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CHEMICALLY BONDED OCTADECYLSILANE AND POLYIMINE STATIONARY PHASES FOR OPEN-TUBULAR MICROCAPILLARY LIQUID CHROMATOGRAPHY

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SUMMARY

The preparation and chromatographic performance of chemically bonded octadecylsilane and polyimine stationary phases in open-tubular microcapillary liquid chromatography were investigated. The retention behaviour on the former stationary phase was similar to that in packed columns, while hydrophobic and electrostatic interactions with solutes were observed on the latter phase. Parameters affecting solute retention were examined on polyimine columns, which selectively (strongly) retained anionic compounds. Temperature- and solvent-gradient elution techniques were applied and the advantages of gradient elution in open-tubular microcapillary liquid chromatography were demonstrated.

INTRODUCTION

Open-tubular microcapillary columns, coated with various liquid phases, have been prepared and their chromatographic performances in liquid chromatography (LC) have been evaluated by some authors[1–4]. Theoretical plate numbers in excess of 100,000 have been obtained on 10–30 μm I.D. columns approximately 20 m long, and the prospects for open-tubular microcapillary columns have been discussed. However, the long-term stability of these physically coated columns is somewhat poor, even if the mobile phase is saturated with the stationary phase. Accordingly, chemically bonded or immobilized stationary phases would be preferred.

In capillary gas chromatography (GC) many kinds of immobilized stationary phases have been prepared, leading to an increase of stability to the effects of temperature and organic solvents[5–9].

A few papers on chemically bonded or immobilized stationary phases for open-tubular microcapillary LC have been published[10–16]. This paper will describe the preparation and evaluation of chemically bonded octadecylsilane (ODS) and polyimine stationary phases for open-tubular microcapillary LC.

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EXPERIMENTAL

Column preparation

Soda-lime glass capillaries of 30–50 μm I.D. were drawn with a glass drawing machine (GDM 1B; Shimadzu, Kyoto, Japan). Capillaries were treated with sodium hydroxide aqueous solution at 45–50°C for 2 days and then washed with methanol until the effluent from the column became neutral as described previously[17]. After washing with methanol, the capillaries were dried under helium at 120°C for 2–4 h, washed with 0.01 *M* hydrochloric acid in methanol, distilled water and then with methanol. Finally, they were dried under helium at 120°C for 2 h. This pretreatment generated a stable silica gel layer on the glass surface and thus substantially increased the surface area of the capillaries. This allows the introduction of larger amounts of stationary phases compared with a smooth glass surface. Octadecyltriethoxysilane (ODTES) (Tokyo Chemical Industry, Tokyo, Japan) was generally employed as a reagent for ODS columns since trichloro- and monochlorodimethyloctadecylsilane reagents sometimes caused clogging of the column. A dry toluene solution (10%, v/v) of ODTES was forced into the pretreated capillaries. The toluene was dried by passing it through a glass column, packed with molecular sieve 4A (8–12 mesh; Yoneyama Yakuhin Kogyo, Osaka, Japan). After the reaction, the capillary columns were washed with dry toluene and dried under helium at 120°C for 2 h. Finally, the columns were washed with methanol, acetonitrile and the mobile phase.

For the preparation of polyimine stationary phases, an aqueous solution of 10% (v/v) γ-aminopropyltriethoxysilane (Tokyo Chemical Industry) was introduced into the capillaries subsequent to the pretreatments. The pH of the reagents was adjusted to 3 with concentrated hydrochloric acid. The reaction took place at 75°C for 4 h. Dialdehyde and diamine solutions were then forced into the capillaries at room temperature, and finally, the polyimine chains were capped with monoamine. A 2.5% (v/v) glutaraldehyde (Tokyo Chemical Industry) aqueous solution, 0.1% (w/v) hexamethylenediamine (Wako, Osaka, Japan) aqueous solution and 1% (w/v) laurylamine (Wako) methanol solution were selected as dialdehyde, diamine and monoamine, respectively for the poly-N-alkyl alkylidenimine columns. For the aromatic polyimine columns, 1% (w/v) methanol solutions of terephthalaldehyde, *p*-phenylenediamine and aniline (all from Wako) were selected as dialdehyde, diamine and monoamine, respectively.

