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Fluidic Behavior of Polymer Compounds in an Open-Tubular Capillary with Ternary Mixed Carrier Solvents under Laminar Flow Conditions

Takahiro NOGAMI, Satoshi FUJINAGA, Masahiko HASHIMOTO, and Kazuhiko TSUKAGOSHI*

Department of Chemical Engineering and Materials Science, Faculty of Science and Engineering, Doshisha University, Kyotanabe, Kyoto 610-0321, Japan

Abstract

An open-tubular capillary chromatography is developed based on tube radial distribution of the ternary mixed carrier solvents of water-hydrophilic/hydrophobic organic under laminar flow conditions. This is called "tube radial distribution chromatography" (TRDC). Fluidic behavior of the polymer compounds, FluoSpheres (1.0 μm diameter), was examined by the TRDC system. The ternary mixture of water-acetonitrile-ethyl acetate, 15:3:2 (water-rich) or 3:8:4 (organic solvent-rich) volume ratio, as a carrier solution was fed into the fused-silica capillary tube under laminar flow conditions. The peak of the FluoSpheres appeared with the apex at the maximum linear velocity and the slope of the non-Gaussian peak did not last longer than the elution time at the average linear velocity with the water-rich carrier solution. With the organic solvent-rich carrier solution FluoSpheres was eluted at near the average linear velocity.

Keywords: Polymer compounds, FluoSpheres, tube radial distribution phenomenon (TRDP), tube radial distribution chromatography (TRDC)

1. Introduction

Microfluidics provides various types of fluidic behavior of the polymer compounds, artificial one such as polymer particles and nature one such as biopolymer, in a microspace or microchannel. The fluidic behavior of the polymer compounds has been examined by changing the channel configuration and the flow rate of the solvents, using aqueous–organic solvent mixtures, and introducing specific modifications into the microchannel.[1-4] Fluidic behavior of the polymer compounds in the microspace is closely related to mixing, separation, diffusion, and reaction of the nano- and micro-particles. Information about the fluidic behavior of the polymer compounds is important and useful to design a microreactor or micro total analysis systems.

Recently, we determined specific distribution of ternary mixed solvents in a microspace under laminar flow conditions.[6-9] When the ternary mixed solvent solution, i.e., water–hydrophilic/hydrophobic organic solvent mixture, is delivered into a microspace, such as a microchannel or a capillary tube, the solvent molecules are radially distributed in the microspace. With an organic solvent-rich solution, an organic solvent-rich major phase is generated around the middle of the microspace as an inner phase, while a water-rich minor phase is formed near the inner wall as an outer phase. In contrast, with a water-rich solution, the water-rich major phase is generated as an inner phase and the organic solvent-rich minor phase is formed as an outer phase. We call this the "tube radial distribution phenomenon" (TRDP).[8-10]

The TRDP is a novel phase-interface concept and can be applied in various technological, engineering, and other scientific fields. We have developed an open-tubular capillary chromatography based on the TRDP. It is called "tube radial distribution chromatography" (TRDC). In contrast to other capillary separation techniques, such as capillary electrophoresis and capillary electrochromatography, the TRDC system works without applying high voltages or using specific columns, e.g., monolithic or packed.

Although various types of analyte were analyzed using the TRDC system,[11] we have not had enough information about fluidic behavior of the polymer compounds in the TRDP. In this study, tentatively, we examined the fluidic behavior of the polymer compounds, FluoSpheres (1.0 μm diameter), through the TRDP in capillary tubes by use of the TRDC system.

2. Experimental

Water was purified with an Elix 3 UV (Millipore Co., Billerica, MA). All reagents used were commercially available and of analytical grade. Acetonitrile, ethyl acetate, lambda-DNA (48502 bp, 32,300,000 molecular weight) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Carboxylated polymer particles prepared from

*Corresponding author.

