TOTALLY ORGANIC STATIONARY PHASE FROM PERFLUOROALKYL ACRYLATE FOR LIQUID CHROMATOGRAPHY

CHUICHI HIRAYAMA*, HIROTAKA IHARA, SHOJI NAGAOKA and KAZUHIRO HAMADA

Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kumamoto 860 (Japan)

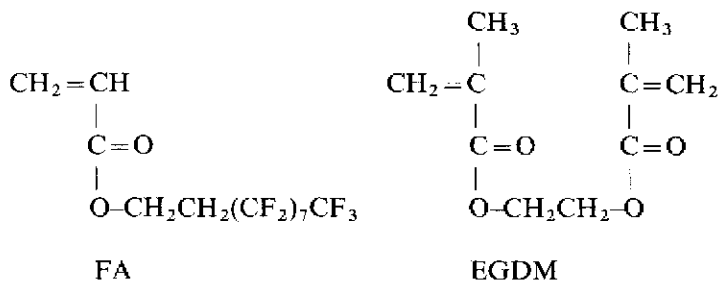(First received September 29th, 1988; revised manuscript received November 28th, 1988)

SUMMARY

Porous and fluorine-containing spherical beads for column packings in high-performance liquid chromatography were prepared by suspension copolymerization of heptadecafluorodecyl acrylate with divinyl monomers as a cross-linking agent. The fluorine content was easily adjustable by changing the monomer ratio. The packings show efficient separation for fluorine-containing compounds, *e.g.*, 5-fluorouracil and uracil, mixtures of fluorinated steroids, etc. In addition, they are highly acid- and alkali-resistant, show no abnormal adsorption for ionic compounds and no excessive retention for large hydrophobic compounds. Therefore, they are applicable also to the chromatographic separation of hydrocarbon substances. These favourable properties are related to the low surface energy based on the higher fluorine content.

INTRODUCTION

Recently, the synthesis of fluorinated stationary phases for liquid chromatography and their specific separation behaviours based on fluorine–fluorine interactions were reported. However, most fluorinated packings are of the silica-bonded type[1–7]. Therefore, these packings have some defects due to the matrix materials.

We have succeeded in making totally organic packings by copolymerization of a perfluoroalkyl monomer (FA). Although modified poly(chlorotrifluoroethylene) is known[8–15], our packings have many advantages: (1) they are made by one-step copolymerization, (2) they are spherical and uniform in size because of suspension

$$
\begin{array}{lll}
 & \overset{\displaystyle CH_3}{|} & \overset{\displaystyle CH_3}{|} \\
CH_2{=}CH & CH_2{=}C & C{=}CH_2 \\
| & | & | \\
C{=}O & C{=}O & C{=}O \\
| & | & | \\
O\text{–}CH_2CH_2(CF_2)_7CF_3 & O\text{–}CH_2CH_2\text{–}O \\
\\
\quad FA & \quad EGDM
\end{array}
$$

polymerization and they become macroporous when a diluent is added in the process of polymerization and (3) the content of perfluoroalkyl group is easily adjustable by changing the molar ratio of FA to comonomer [for example, ethylene glycol dimethacrylate (EGDM) as a cross-linking agent].

In this communication we report the synthesis of perfluoroalkyl polymer packings and the successful separation of hydrophobic or fluorine-containing compounds.

EXPERIMENTAL

Synthesis

Heptadecafluorodecyl acrylate (FA, Nippon Oil & Fats), ethylene glycol dimethacrylate (EGDM) or divinylbenzene (DVB) as a cross-linking agent, diethylbenzene (DEB) as a diluent and benzoyl peroxide as an initiator were mixed at room temperature. The mixture was added to a 1–3% (w/w) solution of aqueous poly(vinyl alcohol) kept at 75°C as a suspension medium, and stirred vigorously at this temperature for 24 h. The spherical polymers produced were collected by filtration and washed with water, hot water and ethanol. By adjusting the stirring speed and sieving, porous spheres of average diameters 5–10 and 25–44 μm were obtained.

Chromatography

The spheres were packed into a stainless-steel column (10–30 cm × 0.4 cm I.D.) under pressure of 60–80 kg cm^{-2}. The chromatograph included a JASCO 880-PU pump, a spectrophotometer UVIDEC-100-IV and a Shodex refracto monitor SE-51. An Unisil Pack (Type 5C18-250A, 25 cm × 0.46 cm I.D., 12 500 theoretical plate numbers, pore size 250 Å; Gasukuro Kogyo) was used as a reference column. All chromatography was carried out in aqueous solutions at 25°C.

