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Synthesis and characterization of a strong cation exchanger based on polymer-coated silica for high-performance liquid chromatography

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Abstract

A strong cation exchanger based on silicone polymer-coated silica (PC-SCX) was synthesized by the following three-step procedure: (1) coating porous silica with a silicone polymer; (2) introduction of epoxy groups with allyl glycidyl ether; (3) introduction of sulfonic groups with sulfanilic acid. The surface structure of PC-SCX was characterized by solid-state ^{13}C-nuclear magnetic resonance spectrometry (NMR) and elemental analysis. In comparison with conventional silica-based cation exchangers, PC-SCX showed an improved durability and a better reproducibility in different synthetic batches. PC-SCX was applied to separation of several different classes of standard cationic compounds, such as biogenic amines, water-soluble vitamins and an antibacterial agent. © 1997 Elsevier Science B.V.

Keywords: Stationary phases, LC; Polymer coatings; Vitamins; Amines; Nucleic acid bases; Benzalkonium chloride

1. Introduction

Ion-exchange chromatography has been used for the separation of various biochemical and pharmaceutical mixtures [1]. Commercially available ion exchangers can roughly be divided into two types, polymeric resins, such as cross-linked polystyrene, and those based on silica [2,3]. Polymeric resins commonly used for ion-exchange chromatography possess a relatively high ion-exchange capacity (4~5 mequiv./g) and usually show a high chemical stability. These resins, however, have several drawbacks, such as a limited pressure stability, and a swelling nature in organic solvents.

Advantages of silica-based ion exchangers are a high efficiency resulting from the high mechanical strength of silica particles, and the absence of the swelling problem. However, the ion-exchange capacity of silica-based ones is usually around 0.3~0.5 mequiv./g, which is much lower than that of the polymeric resins. Also, the silica packings have a limitation in the mobile phase pH. The Si–O–Si linkages are known to be easily hydrolyzed under a water-rich mobile phase at low pH, which is commonly used for the separation of protonated analytes [4,5].

Undesirable secondary effects of residual silanol groups also complicate some applications of silica-based ion exchangers. In addition, it is sometimes difficult to obtain reproducible results with packing materials produced in a different synthetic batch. These problems all seem to come from the wet

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chemistry between silica and the reaction solutions involved in the synthetic procedure; chemical reactions on the silica surface may not be as straightforward as those in homogeneous solutions. In spite of these problems, silica-based cation exchangers are being used in many applications because of their high separation efficiency. Improvements in terms of chemistry, either in their synthetic procedure, or in their performance in applications, are greatly demanded.

A novel polymer-coating technique to form reactive polymethylsiloxane films on metal oxides by chemical vapor deposition of 1,3,5,7-tetramethylcyclotetrasiloxane (H4) was developed by Fukui and coworkers [6]. The polymer-coating technique was utilized to prepare chemically stable C_{18} and other packing materials based on silica [7–11]. These C_{18} silica packings showed an improved resistance at pH 2~10, in comparison with conventional silica packings [7]. Since their original silica surface was totally covered by the silicone polymer, these polymer-coated silica packings were found suitable for analysis of chelating compounds, which had been considered difficult to deal with due to their interaction with the silica surface [12].

In this paper, it was attempted to develop a strong cation exchanger using the polymer-coating technique developed for silica [13], as one of the solutions to the problems found in conventional silica-based strong cation exchangers for liquid chromatography (LC). A synthetic procedure, surface characterization, separation characteristics, and overall performance of the polymer-coated cation exchanger will be discussed.

2. Experimental

2.1. Reagents and materials

High-purity silica (Shiseido, Tokyo, Japan) (particle diameter, 5 μm; pore size, 80 Å; specific surface area, 410 m^2/g; metal impurities, <5 ppm) was used as a starting material for the polymer-coated strong cation exchanger (PC-SCX). 1,3,5,7-Tetramethylcyclotetrasiloxane (H4), a silicone monomer used for the polymer coating, was purchased from Toshiba Silicone (Tokyo, Japan). All reagents and solvents used to synthesize the packing material were of special grade from Nacalai Tesque (Kyoto, Japan) and were used as received. Nucleic acid bases used as standard compounds were obtained from Tokyo Kasei (Tokyo, Japan). Water-soluble vitamins and biogenic amines were purchased from Nacalai Tesque. Water was purified with a Milli-Q system (Nihon Millipore Kogyo, Tokyo, Japan). A Nucleosil 5SA column (250×4.6 mm I.D.), which was purchased from Sensyu Kagaku (Tokyo, Japan) and a Capcell Pak C_{18} UG120 (250×4.6 mm I.D., Shiseido) were used for comparative studies as described later in the text.

