Original

Influences of Analyte Injection Volumes and Concentrations on Capillary Chromatography Based on Tube Radial Distribution of Carrier Solvents under Laminar Flow Conditions

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Abstract

A capillary chromatography called the tube radial distribution chromatography (TRDC) system has been developed using an open capillary tube and a water-hydrophilic/hydrophobic organic solvent mixture carrier solution. In this study, we examined the effects of the analyte injection volume (injection time) and concentration on the chromatograms in the TRDC system using a fused-silica capillary tube (50 μm i.d. and 100 cm effective length) and a water-acetonitrile-ethyl acetate mixture carrier solution (volume ratio, 3: 8: 4). Analyte solutions of 1-naphthol and 2,6-naphthalenedisulfonic acid (1 mM each) were injected into the system with various injection times of 10-1500 s from a height of 20 cm by gravity. They were separated and detected in this order with good reproducibility up to an injection time of 150 s. The analyte solutions (0.075-3.0 mM) were analyzed with the definite injection time of 30 s from a height of 20 cm by gravity. They were separated and detected with a baseline separation and their calibration curves showed linearity up to 1.5 mM. It was confirmed that the TRDC system worked well as a quantitative analysis.

Keywords : chromatography, tube radial distribution, laminar flow conditions, analyte injection volumes, analyte concentration

Introduction

Analytical methods using capillary tubes have attracted a great deal of interest since the last century [1-3]. Well-known methods include capillary electrophoresis, capillary electrochromatography, micellar electrokinetic capillary chromatography, and high-performance liquid capillary chromatography using packed and monolithic columns. However, only a few new concepts concerning capillary chromatography have been reported in the last decade [4,5]. Related research has been discussed in detail in our previous papers [6,7]. We have developed a capillary chromatography system using an open capillary tube and a water-hydrophilic/hydrophobic organic mixture carrier solution [6-11]. This system works under laminar flow conditions and does not require any packed reagents in the capillary tube or application of high voltage to the ends of the tube. It is called a tube radial distribution chromatography (TRDC) system.

In the TRDC system [6,7], 1) various types of capillary tube are available, such as fused-silica, polyethylene, and poly (tetrafluoroethylene), 2) a ternary mixed solution, *i.e.*, water-hydrophilic/hydrophobic organic mixture carrier solution (homogeneous) must be delivered into the capillary tube under laminar flow conditions, 3) the elution times of analytes, hydrophobic and hydrophilic, are easily reversed by changing the component ratios of the carrier solvents, *i.e.*, by using an organic solvent-rich and a water-rich carrier solution, and 4) the first peak appears with almost the average linear velocity and the other peaks are eluted with lower velocities than the average linear velocity under laminar flow conditions in both organic solvent-rich and water-rich carrier solutions.

Separation performance in the TRDC system is explained

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based on the obtained experimental data as follows. First, aqueous and organic solvents in the carrier solution are dispersed non-uniformly at a specific flow in the capillary tube under laminar flow conditions, generating organic solvent-rich and water-rich phases in the tube. An organic solvent-rich carrier solution generates an organic solvent-rich major inner phase and a water-rich minor outer phase in the capillary tube, while a water-rich carrier solution results in a water-rich major inner phase and an organic solvent-rich minor outer phase in the tube. That is, a major inner phase is formed around the center of the tube away from the inner wall and a minor outer or capillary wall phase is generated near the inner wall. The tube radial distribution of the solvent molecules in the carrier solution is thus caused by the flow in the capillary tube under laminar flow conditions. Consequently, the analytes that are delivered through the capillary tube are distributed between the inner and outer phases, and undergo chromatographic separation in the capillary tube.

