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NOVEL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ADSORBENTS PREPARED BY IMMOBILIZATION OF MODIFIED CYCLODEXTRINS

KENJIRO HATTORI*, KEIKO TAKAHASHI and MASAHITO MIKAMI
Department of Industrial Chemistry, Faculty of Engineering, Tokyo Institute of Polytechnics, Atsugi, Kanagawa 243-02 (Japan)
and
HIDEO WATANABE
Tokyo Research Laboratories, Toyo Soda Mfg. Co., Ltd., Hayakawa, Ayase, Kanagawa 252 (Japan)
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SUMMARY

6-Deoxyamino-β-cyclodextrin has been immobilized through its epoxy glyceride group on hydrophilic gel beads. The retention behaviour of some aromatic and ionic compounds on the resulting gels was studied. The capacity factors of compounds such as mandelic acid and N-benzyloxycarbonylalanine having an aromatic ring and a carboxylic group were increased up to values of 150 by virtue of the hydrophobic and ionic interactions between the aminocyclodextrin moiety and the guest molecules in buffer solution of pH 4–12. Compounds having only an aromatic ring showed moderate capacity factors between 0 and 10, and those having only a carboxylic acid, such as DL-aspartic acid, showed low capacity factors between 0 and 2. There was no difference in the retention behaviour of the D- and L-forms of the substrates. The effects of the pH and the organic solvent content in the mobile phase were examined. The results suggest the occurrence of "host–guest chromatography" with multipoint recognition such as ionic interaction and inclusion complex formation.

INTRODUCTION

The specific interaction between aromatic compounds and the hydrophobic cavity of the cyclodextrin (CD) molecule has been successfully utilized in high-performance liquid chromatography (HPLC)[1–6]. Several polyurethane resins were prepared by cross-linking of epichlorohydrin[7] and of diisocyanates[8]. Another method of CD immobilization was through the use of matrix gels such as silica or polymer gels[9–12], CD has also been used in the mobile phase to separate mandelic acid isomers[13,14].

In our present work, a novel packing material for HPLC was prepared by immobilizing 6-deoxymonoamino-β-CD through its epoxy glyceride group on hy-

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drophilic gel beads. The capacity factors of guest molecules having an aromatic ring together with a carboxylic group were increased by a combination of hydropohobic and ionic effects. The results suggest the occurrence of "host–guest chromatography" with multipoint molecular recognition.

EXPERIMENTAL

Materials

β-CD was a gift from Nihon Shokuhin Kako. The hydrophilic gel beads G3000PW (PW) were supplied by Toyo Soda. Substrates including mandelic acids, methyl mandelates and amino acids were commercial grade used without any purification. N-Benzyloxycarbonylalanine was prepared by the usual methods. All the substrates were in their racemic forms, except for the determination of the association constant in Fig. 8.

Apparatus

The chromatography was accomplished on a Toyo Soda HLC-802 chromatograph equipped with an UV detector (Toyo Soda UV-8). The measurement of the association constant was carried out with an UV spectrometer Jasco Uvidec-1 equipped with a thermostat to maintain the cell compartment at 27.0 ± 0.5°C.

Preparation of bead gels

β-CD was immobilized on a matrix of gel beads as shown in Fig. 1. The bead gel (PW) was treated with epichlorohydrin in an alkaline solution at 45°C for 4 h to modify its active primary hydroxyl groups, as described[15]. The product (epoxy-PW) was then treated with β-cyclodextrin in an alkaline solution, pH 12, at 37°C for 24 h to give β-CD immobilized on the bead gel (CD-PW)[16].

The epoxy content of epoxy-PW was determined by titration methods[17]. After reaction with 1.3 *M* sodium thiosulphate, a titration was carried out using 1/10 *M* hydrochloric acid solution. The result indicated that 620 μmol epoxy group per 1 g dry gel had been introduced. Also, the amount of immobilized β-CD was determined

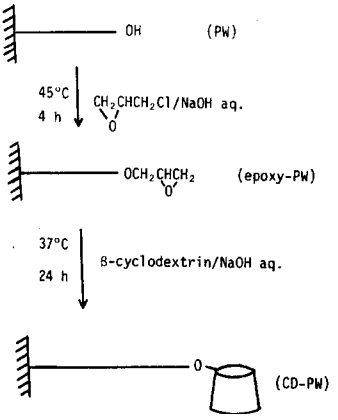


Fig. 1. Preparation of epoxy-PW and CD-PW gels.

