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Development of a New Type of Open Tubular Capillary Liquid Chromatography System Based on Microphase Separation of Mixed Solvents

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This paper describes a new type of open tubular capillary liquid chromatography (OTCLC) which utilizes the microheterogenities and preferential solvation phenomena in organic-water mixed solvents. Several model compounds have been separated by the proposed OTCLC system, and it has been confirmed the separation occurs due to the micro-phase separation of the mobile phase enhanced by salts. The well-known Golay equations were applied to characterize and optimize the OTCLC system.

This paper describes a new type of open tubular capillary liquid chromatography (OTCLC) which utilizes the microheterogenities and preferential solvation phenomena in organic-water mixed solvents. Micro-scale chromatography systems are more attractive than conventional systems because less sample and less mobile phase are consumed. For example, a theoretical study by Knox and Gillbert revealed that if an open tubular 10 μm capillary is used as separation column and the standard deviation of the un-retained solute is kept to less than 1 nL, a peak of $N = 10^6$ theoretical plate number will elute in 2 h compared with 55 h with conventional packed columns.[1] Practicing liquid chromatographic separation with a narrow tube is, however, relatively more difficult compared with its conventional counterparts. The difficulties in construction and operation of very narrow-bored capillary liquid chromatography are: (1) preparation of suitable stationary phase in the very narrow separation column and (2) injection and detection of extremely small sample volumes. In order to increase the diffusivity and to decrease the viscosity of the mobile phase, higher temperatures were used on 50 μm I.D. column by Liu et al.[2] At 200 °C, 10^6 theoretical plates were obtained with a 19.6 m long column. However, the stationary phase of commercially available columns is unstable under high temperature.

The separation of cations using OTCLC on 5–10 μm I.D. column was studied by Simon et al.[3] The capillary column was coated with a strong cation exchanger. To improve selectivity, OTCLC system usually used modified columns.[4,5]

Moreover, open tubular capillary columns are widely used in capillary electrophoresis (CE).[6–8] Electronically driven CE is now achieving a typical several hundred thousands to millions number of theoretical plates and separates the analytes in as little as several seconds. However, there are some limitations of CE, such as aqueous solutions containing high electrolyte concentration must be used to assure a steady electronic current; high voltages (≈10 kV) are used that cause problems concerning safety and interface design when the separation column is attached to other instruments, e.g., mass spectrometry detector.

We propose here a new type of open tubular, mechanically driven capillary liquid chromatography system which could overcome those limitations while preserving the merits: small sample volumes, less solvent consumption and high separation efficiency without using specific columns.

Theoretical Aspects

The proposed open tubular capillary liquid chromatography (OTCLC) system utilizes the recent discoveries of microheterogeneities in some organic–water mixed solvents and salting-out phase-separation phenomena observed in these mixed solvents. Acetonitrile serves as a good example for these organic solvents. Under ambient condition, acetonitrile is miscible with water at any ratio; heterogeneities, however, have been observed at a molecular level in its water mixtures. It has been demonstrated that large acetonitrile molecular clusters as well as water molecular clusters coexist in the mixtures over a wide composition range.[9–12] The solvent clusters preferentially solvate to analytes depending on their solubility in acetonitrile and water. We call it microsolvent clusters extraction mechanism.[13] Such microheterogeneities were further enhanced by addition of electrolytes, such as NaCl, into the mixture.[14] When the salt concentration is high, phase separation occurs in the homogeneous acetonitrile–water mixture, resulting in an acetonitrile-enriched organic phase and a water-enriched aqueous phase. This salted-out two phase system serves as an effective platform for separation/extraction of inorganic as well as organic compounds which could not be extracted by conventional extraction systems.[11,12]

The inner wall of a fused capillary is negatively charged due to the dissociation of its silanol groups. When an acetonitrile–water mixture with suitable salt concentration is pumped into such a capillary, micro-phase separation occurs near the capillary wall, resulting in a water-enriched aqueous phase attached to the capillary inner wall. In other words, a liquid membrane that has considerably different properties from the mobile phase is formed on the inner wall of the capillary. When a mixture of analytes is injected into the capillary, partition of the analytes occurs between the mobile phase and the liquid membrane, causing separation of the analytes in the proposed chromatography system.