The tube radial distribution of the carrier solvents in the TRDC system is also supported by other experimental data using polymer particles as analytes [8], phenylboronic acid or iminodiacetic acid-modified fused-silica capillary tubes [9], and double fused-silica capillary tubes with different inner diameters [10], as well as by visual observation that the dyes dissolved in the carrier solution were radially distributed based on their hydrophilic or hydrophobic nature in the micro-channel in a micro-chip or in the capillary tube [11,12]. We also tried to discuss the mechanism of the formation of the inner and outer phases in the tube radial distribution of the solvents [13].

To date, various mixtures of hydrophilic and hydrophobic analytes have been separated using the TRDC system [6,7]. However, we have not examined the effects of the analyte injection volume or injection time and concentration on the chromatograms in the TRDC system in detail. The analyte injection volume and concentration are fundamental analytical factors in general chromatography separation and flow-injection analysis. In this study, we examined separation performance with various analyte injection volumes and concentrations in the TRDC system to expand our knowledge regarding this new separation system.

Experimental

Chemicals and capillary tubes

Water was purified with an Elix 3 UV (Millipore Co., Billerica, MA). All reagents used here 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). A fused-silica capillary tube (50 μm i.d. and 150 μm o.d.) was purchased from GL Science (Tokyo, Japan).

Apparatus and procedures

The TRDC system equipped with absorption on-line detection was comprised of an open fused-silica capillary tube (50 μm i.d., 120 cm length; 100 cm effective length), micro-syringe pump (MF-9090; Bioanalytical Systems, Inc., West Lafayette, IN), and absorption detector (modified SPD-10AV spectrophotometric detector; Shimadzu Co., Kyoto, Japan). The schematic diagram is shown in Fig. 1. The tube temperature was controlled by immersing the capillary tube in water maintained at a definite temperature (0°C) in a beaker with stirring. A water-acetonitrile-ethyl acetate mixture with a volume ratio of 3: 8: 4 was used as an organic solvent-rich carrier solution. An analyte solution including 1-naphthol and 2,6-naphthalenedisulfonic acid as a model was prepared with the carrier solution.

The analyte solution was introduced directly into the capillary inlet side by the gravity method (from a height of 20 cm). After analyte injection, the capillary inlet was connected through a joint to a micro-syringe. The syringe was set on the micro-syringe pump and the carrier solution was fed into the capillary tube at a definite

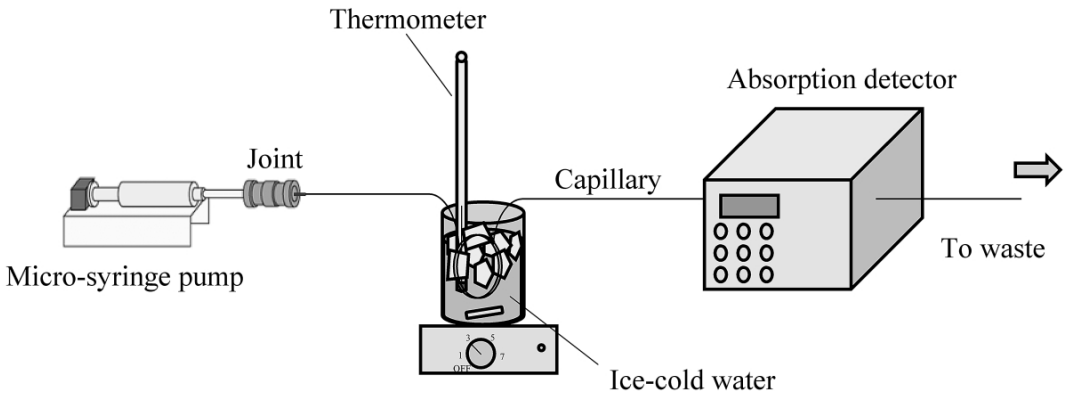

Fig. 1 Schematic diagrams of the TRDC system.

flow rate (0.2 μL min^{-1}) under laminar flow conditions (Reynolds number was calculated roughly to be 0.14 in the present system). On-capillary absorption detection (254 nm) was performed with the detector.

