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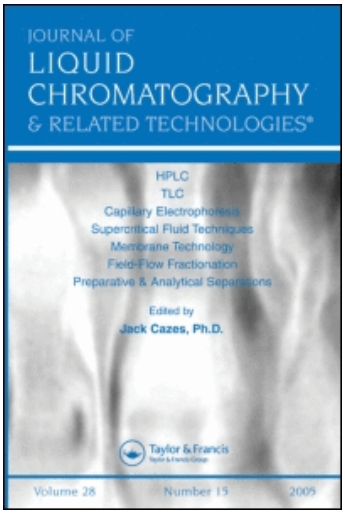

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Ultrashort Open-Tubular Capillary Column with Modified Silica-Gel Thin Layer for Capillary Electrochromatography

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ABSTRACT

A new preparation method has been developed that uses silica-oligomer for the sub-micron silica-gel layer for the open-tubular capillary column (OTC). The silica-gel layer has a unique sub-micron cell structure, which was observed by scanning electron microscope, and this structure contributes to increasing its total surface area. Using ultrashort (2 cm long) OTCs modified with docosyl methyl dichloro silane, separation of neutral compounds has been demonstrated. The column shows a good

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performance, such as theoretical plate number 71,000 plates/m. The column gives a relatively high capacity factor, such as 1.85 for fluorine.

Key Words: Silica-gel thin layer; Sub-micron structure; Open-tubular; Short column; Capillary electrochromatography.

INTRODUCTION

After the initial slow development of capillary electrochromatography (CEC) in the 1980s,[1,2] CEC has become an important tool in separation science in recent years. The capillary columns used in CEC are classified into three types. The most popular type is a capillary column packed with silica-gel or polymer based supports.[3–10] The second type is a capillary column of continuous polymer beds,[11–13] and the third type is an open-tubular capillary column (OTC).[1,14–21]

The capillary packed column has been used practically, while the capillary column of continuous beds is under investigation. Open-tubular capillary column has the simplest design and is easy to use due to the low column flow resistance. Although the OTC has a long history, it lacks efficiency and its sample-loading capacity is relatively low owing to the small surface area of the stationary phase. Several efforts to overcome these deficits have been made in recent years. Reagents, such as sodium hydroxide and ammonium hydrogen difluoride have been used to etch the inner wall of the capillary.[14,15] Colon et al.[16] and Freitag et al.[17] prepared the silica layer using a sol-gel method. The column lengths used were generally from 40 to 100 cm.

One of the current topics in analytical chemistry is the miniaturization of analytical systems, the so-called micro total analytical system (μTAS). In μTAS, a very short OTC may be applicable as a separation unit. In our former report,[23] five biochemical compounds were separated using an ultrashort packed capillary column (the packed length: 1.5 to 2.0 cm; packed with cation exchange supports), and Ericson et al. used a short packed column (effective length 4.5 cm) on a chip.[11]

Short capillary columns and short OTCs are applicable in the fluidic system of μTAS, although short OTCs, such as around 1 cm long, has not been reported.

In this paper, a new preparation method for the silica-gel thin layer on the inner wall of an open tubular capillary is proposed. The new silica-gel thin layer is formed through a polymerizing procedure of silica-oligomers. It is easily modified with alkyl silanes. The newly modified silica-gel stationary phase shows a good separation efficiency, and 2 cm long ultrashort columns modified with docosyl methyl dichloro silane can be used for separations.

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EXPERIMENTAL

Column Preparation

The OTC was prepared by the following procedure. The first step was the pretreatment of the inner wall of the capillary with sodium hydroxide aqueous solution. A fused-silica capillary tubing (i.d. 25 or 30 μm, o.d. 375 μm, supplied from GL Science, Tokyo) was filled with a 1 M sodium hydroxide aqueous solution, and then kept at 80°C for 30 min in a gas chromatograph oven (GC-14A, Shimadzu Ltd., Kyoto). The alkaline solution in the capillary tubing was then washed out with ethanol using a liquid chromatograph pump (LC-8A, Shimadzu), and nitrogen gas was introduced to take ethanol from the capillary.

