

NOVEL HPLC ADSORBENTS BY IMMOBILIZATION OF MODIFIED CYCLODEXTRINS

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1. INTRODUCTION

Cyclodextrin(CD) has been successfully utilized in HPLC for the specific interaction between aromatic compounds and the hydrophobic cavity of the CD molecule.(Ref.1-3) In our present work(Ref.4-5), a novel packing material for HPLC was prepared with 6-deoxy-monoamino- β -CD immobilized through the epoxy glyceride group on hydrophilic gel beads. The capacity factors of the guest molecules having an aromatic ring together with a carboxylic group were increased by the interaction of the hydrophobic effect and the ionic effect. The results suggest "host-guest chromatography" with multi-point molecular recognition.

2. EXPERIMENTAL

2.1 Preparation of bead gels

β -CD was immobilized on a matrix of gel beads. The bead gel(PW) was treated with epichlorohydrin in an alkaline solution at 45°C for 4 h to modify the active epoxy group on the primary hydroxyl group of CD. Consequently, the product was treated with β -cyclodextrin in an alkaline solution of pH 12 at 37°C for 24 hr to give the β -CD immobilized bead gel(CD-PW).

Determination of the epoxy content of the gel was determined by titration methods. The result indicated that there was the introduction of an active epoxy group of 620 μ mole/(1 g dry gel). Also, the amount of immobilized β -CD on CD-PW was determined by HPLC analysis of the glucose produced by the hydrolysis with the acid. It showed that 540 μ mole/(1 g dry gel) of β -CD was immobilized in this preparation.

The immobilization of amino- β -CD and amino-methylated- β -CD on PW gel was carried out. Amino-CD gel (ACD-PW) was obtained by the procedure with the epoxy bead gel and 6-monoazide- β -CD which was prepared by regiospecific tosylation and substitution by sodium azide. The obtained monoazide- β -CD gel was treated with triphenylphosphin in DMF, then with ammonium hydroxide which gave amino- β -CD gel(ACD-PW). Amino-methyl- β -CD gel(A-Me-CD-PW) was prepared starting with the 6-monoazide- β -CD gel. The gel was methylated with dimethylsulfate in the mixed solvent of chloroform and an aqueous sodium hydroxide solution with shaking at room temperature for 72 hrs. Monoazide-methyl- β -CD gel

was treated with triphenylphosphine in DMF, then with ammonium hydroxide which gave A-Me-CD-PW. The amino group content of both resulting gels was determined by neutral titration with a pH stat apparatus. There were amino groups of 530 and 230 μmole per 1 g dried ACD-PW and A-Me-CD-PW, respectively.

2.2 Column Chromatography

The above obtained bead gels were packed by the slurry packing method in a stainless column of 4.0 mm inner diameter and 300 mm length. In the experiment of pH dependency, the eluent was usually a mixture of 10 v/v% acetonitrile and 90 v/v% 1/40 M-phosphate buffer between pH 4-7 or 90 v/v% 1/40 M carbonate buffer between pH 9-11.5. Flow rate was approximately 0.5-0.7 ml/min. In the experiment for the effect of organic solvent, the eluent was a mixture of x v/v% acetonitrile and (100-x) v/v% 1/40 M-phosphate buffer of pH 7.0 at the flow rate of 0.6-0.8 ml/min.

3. RESULT AND DISCUSSION

3.1 Introduction of amino-CD on the gel beads

As the PW gel polymer has hydroxyl groups on the side chain, CD units were able to be introduced by the treatment of the gel with epichlorohydrin using active epoxy groups on the gel surface. It contained 620 $\mu\text{mole}/(1\text{ g dry gel})$. More than 87% of these groups were reacted with CD to prepare CD-PW gel beads. This report is the first for a HPLC adsorbent which contains a CD cavity on the synthetic polymer. In order to enforce the capacity factor, two kinds of modification of CD molecules were carried out. To attach the amino group on CD, the usual preparation methods by way of tosylation and azidification were adopted. This "pre-procedure method" gave a sufficient amount of amino groups on the CD-PW gel of 530 $\mu\text{mole}/(1\text{ g dry gel})$. Another improvement of this HPLC adsorbent was methylation of the hydroxyl group on CD molecules. With the addition of CHCl_3 , dimethylsulfate and an aqueous alkaline solution, the methylation, at least on the C-6 hydroxyl group, was easily performed, though there was a 57% decrease of amino groups through this procedure. Both ACD-PW and A-Me-CD-PW gel beads were new types of HPLC adsorbents. These gel beads can be used at high speed flow in a wide range of pH for the separation of various aromatic acids such as mandelic acid and N-protected phenylalanine.