Results and discussion

Analyte injection times, volumes, and zone lengths

Analyte solution consisting of 1-naphthol and 2,6-naphthalenedisulfonic acid was injected into the capillary tube by gravity from a height of 20 cm at various injection times of 10-1500 s (25 min) in the present TRDC system. To examine the analyte injection volumes and zone lengths in the capillary tube corresponding to the analyte injection times of 10-1500 s, the flow rate by gravity was first estimated with the Hagen-Poiseuille equation theoretically.

The flow rate in the capillary tube with gravity from a height of 20 cm was calculated using the Hagen-Poiseuille equation (Eq. 1), which is shown below: Q is the flow rate, α is the radius, Δp is loss of pressure, μ is viscosity, and L is total length.

$$Q=\frac{\pi\alpha^4}{8\mu}\frac{\Delta p}{L} \quad \text{Eq. 1)}$$

The flow rate for the capillary 50 μm in i.d. (a, 25×10^{-6} m) was calculated to be approximately 2.67×10^{-13} m^3s^{-1}, where Δp, μ, and L were estimated to be 1.3×10^{3} $kgm^{-1}s^{-2}$ (calculated from the difference in height or position between the capillary inlet and outlet, 20 cm), 6.2×10^{-4} $kgm^{-1}s^{-1}$ (measured by a viscosity measurement device for the carrier solution), and 1.2 m, respectively.

Also, the value of the flow rate, Q, was estimated experimentally by the experimental data of gravity method. 1-Naphthol analyte (1 mM) was injected into the capillary tube in the usual way (from a height of 20 cm for 10 s) and then the analyte was delivered with the carrier solution into the tube by gravity (from a height of 20 cm) instead of the micro-syringe pump. The flow rates for the capillary 50 μm in i.d. were calculated with Eq. 2, where t is the elution time and L' is the effective length. The value of Q was calculated to be approximately 2.72×10^{-13} m^3s^{-1} (using t, L', and α of 120 min, 1.0 m, and 25×10^{-6} m, respectively).

$$Q=\frac{\alpha^2\pi L'}{t} \quad \text{Eq. 2)}$$

There was good correspondence in the flow rate between the value calculated with the Hagen-Poiseuille equation and that with the gravity experimental data. The value of the flow rate, 2.7×10^{-13} m^3s^{-1}, was used in the following estimations. The analyte injection volumes were calculated by multiplying the flow rates and the injection times as well as the analyte zone lengths in the tube were calculated by dividing the analyte injection volumes by the tube cross-section. That is, the injection times of 10-1500 s (25 min) corresponded to the analyte injection volumes of 2.7-400 nL and the zone lengths of 1.5-200 mm.

Effects of injection time on the chromatograms

We examined the effects of analyte injection time on separation in the TRDC system with an analyte solution of 1-naphthol and 2,6-naphthalenedisulfuric acid as a model. The experiments were performed with the organic solvent-rich carrier solution because the carrier solution provided better resolution on the chromatograms than the water-rich carrier solution when the fused-silica capillary tube was used in our previous studies [7,10].

The analyte solutions of 1-naphthol and 2,6-naphthalenedisufonic acid (1 mM each) were analyzed with various injection times of 10-1500 s (25 min) by the TRDC system. The typical chromatograms obtained at injection times of 10, 40, 150, 600, 1320, and 1500 s are shown in Fig. 2. As shown in Fig. 2, 1-naphthol and 2,6-naphthalenedisufonic acid were separated and detected in this order with the TRDC system using the organic solvent-rich carrier solution with good reproducibility for their peak shapes and elution times with the injection times of 10-150 s (2.7-40 nL injection volume or 1.5-20 mm zone length). The first peaks of 1-naphthol were eluted with near the average linear velocity in the present system and the second peaks of 2,6-naphthalenedisufonic acid were eluted with lower velocity than the average linear velocity. The capacity factors of the second peaks, which were calculated using the first peaks as the dead (hold-up) times, at injection times of 10, 40, and 150 s were 0.57, 0.61, and 0.58, respectively. With injection times of 150-1350 s (22 min) (40-360 nL injection volume or 20-180 mm zone length), although they were separated on the chromatograms, the elution times of the second peaks became earlier. With injection times of more than 1400 s, even separation was not performed as shown in the typical chromatogram (injection time 1500 s (25 min) in Fig. 2. It is noted here that such experiments treated with the wide ranges of the injection volume (2.7-400 nL) and the zone length (1.5-200 mm) would not be carried out in the usual capillary chromatography using monolithic or packed columns together with an sample injector.