2.2. Synthesis of the polymer-coated strong cation exchanger

2.2.1. Preparation of polymer-coated silica

The H4-coated silica was prepared according to the method of Fukui and coworkers [6]. The H4 molecules were deposited on the silica surface, where they were polymerized to form a thin reactive film. The measured thickness of the homogeneous polymer layer was approximately 7 Å, which corresponds to that of a monolayer. The reactive Si–H groups (2.10 mmol/g) on the silica surface were utilized for the subsequent modifications.

2.2.2. Introduction of epoxy groups

Allyl glycidyl ether (100 g) was reacted with the Si–H groups of the H4-coated silica (100 g) in refluxing 2-isopropanol (200 ml) for 5 h under a nitrogen atmosphere in the presence of hexachloroplatinic acid (50 mg) [9]. When the hydrosilylation was complete, the solvent mixture was removed with a glass filter, and the filtered epoxysilica was washed thoroughly with 2-isopropanol, methanol, and chloroform. The epoxysilica was dried in vacuo for 8 h at 80°C to remove the residual allyl glycidyl ether completely.

2.2.3. Introduction of sulfonic groups

Sulfanilic acid (342 g) was added to water (600 ml), and the solution was heated gently at 50°C. The pH was adjusted to 8.5 with sodium hydroxide. The epoxysilica was added to the solution and the resultant dispersion was refluxed for 4 h [14]. The

