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Separation of Optical Isomers in Capillary Chromatography Using a Poly(tetrafluoroethylene) Capillary Tube and an Aqueous–Organic Mixture Carrier Solution

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Capillary chromatography for the separation of optical isomers was developed using an untreated poly(tetrafluoroethylene) capillary tube and a water–hydrophilic/hydrophobic organic solvent mixture as a carrier solution. The open tubular capillary was 110 cm in length (90 cm effective length) and 100 μm in inner diameter. The carrier solution was prepared with a water–acetonitrile–ethyl acetate mixture (15:3:2 volume ratio) containing 1 mM β-cyclodextrin. A model analyte solution of dansyl-DL-methionine was injected into the capillary tube by a gravity method. The analyte solution was subsequently delivered through the capillary tube with the carrier solution by a microsyringe pump; the system worked under laminar-flow conditions. The analytes were separated through the capillary tube with on-capillary detection by an absorption detector. D-Isomer and L-isomer were eluted in this order with the water–acetonitrile–ethyl acetate carrier solution including β-cyclodextrin.

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Capillary chromatography, including capillary electrochromatography, micellar electrokinetic capillary chromatography, and capillary high-performance liquid chromatography, has attracted a great deal of attention since the last century. However, only a few new concepts concerning capillary chromatography have been reported in the last decade.[1,2] We reported an interesting capillary chromatography system using an untreated open tubular capillary made of fused silica, polyethylene, or poly(tetrafluoroethylene) (PTFE) and a water–hydrophilic/hydrophobic organic solvent mixture carrier solution. This is called the "tube radial distribution chromatography (TRDC) system".[3-5] To date, the TRDC system has mainly been used for the separation and detection of model analyte solutions, including hydrophilic and hydrophobic organic compounds.

The separation performance in the TRDC system is proposed as follows: when a water-rich carrier solution is delivered into the capillary tube, a water-rich phase (major inner phase) is generated around the center of the tube far from the inner wall of the tube, while an organic solvent-rich phase (minor outer phase or capillary wall phase) forms near the capillary inner wall, based on the tube radial distribution of the carrier solvents under laminar-flow conditions. On the other hand, when an organic solvent-rich carrier solution is fed into the tube, an organic solvent-rich phase (major inner phase) is generated around the middle of the tube, while, a water-rich phase (minor outer phase or capillary wall phase) forms near the inner wall. Subsequently, hydrophilic and hydrophobic analytes are distributed between the inner and outer phases in the capillary tube undergoing chromatographic separation. The elution times of the analytes can be easily changed by altering the component ratios of the carrier solvents in all types of capillary tube, such as fused silica, polyethylene, and PTFE.

Optical isomer separation is one of the most important aspects of analytical chemistry and separation science.[6-9] In this study, although investigations regarding the TRDC system are still in the preliminary stages, we attempted to apply the system to the separation of optical isomers to examine its separation potential.

Experimental

Water was purified with an Elix UV 3 (Millipore Co.). All reagents used were commercially available and of analytical grade. 2,6-Naphthalenedisulfonic acid, 1-naphthol, acetonitrile, ethyl acetate, and β-cyclodextrin were purchased from Wako Pure Chemical Industries, Ltd. Dansyl-DL-methionine and dansyl-L-methionine were purchased from Sigma-Aldrich Co. PTFE capillary tubes (100 μm i.d., 200 μm o.d.) were purchased from Yasaka Industries, Inc.

The capillary chromatography system consisted of an open capillary tube, a microsyringe pump (MF-9090; Bioanalytical Systems, Inc.), and an absorption detector (modified SPD-6AV spectrophotometric detector; Shimadzu Co.). The PTFE capillary tube, 110 cm in length (effective length: 90 cm), was set in the system. A mixture of water–acetonitrile–ethyl acetate (15:3:2 volume ratio) containing 1 mM β-cyclodextrin was used as a carrier solution. The analyte solution was prepared with the carrier solution.

The analyte solution was introduced directly into the capillary inlet side for 5 s from a height of 20 cm by the gravity method. After analyte injection, the capillary inlet was connected through the joint to a microsyringe. The syringe was set on the microsyringe pump. The carrier solution was fed in the capillary

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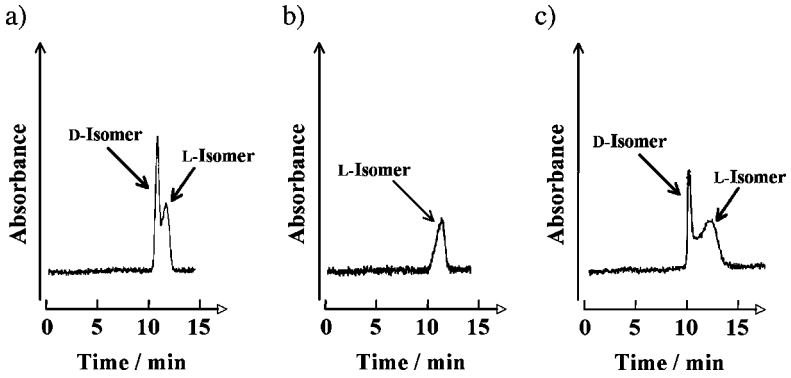