Apparatus

A liquid chromatograph was assembled from a Micro Feeder (Azumadenkikogyo, Tokyo, Japan), equipped with a gas-tight syringe or LC-5A (Shimadzu) as a pump, Micro Valve Injector (0.02 μl; Japan Spectroscopic, Tokyo, Japan), a laboratory-built gradient maker, a column oven, an open-tubular microcapillary column and a UV spectrophotometer UVIDEC 100 (Japan Spectroscopic), equipped with a small-volume (0.04 μl) flow cell as a detector. The Micro Feeder was used in the constant-flow mode, while the LC-5A was used in the constant-pressure mode.

The gradient equipment was consisted of a mixing vessel (inner volume 64 μl) and a magnetic stirrer. The mixing vessel was a modified gas-tight syringe without a plunger. Both ends of the syringe were fitted with needles, as described previously[18]. Folded stainless-steel tubing (0.63 mm O.D.) was placed in the mixing vessel for effective stirring. It was easy to produce this syringe-type of mixing vessel in various

volumes. Connections between the mixing vessel and the injector were formed with fused-silica tubing, 9.5 cm × 0.071 mm I.D. (Scientific Glass Engineering, Melbourne, Australia), stainless-steel tubing (2 cm × 0.13 mm I.D. and 8 cm × 0.17 mm I.D.) and PTFE tubing (0.2 mm I.D.). In this gradient system, the ratio of the flow-rate to the volume of the mixing vessel determines the gradient profile and an exponential gradient with almost linear behaviour in the beginning is obtained.

The constructions of the flow cell and the laboratory-built column oven were the same as in previous papers[19,20].

RESULTS AND DISCUSSION

ODS column

The chromatographic behaviour of ODS glass capillary columns was nearly the same as that of ODS packed columns. The difference in the mobile phase composition between open-tubular and packed columns was due to the difference in the phase ratio (the ratio of the volume of the mobile phase to weight of the stationary phase). More octadecyl groups could be introduced into the glass surface by increasing the temperature of pretreatment with sodium hydroxide solution as in the previous paper[15]. For the present work, 45–50°C was selected as the pretreatment temperature, in order to achieve a stable silica gel layer and to allow the desired amounts of the stationary phase to be introduced.

In the case of a 5 m × 50 μm I.D. column, pretreated at 50°C, at most around 100 μg of ODS could be chemically bonded, the phase ratio being *ca.* 100 ml/g. Sample amounts should be reduced, considering the amount of the stationary phase. Injection of a sample up to 20 ng caused no deterioration of the column efficiency for the 5.4 m × 45 μm I.D. column when the capacity factor, k', of a solute was 0.4–1.3. The permissible amount of sample was decreased with decreasing column bore. Further, the injection volume must be limited. A volume occupying around 1 cm of the column length was a permissible injection volume; with greater volumes the peaks were sometimes skewed.

The measurement of the column efficiency was carried out by stop-flow on-column injection[10] since valve injection led to a deterioration in the column efficiency, especially for narrow and/or short columns. The H–u (H=plate height; u=linear velocity) and h–v (h=reduced plate height; v=reduced linear velocity) relationships were measured for columns with 30–50 μm I.D. Basic equations for open-tubular capillary LC are[21]

$$H = \frac{2D_m}{u} + \frac{(11k'^2+6k'+1)d_c^2}{96\,(1+k')^2 D_m} \cdot u + \frac{2k'd^2}{3\,(1+k')^2} \cdot u \tag{1}$$

$$h = \frac{2}{v} + \frac{(11k'^2+6k'+1)}{96\,(1+k')^2} \cdot v + \frac{2k'}{3\,(1+k')^2}\left(\frac{d}{d_c}\right)^2\left(\frac{D_m}{D_s}\right) v \tag{2}$$

