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Effects of Cross-Linking and Spacer Groups on beta-Cyclodextrin Bonded Liquid Chromatographic Separation

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Abstract−Mesoporous glass beads and 5 μm silica particles were modified with beta-cyclodextrin (β-CD) by means of directly bonding, linking spacer group, and cross-linking agent. The selectivities of the β-CD modified silica particles were measured by simple column chromatography to separate a model mixture of 1- and 2-hydroxy-naphthalene (naphthols) and ortho, meta, and para-xylene. In the packed column chromatography experiments, two major controlling factors (inclusion complex formation effect and steric hindrance effect of the analytes) on the separations were observed. The elusion orders of the β-CD directly bonded glass beads were meta-, para-, ortho-xylene and 1-naphthol, 2-naphthol. The phenomena of β-CD pore blocking and narrowing by spacer groups and cross-linking agent were observed. The spacer group and cross-linking agent decreased inclusion complex formation of 2-naphthol.

Key words: Beta-cyclodextrin, Chromatographic Separation, Inclusion Complex Formation, Cross-linking, Spacer Group

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligopolysaccharides containing from 6 to 13 glucose units bonded through 1,4-likages. In particular, α-CD, β-CD and γ-CD industrially produced have the ability to form inclusion complexes with many substances. β-CD contains 7 glucose units and has a central hydrophobic cavity and a hydrophilic outer shell [Szejtli, 1998]. The most unique character of β-CD is its ability to include selectively various organic and inorganic molecules within hydrophobic cavities. Thus, CDs are good candidates as highly selective bonded phase materials for chromatographic separation.

Even with the difficulty of immobilizing large size cyclodextrin to inorganic surface, Armstrong et al. were successful in immobilization of CD to silica gel by means of a specific silane linkage [Armstrong and DeMond, 1984; Armstrong, 1985; Armstrong et al., 1985]. Also, a porous tubular ceramic membrane was impregnated with a β-CD to obtain a chiral-selective membrane [Krieg et al., 2000]. Although a variety of linking spacers and synthesis procedures are well known, the effects of spacer groups between CDs and inorganic supports on the formation of inclusion complexes were rarely known. Armstrong noted about the length of the linkage chains between silica gel and CDs. With chains as short as three carbon atoms in the silane, coupling CD is more difficult to accomplish because of the relative size of the CD. With a chain as long as eighteen to twenty carbon atoms there is a danger of losing resolution in the separation process [Armstrong et al., 1985]. Armstrong also described that end-capping the residual silica gel surface hydroxyls with reactive silanes such as trimethylchlorosilane can produce a more stable silica gel since the silanols on the surface of the silica are sites most susceptible to dissolution in high aqueous system [Armstrong, 1985]. The possibility of applying simulated moving bed chromatography for the separation of naphthol isomers was investigated by numerical method based on the single column chromatography experimental data and optimum operation conditions were recommended [Kim et al., 2001]. Recently, α-CD bonded phase showed good separation of p-xylene from m- and o-xylene by forming inclusion complex formation [Kim et al., 2002]. Also, directly loaded β-CD on glass beads showed better separation than the loaded β-CD with spacer group and stability of loaded phase in the stream of methanol moving phase [Kim et al., 2003].

In this study another possible reaction between end-capping agents, trimethylchlorosilane and hydroxyl groups will be presented through a simple chromatographic separation based on inclusion complex formation. Similarly, the effects of spacer groups between CD and solid surface, and cross-linking agents such as 1,8-trichlorosilyloctane on the formation of inclusion complexes are studied. The separation efficiency of the CD-modified silica particles was tested by using column chromatography to separate a model mixture of naphthalene isomers, 1- and 2-hydroxy-naphthalene (naphthols) and o-, m-, and p-xylenes.

