

# Electrospray Ionization Ion Mobility Spectrometry

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An improved electrospray ionization source for ion mobility spectrometry was developed by making three salient modifications to the electrospray needle. First, the needle was removed from the ion drift region, reducing neutralization of charged drops and ions on the walls of the ion mobility drift tube. Second, the tip of the spray needle was electrically insulated, extending the voltage range with which a stable electrospray could be achieved. Third, the needle was cooled, eliminating problems associated with solvent volatilization in the spray needle. Ion mobility spectra of cytochrome *c* and Triton X-100 were compared with those obtained by electrospray ionization mass spectrometry. While direct mass/charge comparison with ion mobility was not possible, similar electrospray ion patterns between the IMS and MS data suggest that the electrospray ionization source for IMS behaved in a manner consistent with that of other electrospray ionization sources. In addition, mass assignments of IMS ion peaks, made by matching patterns from mass spectra, fit well with those expected from IMS theory.

The inherent high resolution of capillary electrophoresis (CE) and microbore liquid chromatography (MLC) along with ease of sample preparation has made high-performance liquid-phase separation (HPLPS) the method of choice for many analytical applications. For the most part, however, detection of samples in the liquid phase has been limited to those analytes which absorb or fluoresce in the UV and visible regions of the spectrum. For compounds which absorb poorly, additional sample cleanup or derivatization procedures are required. Thus, development of a simple and dependable detection method for non-UV-absorbing compounds in solution would significantly expand the analytical applications for HPLPS.

In gas chromatography, ionization methods have provided a basis for universal detection. Most of these methods, however, rely on the selective ionization of analytes in the presence of the mobile phase and, with the exception of mass spectrometry, have not been successfully interfaced to HPLPS methods because of solvent interferences. Ion mobility spectrometry (IMS) is a technique for detecting ions in the gas phase which are separated on the basis of differences in cross sectional area. Since most analytes in HPLPS give rise to ions of larger cross section than those of the solvent, IMS would seem a logical choice as an alternative detection method. Furthermore, the feasibility of IMS-based detection for HPLPS has already been demonstrated<sup>1</sup> using an electrospray nebulizer as an ionization source.

Electrospray ionization ion mobility spectrometry (ESI-IMS) was first described in 1972 by Gieniec et al.,<sup>2</sup> who modified a Plasma Chromatograph manufactured by Franklin GNO Corp. (West Palm Beach, FL). In their design, the normal sample entrance and the <sup>63</sup>Ni ion source were removed from the commercial instrument and replaced with an electrospray chamber. Liquid samples were introduced into the enclosed glass spray chamber by a syringe pump via a Teflon tube connected to a 27-gauge hypodermic needle. The spray chamber was separated from the ion drift tube by a brass plate containing a large orifice for the bath gas and ions to enter the drift region. Electrospray was developed by applying a potential between the needle tip and the brass plate. The bath gas was introduced into the spray chamber at the rear of the needle. According to the authors, this heated gas flow served to help evaporate the droplets and to sweep the ions downstream through the orifice in the brass plate. Unfortunately, all of the solvent was swept toward the ion drift region and apparently contaminated this region, decreasing both sensitivity and resolution in the spectrometer.

Smith and co-workers have reported the ESI-IMS spectra for cytochrome *c*<sup>3</sup> and lysozyme.<sup>4</sup> These spectra, obtained at low temperatures (25–90 °C), were similar in resolution, shape, and drift time to those first reported by Gieniec et al.<sup>2</sup> The peaks drifted at relatively long drift times (>50 ms), and only one broad peak (peak width at half-height of about 25 ms) was observed for each compound. An obvious concern with respect to these spectra was presented by Fernandez de la Mora.<sup>5</sup> "... ESI mass spectrum (of cytochrome *c*) generally contains between 15 and 20 charges. Should not then the mobility spectrum contain also six peaks at least for cytochrome *c*?"

Improvement in both sensitivity and resolution was reported by Shumate and Hill by incorporating an electrospray needle directly into a unidirectional flow ion mobility spectrometer.<sup>6</sup> While this design worked well for low molecular weight compounds, high molecular weight, nonvolatile compounds were not detected. In addition, enclosure of the needle in the stainless steel drift tube made it difficult to achieve electrospray conditions, and the most stable operation of the detector

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- (2) Gieniec, J.; Cox, H. L., Jr.; Teer, D.; Dole, M. *Abstracts, 20th Annual Conference on MS and Allied Topics*, Dallas, TX, 1972, 276.
- (3) Smith, R. D.; Loo, J. A.; Loo, R. R. O.; Busman, M.; Udseth, H. R. *Mass Spectrom. Rev.* **1991**, *10*, 359–451.
- (4) Smith, R. D.; Loo, J. A.; Loo, R. R. O.; Udseth, H. R. *Mass Spectrom. Rev.* **1992**, *11*, 434–443.
- (5) Fernandez de la Mora, J. *Mass Spectrom. Rev.* **1992**, *11*, 431–434.
- (6) Shumate, C. B.; Hill, H. H., Jr. *Anal. Chem.* **1989**, *61*(6), 601.

occurred when a corona discharge was visible at the tip of the spray needle. Nevertheless, with this "coronaspray" ionization ion mobility spectrometer (CSI-IMS), a wide variety of compounds could be detected from solution with good sensitivity. Subsequently, potential applications of IMS as a detector for liquid chromatography,<sup>7</sup> as a detector for capillary electrophoretic methods,<sup>8</sup> and as an on-line liquid-stream process sensor were reported.<sup>9</sup>