RESULTS AND DISCUSSION

Perfluoroalkyl polymer beads

Fluorine-containing spherical beads were prepared by suspension copolymerization under the conditions given in Table I. The beads obtained are spherical and unevenly sized as shown in Fig. 1. It was confirmed by elemental analysis and infrared

TABLE I

PREPARATIVE CONDITIONS AND ELEMENTAL ANALYSIS OF PERFLUOROALKYL POLYMERS

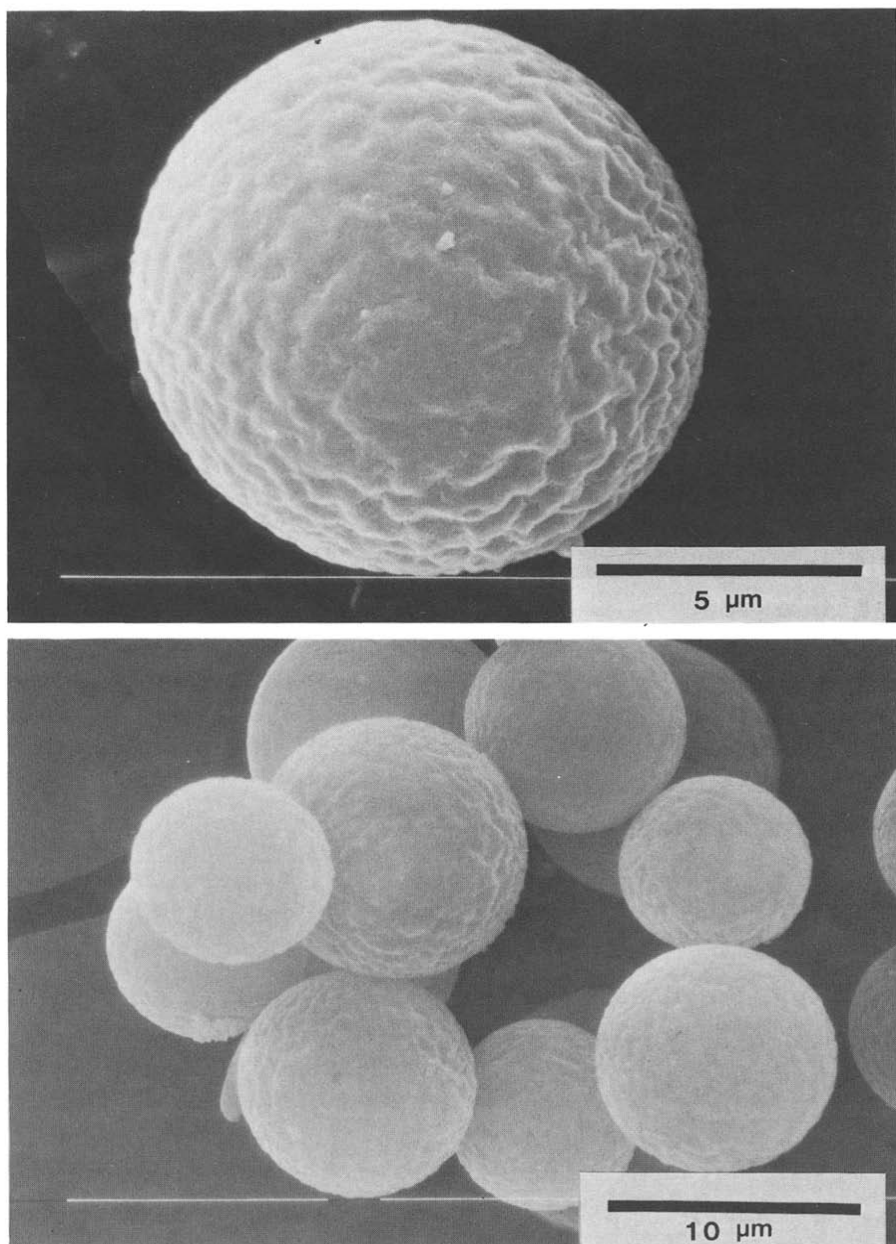| No.	Molar ratio FA:EGDM:DVB	DEB (%, v/v for monomer)	Elemental analysis (%)		
			C	H	F
FA-EGDM-1	70:30: —	100	35.9	2.2	53.5
FA-EGDM-2	50:50: —	100	39.8	3.2	45.1
FA-EGDM-3	30:70: —	100	47.4	4.7	32.9
FA-DVB-1	70: — :30	50	36.8	2.2	56.3
FA-DVB-2	50: — :50	50	44.2	2.9	49.8
FA-DVB-3	30: — :70	50	52.3	3.9	39.3