EXPERIMENTAL SECTION

1. Materials

Silica (particle size 5 μm, pore size 8.0 nm, and surface area 200 m^2/g, Altech) and glass beads (particle size 50-100 μm, pore size 31.6 nm and surface area 97 m^2/g, Trisoperl, Schuller) were used as supports. Beta-CD (Wacker Biochem) was attached to the supports by a direct bonding method, using epoxy spacers, and cross-linking agent. 3-Glycidoxypropyl-trimethoxysilane (Aldrich) was used as a spacer group. 1,8-Trichlorosilyloctane (Petrarch Systems) and epichlorohydrin (Aldrich) were used as cross-linking agents. HPLC grade of dimethylformamide (Aldrich), toluene (Aldrich), methanol (Aldrich), de-ionized water were used as solvents and wash-

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‡This paper is dedicated to Professor Hyun-Ku Rhee on the occasion of his retirement from Seoul National University.

ing agents. A 10mL burette (diameter 0.55 cm, Pyrex Brand, Fisher) was used as a chromatography column. The bed height was 37 cm and the free volume percentage of the packed β-CD bonded glass beads was 53%. The flow rate was 0.25 mL/min.

2. Methods

Epoxy spacer cyclodextrin bonded glass beads were prepared according to the method described [Armstrong, 1985]. The amounts of organic loaded on the supports were estimated by using a thermal gravimetric analyzer (TGA, Seiko) and a linear heating rate of 10 K/min from room temperature to 873 K. In the direct bonding method, 350 mL of dried dimethylformamide, 25 g of dried β-CD salt, and 25 g of dried glass beads or 10 g of silica were kept at 144 °C for 3 hr with stirring. The resulting slurry was put aside for a few minutes in order to allow the β-CD bonded-glass beads/silica to be settled. The upper portion of the slurry was removed and the modified glass beads/silica were filtered with a filter paper, and washed with toluene and methyl alcohol. The β-CD bonded-glass beads/silica were dried in a vacuum oven at 80 °C overnight. A typical β-CD weight loading on the glass beads and silica was 15.6% and 23.8%, respectively.

In order to stabilize the directly bonded cyclodextrin on the surfaces, Twenty grams of directly bonded β-CD glass beads and 60 mL of saturated β-CD salt solution was cross-linked with 1.5 g of epichlorohydrin (or 2.5 g of 1,8-trichlorosilyloctane) in 120 mL of toluene. The mixture was maintained at 90 °C for 50 minutes with stirring. The resulting slurry was put aside for a few minutes. The upper portion of the slurry was removed and the modified glass beads/silica were filtered with a filter paper, and washed with toluene and methyl alcohol.

In order to measure the amounts of xylene isomers adsorbed on β-CD, silica, and glass beads, these adsorbents were cleaned in a flow of helium gas at 473 K for 1.5 hour. The adsorbents were cooled to room temperature 299 K in a flow of helium gas. The flowing gas was switched to helium containing an isomer of xylene vapor. The weight gains were measured with a balance (Cahn TG-131, Cahn Instruments, Inc.) for 5 hr.

Packing materials were packed into the column by slurry packing or dry powder packing. The column chromatographic experiments were carried out at room temperature (295 K). Xylene and naphthol isomers were tested as elute compounds. Pure toluene and methyl alcohol were used isocratic mobile phases. Eluting solvent compositions were analyzed with an HP GC-MS (model 5890/5970).

RESULTS AND DISCUSSION

1. Effects of Pore Sizes of Supports

In the packed column chromatography experiments, two major factors controlling the separations were observed. The first was the possibility of formation of inclusion complexes with the β-CD pores or the pore structures induced by linking between β-CD and supports. The other was the steric hindrance effect between the eluting compounds and the stationary phases. The steric hindrance factor was confirmed from the packed column chromatography tests with unmodified 5 μm silica particles having 8nm pores and unmodified 50-100 μm glass beads having 31.6 nm pores as well. Molecules having smaller cross sectional areas have less steric hindrances in the columns packed with unmodified silica or glass beads. The

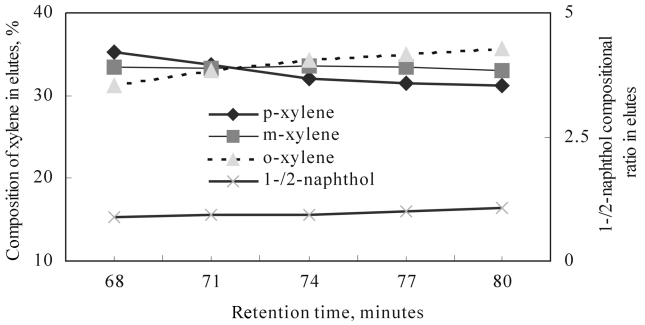

Fig. 1. The composition plots of eluting solutes from the column packed with unmodified glass beads with methanol mobile phase versus time (minutes).