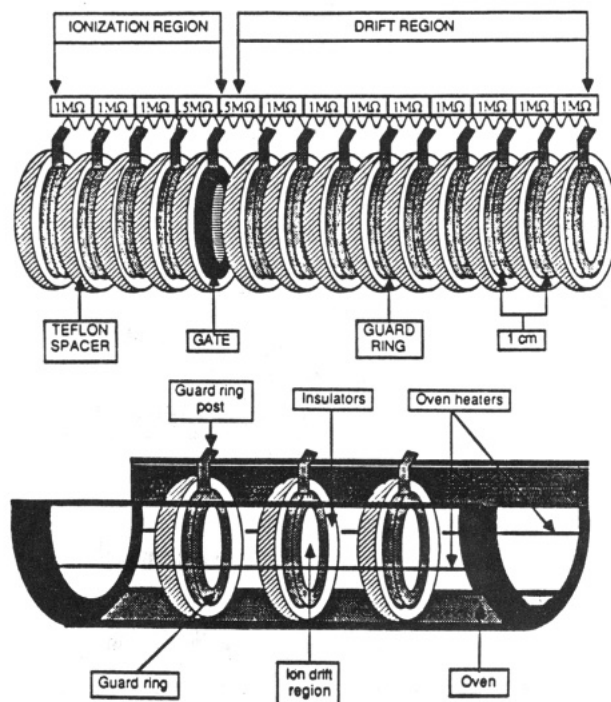
Although initial investigations of the IMS as a detection method for liquid samples were encouraging, lack of response for high molecular weight compounds indicated that the spray source was not behaving in a manner analogous to that observed when electrospray sources were used with mass spectrometry.

Our experience, coupled with that reported by others, led us to further investigations of ESI-IMS for the purpose of identifying the cause of these differences between data observed with ESI-IMS and those expected from ESI-MS. The overall objective was to design an IMS with predictable response characteristics and which could be used for detection after HPLPS.

## EXPERIMENTAL SECTION

Two ion mobility spectrometers were used in these investigations: The first was identified as a coronaspray ion mobility spectrometer (CSI-IMS) since it was primarily operated in that mode. The second was identified as the electrospray ionization ion mobility spectrometer (ESI-IMS) and was the design developed as a result of information gained from experiments with the CSI-IMS instrument. The drift tubes of both spectrometers were based on the standard stacked ring configuration used by a variety of commercial instruments. A series of stainless steel rings alternating with a series of insulator rings were assembled to produce the atmospheric pressure ion drift tube. The insulating rings served to isolate the stainless steel rings from one another while the stainless steel rings, called guard rings, were connected by a series of matched megaohm resistors. Applying a high voltage to the first ring of the tube generated a uniform electric field in the center of the tube, directing the coronasprayed or electrosprayed ions down the tube to a terminal Faraday plate where current was collected and measured.

**The CSI-IMS.** This spectrometer was designed by Shumate<sup>10</sup> and constructed under consignment by ScienTech, Inc. (Pullman, WA). A schematic diagram of the spectrometer is shown in Figure 1. Guard rings in this design had an internal diameter of 2.5 cm and were 0.8 cm in width. Teflon insulators, fitted around each ring, served to isolate the guard rings from the aluminum housing as well as from each other. The oven was constructed from a 10 in. long temperature-controlled aluminum cylinder. Figure 1a provides a view of the entire drift tube assembly which was located inside the aluminum oven, and Figure 1b shows a cut-away schematic of the



**Figure 1.** Cut-away design of the ion mobility spectrometer showing the guard ring and ion gate assembly (a, top) and the aluminum housing which served as the IMS oven (b, bottom).

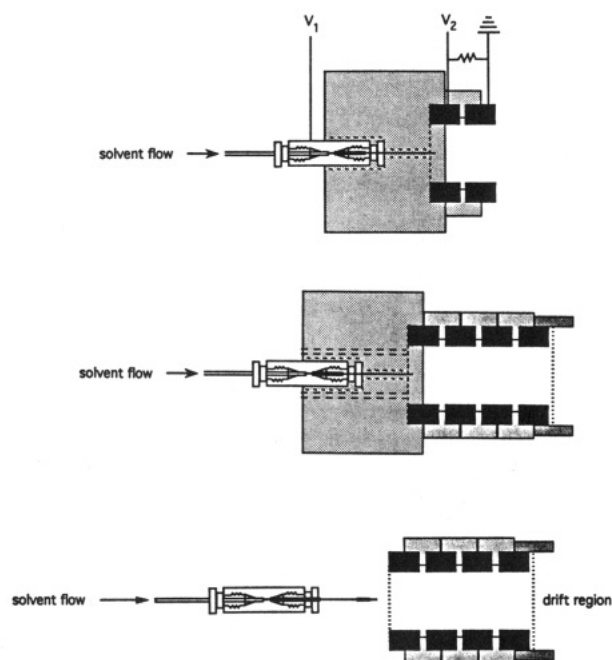
aluminum oven with only three guard rings. In operation, all of the guard rings shown in Figure 1a were inserted into the aluminum oven.

Electronic components of the spectrometer included an IMS controller, a current amplifier, a data acquisition/control system, an interface box, and a temperature controller. The IMS controller was a custom-built device containing electrospray high-voltage supplies (Model 02-150P, Bertan Associates, Inc., Hicksville, NY), drift field high-voltage supplies, and a "gate driver". The electrospray high-voltage supply provided both positive and negative voltage which could be varied up to 15 000 V. The drift field supply provided positive and negative voltages which were variable up to 5000 V.

The coronaspray needle was modified from the design given in refs 6 and 7 and is shown in Figure 2. It consisted of a 316 stainless steel union with ferrules and compression screws (Waters Division of Millipore, Milford, MA). A commercially available 25-gauge polished needle (P/N 80726, Hamilton, Reno, NV) was cut to a length of approximately 1.3 in. The cut end of the needle was inserted into a Teflon sleeve (<sup>1</sup>/<sub>16</sub> in. o.d. 0.006 in. i.d.) and pushed through a loosened compression screw and ferrule in the steel union. The compression screw was tightened and the entire assembly inserted into a Teflon housing which was clamped to a ring stand. The polished needle tip protruded 0.05 in. inside the first of two stainless steel drift tube rings. The spray assembly was fed by a prototype dual syringe micropump via a 3-ft length of PEEK tubing (<sup>1</sup>/<sub>16</sub> in. o.d., 0.006 in. i.d.).

