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High-pressure and supercritical capillary electrophoresis

CLEMENT R. YONKER* and RICHARD D. SMITH
Chemical Methods and Separations Group, Chemical Sciences Department, Pacific Northwest Laboratory (Operated by Battelle Memorial Institute), Richland, WA 99352 (U.S.A.)

ABSTRACT

Preliminary results are reported for high-pressure and supercritical capillary electrophoresis. The fluid system investigated was methanol [critical temperature (T_c) = 240°C, critical pressure (P_c) = 80.9 bar] containing a small concentration of background electrolyte. The temperature and pressure regions studied ranged from 25–280°C and 67.1–295.7 bar, respectively, during the course of experimentation. Initial results of electrophoretic separations of 1-naphthol-4-sulfonic acid sodium salt and thymol blue as a function of temperature and pressure will be described and discussed.

INTRODUCTION

Capillary electrophoresis (CE) has proven itself to be a technique of high separation efficiency and of potentially extreme importance in the area of bioseparations[1–5]. The applications of CE to the separation of peptides, proteins and nucleic acids continue to increase. The enhanced separation efficiencies and decreased analysis times for complex solute molecules have proven advantageous for CE as compared to other separation methodologies.

The separation efficiency ultimately obtainable by CE depends upon the ratio of electrophoretic mobility to the diffusion coefficient. Although very high speed separations ($\approx$ 1.5 s) have been demonstrated by capillary zone electrophoresis[6], these have required extremely small sample volumes and have yet demonstrated only limited numbers of theoretical plates. Continued improvement of high-speed separations encounters fundamental limitations. To obtain short analysis times the applied potential across the length of the capillary column should be high[7]; in the absence of heating effects separations improve in direct proportion to electric field strength. However, an excessively high applied potential can lead to deleterious effects due to heating of the capillar buffer solution, causing decreased separation efficiency and possible vaporization of the buffer solution[8]. Decreased separation efficiencies can occur through an increase of the parabolic nature in the electroosmotic velocity flow profile with the increased radial temperature gradient[8,9]. Decreasing the buffer

conductivity to limit heating effects, proportionally limits sample size, due to practical limits placed on the conductivity difference between the buffer and sample.

An increase in the mass transfer characteristics of a solute molecule could improve the separation efficiency in a mass transfer limited system. In a separation which is not mass transfer limited an increase in the diffusion coefficient could prove problematic, leading to increased zone broadening. In capillary electrophoresis where axial diffusion can define the zone broadening limit in some cases, increasing the diffusion coefficient of the solute would not prove beneficial. This could also be true in supercritical-fluid capillary electrophoresis (SCE) for non mass transfer limited situations.

True plug flow is not achieved in electroosmotic flow[9]. Therefore, the shear layer at the capillary wall can contribute to solute zone broadening in capillar electrophoresis. Increasing radial mass transport through this shear layer could decrease the overall zone broadening in the system. This will be dependent on the interplay between ion molecular diffusion in the radial and axial direction in the capillary. SCE could prove to be an interesting technique to study these physicochemical processes for electroosmotic flow.

One might anticipate that electrophoretic mobilities will increase linearly with molecular diffusion coefficients, providing a basis for faster separations. Other secondary benefits might arise from such an experimental approach. For example, increasing pressure in the system would increase the solvent's boiling point, thus decreasing solvent vaporization. Vapor generation during CE separations generally leads to (often dramatic!) failure of the separation. This approach could allow higher electric fields to be used. Therefore, high-pressure capillary electrophoresis (HPCE) and SCE could prove beneficial in mass transfer limited systems and in obtaining shorter analysis times. From a fundamental viewpoint, SCE and HPCE present the novel opportunity to study the effects of fluid density (pressure and temperature) on solute mass transfer in electroosmotic flow. SCE and HPCE can contribute to the understanding of the effect of fluid viscosity changes on electrophoretic and electroosmotic mobilities. Changes in the capillary surface double layer as a function of pressure and temperature can be studied. Any changes in the zeta potential of the surface and the solute molecule with density which can affect solute migration can be studied. Finally, the thermodynamics of equilibrium for the charged solute species as a function of pressure and temperature can be determined.