The second step was the formation of the silica-gel layer on the surface of the inner wall. A 3–24% (v/v) silica-oligomer $\{(-Si(OEt)_2-O-)_n, n = 10 \sim 20\}$ ethanol solution (silica oligomer was synthesized from ethoxysilane and was kindly supplied by PIATEC, Mie, Japan) was dynamically coated using nitrogen gas (2 atm). Then, the capillary was filled with the 5% ammonium aqueous solution and kept for an hour at room temperature. Silica-oligomer on the capillary inner wall was polymerized with ammonia as the catalyst. After ammonium solution in the capillary was flushed out by nitrogen gas, the capillary was heated at 80°C for 10 min, and then programmed at 3°C/min up to 180°C, where it was kept for 15 min.

In the third step, the silica-gel layer formed on the inner wall of the capillary was modified with 5% docosylmethyldichlorosilane (C22), or octadecyltriethoxysilane (C18) in xylene. The alkyl silane–xylene solution was continuously introduced into the capillary under heating at 130°C in a GC oven for one hour. During this procedure, the newly formed thin silica-gel layer was modified with alkyl silane. Then, the capillary column was washed, in series, with toluene and ethanol.

Capillary Electrochromatography Apparatus

A homemade system for the open tubular capillary electrochromatography was composed of a UV detector (UV-970, Jasco, Tokyo), a high voltage power supply (HCZE-30NPO, Matsusada Precision, Shiga, Japan), and two homemade reservoirs, shown in Fig. 1. A UV cell holder was remodeled to a width of 2 cm length, in which a short capillary column was covered. Each homemade reservoir was composed of a polyethylene syringe (2.5 mL, Terumo, Tokyo) and a platinum wire (o.d. 0.5 mm). These reservoirs and a column were set in line and horizontally.[23] Negative high voltage was applied

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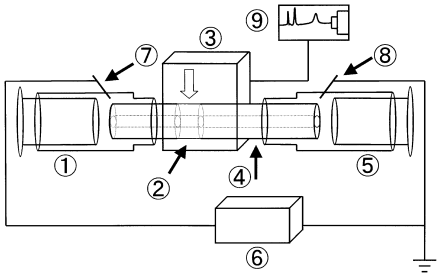

Figure 1. Schematic diagram of miniaturized open-tubular CEC with UV detection. (1) Reservoir with negative electrode; (2) on-column cell window. Outside polyimido coating of fused silica capillary tubing was removed using a small knife; (3) UV detector, home made-cell housing; (4) OTC; (5) reservoir with positive electrode; (6) high voltage power supply; (7) platinum negative electrode being grounded; (8) platinum electrode; (9) recorder. Injection: after changing the reservoir to a polyethylene syringe filled with sample solution, electrokinetic injection was performed.

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to the reservoir at the capillary column outlet, and the electrode of the reservoir at the inlet column was grounded.

Thiourea was used as a non-retained solute. A mixture of phosphate buffer and methanol was used as eluent for the separations of aromatics, and a mixture of citric buffer and acetonitrile for proteins. These eluents were well degassed by an ultrasonic bath before use. All reagents were obtained from Wako Pure Chemical Industry (Osaka, Japan). Electrokinetic injection was performed after exchanging the reservoir, (5) in Fig. 1, to a syringe reservoir filled with the sample solution (called a sample reservoir).

Scanning Electron Microscopic Observation

To observe the formed thin silica-gel layer on the inner wall of the capillary column, the column was split in two pieces along its axis using a sharp knife, or broken into pieces with pliers after it had been cut to lengths of 10 mm. Then, the small pieces of the glass capillaries were placed on the top surface of a sample disk. The top surface of the copper disk was covered with a piece of adhesive electric conductive tape. The glass pieces were spattered first, with carbon and then, with an alloy of platinum and gold. The pieces of the capillary column were observed by a scanning electron microscopy (Type JXA-840 and JSM-6100, JEOL, Tokyo). The scanning electron micrographs obtained are shown in Fig. 2.