Fig. 1. Electron micrographs of perfluoroalkyl polymer beads prepared by suspension polymerization (FA-EGDM-3).

spectra (1100–1250 cm^{-1}) that the fluorine content of the beads increased with increasing monomer ratio of FA to the cross-linking agent: spherical beads with higher fluorine contents from 33 to 56% (w/w) are obtained with ease (21.1% was reported by Berendsen et al.[2] in the case of silica-based types).

The packings became porous by use of diethylbenzene as a diluent in the polymerization process. Organic solvents have little influence on the degree of swelling of FA–EGDM type beads. It was determined by gel permeation chromatography (GPC)[16] in tetrahydrofuran that the porosity and the exclusion molecular weight of the FA–EGDM type beads were in the ranges of 40–60% and 100 000–300 000 (for polystyrene), respectively. However, the corresponding values were not determined for the FA–DVB type beads because of the large swelling in tetrahydrofuran, due to the hydrophobicity of the DVB moiety.

Chromatographic properties

Fig. 2 shows the relationship between the monomer ratio of FA and the capacity factor, k', for benzene and hexafluorobenzene as a solute. It would be expected that an increase in the monomer ratio of FA would lead to an increase in the k' value of hexafluorobenzene due to the fluorine–fluorine interaction between packings and solutes. However, as shown in Fig. 2, k' was independent of the monomer ratio. This may be attributable to the fact that the packing density of FA groups increases with an increase in the monomer ratio of FA, thereby causing a stronger interaction among the fluorocarbon chains than between the fluorocarbon chains and the solutes. This is supported by our observation that the k' value increased with an increase in the monomer ratio of FA when the polymerization was carried out in the presence of a co-monomer, such as propyleneglycol methacrylate, indicating that the orientation of the FA groups is disordered by the dilution due to the presence of the co-monomer. In contrast, the value of k' for hexafluorobenzene is independent of the monomer ratio of FA, but the value for benzene decreased with increasing monomer ratio. This is attributable to a lower surface energy based on the orientation of the perfluoroalkyl groups. The lowering of the surface energy results in an "oilproof" property, which gives limited miscibility between hydrocarbon and fluorocarbon substances. However, this property is not disadvantageous for chromatographic separation of hydrocarbon substances.

The value of k' is always higher with DVB than with EGDM. This indicates that DVB as a cross-linking agent confers sufficient aromaticity upon the packings to result in π–π interactions[17,18] between the packing and the solute.

Fig. 3 shows a comparison with the reversed-phase packing, octadecyl-bonded silica gel (ODS), using perfluorinated benzenes (a) and normal hydrocarbons (b) as the
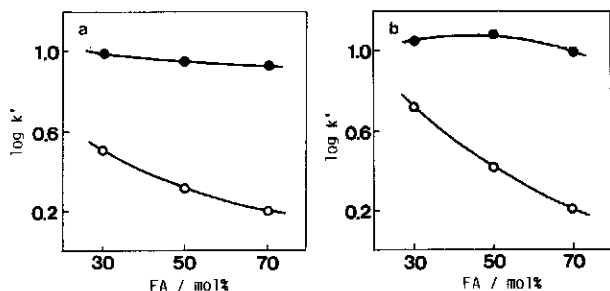

