Temperature Effect on Separation Performance in Capillary Chromatography based on Tube Radial Distribution of Aqueous-Organic Mixture Carrier Solvents

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We have developed a capillary chromatography system using an open capillary tube made of fused-silica, polyethylene, or poly(tetrafluoroethylene), and a water-hydrophilic/hydrophobic organic mixture carrier solution. This so-called tube radial distribution chromatography (TRDC) system works under laminar flow conditions. Previously, the effects of capillary temperature on separation performance in the TRDC system were examined using a fused-silica capillary tube and a water-acetonitrile-ethyl acetate mixture carrier solution; 1-naphthol and 2,6-naphthalenedisulfonic acid in a model mixture were eluted with baseline separation over the temperature range of $5 - 23°C$ with the organic solvent-rich carrier solution. In this study, based on the assumption that the phase distribution of inner and outer phases in the capillary tube induced by the tube radial distribution of the carrier solvents did not change with temperature over a limited range, we attempted to apply the van't Hoff equation to the chromatographic data obtained at various capillary temperatures.

Key words: Capillary chromatography, Laminar flow conditions, Tube radical distribution, Capillary temperature, The van't Hoff equation

1. Introduction

Miniaturization is one of the most active research directions in analytical chemistry. Valuable investigations with respect to miniaturization have in particular been conducted in the field of liquid chromatography; *e.g.,* capillary liquid chromatography or capillary chromatography.[1-3] We developed a capillary chromatography method using an open capillary tube and a water-hydrophilic/hydrophobic organic mixture (homogeneous solution) carrier solution;[4-12] the system worked under laminar flow conditions. We called this system the tube radial distribution chromatography (TRDC) system. To date, various mixture analytes of hydrophilic and hydrophobic molecules have been separated in the TRDC system. Fused-silica, polyethylene, or

poly(tetrafluoroethylene) capillary tubes (50, 200, or 100 μm i.d., respectively) without any pretreatments were used together with a water-acetonitrile-ethyl acetate mixture solution as the carrier.

Aqueous and organic solvents in the carrier solution are dispersed non-uniformly in the capillary tube under laminar flow conditions, generating an organic solvent-rich and a water-rich phase in the capillary tube. An organic solvent-rich carrier solution generates an organic solvent-rich inner phase and a water-rich outer phase, while a water-rich carrier solution results in a water-rich inner phase and an organic solvent-rich outer phase. That is, a major inner phase is formed about the center of the tube away from the inner wall and a minor outer or capillary wall phase is generated near the inner wall, based on the tube radial distribution of the carrier solvents. The analytes that are

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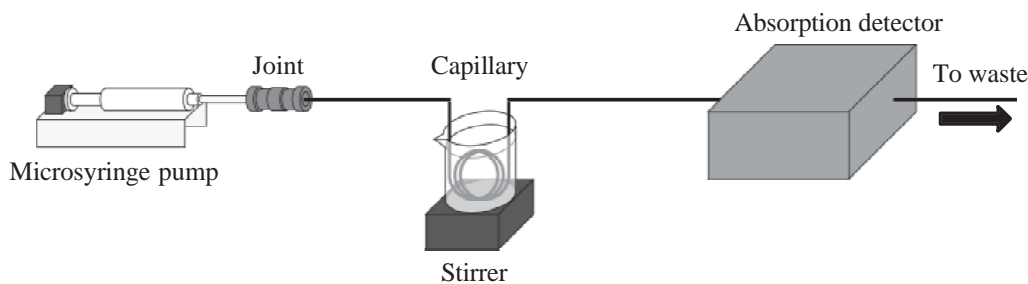
Fig. 1 Schematic diagram of the present capillary chromatography system.

delivered through the capillary tube are distributed between the inner and outer phases undergoing chromatographic separation.

Since the investigation of the TRDC system is still at the preliminary stage, it is important to examine the elution behavior under various analytical conditions in the system to expand our knowledge regarding its separation performance. In our previous study,[12] we examined the analytical conditions of the TRDC system in detail, including tube temperature, tube inner diameter, tube length, and flow rate, using fused-silica capillary tubes and water-acetonitrile-ethyl acetate mixture as the carrier solution. In this study, we tried to examine the effects of capillary temperature on separation performance in the TRDC system through the van't Hoff equation on the assumption that the phase distribution of the inner and outer phases did not change under a certain condition.

