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Note

Polyallylamine-coated silica gel microbore column for liquid chromatography

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Pellicular-type packing materials for liquid chromatography have been prepared by coating and cross-linking the amine or imine compounds on the surface of silica gel beads. Columns coated with polyamide[1] and polyvinylpyrrolidone[2] were applied to the separation of aromatic acids and phenols. Poly(ethyleneimine) (PEI)-coated and cross-linked silica gel columns have been developed and applied to the liquid chromatography of proteins[3] and oligonucleotides with up to 35 bases[4].

In previous papers[5,6], PEI-coated microbore columns have been used for the separation of isomeric derivatives of aromatic acids and phenols. Polyallylamine (PAA) is a recently commercialized compound with amino group side-chains. This paper describes the use of PAA, coated on silica gel columns.

MATERIALS AND METHODS

Chromatography was carried out with a Shimadzu LC-5A pump (Shimadzu, Kyoto, Japan) and a UV detector (a Shimadzu SPD-2AM or a JASCO UVIDEC II, Japan Spectroscopic, Tokyo, Japan). The sample injector was a Rheodyne micro injector 7410 (0.5-μl loop) or a JASCO micro loop injector (0.05 μl).

A solution of PAA hydrochloride was mixed with sodium hydroxide solution to neutralize the hydrochloric acid. To coat the silica gel beads, they were suspended in a sodium hydroxide solution of PAA hydrochloride (35 mg/ml) and dispersed with a sonicator. After leaving for 1 h, the slurry was packed into 0.8 mm I.D. or 0.33 mm I.D. stainless-steel tubes, using a JASCO FLC 700 syringe-type pump under a pressure of 250–400 kg/cm^2. After washing with water until the eluate pH decreased to 7, the column was equilibrated with the eluent. These columns have a porous Teflon frit at the end of the tubes.

PAA hydrochloride (mol.wt. 8500–11 000) was obtained from Nitto Boseki (Osaka, Japan), and spherical silica gel beads (Develosil 10, particle diameter 10 μm, and Develosil 3, 3 μm) were from Nomura Chemicals (Seto, Japan). Other chemicals were purchased from Nakarai Chemicals (Kyoto, Japan).

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RESULTS AND DISCUSSION

PAA could not be coated on to the silica gel surface from an aqueous solution of PAA hydrochloride. Therefore, base was added to the solution to neutralize the hydrochloric acid.

The optimum amount of alkaline reagent for the column preparation was examined. The retention of nitrophenols was used to estimate the column capacity and selectivity. Retention increased with the ratio of sodium hydroxide added and stabilized at a constant value above the equimolar ratio of base to hydrochloric acid in the PAA hydrochloride solution. Therefore, an equimolar amount of base was added to the PAA hydrochloride as a coating solution.

The retention of *p*-hydroxybenzoates is plotted in Fig. 1A as a function of PAA concentration. The retention of methyl and ethyl esters of *p*-hydroxybenzoate increased with the concentration of PAA. On the other hand, the retention of propyl and butyl esters and *o*-phenylphenol was at a maximum at 1% PAA concentration, and decreased as the PAA concentration increased. Above 10% PAA all compounds acquired constant capacity factors (k').

The plots of the k' values of mono- and dinitrophenols against PAA concentration are shown in Fig. 1B. The retention of dinitrophenols were affected more strongly than that of mononitrophenols, and maximum k' values were again attained with 10% PAA solution.

The carbon contents of the PAA-coated Develosil 3 prepared from 1% to 10% PAA concentrations increased from 3.2% to 9.6%; above 10% PAA, the carbon content became constant.