For solvent spray experiments, the first guard ring was biased at +250 V and the second guard ring was at ground as shown in Figure 2a. Solvents evaluated were water, methanol, acetonitrile, tetrahydrofuran, isopropyl alcohol, ethyl acetate, methylene chloride, toluene, hexane, and isooctane.

- (7) McMinn, D. G.; Kinzer, J. A.; Shumate, C. B.; Siems, W. F.; Hill, H. H., Jr. *J. Microcolumn Sep.* **1990**, *2*, 188-192.
- (8) Hallen, R. W.; Shumate, C. B.; Siems, W. F.; Tsuda, T.; Hill, H. H., Jr. *J. Chromatogr.* **1989**, *480*, 233-245.
- (9) Shumate, C. B.; Hill, H. H., Jr. *Electrospray Ion Mobility Spectrometry—Its Potential as a Liquid-Stream Process Sensor In Pollution Prevention in Industrial Processes—The Role of Process Analytical Chemistry*; Breen, J. J., Dellarco, M. J., Eds.; American Chemical Society: Washington, DC, 1992.
- (10) Shumate, C. B. *An Electrospray Nebulization/Ionization Interface for Liquid Introduction into an Ion Mobility Spectrometer*. Ph.D. Thesis, Washington State University, 1989.

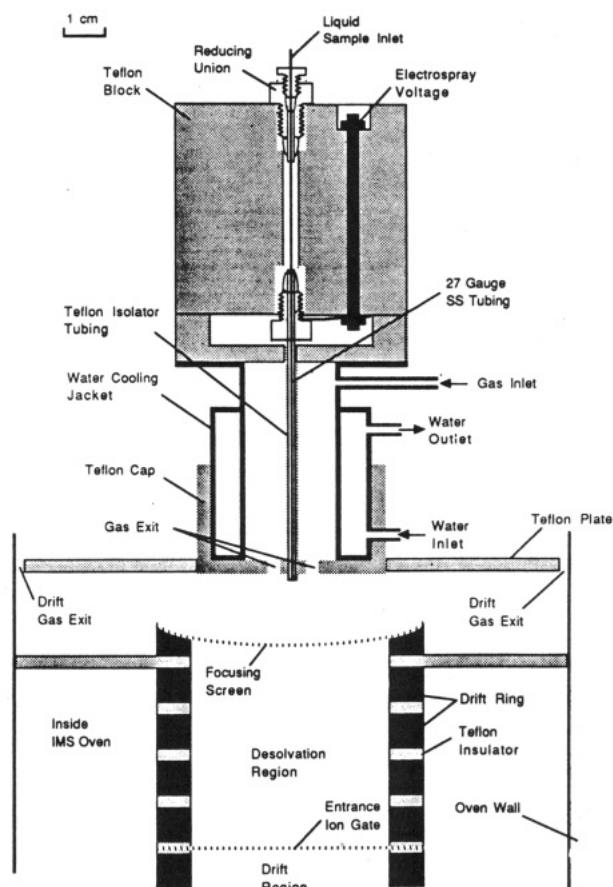


**Figure 2.** Schematic diagrams of spray ionization sources. (a, top) Design used in solvent spray experiments. Needle: 25-gauge polished SS, noninsulated. Solvent flow rate: 10  $\mu\text{L}/\text{min}$ . Voltages: 5–6 kV on needle; 250 V on first guard ring; second guard ring at ground. Temperature: ambient. (b, middle) Design used in coronaspray experiments. Needle: uninsulated 25-gauge polished stainless steel inserted into IMS tube. Solvent flow rate: 10  $\mu\text{L}/\text{min}$ . IMS nitrogen drift gas flow rate: 800 mL/min. Voltages: 5–6 kV on needle; 2–3 kV on first drift ring, generating a drift field of 200–300 V/cm in the IMS tube. Temperature: 60–80  $^{\circ}\text{C}$ . (c, bottom) Open design of electrospray IMS. In this design the needle is located in the ambient temperature of laboratory air and outside the ion drift field. The focusing screen shown in this design served to direct the electrosprayed ions down the center of the ion drift region.

tane in addition to several mixtures typical of HPLC mobile phases. In each case, flow rates were adjusted to 10  $\mu\text{L}/\text{min}$ .

For coronaspray experiments, the IMS was interfaced to the Teflon spray assembly as shown in Figure 2b. The spray assembly was identical to that shown in Figure 2a to which a guard ring stack, shutter grid, and collector assembly were added to form a typical IMS drift tube as shown in Figure 1a. Four  $1/8$ -in. holes were drilled in the Teflon block which housed the needle assembly, allowing the drift gas to exit the tube. An injector (Rheodyne 7000, Rheodyne, Cotati, CA) with 20- $\mu\text{L}$  loop was installed between the pump and the needle, leaving 3 ft of PEEK tubing between the needle and injector. The drift tube field strength was set at 200–300 V/cm. The drift gas was nitrogen with a flow rate of 800 mL/min. The drift tube temperature was set at 60 or 80  $^{\circ}\text{C}$ . Needle voltage was established for each set of drift tube conditions by the following procedure: The IMS was turned on, and spectral scans were monitored with no solvent flow to the needle. Needle voltage was initially set to equal that of the first guard ring and was successively increased until reactant ion spectra were observed, indicating a corona discharge at the needle tip.<sup>6</sup> Solvent flow rate was then set at 8  $\mu\text{L}/\text{min}$ , and samples were injected. The solvent used for this study was 50% methanol/water.

