a vacuum oven was added to the solvent (DMF). 50 mL of azeotrope was removed from the boiling mixture in the three-neck flask by a Dean-Stark type trap. The mixture was cooled down to 140 °C. 25 g of β-CD sodium salt dried in a vacuum oven overnight was added to the previous mixture. The resulted slurry was kept at 144 °C for 3 hr with stirring. The resulting slurry was put aside for a few minutes in order to precipitate β-CD bonded glass beads. The upper portion of the

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slurry was removed and the precipitated glass beads were filtered with a filter paper, washed with toluene and methyl alcohol. The β-CD bonded glass beads were dried in a vacuum oven at 80 °C overnight and were tested as packing materials in a column chromatography.

In order to cross-link the directly bonded β-CD glass beads, 15 g of β-CD bonded phases was dried in a vacuum oven at 80 °C, and then put into 120 mL of dried toluene in a three-neck flask. 20 mL of azeotrope was removed from the boiling mixture by a Dean-Stark type trap. The mixture was cooled down to 90 °C and in the range from 1.5 g to 8 g of 1,8-trichlorosilyloctane or epichlorohydrin was added to the mixture with stirring for 10 minutes. 60 mL of β-CD salt solution was added to the mixture, and then maintained at 90 °C for 50 minutes with stirring. The mixtures were filtered and washed with 100 mL of toluene and methanol, respectively.

As an end-capping agent, trimethylchlorosilane was added to the directly bonded β-CD glass beads, and kept at room temperature with stirring for 24 hr. The mixture was filtered and washed with 100 mL of toluene and methanol, respectively.

The amounts of loading on glass beads were measured with a thermal gravimetric analyzer (TGA). Column packing materials were packed into a 10 mL burette by slurry packing or dry powder packing. Naphthol isomers were tested as elute compounds. The chromatographic column was washed in the stream of methanol flow driven by gravitational force of the methanol head for 30 min. After the column was washed, 0.5 mL equimolar mixture of naphthol isomers diluted with methanol (20% in weight) was injected at the top of the column. Methanol head was applied to the top of the column after all the sample mixture went to the column bed. The flow rate of carrier methanol was from 0.05 to 0.07 g/min. The column chromatographic experiments were carried out at room temperature (22 °C). Eluting liquid mixtures were collected at an equal time interval and analyzed with an HP GC-MS (model 5890/5970).

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 as the differences in sample weight percents between 150 °C and 600 °C 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.

When Armstrong's method [Armstrong, 1985] was used, where 3-glycidoxypropyltrimethoxysilane was used as a spacer group, up to 11.6 wt% of organic was loaded on glass beads. This spacer group linked β-CD was more stable than the directly loaded one in polar mobile phases like water. The spacer group linked sample lost up to 60% of the loading when washed with water. This means that the sample synthesized contained organic not bonded to the glass beads. By comparison, the directly loaded sample lost most of the organic loaded when the sample was washed with water, although the direct loading method has merits of simple synthetic procedures and high loading of cyclodextrin. However, the directly loaded sample was very stable in toluene and methanol flow because the synthesized sample was fully washed with toluene and methanol. Typically, when the sample 15.2 wt% β-CD loaded was used in the methanol flow at the chromatography experiment, organic loading of the sample was slightly diminished to 14.4 wt%.

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 22.4 wt% loading. The column packed with β-CD glass beads cross-linked by epichlorohydrin showed a high back pressure in the 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].

Compared 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 wt% 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. The hydrophobic part of 2-naphthol is easy to be a guest molecule to the host β-CD cavity 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 the 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

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/epichlorohydrin	22.4	2.2
β-CD/glass beads/1,8-trichlorosilyloctane (1.5 g)	13.7	7.3
β-CD/glass beads/1,8-trichlorosilyloctane (8.0 g)	20.2	10.4
β-CD/glass beads/trimethylchlorosilane	14.1	2.8
β-CD/glass beads/bromomethane	16.3	1.7

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)	1.4	1.2	1.1	0.9	0.7
Cross-linked phase (1,8-trichlorosilyloctane)	1.0	1.0	1.0	1.0	1.0
End-capped phase (trimethylchlorosilane)	1.0	1.0	1.0	1.0	1.0

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 11.6 wt%. This result means that formation of inclusion complexes of 2-naphthol were limited extremely when the spacer group was introduced. It is inferred that the spacer group between glass bead surface and β-CD narrows or blocks the β-CD cavity.

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 comes 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 which 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 did not show any separation selectivity between naphthol isomers. It is confirmed 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.

CONCLUSIONS

The directly loaded β-CD on glass beads was unstable in polar mobile phases but considerably stable in methyl alcohol mobile phase. Direct loading method has merits of simple synthetic procedures and high organic loading. Cross-linking agents such as 1,8-trichlorosilyloctane could stabilize the directly loaded organic. Both epichlorohydrin and trimethylchlorosilane could stabilize slightly the organic loaded by the direct method.

The β-CD directly bonded glass beads packed column could give good separation between naphthol isomers through the inclusion complex formation mechanism. However, the epoxy spacer β-CD glass beads or cross-linked β-CD directly bonded glass beads showed lower or negligible selectivities between naphthol isomers. These results can be inferred that β-CD pores blocked by spacer group or cross-linking agent were too narrow to make a inclusion complex with 2-naphthol isomer especially. 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 group.

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