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Chemical Standards in Ion Mobility Spectrometry

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Abstract

In ion mobility spectrometry (IMS), reduced mobility values (K0) are used as a qualitative measure of gas phase ions, and are reported in the literature as absolute values. Unfortunately, these values do not always match those collected in the field. One reason for this discrepancy is that the buffer gas may be contaminated with moisture or other volatile compounds. In this study, the effect of moisture and organic contaminants in the buffer gas on the mobility of IMS standards and analytes was investigated for the first time using IMS directly coupled to mass spectrometry. 2,4-dimethylpyridine, 2,6-di-*tert*-butyl pyridine (DTBP), and tetrabutylammonium, tetrapropylammonium, tetraethylammonium, and tetramethylammonium chlorides were used as chemical standards. In general, the mobility of IMS standard product ions was not affected by small amounts of contamination while the mobilities of many analytes were affected. In the presence of contaminants in the buffer gas, the mobility of analyte ions is often decreased by forming ion-molecule clusters with the contaminant. To ensure the measurement of accurate reduced mobility values, two IMS standards are required: an instrument and a mobility standard. An instrument standard is not affected by contaminants in the buffer gas, and provides an accurate measurement of the instrumental parameters, such as voltage, drift length, pressure, and temperature. The mobility standard behaves like an analyte ion in that the compound's mobility is affected by low levels of contamination in the buffer gas. Prudent use of both of these standards can lead to improved measurement of accurate reduced mobility values.

INTRODUCTION

Ion mobility spectrometry (IMS) is an analytical technique that separates gas-phase ions according to their size to charge ratios, and is used in a growing number of applications. Initially developed in the 1970s as an inexpensive method for quantifying trace organic compounds and for estimating their mass,[1] IMS has grown into the analytical method of choice for the detection of chemical warfare agents,[2] toxic industrial chemicals,[3] drugs of abuse,[4–6] and explosives.[6–7] Ion mobility spectrometers have been coupled to mass spectrometers, and employed for separation and detection of biomolecules such as proteins,[8] peptides,[9–10] carbohydrates,[11] and lipids.[12] When coupled to mass spectrometry, ion mobility spectrometry offers value-added information of size, shape, and charge number.[13] When mass spectra are spread out along the mobility axis, noise reduction, isomer separation, and charge identification are possible.[14] In addition, mobility-mass correlation curves aid in class identification of

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unknowns.[15,16] For all these applications, it is critical that the ion mobilities measured are accurately and reproducibly reported.

Ion mobility spectrometry differs from mass spectrometry in that the separation of gas phase ions occurs by interaction of the ions with a buffer gas in an electric field. While there are several types of ion mobility spectrometers, the traditional drift time instrument measures the velocity of an ion in a buffer gas under the influence of a homogeneous electric field. Under ideal conditions, the velocity of these ions is proportional to the electric field strength and dependent on the ion's identity. The proportionality constant between ions' velocity and electric field strength, known as the ion mobility constant (K), becomes a qualitative measure of the ion:

$$K=\frac{v}{E}=\frac{L^2}{V\cdot t_d} \quad (1)$$

where v is the velocity of the ion in cm s^{-1}, E the electric field in the drift region in V cm^{-1}, L the length of the drift region in cm, V the total voltage drop in volts across the drift region, and t_d the time the ion spends traveling the distance L in seconds. As early as 1892, Ernest Rutherford measured the mobility of ions formed by x-ray ionization,[17] and characterized the ions using ion mobilities.[18] Because the velocity of the ion varies with both temperature and pressure, measured mobility constants are commonly corrected to standard temperature and pressure to produce a reduced mobility constant (K_0):

$$K_0=K\frac{P}{760}\frac{273}{T}=\frac{L^2}{V\cdot t_d}\frac{P}{760}\frac{273}{T} \quad (2)$$

where P is the pressure in the drift region in Torr and T is the buffer gas temperature in Kelvin.[19] Equation 2 holds for small molecules; large molecules, such as proteins, may undergo changes in their collision cross sections with temperature that are not corrected with this equation. Collision cross sections depend on the masses of the ion and the buffer gas molecules, the ion-buffer gas interactions, and the ion's shape. Therefore, even with very accurate ion mobility spectrometers capable of precisely measuring mobilities, the mobilities of chemical standards would depend on the buffer gas and degree of contamination. In 1928, Dusault and Loeb expressed the necessity of using chemical standards to calibrate the mobility values obtained in their laboratory.[20]

