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DYNAMICALLY MODIFIED SILICA COLUMNS IN OPEN-TUBULAR CAPILLARY LIQUID CHROMATOGRAPHY

TOYOHIDE TAKEUCHI* and DAIDO ISHII

Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya-shi 464 (Japan)

and

AKIRA NAKANISHI

Research and Development Centre, Daikyo Oil Co., Yokkaichi-shi 510 (Japan)

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SUMMARY

Open-tubular liquid chromatography using dynamically modified columns has been investigated. Chromatographic separations are achieved with silica gel as the stationary phase and a mobile phase containing quaternary ammonium bromide as a hydrophobic layer-forming reagent. Parameters that affect the retention of solutes are examined.

INTRODUCTION

High-performance liquid chromatography (HPLC) using dynamically modified columns in which silica gel packings are generally employed as the stationary phase and cationic or non-ionic detergents are present in the mobile phase as a hydrophobic layer-forming reagent has been investigated[1–8]. Silica columns dynamically modified with long-chain quaternary ammonium ions worked like chemically bonded ODS silica columns. The advantage of the dynamic modification over chemically bonded columns is only a slight variation in selectivity for different brands of column material.

In open-tubular capillary liquid chromatography (LC), immobilized or chemically bonded stationary phases are preferred owing to the long-term stability of the column efficiency. The column bore significantly affects the column efficiency in open-tubular capillary LC, which forces us to employ narrow-bore columns. However, it is difficult to prepare narrow-bore chemically bonded open-tubular capillary columns. As a silica gel layer can be easily formed on a glass surface[9], silica columns dynamically modified with long-chain quaternary ammonium ions are expected to replace chemically bonded ODS capillary columns. This paper describes the prospects for open-tubular capillary LC using dynamically modified silica columns.

EXPERIMENTAL

The liquid chromatograph consisted of a Micro Feeder (Azumadenkikogyo, Tokyo, Japan) equipped with an MS-GAN 025 0.25-ml gas-tight syringe (Terumo, Tokyo, Japan) as a pump, a 0.02-μl micro valve injector [Japan Spectroscopic (JAS-CO), Tokyo, Japan], an open-tubular capillary column, a home-made column oven and a UVIDEC-100 UV spectrophotometer (JASCO) equipped with a modified flow cell (0.04 μl).

Open-tubular capillary silica gel columns were prepared as follows. Soda-lime glass capillaries were drawn with a GDM-1B glass-drawing machine (Shimadzu, Kyoto, Japan) and treated with a 1 N sodium hydroxide solution at 40–50°C for 2 days. The capillaries were then washed with methanol until the effluent became neutral and dried under helium at 120°C for 2 h. Subsequently, the capillaries were washed with 0.01 N methanolic hydrochloric acid until the effluent became acidic and then with distilled water and methanol. Finally, the capillaries were dried under helium at 120°C for 2 h. This pre-treatment produced a stable silica gel layer on the glass surface. The glass surface was observed with an EMX-SM7 electron microprobe X-ray analyser (Shimadzu).

Subsequent to the above treatment, the mobile phase containing a detergent was passed into the capillary for conditioning until the retention of solutes became constant. Cetyltrimethylammonium bromide (cetrimide) was obtained from Wako (Osaka, Japan). *n*-Tetradecyltrimethylammonium bromide and *n*-dodecyltrimethyl-ammonium bromide were obtained from Tokyo Chemical Industry (Tokyo, Japan).

RESULTS AND DISCUSSION

Fig. 1 shows scanning electron micrographs of a glass surface treated with alkaline solution. It can be seen that the surface area is greater than that of bare glass and that the shape of the deposits changes slightly with the progress of the treatment. The final products were hydrodynamically stable, *i.e.*, clogging problems were decreased. The density of deposits was dependent on the treatment temperature and period.

