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Capillary chromatography based on tube radial distribution of aqueous–organic mixture carrier solvents

Naoya Jinno, Minoru Itano, Masahiko Hashimoto, Kazuhiko Tsukagoshi*

Department of Chemical Engineering and Materials Science, Faculty of Science and Engineering, Doshisha University, 1-3 Miyakodani, Tatara, Kyotanabe, Kyoto 610-0321, Japan

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ABSTRACT

A capillary chromatography system was developed using open capillary tubes made of fused-silica, polyethylene, or poly(tetrafluoroethylene), and an aqueous–organic mixture (water–acetonitrile–ethyl acetate mixture) as a carrier solution. Model analyte mixture solutions, such as 2,6-naphthalenedisulfonic acid and 1-naphthol, Eosin Y and perylene, bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein and 1,1'-bi-2-naphthol, and 2,7-naphthalenedisulfonic acid and *p*-nitroaniline, were injected into the capillary tube by a gravity method. The analyte solutions were subsequently delivered through the capillary tube with the carrier solution by a micro-syringe pump. The system worked under laminar flow conditions. The analytes were separated through the capillary tube and detected on-capillary by an absorption detector. For example, 2,6-naphthalenedisulfonic acid and 1-naphthol were detected in this order with a carrier solution of water–acetonitrile–ethyl acetate (volume ratio 15:3:2), while they were detected in the reverse order with a carrier solution of water–acetonitrile–ethyl acetate (volume ratio 2:9:4). The other analyte solutions were similarly separated by the system. The elution times of the analytes could be easily reversed by changing the component ratio of the solvents in the carrier solution.

1. Introduction

Capillary chromatography including capillary electrochromatography [1,2], micellar electrokinetic capillary chromatography [3,4], and capillary high-performance liquid chromatography using packed and monolithic capillary columns [5,6] have attracted great attention in the analytical chemistry field and in separation science ever since the last century. Most capillary chromatography systems feature rapid measurements, easy procedures, inexpensive and small apparatus, small sample volumes, and low costs. However, only a few new concepts concerning capillary chromatography have been proposed in the last decade. In one example, wide-bore hydrodynamic chromatography [7,8] was carried out using an open capillary tube of fused-silica. Diffusive and non-diffusive analytes showed quite different elution behavior in the capillary tube under laminar flow conditions. However, these analytes were not completely separated using the principle of the method, i.e., the diffusive and non-diffusive analytes were overlapped on the chromatogram. In a second example, Tabata et al. has recently reported a capillary chromatography system based on microphase separation of mixed solvents [9]. They described that when an acetonitrile–water mixture with suitable salt concentration was pumped into a fused-silica capillary in which the inner wall was negatively charged due to the dissociation of its silanol group, microphase separation occurred near the capillary wall, resulting in a water-enriched aqueous phase attached to the capillary inner wall. The mixture of hydrophilic and hydrophobic analytes was separated in this order by chromatography.

This paper reports a capillary chromatography system using open capillary tubes made of either fused-silica, polyethylene, or poly(tetrafluoroethylene) (PTFE), and a salt-free water–hydrophilic–hydrophobic organic solvent mixture carrier solution. In our previous communication, we briefly described the results of a fused-silica or polyethylene capillary tube that was applied to the system [10], and here we mainly report on the results concerning a PTFE capillary tube. Four analyte solution mixtures of hydrophilic and hydrophobic molecules (2,6-naphthalenedisulfonic acid and 1-naphthol, Eosin Y and perylene, bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein and 1,1'-bi-2-naphthol, and 2,7-naphthalenedisulfonic acid and *p*-nitroaniline) were examined in the present system and found to separate well. Separation in the capillary chromatography system was performed without the use of any specific packed capillary tubes, additives of gels, surfactants or salts, or high-voltage supply devices. Furthermore, the elution order of the analytes could be changed easily by altering the component ratio of the solvents in the carrier solution. The separation performance of the system is discussed based on the results obtained using fused-silica, polyethylene, and PTFE capillary tubes. The elution behavior of the

* Corresponding author. Tel.: +81 774 65 6595; fax: +81 774 65 6803.
E-mail address: ktsukago@mail.doshisha.ac.jp (K. Tsukagoshi).