In theory, K_0 values are constant for a given compound in a given buffer gas, and are a qualitative indicator of the ion's identity. The primary advantage of K_0 values in IMS is that they are fundamentally related to the ion collision cross sections through the Mason Schamp Equation and to the ion's diffusion coefficient through the Einstein relation.[21] A compilation of reduced mobility values for a variety of gas phase ions was published in 1986.[22] In general, published K_0 values are considered to match one another if their uncertainties are within 2% (~0.02 cm^{2}V^{-1}s^{-1}).

In practice, however, K_0 values do not always match those reported in the literature. These variations are generally attributed to instrumental parameters, such as inhomogeneities in temperature and electric field, which are often not well characterized. In 1931, Loeb started using the term reduced mobility constant and proposed air ions as a calibration gas.[23] To calibrate instrumental parameters, Karpas suggested the use of chemical standards to correct reduced mobility values. He specifically suggested 2,4-lutidine, with a known and well characterized K_0 value of 1.95 cm^{2}V^{-1}s^{-1}, because it has a high proton affinity and produced

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a single peak at his experimental conditions.[24] Viidanoja et al. defined an ideal chemical standard for ESI-IMS as "a compound that produces only a single ion mobility peak, and for which the IMS spectrum and drift behavior are insensitive to solvent composition and gaseous impurities within the ion source and the drift tube".[25] Using an accepted standard, reduced mobility values can be calculated from measured mobility values by the following relation: [26]

$$\frac{K_{o\,(\text{unknown})}}{K_{o\,(\text{standard})}}=\frac{t_{d\,(\text{standard})}}{t_{d\,(\text{unknown})}} \tag{3}$$

Berant and Karpas corrected uncertainties in the measurement of electric field strength, temperature, and pressure in IMS experiments using this method.[27] Rearden et al. used the proton-bound dimer peak of 2,4-lutidine as an external standard to calibrate the reduced mobility scale[28] because the K_0 value (1.43 $cm^2V^{-1}s^{-1}$) has been reported to be unaffected by humidity at the temperatures used in the study.[26]

Protonated dimethyl methylphosphonate $(DMMP)H^+$ and proton-bound dimer $(DMMP)_2H^+$ were investigated as chemical standards for IMS, but changes in mobility were found between −13 to 207 °C for these compounds.[29] Tabrizchi proposed the reactant ion as an internal standard for IMS.[30] However, Eiceman et al. considered that to use the reactant ion as an internal standard was not acceptable.[26] Reactant ions are often ion clusters and their mobility values change as a function of temperature and moisture. Eiceman et al. also considered 2,4-lutidine · H^+ and $(DMMP)H^+$ unsuitable as chemical standards for IMS due to significant changes in their reduced mobilities between ambient temperature and 250°C. They showed that the reduced mobilities of the proton-bound dimer of 2,4-lutidine $(2,4\text{-}DMP)_2H^+$ and $(DMMP)_2H^+$ were almost unchanged between ambient temperature and 250 °C. These proton-bound dimers, however, were not considered good standards because high concentrations of 2,4-lutidine and DMMP were required to see the dimers. The presence of high concentrations of these high-proton-affinity compounds would be detrimental to the observation of other analytes.[26] In 2006, Ewing et al. found that the reduced mobilities of $(DMMP)_2H^+$ were stable from 290 to 490 K at concentrations of 6.0, 5.0×10^2, and 2.0×10^3 ppmv of water; they also observed the reduced mobilities of $(DMMP)H^+$, 2,4-lutidine, and $(H_2O)_nH^+$ to increase with temperature, which they attributed to loss of water of hydration.[31]

Di *tert*-butyl pyridine (DTBP) was first used as a chemical standard for IMS in 2002 by Eiceman et al.[32] In 2003, they recommended the use of this compound as a chemical standard because its mobility was independent of buffer gas temperature and moisture in the buffer gas.[26] Pedersen et al. used DTBP as an internal standard to minimize the influence of temperature, pressure, and electrical field in the characterization of proton-bound acetate dimers.[33] DTBP also has been used to correct mobilities in IMS[34] and to demonstrate the performance of ion mobility spectrometers coupled to mass spectrometers.[35,36]