Large injection times or analyte injection volumes led to non-Gaussian peaks or trapezoid-type peaks, as shown in Fig. 2. The relationships between the injection times and peak heights or peak areas for both analytes were examined for injection times of 10-150 s. The obtained relationships are shown in Fig. 3. The relationships between the injection times and peak areas for both analytes indicated good linearity, while those between injection times and peak heights indicated linearity up to *ca.* 40 s for 1-naphthol and up to *ca.* 60 s for 2,6-naphthalendisulfonic acid. The inflection point (*ca.* 40 s) of 1-naphthol appeared earlier than that of 2,6-

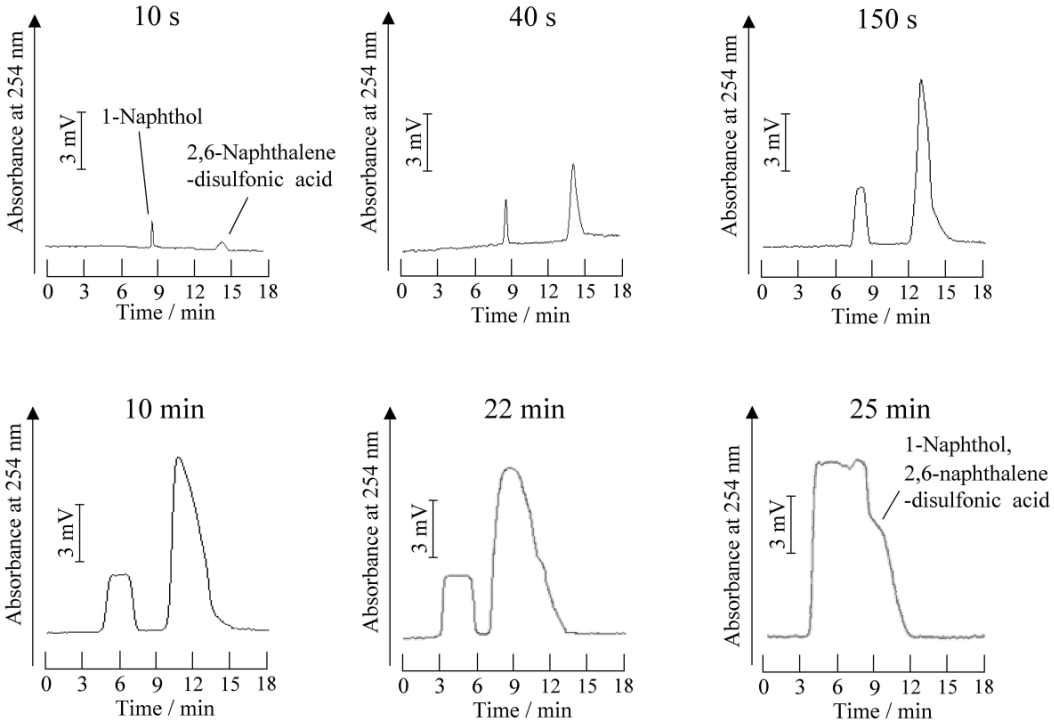

Fig. 2 Chromatograms of a mixture of 1-naphthol and 2,6-naphthalenedisulfonic acid obtained by the TRDC system with various injection times.
Conditions: Capillary tube, 120 cm (effective length: 100 cm) of 50 μm i.d. fused-silica; carrier, water-acetonitrile-ethyl acetate (3: 8: 4 v/v/v) mixture solution; sample injection, 20 cm height (gravity)×10, 40, 150, 600, 1320, and 1500 s; flow rate, 0.2 μL min^{-1}; tube temperature, 0°C; and analyte concentration, 1 mM.