2. Experimental

Water was purified with an Elix UV 3 (Millipore Co., Billerica, MA). All reagents used were commercially available and of analytical grade. 1-Naphthol, 2,6-naphthalenedisulfonic acid, acetonitrile, and ethyl acetate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fused-silica capillary tube was purchased from GL Science (Tokyo, Japan).

A schematic diagram of the present capillary chromatography system comprising an open fused-silica capillary tube, 120 cm (effective length of 100 cm) of

75 μm i.d., microsyringe pump (MF-9090; 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 preset at a definite temperature in a beaker with stirring. A water-acetonitrile-ethyl acetate mixture with volume ratio of 3:8:4 was used as the carrier solution. Analyte solutions were prepared with the carrier solutions.

The analyte solution was introduced directly into the capillary inlet by the gravity method (20 cm height × 30 s). After the 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 flow rate (0.8 μL min^{-1}) under laminar flow conditions. On-capillary absorption detection (254 nm) was performed with the detector.

3. Results and discussion

We previously examined the effects of tube temperature on separation in the TRDC system with a mixture analyte solution of 1-naphthol and 2,6-naphthalenedisulfuric acid as a model.[12] The experiments were performed with the organic solvent-rich carrier solution (water-acetonitrile-ethyl acetate; 3:8:4 v/v/v) because the carrier solution provided better resolution on the chromatograms than the water-rich carrier solution (water-acetonitrile-ethyl acetate; 15:3:2 v/v/v). The obtained chromatograms in
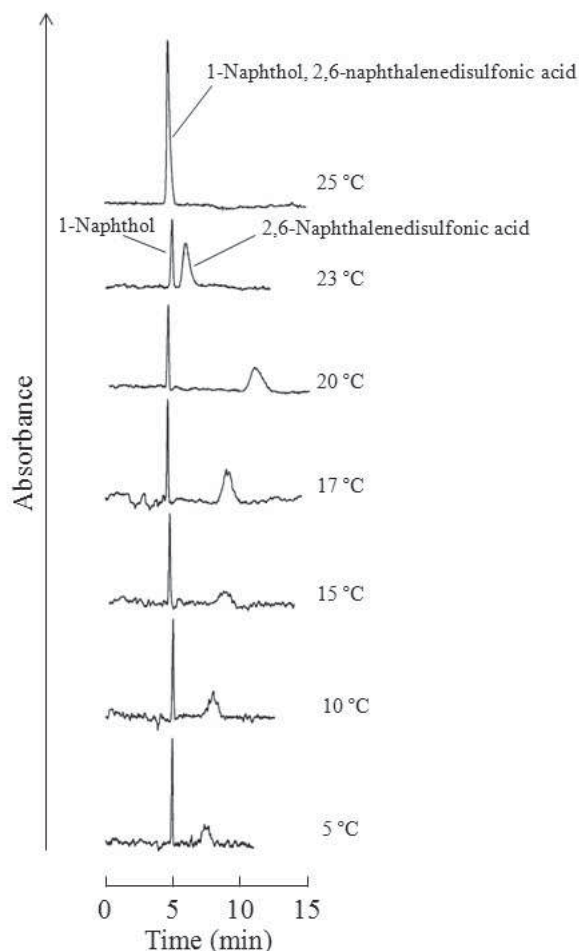
Fig. 2 Chromatograms obtained at various capillary temperatures in the TRDC system.[12]
Conditions: Capillary tube, 120 cm (effective length of 100 cm, the part of it (*ca*. 80 cm) was dipped in the temperature-controlled water) of 75 μm i.d. fused-silica; carrier, water-acetonitrile-ethyl acetate (3:8:4 v/v/v) mixture solution; sample injection, 20 cm height (gravity) × 30 s; flow rate, 0.8 μL min^{-1}; tube temperature, 5 – 25°C; and 2,6-naphthalenedisulfonic acid and 1-naphthol, 1 mM each.

our previous work[12] are shown in Fig. 2 together with analytical conditions as a reference.

As can be seen in Fig. 2, 1-naphthol and 2,6-naphthalenedisulfuric acid in the mixture solution were detected with baseline separation in the temperature range of 5 – 23°C, while they were not separated at all at a temperature of 25°C. More specifically, the resolution was improved with an increase in temperature from 5°C to 20°C but suddenly decreased at 23°C. The data clearly indicated that the tube temperature had a significant and critical influence on the separation performance in the TRDC system.