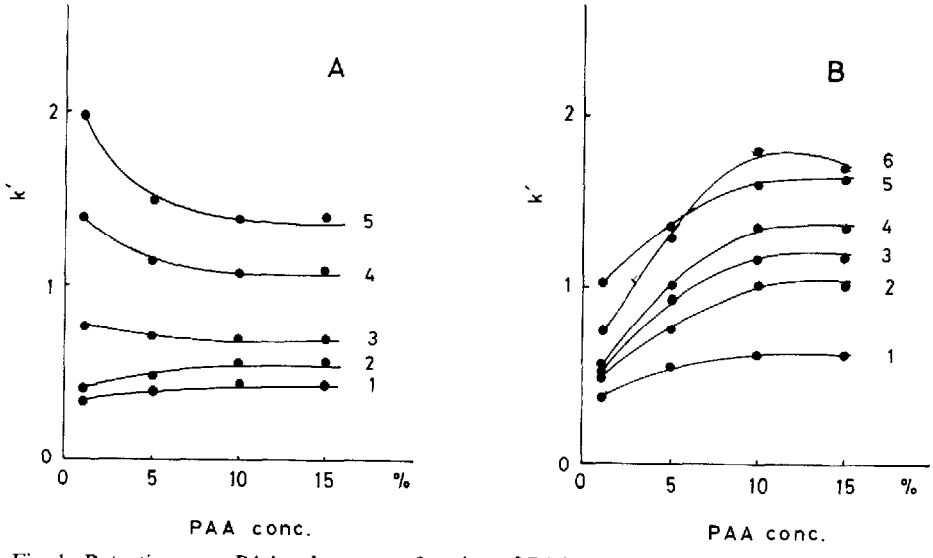

Fig. 1. Retention on a PAA column as a function of PAA concentration in the coating solutions. (A) *p*-Hydroxybenzoate esters and *o*-phenylphenol. Curves: 1 = methyl *p*-hydroxybenzoate; 2 = ethyl *p*-hydroxybenzoate; 3 = *n*-propyl *p*-hydroxybenzoate; 4 = *o*-phenylphenol; 5 = *n*-butyl *p*-hydroxybenzoate. (B) Nitrophenols. Curves: 1 = *m*-nitrophenol; 2 = *p*-nitrophenol; 3 = 2,5-dinitrophenol; 4 = 2,4-dinitrophenol; 5 = *o*-nitrophenol; 6 = 2,6-dinitrophenol. Temperature, ambient; eluent, 5 m*M* sodium hydrogen carbonate.

Silanol groups on the silica gel surface, which carry a negative charge, form ion pairs with the free amino groups in PAA, so that PAA molecules will cover the surface of silica gel. At lower concentrations, PAA molecules can spread completely over the silica surface, and most of the amino groups pair with silanol groups with the hydrocarbon chain on the outside. In this manner, the silica gel is covered by a monolayer of PAA and behaves as a reversed-phase stationary matrix. Therefore, *p*-hydroxybenzoates with large hydrocarbon groups, such as propyl and butyl esters and *o*-phenylphenol, are more strongly retained. As the concentration of PAA increases, some of the amino groups in PAA cannot form ion pairs with silanol groups. Hence the PAA molecule attaches itself partially to the silica gel surface with the remaining amino groups free in solution. The polarity of the stationary phase then increases as the PAA concentration increases. The retention of propyl and butyl esters and *o*-phenylphenol decreased with increasing PAA concentration in the coating solution, whereas more polar compounds, such as methyl and ethyl esters, were oppositely affected. Nitrophenols may be adsorbed by hydrogen bonding and/or charge-transfer interaction at the hydroxyl and nitro groups in the solute molecules on the amino groups of the stationary PAA molecule.

A chromatogram for hydroxybenzoic acid isomers obtained with a 170 × 0.8 mm I.D. column and a 5 m*M* sodium hydrogen carbonate eluent is shown in Fig. 2A. Mono-, di- and trinitrophenols were separated under the same conditions. Retention of nitrophenols increased with the number of nitro groups in the solute molecules (Fig. 2B).

PAA-coated silica gel beads with a particle diameter of 3 μm was packed into a 50 × 0.33 mm I.D. column. The separations of dinitrophenols and nitrophenols are depicted in Figs. 3A and B, respectively.