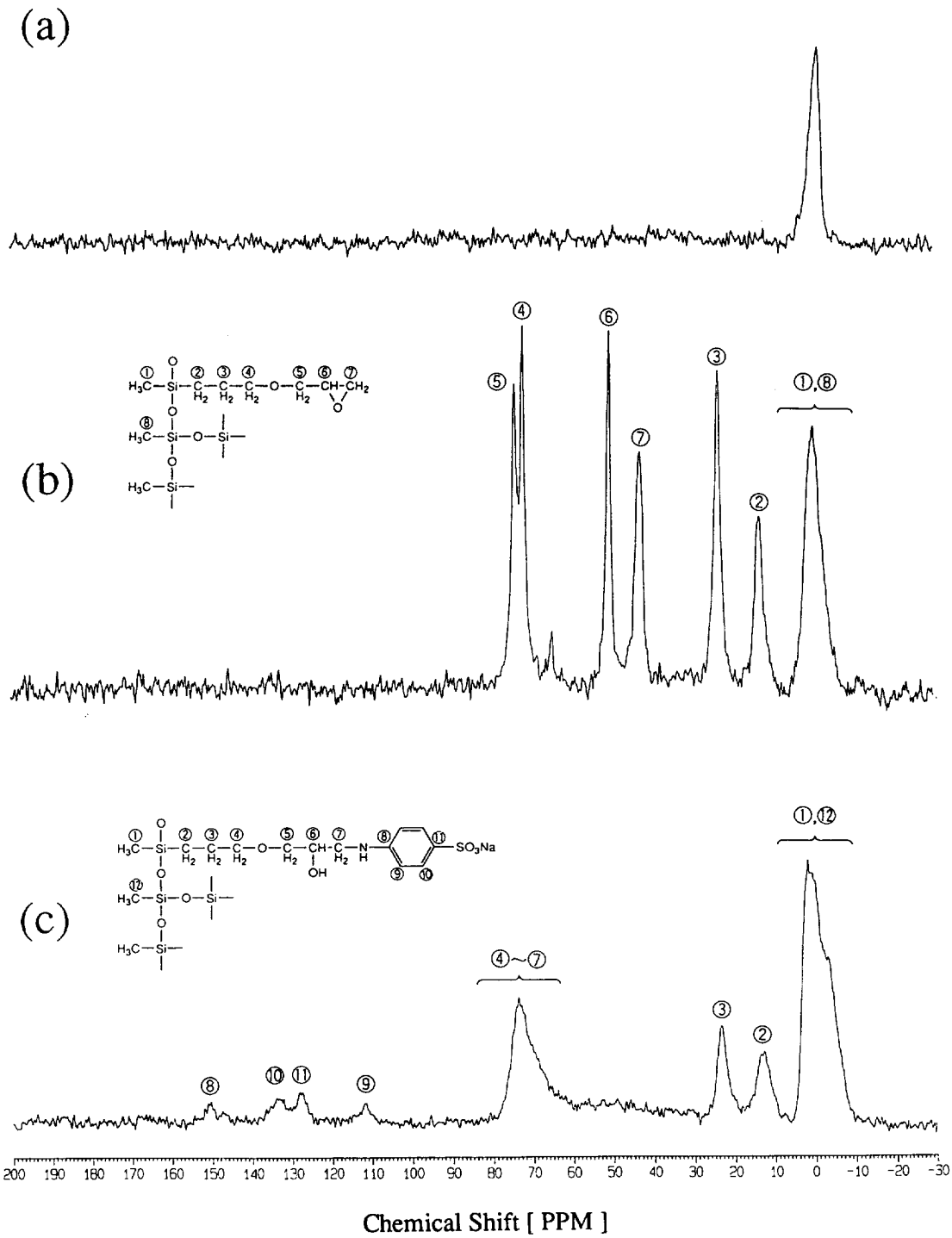

Fig. 1. ^{13}C NMR CP/MAS spectra of the modified silica: (a) polymer-coated silica; (b) modified silica by allyl glycidyl ether; (c) modified silica by sulfanilic acid.

solvent was filtered out while hot, and the silica was washed with water and methanol. The obtained sulfonic silica was dried in vacuo for 8 h at 80°C.

2.3. Characterization of the polymer-coated strong cation exchanger

The amount of reacted groups was evaluated by carbon content measured with a Model 2400 CHN elemental analyzer (Perkin–Elmer Japan, Yokohama, Japan). Ion-exchange capacity was calculated by converting the value of the sulfur content into the amount of the sulfonic group. The sulfur content was also measured in the elemental analysis at the institute of physical and chemical research (Saitama, Japan). Solid-state ^{13}C NMR spectra were measured with an EX-400 (JEOL, Tokyo, Japan) equipped with a cross polarization/magic angle spinning (CP/MAS) module.

The PC-SCX was packed into a stainless-steel column (250×4.6 mm I.D.). Its durability and reproducibility was evaluated by observing the separation of nucleic acid bases in the conditions described later in the text. Chromatographic conditions for other applications will be given in the corresponding figure captions.

2.4. Instrumentation

The HPLC system consisted of a DGU-4A degasser, an LC-9A pump, a SIL-6B autoinjector, an SPD-6AV UV detector, an SCL-6B system controller, and a C-R4AX data processor (all from Shimadzu, Kyoto, Japan).

3. Results and discussion

3.1. Surface structure as a fundamental feature of the cation exchanger

The ^{13}C NMR spectrum of the product at each step of the synthetic procedure is shown in Fig. 1. The peak assignment in the figure was made with the aid of the previous report [7]. The upper spectrum was obtained with the H4-treated (polymer-coated) silica. The broad signal observed around −4 to 0 ppm corresponds to methyl carbons of the polymer

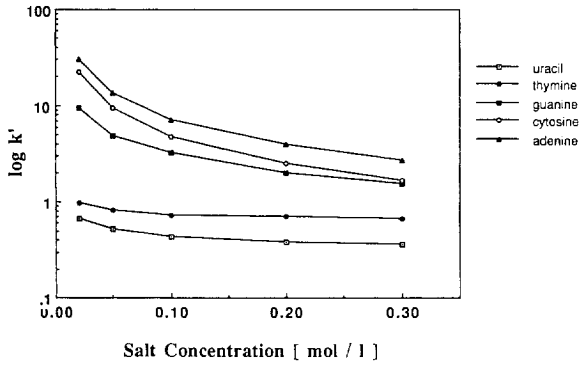

Fig. 2. Effect of salt concentration on retention of five nucleic acid bases. HPLC conditions: column size, 250×4.6 mm I.D.; mobile phase, $NH_4H_2PO_4$ (pH 3.50 adjusted with H_3PO_4); detection, UV 254 nm; temperature, 40°C; flow-rate, 1.0 ml/min.

film formed by H4 molecules. The middle spectrum was obtained with the epoxysilica, the intermediate product. The bottom spectrum was obtained with the final product. It was confirmed that the epoxy carbons seen in the middle spectrum were completely gone in the final spectrum, and that *p*-substituted aromatic carbons were clearly recognized in the final product.

