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PRELIMINARY INVESTIGATION OF ION MOBILITY SPECTROMETRY AFTER CAPILLARY ELECTROPHORETIC INTRODUCTION

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SUMMARY

Using standard capillary electrophoretic and ion mobility methods, several electrospray interface designs were investigated for the capillary electrophoretic introduction of samples into the ion mobility spectrometer. Of the interfaces investigated, the flow assisted interface and the direct coupled interface showed the most promise. These preliminary experiments were encouraging. The ion mobility spectrometer coupled with a capillary electrophoretic introduction system operated with excellent separation efficiency and ion mobility reproducibility. Using tetrabutylammonium iodide, the number of theoretical plates for the spectrometer was calculated to be $3 \cdot 10^3$ and reduced mobilities were found to be reproducible with a relative standard deviation of 1.43%. Because of the desire to hold the spectrometer as hot as possible, the solvent would often vaporize in the interface, creating an unstable spray and inhomogeneities in the electrophoretic field. More work is needed to improve the spray process which contributed to the overall noise of the system and to eliminate the phenomenon of solvent vaporization which limited the reproducibility of electrophoretic migration times.

INTRODUCTION

For some time now, our laboratory has been interested in the development of ion mobility spectrometry (IMS) as a detection method after high-resolution separations. IMS is an analytical method by which gas-phase ions at atmospheric pressure are separated in time according to their mobilities as they travel through an electrical field. The high efficiencies of many gas-phase ionization methods such as chemical ionization, electron capture and photoionization coupled with the arrival time spectra of these ions provide both qualitative and quantitative information for the analytical chemist. One application of IMS for analytical chemistry has been as a detection method after chromatography.

As a capillary gas chromatographic detector it has the combined versatility of detectors such as the flame ionization detector, the electroncapture detector, and several selective detectors[1,2]. As a detector for supercritical fluid chromatography it

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has potential for the detection of high-molecular-weight compounds and for use with modified mobile phases[3,4]. More recently, we have become interested in the use of IMS as a detection method for liquid samples[5]. Following the lead of Thomson *et al.*[6] and Whitehouse *et al.*[7] with electrospray mass spectrometry, we reinvestigated the work of Gieniec *et al.*[8] and demonstrated that an electrospray-related method which we called corona-spray IMS had potential application for liquid chromatography and flow injection analysis[9].

Our initial interest in capillary zone electrophoresis (CZE) was as the liquid phase analogue to gas-phase IMS, although there are some salient differences. From the work of Smith and co-workers[10,11] on CZE–electrospray mass spectrometry and our previous experience in corona-spray IMS, it seemed feasible to couple the liquid-phase electrophoretic technique with the gas-phase ion mobility technique to provide a two-dimensional open tubular electrophoretic separation.

In this preliminary investigation, the primary objectives were to evaluate the quality of the ion mobility spectra after electrophoretic injections and to investigate various electrospray interface assemblies that have been used in other work.

EXPERIMENTAL

Instrumentation

A schematic of the overall CZE-IMS apparatus is provided in Fig. 1. The ion mobility spectrometer utilized in this study was purchased from Scientech, Pullman, WA, U.S.A. It was a stacked ring spectrometer with a corona-spray nebulization/ionization source similar to that reported recently by Shumate and Hill[9]. In the normal operation of the spectrometer, liquid samples were delivered through the corona-spray needle at flow-rates between 1 and 20 μl/min. The corona-spray needle potential was 3000–4000 V above the potential of the drift tube. In a nitrogen or air atmosphere this potential was sufficient to produce a corona discharge. As the liquid sample was forced through this discharge, it became charged and liquid droplets burst apart into a fine aerosol spray from the force of coulombic repulsion. As the solvent evaporated from this electrofied aerosol, charge density on the charged drops increased and further coulombic repulsion dissociations occurred until stable ionic species were achieved.

