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Detection of Biological Materials Using Ion Mobility Spectroscopy

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Abstract

Traditionally, Ion Mobility Spectroscopy has been used to examine ions of relatively low molecular weight and high ion mobility. In recent years, however, biomolecules such as bradykinin, cytochrome c, bovine pancreatic trypsin inhibitor (BPTI), apomyoglobin, and lysozyme, have been successfully analyzed, but studies of whole bio-organisms have not been performed. In this study an attempt was made to detect and measure the mobility of two bacteriophages, λ -phage and MS2 using electrospray methods to inject the viruses into the ion mobility spectrometer. Using data from Yeh, et al.,¹ which makes a comparison between the diameter of non-biologic particles and the specific particle mobility, the particle mobility for the MS2 virus was estimated to be 10^{-2} cm²/volt-sec. From this mobility the drift time of these particles in our spectrometer was calculated to be approximately 65 msec. The particle mobility for the λ -phage virus was estimated to be 10^{-3} cm²/volt-sec. which would result in a drift time of 0.7 sec.

Spectra showing the presence of a viral peak at the expected drift time were not observed. However, changes in the reactant ion peak that could be directly attributed to the presence of the viruses were observed. Virus clustering, excessive collisions, and the electrospray injection method limited the performance of this IMS. However, we believe that an instrument specifically designed to analyze such bioagents and utilizing other injection and ionization methods will succeed in directly detecting viruses and bacteria.

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Background

Ion Mobility Spectrometers (IMS) have been used by the military to detect chemical agents for many years. A growing area of concern to the military, however, is the ability to detect biological organisms, and to identify the species in near-real time so those proper countermeasures can be employed. In addition to the time constraints, the wide variety of biological and viral agents that could be used in terrorist activities makes identification very difficult. Also, the toxic agents are often masked by the presence of innocuous materials that are always present in our atmosphere. In many instances, the innocuous materials can be present in concentrations that are a thousand fold greater than the target materials.

A limited number of laboratory studies have used pyrolysis gas chromatography and pyrolysis gas chromatography / mass spectroscopy in an attempt to identify various biological materials. Pyrolysis combined with Ion Mobility spectroscopy has been attempted in at least one instance, and like the pyrolysis-GC and pyrolysis GC/MS analyses, species identification has not been successful. Pyrolysis results in the scission of the carbon-carbon bonds in the molecule. Even though different substituent groups may be attached to these carbon-carbon bonds, the bond strengths are essentially identical. Therefore, the resulting fragments are similar, and in some cases identical, which makes speciation very difficult if not impossible. In addition, the equipment required for the pyrolysis / GC or pyrolysis GC/MS does not lend itself well to rapid or easy deployment in the field, especially under battlefield conditions. IMS has been used in the field under battlefield conditions as chemical agent detectors, and has a long history of success in this application. Direct analysis (without pyrolysis) of biological species is theoretically possible using IMS.

IMS Analysis of Biological Materials

Traditionally, IMS has been used to examine ions of relatively low molecular weight and high mobility. However, lately, great interest has developed for the study of analytes which possess high molecular weight and low mobility. The molecules of interest have been bio-molecules like bradykinin, cytochrome c, bovine pancreatic trypsin inhibitor (BPTI), apomyoglobin, and lysozyme^{2,3}, for which electrospray / Ion Mobility Spectroscopy (ES/IMS) measurements have been successfully acquired. Although bio-molecular studies have been successful, studies of bio-organisms have not yet been performed.

In theory, however, if a molecule can be ionized, and if the resulting ion has a sufficient lifetime to allow it to be detected after moving through a drift region, one can perform ion mobility studies of that ion. Normally, these mobility studies are applied to relatively small molecules (<1000 Daltons). In the Ion Mobility Spectrometer, molecules are ionized in the source region, typically by using ⁶³Ni, or, less commonly, by photoionization or corona discharge. The molecules, which are normally singly ionized, are then injected into the drift region where their transit time is governed by their

mobility. The reduced mobility (K_0), defined in equation 1, is used to normalize the drift time so that spectra collected under various conditions are comparable.

$$K_0 = (d/Et)(P/760)(273/T) \quad (1)$$

K_0 (units are $\text{cm}^2\text{V}^{-1}\text{s}^{-1}$) relates the distance the ion drifts from the shutter grid to the detector (d , in cm), the electric field strength (E , in volts/cm), the time it takes to traverse this distance (t , in seconds), the ambient pressure (P , in torr), and the drift tube temperature (T , in $^\circ\text{K}$). Note that this is a greatly simplified treatment of the ion mobility, and makes assumptions that only approximate actual ion behaviors. A complete, comprehensive treatment can be found in Eiceman, et. al.⁴

The viral and biological species of interest in this study have dimensions of 0.1 microns to about 3 microns. Assuming the charged species will exist as singly ionized entities, their mobilities would be 3 to 4 orders of magnitude lower than the molecules that are typically analyzed by IMS. Consequently, the IMS instrumentation and control software needed to be modified to be able to measure the long drift times. The expected drift times for these species in the IMS we used range from about 100 milliseconds to hundreds of seconds.