The ion-exchange capacity of PC-SCX was calculated as 0.23 meq/g by converting the sulfur content (0.75%) measured in the elemental analysis. This value was slightly smaller than that obtained with

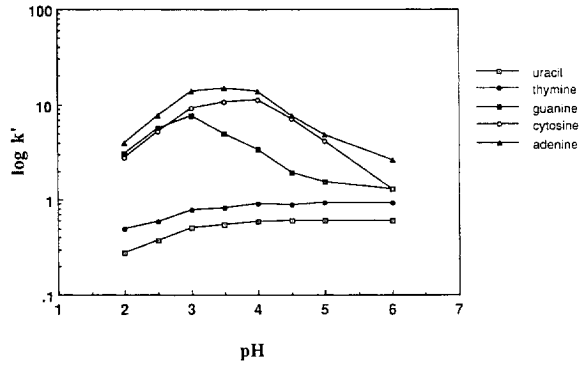

Fig. 3. Effect of pH on retention of five nucleic acid bases. HPLC conditions: column size, 250×4.6 mm I.D.; mobile phase, 0.02 *M* $(NH_4)_2HPO_4$; detection, UV 254 nm; temperature, 40°C; flow-rate, 1.0 ml/min.

Nucleosil 5SA (0.29 mequiv./g, S%=0.93%), a packing material chosen for comparative studies [15].

Retention behaviors of five nucleic acid bases was studied to confirm the function of PC-SCX as a strong cation exchanger for LC. Capacity factors (k') of five bases were plotted versus the salt concentration in the mobile phase. The k' values of all the analytes decreased with the increasing salt concentration (Fig. 2). The retention behaviors observed here seem common to all cation exchangers; the opposite behavior would be expected in reversed-phase LC. The influence of the pH value on the retention behavior of these bases was also studied (Fig. 3). While the k' values of uracil and thymine did not change very much within the pH range, those of guanine, cytosine and adenine changed drastically. These behaviors could be explained by the pK_a values of the analytes.

Since it was prepared from silica, coated with the organic silicone polymer, PC-SCX was expected to possess a reversed-phase nature in addition to that of the cation exchanger, benzalkonium chloride, one of

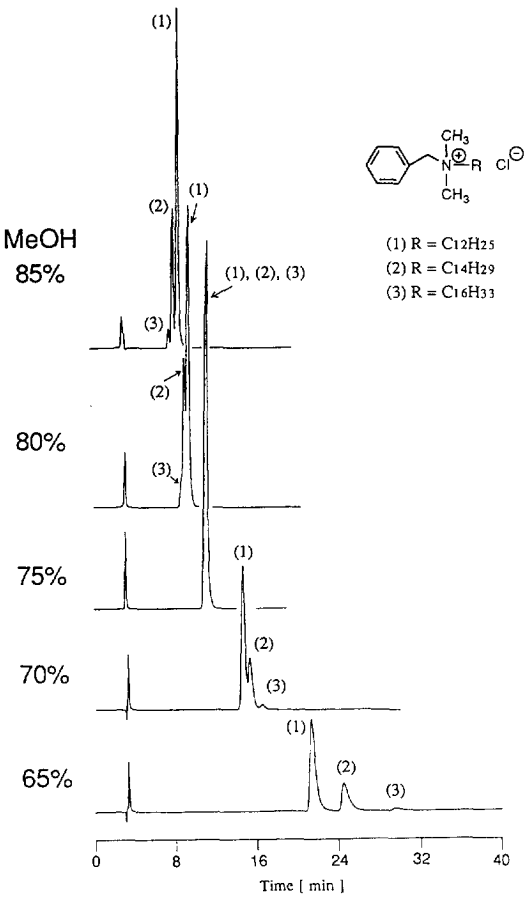

Fig. 4. Effect of organic solvent concentration on separation of benzalkonium chloride homologues. HPLC conditions: column size, 250×4.6 mm I.D.; mobile phase, 50 mM $NaClO_4$ in methanol–water (65:35, 70:30, 75:25, 80:20, 85:15); detection, UV 254 nm; temperature, 40°C; flow-rate, 1.0 ml/min; sample, benzalkonium chloride homologous.