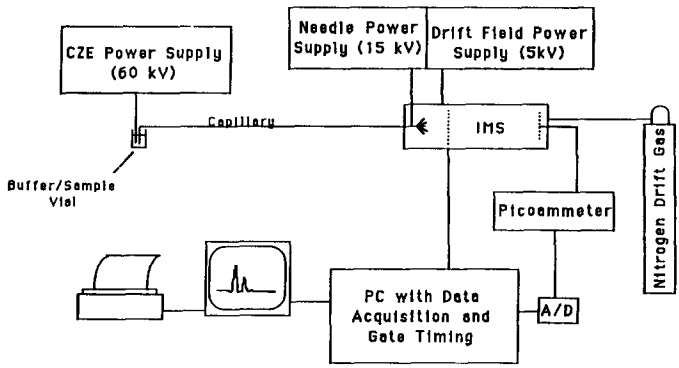

Fig. 1. CZE–IMS system diagram. A/D = Analog-to-digital converter; PC = personal computer.

These ions (or ion clusters) migrated in the electric field of the drift tube to the ion entrance gate of the spectrometer's drift region. When the gate was closed, ions were neutralized at the gate but when it was opened, ions were allowed to migrate into the separation region of the spectrometer. Typically the entrance gate was pulsed open about 1% of the time. After a pulse of ions were admitted to the drift region of the spectrometer, they separated in time through a fixed distance according to their mobilities. The waveform produced by the separated ions was tracked and amplified using a fast electrometer and then digitized with a 12-bit A/D and stored in a computer. Custom-written software was used to control the instrument and collect data.

Operating parameters for the ion mobility spectrometer were selected for this work based on previous experience with the system. No optimizations were performed for these initial investigations of CZE–IMS. In this study, the drift gas for the spectrometer was 99.98% nitrogen or air (Liquid Air Corp., Walnut Creek, CA, U.S.A.) supplied at a flow-rate of 600 ml/min. Operating temperatures ranged from 80 to 120°C and the pressure was ambient, varying from 695 to 700 Torr. The drift length of the spectrometer was 7 cm and the ionization region was 5 cm in length. A voltage of +3600 V was placed across the entire 12 cm length of the tube to create an electric field of 300 V/cm.

The electrophoretic portion of the apparatus was a laboratory constructed device. A plexiglass sample holder and injector was constructed by WSU technical services. A 60-kV power supply was constructed from a "power pack" (Model 60A, Hipotronics, Brewster, NY, U.S.A.) and a 120-V autotransformer.

As with the operating conditions of the IMS system, the electrophoretic conditions were not optimized but rather selected on the basis of previously published literature settings. The columns used were 50–100 cm lengths of untreated fused-silica capillary tubes of 100 μm I.D. The electric field placed on the column was generally around 200 V/cm and the current observed through the column ranged between 10 and 25 μA. Buffer concentrations ranged from 1–10 m*M* and sample concentrations were generally on the order of the buffer concentrations.

Buffer systems used in this work were Na_2HPO_4–KH_2PO_4 at pH 6.8 and ammonium acetate at pH 7.2. Sample compounds tested included caffeine, tetramethyl ammonium iodide, trimethylphenyl ammonium iodide and tetrabutyl ammonium iodide.

Samples were introduced into CZE columns by discontinuous electrophoretic injection and by continuous electrophoretic injection. The discontinuous method was similar to the standard electrophoretic method used in CZE. With the voltage off, the column was removed from the buffer solution and inserted into the sample solution. The voltage was turned on for a timed electrophoretic injection and then off again. Injection times were typically 15–30 s. The column was removed from the sample solution, reinserted in the buffers and voltage was reestablished to the column. In the continuous electrophoretic injection method, the column was filled with buffer and inserted into the sample solution. Voltage was then applied to the sample and migration of the sample was monitored.

Figs. 2–4 show schematic diagrams of the interfaces investigated. Fig. 2 shows a nebulization-assisted spray (NAS) similar to that described by Bruins *et al.*[12]. In this approach a double "T" connector was used to construct the interface. In the first "T", the CZE capillary was butt connected to a fused-silica transfer capillary. This capillary

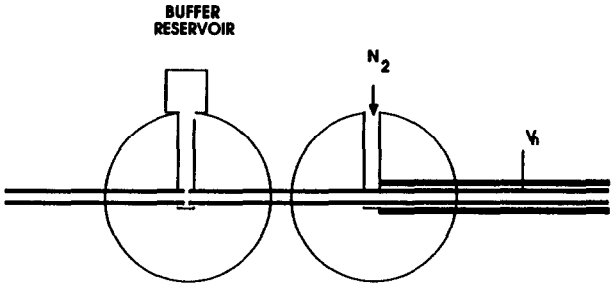

Fig. 2. Nebulization-assisted spray interface. V_n = Potential on the electrospray needle.

connection was not sealed but rather surrounded by a buffer solution that was referenced to ground, establishing the electrophoretic potential drop across the column. The second "T" connector introduced a nebulizing flow of nitrogen concentric to the capillary transfer line. Both the capillary transfer line and the nitrogen were passed through a metal needle with an applied potential to provide the electrospray voltage.