The Golay equations[15] are readily applied to the proposed OTCLC system, and the height equivalent to a theoretical plate (HETP) is:

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$$H = H_d + H_s + H_m = \frac{2D_m}{u} + \frac{2k'd^2u}{3(1+k')D_s} + \frac{(11k'^2+6k'+1)r_c^2u}{24(1+k')^2D_m}, \quad (1)$$

where H_d, H_s, and H_m stand for longitudinal diffusion and resistance to mass transfer in stationary and mobile phases, respectively. D_m and D_s are the diffusion coefficients of a solute in mobile and stationary phases, respectively. d is the thickness of the stationary phase, and r_c is the radius of the capillary. k' is the capacity factor of the solute, and u is the flow rate of the mobile phase.

Experimental

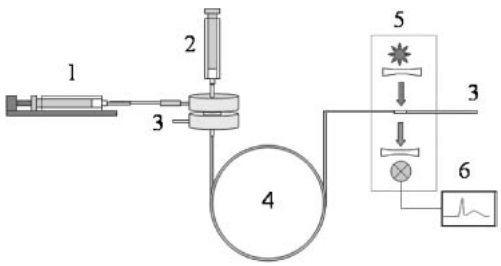
Apparatus. The OTCLC employs a syringe driver (syring Pump Controller MF-9090, 0.1 μdm^3 (= 0.1 to 100 μL min^{-1} adjustable, BAS, U.S.A.) together with a Hamilton Gastight 1 cm^3 (=1 mL) syringe for mobile phase pumping. A 0.2 μL microsample injector (Model 7520, Rheodyne, U.S.A.) working in stopped-flow mode is used for sample injection. By controlling the sampling time, much smaller sample volume than 0.2 μL could be injected. Fused silica capillary tubing with an I.D. of 10 μm and an O.D. of 375 μm of ≈30 cm long (from the injection port to detection window) was employed. An intelligent UV–vis detector of capillary (CE-970, JASCO, Japan) was used for detection. The detector is connected to a personal computer through JASCO LC-NET. Data collection and chromatogram analysis were accomplished by JASCO-Borwin®. The layout of the OTCLC system is shown in Scheme 1.

Results and Discussion

Separation of Several Model Compounds. OTCLC was employed to study model compounds between neutral and charged compounds. Figure 1 clearly shows the ability to separate neutral compounds from charged compounds by using the OTCLC system. Moreover, compounds of similar structure with different charges also were separated. The elution orders in Fig. 1 are explained by extraction behavior using the mixed solvent. Extraction of *p*-nitroaniline and 2,7-naphthalenedisulfonic acid (2,7-NDS) into acetonitrile–water mixed solvents by salting-out showed that *p*-nitroaniline is effectively extracted to the upper (acetonitrile-enriched) phase while 2,7-NDS remained in the lower (water-enriched) phase. This supports the observed elution order as shown in Fig. 1d.

Factors that Affect the Separation. Several factors including mobile phase composition, flow rate and sampling volume were studied to improve the separation.

Effect of Mobile Phase Composition. Figure 2 shows that increasing of acetonitrile in the mobile phase slightly increases the elution time of 1-naphthol but significantly increases the elution time of 2,6-NDS; hence, it improves the separation of 1-naphthol from 2,6-NDS. Moreover, in the absence of NaCl in the mobile phase, no separation in both cases (AN/H_2O 9/1 (v/v) and AN/H_2O 8/2 (v/v)) was observed, demonstrating the necessity of addition of electrolytes for the micro-



Scheme 1. Schematic layout of the proposed OTCLC system. 1: Syringe pump; 2: sample injector; 3: drain out; 4: capillary tube; 5: detection unit; 6: recorder.