In this manuscript, we present initial data involving our preliminary experiments with high-pressure and supercritical capillary electrophoresis. The discussion centers on the effects of pressure and temperature on solute selectivity and separation efficiency.

THEORY

Capillary electrophoresis involves the electrophoretic migration of a solute species in a capillary in the presence of an electric field. In the high-pressure or supercritical fluid capillary electrophoresis arrangement we have selected for initial investigation, the electrophoretic migration of the solute occurs in a capillary connected between two high-pressure solvent reservoirs. Therefore, the total velocity of a solute species can be described as

$$v_T^1 = v_{ep}^1 + v_{eo}^1 + v_p^1 \tag{1}$$

where v_T is the total velocity of the solute: v_{ep} and v_{eo} are the electrophoretic and electroosmotic velocity of the solute, respectively: v_p is the velocity of the solute due to a pressure difference (potentially caused by any leakage in the pressurized system and would be detrimental to obtaining high efficiencies): and 1,2... refers to the number of solutes. The electrophoretic velocity of the solute is

$$v_{ep} = \mu_{ep} E \tag{2}$$

where μ_{ep} is the electrophoretic mobility of the solute and E is the electric field strength. The electric field strength is the ratio of the applied voltage to the capillary length (V/L). The electrophoretic mobility of the solute is

$$\mu_{ep} = [\varepsilon\zeta/4\pi\eta] \tag{3}$$

where ε is the dielectric constant, η is the viscosity of the solvent, and ζ is the zeta potential at the solute/solvent interface. These molecular parameters control the electrophoretic mobility for a specific solute molecule. A similar relationship can be described for the electroosmotic mobility; in this case, the zeta potential refers to the potential at the liquid/solid interface on the capillary surface[1,10,11]. In the case of HPCE and SCE, the physicochemical parameters of ε, ζ and η will be a function of temperature, pressure, and fluid density. The dielectric constant and viscosity of a supercritical fluid are clearly a function of temperature and pressure[12–14]. Thus, one can expect to observe changes in the electrophoretic and electroosmotic velocity of the system when using a supercritical fluid compared to conventional liquids.

The separation of two solute molecules will be dependent on differences in their migration velocity. Eqn. 1 describes the total velocity of the solute in a CE experiment. The difference in migration velocities for two solutes (Δ) is determined by subtracting their respectively total velocities. Assuming $v_{eo}^2 = v_{eo}^1$ and $v_p^2 = v_p^1$, then at constant pressure or temperature,

$$\Delta \equiv v_T^2 - v_T^1 = v_{ep}^2 - v_{ep}^1 = \mu_{ep}^2 E - \mu_{ep}^1 E = (\zeta^2 - \zeta^1)E\varepsilon/4\pi\eta \tag{4}$$

The difference in migration velocity (selectivity) is dependent on field strength (E) and two other terms; one term is dependent on molecular differences ($\zeta^2 - \zeta^1$), and the second term is dependent on the solvent ($\varepsilon/4\pi\eta$). The change in selectivity, Δ, as a function of pressure and/or temperature in the supercritical-fluid region could be a sensitive indicator of the change in physical or chemical environment about the solute molecules.

The difference in migration time for two solutes as a function of Δ can be written as,

$$\Delta t = \frac{\Delta L}{\mu^1 \mu^2 E^2} \tag{5}$$

where μ^1 and μ^2 represent the mobility for the two solutes, respectively. The difference in migration time can be related to the fundamental parameters of ζ, E, η and V on appropriate substitution and rearrangement of eqn. 5 yielding,

$$\Delta t = \frac{(\zeta^2 - \zeta^1)\ 4\pi\eta\ L^2}{(\zeta^2\zeta^1)\ \varepsilon V} \tag{6}$$

Therefore, the difference in migration time can be related to the applied potential and specific solute or solvent effects as stated in the above discussion. While the determination of Δt is an oversimplification of the parameters governing solute migration, some insight into the effects of pressure, temperature and applied potential in HPCE and SCE can be obtained from this value.