With the same drift tube described above, the coronaspray source was modified as shown in Figure 2c to produce a stable electrospray. The needle assembly described above was



**Figure 3.** Closed design of the electrospray ion mobility spectrometer showing a cooled electrospray needle, insulated electrospray needle, and spray focusing screen.

removed from the Teflon housing and directly attached to a ring stand with an insulated clamp. A grid consisting of nickel mesh was fused to the first guard ring of the drift tube to direct ionized spray into the tube. Finally, a double-layer Teflon sleeve, fabricated from shrink-tubing, was slipped onto the needle tip. In operation, the insulated tip was placed outside of the drift tube,  $1/8$ – $1/4$  in. from the focusing grid. Conditions for experiments, unless otherwise specified, were as follows: drift mode, positive; drift gas, nitrogen at 800 mL/min; pressure, barometric; desolvation region, 4 cm; drift region, 10 cm; field strength, 310 V/cm; drift temperature, 167  $^{\circ}\text{C}$ ; pulse width, 0.5 ms; gain,  $10^9$  V/amp; scan time, 50 ms; scans averaged, 50 or 100.

**The ESI-IMS.** A schematic diagram of this instrument is shown in Figure 3. As with the IMS described previously, the ion drift tube was constructed from stacked stainless steel rings, separated by Teflon insulators, and electrically connected by a series of megaohm resistors. This configuration provided a uniform electric field inside the drift region. The inside diameter of the tube was 4.22 cm, and the outside diameter was 5.71 cm. All of the characteristics required for stable electrospray described for the previous design were incorporated into the closed design. First, the spray needle was removed from the drift tube of the spectrometer, minimizing the electrical field strength between the needle and the drift tube wall. Second, a focusing screen was added to the first guard ring of the drift tube, directing the sprayed ions into the desolvation region of the ion drift tube. And third, the

27-gauge SS spray needle was insulated with 0.4 mm i.d. Teflon tubing with a wall thickness of 0.2 mm.

The salient difference between this design and the one described above was that the spray needle was completely enclosed in the spectrometer, eliminating contamination from laboratory air and stabilizing the temperature inside the drift tube. As a result of enclosing the needle in the spectrometer, the spray needle had to be externally cooled in order to avoid volatilization of the liquid stream in the needle. When the liquid stream vaporized before it reached the tip of the needle, the spectra became unstable. As shown in the figure, external cooling was applied to the needle via a water-cooled/air-cooled jacket. The entire assembly consisted of a water chamber surrounding an air chamber which housed the insulated spray needle.

The electronics of the electrospray IMS included a high-voltage supply for the spray needle, a high-voltage supply for the drift field, a temperature controller for the IMS oven, a gate driver under software control, and a current amplifier to amplify the ion signal. The spray voltage was supplied by two high-voltage supplies, one for positive and one for negative. Both were Model 602B-200P (Bertan Associates, Inc. Hicksville, NY) with a maximum potential of 20 000 V. Typically, 10 000 V was applied to the needle for generating the electrospray. The drift field potential was provided by a single 5000-V high-voltage supply (Model PMT-50 A, Bertan Assoc. Inc., Hicksville, NY). The current limit of the power supply was 0.5 mA. At the total drift tube resistance of about 16 M $\Omega$ , only about 0.31 mA was flowing through the resistor chain connecting the guard rings.

The gate driver was controlled by a CACTUS PC/XT computer (Cactus Computer Co., Moscow, ID). The computer was equipped with a Burr-Brown PCI-20000 data acquisition/control system (Burr-Brown Corp., Tucson, AZ) which included a high-performance carrier (PCI-20041C carrier with DMA capability, plugged into the I/O channel of the computer), a high-speed A/D module (PCI-20019M), a counter timer (PCI-20007M), and PCI-200021S high-speed and DMA software. The high-speed A/D module (12-bit dynamic range, 54-kHz maximum sampling rate) was used to measure 0–1 voltage of the electrometer output and convert this analog signal to a digital one. The counter-timer module of the Burr-Brown board, controlled entirely by lab-designed software, provided timing for digitization and gates.

The solvent used throughout this portion of the investigation was a mixture of water, methanol, and acetic acid in a volume ratio of 47.5/47.5/5. The liquid was pumped to the electrospray needle by a dual piston syringe pump (Microgradient System, BrownLee Labs, Applied Biosystems, Santa Clara, CA). The syringe pump provided pulseless flow at low flow rates (1–10  $\mu$ L/min.). The injector (C6W, Valco Instruments Co., Houston, TX) was a six-port valve with 6- $\mu$ L external injection volume. A 100  $\mu$ m i.d. and 200  $\mu$ m o.d. deactivated vitreous silica tubing (J&W Scientific, Folsom, CA) of approximately 1 m in length was used as a transfer line. One end of the tubing was connected to the pump through a  $1/16$ – $1/32$ -in. zero dead volume internal reducer (1ZR1.5T, Valco Instru. Co. Inc.). The other end of the transfer line was inserted through the 27-gauge stainless steel electrospray needle. A  $1/16$ – $1/32$ -in. zero dead volume internal reducer (1ZR1.5T,

Valco Instr. Co. Inc.) was used to hold the transfer line, so that the exit of the fused silica transfer line was recessed about 1 mm from the end of the electrospray needle.

**Electrospray Ionization Mass Spectrometry.** A previously characterized lab-made mass spectrometer with an electrospray interface was used to obtain mass spectra.<sup>11</sup> For these studies, however, there was no grid between the needle and the inlet nozzle, and the needle was maintained at a constant positive voltage rather than pulsed as was described in ref 11. Mass spectra were acquired using a Quadstar (Balzers) data system. Resulting data files were converted to ASCII format and further reduced using Microsoft Excel (Microsoft). Finished spectra were generated using Deltagraph (Deltapoint).