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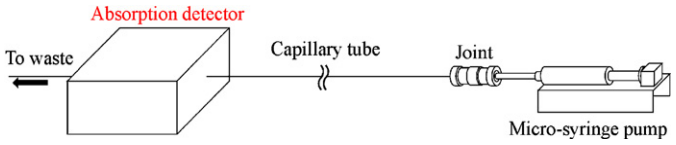

Fig. 1. Schematic diagram of the capillary chromatography system.

analytes in the system provided some novel information that will be useful in separation science.

2. Experimental

2.1. Reagents and capillary tubes

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, perylene, Eosin Y, 1,1'-bi-2-naphthol, *p*-nitroaniline, 2,7-naphthalenedisulfonic acid, acetonitrile, and ethyl acetate were purchased from Wako Pure Chemical Industries, Ltd. Bis[*N*,*N*-bis(carboxymethyl) aminomethyl]fluorescein was purchased from Dojindo Laboratories. A fused-silica capillary tube (50 μm i.d., 150 μm o.d.), a high-density polyethylene capillary tube (200 μm i.d., 500 μm o.d.), and a PTFE capillary tube (100 μm i.d., 200 μm o.d.) were purchased from GL Science, Natume Co., and Yasaka Industries, Inc., respectively.

2.2. Apparatus and procedures

A schematic diagram of the present capillary chromatography system comprised of an open capillary tube, micro-syringe pump (MF-9090; Bioanalytical Systems, Inc.), and absorption detector (modified SPD-10AV spectrophotometric detector; Shimadzu Co.) is shown in Fig. 1. A fused-silica or polyethylene capillary tube, 120 cm in length (effective length: 100 cm), and a PTFE capillary tube, 90 cm in length (effective length: 70 cm), were placed in the system. Water–acetonitrile–ethyl acetate mixtures with volume ratios of 15:3:2, 4:7:9, 2:5:9, 2:7:4, and 2:9:4 were used as carrier solutions. Analyte solutions were prepared with the carrier solutions.

The analyte solution was introduced directly into the capillary inlet side for 20 s from a height of 20 cm for the fused-silica tube, for 5 s from a height of 20 cm for the polyethylene tube, or for 5 s from a height of 20 cm for the PTFE tube by the gravity method. After analyte injection, the capillary inlet was connected through a joint to a micro-syringe. The syringe was set on the micro-syringe pump. The carrier solution was fed in the capillary tube at a flow rate of 0.2 μL min^{-1} for fused-silica, 8.0 μL min^{-1} for polyethylene, or 0.8 μL min^{-1} for PTFE under laminar flow conditions. On-capillary absorption detection (232, 254, or 280 nm) was performed with the detector.

3. Results and discussion

3.1. Properties of PTFE capillary tube

Although a PTFE capillary tube is useful and available for microflow separation systems because of its inert inner surface and flexibility [11,12], it should be noted that the crystallinity of the PTFE causes light scattering. Therefore, we first examined the optical properties of the PTFE capillary tube as follows. The PTFE capillary was fixed to the modified absorption detector. The capillary was filled with water, and absorption in the capillary tube was examined at 200–650 nm. The obtained results are shown in Fig. 2. The capillary showed maximum absorption at 200 nm which decreased

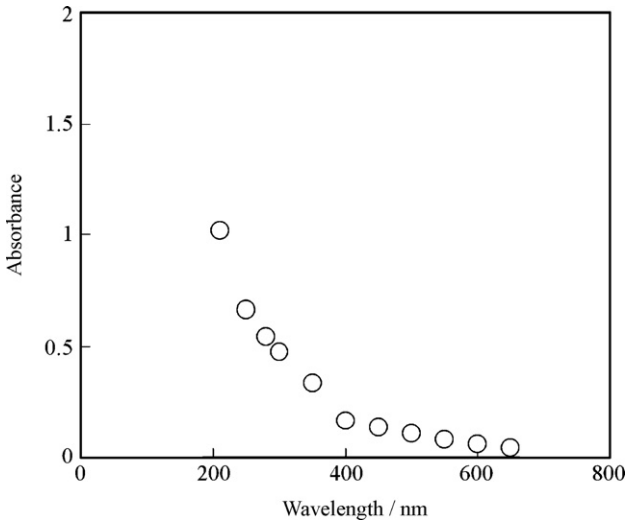

Fig. 2. Relationship between detection wavelength and absorption in the PTFE capillary tube.

with increasing wavelength. Absorption detection in the capillary chromatography system, particularly in the ultraviolet region, was disturbed by the light scattering properties of PTFE. However, by adjusting the apparent absorption due to the scattering of the tube to a baseline for measuring the absorption profile, compounds with absorption behavior such as the analytes used here were detected without any problems at wavelengths of 232, 254, or 280 nm.