The second approach, shown in Fig. 3, was a flow-assisted spray (FAS) method similar to that used by Smith and co-workers[10,11]. In this design the separation column was inserted through the "T" fitting to the end of the electrospray needle. A sheath flow of a secondary buffer was introduced by the "T" connector, establishing contact with the electrophoretic solution at the end of the column. Potential was established

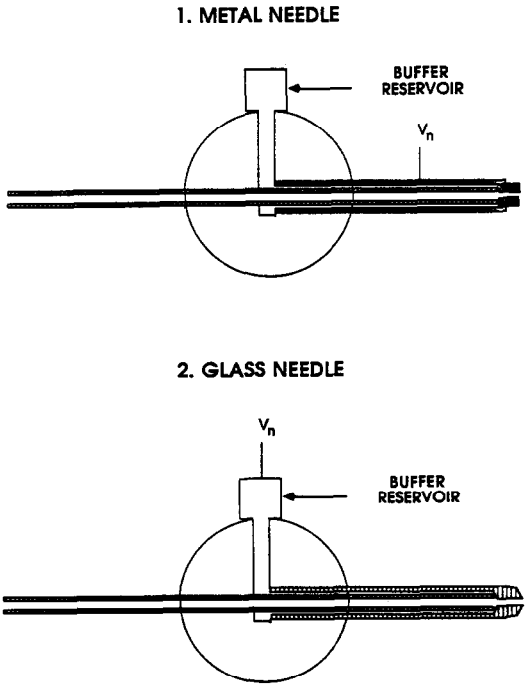

Fig. 3. Flow-assisted spray interface.

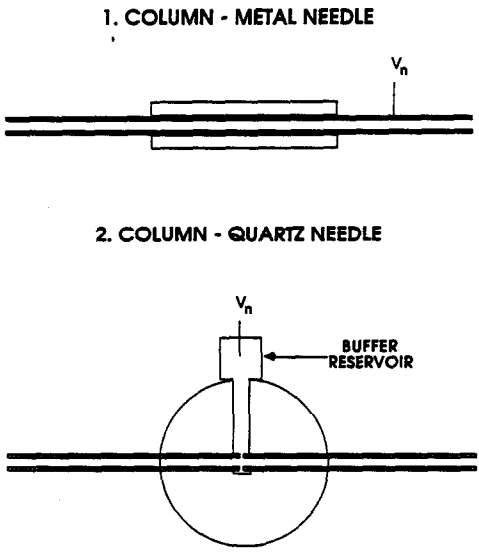

Fig. 4. Direct-coupled spray interface.

either through a metal needle or by placing an electrode in the sheath flow buffer. Establishment of the terminal potential through the buffer was required when glass was used as the electro-spray needle.

The final interface design investigated was a simple direct-coupled spray (DCS) method similar to that used for pumped flow systems[9]. Shown in Fig. 4, this approach took two forms, one with a metal needle and one with a fused-silica needle. With the metal needle, the terminal voltage was applied directly to the needle. With the fused-silica needle, the terminal voltage was applied through the connector joint by an external buffer solution.

Table I provides a list of typical potentials used for each interface system.

TABLE I

TYPICAL VOLTAGES APPLIED

V_s = potential of the sample end of the column; V_r = reference voltage (potential at the end of the electrophoretic column); V_n = potential on the electrospray needle; V_d = voltage on the first ring of the ion mobility spectrometer.