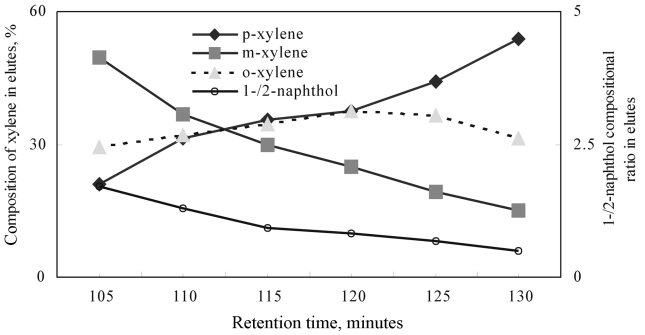

Fig. 2. The composition plots of eluting solutes from the column packed with directly loaded β-CD silica with methanol mobile phase versus time (minute).

elution orders from unmodified packing were generally para-, meta-, ortho-xylene among xylene isomers and 2-naphthol, 1-naphthol between naphthol isomers as shown in Fig. 1. However, the column packed with the directly bonded β-CD silica (its loading 23.8%) has a different elution order meta-, ortho-, para-xylene among xylene isomers, and 1-naphthol, 2-naphthol between naphthol isomers as shown in Fig. 2. These results suggest that p-xylene forms strong inclusion complexes with the β-CD pore; as a result, p-xylene has the longest retention period among xylene isomers. The separation between m- and o-xylene is determined by differences in the capability of inclusion complex formation and in the steric hindrance effect of two isomers as well. Adsorption capacities of the β-CD and unmodified silica for the vapor of p-, m-, and o-xylene in the stream of helium are 1.49%, 0.63%, 0.83% and 9.42%, 6.73%, 9.21%, respectively.

When the β-CD directly bonded glass beads (its loading 15.6%) were used, the xylene elution order was m-, p-, o-xylene as shown in Fig. 3. This result is interpreted as that the portion of larger pores of the β-CD bonded glass beads bed is much larger than that of the β-CD bonded silica bed because of the larger pores (31.6 nm) and particles sizes (5-100 μm) of the glass beads. Thus, o-xylene is more strongly bound rather than p-xylene to the β-CD bonded glass beads. This trend was confirmed in the xylene adsorption experiment to the glass beads. The adsorption capacities of the glass beads for the vapor of p-, m-, and o-xylene in the stream of helium are 3.30%, 3.07%, and 3.59%, respectively. It is known that separation selectivity is determined by bonded phase and support material as well.

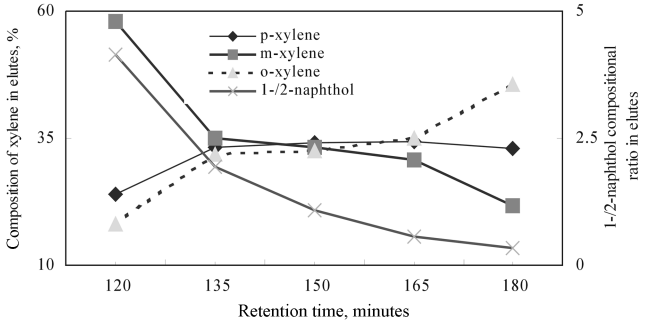

Fig. 3. The composition plots of eluting solutes from the packed with directly loaded β-CD glass beads with methanol mobile phase versus time (min).