3.2. Effects of the component ratio of the carrier on separation

In our previous communication [10], a solution of 2,6-naphthalenedisulfonic acid (hydrophilic) and 1-naphthol (hydrophobic) was analyzed using the capillary chromatography system with open capillary tubes of fused-silica or polyethylene. When using a water-rich carrier solution of water–acetonitrile–ethyl acetate with a volume ratio of 15:3:2 for both capillary tubes, the mixture of 2,6-naphthalenedisulfonic acid and 1-naphthol was separated through the open capillary tube and they were detected in this order. On the other hand, using an organic solvent-rich carrier solution of water–acetonitrile–ethyl acetate with a volume ratio of 2:7:4 or 2:5:9 for fused-silica or polyethylene, respectively, they were detected with inverse elution times, i.e., 1-naphthol and 2,6-naphthalenedisulfonic acid were detected in this order (data not shown).

A solution of 2,6-naphthalenedisulfonic acid and 1-naphthol was also analyzed using the present system with an open PTFE capillary tube and an aqueous–organic carrier solution. The obtained absorption profiles are shown in Fig. 3 using three carrier solutions of water–acetonitrile–ethyl acetate with volume ratios of 15:3:2, 4:7:9, and 2:9:4. When using a water-rich carrier solution of water–acetonitrile–ethyl acetate with a volume ratio of 15:3:2, 2,6-naphthalenedisulfonic acid and 1-naphthol were separated through the open capillary tube and detected at *ca.* 6.8 and 8.5 min, respectively (Fig. 3a). The components of the analytes, 2,6-naphthalenedisulfonic acid and 1-naphthol, in the profile were confirmed with individual absorption signals. The confirmation was performed for all experimental data in Figs. 3–5.

On the other hand, using the organic solvent-rich carrier solution of water–acetonitrile–ethyl acetate with volume ratios of 4:7:9 and 2:9:4, the analytes were detected with inverse elution times. 1-Naphthol and 2,6-naphthalenedisulfonic acid were detected in this

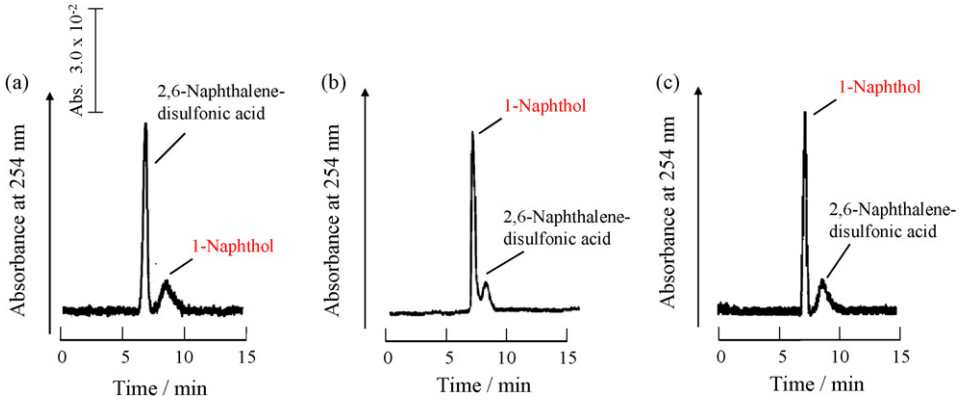

Fig. 3. Absorption profiles of a mixture of 2,6-naphthalenedisulfonic acid and 1-naphthol by the present system using a PTFE capillary tube. Conditions: capillary tube, 90 cm (effective length: 70 cm) of 100 μm i.d. PTFE; carrier, (a) water–acetonitrile–ethyl acetate (15:3:2) mixture solution, (b) water–acetonitrile–ethyl acetate (4:7:9) mixture solution, and (c) water–acetonitrile–ethyl acetate (2:9:4) mixture solution; sample injection, 20 cm height (gravity) × 5 s; flow rate, 0.8 μL min^{-1}; and analyte concentration, 1 mM each.