Interface	*Needle*	V_s (kV)	V_r (kV)	V_n (kV)	V_d (kV)
NAS	Conducting	+16	0	+8	+3.6
FAS	Conducting	+18	+9.3	+9.3	+4.9
	Non-conducting	+18	+9.9	+9.9	+4.0
DCS	Conducting	+22.5	+7.5	+7.5	+4.5
	Non-conducting	+22.5	+7.5	+7.5	+4.5

RESULTS AND DISCUSSION

Nebulization-assisted spray approach

The NAS approach had a number of advantages which would have been beneficial for the introduction of liquids into the IMS system had this interface method been compatible with our spectrometer. First, since the buffer solution was held at ground, the CZE potential and the potential on the electrospray needle could be varied independently of one another. Secondly, when the electrospray was observed outside of the spectrometer, the nebulization process was found to enhance the ability of the interface to produce a fine spray. Unfortunately, the gas flow-rates required to produce the spray were so large and the spray orifice so small that the gas velocities produced by the interface were too large for the IMS system to handle. The primary effect observed with the NAS interface was the presence of a large standing current, even when the ion gate was closed. Opening and closing the ion gate indicated that about half of the total ion current was being blown through the gate. Also, with the entrance gate closed, the standing current in the spectrometer could be varied as

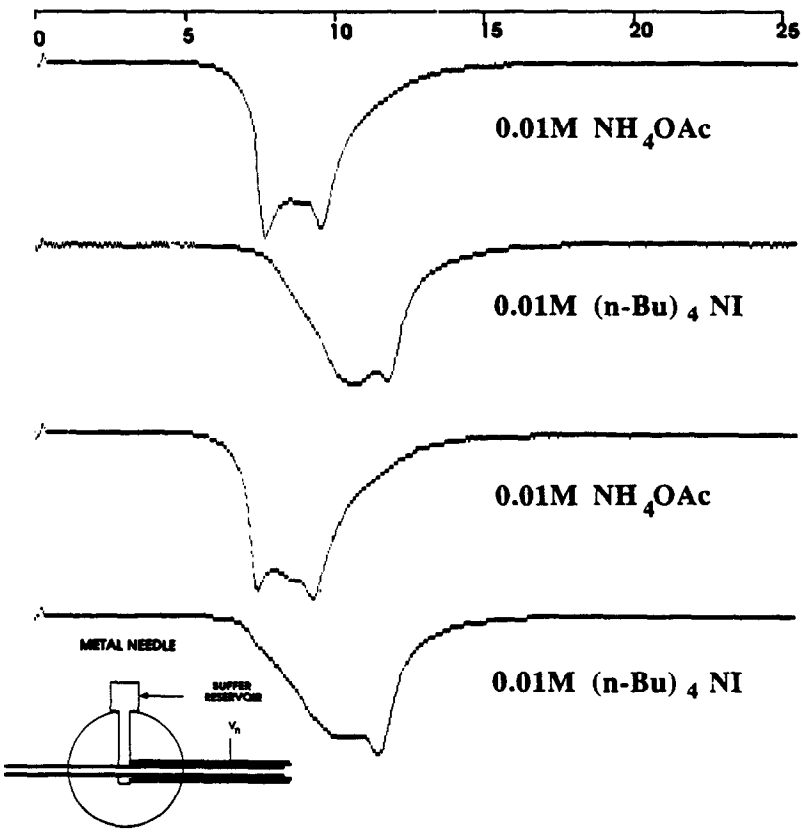

Fig. 5. Continuous electrophoretic introduction with metal-needle FAS at about 30 μl/min sheath buffer flow. Conditions: 50 cm × 100 μm I.D. column, capillary voltage 18 kV, needle voltage 9.3 kV, IMS drift voltage 4.9 kV, detector temperature 110°C. Ac = CH_3CO; Bu = C_4H_9. Scale at top: time in ms.

a function of the flow-rate of the nebulizing gas. Perhaps there is some way in which the ion gate or the spectrometer could be modified to accept the NAS, but without modification it was not possible to use the NAS interface with IMS.

Flow-assisted spray

FAS showed promise as a method for interfacing CZE to IMS. The advantages of this approach are the same as they are for mass spectrometry: the potential is placed at the end of the column so that electrophoretic separation occurs throughout the column and the sheath buffer can be modified to optimize the electrospray process.