The surfactant concentration affects the retention of solutes in dynamically modified LC[1–8]. The effect of the surfactant concentration on the retention of solutes was examined by using methanol–water (1:1) and cetrimide as the modifying reagent. The results are shown in Table I. The capacity factors (k') of the solutes did not depend on the surfactant concentration in the range 0.005–0.1% (w/v). At a concentration of 0.5%, chromatographic peaks were skewed. These concentrations are lower than the critical micellar concentration (0.58%)[7]. After washing the column with 200 μl of 0.01 N methanolic hydrochloric acid, the solutes were not retained, which indicated that the washing process apparently gave a silica surface similar to the original one and the detergent attached to the surface played an important role in the retardation of solutes. In addition, aromatic hydrocarbons were not retained on the column before passing the mobile phase containing the detergent. This result indicates that reagents are irreversibly adsorbed on the glass surface when using a methanol–water mobile phase.

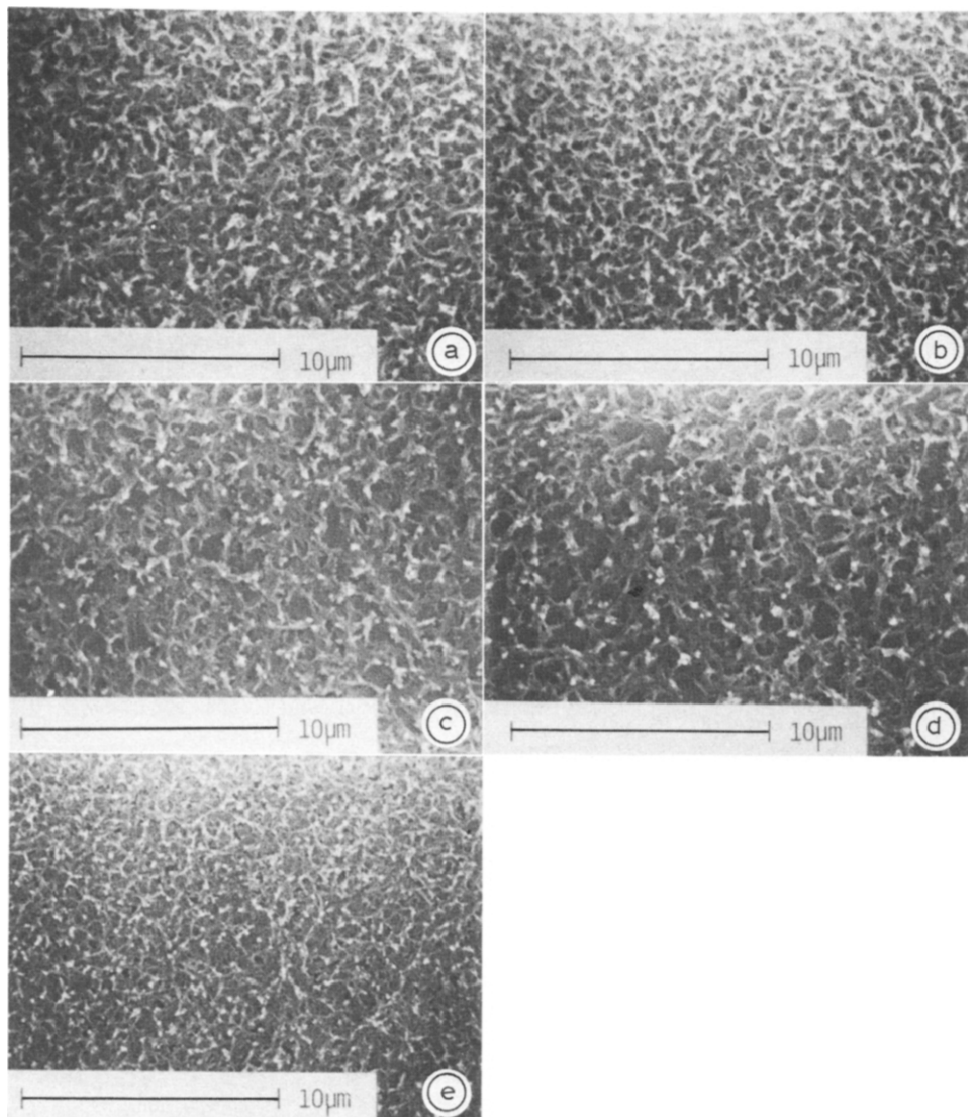Typical separations of aromatic hydrocarbons on a dynamically modified silica