3.2 pH Dependence of Capacity Factors

These four types of adsorbents, i.e., PW, CD-PW, A-CD-PW and A-Me-CD-PW were compared with capacity factors $k' (=t_R/t_0 - 1)$ towards the various guest molecules by changing the pH of the eluent in order to elucidate the interaction between the amino group on CD and the carboxylic group on the substrates. There was no retention for Asp, and only by A-Me-CD-PW was there a slight retention in the eluent of the alkaline region. In the case of methyl mandelate (Me-M), the PW gel showed no retention. For the gels of ACD-PW and A-Me-CD-PW, there was a tendency of increased retention in the alkaline region. In the case of the Phe substrate, PW and CD-PW guests did not show any retention, but ACD-PW gel showed retention factors up to 1.0 in alkaline solution.

Mandelic acid (MA) was not thoroughly retained by PW gel, but a

slight retention by the CD-PW gel was indicated. In the case of ACD-PW gel, the capacity factor decreased at pHs of 3.6 and 10.1, near the pKa values of MA and ACD-PW. This result indicated an ion-exchange type of retention behavior and a strong electrostatic interaction between the amino group and the carboxylic group which was not the case for the Asp substrate. A-Me-CD-PW gel then showed an extraordinary high capacity factor especially at acidic regions. The enhanced hydrophobic interaction by the methylation at the hem of CD ring was mainly at the C-6 positions of the glucose residue. Another substrate, benzyloxycarbonyl(Z)-Ala, is similar in behavior to MA. The k' values for ACD-PW and A-Me-CD-PW was much higher up to 150 at acidic regions.

3.3 Dependence of Capacity Factor on Organic Eluents

In order to clarify the interaction between the hydrophobic CD ring and each guest molecule, the concentration of organic solvent was changed and the dependency of the retention behavior was examined. In these experimental runs, an aqueous buffer of pH 7 was mixed with the organic solvents. The concentration of acetonitrile was changed in the range of 0 to 30 v/v%, and the capacity factors for the guest molecules of Me-M, Phe and Asp were examined.

For the guest molecules Me-M, three CD-immobilized gels, CD-PW, ACD-PW, and A-Me-CD-PW, showed a slight retention change from 0 to 3.5 depending 30 to 0 v/v% of acetonitrile content, although the matrix gel PW showed no change. This change of k' seemed to reflect the change of merely hydrophobic interaction caused by the CD ring. Asp, which has no aromatic moiety and is not expected to interact with the CD cavity, showed no retention change for all the gel adsorbents. This suggests that the presence of a phenyl ring was the key point for the interaction with CD's hydrophobic cavity.

In the case of the MA substrate, the organic solvent effect on the capacity factor was observed with a CD-PW gel compared with a PW gel which showed no effects. This indicated that MA effectively interacted with CD's hydrophobic cavity. With ACD-PW gel, larger changes of capacity factor were observed. Also, the result suggested an interaction between the CD cavity and the guest. The extraordinary large effect was observed for a A-Me-CD-PW gel which was obtained by the methylation of the β -CD ring, and CD cavity was improved to the more hydrophobic circumstance. These results supported the idea that the hydrophobic cavity involved the retention behavior and also the amino group increased the retention behavior.

In the case of Z-Ala, for a PW gel, there was no change in retention. For CD-PW and ACD gels, a small change was observed. For the ACD-PW gel, a larger change in retention than for the CD-PW gel was observed. The A-Me-CD-PW gel had the highest capacity factor and was the most sensitive to the change of organic content.

From the above solvent effect on the retention capacity, it is presumed that the hydrophobic cavity of CD evidently interacted with guest molecules.

3.4 N-Substitution Effect of the Guest Molecules

Aromatic amino acids showed low capacity factors because of ionic repulsive force between the amino moiety of amino acid such as Phe, Tyr and dihydroxyphenylalanine, the inclusion effect was not observed. But

N-substituted amino acids showed high capacity factors; N-acetylated Phe and N-formylated Phe gave 6 and 10 times higher capacity factors than Phe in ACD-PW respectively, and 30 and 50 times higher in A-Me-CD-PW. These N-substitution effects on retention behavior were also observed for a nonaromatic amino acid such as Ala. The capacity factors of N-substituted amino acids depended on the ionic strength of eluent. The dependency was observed clearly in A-Me-CD-PW. Methylation of hydroxyl groups on the CD ring enhanced the interaction with amino acids.

The inclusion effect was observed clearly in A-Me-CD-PW gel. The aromatic guests such as Z-Phe and Z-Ala showed high capacity factors than those in ACD-PW.

4. Conclusion

According to the CPK scale molecular model, an amino group is embedded inside the CD ring. So it may be presumed that the ionic interaction between carboxylic group and amino group should be difficult from the outside of the CD ring. Thus, it is necessary that the guest molecule is included into the cavity by hydrophobic interaction, then the included molecule can be held tightly by both ionic and hydrophobic interaction.

All these observations supported the operational interaction in the present packings which were prepared by immobilization of CD derivatives on the hydrophilic polymer gel. Especially in the case of Z-Ala and MA, as they have both phenyl and carboxylic groups, a stable complex can be formed by the double forces through the hydrophobic and ionic interactions. This caused the high retention power. It may be concluded that the present amino-CD gel and amino-methyl-CD gel indicated both hydrophobic and ionic interactions, and it showed novel and unique adsorbent properties for practical HPLC use.

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