Fig. 5 shows ion mobility spectra that were obtained with continuous electrophoretic introduction when the column was alternated between the buffer and the sample. The top spectrum shown in this Figure was of a 10 m*M* solution of ammonium acetate. In the spectrum there were two peaks occurring at drift times of 7.70 and 9.60 ms. Unfortunately these two peaks were poorly resolved and it appeared that the efficiency of the ion mobility separation was considerably reduced over that which is possible after liquid chromatography with direct coupled electrospray ionization. Nevertheless, when the sample, a 10 m*M* solution of tetrabutylammonium iodide, was introduced into the spectrometer, a definite change in the spectral pattern was observed. The second spectrum in Fig. 5 shows the disappearance of the reactant ion at 7.70 ms and the appearance of a product ion at 11.85 ms. A little over 4 min after the column was returned to the ammonium acetate solution, the spectrum reverted to the initial pattern, as indicated by the third trace in Fig. 5. These two patterns would reproducibly alternate as a function of whether the buffer or sample was electrophoretically introduced into the spectrometer. A delay of about 4 min was observed each time the column was switched between the buffer and the sample before the appropriate spectrum appeared. The ion mobility spectra are of lower resolution than is normal for IMS, but the reproducible pattern changes indicate that a sample can be electrophoretically introduced into the ion mobility spectrometer and that characteristic spectra can be obtained.

The broadness of the peaks shown in Fig. 5 could be attributed to insufficient solvent evaporation prior to the ions entering the drift region of the spectrometer. Continued solvent evaporation and ion molecule reactions in the drift region may contribute significantly to the band broadening process. One attempt to eliminate this source of band broadening was to reduce the flow of the sheath buffer by restricting the orifice of the electrospray needle. This was accomplished by using a glass capillary needle which had been pulled to a narrow tip. The fused-silica capillary column was butted against the restricted end of the glass needle as shown in Fig. 3. The column–needle connection was tight enough to significantly restrict the sheath buffer flow while maintaining electrical contact with the CZE buffer. During a continuous run of about 2 h, a drop in the buffer reservoir was observed which indicated that the flow-rate was about 1 μl/min.

Fig. 6 shows spectra obtained using FAS with a glass needle, in a capillary isotachophoresis (CITP) experiment[13]. The capillary column was filled with 10 m*M* ammonium acetate–methanol (90:10) and a sample mixture of $(CH_3)_4NI$ and $(CH_3)_3C_6H_5NI$ (both at 1 mg/ml) was introduced by applying 16 kV for 30 s. The CITP separation was begun by placing the capillary in 0.01 *M* $(n\text{-}C_4H_9)_4NI$–methanol (90:10) and applying an electrophoresis potential of 8.1 kV (18 kV at the buffer