$$\equiv \frac{B}{v} + C_m v + C_s v \tag{3}$$

where D_m and D_s are the diffusion coefficients of a solute in the mobile and the stationary phase, d_c is the column diameter and d is the thickness (or depth) of the stationary phase, respectively. Theoretically, h is proportional to v when v is larger than 20[21]. Linear relationships were observed in the region of u=0.2–2 cm/sec or v=100–1000. When k' is 1–2, the H values and the slopes of the H–u plot decreased with decreasing column bore. Contrarily, nearly the same linear relationships between h and v were obtained with respect to values and slopes. These results indicate that extra-column effects can be neglected and that a stationary phase with the equivalent efficiency can be prepared independent of the column bore. C_m values can be calculated from eqn. 2. Observed C values (C_m plus C_s) were 1.1–1.4 times larger than C_m values, which indicates that mass transfer resistance in the mobile phase is dominant.

Fig. 1 shows an isocratic separation of indole derivatives on a 5.3 m × 38 μm I.D. ODS column. The employment of a mobile phase adjusted to low pH did not cause any problem as far as clogging of the column is concerned. Prepared ODS capillary columns were very stable and their performance was unchanged over several months.

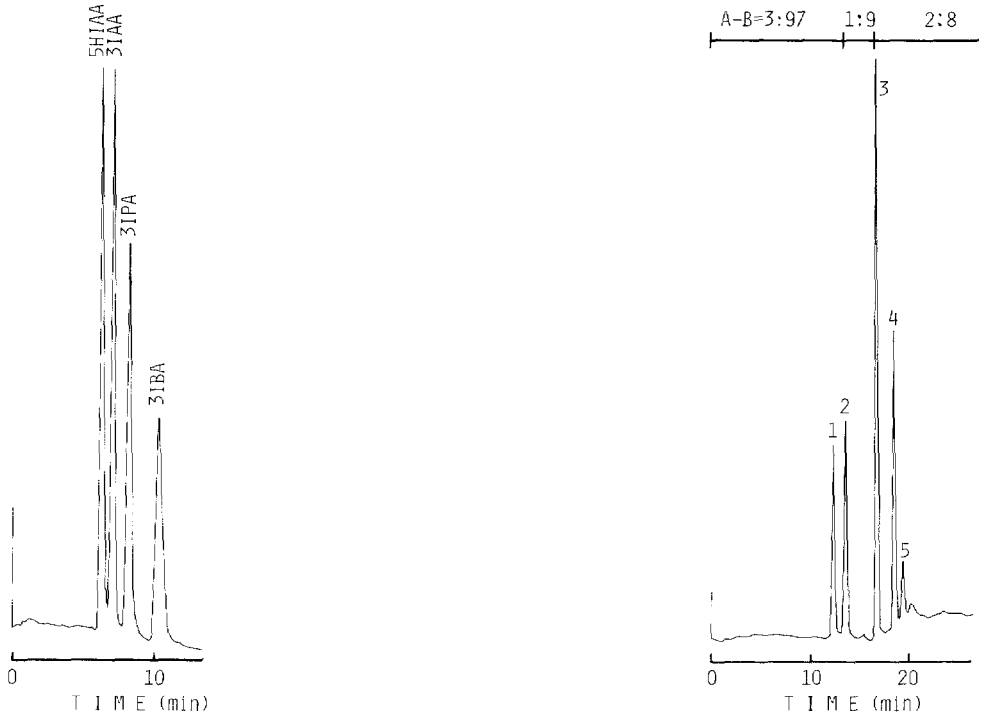


Fig. 1. Separation of indole derivatives. Column: ODS, 5.3 m × 38 μm I.D. Mobile phase: acetonitrile–1 *M* acetic acid aqueous solution (1:9, v/v), pH 2.4. Flow-rate: 1.1 μl/min. Sample: 5-hydroxyindole-3-acetic acid (**5HIAA**); 3-indoleacetic acid (3IAA); 3-indolepropionic acid (3IPA) and 3-indolebutyric acid (3IBA). Wavelength of UV detection: 266 nm.