In 2005, Viidanoja et al. proposed tetraalkylammonium ions as chemical standards. These compounds are inherently ionic, which guarantees no charge competition, and are detected with high sensitivity in ESI-IMS. Tetraalkylammonium ions produce only a single ion mobility peak and have a low clustering tendency, which makes them insensitive to contaminants in the buffer gas. However, their reduced mobilities are not well established.[25] Jafari used tetrabutylammonium bromide as an external standard to test the performance of a new mobility spectrometer design.[37]

In the early 20th century, it was noted that K_0 values were influenced by parameters other than pressure and temperature. These were most notably contaminants in the buffer gas. In 1910,

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at the suggestion of J.S. Townsend, Lattey investigated the effects of moisture on the mobility of ions.[38] Lattey also reported the influence of other contaminants, such as traces of air and carbon dioxide.[39] Erikson also found in 1927 that adding CO_2 and water vapor to the buffer gas (air) decreased the mobility of ions but adding hydrogen increased it.[40] Eiceman et al. reported that the drift time of the reactant ions peaks (RIPs) in IMS increased ~4% with the increase of moisture from 0 to 2030 ppm,[41] and Ewing et al. found reductions in mobilities of 12% and 7.3% for $(DMMP)H^+$ and 2,4-lutidine, respectively, when increasing water content from 6 ppmv to 2.0×10^3 ppmv in the mobility spectrometer. Similar effects of moisture on the mobility of ions have been reported.[42–45].

Although standards are becoming generally accepted and useful in IMS, little work has been conducted on the influence of contaminants in the buffer gas on the mobilities of these chemical standards for IMS. In this work, the mobility behavior of chemical standards was analyzed when contaminants, such as moisture, solvents from electrospray ionization, volatiles from out gassing of instrumental components, or trace organics were introduced into the buffer gas.

EXPERIMENTAL

Instrument

An electrospray ionization atmospheric-pressure ion mobility spectrometer (ESI-APIMS) interfaced through a 40-μm pinhole to a quadrupole mass spectrometer (Figure 1) was used in this work. Typical operating parameters used with this instrument are shown in Table 1.

The IMS instrument was built at Washington State University, and consisted of a drift tube and an electrospray ionization source. The drift tube consisted of two sections: a desolvation and a drift region separated by a Bradbury-Nielsen ion gate. The ion gate comprised 80 parallel 75-μm Alloy-46 stainless steel wires (California Fine Wire Co., Grove Beach, CA) 0.6 mm apart. Ions were gated into the drift region with a 0.1 ms pulse. When the gate was closed, ions were stopped from passing into the drift region by applying a closure potential that was 40 V higher for one set of wires (positive wires) and 40 V lower for the other set (negative wires) than the drift voltage in the position of the gate. Positive and negative wires were alternated in the gate. Both desolvation and drift regions had stainless steel rings, alternating with ceramic insulating rings, connected in series by high temperature resistors (Caddock Electronics Inc., Riverside, CA, ±1%). The rings were 0.5 cm wide and 5 cm in diameter. A counterbore on the outer surface of each metal ring allowed the fitting of neighboring insulating rings in a horizontal stacking arrangement. Metal rings were 2 mm apart in this arrangement. An electric field of 432 V cm^{-1} was formed in the drift tube when an electrical potential was applied to the first ring.[46] An ESI target screen was made out of 2-mm stainless steel mesh with a 0.5-cm round orifice in the center. The target screen was located in the first ring of the drift tube. Preheated N_2 buffer gas was introduced through a stainless-steel tube at the low voltage end of the drift tube at a flow rate of 0.9 L/min countercurrent to ion motion to aide in the desolvation of the ions. The buffer gas was heated by passing it through a 2-m long stainless-steel tube coiled inside a heated aluminum block (Figure 1). The mobility spectrometer was operated at ambient pressure (680–710 Torr in Pullman, WA).