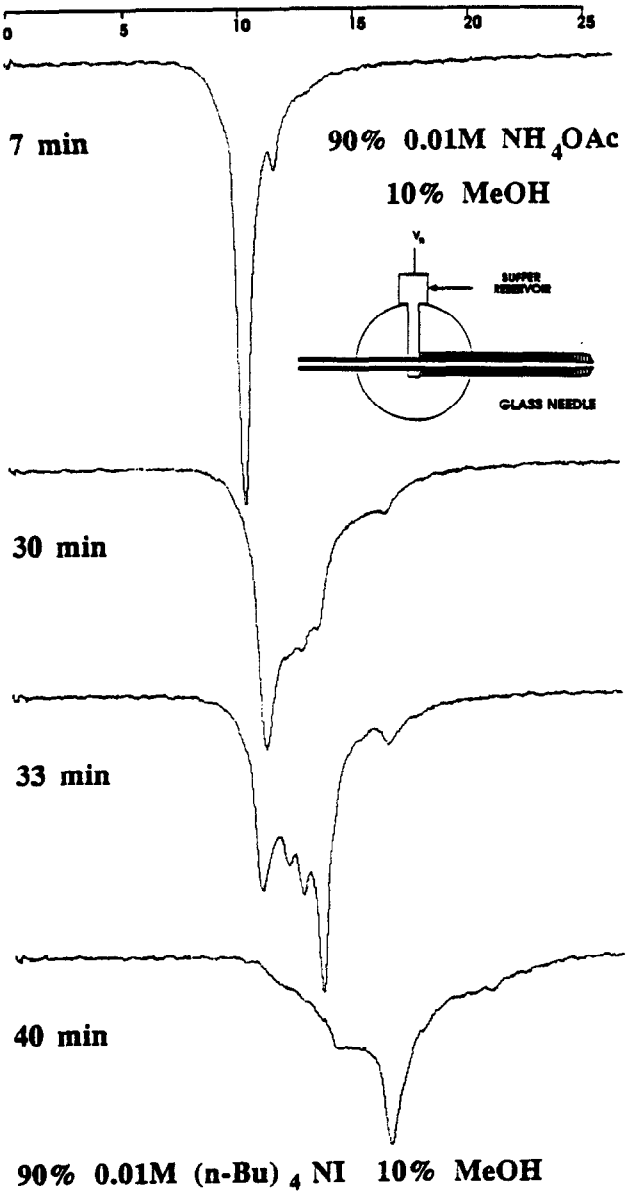

Fig. 6. Capillary isotachophoresis with glass-needle FAS at 1 μl/min sheath buffer flow. Conditions: 100 cm × 100 μm I.D. column, capillary voltage 18 kV, needle voltage 9.9 kV, IMS drift voltage 4 kV, detector temperature 20°C. MeOH = Methanol. Scale at top: time in ms.

reservoir, 9.9 kV at the needle). After about 30 min the spectrum began to show a marked change. Spectral change continued until about 40 min, after which time a spectrum characteristic of $(n\text{-}C_4H_9)_4NI$ was observed (compare the bottom trace of Fig. 6 with the bottom trace of Fig. 5). Spectra obtained between 30 and 40 min after

starting the separation were clearly different from the initial and final spectral patterns, as shown in the middle two traces in Fig. 6. We were probably observing a partially developed separation of $(CH_3)_3C_6H_5N^+$ and $(CH_3)_4N^+$.

Throughout these experiments we had difficulty maintaining stable spray conditions. As sheath buffer flows increased, arcing was increasingly observed in the high electric field of the IMS system. At buffer flows greater than about 5 μl/min the spectral instability due to these arcs made the detector essentially unuseable. At low buffer flows electrical contact between the sheath and CZE buffers was unreliable. In practice, only a narrow window of flow-rates (about 0.5 to 2.0 μl/min) was useful. The flow problems were compounded by trying to raise the temperature inside the IMS system. Elevated temperatures generally improve IMS performance, and certainly assist volatilization of both solvent and ammonium acetate buffer. However, above 100°C bubble formation within the CZE capillary was increasingly a problem. In the worst case, vapor locks occurs, destroying electrical continuity in the CZE capillary. In practice temperature in the IMS system had to be kept below 100°C for continuously stable operation.

Direct coupled spray

Fig. 7 (top) shows a background spectrum obtained with direct coupling in which the buffer solution was a 10 m*M* PO_4^{3-} at pH 7.1. Individual injections of $(CH_3)_3C_6H_5NI$, $(n\text{-}C_4H_9)_4NI$, and caffeine provided characteristic ion mobility spectra which were observed 4.5, 6 and 10 min, respectively, after injection. The major problem experienced with this method of interfacing was premature volatilization of the solvent, creating vapor locks in the column. Also, in these experiments plugging the column with precipitated buffer was experienced. This was particularly true because of the very low buffer flow-rates available from the electroendosmotic effects.

Separation efficiency

With the 10^6 plate efficiency that can be achieved with CZE, the ion mobility spectrometer offers additional separation information. Using equations and relationships which have been developed for CZE and applying them to IMS, we can say that the maximum efficiency (N_{max}) possible for IMS is defined by the relation

$$N_{max} = KV/2D$$

where K is the ion mobility constant, V is the potential drop across the drift region of the spectrometer and D is the gas phase diffusion constant of the ion or ion cluster. For the ideal ion mobility separation, mobility is controlled by diffusion and is related through the Einstein equation.

$$K = qD/kT$$

where q is the charge on the ion, k is the Boltzman constant and T is the temperature in Kelvin. Substituting into the equation above, N_{max} becomes independent of both mobility and the diffusion constant.