The PAA column retained phenol isomers such as nitrophenols, chlorophenols and *p*-hydroxybenzoate esters and showed excellent selectivity. However, the column showed very weak interaction with benzoic acids over an eluent pH range from 4 to 9. These results may indicate that the major interaction forces between the phenolic

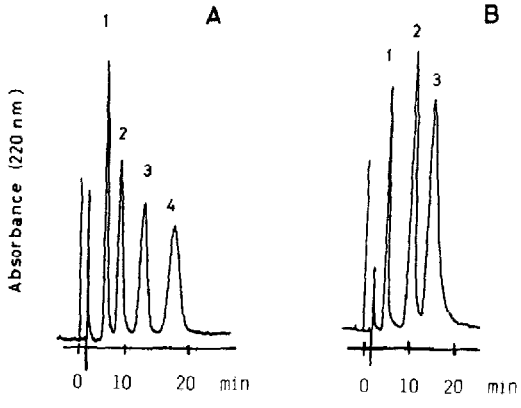

Fig. 2. Chromatograms of hydroxybenzoic acids and nitrophenols. (A) Hydroxybenzoic acid isomers. Peaks: 1 = benzoic acid; 2 = *o*-hydroxybenzoic acid; 3 = *m*-hydroxybenzoic acid; 4 = *p*-hydroxybenzoic acid. (B) Nitrophenols. Peaks: 1 = *m*-nitrophenol; 2 = 2,4-dinitrophenol; 3 = 2,4,6-trinitrophenol. Column, polyallylamine-coated Develosil 10, 170 × 0.8 mm I.D.; eluent, 5 m*M* sodium hydrogen carbonate; flow-rate, 50 μl/min; temperature, ambient; sample volume, 0.5 μl; detection wavelength, 220 nm.

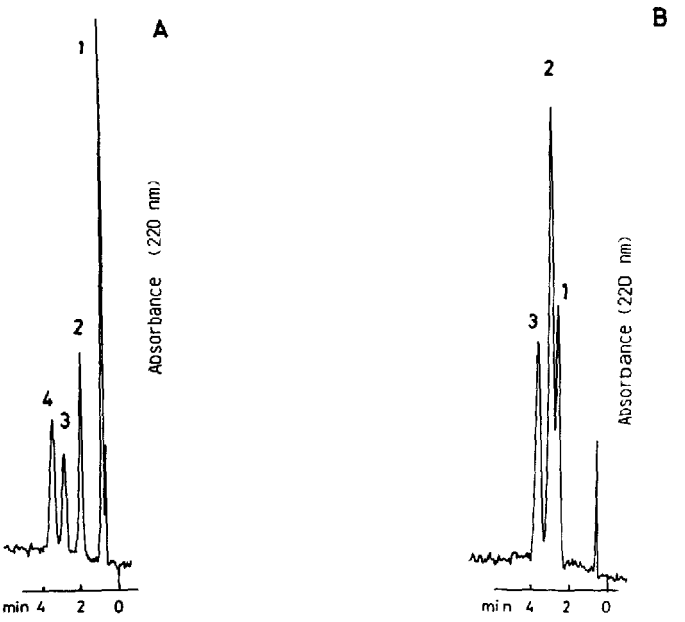

Fig. 3. Chromatograms of nitrophenols and dinitrophenols. (A) Nitrophenol isomers. Peaks: 1 = phenol; 2 = *m*-nitrophenol; 3 = *p*-nitrophenol; 4 = *o*-nitropenol. (B) Dinitrophenol isomers. Peaks: 1 = 2,5-dinitrophenol; 2 = 2,4-dinitrophenol; 3 = 2,6-dinitrophenol. Column, polyallylamine-coated Develosil 3, 50 × 0.33 mm I.D.; PAA hydrochloride concentration in coating solution, 10%; eluent, 5 m*M* sodium hydrogen carbonate (pH 8); flow-rate, 8 μl/min; pressure, 40 kg/cm²; temperature, 40°C; detection wavelength, 220 nm; sample volume, 0.05 μl.

compounds and the PAA column are hydrogen bonding and charge-transfer interaction which cooperate with hydrophobic interactions. On the other hand, ion-exchange interaction does not take part in the retention mechanisms of these compounds.

The PAA-coated pellicular-type column was easy to prepare and will be useful for the separation of isomeric phenolic compounds. The columns were stable for more than three weeks with carbonate eluents.

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