E-mail: ktsukago@mail.doshisha.ac.jp

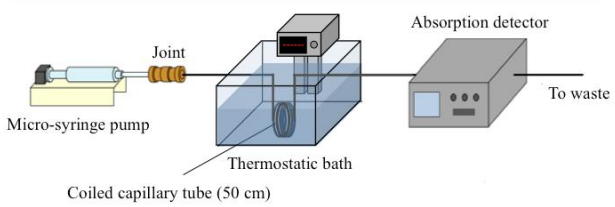

Fig. 1 Schematic diagram of the present capillary chromatographic system.

polystyrene matrix, FluoSpheres (1.0 μm diameter), from Molecular Probes Inc. FluoSpheres were provided as suspension (2% solids, 4×10^{10} particles mL^{-1}) and diluted with a carrier solution as necessary. Fused-silica capillary tubes (50 μm inner diameter) were purchased from GL Science (Tokyo, Japan).

A schematic diagram of the present chromatography system comprised of an open capillary tube, microsyringe pump (55-1111, Harvard Apparatus; Bioanalytical Systems, Inc., West Lafayette, IN), and absorption detector (modified SPD-10AV spectrophotometric detector; Shimadzu Co., Kyoto, Japan) is shown in Fig. 1. The tube temperature was controlled by dipping the capillary tube in water maintained at a definite temperature of 20 °C in a vessel. Water-acetonitrile-ethyl acetate mixtures with volume ratios of 15:3:2 and 3:8:4 were used as water-rich and organic solvent-rich carrier solutions in the TRDC system. The component ratios of 15:3:2 and 3:8:4 were recommended in our previous reports.[9-11] Analyte solutions were prepared with the carrier solutions. In this study, 200 times-diluted FluoSheres and 0.8 nM lambda-DNA (MW 32,300,000; 48502bp) were used as analyte solutions.

The analyte solution was introduced directly into the capillary inlet side by the gravity method.[12] After analyte injection, the capillary inlet was connected through a joint to a microsyringe. The syringe was set on the microsyringe pump. The carrier solution was fed into the capillary tube at a definite flow rate under laminar flow conditions. On-capillary absorption detection (260 nm) was performed with the detector.

3. Results and Discussion

When the present capillary chromatography is carried out with a water carrier solution, the chromatographic system inevitably works as a wide-bore hydrodynamic chromatography for polymer compounds. The wide-bore hydrodynamic chromatography works by a different

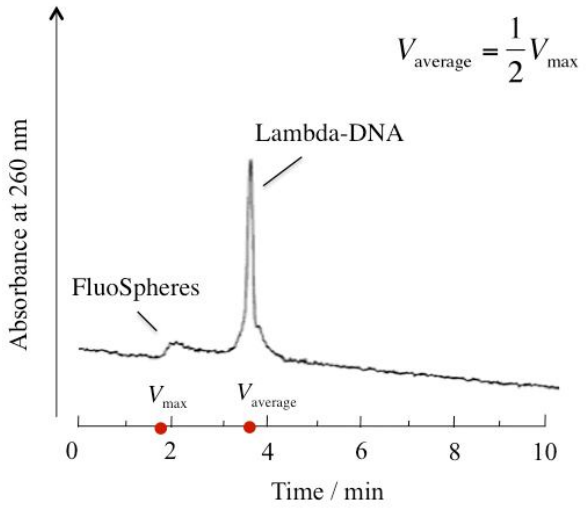

Fig. 2 Chromatograms of Fluospheres and lambda-DNA obtained by wide-bore hydrodynamic chromatography. Conditions: Capillary tube, 110 cm (effective length: 90 cm) of 50 μm i.d. fused-silica; carrier, water; sample injection, 50 cm height (gravity) × 20 s; flow rate, 0.5 μL min^{-1}; tube temperature, 20 °C; and 200 times-diluted FluoSheres and 0.8 nM lambda-DNA. *V*max and *V*average mean the maximum linear velocity and the average linear velocity under the present laminar flow conditions, respectively. The plots on the horizontal line mean the estimated elution times with *V*max and *V*average.

mechanism originating from the solute distribution coupled with the laminar flow conditions.[13-15] A diffusive solute gives an elution curve with an apex at the time required for the average linear velocity to the detector (i.e., such a curve is characterized by a Gaussian peak), whereas a non-diffusive solute gives an asymmetric elution curve with the apex at the maximum linear velocity in a capillary (i.e., such a curve is characterized by a non-Gaussian peak).