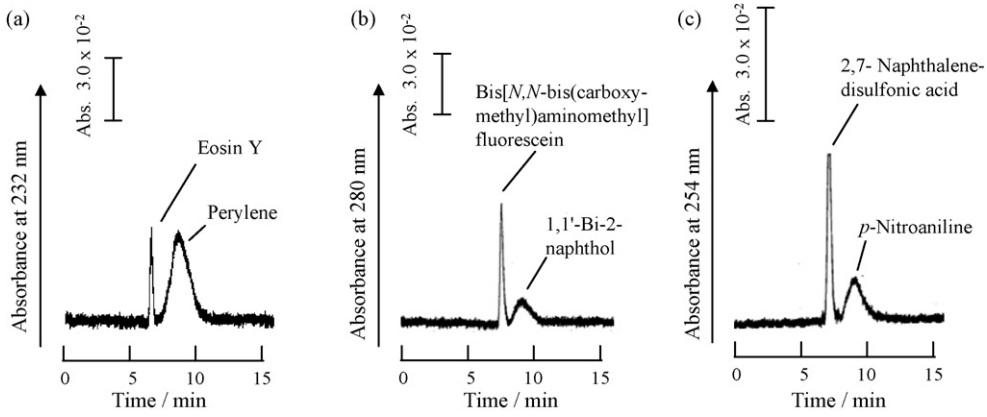

Fig. 4. Absorption profiles of the mixtures by the present system with a water-rich carrier solution. (a) Eosin Y and perylene, (b) bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein and 1,1'-bi-2-naphthol, and (c) 2,7-naphthalenedisulfonic acid and *p*-nitroaniline. Conditions: capillary tube, 90 cm (effective length: 70 cm) of 100 μm i.d. PTFE; carrier, water–acetonitrile–ethyl acetate (15:3:2) mixture solution; sample injection, 20 cm height (gravity) × 5 s; flow rate, 0.8 μL min^{-1}; and analyte concentration, 1 mM each.

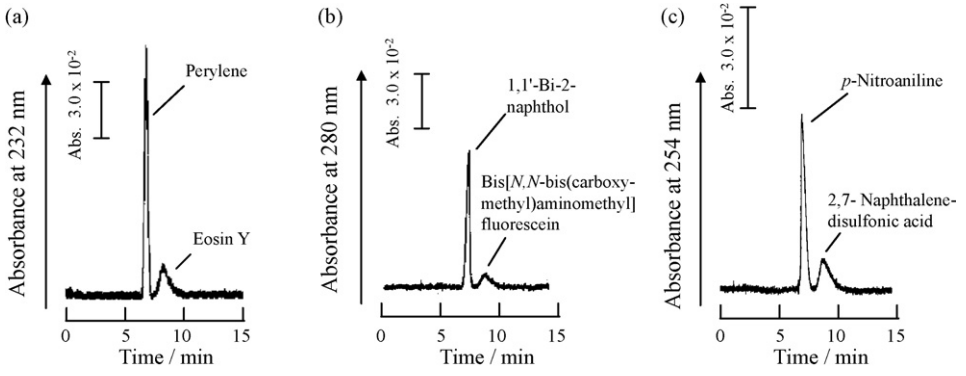

Fig. 5. Absorption profiles of the mixtures by the present system with an organic solvent-rich carrier solution. (a) Eosin Y and perylene, (b) bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein and 1,1'-bi-2-naphthol, and (c) 2,7-naphthalenedisulfonic acid and *p*-nitroaniline. Conditions: capillary tube, 90 cm (effective length: 70 cm) of 100 μm i.d. PTFE; carrier, water–acetonitrile–ethyl acetate (2:9:4) mixture solution; sample injection, 20 cm height (gravity) × 5 s; flow rate, 0.8 μL min^{-1}; and analyte concentration, 1 mM each.

order at *ca.* 7.0 and 8.3 min for the carrier with a volume ratio of 4:7:9 (Fig. 3b) and at *ca.* 7.1 and 8.9 min for the carrier with a volume ratio of 2:9:4 (Fig. 3c).

The separation behavior of the mixture of 2,6-naphthalenedisulfonic acid and 1-naphthol, including reversibility of the elution times by changing the component ratio of the solvents in the carrier solution, was observed for fused-silica, polyethylene, and PTFE capillary tubes. Based on the obtained results, the properties of the capillary tube material, i.e., fused-silica, polyethylene, and PTFE, seemed to have little influence on the separation behavior of the analytes in the present capillary chromatography system.