The mass spectrometer was an ABB Extrel (Pittsburgh, PA) 150-QC quadrupole (m/z 0–4000). A Keithley model 427 amplifier (Keithley Instruments, Cleveland, OH) amplified the electron multiplier detector signal of the mass spectrometer and sent it to the data acquisition systems. Merlin software (ABB Extrel, Pittsburgh, PA) controlled the mass spectrometer and collected the mass spectral data. Custom LabView software (National Instruments, Austin, TX) was used to collect the IMS data and controlled the ion gate. The electronics for controlling the gate and IMS data acquisition were built at WSU.[47]

Spectra in ion mobility spectrometers coupled to quadrupole mass spectrometers can be acquired in single ion monitoring IMS (SIM-IMS), radiofrequency-only IMS (IMS), and mass spectrometry modes. In SIM-IMS mode, the mass spectrometer voltages are set so that only ions of a given mass to charge ratio or a range of ions are detected. These settings avoid the interference of other ions when analyzing a specific compound. In SIM-IMS mode, the mobility spectra of a specific ion or ions are collected. In IMS mode, ions are pulsed into the drift region and introduced into the mass spectrometer, where they are all detected without scanning; the mobility spectrum of all ions is collected in this mode. In mass spectrometry mode, all ions are detected by the mass spectrometer, and the mobility spectrometer is used as a desolvation region with the gates always open.

Materials and reagents

Methionine, phenylalanine, serine, threonine, tyrosine, tryptophan, ethanolamine, tribenzylamine, tributylamine, valinol, 2,4-dimethylpyridine (2,4-lutidine), 2,6-di-*tert*-butyl pyridine (DTBP), methyl 2-chloropropionate (MCP), and α-trifluoromethyl benzyl alcohol (tFMBA), and tetrabutylammonium (TBA), tetrapropylammonium (TPA), tetraethylammonium (TEA), and tetramethylammonium (TMA) chlorides were ACS reagent grade (≥98% purity), and were purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI). Amino acids were selected as analytes to provide a series of compounds with different molecular weights and steric effects. Ethanolamine, tribenzylamine, and tributylamine, selected as analytes, also provided compounds with steric effects different to those of amino acids. The chemical standards selected are the most often used in IMS.[25,26] Additionally, tetraalkylammonium ions were selected as chemical standards because they are ionic compounds, and no charge competition was expected in ESI-IMS, which guaranteed a high sensitivity for these standards. MCP and tFMBA, an ester and an alcohol, were selected as buffer gas contaminants to mimic contamination with organic compounds. MCP and tFMBA, are representatives of common contaminants such as volatile esters and alcohols. Water and organic contaminants are the most common buffer gas impurities in IMS.

Contaminant introduction

Test contaminants were continuously pumped through a 10-cm-long, 50-μm ID silica capillary (Polymicro Technologies, Phoenix, AZ) in the liquid state and introduced into the buffer gas line using a Swagelok T-junction. Gas tight syringes (Hamilton, Reno, NV) were used to ensure no leaking of contaminants during injection. The temperature of the junction was increased to 150 °C using a heating tape (OMEGA Engineering, Stamford, CT) to help vaporize the contaminant. To verify that the nominal amount of water contaminant injected was effectively introduced, the water content in the buffer gas was measured with a GE Moisture Image Series 1 instrument (Billerica, MA). Eiceman et al. studied the effect of moisture on the reduced mobilities of dimethyl methylphosphonate, 2,4-lutidine, DTBP, and the reactant ions. They found no significant effects on the mobilities of these ions by increasing the moisture content up to 161 μmol m^{-3} (2.9 mg m^{-3}).[26] In our investigation, experiments were performed at higher concentrations of water, up to 8.8×10^2 mmol m^{-3} (3.3×10^4 ppmv). These are concentrations that might be reached at field conditions and are still below water vapor saturation conditions.

Other contaminants were run using the smallest flow rate that produced a measurable change in K_0 to a flow rate where a plateau was found in K_0 values.

Sample preparation and introduction

50 μM standard solutions of the analytes were prepared in ESI solution (47.5 % methanol: 47.5 % water: 5 % acetic acid). Electrospray ionization (ESI) was used to inject 3 μl min^{-1} of liquid samples or solvent (ESI solution) using 250-μl syringes into 40-cm long, 100-μm ID capillaries. Stainless steel unions (Valco, Houston, TX) connected these capillaries to 50-μm ID capillaries.

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The ends of these capillaries were placed in the center of the target screen at the entrance of the mobility spectrometer. The stainless steel unions received a high voltage of 15.6 kV, with a 3.5 kV bias with respect to the target screen in the first ring, to produce positive ions.