Fig. 2. Relationship between the monomer ratio, $\{[FA]/([FA] + [cross\text{-}linking\ agent])\} \cdot 100$, and the value of k' for benzene ($\bigcirc$) and hexafluorobenzene ($\bullet$) on the FA–EGDM column (a) and the FA–DVB column (b). Mobile phase: acetonitrile–water (50:50, v/v).
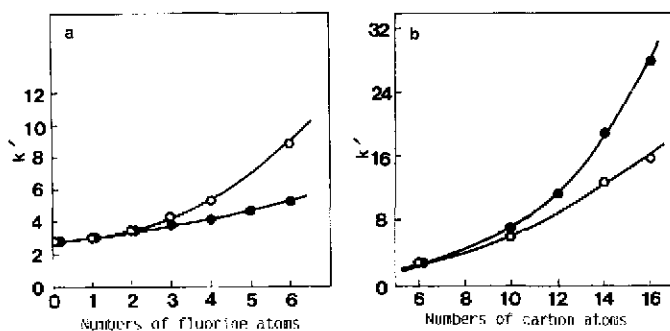
Fig. 3. Retention of fluorinated benzenes (a) and aromatic compounds (b) on FA-EGDM-3 (○) and ODS (●) columns. Mobile phases: acetonitrile–water (50:50, v/v) (a) and (90:10, v/v) (b). Solutes: (a) benzene, fluoro-, 1,2-difluoro-, 1,2,4-trifluoro-, 1,2,3,5-tetrafluoro-, pentafluoro- and hexafluorobenzene; (b) benzene, naphthalene, biphenyl, anthracene and pyrene.

solutes. It is clear that sufficiently large capacity factors are obtained for both types of solutes in the case of the FA–EGDM column. In the case of the ODS column, lower capacity factors are obtained for perfluorinated benzenes and increased retention is observed for hydrocarbons, especially long-chain alkyl compounds. The advantages of the FA–EGDM beads are attributable as follows: for fluorinated solutes, the fluorine–fluorine interaction between the stationary phase and the solutes plays a significant role in producing large capacity factors and the FA–EGDM beads contain a sufficient amount of fluorine atoms. For hydrocarbon solutes, the ODS packings show higher affinity than do the FA–EGDM packings. Therefore, the ODS column is unsuitable for isocratic separation of solutes with significantly different hydrophobicities and gradient elution should be applied in this case. On the contrary, FA–EGDM packings enable chromatographic separation in the isocratic mode even for such solutes because of the retention of large hydrophobic compounds is not excessive. This is also due to the "oilproof" property based on the lower surface energy of the perfluoroalkyl groups.

A similar limited miscibility between hydrocarbon and fluorocarbon substances is observed in micellar systems. Long-chain alkyl groups are difficult to incorporate into the micelles from a surfactant with an heptadecafluorodecyl chain, or are phase-separated in the micelles (Fig. 4)[19,20].
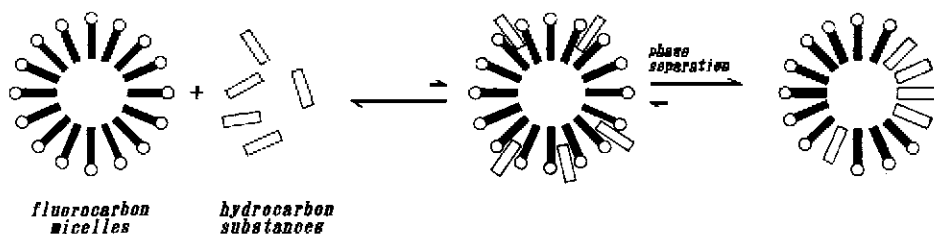