EXPERIMENTAL

The supercritical capillary electrophoresis system is basically similar to low pressure systems published in the literature[4]. A schematic of the SCE system is shown in Fig. 1. This system has two high-pressure SS316 solvent reservoirs; one at the anode end of the capillary column and one at the grounded end of the column. The system is pressurized using an high-performance liquid chromatography (HPLC) syringe pump (Varian 8500) under microprocessor control. A variable-wavelength UV–visible detector (ISCO) was used for on-line detection of the solute after a section of the polyimide coating of the capillary had been removed. A high-voltage power supply (Glassman) was used to supply the positive potential to the anodic end of the capillary column. A muffle furnace with a temperature controller was used to hold the capillary column at a constant temperature. A hydrostatic line was used between the two high-pressure reservoirs to facilitate sample loading and pressurization. The hy-

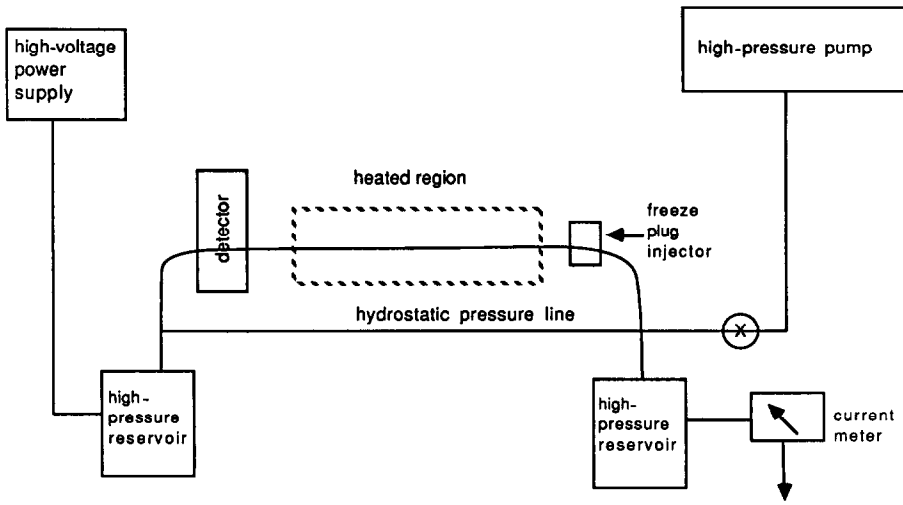

Fig. 1. Schematic of supercritical-fluid capillary electrophoresis system.

drostatic line was connected to the pressurized solvent reservoirs through two high-pressure 1/16-in. stainless-steel 316 tees. These fittings contained connections for the analytical CE column, the hydrostatic line and the solvent delivery line from the pump (see Fig. 1).

Sample injection was accomplished by dipping the end of the analytical capillary column in the solute–electrolyte solution. Then a small section (< 1.5 cm) of the column was frozen using liquid nitrogen. The contraction of the solvent in the capillary pulled a sample plug into the column. The column segment was kept frozen while the system was reconnected to the high-pressure reservoir and then pressurized with the HPLC pump. The hydrostatic line was needed to equalize the pressure on both sides of the frozen column plug, such that on thawing the sample plug would remain positioned at the end of the analytical column. The system temperature and pressure could be equilibrated to the appropriate conditions chosen for study while the column segment was frozen. The "freeze plug" injection technique was necessary because the large volume of typical HPLC sample valves and difficulties arising from the metal and graphite surfaces in these valves which affected the potential gradient down the column. Work is proceeding on the design and construction of more appropriate high-pressure, low dead volume valves for use in the SCE system.