3.3. Separation of the other analyte mixtures using a PTFE capillary tube

Other mixtures of Eosin Y and perylene, bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein and 1,1'-bi-2-naphthol, and 2,7-naphthalenedisulfonic acid and *p*-nitroaniline were subjected to separation with the present system using the PTFE capillary tube with a water-rich carrier solution of water–acetonitrile–ethyl acetate (volume ratio 15:3:2). The obtained absorption profiles are shown in Fig. 4. In each profile, the hydrophilic Eosin Y, bis[*N*,*N*-bis(carboxymethyl) aminomethyl]fluorescein, or 2,7-naphthalenedisulfonic acid was eluted first, followed by the hydrophobic perylene, 1,1'-bi-2-naphthol, or *p*-nitroaniline, with good separation.

In addition, these mixtures were subjected to separation using the present system with an organic solvent-rich carrier solution of water–acetonitrile–ethyl acetate (volume ratio 2:9:4). The obtained absorption profiles are shown in Fig. 5. In each profile, the hydrophobic perylene, 1,1'-bi-2-naphthol, or *p*-nitroaniline was eluted first, followed by the hydrophilic Eosin Y, bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein, or 2,7-naphthalenedisulfonic acid, with good separation.

3.4. Elution times of the two analytes in the mixture

A similar separation behavior for a mixture of 2,6-naphthalenedisulfonic acid and 1-naphthol was observed when open capillary tubes made of fused-silica, polyethylene, or PTFE were used. In addition, the four mixed analyte solutions, i.e., 2,6-naphthalenedisulfonic acid and 1-naphthol, Eosin Y and perylene, bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein and 1,1'-bi-2-naphthol, and 2,7-naphthalenedisulfonic acid and *p*-nitroaniline, were confirmed to be similarly separated with the capillary chromatography system using the PTFE capillary tube. That is, in all the experiments using the three different types of capillary tubes and four different analyte mixtures, when a water-rich carrier solution, such as water–acetonitrile–ethyl acetate (volume ratio 15:3:2) was used, the hydrophilic compound was eluted earlier than the hydrophobic compound. Conversely, when a carrier solution with a large organic solvent component, such as water–acetonitrile–ethyl acetate (volume ratio 2:9:4) was used, the hydrophobic compound was eluted earlier than the hydrophilic compound. Thus, the elution order can be easily reversed by changing the component ratio of the solvents in the carrier solution in the three capillary tubes.

The Reynolds number was estimated to be roughly <1 under the present analytical conditions, confirming that the system worked under laminar flow conditions. Under these conditions, the linear velocity of the fluid in the capillary tube showed a parabolic curve; the linear velocity showed a maximum value around the middle of the capillary tube and decreased approaching the inner wall of the tube. Solutes that are diffusive or of low molecular weight are eluted from the tube with an average linear velocity and a Gaussian distribution [7,8]. The average linear velocities using the fused-silica, polyethylene, and PTFE capillary tubes were estimated to be 1.7, 4.2, and 1.7 mm s^{-1}, respectively, under the corresponding analytical conditions. As shown in Figs. 3–5, as well as the results described in our previous communication, the elution times of the first peaks in the fused-silica, polyethylene, and PTFE capillary tubes were *ca.* 9.6, 4.0, and 6.9 min, respectively, showing no change in the carrier solution for the same capillary tube. Moreover, the elution times of the first peaks nearly corresponded to those calculated with the average linear velocities, and the second peaks were eluted with smaller velocities than the average linear velocities. However, the elution times of the second peaks lacked reproducibility compared to those of the first peaks. The elution times of the second peaks will be also examined by use of other capillary tubes of fused-silica and polyethylene and discussed from the viewpoint of parabolic flow under laminar flow conditions.