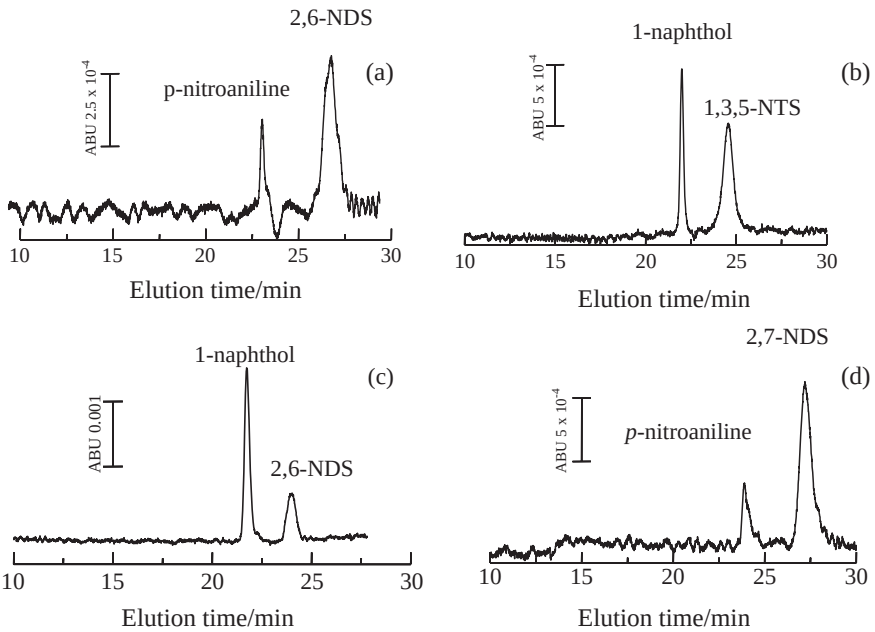


Fig. 1. Separation of the mixtures of (a) *p*-nitroaniline with 2,6-naphthalenedisulfonic acid sodium salt (2,6-NDS); (b) 1-naphthol with 1,3,5-naphthalenetrisulfonic acid sodium salt (1,3,5-NTS); (c) 1-naphthol with 2,6-NDS; (d) *p*-nitroaniline with 2,7-naphthalenedisulfonic acid sodium salt (2,7-NDS). Mobile phase: AN/H_2O 8/2 (v/v) containing 0.1 M NaCl (a,d) and 0.1 M CH_3COONa (b,c). Detection: (a) 254; (b,d) 233; (c) 224 nm. Room temperature.

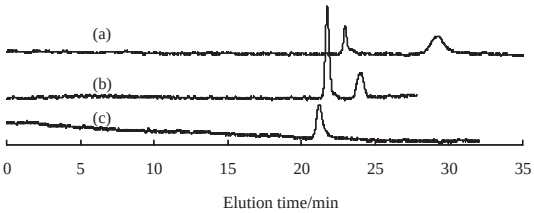


Fig. 2. The effect of mobile phase composition on separation. (a) AN/H_2O 9/1 (v/v) containing 0.1 M NaCl; (b) AN/H_2O 8/2 (v/v) containing 0.1 M NaCl; (c) AN/H_2O 8/2 (v/v) without salt. Flow rate: 0.2 μL min^{-1}; sampling time: 3 s; sample: 0.1 mM 1-naphthol (first peak), 2,6-NDS (second peak); detection: 224 nm.