**Computer Simulation.** Computer modeling of the electric fields generated by the application of bias voltages was accomplished using the MacSimion (Montech PTY, Ltd., Clayto, Victoria, Australia) ion trajectory simulation program performed on an Apple Macintosh personal computer (Apple Computer, Cupertino, CA). Although the program was designed to calculate and display ion trajectories in a vacuum, the program module also calculated the electrical fields and displayed equipotential contours imposed by the IMS geometry. With the electric fields accurately represented, the electrostatic forces experienced by the ions were described. Ion trajectories under atmospheric conditions are orthogonal to equipotential contour lines of the electric fields.

## RESULTS AND DISCUSSION

As stated in the introduction, the objective of these investigations was to identify problems associated with CSI-IMS with the final goal of constructing a stable electrospray ionization source for IMS which behaved in a manner consistent with electrospray ionization sources commonly used in mass spectrometry.

Initial experiments served to standardize hardware and operating conditions, comparing results with those achieved previously. The first experiments were performed without the drift tube in order to observe the spray and evaluate the spray of several solvents. Through the use of the configuration shown in Figure 2a, several solvents were sprayed. These experiments permitted visual inspection of the needle tip, as well as the spray plume generated under field conditions similar to that of a stacked ring IMS drift tube. For each solvent, needle voltage was successively increased until a typical plume was observed, characterized by the appearance of the "Taylor cone" at the needle tip. When voltage was increased beyond that of the Taylor cone formation, this single cone disappeared and several smaller jets could be observed. Most of the spray generated in this experimental configuration collided with one or both of the rings. At 5–6 kV, a corona electrical discharge was clearly visible at the needle tip. Solvents investigated in this study included water, methanol, acetonitrile, tetrahydrofuran, isopropyl alcohol, ethyl acetate, methylene chloride, toluene, hexane, and isooctane. Results of the test indicated that dry hexane and isooctane were not amenable to electrospray. A spray for dry toluene was

(11) Tomany, M.; Wittmer, D.; Gabeler, S.; Jarrell, A. *Design and Performance Characteristics of a Particle Beam Interface and an Electrospray Interface*; Proceedings of the 40th ASMS Conference on Mass Spectrometry and Allied Topics; Washington, DC, May 31–June 5, 1992.

**Table 1. Spectral Detection Limits of CSI-IMS for Selected Compounds Using the Signal Averaging Mode**

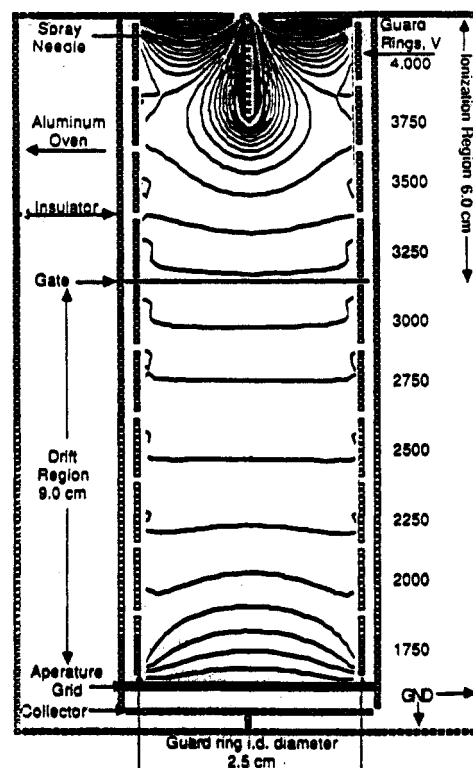
compd	class	MW	pg/ $\mu$ L	pmol/ $\mu$ L
methiocarb	pesticide	225	600	2.7
tributylamine	industrial	185	1.2	0.006
streptomycin sulfate	antibiotic	1457	1600	1.1
phenylalanine	amino acid	165	300	1.8

observed but only at voltages well into the corona regime. However, when a solvent of higher dielectric such as methanol, acetonitrile, or THF was added in a proportion of 5% (v/v) to any of these, the mixture readily electrosprayed at voltages somewhat higher (0.5–1 kV) than that required for the pure modifier. All other solvents produced electrospray within the range of 2–3 kV. Stability of the electrospray plume varied from solvent to solvent at a given voltage. It generally appeared that plume stability increased with volatility and polarity of the solvent, and decreased with viscosity. A stable spray could be obtained by adjusting the needle voltage.

Initial investigations spraying into the ion mobility spectrometer were conducted using the configuration shown in Figure 2b. In this configuration, IMS spectra for several compounds were obtained and good sensitivities observed. Examples of detection limits for several compounds are given in Table 1. With this method, detection of amines looked particularly promising. Thus a series of amines were evaluated, and all provided good IMS response although  $K_0$  values did not match those in the literature. Other problems associated with this design were also encountered. At low temperatures, stable spectra were obtained for low molecular weight compounds. When a homologous series of organic acids successively were observed, the signal decreased drastically for hexanoic acid, and no signal was observed for heptanoic acid. A signal for heptanoic acid was observed at higher (80 °C) temperature and appeared to be the result of well-resolved clusters. At temperatures greater than 80 °C, the reactant ion peak became unstable, presumably as a result of outgassing or boiling of the solvent in the needle. Several small changes were made to the hardware, each with an increase in performance, but the problems inherent at low drift tube temperatures proved too troublesome to continue.

