doi:10.1246/cl.2012.855
Published on the web August 11, 2012

Influence of Adding Surfactants to an Analyte Solution on Separation Performance in Open-tubular Capillary Chromatography Based on the Tube Radial Distribution of Ternary Mixed Carrier Solvents

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An open-tubular capillary chromatography system has been developed using a ternary mixed solvent mixture, i.e., water–hydrophilic/hydrophobic organic solvent mixture as a carrier solution. In this study, the influence of adding surfactants to an analyte solution on separation performance was examined in a chromatographic system using a fused-silica capillary tube (75 μm inner diameter and 100 cm effective length) and a ternary mixture of water–acetonitrile–ethyl acetate (3:8:4 volume ratio) carrier solution. Sodium dodecyl sulfate (anionic), ethylhexadecyldimethylammonium bromide (cationic), and Triton X-100 (nonionic) were used as surfactants. Model analytes, 1-naphthol and 2,6-naphthalenedisulfonic acid, were separated in this order by adding the anionic and nonionic surfactants. These surfactants in the analyte solution greatly improved the separation performance (theoretical plate numbers for 2,6-naphthalenedisulfonic acid; >10000) compared with the same separation performed in the absence of surfactants. On the other hand, the analytes were not separated at all using a cationic surfactant.

Since the nineteenth century, microfluidic flow has been known to exhibit interesting and useful physical or hydrodynamic phenomena such as electroosmotic and laminar flow. In the last century, it was discovered that electroosmotic flow in a capillary tube promotes capillary electrophoresis[1,2] and capillary electrochromatography,[3] while laminar flow conditions enable hydrodynamic chromatography.[4,5] Our group reported the tube radial distribution phenomenon (TRDP) of carrier solvents under microfluidic flow conditions in 2009.[6–8] When the ternary mixed solvents mixture of water–hydrophilic/hydrophobic organic solvent mixture are delivered into a microspace, such as a microchannel or a capillary tube, the solvent molecules are radially distributed in the microspace generating inner and outer phases.[7–9] A capillary chromatography system based on the TRDP in which the outer phase acts as a pseudostationary phase under laminar flow conditions has been developed. We call the separation method "tube radial distribution chromatography" (TRDC).[6,10,11]

Various types of analytes, such as miscellaneous organic compounds, amino acids, peptides, proteins, nucleosides, lambda-DNA, metal ions, metal complexes, fluorescent compounds, polymer compounds, and optical isomers,[10,11] were analyzed using the TRDC system. Through all the experimental data, we notice a tendency for the analytes distributed in the outer phase or pseudostationary phase to possess lower elution velocity and consequential broadening of their peak shapes. In this study, we tried to improve the separation performance for the later peaks by adding surfactants to the analyte solution. It was observed that the addition of anionic and nonionic surfactants could improve the resolution and theoretical plate numbers for the model analytes.

Water was purified with an Elix 3 UV system (Millipore Co., Billerica, MA). All reagents were obtained commercially and were of analytical grade. 1-Naphthol, 2,6-naphthalenedisulfonic acid, sodium dodecyl sulfate (SDS), ethylhexadecyldimethylammonium bromide (EHDAB), acetonitrile, and ethyl acetate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Triton X-100 was purchased from Nacalai Tesque (Kyoto, Japan). Fused-silica capillary tubes (75 μm inner diameter) were purchased from GL Science (Tokyo, Japan).

The TRDC system comprised a fused-silica capillary tube (120 cm total length and 100 cm effective length), a microsyringe pump (MF-9090; Bioanalytical Systems, Inc., West Lafayette, IN, USA), and a fluorescence detector (modified FR-535 fluorescence detector; Shimadzu Co., Kyoto, Japan). The tube temperature was controlled by immersing the capillary tube (40 cm) in water maintained at 15 °C in a beaker with stirring. A water–acetonitrile–ethyl acetate mixture (3:8:4 volume ratio) was used as the carrier solution. Analyte solutions of 1-naphthol and 2,6-naphthalenedisulfonic acid (1 mM each) were prepared using the carrier solution either with or without surfactants.

The analyte solution was introduced directly into the capillary inlet side by gravity (30 cm height for 25 s). 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 constant flow rate (0.5 $\mu L\,min^{-1}$) under laminar flow conditions. On-capillary fluorescence detection of the analytes was performed with the detector; ex. 290 nm and em. 355 nm.

Figure 1 shows the chromatograms obtained with the present TRDC system, where the analytes were prepared with the carrier solution containing the surfactants, 20 mM SDS (anionic), 10 mM EHDAB (cationic), and 2.0 wt% Triton X-100 (nonionic). On the other hand, the analytes prepared with a surfactant-free carrier solution were used as a reference. Hydrophobic 1-naphthol and hydrophilic 2,6-naphthalenedisulfonic acid were separated in this order in the absence and presence of surfactants (SDS and Triton X-100). The first peak (1-naphthol) was eluted with an average linear velocity, while the second peak (2,6-naphthalenedisulfonic acid) was detected with a velocity lower than the average linear velocity under the laminar flow conditions. The elution behavior of the analytes was reasonable because of their hydrophobic and hydrophilic nature and also because of the TRDP created by the organic solvent-rich carrier solution generating major organic solvent-rich inner phase and minor water-rich outer phase.[6–10] However, the analytes were not separated with the carrier solution containing EHDAB.