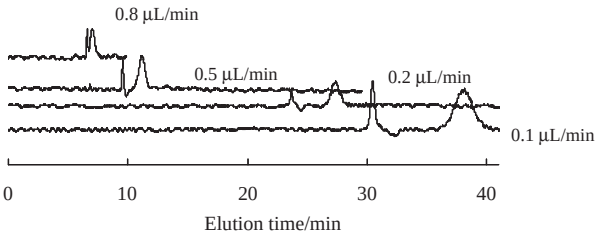


Fig. 3. The effect of flow rate of mobile phase on separation. Mobile phase: AN/H_2O 8/2 (v/v) containing 0.1 M NaCl; sampling time: 3 s; samples: 0.1 mM *p*-nitroaniline (first peak), 2,6-NDS (second peak); detection: 254 nm.

phase separation in capillary tubing.

Effect of Flow Rate of Mobile Phase. Figure 3 indicates that if the linear velocity of the mobile phase is slower, better separation of *p*-nitroaniline and 2,6-NDS is achieved. The values of the separation factor (α) and resolution factor (R) of the above two compounds were 1.16, 1.3; 1.15, 2.8; 1.25, 3.5 with a flow rate of 0.5, 0.2, and 0.1 μL min^{-1}, respectively. The theoretical plates (N) of *p*-nitroaniline and 2,6-NDS were 2947, 860; 36545, 2684; 14884, 2143, respectively, for flow rates of 0.5, 0.2, and 0.1 μL min^{-1}, respectively. The values of HETP increased with the flow rate. For example, the H values for *p*-nitroaniline and 2,6-NDS for the flow rates of 0.1, 0.2, and 0.5 μL min^{-1} were 0.02, 0.13; 0.08, 0.11; 0.10, 0.35 mm, respectively. Thus, H_s and H_m terms in Eq. 1 contributed mainly to the overall H.

Effect of Sampling Volume. Over loading could significantly lower the separation performance of the chromatography system, and it is especially serious for open tubular systems because the surface area of the stationary phase is small. As shown in Fig. 4, a sampling time of 9 s greatly lowered the separation of *p*-nitroaniline from 2,6-NDS. On the other hand, sampling time of 3 s gave good separation of *p*-nitroaniline and 2,6-NDS; however, the signal response was not very strong. Retention time and peak height depended on flow rate, solvent composition and injected sample volume. However, if these factors are kept constant, recovery of the retention time and peak height is expected. The retention times (min) were 23.6 and 23.9 for *p*-nitroaniline, Figs. 1a and 1d, and 22.0 and 21.8 for 1-naphthol, Figs. 1b and 1c, with an error of less than 1%. The injected sample volume was adjusted with a constant injection period (3 s) using a micro-injector (0.2 μL). The

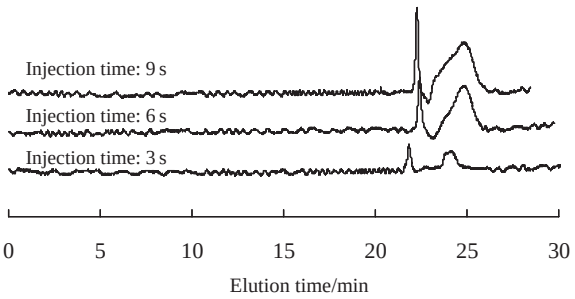


Fig. 4. The effect of sample volume on separation. Mobile phase: AN/H_2O 8/2 (v/v) containing 0.1 M NaCl; sampling time: from upper to bottom, 9, 6, 3 s; samples: 0.1 mM *p*-nitroaniline (first), 2,6-NDS (second); detection: 254 nm.

precision of injected volume was estimated from the peak heights (a.u.) of the 1-naphthol in Figs. 1b and 1c, which were 6.1 and 5.8, respectively.

Conclusion

Utilizing the recent discovery of microheterogeneities, preferential solvation and salting-out phase separation in organic-water mixed solvents, such as acetonitrile–water,[9–14] a new type of open tubular capillary liquid chromatography has been proposed and tested without needing a special separation column. Several model compounds have been separated by the proposed OTCLC system. The separation power of the present chromatography system is due to salt enhanced micro-phase separation of the mobile phase between the capillary wall and the center of capillary tube.

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