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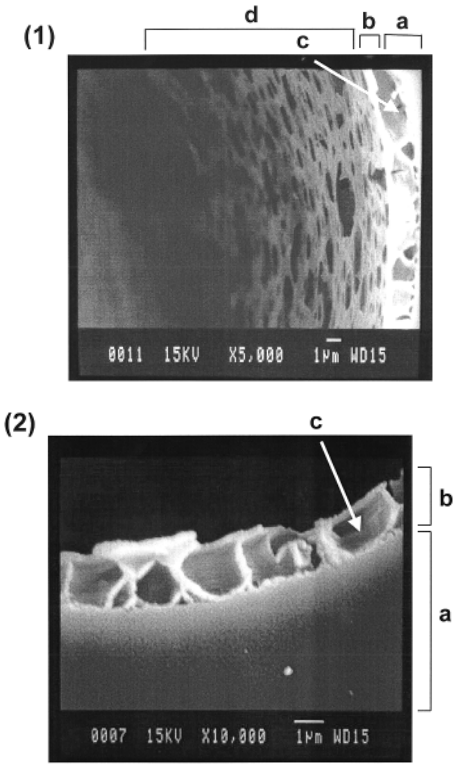

Figure 2. Scanning electron micrographs of silica-gel thin layer formed on the inner wall of capillary tubing. (1) Surface (magnification: 5000) and (2) cross-section of silica-gel thin layer (magnification: 10,000). (a) Fused-silica capillary glass body; (b) cross-sectional view; (c) micro-structure of nano-cells in the layer; (d) surface of the silica-gel layer.

RESULTS AND DISCUSSION

Preparation of Thin Silica-Gel Layer

One of the drawbacks to using an OTC is that the surface area of the stationary phase is insufficient. Therefore, its sample loading capacity is limited and it does not generally give a capacity factor of more than 2. Although, many studies have aimed to form a stationary phase with high surface area, it has not yet been achieved.[1,14–21] Among these attempts, the sol-gel method seems to produce a bed similar to silica-gel supports.

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Constantin and Freitag[17] mixed two silanes (tetraethoxysilane and *n*-octyltriethoxysalane) and had them react for 1 hour at 80°C prior to their introduction into the capillary tubing. Then, the reaction mixture, namely gel, was dynamically coated on the inner wall of the fused-silica capillary tubing, followed aging. Their protocol included the formation of gel outside the capillary tubing. In the present study, a known polymerized silica, oligo-silica, was used. The oligo-silica was the intermediate product in silica-gel supports. Its polymerized number was carefully controlled at the silica-gel producer. It was found, that oligo-silica gel was easily polymerized on the inner capillary wall and produced a fine thin layer on the wall.

In the preparation procedure, first the concentration of oligo-silica gel in ethanol, which was used as a reagent for dynamic coating on the inner wall of capillary tubing, was varied from 3 to 24% (v/v), and best results were obtained by using 9% (v/v) oligo-silica solution for a 30 μm i.d. capillary tubing. The oligo-silica is polymerized by the catalytic action of the ammonia aqueous solution.

The period of ammonia treatment was varied from 1 to 24 hours at room temperature. It was observed that longer ammonia treatment gave larger holes on the surface of the silica-gel thin layer. The scanning electron micrograph, shown in Fig. 2, is of a silica-gel surface formed by 24 hour ammonia treatment. The hole size was not found to be related to the capacity factor or column efficiency in the present experiment. The capillary columns used in Figs. 3 and 4 were treated for 1 hour with ammonia solution.

Dynamic coating with a silica-oligomer solution was attempted one or two times. The silica-gel thin layer, obtained by coating twice, had a higher capacity factor compared with that obtained with a single coating at 75 μm i.d. capillary. Silica-gel thin layers could be formed in every capillary with an inner diameter of 25 μm to 75 μm. The capillary column with a 75 μm i.d. gave a broader peak. Therefore, capillary columns under 50 μm in i.d. may be used for the separation. The advantageous inner diameter of the capillary is 25–30 μm. Silica-gel thin layers in the capillaries with 2–30 μm i.d. were formed with good reproducibility.

Thin Silica-Gel Layer of Sub-micro Structure

Photographs taken by scanning electron microscopy are shown in Fig. 2. These photographs clearly show the structure of the surface and cross section of the thin silica-gel layer formed on the inner wall of the capillary. The thickness of the layer was about 1.0 μm, and small rooms (which we called nano-cells) are aligned up inside the layer, shown at Fig. 2c. There are many holes on the surface [Fig. 2(c)], which may link with the inside nano-cell. The layer of nano-cell microstructure may contribute to increasing the surface area

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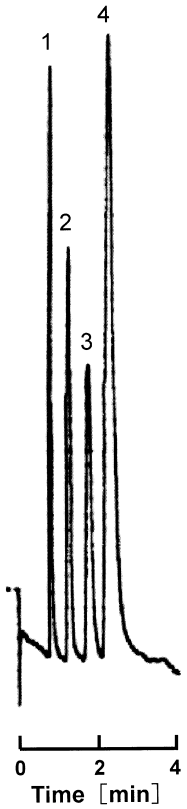