Calibration

Calibration of the mobility spectrometer was obtained applying Equation 3 and using DTBP and tetraalkylammonium ions as chemical standards. These chemical standards allowed the calculation of reduced mobilities without introducing errors acquired when measuring instrumental parameters such as temperature, drift tube length, pressure, and drift field.

Identification of analytes

Analytes were identified by comparing the molecular weight of their protonated molecules or clusters with their m/z signal produced in the mass spectrometer. Also, reduced mobilities of protonated analyte ions were compared with those from literature.

RESULTS AND DISCUSSIONS

1. Effect of moisture contamination in the drift region on the ion mobility of analytes

The mobility spectra in Figure 2a illustrates how the mobility of valinol response ions was affected when water was introduced into the buffer gas of the ion mobility spectrometer. The drift time for valinol increased greater than 1 ms as moisture increased in the buffer gas from 0.03 mmol m^{-3} (10 ppmv, the average water concentration in nitrogen buffer gas in "N_2-only" conditions) to 8.8×10^2 mmol m^{-3}. This drift time increase corresponded to a reduction in mobility of 7.1% (Table 2). The reduction in mobility with moisture was due to formation of large analyte-water clusters, as demonstrated in the mass spectrum of valinol (Figure 3) obtained under conditions used in Figure 2a. Figure 3 shows clusters of protonated valinol with one to eleven water molecules occurring at m/z 122, 140, 158, 176, 194, 212, 230, 248, 266, 284, and 302.

The mobilities of other analytes also decreased with moisture in the buffer gas; ethanolamine, valinol, threonine, methionine, phenylalanine, tyrosine, tryptophan, serine, tributylamine, and tribenzylamine exhibited percentage reductions in mobility (%ΔK_0) of −9.2%, −7.1%, −6.6%, −5.5%, −5.1%, −5.1%, −2.2%, −7.9%, −2%, and −1 %, respectively, as moisture increased in the buffer gas from 0.03 mmol m^{-3} to 8.8×10^2 mmol m^{-3}. %ΔK_0 is defined as the percentage difference between K_0 in N_2-only buffer gas and K_0 when a contaminant is introduced into the buffer gas at a given concentration. In general, changes in K_0 values were smaller with increasing molecular weight of the ion when water was introduced into the buffer gas; for these compounds, this relation was linear with a correlation coefficient of −0.94. This trend may be due to the small effect on ion size when a water molecule clusters to large ions. The other ions were not included to calculate this and other trends because of steric effects explained later. Table 2 summarizes the percentage reduction in mobilities (%ΔK_0) for the test compounds when contaminants were introduced into the buffer gas. In this work the reactant ions experienced a large effect of moisture. However, valinol and most amino acids had greater changes with water as contaminant than reactant ions. It looks like the greater number of sites for attachment of water molecules in valinol and amino acids (three sites corresponding to three hydrogens) than in the reactant ion peaks (water ions with only two sites, the two hydrogens) produced a larger accumulation of water molecules in valinol (see Figure 3) and amino acids (which decelerated these ions) than in the reactant ions.

The decrease in mobility of all ions with water concentration in the buffer gas agreed with earlier reports that moisture in the buffer gas decreased ion mobility.[38–45] In those works, ions in dry air were faster than those in moist air. In other studies by Eiceman et al., moisture was

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not found to produce significant effects on the mobility of dimethyl methylphosphonate, 2,4-lutidine, DTBP, and reactant ions.[26] However, Eiceman et al. only explored concentrations of water up to 0.16 mmol m^{-3}, well below the levels investigated in this study.

2. Effect of organic contamination in the drift region on the ion mobility of analytes

To simulate organic contamination of the buffer gas, a volatile alcohol and an organic ester were selected: α-trifluoromethyl benzyl alcohol (tFMBA) and methyl 2-chloropropionate (MCP).

a. Effect of tFMBA contamination in the drift region on the ion mobility of analytes—Figure 2b shows that as the concentration of tFMBA increased in the buffer gas from 0 to 2.3 mmol m^{-3} (86 ppmv) at 150°C the drift time of serine response ions increased 2.3 ms (11.7%). This mobility decrease as tFMBA was introduced into the buffer gas was due to formation of large analyte-tFMBA clusters as demonstrated in the mobility and mass spectra data of Figure 4 obtained under conditions used in Figure 2b. In Figure 4a, protonated molecules of serine and serine-tFMBA clusters appeared as a single and broad peak at 21.8 ms in the mobility spectrum, indicating fast equilibria between these species.[25] Figure 4b displays serine clusters with one and two molecules of tFMBA in the mass spectrum occurring at m/z 282 and 457.