Fig. 4. Schematic illustration of the limited miscibility between fluorocarbon micelles and hydrocarbon substances.
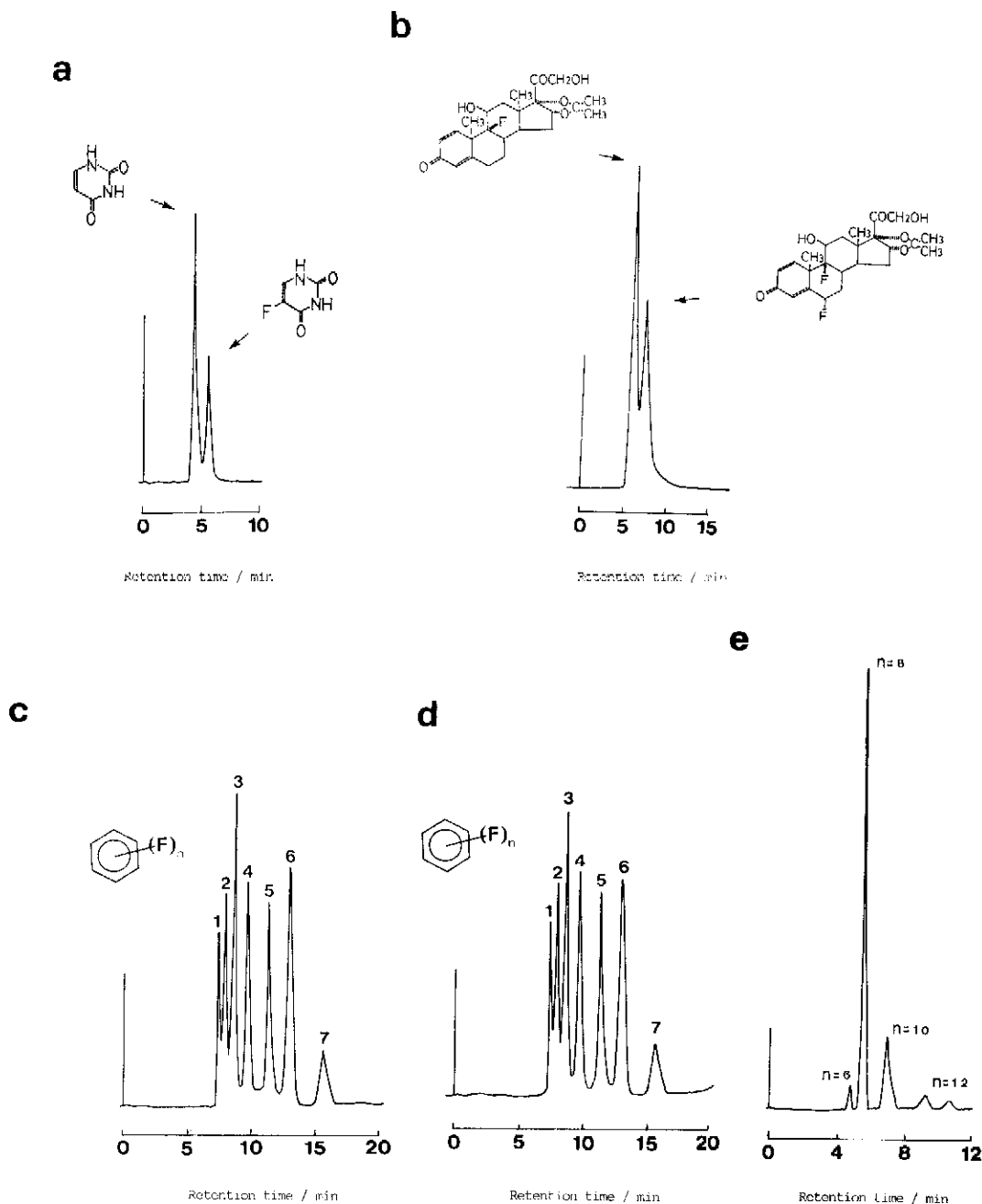
Fig. 5. Typical chromatograms using the FA-EGDM-3 column. Mobile phases and solutes: (a) water for uracil and 5-fluorouracil; (b) methanol–water (60:40, v/v) for steroids; (c) acetonitrile–water (50:50, v/v) for fluorinated benzene (1 = benzene, 2 = fluorobenzene, 3 = 1,2-difluorobenzene, 4 = 1,2,4-trifluorobenzene, 5 = 1,2,3,5-tetrafluorobenzene, 6 = pentafluorobenzene and 7 = hexafluorobenzene); (d) as (c) after treatment with 0.1 M NaOH for 1 day; (e) ethanol for perfluoroalkyl alcohols $CF_3(CF_2)_{n-1}CH_2CH_2OH$.

TABLE II

RESISTANCES TO ACID AND ALKALI OF FA-EGDM-3 PACKINGS

Time[a] (day)	0.1 M HCl (aq.)		0.1 M NaOH (aq.)	
	%C	%H	%C	%H
0	47.4	4.7	47.4	4.7
60	47.4	4.7	47.4	4.8
120	47.4	4.8	47.5	5.0

[a] Time of standing at room temperature.

TABLE III

RETENTION BEHAVIOURS FOR BASIC SUBSTANCES

Mobile phases: acetonitrile water (80:20, v/v).