The analytical capillary column used was fused silica, 150 μm I.D. by 360 μm O.D. and *ca.* 100 cm in length. The hydrostatic column was 50 μm I.D. × 210 μm O.D. fused silica which had a bonded coating of SE-54 on the surface. This organic coating precluded any electroosmotic flow but maintained the two high-pressure reservoirs at a similar pressure value, independent of any electroosmotic flow in the analytical capillary when the system was pressurized. The solvent used was methanol (T_c = 240°C, P_c = 80.9 bar) having an electrolyte concentration of 10 m*M* acetic acid and 10 m*M* sodium acetate. This electrolyte system had a current of *ca.* 40 μA at room temperature and 296 bar at +30 kV applied across the column. The solutes used were 1-naphthol-4-sulfonic acid sodium salt (Kodak Chemical) and thymol blue (Aldrich). Both molecules were soluble in the methanol electrolyte solvent and showed electrophoretic migration at room temperature and pressure. After the determination of the net electroosmotic and electrophoretic flow velocities in the system at room temperature and pressure, initial experiments were undertaken to determine the effect of high-pressure and supercritical conditions on the electrophoretic separation.

RESULTS AND DISCUSSION

There have been several reports of electrochemistry and ion-mobility in supercritical fluids which demonstrate that electrophoretic separations in a supercritical fluid should be feasible[15–20]. This work has entailed voltammetry studies in supercritical carbon dioxide by Wightman and co-workers[16–18] and electrochemical studies in supercritical water and other non-aqueous solvents by Flarsheim *et al.*[19] and Crooks[20]. These studies demonstrate that a supercritical fluid can dissolve an electrolyte and can have sufficient ionic strength (*i.e.*, carry sufficient current) needed for supercritical-fluid capillary electrophoresis.

SCE presents the novel opportunity to study directly the effect of fluid density (*i.e.*, pressure and temperature) on the capillary surface double layer, the zeta potential of the capillary surface and the solute molecule, the effect of changing viscosity on the

mass transport properties of the charged solute in the supercritical fluid, the electrostriction of the solvent about the solute ion[21,22], and the change in equilibrium of the charged solute. In addition, use of high pressures would allow higher electric field gradients to be applied to the analytical column for potentially obtaining shorter analysis times. We note potential difficulties could still arise due to the increased resistive heating of the buffer solution and the effect of the radial temperature gradient on the fluid density and flow velocity profile. In mass transfer limited electrophoretic separations in liquids where the slight parabolic nature of the flow velocity profile leads to decreased separation efficiencies, the use of a supercritical fluid could prove beneficial as shown in supercritical-fluid chromatography. The study of the physicochemical parameters governing SCE could lead to an improved understanding of ion solvation and capillary electrophoresis as practiced today.

Initial data for high-pressure and supercritical capillary electrophoresis are listed in Table I. The change in migration time between the two solutes, Δt, is given as a function of pressure, temperature and voltage potential. At constant pressure and temperature, as the applied potential increases Δt between the two peaks decreases. This decrease in the difference in the migration time between the peaks is due to the increase in potential applied to the system. As shown in eqn. 6, there is a inverse relationship between applied potential (V) and Δt. Therefore, as the potential is increased at constant pressure and temperature, the migration time difference between the solutes would be expected to decrease which is seen in Table I. For HPCE at constant temperature and applied potential, a slight decrease in Δt is noted with increasing pressure. Pressure can effect the solvent viscosity and dielectric constant or the zeta potential between the solute–solvent and solvent–surface. Over the pressure range studied at this temperature, these effects could be expected to be either slight or

TABLE I

SOLUTE SELECTIVITY (Δt) AS A FUNCTION OF POTENTIAL, PRESSURE AND TEMPERATURE

Conditions	*Δt (s)*	*Potential (kV)*	*Atm*	*T(°C)*	*i (μA)*
136 bar, 21°C	270 ± 10	+15			33
	282 ± 20	+20			45
	180 ± 20	+25			57
	180 ± 10	+30			70
21°C, + 20 kV	264 ± 20		67		45
	252 ± 20		137		45
	216 ± 20		206		45
	204 ± 20		278		43
296 bar, + 30 kV	249 ± 9			21	41
	265 ± 9			55	44
	234 ± 9			105	48
	238 ± 9			150	49
	204 ± 9			200	50
	180 ± 9			217	48
	160 ± 18			250	43
	189 ± 9			280	31