Electrospray Ionization

Electrospray ionization (ESI) was developed in the 1980s, and is often used to form ions of high molecular weight analytes, especially if they are in solution. The electrospray source produces ions as shown in Figure 1. The analyte is introduced into the electrospray system through a conductive capillary column or syringe needle in section "A." A nebulizer gas flows through a cylindrical electrode maintained at high voltage. This electrode serves to shape the distribution of the potential to the needle and helps guide the discharge of the needle. These fields will charge the surface of the forming droplet and disperse it by coulombic forces into a mist (B). As the solvent evaporates, the electric field increases (C). When the droplet size decreases to the point that the ions reach a critical field strength, ions are emitted from the droplet (D).⁵ These ions can then be separated and introduced into the analyzer by using a screen with a potential applied upon it.

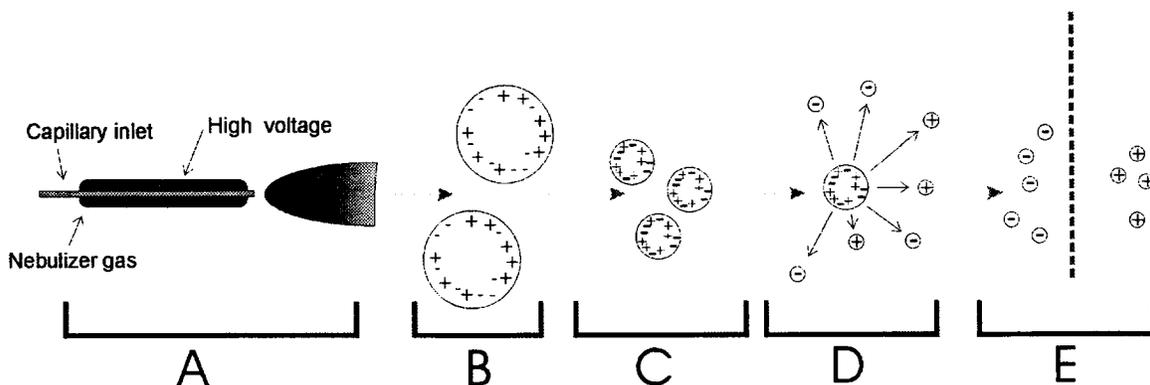


Figure 1. *Electrospray Ionization Source*

One of the major differences between the electrospray system and the more typical ^{63}Ni ionization is the formation of multiply charged moieties in the electrospray system. For example, the protein apo-myoglobin (16,951.5 Daltons) produces a series of ions with charge states from +8 to +27, resulting in m/z values in the 600 to 2000 Dalton range.⁶ As will be explained in more detail below, the presence of these multiply charged species were not observed during our work with the λ -phage and MS2. However, modifications of our operating conditions may be needed to allow these species to be observed. The patterns observed could then be used as a “fingerprint” and provide additional specificity for identification of the biological species. The work performed here did not include efforts to produce the multiple-charged ions, or to interpret any resulting fingerprints.

Equipment

The ion mobility spectrometer used for the investigation was fabricated and assembled at New Mexico State University.

The drift cell, shown in Figure 2, was built in a stacked ring design, composed of alternating stainless steel and Teflon rings. The Teflon rings (5.5 mm thick, 35 mm diameter bore) functioned as insulators between the stainless steel rings. The stainless steel rings (5.0 mm thick, 35 mm diameter bore) were connected by a series of resistors to provide a uniform electrical field in the ion drift tube.