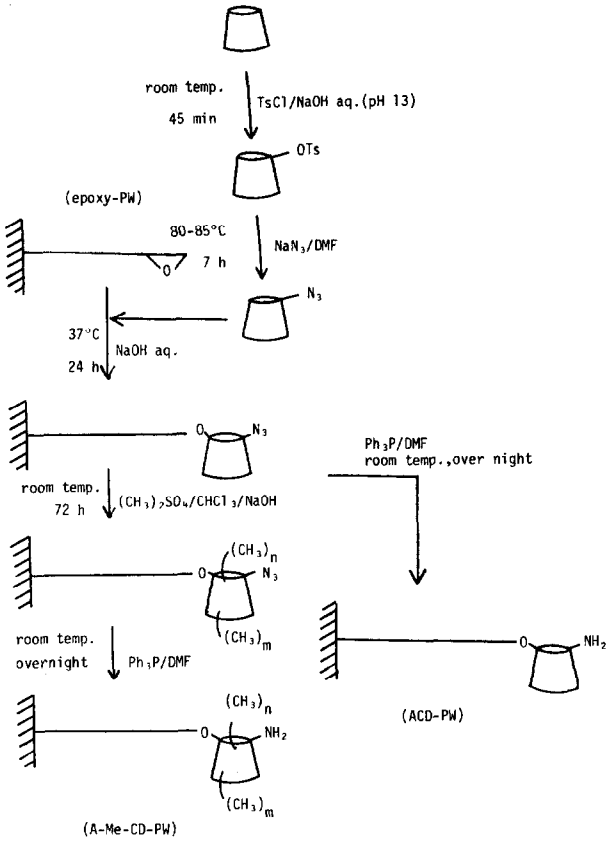


Fig. 2. Preparation of ACD-PW and A-Me-CD-PW gels.

by HPLC analysis of the glucose produced upon hydrolysis with hydrochloric acid. It was shown that 540 μmol of β-CD per 1 g dry gel were immobilized in this preparation.

The immobilization of amino-β-CD and amino-methylated-β-CD on PW gel was carried out as summarized in Fig. 2. The amino-CD gel (ACD-PW) was obtained from epoxy-PW and 6-monoazido-β-CD; the latter was prepared by regiospecific tosylation[18] and substitution by sodium azide[19]. The monoazido-β-CD gel thus obtained was treated with triphenylphosphine in dimethylformamide (DMF), then with ammonium hydroxide to give the desired product. Aminomethyl-β-CD gel (A-Me-CD-PW) was prepared from the 6-monoazido-β-CD gel. This gel was methylated with dimethyl sulphate in chloroform–aqueous sodium hydroxide solution with shaking at room temperature for 72 h. The resulting monoazido-methyl-β-CD gel was treated with triphenylphosphine in DMF, then with ammonium hydroxide to give the desired product. The amino content of the two gels was determined by titration with a pH-stat apparatus as 530 and 230 μmol per 1 g of dried ACD-PW and A-Me-CD-PW, respectively.

Column chromatography

The obtained bead gels were packed by the slurry method in a stainless-steel

column (300 × 4.0 mm I.D.). In the experiments of different pH values, the eluent was usually a mixture of 10% (v/v) acetonitrile and 90% (v/v) 1/40 *M* phosphate buffer between pH 4 and 7 or 90% (v/v) 1/40 *M* carbonate buffer between pH 9 and 11.5. The flow-rate was approximately 0.5–0.7 ml/min. In the experiments on the effect of the organic solvent, the eluent was a mixture of *x*% (v/v) acetonitrile in 1/40 *M* phosphate buffer, pH 7.0, at a flow-rate of 0.6–0.8 ml/min.

Determination of association constant

6-monoamino-β-CD was prepared according to the literature[20] and recrystallized twice. Its elemental analysis, titration of amino group content and ^{13}C NMR spectrum were in accorded with the expected structure.

Benesi–Hildebrand plots were used to determine the binding constant between D- or L-mandelic acids and β-CD or 6-amino-β-CD at 27.0°C. The concentration of mandelic acid was kept constant at $6.00 \cdot 10^{-5}$ *M*. The concentration of β-CD was varied between $1.67 \cdot 10^{-3}$ and $1.00 \cdot 10^{-2}$ *M*. The solutions used were as follows: 1/10 *M* acetate buffer at pH 4.0; 1/15 *M* phosphate buffer at pH 7.0; 1/20 *M* sodium tetraborate–hydrochloric acid buffer in the range pH 8.0–9.2; 1/20 *M* sodium tetraborate–sodium hydroxide buffer in the range pH 9.5–11.2.