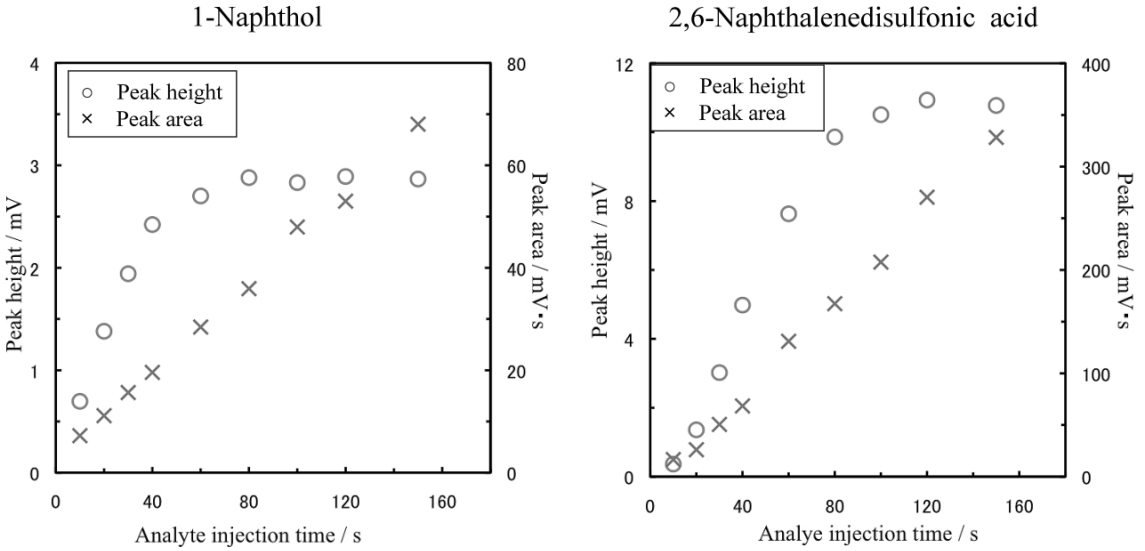

Fig. 3 Relationships between injection times and peak heights or peak areas for 1-naphthol and 2,6-naphthalenedisulfonic acid in the TRDC system.
Conditions: Capillary tube, 120 cm (effective length: 100 cm) of 50 μm i.d. fused-silica; carrier, water-acetonitrile-ethyl acetate (3: 8: 4 v/v/v) mixture solution; sample injection, 20 cm height (gravity)×10-150 s; flow rate, 0.2 μL min^{-1}; tube temperature, 0°C; and analyte concentration, 1 mM.

naphthalendisulfonic acid (*ca.* 60 s). The difference in inflection points may have been due to the differences in elution time or diffusion time between 1-naphthol and 2,6-naphthalenedisulfonic acid.

Effects of analyte concentrations on the chromatogram

We examined the effects of analyte concentrations (0.075-3.0 mM) on separation in the TRDC system with an analyte solution of 1-naphthol and 2,6-naphthalenedisulfuric acid as a model. The analyte solution was injected into the capillary tube by gravity from a height of 20 cm for 30 s. The typical chromatograms obtained with concentrations of 0.1, 0.5, 1.5, 2.0, and 3.0 mM are shown in Fig. 4. 1-Naphthol and 2,6-naphthalenedisulfuric acid were separated with a base-line separation and detected up to 1.5 mM. The capacity factors at concentrations of 0.1, 0.5, and 1.5 mM were 0.54, 0.59, and 0.57, respectively. At concentrations higher than 1.5 mM, the resolutions gradually decreased and finally they could not be separated on the chromatogram at all at about 3.0 mM, as shown in Fig. 4.