Figure 3. Separation of neutral compounds using a C22 modified OTC. Column: effective length 2.3 cm, whole length 5.0 cm, inner diameter 30 μm, stationary phase C24; Eluent: mixture of 20 mM phosphate buffer (pH 7.0) and methanol (1 : 1); Applied voltage: −1.8 kV; Current: 1.9 μA; Detection: UV 254 nm. Sample: thiourea (1), naphthalene (2), diphenyl (3), and fluorene (4).

of the stationary phase. This nano-cell is a unique characteristic of our newly formed silica-gel layer. The reproducibility of this layer was quite good. After preparation of the new silica-gel layer, it was modified with alkyl silane.

Typical Chromatograms Obtained by Using Ultrashort Open-Tubular Capillary Column

Typical chromatograms obtained using ultrashort OTCs are shown in Figs. 3 and 4. The effective lengths of the OTCs used in Figs. 3 and 4 are

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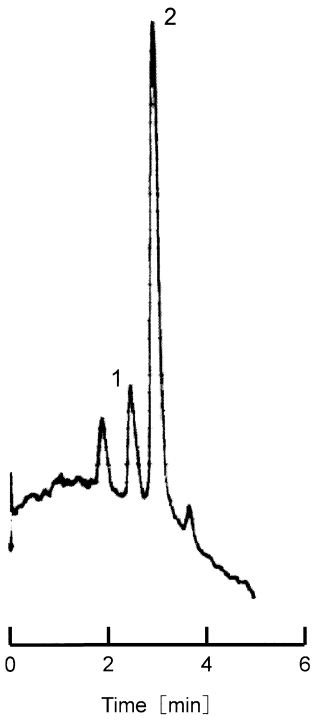

Figure 4. Separation of proteins using a C22 modified short OTC. Peaks 1 and 2 are α-chymotrypsin and lysozyme, respectively. Column: effective length 2.6 cm, whole length 5.2 cm, inner diameter 30 μm; Eluent: mixture of 30 mM citric buffer (pH 3.8) and acetonitrile (8 : 2); Applied voltage: −2.0 kV.

2.3 cm and 2.6 cm, respectively. In Fig. 3, thiourea, naphthalene, diphenyl, and fluorene are separated at the baseline level within 3 min. Even though the columns used are quite short, the chromatogram shown is fine. Thiourea was assumed to be a non-retained solute and its retention time was used for estimating capacity factor. The diphenyl peak gives the theoretical plate number per meter, N, 71,000, and the value of the height equivalent to a theoretical plate, H, is 14 μm. The other unique characteristic is that the present OTC has a relatively large capacity factor. Namely, the capacity factor of fluorene in the present work is 1.85 with the following conditions: inner diameter of the column: 30 μm; eluent: the mixture of methanol and phosphate buffer (1 : 1). The present OTC with silica-gel thin layer provides good performance. These characteristics might be derived by the unique structure of the nano-cells in the silica layer, which have a relatively large surface area.

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A mixture of proteins was separated by using ultrashort OTCs. The separation of α-chymotrypsin and lysozyme using the C22 modified short OTC (effective column length: 2.6 cm) is shown in Fig. 4. The peaks shown in Fig. 4 are sharp and symmetrical. Because the pI values of α-chymotrypsin and lysozyme are 8.1 to 8.6 and 11.0 to 11.4, respectively,[24] both proteins have positive charges in solution of pH 3.8 under the present separation conditions.

CONCLUSION

A new preparation method for OTCs using silica-oligomers was proposed. A unique nano-cell microstructure in the silica-gel layer on the inner wall of the OTC was observed by a scanning electron microscope. From photographs taken by scanning electron micrographs, the surface area of a newly formed silica-gel thin layer appears to have a quite large surface area. Chromatographic runs support this speculation. With very short OTCs, such as 2-cm-long columns, the present OTC separates the media very well. Although the number of theoretical plate per ultrashort OTC is less than that of an ultrashort packed capillary column,[23] the former column has the advantage of easy handling when it is installed in the miniaturized electrochromatographic system. The present procedure for making silica-gel thin layers on the inner wall of the open-tubular capillary will also be applicable to miniaturized separation systems, such as μTAS.

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