Fig. 1 Chromatograms of the mixture of a) 500 μM dansyl-DL-methionine, b) 250 μM dansyl-L-methionine, and c) 500 μM dansyl-DL-methionine plus 250 μM dansyl-L-methionine mixture. Conditions: capillary tube, 110 cm (effective length, 90 cm) of 100 μm i.d. PTFE; carrier, water–acetonitrile–ethyl acetate (15:3:2 volume ratio) mixture solution containing 1 mM β-cyclodextrin; sample injection, 20 cm height (gravity) × 5 s; flow rate, 0.8 μL min^{-1}.

tube at a flow rate of 0.8 μL min^{-1} under laminar-flow conditions. On-capillary absorption detection (254 nm) was performed with the detector.

Results and Discussion

We applied the TRDC system to optical isomer separation. A combination of β-cyclodextrin as a host molecule (chiral selector) and dansyl-DL-methionine as a guest molecule (optical isomers) was adopted as a model for optical isomer separation with reference to previous reports.[8,9] As preliminary experiments, a solution of 2,6-naphthalenedisulfonic acid (1 mM) and dansyl-DL-methionine (500 μM) was analyzed using a normal TRDC system with the water–acetonitrile–ethyl acetate (15:3:2 volume ratio) carrier solution not including β-cyclodextrin. 2,6-Naphthalenedisulfonic acid and dansyl-DL-methionine were eluted with baseline separation in this order, although the optical isomer separation of dansyl-DL-methionine was not performed. With the water-rich carrier solution, 2,6-naphthalenedisulfonic acid (hydrophilic) was eluted with near the average linear velocity and dansyl-DL-methionine (comparatively hydrophobic) was fed with a lower velocity than the average linear velocity under laminar-flow conditions.

Next, a solution of 2,6-naphthalenedisulfonic acid (1 mM) and 1-naphthol (1 mM), typical model analytes for the TRDC system, was analyzed with the TRDC system using the water–acetonitrile–ethyl acetate (15:3:2 volume ratio) carrier solution not including or including β-cyclodextrin (1 or 2 mM), in order to know any influence of β-cyclodextrin as an amphiphilic molecule in the carrier solution on the system. 2,6-Naphthalenedisulfonic acid (hydrophilic) and 1-naphthol (hydrophobic) were separated and detected in this order based on the TRDC separation performance with the carrier solution not containing or containing 1 mM β-cyclodextrin, although the presence of β-cyclodextrin (1 mM) slightly made the peak of 1-naphthol broader. On the other hand, they were not separated at all with the carrier solution including 2 mM β-cyclodextrin; both of them were eluted with near the average linear velocity. For the moment, it is difficult to exactly discuss an interaction between 1-naphthol and β-cyclodextrin in the capillary tube. An excess of β-cyclodextrin, which is an amphiphilic molecule, in the carrier solution may disturb the formation of the inner and outer phases in the capillary tube in the TRDC system. However, at least, it was confirmed that TRDC separation could be brought out with the carrier solution containing 1 mM β-cyclodextrin.

The solutions of 500 μM dansyl-DL-methionine, 250 μM dansyl-L-methionine, and 500 μM dansyl-DL-methionine plus 250 μM dansyl-L-methionine were subjected to the present TRDC system with the water–acetonitrile–ethyl acetate (15:3:2 volume ratio) carrier solution containing 1 mM β-cyclodextrin. The chromatograms obtained for the above three analyte solutions are shown in Fig. 1. The elution times and the peak shapes observed on the chromatograms clearly showed that the D-isomer and L-isomer in dansyl-DL-methionine were separated and detected in this order by the present TRDC system.

As shown in Fig. 1, the D-isomer was eluted with near the average linear velocity and the L-isomer was eluted with lower velocity than the average linear velocity. As shown in preliminary experiments, dansyl-DL-methionine was eluted with a lower velocity than the average linear velocity based on the nature of hydrophobicity with the carrier solution not including β-cyclodextrin; that is, dansyl-DL-methionine was comparatively hydrophobic. In the water-rich carrier solution containing 1 mM β-cyclodextrin, β-cyclodextrin was mainly distributed in the major inner phase or the water-rich phase. Dansyl-D-methionine has a larger interaction with β-cyclodextrin as a chiral selector than dansyl-L-methionine.[9] The interaction between dansyl-D-methionine and β-cyclodextrin must alter the distribution of the D-isomer from the outer phase to the inner phase, leading to an earlier elution time of the D-isomer than the L-isomer for separation.

In conclusion, the optical isomer separation of dansyl-DL-methionine as a model was performed by the TRDC system with a water-rich carrier solution containing 1 mM β-cyclodextrin. The observed elution order of the D-isomer and L-isomer was successfully explained based on the tube radial distribution of the carrier solvents, providing the major water-rich phase and the minor organic solvent-rich phase. The results obtained here regarding optical isomer separation provide important insight to expand the TRDC system for future research.

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