The calibration curves were examined up to 1.5 mM for both analytes with peak heights and peak areas. The obtained calibration curves are shown in Fig. 5. Good linearity was observed in the curves shown in Fig. 5. Correlation coefficients were 0.996-0.998 for all calibration curves. Relative standard deviations of 1-naphthol were 2.5% (n=8) for both of peak heights and areas as well as those of 2,6-naphthalenedisulfuric acid were 3.2% (n=8) for peak heights and 5.4% (n=8) for peak areas. It was confirmed from the obtained data in Fig. 5 that the TRDC system worked well as a quantitative analysis.

Conclusions

We developed a novel capillary chromatography method called the tube radial distribution chromatography (TRDC) system, which uses an open capillary tube and an aqueous-organic solvent carrier solution under laminar flow conditions. The separation performance in the system was explained based on the tube radial distribution of the carrier solvents in the capillary tube. In this study, we examined the effects of analyte injection volume and concentration on the chromatograms in the TRDC system. The injection volumes and concentrations are fundamental analytical factors in chromatographic research. The analyte solution of 1-naphthol and 2,6-naphthalene as a model was separated and detected in this order up to the injection time of 150 s (injection volume 40 nL or zone length 20 mm) and they were also analyzed up to 1.5 mM with

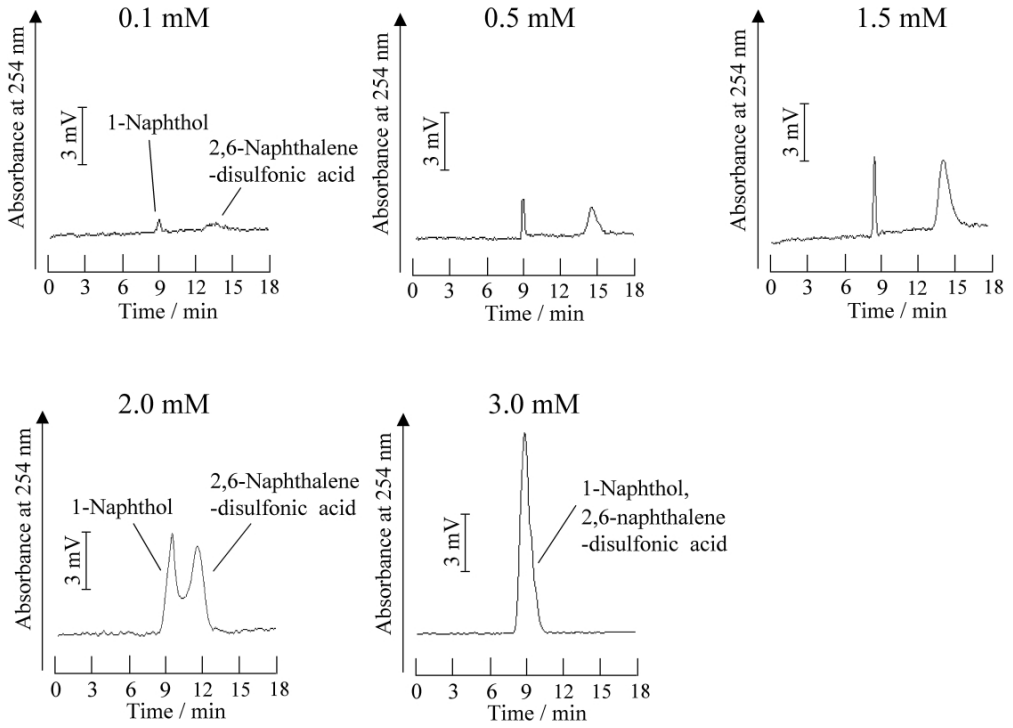