Fig. 2. Stepwise gradient separation of typical components in a pharmaceutical preparation. Column: ODS, 4.9 m × 52 μm I.D. Mobile phase: A, acetonitrile; B, 0.05% ammonium carbonate, composition as indicated. Flow-rate: 1.1 μl/min. Sample: 1 = 7.0 ng of barbital; 2 = 6.7 ng of acetaminophenol; 3 = 9.1 ng of caffeine; 4 = 9.6 ng of phenacetin; 5 = 2.0 ng of *p*-chloroacetanilide. Wavelength of UV detection: 225 nm.

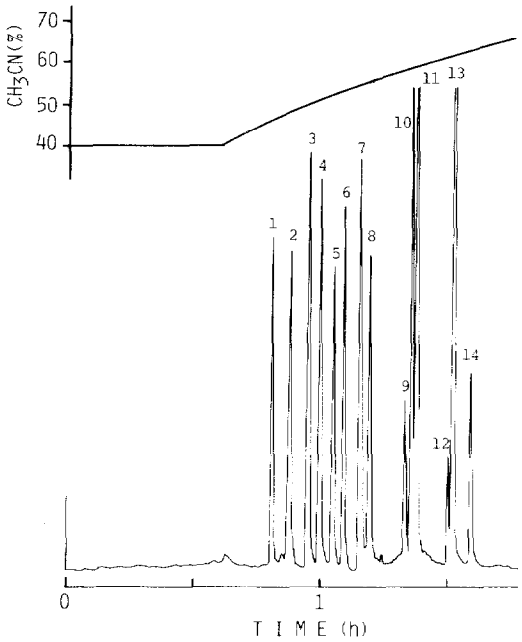


Fig. 3. Continuous solvent-gradient separation of polynuclear aromatic hydrocarbons. Column: ODS, 22 m × 31 μm I.D. Mobile phase: as indicated. Flow-rate: 0.52 μl/min. Sample: 1=benzene; 2=naphthalene; 3=biphenyl; 4=fluorene; 5=phenanthrene; 6=anthracene; 7=fluoranthene; 8=pyrene; 9=*p*-terphenyl; 10=chrysene; 11=9-phenylanthracene; 12=perylene; 13=1,3,5-triphenylbenzene; 14=benzo[*a*]pyrene. Wavelength of UV detection: 250 nm. Column temperature: 36°C.

Gradient elution is a promising technique, which reduces the analysis time and improves the selectivity in chromatography. Solvent-gradient elution is the most convenient method in LC, while temperature-gradient elution is the most successful method in GC. In open-tubular capillary LC, both solvent-gradient and temperature-gradient elutions are useful.

Stepwise solvent-gradient elution is a simple technique. Its usefulness was pointed out in a previous paper on ultra-micro high-performance LC[22]. The stepwise gradient separation of typical components in a pharmaceutical preparation is demonstrated in Fig. 2.

Figs. 3 and 4 show solvent-gradient and temperature-gradient separations, respectively, of polynuclear aromatic hydrocarbons on a 22 m × 31 μm I.D. ODS column. The former was operated at constant flow-rate (0.52 μl/min), while the latter was operated at constant pressure (40 kg/cm^2). The flow-rate increased with increasing column temperature in the latter case. Both gradient modes were useful for the elution of solutes that were strongly retarded on the column and for improved selectivity.

Fig. 5 shows a solvent-gradient separation of epoxy resin oligomers. The characterization of by-products as well as main peaks is of practical importance, since the properties of the epoxy resin are highly affected by them. Both ends of the molecules occurring as main products are epoxide groups, while by-products have functional groups other than epoxide groups either as end groups or as side chains pendant to the