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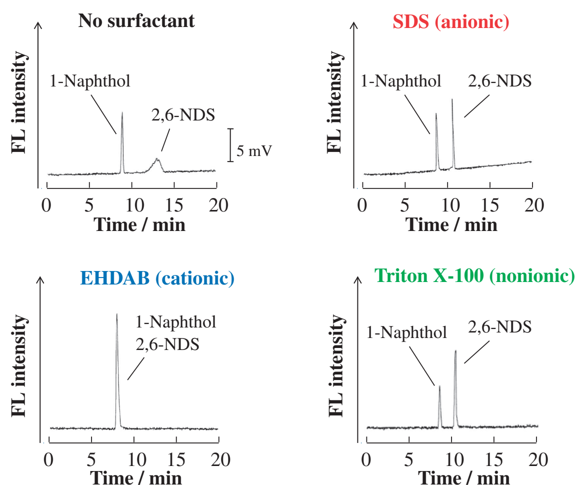

Figure 1. Chromatograms of 1-naphthol and 2,6-naphthalene-disulfonic acid (2,6-NDS) obtained with the present TRDC system using analyte solutions with or without surfactants.

Table 1. Experimental data of the elution times (t_1 and t_2), the peak widths (W_1 and W_2), the capacity factors (k'), the resolutions (R_s), the theoretical plate numbers (N), and the height of equivalent theoretical plate (H) in the present TRDC system[a]

Surfactant	t_1 /min	t_2 /min	W_1 /min	W_2 /min	k'	R_s	N	H /mm
None	8.85	13.00	0.42	2.08	0.47	3.32	625	1.60
SDS	8.65	10.58	0.38	0.28	0.22	5.84	22800	0.04
EHDAB	8.80	—	0.60	—	—	—	—	—
Triton X-100	8.50	10.29	0.41	0.41	0.21	4.37	10100	0.10

[a]The subscript 1 and 2 are for 1-naphthol and 2,6-naphthalenedisulfonic acid, respectively.

Furthermore, as clearly shown in Figure 1, the addition of SDS and Triton X-100 to the analyte solutions dramatically improved the separation of the analytes. The resolution and theoretical plate numbers are calculated in the usual manner and summarized in Table 1 together with other experimental data. The theoretical plate numbers for 2,6-naphthalenedisulfonic acid with added SDS and Triton X-100 were 10100 and 22800, respectively. These data are generally competitive with those obtained using capillary electrophoresis. However, it is significant that the present TRDC system featured such superior separation performance without applying high voltages or using any specific columns, such as monolithic or packed varieties.

The reason why the addition of surfactants to the analyte solutions provided such high resolution has not yet been determined. However, we tentatively suggest the following explanation of our findings. The tentative suggestion is illustrated in Figure 2. First, the broadening of the second peak in the general system without surfactant might be caused by the greater thickness, micrometer order, of the outer phase that functions as a pseudostationary phase. The analyte having an anionic nature, 2,6-naphthalenedisulfonic acid, is easily surrounded by the surfactants, SDS (anionic) and Triton X-100

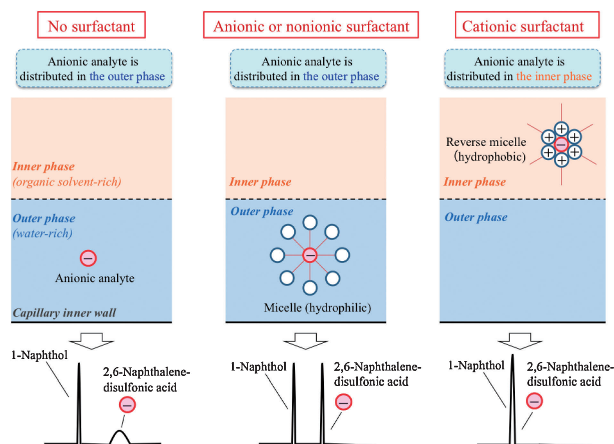

Figure 2. Illustration of the analyte distribution between the inner and outer phases with or without surfactants.

(nonionic), to generate a micellar assembly. The formation of hydrophilic micelles might reduce adsorption on the inner wall resulting in very sharp peaks. On the other hand, the anionic analyte was strictly surrounded with the cationic surfactant, EHDAB, generating reverse micellar assemblies. The hydrophobic micelle containing the analyte must behave similar to the hydrophobic analyte, 1-naphthol, in the capillary tube leading to no separation. As another suggestion, there is some turbulence near the interface between the two phases moving down in the capillary tube, since a shear stress is generated due to differences in physical properties of these phases. Addition of surfactants may suppress the influence of the turbulence by the decrease in a friction coefficient between the two phases, leading to decrease in peak broadening or solute diffusion.

This work was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. It was also supported by "Advanced Study for Integrated Particle Science and Technology," Strategic Development of Research Infrastructure for Private Universities, the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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