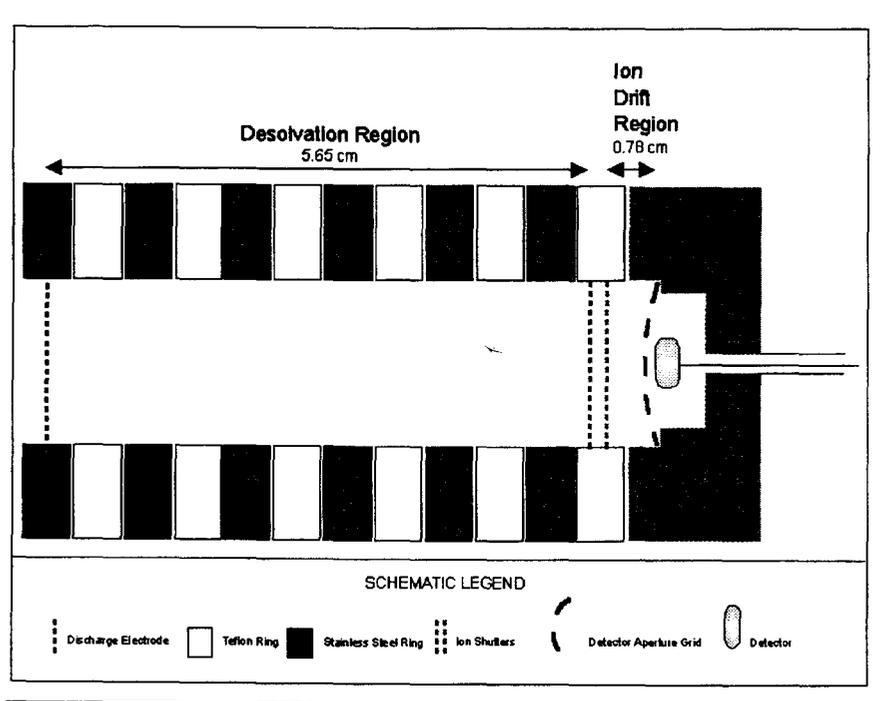


Figure 2. Drift cell design

The ion drift tube was composed of two regions. The first region, used for desolvation, was 5.65 cm while the second region, the analytical region, was 0.78 cm.

A wire mesh ring (labeled discharge electrode in Figure 2) replaced the standard ^{63}Ni ion source used in conventional IMS systems. This mesh functioned as a counter electrode for the formation and guidance of ions from the Electrospray Ionization Source (ESI) (SCIEX, Toronto, Canada). The ESI was biased +3.5kV to +5kV with respect to the mesh counter electrode. The voltage supplied to the ion drift tube in the IMS, counter electrode and electrospray ion source originated from different power supplies. A schematic drawing of the electrical connections between the electrospray ion source, counter electrode, and ion drift tube is shown in Figure 3.

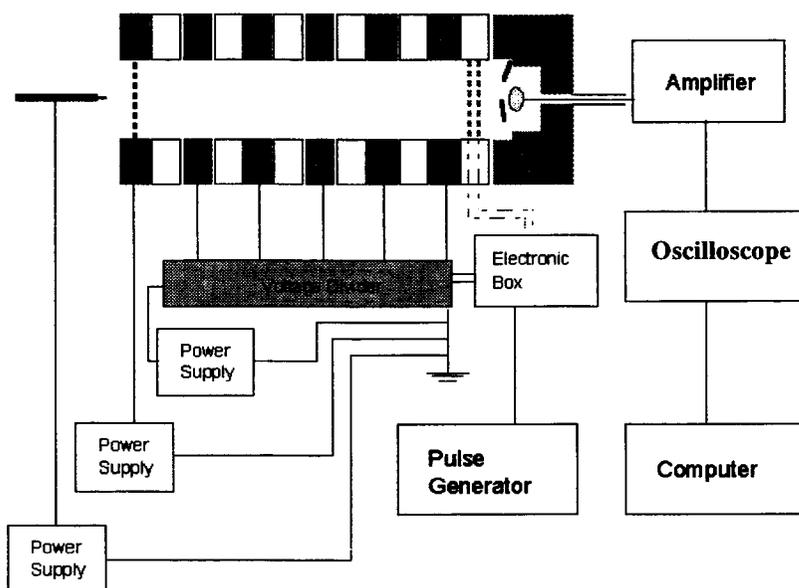


Figure 3. System Schematic

Electronic components of the spectrometer included a current amplifier, oscilloscope, computer acquisition system, wave generator, custom electronic boxes, and three separate power supplies. The first primary component, the ESI, was powered by a Fluke 410B High Voltage Power Supply. The mesh counter electrode and the drift tube were powered by two separate Standard Research Systems, Inc., Model PS350 power supplies. A Hewlett Packard Model 8116A Pulse/Function generator produced a wave pulse that was fed into custom made electronic boxes. These electronic boxes were designed to pulse the shutter in the ion drift tube. A schematic of these pulse circuits is shown in Figure 4.