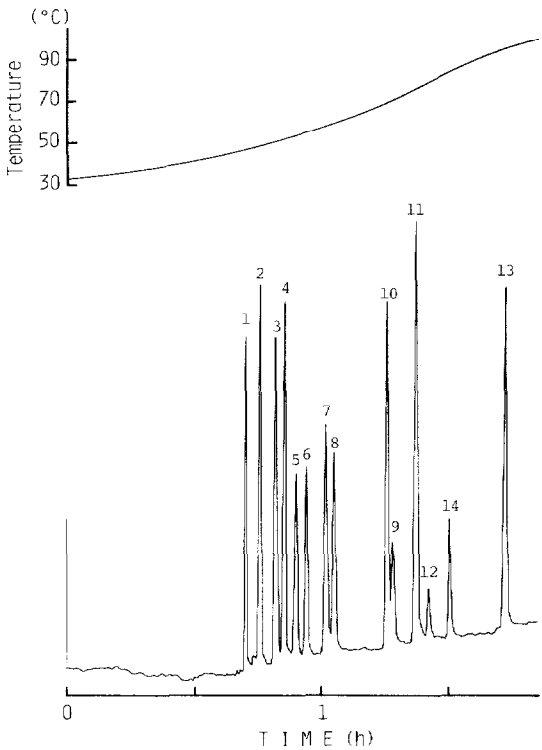


Fig. 4. Temperature-gradient separation of polynuclear aromatic hydrocarbons. Mobile phase: acetonitrile–water (40:60, v/v). Inlet pressure: 40 kg/cm^2. Wavelength of UV detection: 254 nm. Column temperature: as indicated. Other operating conditions as in Fig. 3.

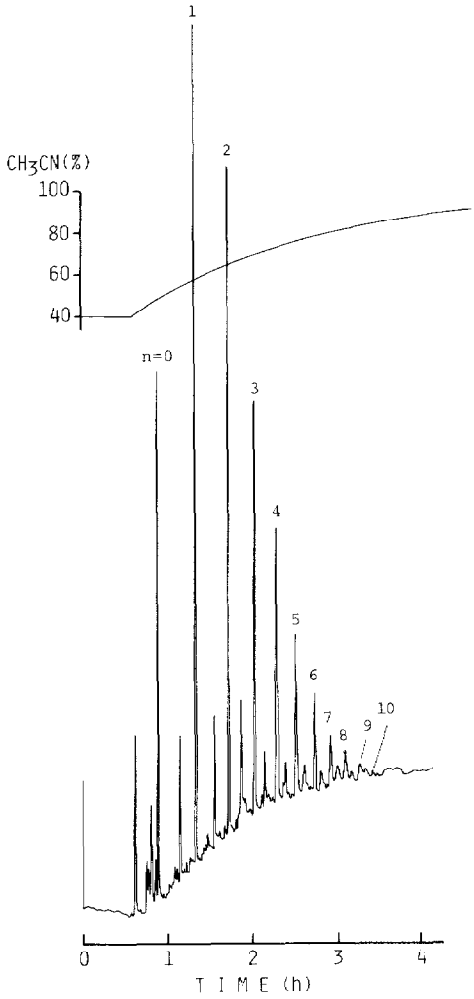
main chain. The structure of the main products is shown in Scheme 1. Besides the main peaks, many of the by-products are resolved on the ODS capillary column. The resolution of these substances is similar to that obtained on packed columns[23–25].

Polyimine column

The phase ratio of the open-tubular capillary column unlike packed columns, is large enough to cause overloading. Bonding of long chain groups to the surface is expected to reduce the phase ratio.

The molecular chain length of polyimine stationary phases can be varied by changing the reagents and/or the number of reaction cycles. The expected structures of the polyimine stationary phases are shown in Scheme 2. Fig. 6 shows a typical separation of polynuclear aromatic hydrocarbons on a poly-N-alkyl alkylidenimine column. The retention behaviour of non-polar solutes on this column was similar to that on an ODS column. For the aromatic polyimine columns, hydrophobic interaction between the solutes and the stationary phase was quite small.