Fig. 1. Scanning electron micrographs of a treated surface. Preparation procedures: 1, treated with 1 N NaOH for 2 days at 45°C; 2, washed with 0.2 ml of methanol (a); 3, dried under helium for 3 h at 120°C (b); 4, washed with 0.2 ml of 0.01 N methanolic HCl (c); 5, washed with 0.1 ml of water (d); 6, washed with 0.2 ml of methanol; 7, dried under helium for 2 h at 120°C (e).

column are shown in Fig. 2. Acetonitrile–water was superior to methanol–water as the mobile phase as the former has a lower viscosity[10]. However, the solubility of cetrimide in acetonitrile was so small that a small amount of methanol was present in the mobile phase in the former case.

The chromatographic behaviour of silica columns dynamically modified with cetrimide is similar to that of chemically bonded open-tubular ODS columns. Linear

TABLE I

EFFECT OF CONCENTRATION OF CETRIMIDE ON CAPACITY FACTORS

Column: 5.3 m × 44 μm I.D. (treated at 50°C). Mobile phase: methanol–water (1:1) containing cetrimide.

Concentration of cetrimide (%, w/v)	Capacity factor	
	Anthracene	Pyrene
0.005	0.55	1.02
0.01	0.61	1.20
0.03	0.61	1.17
0.1	0.66	1.25

relationships between the logarithm of the capacity factors and the mobile phase composition are illustrated in Fig. 3. The capacity factors of dynamically modified silica columns are lower than those of chemically bonded ODS columns, which may be due to the smaller surface coverage and shorter chain length of the modifier for the former stationary phase. It is reported that the surface coverage by dynamic modification is less than that of the best commercial bonded-phase packings and the maximum possible coverage is one sixth[2]. The amounts of adsorbed reagents can be roughly estimated from previous work[11], viz., a few micrograms of cetrimide per metre of the column. It will be possible to determine the amounts of adsorbed re-agents by colorimetry[1].

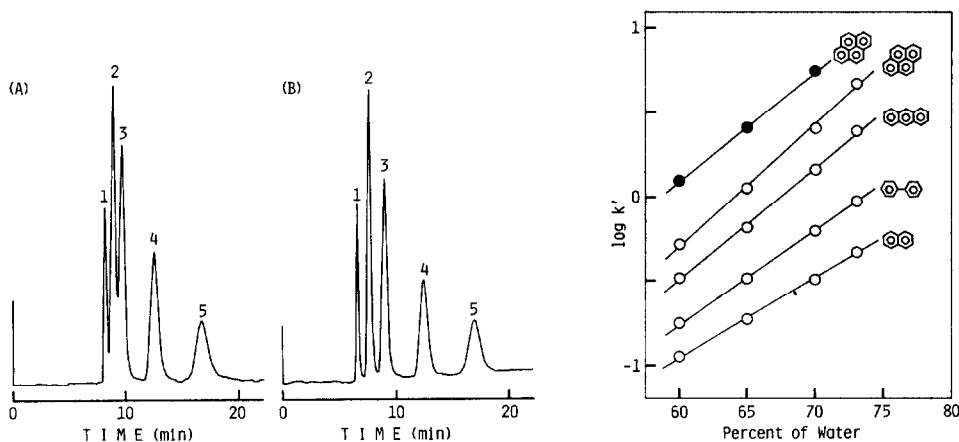The logarithm of the capacity factor was proportional to the reciprocal of the

Fig. 2. Separation of aromatic hydrocarbons on a dynamically modified silica column. Column: 5.3 m × 44 μm I.D. (treated at 50°C). Mobile phases: (A) methanol–water (1:1) containing 0.01% (w/v) of cetrimide; (B) acetonitrile–methanol–water (29:1:70) containing 0.01% (w/v) of cetrimide. Flow-rates: (A) 1.04; (B) 1.39 μl/min. Wavelength of UV detection: 254 nm. Samples: 1, benzene; 2, naphthalene; 3, biphenyl; 4, anthracene; 5, pyrene.