The separation selectivity of the β-CD bonded glass beads between naphthol isomers is much larger than that of β-CD bonded silica as shown in Fig. 2 and Fig. 3. The moving velocity of the naphthol isomers through the β-CD bonded silica column was at least three times lower than that through β-CD bonded glass beads column. It is supposed that the molecular size of naphthol is too big to go through the pore structure of the β-CD bonded silica bed. Thus, the difference in retention periods between 2-naphthol forming inclusion complexes and 1-naphthol by-passed is diminished.

2. Effect of Spacer Group

The β-CD directly bonded silica has a strong binding force for p-xylene. From the column chromatography data, we speculate that the pore openings of the cyclodextrin host can be narrowed or blocked by spacer group, 3-glycidoxypropyltrimethoxysilane. The spacer β-CD silica (its loading 10.7%) has a greater affinity for o-xylene rather than p-xylene possibly due to weaker inclusion complex formation by pore narrowing and pore blocking, which could be offset by a greater steric hindrance effect from the spacer groups as shown in Fig. 4. In addition, steric hindrance of o-xylene was increased by the spacer groups between the silica surface and β-CD. When glass beads were used as a support, the spacer group effect was negligible in the separation of xylene isomers between the β-CD directly bonded glass beads and the spacer β-CD glass beads because of the large pores of the glass beads. However, a large difference in the separation of naphthol isomers was observed between the β-CD directly bonded glass beads and spacer β-CD glass beads.

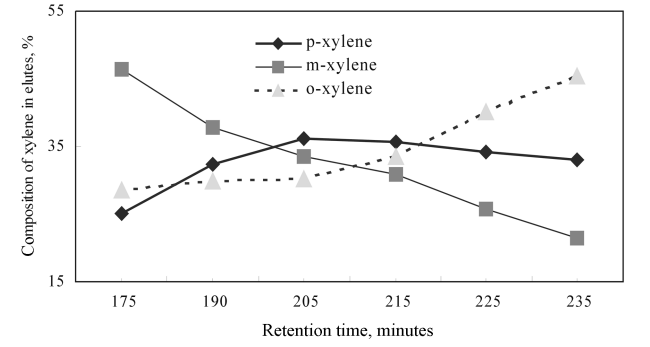

Fig. 4. The composition plots of eluting solutes from the column packed with 3-glycidoxypropyltrimethoxysilane linked β-CD silica with methanol mobile phase versus time (min).

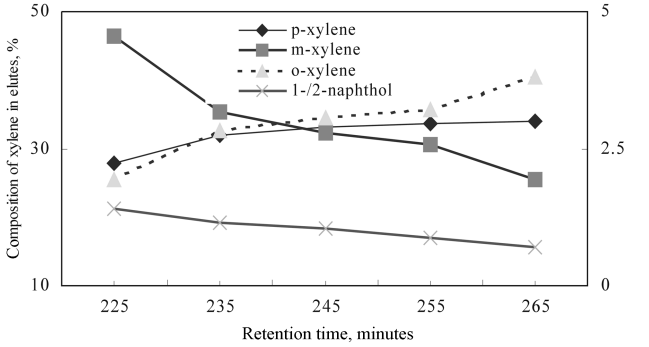

Fig. 5. The composition plots of eluting solutes from the column packed with 3-glycidoxypropyltrimethoxysilane linked β-CD glass beads with methanol mobile phase versus time (min).

The β-CD directly bonded glass beads having a strong binding force for 2-naphthol due to inclusion complex formation mechanism. In comparison, the inclusion complex formation of 2-naphthol was lowered because of increasing steric hindrance contribution as shown in Fig. 5. Thus, selectivity of the spacer β-CD glass beads for naphthol isomers was decreased.