$$N_{max} = qV/2kT$$

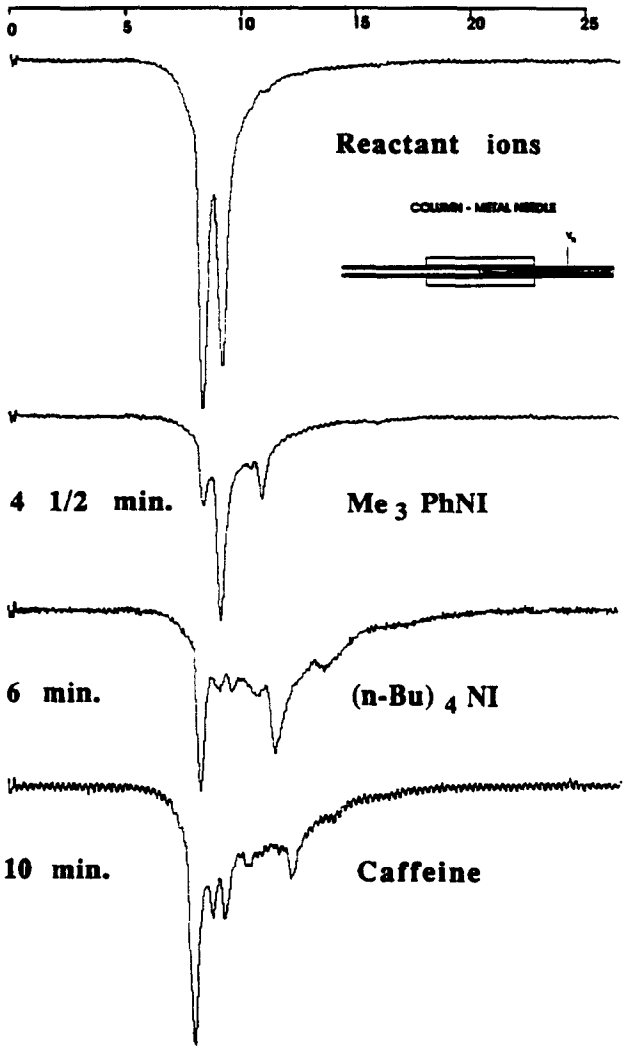

Fig. 7. CZE of single compounds with metal-needle DCS interface, 10 m*M* PO_4^{3-} buffer, 60 cm × 100 μm I.D. column, capillary voltage 22.5 kV, needle voltage 7.5 kV, IMS drift voltage 4.5 kV, detector temperature 90°C. Me = CH_3; Ph = C_6H_5. Scale at top: time in ms.

Thus the maximum number of plates possible for the ion mobility separations performed in this study (where $V = 2100$ V and $T = 373$ K) was $N_{max} = 3.3 \cdot 10^4$.

Table II provides a comparison of the efficiency of the spectrometer for $(n\text{-}C_4H_9)_4NI$ with several of the interface methods. In this table N was calculated from the spectra shown in this paper using the relation

$$N = 5.54\ (t/w)^2$$

where t is the drift time of the ion and w is the width of the peak in time at one half the maximum height.

TABLE II
IMS SEPARATION EFFICIENCY FOR TETRABUTYLAMMONIUM IODIDE

Interface	*N*	*Fig.*
FAS (high flow)	390	5 (4th panel)
FAS	650	6 (4th panel)
FAS (low flow)	3200	8 (2nd panel)
DCS	1100	7 (3rd panel)
DCS[a]	3500	8 (3rd panel)

[a] Liquid chromatography system.

Reproducibility

Vapor locks within the column and interface occurred frequently with all designs. When they occurred electrical discontinuity perturbed the electric field and the migration velocity of the ions. Except for certain short periods of time when these vapor locks were avoided, retention data was generally nonreproducible. On occasion, the system would operate for several hours without the formation of vapor locks and during those periods retention data were reasonably reproducible although statistical information was not obtained.

With respect to gas-phase ion mobility data, however, reproducibility was excellent. Fig. 8 shows two spectra at different temperature of tetrabutylammonium iodide using the glass-needle FAS interface and one spectrum using the DCS interface with a pumped flow (liquid chromatography–IMS). Although drift times of the test compound in each of these spectra are different, reduced mobilities match very well.