Fig. 3. Log *k'* versus mobile phase composition. Column: as in Fig. 2. Mobile phase: acetonitrile–water containing 1% (v/v) of methanol and 0.01% (w/v) of cetrimide. Column temperature: 26.5°C. Closed symbols: results obtained with an ODS column (5.3 m × 38 μm I.D.) and acetonitrile–water as the mobile phase.

TABLE II

EFFECT OF THE TEMPERATURE OF TREATMENT WITH SODIUM HYDROXIDE SOLUTION ON CAPACITY FACTORS

Mobile phase: acetonitrile–methanol–water (29:1:70) containing 0.01% (w/v) of cetrimide. Column temperature: 24°C.

Treatment temperature (°C)	Capacity factor	
	Anthracene	Pyrene
40	0.45	0.80
45	0.64	1.05
50	1.43	2.57

TABLE III

EFFECT OF THE CHAIN LENGTH OF THE MODIFIER ON THE RETENTION OF SOLUTES

Column: 5.3 m × 44 μm I.D. (treated at 50°C). Mobile phase: acetonitrile–methanol–water (29:1:70) containing 0.01% (w/v) of alkyltrimethylammonium bromide. Column temperature: 24°C. Modifier: $CH_3(CH_2)_nN(CH_3)_3Br$.

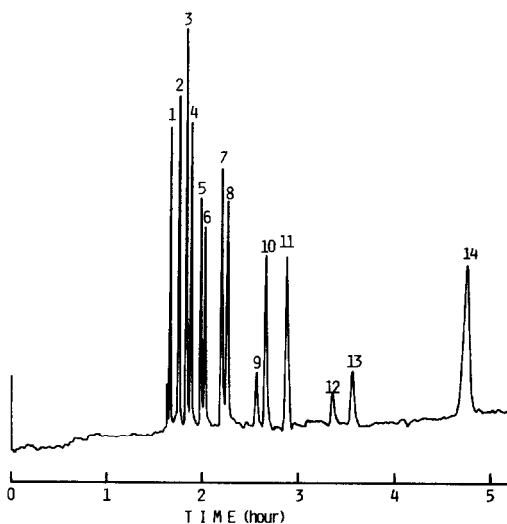| Modifier	Capacity factor	
	Anthracene	Pyrene
$n = 11$	0.53	0.87
$n = 13$	0.66	1.06
$n = 15$	1.43	2.57

Fig. 4. Separation of polynuclear aromatic hydrocarbons on a long column. Column: 39 m × 38 μm I.D. (treated at 45°C). Mobile phase: acetonitrile–methanol–water (39:1:60) containing 0.01% (w/v) of cetrimide. Inlet pressure: 40 atm [pumped with LC-5A (Shimadzu)]. Column temperature: 41°C. Wavelength of UV detection: 254 nm. Samples: 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, benzo[a]pyrene; 14, 1,3,5-triphenylbenzene.
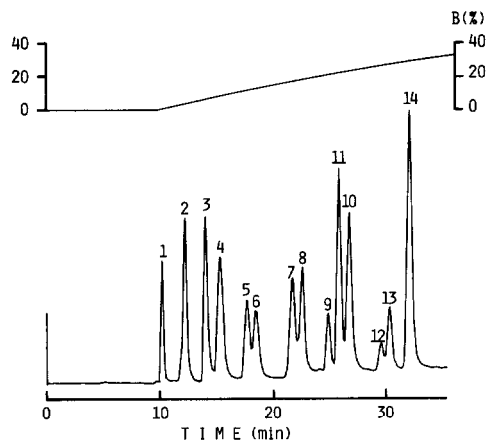
Fig. 5. Gradient separation of polynuclear aromatic hydrocarbons. Column: 7.8 m × 35 μm I.D. (treated at 45°C). Mobile phase: (A) acetonitrile–methanol–60 mM phosphate solution (20:10:70) containing 0.06% (w/v) of cetrimide, pH = 7.37; (B) acetonitrile–methanol (75:25) containing 0.06% (w/v) of cetrimide, gradient profile as indicated. Flow-rate: 1.04 μl/min. Column temperature: 31°C. Wavelength of UV detection: 254 nm. Samples: as in Fig. 4.

absolute temperature for dynamically modified silica columns in the range 17–89°C.