Results of computer-simulated studies of the CSI-IMS design are shown in Figure 4. Equipotential lines at 250-V intervals were drawn inside the drift tube. The collecting electrode, located below the aperture grid at the bottom of the figure, was at ground potential. The aperture grid served to shield the collection from inductive effects of the ion cloud as it migrated down the drift tube toward the collector. Just above the aperture grid was a relatively steep electrical gradient which focused the ions to the collecting electrode. In the main portion of the drift tube, the field gradient was found to be relatively smooth with a simulated field of 250 V/cm. Above the ion entrance gate, equipotential lines indicated that ions were directed toward the walls of the detector. This simulation, coupled with results found using the spray configuration in Figure 2a, indicated that when the needle was located inside the drift tube, sensitivity was lost due to neutralization of ions on the guard rings of the spectrometer.

In order to focus ions into the center of the ion drift region where the field was uniform and to avoid problems associated



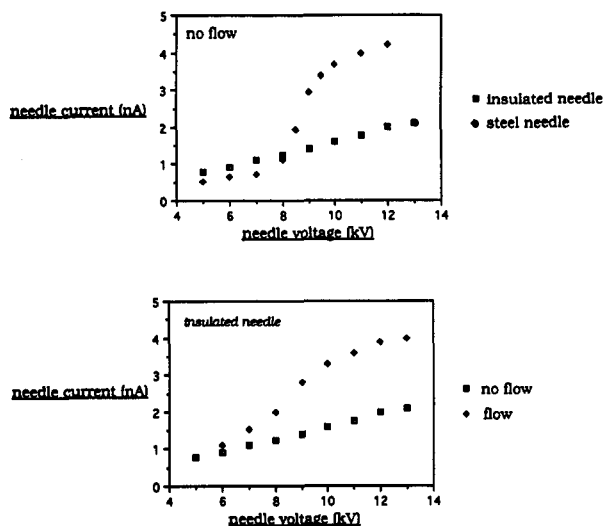
**Figure 4.** Computer simulation of coronaspray ion mobility spectrometer showing the contour profile of the equipotential field lines. Each equipotential field line in the figure represents a potential drop of 250 V. Note the circular nature of the equipotential lines near the spray needle. Since ions travel perpendicular to field lines, most were directed radially to guard rings instead of longitudinally to the center of the drift tube.

with heating the needle, the needle assembly was removed from the drift tube as shown in Figure 3c. A grid consisting of nickel mesh was fused to the first guard ring to direct the spray into the drift tube. With the needle removed from the drift tube, temperature of the drift gas could be increased to about 170 °C, permitting efficient desolvation of the spray drops prior to reaching the ion entrance gate. In addition, the needle remained cool, avoiding buildup of nonvolatile material at the needle tip.

Because the needle was located outside of the drift tube, the ion spray could be observed. Under these conditions a corona discharge could be seen similar to that observed in the experiments described for Figure 3a. Insulating the tip of the spray needle with Teflon tubing eliminated visual observation of the corona discharge. With the insulated needle, there was less tendency for arcing from the needle tip to the ring or grid, allowing a higher field strength to be generated at the origin of the plume, either by moving the insulated spray tip closer to the grid or by increasing needle voltage. With increasing needle voltage, it was found that the maximum flow rate at which a stable plume and spectra were obtained had increased from a typical 6–8  $\mu$ L/min to a typical 16  $\mu$ L/min. Minimum flow remained 1  $\mu$ L/min, the limit of the pump.

Under this configuration, electric current from both a noninsulated and an insulated needle was monitored and recorded as a function of voltage with no solvent flow passing through the needles. Results of this experiment are shown in Figure 5a. With the noninsulated steel needle, onset of the corona discharge could be clearly seen as a sharp increase in





**Figure 5.** Electro spray needle current. (a, top) Comparison of total ion current from insulated and noninsulated needles. (b, bottom) Comparison of total ion current from an insulated needle with and without solvent flow. These data illustrate the fact that the insulated needle increases the potential range in which a stable electrospray can be obtained.

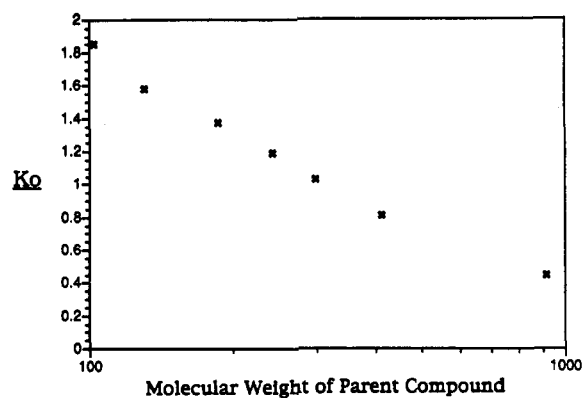
**Table 2. Reduced Ion Mobilities ( $K_0$ ) of Organic Amines from ESI-IMS Compared with  $^{63}\text{Ni}$  Source Literature Values**

amine	MW	$K_0^a$ (ESI)	$K_0$ (NOR)	$K_0$ ( $^{63}\text{Ni}$ )
ammonia	17	1.86	1.97	3.02
methylamine	31	1.75	1.85	2.65
<i>n</i> -butylamine	73	1.86	1.97	1.98
triethylamine	101	1.85	1.96	1.95
lutidine	107	1.84	1.95	1.95
isoquinoline	129	1.73	1.83	1.85
dibutylamine	129	1.59	1.68	1.64
tributylamine	185	1.36	1.44	1.38
tetrabutylammonium	242	1.19	1.26	
tetrapentylammonium	298	1.03	1.09	
tetraheptylammonium	410	0.81	0.86	
tetraoctadecylammonium	916	0.44	0.47	