3.5. Consideration of separation performance

Tabata et al. [9] reported a capillary chromatography system using an open fused-silica capillary tube, in which the inner wall was negatively charged due to the dissociation of silanol groups, and a water–acetonitrile mixture including sodium chloride with high concentration as the carrier. Although they separated the hydrophilic and hydrophobic molecules in this order, but could not change the order of elution, we have demonstrated the separation of a mixture of hydrophilic and hydrophobic molecules by capillary chromatography using open capillary tubes made of fused-silica, polyethylene, or PTFE, and a salt-free water–hydrophilic–hydrophobic organic solvent mixture carrier solution. Thus, we determined that the negative charge due to the silanol groups on the inner wall plays no major role in creating a water-rich phase near the inner wall, at least in our present system. Furthermore, the elution times of the analytes in the present system can be easily reversed by changing the component ratio of the carrier solvents in all types of capillary tubes. In addition such a salt-free solvent mixture is easy to use in a capillary tube without any problems of salt deposition.

Based on our results, we proposed that separation in the present capillary chromatography system was performed using the tube radial distribution of the aqueous–organic mixture solvents under laminar flow conditions, known here as "a tube radial distribution chromatography (TRDC)" system. Possible separation performance in the TRDC system using an open capillary tube and an aqueous–organic solvent mixture carrier solution is described as follows and illustrated in Fig. 6 (water-rich case in (a) and organic solvent-rich case in (b)). The linear velocity of the fluid in the capillary tube under laminar flow conditions is expressed as a parabolic curve in Fig. 6; however, the results in a water–organic solvent mixture carrier solution may deviate from an ideal parabolic curve.

First, water and organic solvents in the carrier solution are not dispersed uniformly in the capillary tube. The tube features an extremely large specific surface area of the inner wall relative to the inside volume, leading to the generation of a water-rich phase and an organic solvent-rich phase based on a tube radial distribution of the aqueous–organic solvent mixture under laminar flow conditions. A major solvent phase forms around the middle of the tube, while a minor solvent phase forms near the inner wall. When a water-rich carrier solution is used, a major solvent phase or a water-rich phase forms around the middle of the tube (Fig. 6a). However, when an organic solvent-rich carrier solution is used, a major solvent phase or an organic solvent-rich phase forms around the middle of the tube (Fig. 6b). Such tube radial distribution of the solvent molecules in the carrier solution must be caused through a specific flow in the capillary tube under the laminar flow conditions.

Subsequently, as the analyte mixtures consisted of typical hydrophilic and hydrophobic molecules, the former (2,6-naphthalenedisulfonic acid, Eosin Y, bis[*N*,*N*-bis(carboxymethyl) aminomethyl]fluorescein, and 2,7-naphthalenedisulfonic acid) is mainly dissolved or dispersed in the water-rich phase, while the latter (1-naphthol, perylene, 1,1'-bi-2-naphthol, and *p*-nitroaniline) is dissolved in the organic solvent-rich phase, leading to tube radial distribution of analyte molecules in the capillary tube.

In the case of a water-rich carrier solution, the hydrophilic analyte, which is dispersed in the water-rich phase of the major solvent phase (around the middle of the capillary tube), is eluted with