The temperature of the treatment with alkaline solution also affected the retention of solutes on a dynamically modified column. The capacity factor increased with increasing treatment temperature owing to the increase in the density of the silica gel, as shown in Table II. Clogging problems were encountered at treatment temperatures higher than 55°C.
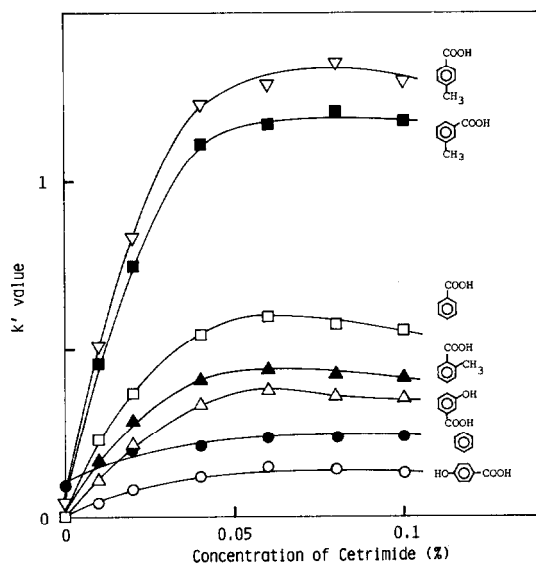

Fig. 6. Dependence of capacity factors on concentration of cetrimide. Column: 7.8 m × 35 μm I.D. (treated at 45°C). Mobile phase: acetonitrile–methanol–60 mM phosphate solution (17:10:73) containing cetrimide, pH = 8.38. Column temperature: 29–31°C.
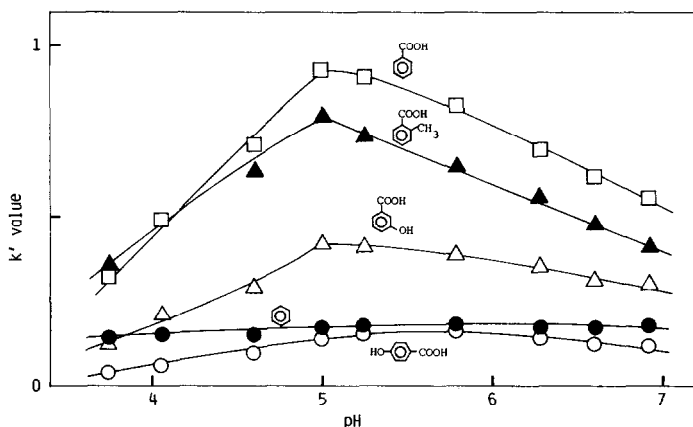
Fig. 7. Effect of pH of the mobile phase on the retention of solutes. Column: as in Fig. 6. Mobile phase: acetonitrile–methanol–60 mM phosphate solution (20:10:70) containing 0.06% (w/v) of cetrimide. Column temperature: 31°C.

Table III shows the effect of the chain length of the surfactants on the retention of solutes. Higher capacity factors were observed for modifiers with longer chain groups. The efficiencies of dynamically modified silica columns were nearly the same as those of chemically bonded ODS columns[12].

Fig. 4 demonstrates the separation of polynuclear aromatic hydrocarbons on a long dynamically modified silica column. Theoretical plate numbers of 30,000 are produced for the last peak on this column. As linked detergents cannot easily be released from the silica surface, it is possible to carry out gradient elution. A gradient separation of polynuclear aromatic hydrocarbons on a dynamically modified silica column is shown in Fig. 5. The difference in the elution order between Figs. 4 and 5 is mainly due to the difference in column temperatures.

It is reported that dynamically modified chromatography using a long-chain detergent such as cetrimide can separate ionizable compounds owing to ion-pair formation[1,5]. Dynamically modified open-tubular silica columns were applied to the separation of ionizable compounds and the effects of the concentration of cetrimide in the mobile phase and the pH of the mobile phase were examined.