Polyimine columns strongly retarded anionic compounds, and this seemed to be due to electrostatic interaction between the anionic compounds and nitrogen atoms in the stationary phase. Fig. 7 shows separations of benzoic acids on poly-N-alkyl alkylidenimine (n=2 in Scheme 2a), where the retention of solutes is dependent on the pH



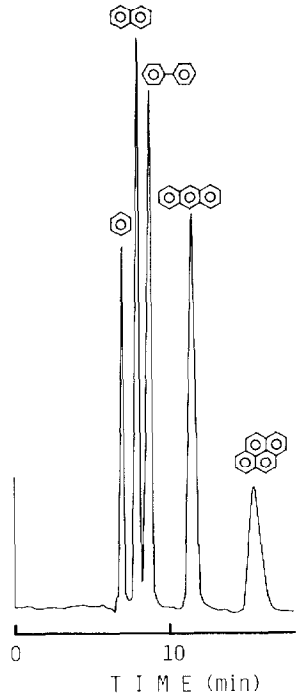
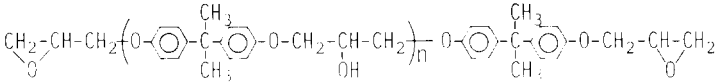


Fig. 5. Solvent-gradient separation of epoxy resin oligomers. Operating conditions as in Fig. 3, except for the following: sample, 160 ng of Epikote 1001; column temperature, 44°C; wavelength of UV detection, 225 nm.

Fig. 6. Separation of polynuclear aromatic hydrocarbons on a polyimine column. Column: poly-N-alkyl alkylidenimine (n=2), 5.3 × 38 μm I.D. Mobile phase: acetonitrile–water (35:65, v/v). Flow-rate: 1.1 μl/min. Wavelength of UV detection: 254 nm.

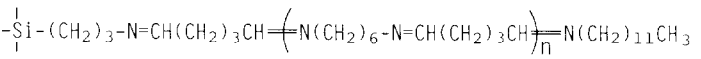
of the mobile phase and the degree of dissociation of the solutes. A larger degree of dissociation of solutes is essential for their retardation. The content of the organic solvent in the mobile phase scarcely affected solute retention when the organic portion was so large that hydrophobic interaction between the solutes and the stationary phase could be neglected. Solute retention decreased with decreasing pH.

The influence of column temperature on column efficiency and solute retention is shown in Fig. 8. The k' values of the solutes are slightly decreased with increasing column temperature, while the H values are decreased. These results point to the use of higher column temperatures.

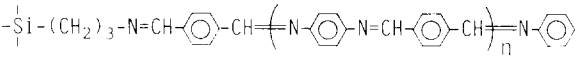


Scheme 1. Structure of main products of epoxy resin oligomers.

a) ALKYL



b) AROMATIC



Scheme 2. Expected structures of polyimine stationary phase.

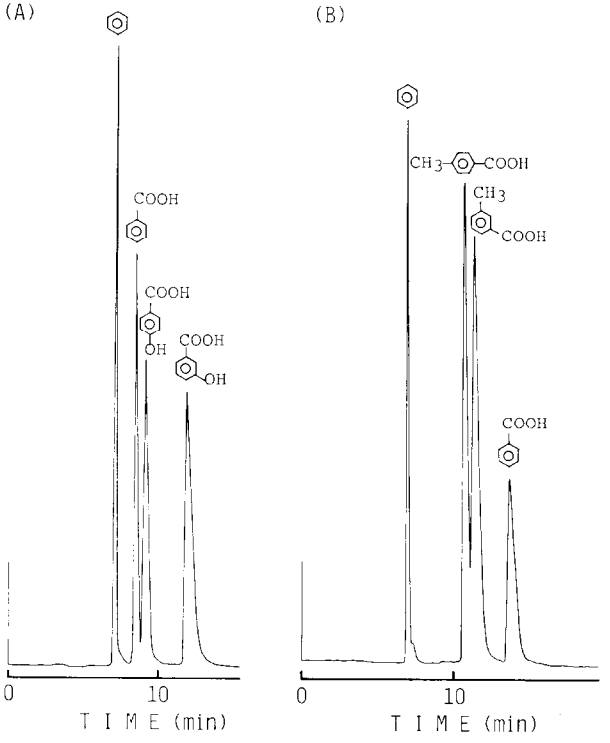


Fig. 7. Separations of benzoic acids. Column: poly-N-alkyl alkylidenimine (n=2), 5.4 m × 43 μm I.D. Mobile phase: acetonitrile-water-acetic acid 84.5:15:0.5, pH=3.8 (A); 90:9.94:0.06, pH=5.5 (B). Flow-rate: 1.1 μl/min. Wavelength of UV detection: 233 nm.