In chromatographic investigation, enthalpy (ΔH) is often calculated by using the van't Hoff equation as a standard method, leading to thermodynamical discussion, such as exothermic ($\Delta H<0$) or endothermic ($\Delta H>0$) in the separation system. We attempted to apply the van't Hoff equation to the chromatographic data of TRDC system (Fig. 2), for the first time. The equation is shown below, where ΔH, ΔS, T, R, and Φ are enthalpy, entropy, absolute temperature, gas constant, and phase ratio, respectively.

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \Phi$$

In this study the capacity factor (k') was calculated using the first peak as the dead (hold-up) time. In addition, we assumed that the phase distribution of the inner and outer phases of the carrier solution in the tube due to the tube radial distribution of the solvents did not change with temperature over the range of 5 – 20°C.

The values of $\ln k'$ were plotted against $1/T$ for the data obtained with the organic solvent-rich carrier solutions (Fig. 3). Obviously, Fig. 3 does not show a simple linear plot based on the separation performance observed on the chromatograms in Fig. 2. Tentatively, we examined the value of enthalpy (ΔH) from the line over the temperature range of 10 – 20°C where separation was observed with increasing resolution. The value of enthalpy was roughly estimated to be 37.1 kJ mol^{-1}. The enthalpy is generally influenced by various types of heat, such as dissolution, adsorption, desorption, and solvation. At the present it is difficult to discuss an interaction between the fused-silica inner-wall surface and the analytes that may contribute to separation performance in the TRDC system. However, the obtained data for the fused-silica capillary tube would give a clue to further account of the separation performance (including contribution of the activity on the inner-wall surface) of the TRDC system from the viewpoint of thermodynamics, comparing the theory with the data for polyethylene or poly(tetrafluoroethylene) capillary tube.
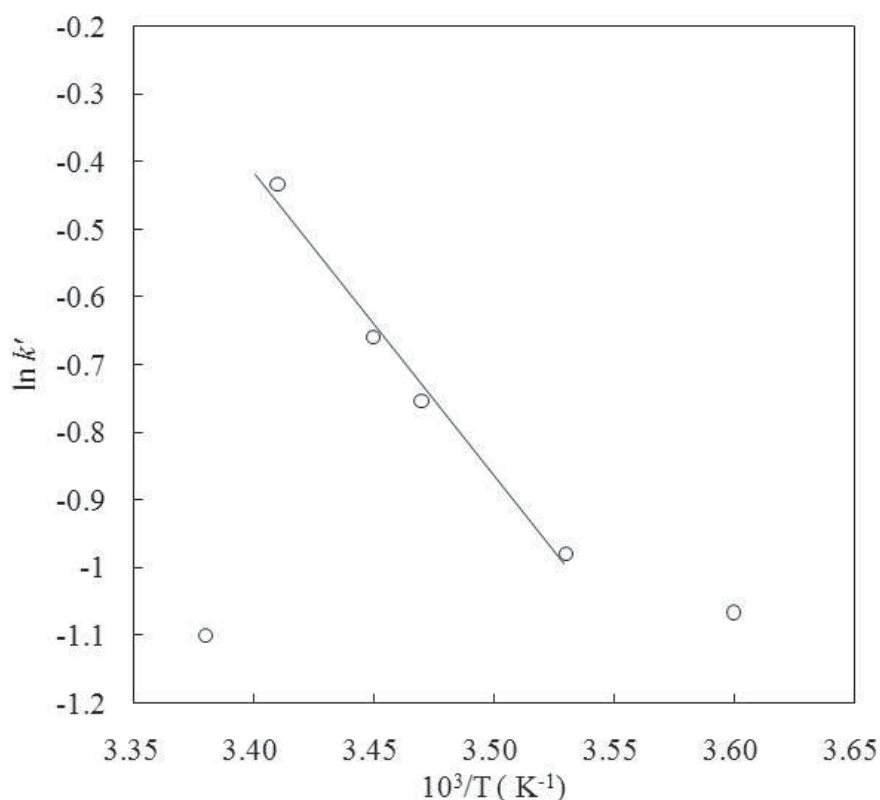
Fig. 3 The van't Hoff plots obtained in the TRDC system.
Conditions: Capillary tube, 120 cm (effective length: 100 cm) of 75 μm i.d. fused-silica; carrier, water-acetonitrile-ethyl acetate (3:8:4 v/v/v) mixture solution; sample injection, 20 cm height (gravity) × 30 s; flow rate, 0.8 μL min^{-1}; tube temperature, 5 – 23°C; and 2,6-naphthalenedisulfonic acid and 1-naphthol, 1 mM each.

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