RESULTS AND DISCUSSION

Introduction of ACD on the gel beads

As the PW gel polymer has hydroxyl groups in its side chain[21–23]. CD units could be introduced after treatment of the gel with epichlorohydrin, as shown in Fig. 1. More than 87% of the resulting epoxy groups reacted with CD to give the CD-PW gel beads. This is the first report of an HPLC adsorbent which contains a CD cavity on a synthetic polymer.

In order to influence the capacity factor, two kinds of modifications of CD molecules were carried out. An amino group was attached to CD by the usual methods of tosylation and azidification as shown in Fig. 2. This "pre-procedure method" gave a sufficient amount of amino groups on the CD-PW gel. Another improvement was achieved by methylation of the hydroxyl group on the CD molecules. By the addition of chloroform, dimethyl sulphate and an aqueous alkaline solution, the methylation, at least on the C-6 hydroxyl group, was easily performed, though there was a 57% decrease in the amino groups.

The resulting ACD-PW and A-Me-CD-PW gel beads are new types of HPLC adsorbents. They can be used at high flow-rates over a wide range of pH for the separation of various aromatic acids such as mandelic acid and N-protected phenylalanine, as will be shown later.

For all of the chiral substrates used in the present experiments, there was no difference in the retention behaviour between the D- and L-forms.

pH dependence of capacity factors

The four types of adsorbents, *i.e.*, PW, CD-PW, A-CD-PW and A-Me-CD-PW, were compared with respect to the capacity factors, k' ($= t_R/t_0 - 1$), of various guest molecules. The pH of the eluent was varied in order to elucidate the interaction between the amino group of CD and the carboxylic group of the substrates.

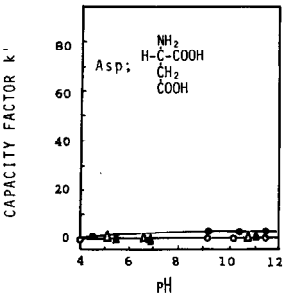


Fig. 3. pH Dependence of the capacity factor, k', of DL-aspartic acid on PW (△), PW, ACD-PW and A-Me-CD-PW with CD-PW (▲), ACD-PW (○) and A-Me-CD-PW (●) gels. Column: 300 × 4.0 mm I.D. Eluent: pH 4–7, acetonitrile–1/40 *M* phosphate buffer (10:90); pH 9–11, acetonitrile–1/40 *M* carbonate buffer (10:90); flow-rate 0.5–0.7 ml/min.

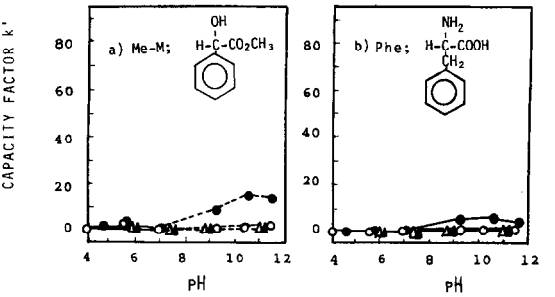


Fig. 4. pH Dependence of the capacity factor, k', of methyl DL-mandelate (a) and DL-phenylalanine (b) on PW (△), CD-PW (▲), ACD-PW (○) and A-Me-CD-PW (●). Other details as in Fig. 3.

The first guest molecule was DL-aspartic acid (Asp). In Fig. 3 the pH dependence of the capacity factor, k', is shown. At neutral pH, this anionic substrate was expected to show an interaction with the positively charged amino group on the CD ring and to exhibit a larger retention volume; however, this was not the case. In the case of PW, CD-PW and ACD-PW, $k' = 0$ at all pH values. Asp was not retained except weakly by A-Me-CD-PW in alkaline solution.

Methyl DL-mandelate (Me-M) and DL-phenylalanine (Phe), which are electrostatically neutral but have a phenyl group, were then examined. The results are shown in Fig. 4. Me-M was not retained on the PW gel. On ACD-PW and A-Me-CD-PW, there was increased retention at alkaline pH, but at strongly alkaline pH the Me-M was probably hydrolyzed to mandelic acid, as will be shown later. Phe was not retained on PW and CD-PW, but on ACD-PW gel showed retention factors of up to 1.0 in alkaline solution. At pH >9.2, which is the pK_a of the guest molecule, Phe became anionic and was able to interact electrostatically with the amino group in the gels. In addition to the ionic interaction, the A-Me-CD-PW column shows a stronger hydrophobic interaction because of the O-methylation of the CD ring. It exhibited a stronger interaction (k' up to 5.5) than the other gels.