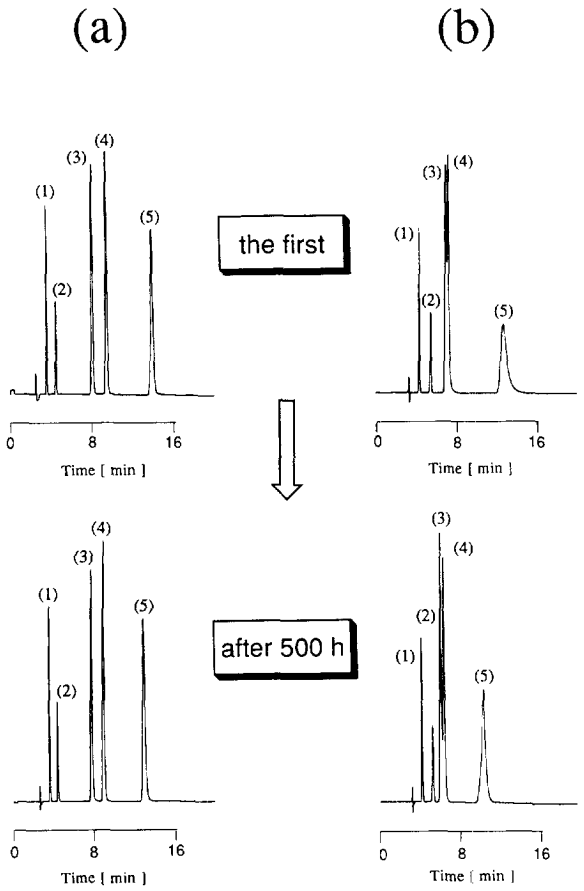

Fig. 5. Chromatograms of the first run and then after 500-h use comparing (a) the PC-SCX with (b) the typical chemical bonded SCX 'Nucleosil 5SA'. HPLC conditions: column size, 250×4.6 mm I.D.; mobile phase, 0.2 M $NH_4H_2PO_4$ (pH 3.50 adjusted with H_3PO_4); detection, UV 254 nm; temperature, 40°C; flow-rate, 1.0 ml/min; peaks: 1=uracil (5 ppm), 2=thymine (5 ppm), 3=guanine (15 ppm), 4=cytosine (30 ppm), 5=adenine (15 ppm); injection volume, 10 μl.

the antibacterial agents used for various products, was chosen as a model compound to observe the separation characteristics of PC-SCX.

Benzalkonium chloride used here consisted of three homologues differing in their alkyl chain length (C_{16}, C_{14}, and C_{12}). All the homologues possess a cationic charge of unity, being a quaternary ammonium ion. Influence of the organic content in the mobile phase on the separation of these homologues is shown in Fig. 4. At low organic (methanol) content, the homologues were eluted in the order of smallest hydrophobicity, which basically follows the rationale of reversed-phase LC. In contrast, when the methanol content was raised, the order of elution was reversed.

The hydrophobic interaction between the benzalkonium homologous and the surface of PC-SCX seemed to decrease when the amount of competing methanol molecules was increased. The separation at high organic contents was thought to be governed by the ion-exchange interaction. The charge-per-weight ratio of the three homologues increases in the order of C_{16} to C_{12}, and the separation at high organic contents seems analogous to that of electrophoresis. It is interesting that the three peaks are bundled together at 75% methanol, where hydrophobic and ion-exchange interactions seem balanced.

3.2. Durability in application and reproducibility in the synthetic procedure

Chromatograms at the initial sample injection and after 500 h under an acidic mobile phase are shown together with those obtained with Nucleosil 5SA (Fig. 5). PC-SCX showed no significant change in the separation profile, while noticeable decreases in retention time and changes in peak shape were observed in Nucleosil 5SA [16]. An improved chemical stability seems to be attained in PC-SCX in comparison with the conventional chemically-bonded silica, whose Si–O–Si linkages are thought to be easily hydrolyzed under acidic conditions [4,5].

Reproducibility in synthetic procedure was evaluated by comparing products of three different batches. A limited reproducibility in different synthetic batches has been one of the major problems in conventional silica-based cation exchangers, which is probably caused by different surface activities in the different silicas used as a starting material [17]. An adequate reproducibility was obtained among PC-SCX lots synthesized from three different silica batches (Fig. 6). It seems that the modification process on the reactive silicone polymer surface is more controllable and reproducible than those on the bare silica surface.