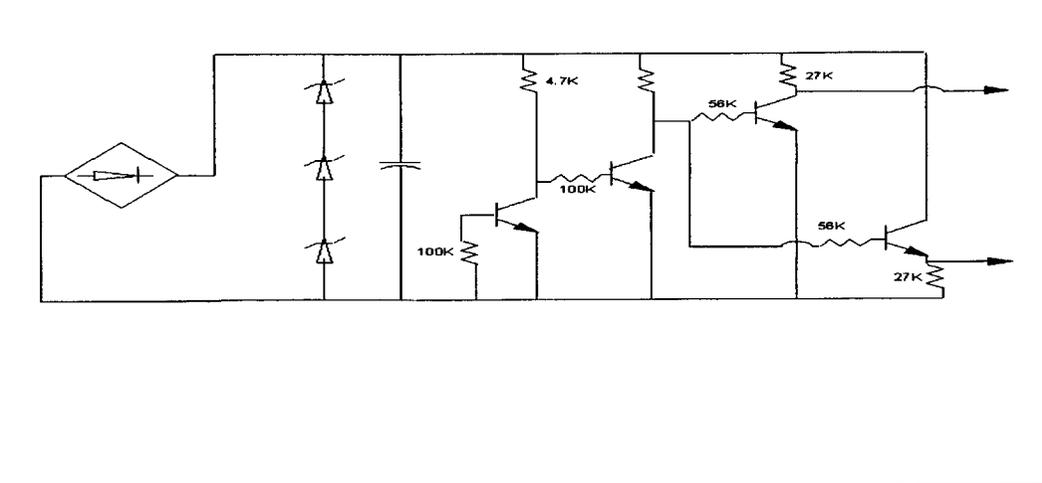


Figure 4. Schematic of Shutter Pulse Generator

A Keithley current amplifier model 428 was used to feed the detector signal into a Hewlett Packard Model 54522A oscilloscope. The oscilloscope was interfaced to a Power Macintosh 6200/225 using a GPIB card. LabView for Mac graphical programming was used for the data acquisition.

The standard operating conditions used for the experiments are listed in Table 1.

Table 1 Standard Operating Conditions

ESI Power Supply	8.9 kV
Counter Electrode Power Supply	4.5 kV
Ion Drift Tube Power Supply	4.5 kV
ESI Solvent Delivery rate	20 ml/hr
Amplification	1×10^8 V/A
Filtering	10 μ s
Pulse Frequency	6.70 Hz
Pulse Width	7 ms
Signal Amplitude	5 V
Signal Offset	2.5 V
Oscilloscope Averaging	8
ESI to Counter Electrode distance	8.5 mm

Experimental Methods

In this study two bacteriophages were used, λ -phage and MS2. Table 2 summarizes the properties of both bacteriophages.

Table 2 Bacteriophages Used

λ -phage		
Genetic Material	DNA	Linear double-stranded
Molecular Weight	Weight	33 X 10 ⁶ Daltons
Size	Head	60 nm Diameter
	Tail	570 nm Length
MS2		
Genetic Material	DNA	Linear single-stranded
Molecular Weight	Weight	1.2 X 10 ⁶ Daltons
Size	Head	24 nm Diameter
	Tail	N/A

According to a paper by Yeh et al. (1980),¹ that makes a comparison between inorganic particle diameter and the specific particle mobility, the particle mobility for the MS2 should be $10^{-2} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. From this data, the drift time was calculated to be approximately 65 msec. The particle mobility for the λ -phage virus was calculated to be $10^{-3} \text{ cm}^2 \text{ Volts}^{-1} \text{ Seconds}^{-1}$,¹ which corresponds to a drift time of 0.7 sec.

The voltage drops between successive rings in the desolvation and drift region were between 400.0 and 421 volts. Voltage drops were obtained by using a voltage divider, and high precision was not required. Therefore, standard 5% resistors were used. A difference of 816 volts was present between the shutter and detector, which provided a gradient of 1046 V/cm. Standard cell voltage was 816 volts.

The data system was programmed to collect data from 400 to 800 milliseconds. In addition, the reactant ion peak due to low molecular weight species such as ethanol and water was monitored for changes. This peak occurs at very short times, 0-10 msec.