The pH dependence of the capacity ratio of DL-mandelic acid (MA) and N-benzyloxycarbonyl-DL-alanine (Z-Ala), which have both anionic and phenyl groups, was examined (Fig. 5). MA was not retained at all by the PW gel, but was slightly retained by the CD-PW gel. On ACD-PW gel, the capacity factor decreased at pH 3.6 and 10.1, near the pK_a values of MA and ACW-PW. This result indicated an ion-exchange type of retention behaviour and a strong electrostatic interaction between the amino group and the carboxylic group, in contrast to the Asp substrate. The A-Me-CD-PW gel yielded extraordinarily high capacity factors especially at acidic pH. The enhanced hydrophobic interaction was observed by the methylation at the hem of CD ring.

Another substrate, Z-Ala, exhibits similar behaviour to that of MA. The k' values on ACD-PW and A-Me-CD-PW were much higher, up to 150 at acidic pH.

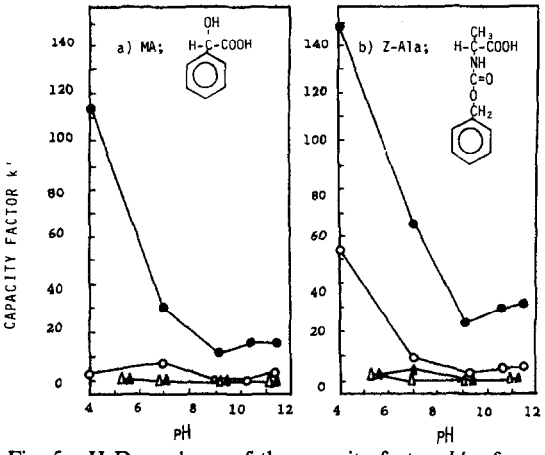


Fig. 5. pH Dependence of the capacity factor, k', of DL-mandelic acid (a) and benzyloxycarbonyl-DL-alanine (b) on PW (△), CD-PW (▲), ACD-PW (○) and A-Me-CD-PW (●). *Other details as in Fig. 3.*

Dependence of capacity factor on organic eluents

In order to clarify the interaction between the hydrophobic CD ring and each guest molecule, the effect of the concentration of the organic solvent on the retention behaviour was examined. In these experiments, an aqueous buffer of pH 7 was mixed with the organic solvents.

The concentration of acetonitrile was varied in the range 0–30% (v/v), and the capacity factors of Me-M, Phe and Asp were examined. The results are summarized in Fig. 6. On the gels CD-PW, ACD-PW and A-Me-CD-PW, Me-M (Fig. 6a) showed a slight change in k' from 0 to 3.5 upon changing the acetonitrile content from 30 to 0%, but on the gel PW there was no change. This change in k' seemed to reflect the change in hydrophobic interaction caused by the CD ring. However, in the case of the Phe substrate (Fig. 6b), there was no change in k' on all the gels. There may be a repulsion between the internal salt of Phe and the hydrophobic cavity of CD. Asp, which has no aromatic moiety and is not expected to interact with the CD cavity, also showed no change in k' on all the gels (Fig. 6c). This suggests that the presence of a phenyl ring is decisive for the interaction with the hydrophobic cavity of CD.

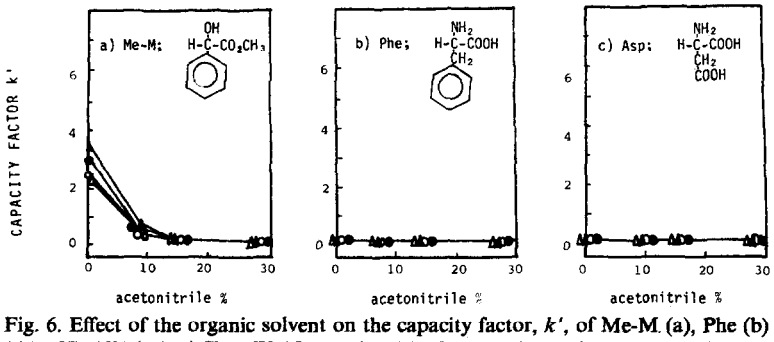


Fig. 6. Effect of the organic solvent on the capacity factor, k', of Me-M (a), Phe (b) and Asp (c) on PW (△), CD-PW (▲), ACD-PW (○), and A-Me-CD-PW (●) gels. Column: 300 × 4.0 mm I.D. Eluent: acetonitrile–1/40 *M* phosphate buffer pH 7.0 (x: 100 − x); flow rate 0.6–0.8 ml/min.