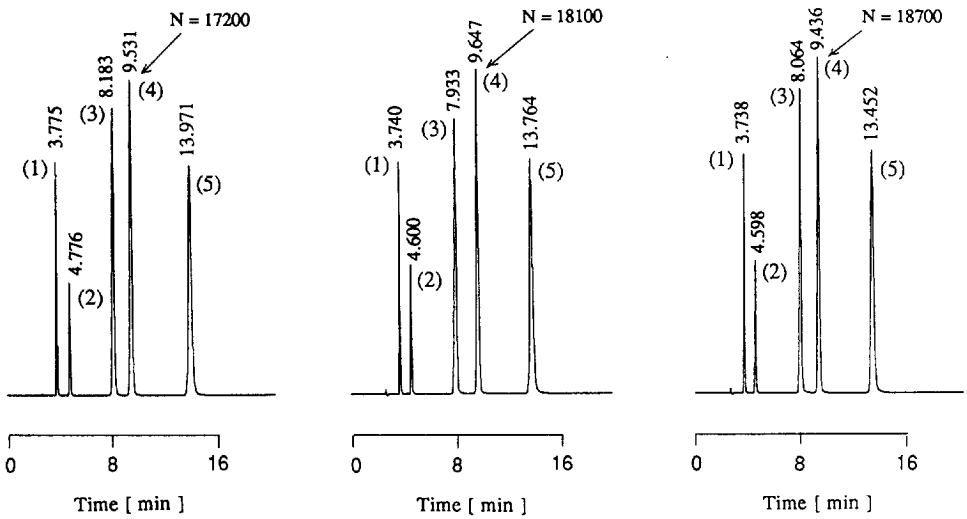

Fig. 6. Chromatograms of the difference batches of synthesis. HPLC conditions: column size, 250×4.6 mm I.D.; mobile phase, 0.2 *M* $NH_4H_2PO_4$ (pH 3.50 adjusted with H_3PO_4); detection, UV 254 nm; temperature, 40°C; flow-rate, 1.0 ml/min; peaks: 1 = uracil, 2 = thymine, 3 = guanine, 4 = cytosine, 5 = adenine.

3.3. Application to several classes of cationic compounds

Separation of eleven biogenic amines was attempted on PC-SCX under neutral pH [18]. The same sample mixture was run on Nucleosil 5SA, and both results are shown in Fig. 7. While most of the amines were base-line separated on PC-SCX, five amines were missing in the chromatogram obtained with Nucleosil 5SA. The missing five amines (DOPA, norepinephrine, epinephrine, dopamine and isoproterenol) were found to share a catechol structure (*o*-dihydroxy structure). Since an *o*-dihydroxy group is highly expected to form a complex with metal atoms at neutral pH, the five amines were probably adsorbed on Nucleosil 5SA in an irreversible manner. PC-SCX seems advantageous in applications for these *o*-dihydroxy compounds with the original silica