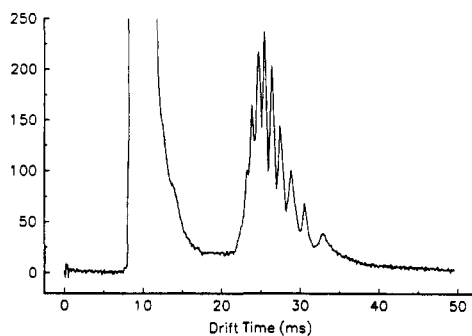
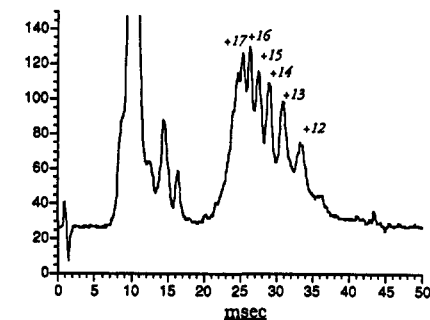
<sup>a</sup>  $K_0 = (d^2/Vt)(P/760)(273/T)$  where  $d$  is the drift distance,  $V$  is the potential across the drift distance,  $t$  is the ion drift time,  $P$  is the pressure in Torr, and  $T$  is the temperature of the drift region in degrees Kelvin.

needle current around 8–10 kV. While a small current was observed for the insulated needle, no sharp increase in current was noted in this region, indicating that background ionization from the corona was significantly reduced with the insulated needle. Figure 5b compared the needle current observed for 8  $\mu\text{L}/\text{min}$  of solvent (50% methanol/water) flowing through the insulated needle with that observed for the no solvent flow condition. In similar experiments reported in ref 6, it was found that, with a noninsulated needle located inside the drift tube, the region in which solvent flow enhanced current over the no flow condition was quite limited. Thus the primary advantage of the insulated needle was to provide an increased range of needle voltage and flow rate over which true electrospray ionization spectra could be obtained.

To investigate the extent of desolvation with this design, a series of amines were electrosprayed into the ion mobility spectrometer and their  $K_0$  values calculated. Inadequate desolvation resulted in ions of lower than expected mobilities. As shown in Table 2, most  $K_0$  values compared well with those obtained by Karpas<sup>12</sup> using a conventional  $^{63}\text{Ni}$  ionization source. This was especially true when  $K_0$  values were



**Figure 6.** Log MW vs ion mobility constants ( $K_0$ ) for quaternary and tertiary amines. Data were obtained with ESI-IMS configuration shown in Figure 2c. Data plotted are shown in Table 2, demonstrating that tetraalkylammonium ions apparently do not dissociate during soft electrospray ionization.

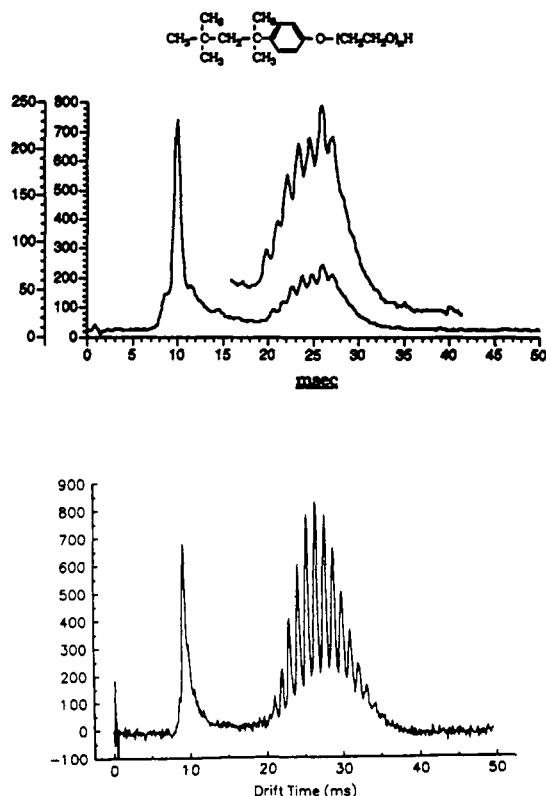


**Figure 7.** Electro spray ion mobility spectrometry of cytochrome c. (a, top) Open electrospray design and (b, bottom) closed electrospray design. By comparison with ESI-MS spectra in Figure 9, this figure illustrates the first example of multiply charged ions separated by IMS.

normalized to 1.95, the  $K_0$  value for lutidine. The exceptions were those of low molecular weight, which appeared in the region of the background solvent (reactant ion) peak. With regard to organic amines, electrospray ionization did have at least one distinct advantage over the  $^{63}\text{Ni}$  source. The  $K_0$  values of quaternary amines have been reported as difficult to measure, presumably due to dissociation in the IMS cell. This appeared not to be the case with this electrospray configuration. Figure 6 shows a relatively smooth curve when log MW was plotted as a function of  $K_0$  for quaternary and tertiary amines together.

Figure 7a and Figure 8a are ion mobility spectra of high molecular weight compounds. Detection of these compounds

(12) Karpas, Z. *Anal. Chem.* **1989**, *61*, 684.