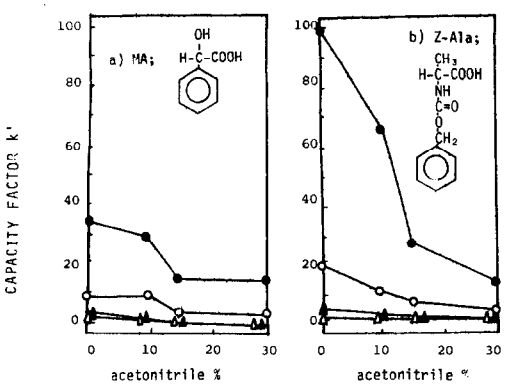


Fig. 7. Effect of the organic solvent on capacity factor, k' of MA (a) and Z-Ala (b) on PW (△), CD-PW (▲), ACD-PW (○) and A-Me-CD-PW (●) gels. Other details as in Fig. 6.

The effects of a mixed organic solvent in the eluent on the capacity factor k' of the guest molecules MA and Z-Ala were examined. The results are summarized in Fig. 7. In the case of MA (Fig. 7a), an effect on the capacity factor was observed on the CD-PW gel but not on the PW gel. This indicated that MA interacts with the hydrophobic cavity of CD. Similar changes in k' were found for Me-M and Phe. On ACD-PW gel, larger changes in capacity factor were observed, again suggesting an interaction between the CD cavity and the guest molecule. An extraordinarily large effect was observed on the A-Me-CD-PW gel, indicating the higher hydrophobicity of the CD cavity upon methylation.

In the case of Z-Ala (Fig. 7b), on PW gel, there was no change in retention. On CD-PW and ACD-PW gels, a small change was observed, being larger with the latter. The A-Me-CD-PW gel yielded the highest capacity factor and was the most sensitive to changes in the content of organic solvent.

From the above results it is presumed that the hydrophobic cavity of CD interacts with the guest molecules.

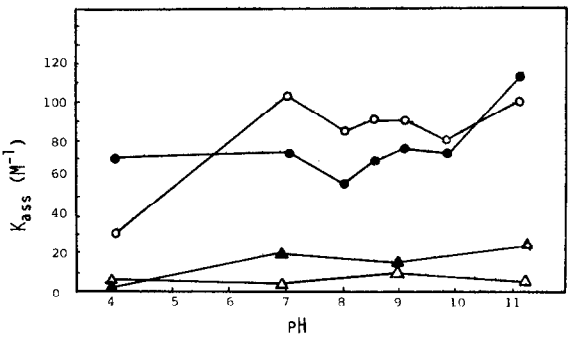
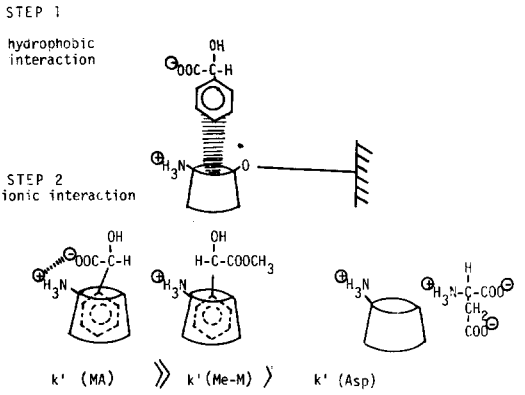


Fig. 8. pH Dependence of the association constannts, K_{ass}, of CD and ACD with D- and L-mandelic acid. [CD] = $1.67 \cdot 10^{-3}$–$1.00 \cdot 10^{-2}$ M, [ACD] = $1.33 \cdot 10^{-3}$ M, [D-, L-mandelic acid] = $6.00 \cdot 10^{-5}$ M; 27 ± 1°C. UV detection: pH 4.0, 225 nm; pH 7.0–11.4, 216 nm. ▲—▲, CD with D-mandelic acid; △—△, CD with L-mandelic acid; ●—●, ACD with D-mandelic acid; ○—○, ACD with L-mandelic acid. Solvents: pH 4.0, 1/10 M acetate buffer; pH 7.0, 1/15 M phosphate buffer; pH 8.0–9.2, 1/20 M sodium tetraborate–hydrochloric acid buffer; pH 9.5–11.2, 1/20 M sodium tetraborate–sodium hydroxide buffer.