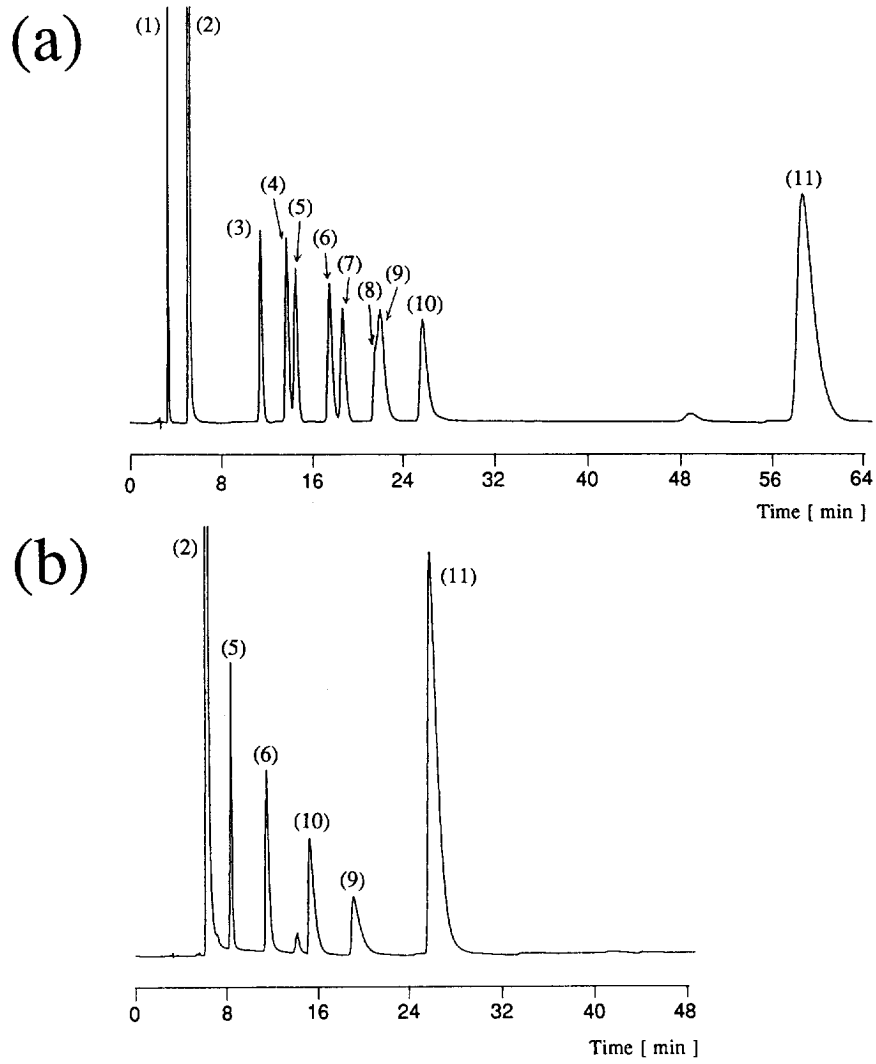

Fig. 7. Chromatograms of the determination of biogenic amines under neutral pH in the comparison of (a) the PC-SCX with (b) the typical chemical bonded SCX 'Nucleosil 5SA'. HPLC conditions: column size, 250×4.6 mm I.D.; mobile phase, 0.2 *M* NaOAc+0.02 *M* AcOH (pH 5.61); detection, UV 254 nm; temperature, 40°C; flow-rate, 1.0 ml/min; peaks: 1=DOPA, 2=creatinine, 3=norepinephrine, 4=epinephrine, 5=octopamine, 6=normetanephrine, 7=dopamine, 8=isoproterenol, 9=metanephrine, 10=tyramine, 11=serotonine.

surface totally eliminated by the polymer coating process.

Ion-pair chromatography based on reversed-phase is another alternative for separation of charged analytes [19]. Ion-pair chromatography is sometimes preferred because of the chemical stability of reversed-phase columns. Separation of water-soluble vitamins by the ion-exchange method with PC-SCX and the ion-pair method with Capcell Pak C_{18}, one of the columns used for reversed-phase chromatography, was compared (Fig. 8). Mobile phases of both methods were arranged so that all of the standard vitamins were eluted within 30 min. Although both separation methods use the cationic nature of analytes, they resulted in very different separation profiles. These two methods will be complementary to each other in optimizing a separation of various cationic compounds when silica-based cation exchangers become as reliable as reversed-phase columns.

A comparison between ion-exchange and ion-pair methods was made from another aspect. Benzalkonium chloride (C_{14} and C_{12}) contained in a commercial antiperspirant as an antibacterial agent was analyzed in both methods (Fig. 9). It is obvious that the better selectivity toward the analyte was obtained in the cation exchanger separation. Cationic ingredients detectable in UV absorption are relatively rare in commercial products, in comparison with non-ionic and anionic counterparts [20]. Since ion-pair chromatography basically cannot discriminate non-ionic components as shown in Fig. 9, the