negligible. Therefore, the change in Δt would be expected to be very small. At constant pressure and applied potential Δt changes with increasing temperature. This change in Δt is significant and falls outside the experimental error (listed in Table I). In the sub-critical and near-critical regions an increase in temperature would be expected to decrease solvent viscosity which should lead to a decrease in Δt. As temperature increases Δt shows a monotonic decrease. At constant pressure, above the critical temperature, further increases in temperature will decrease solvent viscosity. Therefore, Δt would be expected to decrease based on solvent viscosity arguments as shown in Table I. Density variations in the critical region would affect the zeta potential of the molecules and the viscosity and dielectric constant of the fluid. All these physicochemical parameters would affect the selectivity obtainable between peaks, but at the crude present level of experimental sophistication it is difficult to isolate these individual parameters. Further studies at constant density are planned which will better characterize these physical parameters in HPCE and SCE, but there are some interesting preliminary trends.

The observed electrical current in the analytical column as a function of potential, pressure and temperature is also listed in Table I. Perhaps the most interesting behavior involves the change in current which occurs as a function of temperature at constant pressure. Column current increases with increasing temperature, with a maximum being reached at *ca.* 200°C. Beyond this value the current decreases as one proceeds into the critical region for methanol. This decrease in current could be due to a decrease in solubility of the background electrolyte as temperature increases. In the critical region, the background electrolyte solubility (or dissociation to ionic species for weaker electrolytes) will be a function of fluid density. As the fluid density decreases with increasing temperature at constant pressure, the background current was seen to drop very rapidly.

A representative separation in SCE is shown in Fig. 2. This figure shows the separation of 1-naphthol-4-sulfonic acid sodium salt and thymol blue at room temperature and 280°C at 296 bar and a blank run at 280°C, 296 bar. The blank experiment under supercritical conditions shows a possible "system" peak. If this is

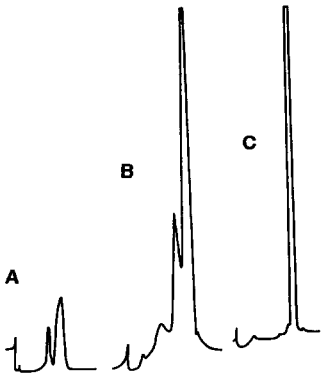

Fig. 2. Supercritical fluid electrophoretic separations of 1-naphthol-4-sulfonic acid sodium salt (first peak) and thymol blue (second peak) at atm 296 and +30 kV; (A) 21°C, 42 μA; (B) 280°C, 30 μA; and (C) blank 280°C, 31 μA.

indeed the case, a systematic study of the "system" peak's migration velocity and its dependence on temperature and pressure could provide information about the surface double layer in the supercritical region. At present it is unclear as to the true nature of this "system" peak, *e.g.*, the "system" peak is not seen at ambient temperatures only at higher temperatures. Further experimental characterization of this "system" peak is being undertaken at this time to determine its exact nature and behavior. Another additional aspect of the results shown in Fig. 2 is the broadening of the peaks in the supercritical fluid as compared to the HPCE separation. One would expect this behavior due to enhanced axial molecular diffusion in a non mass transfer limited separation.

CONCLUSIONS

The preliminary results discussed in this manuscript demonstrate the feasibility of supercritical-fluid electrophoresis. This technique could potentially be used to enhance resolution, separation efficiency, or speed in mass transfer limited systems. Of major interest is the physicochemical study of the surface double layer as a function of pressure and temperature. Specific changes in the zeta potential of the capillary surface and probe molecule with density can also be studied. The effects on the electrophoretic and electroosmotic mobilities by changing fluid viscosity and dielectric constant, and the electrostriction of solvent about an ion in a supercritical fluid can be investigated. Studies of these types should be amenable with SCE, leading to a better understanding of the physicochemical parameters governing solute separation by capillary electrophoresis.

HPCE and SCE are techniques in their infancy and their ultimate usage as analytical separation methodologies is still in question. But, they could prove useful in certain separation schemes. Their use in the investigation of density effects on the physicochemical parameters governing ion mobility in a liquid could prove to be important. Further research characterizing these parameters is continuing at this time.

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