The mobilities of other analytes also decreased when tFMBA was introduced into the buffer gas; valinol, threonine, methionine, phenylalanine, tyrosine, tryptophan, serine, tribenzylamine, tributylamine, and ethanolamine showed $\%\Delta K_0$ values of −5.1%, −8.6%, −4.6%, −7.3%, −7.0%, −4.0%, −10.6%, −3.1%, −6.2%, and −13% respectively, as tFMBA concentration increased in the buffer gas from 0 mmol m^{-3} to 2.3 mmol m^{-3} (Table 2). $\%\Delta K_0$ values decreased with molecular weight of the ion; for these compounds, this relation was linear with a correlation coefficient of −0.77.

b. Effect of methyl 2-chloropropionate contamination in the drift region on the ion mobility of analytes—When the concentration of an organic ester, methyl 2-chloropropionate (MCP), was increased in the buffer gas from 0.00 to 0.93 mmol m^{-3} (35 ppmv), the test compounds showed the following $\%\Delta K_0$ values: valinol (−31%), methionine (−14%), phenylalanine (−21%), serine (− 29%), threonine (−23%), tyrosine (−16%), tryptophan (−15%), tribenzylamine (6.1%), tributylamine (15%), and ethanolamine (−36%) (Table 2). The mobilities of both test compounds decreased with MCP concentration in the buffer gas. As with tFMBA, $\%\Delta K_0$ values decreased with the molecular weight of these compounds when MCP was introduced into the buffer gas; this relation was linear with a correlation coefficient of −0.89.

In general, all analytes investigated experienced a decrease in mobilities when organic contamination was introduced into the buffer gas. These results agreed with earlier reports that organic molecules in the buffer gas decreased the mobility of ions.[48–51]. Nevertheless, effects of water on ion mobility were lower than the effects of organic contaminants perhaps due to the small size of water clusters.

As discussed in the introduction, 2,4-lutidine, DTBP, and tetraalkylammonium salts have been recommended as chemical standards for IMS.[25,26] These compounds were investigated with respect to the effects that contamination of the buffer gas with water or organic compounds have on ion mobility.

3. Effect of moisture contamination in the drift region and ion mobility of chemical standards

In general, the mobility of chemical standards did not significantly change as a function of water concentration in the buffer gas. When water concentration was increased in the buffer gas from 0.03 mmol m^{-3} to 2.3 mmol m^{-3}, no change in mobility was observed. However, when water concentration was increased to 8.8×10^2 mmol m^{-3} (at 150°C), changes in mobilities were observed as follows: 2,4-lutidine (−3.8%), TMA ions (−4.0%), TEA ions (−2.9%), DTBP (−2.1%), TPA ions (−1.8%), and TBA ions (−0.9%). The reduction in mobility of DTBP was smaller than that of 2,4-lutidine. This difference in mobility may be due to the larger size of DTBP and the steric hindrance caused by the large t-butyl substituents on DTBP, located at positions 2 and 6 on the ring, shielding the protonated pyridine nitrogen from clustering. The two smaller methyl groups of 2,4-lutidine, which were located at positions 2 and 4 on the ring, shielded the protonated nitrogen less effectively from interacting with moisture. This interaction with water molecules produced clusters of 2,4-lutidine with water. Again, as demonstrated with analytes, $\%\Delta K_0$ values decreased with molecular weight of the chemical standards; for chemical standards, this relation was linear with a correlation coefficient of −0.98.