In order further to elucidate this interaction, the equilibrium constants, K_{ass}, for binding of β-CD and amino-β-CD to D- and L-mandelic acids were measured in the range pH 4–11. The results (Fig. 8) yielded K_{ass} values at pH 7 of about 16 and 90 M^{-1} for binding of β-CD and of amino-β-CD, respectively, in accord with the capacity factors obtained on the immobilized CD gels.

According to the Pauling–Corey–Koltun steric molecular models, an amino group is embedded inside the CD ring. So it may be presumed that the ionic interaction between a carboxylic group outside the CD ring and this amino group is hindered. 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 as suggested[24].



Scheme 1. Suggested mechanism.

Scheme 1 illustrates this mechanism for the present packings. In step 1, the hydrophobic moiety of the guest molecule is included in the hydrophobic cavity of CD, forming a host–guest complex. In step 2, the carboxylic moiety participates in a stronger interaction with the amino group. Especially in the case of MA, which has both phenyl and carboxylic groups, a stable complex can be formed by the combined effect of the hydrophobic and ionic interactions, resulting in increased retention.

It may be concluded that the present amino-CD and aminomethyl-CD gels exhibit both hydrophobic and ionic interactions, making them novel adsorbents for HPLC.

REFERENCES

1 W. L. Hinze, *Sep. Purif. Methods*, 10 (1981) 159.
2 E. Smolková-Keulemansová, *J. Chromatogr.*, 251 (1982) 17.
3 D. W. Armstrong, W. DeMond and B. P. Czeck, *Anal. Chem.*, 57 (1985) 481.
4 W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, A. Alak and T. Wand, *Anal. Chem.*, 57 (1985) 237.
5 D. W. Armstrong, W. DeMand, A. Alak, W. L. Hinze, T. E. Riehl and K. H. Bui, *Anal. Chem.*, 57 (1985) 234.
6 D. W. Armstrong and W. DeMand, *J. Chromatogr. Sci.*, 22 (1984) 411.
7 N. Wiedenhof, J. N. J. J. Lammers and C. L. van Panthaleon van Eck, *Staerke*, 21 (1969) 119.

8 J. L. Hoffmann, *J. Macromol. Sci., Chem.*, A7 (1973) 1147.
9 A. Harada, M. Furue and S. Nozakura, *J. Polym. Sci., Polym. Chem. Ed.*, 16 (1978) 189.
10 K. G. Feitsma, B. F. H. Drenth and R. A. de Zeeuw, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10464 (1984) 147.
11 Y. Mizobuchi, M. Tanaka and T. Shono, *J. Chromatogr.*, 208 (1981) 35.
12 M. Tanaka, Y. Mizobuchi, T. Sonoda and T. Shono, *Anal. Lett.*, 14 (181) 281.
13 J. Dębowski, D. Sybilska and J. Jurcrzak, *J. Chromatogr.*, 237 (1982) 303.
14 Y. Nobuhara, S. Hirano and Y. Nakanishi, *J. Chromatogr.*, 258 (1983) 276.
15 I. Matsumoto, Y. Mizuno and N. Seno, *J. Biochem. (Tokyo)*, 85 (1979) 1091.
16 R. Uy and F. Wold, *Anal. Biochem.*, 81 (1977) 98.
17 L. Sundberg and J. Porath, *J. Chromatogr.*, 90 (1974) 87.
18 K. Takahashi, K. Hattori and F. Toda, *Tetrahedron Lett.*, 25 (1984) 3331.
19 J. Boger, D. G. Brenner and J. R. Knowles, *J. Am. Chem. Soc.*, 101 (1979) 7630.
20 J. Boger, R. Corcoran and J.-M. Lehn, *Helv. Chim. Acta*, 61 (1978) 2190.
21 T. Hashimoto, H. Sasaki, M. Aiura and Y. Kato, *J. Polym. Sci.*, 16 (1978) 1789.
22 Y. Kato, H. Sasaki, M. Aiura and T. Hashimoto, *J. Chromatogr.*, 153 (1978) 546.
23 T. Hashimoto, H. Sasaki, M. Aiura and Y. Kato, *J. Chromatogr.*, 160 (1978) 301.
24 Y. Matsui and A. Okimoto, *Bull. Chem. Soc. Jpn.*, 51 (1978) 3030.