Solute	k'	
	FA-EGDM-3	ODS
Pyridine	0.08	0.27
Quinoline	0.39	0.70
α-Naphthoquinoline	1.19	1.66
Benzylamine	0.18	Adsorption (not eluted)

As shown in Figs. 2 and 3a, the FA EGDM column is highly efficient for the separation of fluorinated compounds. Fig. 5 shows typical chromatograms for mixtures of fluorine-containing compounds. 5-Fluorouracil, a carcinogenic substance, could not be separated from uracil on the ODS column under the same conditions.

Resistances to acid and alkali

The FA moiety in the packings is also a typical "waterproof" group. The FA–EGDM packings are remarkably acid- and alkali-resistant on the basis of this property. As shown in Table II, the packings can withstand use at pH 1 and 13. The chromatograms c and d in Fig. 5 show that the retention time does not change after treatment of the column with an alkaline solution.

In addition, the FA-EGDM packings are composed of a totally organic and non-ionic polymer. Abnormal adsorptions are often observed for ionic substances in the case of silica-bonded packings[21,22]. This is due to undesirable silanol residues in the packing. Such adsorption behaviours are not detected with the FA–EGDM packings (Table III).

CONCLUSIONS

(1) Totally organic and non-ionic packings were prepared by suspension copolymerization using perfluoroalkyl acrylate.

(2) The packings are spherical and the size is adjustable with ease.

(3) The fluorine content of the packings is adjustable by changing the monomer ratio.

(4) The packings enable efficient separation of fluorine-containing substances.

(5) The packings show no excessive retention for large hydrophobic hydrocarbons.

(6) The packings are highly acid- and alkali-resistant and show no ionic adsorption for ionic substances.

REFERENCES

1   W. H. Pirkle, D. W. House and J. M. Finn, *J. Chromatogr.*, 192 (1980) 143.
2   G. E. Berendsen, K. A. Pikaart, L. Galan and C. Olieman, *Anal. Chem.*, 52 (1980) 1990.
3   G. E. Berendsen and L. De Galan, *J. Chromatogr.*, 196 (1980) 21.
4   H. A. H. Billiet, P. J. Shoenmakers and L. De Galan, *J. Chromatogr.*, 218 (1981) 443.
5   P. C. Sadek and P. W. Carr, *J. Chromatogr.*, 288 (1984) 25.
6   J. Kip, P. van Haperen and J. C. Kraak, *J. Chromatogr.*, 356 (1986) 423.
7   M. K. L. Bicking, M. L. Bicking and W. E. Longman, *Anal. Chem.*, 58 (1986) 499.
8   R. L. Pearson, J. F. Weiss and A. D. Kermers, *Biochim. Biophys. Acta*, 228 (1971) 770.
9   B. Z. Egan, *Biochim. Biophys. Acta*, 299 (1973) 245.
10 N. Tanaka, H. Goodell and B. L. Karger, *J. Chromatogr.*, 158 (1978) 233.
11 T. A. Zwier and M. F. Burke, *Anal. Chem.*, 53 (1981) 812.
12 J. A. Huth, N. D. Danielson, *Anal. Chem.*, 54 (1982) 930.
13 R. W. Siergiej and N. D. Danielson, *Anal. Chem.*, 55 (1983) 17.
14 M. Kruempelman and N. D. Danielson, *Anal. Chem.*, 57 (1985) 340.
15 N. D. Danielson, S. Ahmed, J. A. Huth and M. A. Targrove, *J. Liq. Chromatogr.*, 9 (1986) 727.
16 H. Ihara, T. Yoshinaga and C. Hirayama, *J. Chromatogr.*, 362 (1986) 197.
17 T. Hanai and J. Hubert, *J. Chromatogr.*, 291 (1984) 81.
18 K. Jinno, T. Nagoshi, N. Tanaka, M. Okamoto, J. C. Fetzer and W. R. Biggs, *J. Chromatogr.*, 392 (1987) 75.
19 H. Ihara, Y. Hashiguchi and T. Kunitake, *Chem. Lett.*, (1983) 733.
20 T. Kunitake, H. Ihara and Y. Hashiguchi, *J. Am. Chem. Soc.*, 106 (1984) 1156.
21 N. Tanaka, *Kagaku no Ryoiki*, 37 (1983) 33.
22 A. M. Krutulovic and P. R. Brown, *Reversed-phase High-performance Liquid Chromatography*; Japanese Translation, H. Hatano, K. Makino and K. Nakano, *Tokyo Kagaku Dojin*, Tokyo, 1985, p. 85.