4. Effect of organic contamination in the drift region on the ion mobility of chemical standards

a. Effect of tFMBA contamination on the ion mobility of chemical standards—When tFMBA concentration in the buffer gas was increased from 0.0 to 2.3 mmol m^{-3} at 150°C, the mobility of 2,4-lutidine decreased by 0.9% and that of DTBP by 1.3%; the mobility tetraalkylammonium ions did not change.

b. Effect of MCP contamination on the ion mobility of chemical standards—The mobility of the chemical standards decreased as MCP concentration was increased in the buffer gas from 0.00 to 0.93 mmol m^{-3} at 150°C as follows: 2,4-lutidine (−19%), DTBP (−0.9%), TMA (−0.4%), TEA (−0.1%), TPA (−0.1%), TBA (−0.1%). The reduction in mobility of DTBP was lower than that of 2,4-lutidine due to steric hindrance

Mobility and mass spectra were studied to understand the stability of tetraalkylammonium ions' mobility values in the presence of contaminants in the buffer gas. In IMS mode, single peaks were detected for each tetraalkylammonium ion with all contaminants; single peaks indicated that no fragments, adducts, or clusters of tetraalkylammonium ions occurred at the temperatures and concentrations of contaminants used, or, at least, they decomposed in the desolvation region.[25] Figure 4c shows single peaks in the IMS spectrum of tetraalkylammonium ions when 8.8×10^2 mmol m^{-3} of water were introduced into the buffer gas. Mobility and mass spectra also produced single, defined peaks for each tetraalkylammonium ion when MCP and tFMBA contaminants were introduced into the buffer gas; cluster peaks were not seen for TEA, TPA, and TBA ions when high concentrations of water contaminant (8.8×10^2 mmol m^{-3})were introduced into the buffer gas (Figure 4d); a small peak for the TMA ion-water cluster may be indistinguishable from the peak at m/z 91, $(H_2O)_5H^+$. The formation of clusters was expected for tetraalkylammonium ions due to the permanent positive charge situated at the quaternary nitrogen atom; however, the steric hindrance exerted by the four alkyl chains enclosing the nitrogen[25] kept contaminant molecules away, which hindered the formation of ion-ligand bonding.

Similarly, when other compounds, such as DTBP, were not affected by a particular contaminant, they did not cluster with that contaminant; when they were affected, analyte-contaminant clusters appeared in the mass spectra (Figures 4a and 4b) in a number and intensity proportional to the extent of change in K_0. Also, when ion mobilities were affected by introduction of buffer gas contaminants, protonated ions disappeared or their intensity decreased. The mass spectra of methionine in Figure 5 illustrates this point; this figure shows

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that the ratio of the intensities of methionine-tFMBA peak to methionine protonated peak ($Met.tFMBA.H^+$:$Met.H^+$) increased when tFMBA concentration in the buffer gas increased. These ratios were 0.00, 0.15, and 0.33 at tFMBA concentrations of 0.0, 1.1, and 1.7 mmol m^{-3}, respectively, demonstrating increasing clustering with tFMBA concentration.

Figure 6 plots K_0 values for test chemical standards and analytes as a function of contaminant concentration in the buffer gas. In general, when moisture contaminated the buffer gas at low concentrations, similar to those used for organic contaminants, the mobility of analyte ions did not shift significantly; however, at high moisture concentrations some mobility shifts were observed. For the standards, only 2,4-lutidine exhibited a significant mobility shift as a function of organic contamination. In the presence of water, none of the standards exhibited a significant change in mobility until high concentrations of water were introduced into the buffer gas.

5. Mobility standards and instrument standards

As demonstrated above, mobility calculations using chemical standards may produce inaccurate values when the buffer gas is contaminated with water or an organic compound. The mobility of either the standard or the analyte may shift as a function of buffer gas contamination. For example, if the chemical standard 2,4-lutidine is used to calculate the mobility of ethanolamine in the presence of MCP, the calculation may be off by 17% or more. This error is due to 2,4-lutidine's product ion mobility value changing by −19% and the mobility of ethanolamine product ion changing by −36% when MCP concentration was varied from 0.00 to 0.93 mmol m^{-3} in the buffer gas (Table 2). Calculating mobilities with DTBP or tetraalkylammonium ions would yield larger errors than calculating the mobilities with 2,4-lutidine because changes in mobility values for those ions were smaller than those for 2,4-lutidine when MCP was introduced into the buffer gas.

To improve mobility calculations using standards, the chemical standards used in this study were classified based on their response to contaminants in the buffer gas. Chemical standards which have mobility values sensitive to the presence of contaminants in the buffer gas were called "mobility standards," and standards for which mobility values did not change as a function of buffer gas contamination were called "instrument standards."