Several factors were taken into consideration when choosing the solvent used for the ESI. The first of these factors was the production of an observable reactant ion peak (RIP). The second of these factors dealt with solubility of the analyte in the solvent. Because of the presence of basic, acidic, hydrophobic, and hydrophilic regions on the bio-organisms,

the solvent used would need chemical properties which would allow it to solvate the analyte. Solvents evaluated in this experiment were water, methanol, and ethanol. In addition, various combinations of these solvents were examined. In all cases, acetic acid was added to each solvent to help protonate all of the basic sites in the bio-agent. This added protonation gives the molecule a higher positive overall charge.

The optimal solvent mixture for the ESI was obtained by balancing the ability of the solvent to produce a satisfactory reactant peak with the solubility of the bacteriophages in the solvent system. Optimal reactant peak height was obtained when using a 50/50 mixture of water and methanol plus 5% acetic acid. Unfortunately, the bacteriophages selected for this demonstration were not soluble in this solution. Accordingly, the solution was modified to obtain the required solubility for the bacteriophages. The solution used for all subsequent tests consisted of ethanol – water (90/10) plus 5% acetic acid.

The optimal concentration of bacteriophages in the ESI solvent mixture was 0.7 μ M. The solvent containing the bacteriophage was introduced into the IMS at a relatively high flow rate of 300 μ l/min. We estimate that 30% to 40% of this flow was trapped on the discharge electrode, effectively reducing the flow rate into the desolvation region of the IMS to the more conventional 180 to 210 ml/minute flow.

Results

Throughout the course of this experiment, some corrosion was observed on various parts of the IMS. This was particularly evident on the electrical connections to the ring electrodes. It is believed that the harsh conditions in the IMS contributed to this corrosion. The bulk materials selected to build the IMS (stainless steel, Teflon®) did not corrode, but connections that were made using common materials such as copper and high carbon steel did show evidence of corrosion. Material selection appears to be more critical for an electrospray IMS than for a conventional IMS.

Although there was no direct observation of a viral peak on the spectra acquired from the IMS, direct effects on the spectra were observed. The most observable and evident of these was the change in the reactant ion peak. As seen in Figure 5, there is a drastic change in the RIP intensity as a result of the viral injection. This change is consistent over relatively long periods when MS2 is being aspirated into the IMS, as well as when discrete syringe injections are made. This phenomenon was observed whenever injections of the virus MS2 or the λ -phage were made. The drifting baseline is thought to have been caused by solvent droplets collecting on the discharge electrode.

To verify that the sharp RIP peak drop was due to the presence of the virus, several control experiments were performed. The first of these experiments involved the injection of a 5.0 μ l air bubble into the solvent line. At first it was suspected that the reason for the sharp drop was due to the inclusion of air bubbles when the viral sample was injected. These air bubbles interrupt the continuous solvent flow, causing a sharp

drop on the RIP. However, several deliberate injections of air did not produce any decrease in the RIP intensity, indicating that the inadvertent injection of air was not the causative mechanism.