3. Effect of Cross Linking

The β-CD direct bonded glass bead packing gives a good selectivity among xylene isomers (elution order m-, p-, o-xylene) and a high selectivity to naphthol isomers (elution order 1-naphthol, 2-naphthol) as shown in Fig. 3. These results show inclusion complex formations of 2-naphthol with the β-CD pores. By comparison, the cross-linked β-CD directly bonded glass beads by 1,8-trichlorosilyloctane lose the selectivity between naphthol isomers due to pore narrowing and blocking by the cross-linking agent. In contrast, the selectivity between xylene isomers is clearly increased by cross-linking maintaining the same elution order (m-, p-, o-xylene) as shown in Fig. 6. In particular, the binding force for o-xylene is clearly increased and the one for p-xylene is slightly decreased. A possible explanation for this result is that cross-linking narrows the β-CD pore openings; thus, inclusion complex formation of p-xylene is limited. Conversely, large pores induced by cross-linking agent among β-CDs and glass beads give a better binding force for o-xylene. The cross-linked β-CD direct bonded glass beads by epichlo-

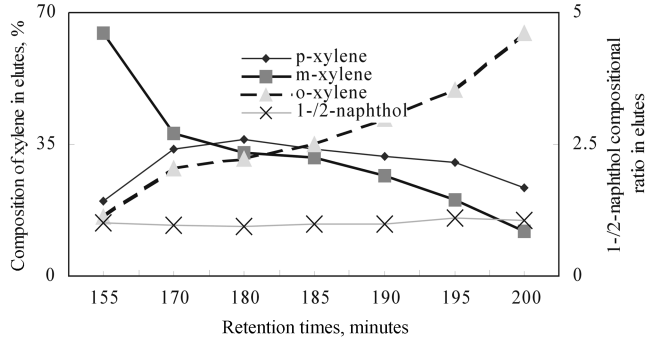

Fig. 6. The composition plots of eluting solutes from the column packed with β-CD glass beads cross-linked by 1,8-trichlorosilyloctane with methanol mobile phase versus time (min).

rohydrin lose the selectivities between naphthol isomers and among xylene isomers. This result means that a three atom chain is too short to cross-link between β-CDs, and the pore opening of β-CD is narrowed or blocked by the reaction of epichlorohydrin with hydroxyl groups of β-CD in an alkaline medium.

CONCLUSIONS

In the packed column chromatography experiments, two major controlling factors on the separations were observed. The first was the possibility of formation of inclusion complexes with β-CD pores or the pore structures induced by linking between β-CD and supports, and the other observed from the unmodified silica or glass beads beds was the steric hindrance effect between eluting molecules and the stationary phases. Where a molecule having smaller cross sectional area has less steric hindrance in the packed column (elution orders: p-, m-, o-xylene, 2-naphthol, 1-naphthol).

The β-CD bonded silica showed the same elution order of m-, o-, p-xylene as the xylene adsorption capacity order, and 1-naphthol, 2-naphthol. This trend can be interpreted as that the β-CD bonded silica has strong binding forces to p-xylene and 2-naphthol due to strong inclusion complex formation with β-CD pores. The separation between m- and o-xylene is determined by the degree of inclusion complex formation and steric hindrance differences. However, a different elution order (m-, p-, o-xylene) resulted from the column packed with the β-CD bonded glass beads due to the large pore and particle sizes of glass beads. It is known that separation selectivity is determined by bonded phase and support material as well. The separation selectivity of the β-CD bonded glass beads between naphthol isomers was much larger than that of β-CD bonded silica.

The column packed with the 3-glycidoxypropyltrimethoxysilane spacer linked β-CD silica showed a different elution order (m-, p-, o-xylene) due to pore narrowing and pore blocking by the spacer group and the large pores induced by spacer groups and the surface of silica. Thus, the spacer group decreased the binding force to p-xylene and increased the binding force to o-xylene. Similarly, pore narrowing and pore blocking by the spacer group decreased the degree of inclusion complex formation of 2-naphthol when the spacer β-CD glass beads were used.

The cross-linked β-CD directly bonded glass beads by 1,8-trichlorosilyloctane lose selectivity between naphthol isomers due to pore narrowing and blocking by the cross-linking agent. In contrast, the selectivity between xylene isomers is clearly increased by cross-linking maintaining the same elution order (m-, p-, o-xylene). Cross-linking narrows the β-CD pore openings, thus, inclusion complex formation of p-xylene is limited. Conversely, large pores induced by cross-linking agent among β-CDs and glass beads give a better binding force for o-xylene.

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