As preliminary experiments, FluoShperes as artificial polymer particles and lambda-DNA as nature biopolymer, were examined by the wide-bore hydrodynamic chromatography mode using a water carrier solution (not containing any chemicals or additives). The obtained chromatogram is shown in Fig. 2 together with analytical conditions. FluoSpheres were eluted first at maximum flow rate under the laminar flow conditions with non-Gaussian peak and then lambda-DNA was eluted with near the average linear velocity with Gaussian peak. They showed typical elution patterns on the chromatograms by the wide-bore hydrodynamic

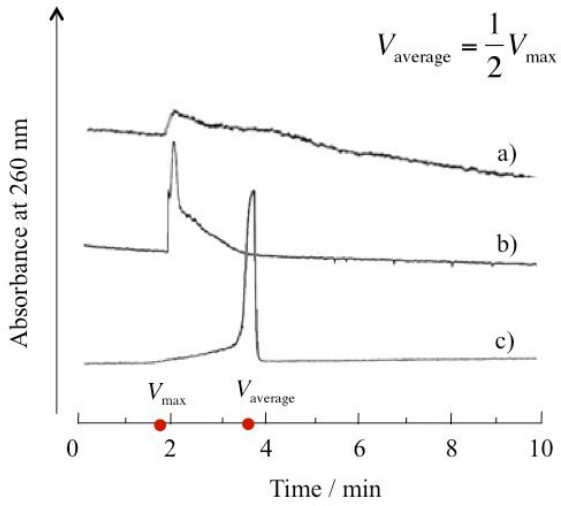

Fig. 3 Chromatograms of FluoSpheres obtained by the present chromatographic system. a) Wide-bore hydrodynamic chromatography mode, b) TRDC mode with the water-rich carrier solution, and c) TRDC mode with the organic solvent-rich carrier solution. Conditions: Capillary tube, 110 cm (effective length: 90 cm) of 50 μm i.d. fused-silica; carrier, a) water, b) water-rich carrier solution (water-acetonitrile-ethylacetate, 15:3:2 volume ratio), c) organic solvent-rich carrier solution (water-acetonitrile-ethylacetate, 3:8:4 volume ratio); sample injection, 50 cm height (gravity) × 20 s; flow rate, 0.5 μL min^{-1}; tube temperature, 20 °C; and 200 times-diluted Fluosheres. *V*max and *V*average mean the maximum linear velocity and the average linear velocity under the present laminar flow conditions, respectively. The plots on the horizontal line mean the estimated elution times with *V*max and *V*average.

chromatography as described above. FluoroShere was non-diffusive and lambda-DNA was diffusive solutes in the water carrier.

Lamda DNA that is rather large molecular weight features hydrophilic and partially hydrophobic character, although it depends on the conditions such as temperature, solved solvent, and added additives. The solubility of lambda-DNA was examined in the carrier solutions for the TRDC system. Lambda-DNA was dissolved with the water-rich carrier solution but was not dissolved with the organic solvent-rich carrier solution due to its hydrophilic nature. In addition the absorption spectra of lambda-DNA gradually changed in the water-rich carrier solution. Lambda-DNA would change from double helix conformation to single coil one with ethyl acetate. Such a change was not observed with a water-acetonitrile mixture solution.