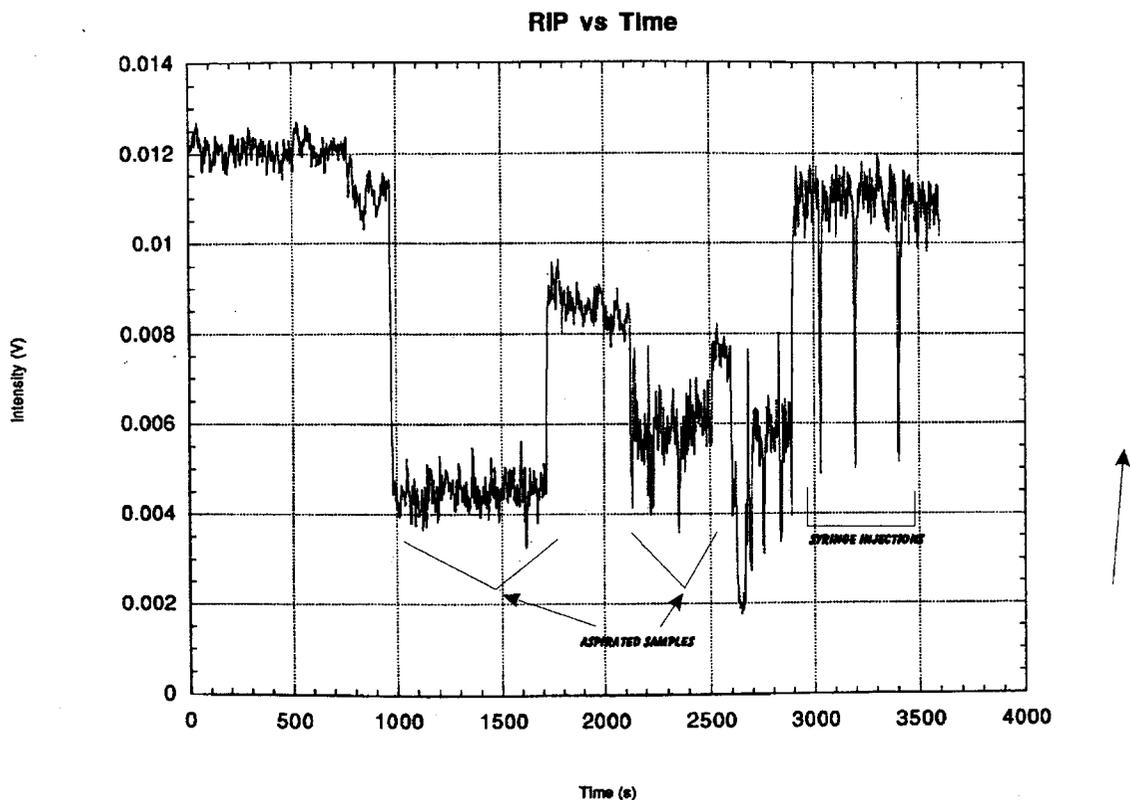


Figure 5. Decrease in Reactant Ion Intensity due to Presence of Biological materials.

The second control experiment involved injecting 5 μ l of the solvent used to dissolve the viral samples, this solvent is the same as is used for the formation of the RIP. The reasoning behind this control test was twofold. First, there was a possibility that the RIP intensity drop was due to formation of large size droplets. Because of its larger than normal size, the drop moves more slowly and could not reach the drift region, causing a decrease of ions reaching the detector. Also, there existed a possibility that the RIP drop was caused by a difference in the composition of the solvent in the viral sample mixture compared to the background solvent. By injecting some solvent used to form the viral samples it might be possible to reproduce this phenomena. However, this experiment did not produce any observable drops on the RIP intensity.

From these two control experiments, it was concluded that the RIP drop was due to the presence of the virus in the injected mixture. The drop in intensity of the RIP comes about from the transfer of charge from the reactant ions to the viral species or clusters of such species. This then raises the question as to why no peak associated with the virus was observed at the expected time.

A number of possibilities come to mind. The low mobility of the viruses in combination with the particular structure of this IMS, a short drift region relative to the width of the shutter grids, results in the viruses not being able to pass through the shutter region in the time set by the shutter pulse. This will result in the viruses stagnating in the shutter region, where the viral sample is more susceptible to collisions with molecules from the drift gas, small ions composing the RIP, and other viral molecules. These collisions could strip the viral molecules of charge making them neutral. Once the viral molecule loses its charge, it would be taken out of the drift region by the drift gas, giving no detectable response. Other possibilities are clustering of the viruses or multiple charging resulting in charge to mass ratios different than the singly charged species. These would contribute to signals in other unexpected parts of the IMS spectrum and reduce the magnitude of any particular peak. This clustering phenomenon was not observed, but, this particular IMS was not optimized to obtain the best signal to noise ratio, thus limiting the sensitivity to the expected small number of charged biologicals.

Work that was performed at New Mexico State University during the course of these studies demonstrated that it is possible for intact bacteria to pass through an electrospray IMS. Using equipment similar to that used in our studies, viable bacteria were sprayed through an electrospray ionization source, down a drift region, and collected on an agar plate. After incubation, it was easily demonstrated that the bacteria survived their passage through the high electric fields. Work to demonstrate the ability of the biological materials to transit a complete IMS system is underway.⁷

Summary

These results show that it is possible to inject viruses into an IMS using electrospray methods and observe a decrease in the reactant ion peak. The lack of a discrete viral peak could be due to a variety of effects ranging from clustering and multiple charging of the viruses to limitations on the injection process due to the low mobility of such virus particles. In the limited time available, it was not possible to redesign the IMS to better match the mobility characteristics of the viral species that we wished to detect directly.

These experiments also show that electrospray is able to successfully inject large biological ions from solution into an IMS. It is not an ideal system when used with the IMS to detect viral particles. The large voltages used in the electrospray process and the nature of the process itself results in significant noise problems. It appears to us that a vapor phase injection method would provide a simpler process to optimize and interpret.

While this 6-month project did not result in the direct detection of viruses by an IMS, the lessons learned have raised our level of confidence that such direct detection of both viruses and bacteria with an IMS is possible.

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