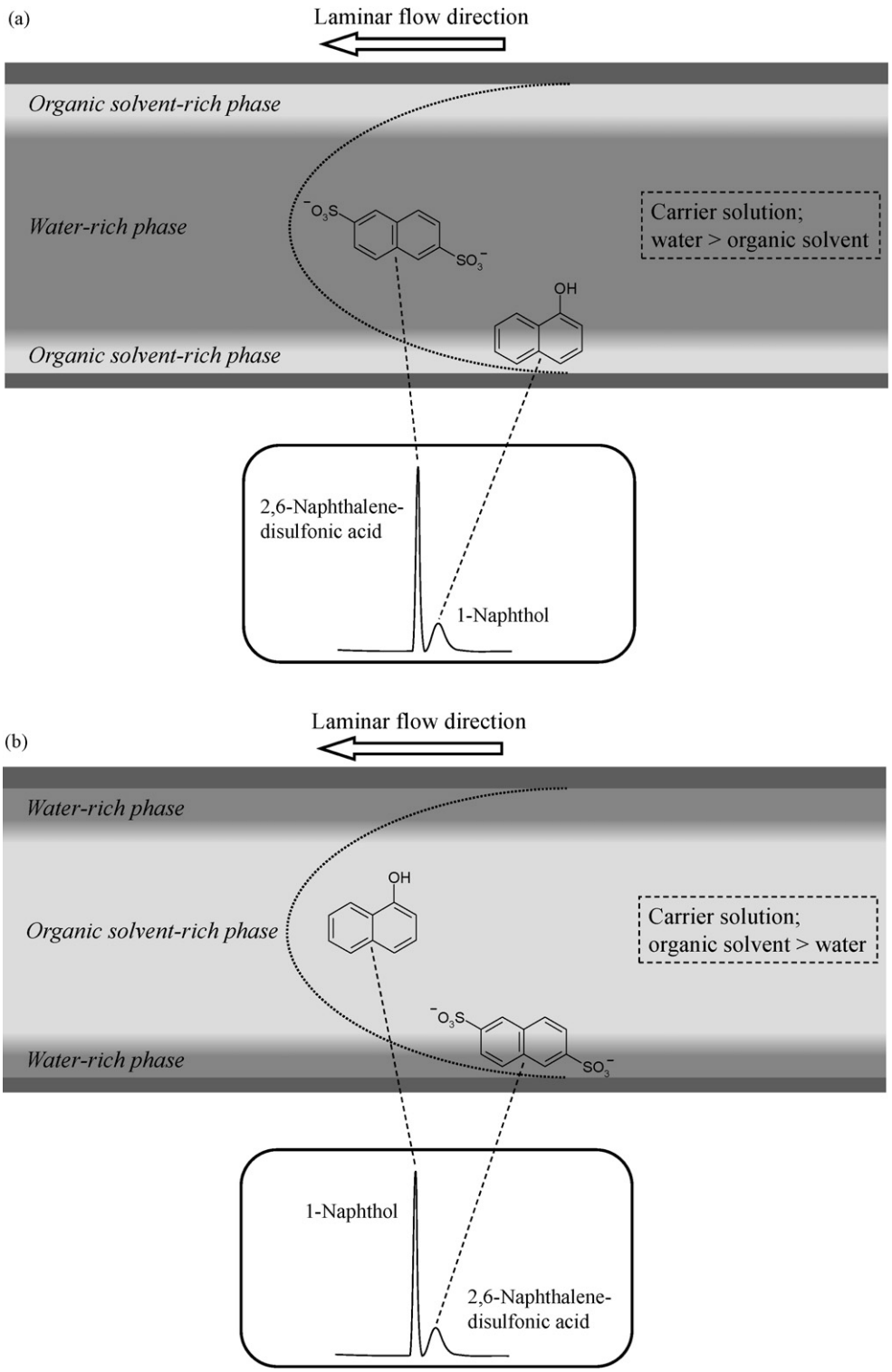

Fig. 6. Illustration of the separation performance in the capillary chromatography system. (a) Water-rich carrier solution and (b) organic solvent-rich carrier solution.

nearly average linear velocity. The hydrophobic analyte, which is dispersed in the organic solvent-rich phase of the minor solvent phase near the inner wall of the tube (pseudo-stationary phase), is eluted with a smaller velocity than the average linear velocity (Fig. 6a). In the case of an organic solvent-rich carrier solution, the hydrophobic analyte dispersed in the organic solvent-rich phase of the major solvent phase (around the middle of the capillary tube) is eluted with nearly average linear velocity. The hydrophilic analyte dispersed in the water-rich phase of the minor solvent phase near the inner wall of the tube (pseudo-stationary phase) is eluted with a smaller velocity than the average linear velocity (Fig. 6b). Therefore, the elution times of the analytes can be easily reversed by altering the component ratio of the solvents in the carrier solution. The separation in the TRDC was performed without using any special

materials such as packed and monolithic capillary tubes or applying a high-voltage for electrophoresis, as for conventional capillary chromatography.

4. Conclusions

We developed a capillary chromatography system using an open capillary tube and an aqueous–organic mixture carrier solution that worked under laminar flow conditions. In contrast to conventional capillary electrochromatography, capillary electrophoresis, and capillary liquid chromatography, separation in this system was performed without the use of any specific materials such as packed capillary tubes, additives such as gels, surfactants, host molecules or salts, or high-voltage supply devices. We examined various mixtures consisting of hydrophilic and hydrophobic molecules in this chromatography system using fused-silica, polyethylene, or PTFE capillary tubes and a water–acetonitrile–ethyl acetate mixture carrier solution. The analytes were well separated through the capillary tubes and detected on-capillary by an absorption detector, and the elution times of them could be easily reversed by changing the component ratio of the solvents in the carrier solution. The separation performance in the system was explained based on the tube radial distribution of the aqueous–organic mixture carrier solvents under laminar flow conditions.

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