Only FluoSpheres were examined by the system as shown in Fig. 1 with a water carrier solution (wide-bore hydrodynamic chromatography mode), a water-acetonitrile-ethylacetate mixture solution (15:3:2; volume ratio) (TRDC mode using the water-rich carrier solution), and a water-acetonitrile-ethylacetate mixture solution (3:8:4; volume ratio) (TRDC mode using the organic solvent-rich carrier solution). The obtained chromatograms are shown in Fig. 3 together with analytical contitions. The conditions were determined referring to our previous papers.[6,7,11)]

With the water carrier solution FluoSpheres showed typical chromatogram of non-diffusive solute in a wide-bore hydrodynamic chromatography (Fig. 3 a)). The data of FluoSpheres obtained with the TRDC system (Fig. 3 b) and c)) were different from that obtained by the wide-bore hydrodynamic chromatography (Fig. 3 a)). In the TRDC system using the water-rich carrier solution, although the peak of the FluoSpheres appeared with the apex at the maximum linear velocity in the capillary tube, the tailing slope of the non-Gaussian peak did not last longer than the elution time at the average linear velocity in the tube (Fig. 2 b)). FluoSpheres were comparatively hydrophilic and dispersed around the middle of the tube in the water-rich phase and little in the organic solvent-rich outer phase. On the other hand, with the organic solvent-rich carrier solution FluoSpheres were eluted at near the average linear velocity with almost Gaussian peak. It might be attributed to few dissociated carboxyl groups of FluoSpheres in the organic solvent-rich solution and higher diffusion coefficient of organic solvents (acetonitrile and ethyl acetate) to the solutes than that of water. We will try to consider the chromatographic data together with computer simulation and fluorescence microscopy observation in the future.

4. Conclusions

We tried to examine the fluidic behavior of the polymer compounds, FluoSheres (1.0 μm diameter), with the TRDC system based on the TRDP. FluoSheres showed the specific fluidic behavior with the TRDC system compared to the chromatogram obtained the wide-bore hydrodynamic chromatography. The data also meant that the TRDC system had potential to deal with various types of analyte, from low molecular weight ones to polymer compounds, with the same system and similar procedure. Information about the fluidic behavior of the polymer compounds obtained here is interesting in the TRDP or TRDC study, and will be useful to

design a microreactor or micro total analysis systems related with polymer compounds.

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References

[1] M. Yamada, M. Nakashima, M. Seki, Anal. Chem., **76**, 5465 (2004).
[2] M. Yamada, M. Seki, Anal. Chem., **78**, 1357 (2006).
[3] Y. Yamaguchi, D. Ogura, K.Yamashita, M. Miyazaki, H. Nakamura, H. Maeda, Talanta, **68**, 700 (2006).
[4] J. Zheng, H.-W. Li, E. S. Yeung, J. Phys. Chem. B., **108**, 10357 (2004).
[5] N. Kaji, Y. Tezuka, Y. Takamura, M. Ueda, T. Nishimoto, H. Nakanishi, Y. Horiike, Y. Baba, Anal. Chem., **76**, 15 (2004).
[6] N. Jinno, M. Itano, M. Hashimoto, K. Tsukagoshi, Talanta, **79,** 1348 (2009).
[7] N. Jinno, M. Murakami, M. Hashimoto, K. Tsukagoshi, Anal. Sci., **26**, 737 (2010).
[8] N. Jinno, M. Murakami, K. Mizohata, M. Hashimoto, K. Tsukagosi, Analyst, **136**, 927 (2011).
[9] N. Jinno, M. Hashimoto, K. Tsukagoshi, Anal. Sci., **27**, 259 (2011).
[10] M. Murakami, N. Jinno, M. Hashimoto, K. Tsukagoshi, Anal. Sci., **27**, 793 (2011).
[11] S. Fujinaga, N. Jinno, M. Hashimoto, K. Tsukagoshi, J. Sep. Sci., **34**, 2833 (2011) and the references cited therein.
[12] Y. Tanigawa, N. Jinno, M. Hashimoto, K. Tsukagoshi, Chromatography, **32**, 135 (2011).
[13] T. Okada, M. Harada T. Kido, Anal.Chem., **77**, 6041 (2005).
[14] T. Masudo, T. Okada, J. Chromatogr. A., **196,** 1106 (2006).
[15] M. Harada, T. Kido, T. Masudo, T. Okada, Anal. Sci., **21**, 491 (2005).

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