Reduced mobility is simply the ratio of the ion velocity to the electric field which has been corrected for temperature and pressure. They are calculated from the relation

$$K_0 = (d/Et)\ (273/T)\ (P/760)$$

where K_0 is the reduced mobility, d is the length of the ion drift region in cm, E is the electric field in V/cm, t is the ion drift time in seconds, T is the temperature of the drift

TABLE III
REDUCED MOBILITY VALUES FOR TETRABUTYLAMMONIUM IODIDE

Interface	*E (V/cm)*	*T (°C)*	*Reduced mobility (K_o)*	*Fig.*
FAS	355	110	1.40	5 (2nd panel)
FAS	355	110	1.43	5 (4th panel)
FAS	294	80	1.40	6 (4th panel)
FAS	294	80	1.39	8 (1st panel)
FAS	294	110	1.39	8 (2nd panel)
DCS[a]	327	175	1.38	8 (3rd panel)
		Average K_0	1.40 ± 1.43% RSD	

[a] Liquid chromatography system.

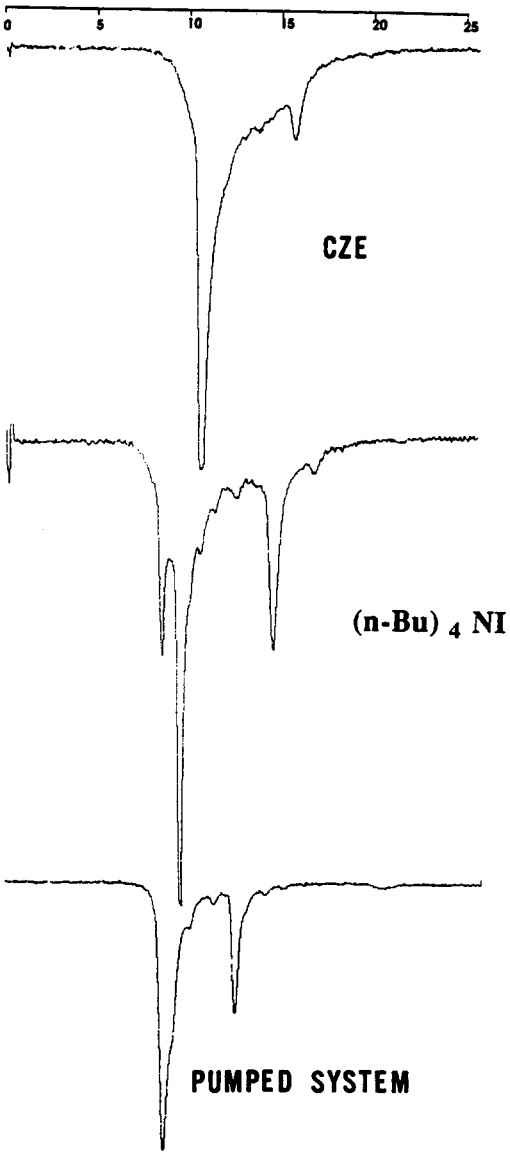

Fig. 8. Tetrabutylammonium iodide at different conditions. (top) CZE–IMS with FAS interface, 12 m*M* ammonium acetate buffer, continuous introduction of 1 m*M* tetrabutylammonium iodide, 100 cm × 100 μm I.D. column, capillary voltage 24 kV, needle voltage 9 kV, IMS drift voltage 4 kV, detector temperature 80°C. (middle) CZE–IMS with FAS interface, 10 m*M* ammonium acetate buffer, continuous introduction of 1 m*M* tetrabutylammonium iodide, 100 cm × 100 μm column, capillary voltage 24 kV, needle voltage 9.5 kV IMS drift voltage 4 kV, detector temperature 110°C. (bottom) Liquid chromatography–IMS system with metal-needle DCS interface, needle voltage 10 kV, IMS drift voltage 5 kV, detector temperature 175°C.

gas in Kelvin, and P is the pressure of the drift gas in Torr. Table III shows that for a variety of conditions and interfaces, K_0 varied by only 1.43%.

While the glass-needle FAS and the DCS interfaces between the CZE column and the IMS system showed some promise, there are important problems, all focussed on the tip of the needle, which must be solved. The IMS needs low buffer flow-rates and high temperature to exhibit its usual stable performance, and it is difficult to see how these conditions can be obtained simultaneously without special means for cooling the needle. The electrophoretic separation requires stable electrical connection to the column end, a condition which also seems to require that the needle end be cool.

ACKNOWLEDGEMENT

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