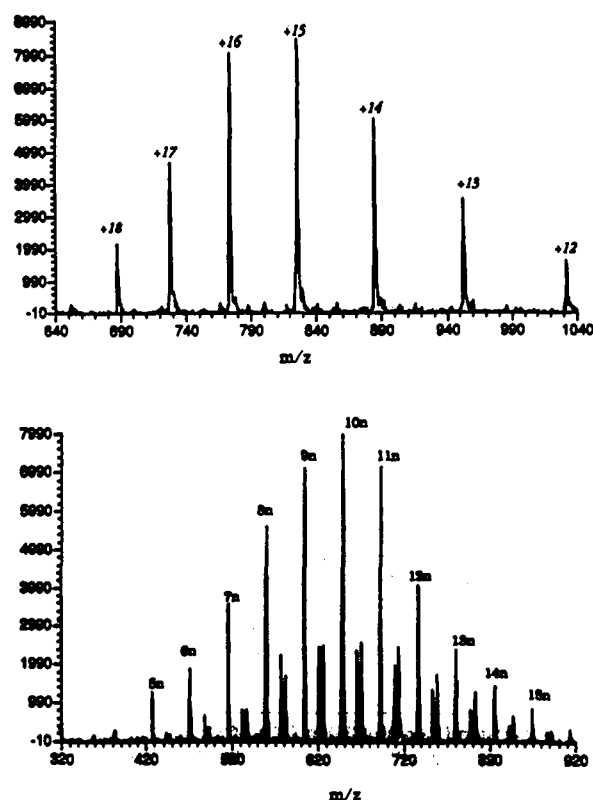


**Figure 8.** Electrospray ionization ion mobility spectrometry of Triton X-100. (a, top) Open electrospray design and (b, bottom) closed electrospray design. By comparison with the ESI-MS data presented in Figure 9, this figure represents singly charged ions of Triton X-100 oligomers. Note that the IMS resolution is sufficient to separate one gas-phase ion oligomer from the next. Each oligomer ion peak is separated by 44 mass units.

was not possible with CSI-IMS. Figure 7a is an ESI-IMS spectrum of a protein, cytochrome *c*, and Figure 8a is the ESI-IMS spectrum of a polymer, Triton X-100. Both spectra produced a response for ions drifting from about 20 ms to about 35 ms. In each case, some resolution of individual ion peaks can be observed.

Figures 7b and 8b show ESI-IMS for the same compounds with the closed design shown in Figure 3. This instrument differed from the prototype in that the electrospray needle assembly was sealed from the laboratory atmosphere and the internal diameters of the guard rings were increased from 2.5 to 4.22 cm. To prevent volatilization of the solvent in the spray needle, the needle assembly was water cooled. Advantages of the sealed configuration were that the temperature in the drift region of the spectrometer remained uniform throughout the desolvation and drift region and contaminants from the laboratory air were excluded from the ionization region. Spectra obtained with the sealed source appeared to exhibit both higher sensitivity and higher IMS resolution.

Figure 9a,b shows ESI-MS spectra of cytochrome *c* and Triton X-100. For cytochrome *c*, seven ions were observed, corresponding to multiple charged ions containing +12 to +18 charges. Multiple charged ions are characteristic of ESI-MS of proteins which have a number of basic sites. Comparing the MS spectrum with the IMS spectra, it is possible to speculate that the multiple peaks observed in the IMS spectrum were also due to multiple charged ion species. If this is true, it is the first time that multiple charged peaks have been reported in IMS.



**Figure 9.** Electrospray ionization mass spectrometry. (a, top) Cytochrome *c* and (b, bottom) Triton X-100. Both spectra are similar to those reported for other ESI-MS systems and were used to make the mass/charge assignments in Figures 7 and 8.

The ESI-MS spectrum of Triton X-100 was of singly charged ions corresponding to 11 different oligomers ranging in degree of polymerization (*n*) from 5 to 15. In the IMS spectrum using the open needle design, a total of seven ion peaks were detected and with the closed design 14 peaks were observed. In both cases the patterns of IMS spectra were similar to those obtained by ESI-MS.

## CONCLUSIONS

True electrospray ion mobility spectrometry has been achieved. To achieve electrospray conditions, three important problems which occurred in previous designs of ion spray ion mobility spectrometers were identified and corrected. The problems were the following: (1) background contribution and dissociative ionization from corona discharge, (2) reduced response due to ion collection on the walls of the drift tube, and (3) prespray precipitation of nonvolatile compounds in the needle due to solvent volatilization. In addition, volatilization of the solvent in the needle prior to spraying led to unstable spray due to bubbles of vapor sputtering from the tip of the needle. Thus, successful operation of IMS in the electrospray ionization regime requires three specific design modifications of the spray needle from that reported in previous studies:

First, ion contribution of corona discharge to the overall current of IMS can be eliminated by enclosing the needle in a nonconductive (e.g. Teflon) sleeve. Under these conditions, ions are only produced as a result of electrospraying a solvent. When no solvent flow is present through the needle, no ions in the IMS will be observed.

Second, when a spray needle is inserted into the drift region of the IMS, electric field lines are produced such that trajectories of the electrosprayed ions are toward the walls of the drift tube, reducing the current directed down the tube. Creating the spray outside of the drift region and adding a focusing screen which is positioned across the entrance to the ion drift region and a few centimeters in front of the tip of the spray needle directs the electrosprayed ions into the center region of the drift tube. With this modification, more of the ions reach the ion drift region and the sensitivity of the instrument is substantially enhanced.

Finally, desolvation of the sprayed ions requires elevated temperatures of the drift gas to evaporate solvent from the ionized spray drops as they migrate down the drift tube and before they reach the first ion gate of the spectrometer. When the electrospray source is incorporated as an integral part of the instrument in a closed design, heat transfer from the drift gas to the needle is sufficient to volatilize the solvent in the needle prior to electrospray. To prevent solvent volatilization in the spray needle, the needle must be cooled.

With a cooled and electrically insulated ion spray needle, and with the electrosprayed drops focused into the desolvation region of the IMS, stable and well-resolved response ions for nonvolatile high molecular weight compounds can be achieved.

Moreover, the ion patterns produced with this electrospray IMS were similar to those observed in electrospray mass spectrometry, indicating that the response and behavior of an electrospray ionization source for IMS can be predicted from mass spectral data. Although optimized sensitivity studies were not conducted in this investigation, estimates made from these studies predict that the detection limit for IMS response will be similar to or less than that achieved for most optical detection methods. Thus, the electrospray IMS appears to offer a viable complementary detection method for liquid chromatography and capillary electrophoresis. Further investigations of this design with respect to liquid-phase detection are currently being conducted.

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