Fig. 4 Chromatograms of a mixture of 1-naphthol and 2,6-naphthalenedisulfonic acid obtained by the TRDC system with various analyte concentrations.
Conditions: Capillary tube, 120 cm (effective length: 100 cm) of 50 μm i.d. fused-silica; carrier, water-acetonitrile-ethyl acetate (3: 8: 4 v/v/v) mixture solution; sample injection, 20 cm height (gravity)×30 s; flow rate, 0.2 μL min^{-1}; tube temperature, 0℃; and analyte concentration, 0.1, 0.5, 1.5, 2.0, and 3.0 mM.

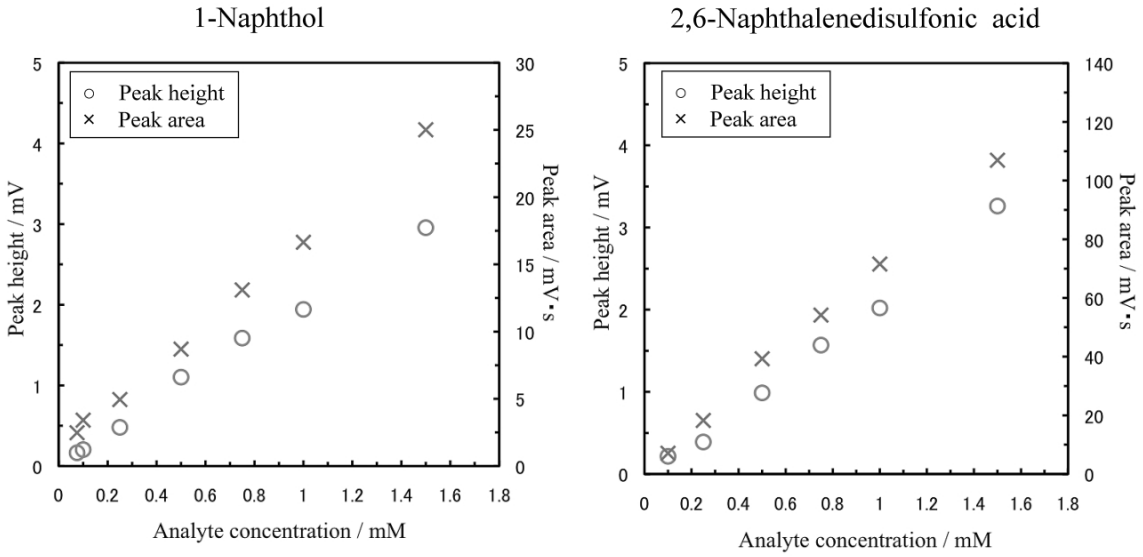

Fig. 5 Calibration curves of 1-naphthol and 2,6-naphthalenedisulfonic acid obtained by the TRDC system. Conditions: Capillary tube, 120 cm (effective length: 100 cm) of 50 μm i.d. fused-silica; carrier, water-acetonitrile-ethyl acetate (3: 8: 4 v/v/v) mixture solution; sample injection, 20 cm height (gravity)×30 s; flow rate, 0.2 μL min^{-1}; tube temperature, 0°C; and analyte concentration, 0.1-1.5 mM.

good reproducibility. Separation in the present TRDC system became difficult at analyte injection times of more than 150 s or analyte concentrations of more than 1.5 mM. The difficulty may be due to instability or collapse of the inner and outer phase formation based on the tube radial distribution of the carrier solvents in the capillary tube with extremely large analyte injection volumes or concentrations. Also, the ranges of analyte injection volume and concentration that provide quantitative analysis might be influenced by analytical conditions, such as capillary inner diameter, flow rates, and component ratios of the carrier solvents.

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