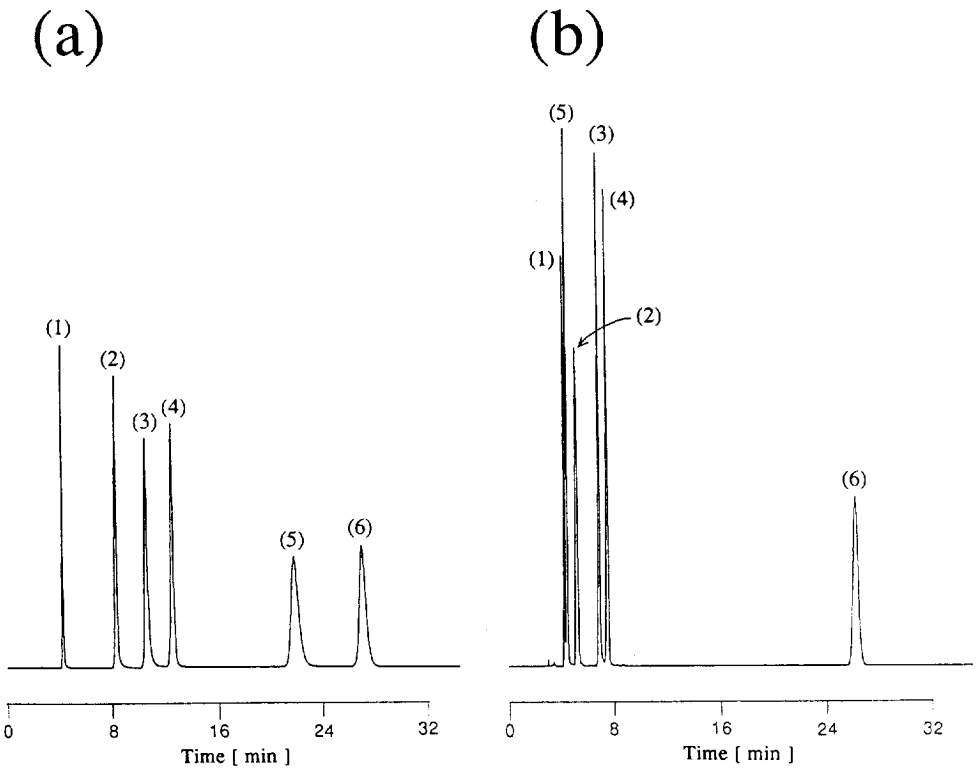

Fig. 8. Chromatograms of standard water-soluble vitamins in the comparison (a) the ion-exchange method by using the PC-SCX column with (b) the ion-pair method by using the reversed phase column. HPLC conditions: column, (a) PC-SCX, (b) Capcell Pak C_{18} UG120; column size, 250×4.6 mm I.D.; mobile phase, (a) 0.2 *M* KH_2PO_4 (pH 3.00 adjusted with H_3PO_4), (b) 10 m*M* 1-octanesulfonic acid sodium salt in acetonitrile–water (18:82) (pH 2.30 adjusted with H_3PO_4); detection, UV 270 nm; temperature, 40°C; flow-rate, 1.0 ml/min; peaks: 1 = nicotinic acid, 2 = nicotinamide, 3 = pyridoxal, 4 = pyridoxine, 5 = riboflavin, 6 = pyridoxamine.

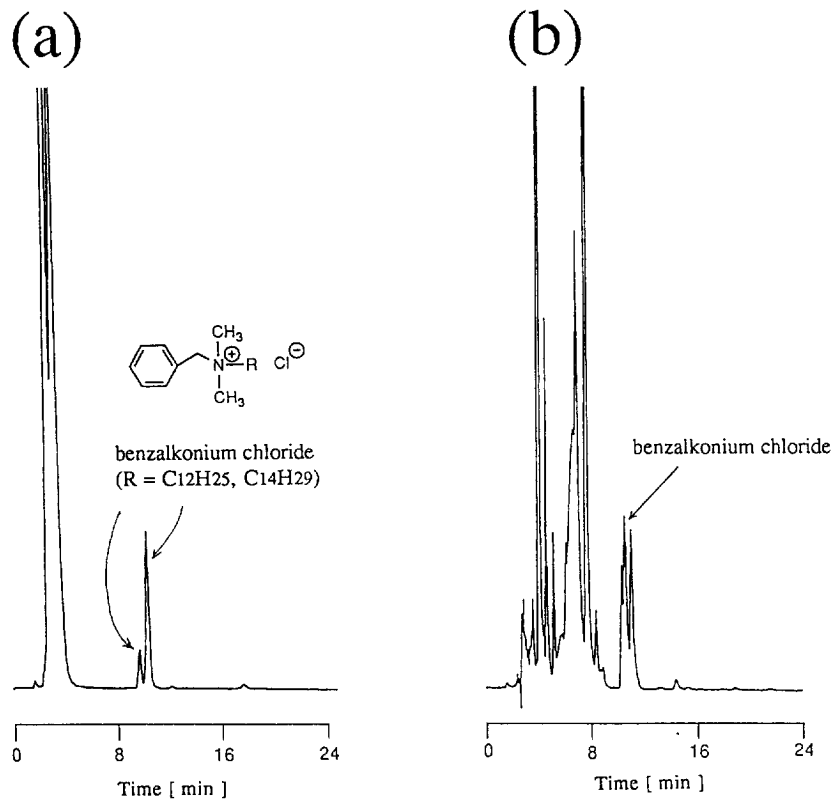

Fig. 9. Chromatograms of the cationic surfactant in the comparison (a) the ion-exchange method by using the PC-SCX column with (b) the ion-pair method by using the reversed-phase column. HPLC conditions: column, (a) PC-SCX, (b) Capcell Pak C_{18} UG120; column size, 250×4.6 mm I.D.; mobile phase, (a) 40 m*M* $NaClO_4$ in methanol–water (85:15), (b) 10 m*M* SDS in methanol–water (78:22); detection, UV 260 nm; temperature, 40°C; flow-rate, 1.0 ml/min; sample, four times diluted solution of the antiperspirant.

chemically stable and lot-reproducible cation exchanger seems advantageous in quality control type of analysis in industry.

4. Conclusions

The surface structure of PC-SCX was characterized by solid-state ^{13}C NMR and elemental analysis. Its fundamental feature as a cation exchanger was confirmed by studying retention behaviors of several standard cationic compounds. PC-SCX showed an improved durability and reproducibility in synthetic procedure, in comparison with the conventional silica-based cation exchanger. The chemically stable cation exchanger seems to be a competitive tool in the separation of various cationic compounds.

Acknowledgments

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