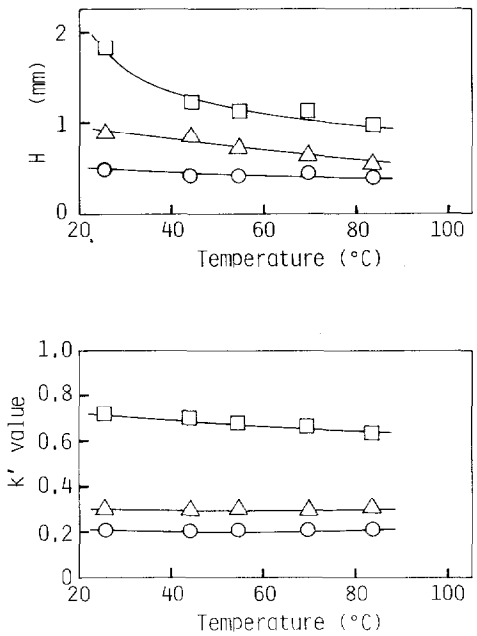


Fig. 8. Influence of column temperature on column efficiency and solute retention. Operating conditions as in Fig. 7A except column temperature. Sample: □ = *m*-hydroxybenzoic acid; △ = *p*-hydroxybenzoic acid; ○ = benzoic acid.

The number of nitrogen atoms could be estimated by the amount of benzoic acid adsorbed on the surface. An eluent with a known concentration, C, of benzoic acid was forced into a column. If benzoic acid was adsorbed on the surface, the breakthrough volume, V_t, would exceed the void volume, V_0, of the column. The amount of benzoic acid adsorbed would be $(V_t-V_0)C$. The breakthrough volume was determined by measuring the UV absorption of the effluent. The results are listed in Table I. Assuming that each nitrogen site adsorbs one benzoic acid molecule, the phase ratio can be estimated from the data in Table I. The phase ratios were 950–3100 ml/g, de-

TABLE I

HYDROPHILIC PROPERTIES OF POLYIMINE COLUMNS

Column				*No. of adsorbed benzoic acid molecules*	*k' of m-hydroxybenzoic acid***
*n**	*diameter (μm)*	*length (m)*	*inner volume (μl)*		
Alkyl					
0	37	5.2	5.6	$0.72 \cdot 10^{16}$	0.04
1	45	5.0	8.0	$1.8 \cdot 10^{16}$	0.31
2	43	5.4	7.8	$3.5 \cdot 10^{16}$	0.73
3	42	5.4	7.6	$4.6 \cdot 10^{16}$	1.19
Aromatic					
2	45	5.4	8.5	$1.5 \cdot 10^{16}$	0.03

* See Scheme 2.

** Mobile phase: acetonitrile–water–acetic acid (84.5:15:0.5).

FIGURE CAPTIONS

pending on chain length, *i.e.*, they are larger than for the ODS capillary columns. The conditions of the reaction between γ-aminopropyltriethoxysilane and silica gel could be changed so as to increase the surface coverage.

In addition, adsorbed anionic compounds could be eluted with a solution containing an acid, such as acetic acid. The retention behaviour of aromatic hydrocarbons in the reversed-phase mode after the use of acidic eluents was different from the one observed using basic eluents. This may be due to irreversible adsorption of ions on the inner column surface.

CONCLUSION

Chemically bonded open-tubular microcapillary columns had long-term stability and could be applied to the separation of anionic and hydrophobic compounds. The narrow-bore ODS column had a high efficiency similar to that achievable with packed columns.

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