Fig. 6 illustrates the dependence of the capacity factors of the solutes on the concentration of cetrimide. The pH was adjusted to 8.38 with 60 mM potassium dihydrogen orthophosphate and dipotassium hydrogen orthophosphate. The capacity factors increase with increasing concentration of cetrimide up to ca. 0.06% and remain nearly constant at concentrations above 0.06%. The degree of variation of the capacity factors versus concentration of cetrimide for ionizable compounds is larger than that for benzene, which suggests that the retention mechanism involves ion-pair formation. Benzoic acid is eluted later than o-toluic acid, which can be explained by steric hindrance by the methyl group, preventing good ion-pair formation. In addition, slight retardation of some solutes on the column without the detergents in the mobile phase indicates that a portion of the detergent is strongly linked on the surface.

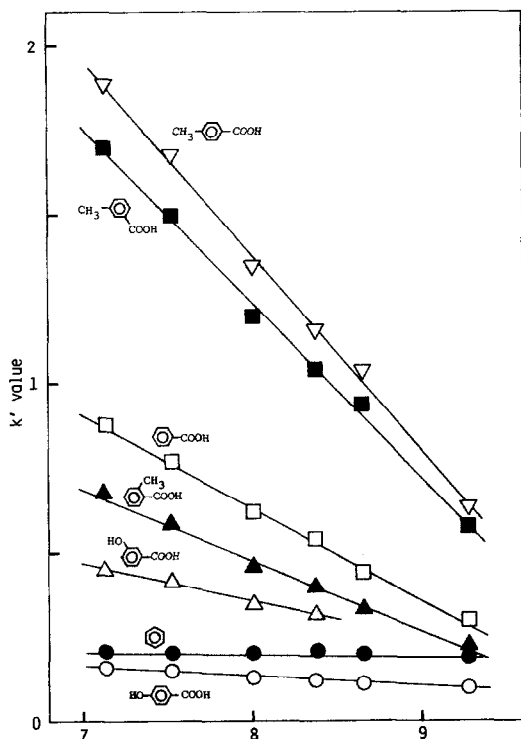Figs. 7 and 8 show the effect of the pH of the mobile phase on the retention

Fig. 8. *Effect of pH of the mobile phase on the retention of solutes.* Column: as in Fig. 6. Mobile phase: acetonitrile–methanol–60 mM phosphate solution (17:10:73) containing 0.06% (w/v) of cetrimide. Column temperature: 30°C.
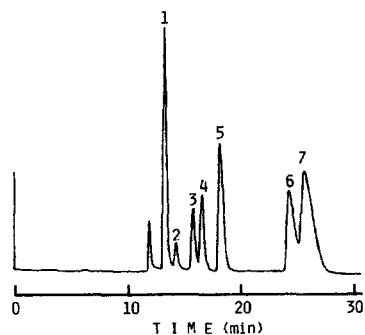

Fig. 9. *Separation of ionizable compounds.* Column: as in Fig. 6. Mobile phase: acetonitrile–methanol–60 mM phosphate solution (17:10:73) containing 0.06% (w/v) of cetrimide, pH = 8.38. Flow-rate: 0.69 μl/min. Column temperature: 29°C. Wavelength of UV detection: 233 nm. Samples: 1, *p*-hydroxybenzoic acid; 2, benzene; 3, *m*-hydroxybenzoic acid; 4, *o*-toluic acid; 5, benzoic acid; 6, *m*-toluic acid; 7, *p*-toluic acid.

of solutes. A pH of *ca.* 5 gave the maximum capacity factors for ionizable solutes, while the variation of the capacity factors of benzene was small in the pH region examined. The pH of the mobile phase affects the degree of ion formation by the detergent with ionizable solutes and silanol groups. These phenomena seem to be very complex and will require further study before an explanation can be given.

Fig. 9 shows a separation of ionizable compounds. Isomers are well separated in this system.

CONCLUSION

Open-tubular dynamically modified silica columns worked like chemically bonded ODS columns and could separate polynuclear aromatic hydrocarbons and ionizable compounds. Open-tubular dynamically modified silica columns will replace chemically bonded columns when narrow-bore columns are to be employed.

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