Properties of mobility and instrument standards—Mobility standards should be small ions without steric hindrance at the charge site to enable the ions' sensitivity to the presence of contamination in the buffer gas; the small size of the mobility standard would allow clustering to change significantly ion size and affect its mobility, indicating that the instrument is contaminated. Other ideal properties of mobility standards should be the production of a single mobility peak and high sensitivity. On the other hand, instrument standards should have steric hindrance at the charge site and a large size. Steric hindrance would deter the attachment of contaminants to the ion charge, and the mobility of the instrument standards would be unaffected by buffer gas contamination. Large ionic size would limit the effect on mobility if some clusters were formed. Thus, a standard with these attributes would only be affected by errors in instrumental parameters, such as voltage, pressure, length, and temperature. Other ideal properties of instrument standards are the production of a single mobility peak, high sensitivity, and stability of reduced mobility values with changes in temperature, moisture, composition of the ESI solvent, and drift field.[25,26] Figure 6 shows that 2,4-lutidine is more responsive to MCP than to tFMBA. In general, compounds' response to clustering will depend on their size and structure. Therefore, mobility standard response will be different with different contaminants in different laboratory or field conditions. Consequently, the ideal mobility standard would be one with a size and structure similar to the analyte. For these reasons, the mobility constants of a wide set of representative mobility standards need to be established; 2,4-lutidine was used as a mobility standard in this work because its mobility is well known

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and, in preliminary tests, was the one with the largest mobility changes upon clustering among current standards.

DTBP as an instrument standard—DTBP was ruled out as a good mobility standard because its $\%\Delta K_0$ values were small in the presence of contaminants in the buffer gas. However, the stability of DTBP's K_0 values would make it a good instrument standard. Other reasons to use DTBP as an instrument standard are high sensitivity due to its high proton affinity, production of a single mobility peak, and relative stability of reduced mobilities with temperature, moisture, and electric field.[26]

Tetraalkylammonium ions as instrument standards—K_0 values for tetraalkylammonium ions also were found to be stable in the presence of contaminants in the buffer gas (Table 2); K_0 values for TBA and TPA ions were more stable than those of DTBP. This stability makes tetraalkylammonium ions excellent instrument standards. Additional reasons to use tetraalkylammonium ions as instrument standards are the production of a single mobility peak, high sensitivity, and stability of reduced mobilities with temperature, moisture, composition of the ESI solvent, and drift field.[25] However, although TBA and TPA ion mobilities were more stable than those of DTBP in the presence of contaminants, their reduced mobilities are not well established, and more investigations are required to determine accurate and precise mobilities of these ions to replace DTBP as the instrument standard of choice in IMS. The reduced mobility values reported for TBA ions ranged from 1.19 to 1.40 $cm^2V^{-1}s^{-1}$ with an average value of 1.30 $cm^2V^{-1}s^{-1}$;[25,37,47,52–53] this variability may be due to inaccurate measurement of instrumental parameters.

Recommended method for ion mobility calibration—For accurate calibration of an ion mobility spectrometer, both an instrument and a mobility standard should be used. An instrument standard would determine the instrument constant (C_i) by rearranging Equation 2.

$$K_{0,\text{standard}} \cdot t_{\text{d,standard}} = \frac{L^2}{V}\frac{P}{760}\frac{273}{T} = C_i \quad (4)$$

The value of C_i should be calculated every time L, P, T, or V might have changed.

After an instrument standard is used to determine C_i, a mobility standard such as 2,4 lutidine, should be employed to determine if the spectrometer is contaminated. If the spectrometer is free of contamination, the product of the measured drift time of the mobility standard and its reduced mobility constant equals the instrument constant, which is calculated using the instrument standard, and the reduced mobility values of unknowns can be accurately measured with the following relation:

$$K_{0,unknown} = \frac{C_i}{t_{d,unknown}} \quad (5)$$

If contamination cannot be removed from the spectrometer, a correction factor might be calculated to take into account the effect of the contaminant on ion mobility. This correction factor would be unique for every analyte, temperature (which affects clustering), and level of contamination, for which such factor may be usable only for routine measurements such as continuous monitoring of a single compound using the same instrumental conditions in steady contamination levels.

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CONCLUSION

The mobilities of selected analytes and chemical standards were measured by electrospray ionization ion mobility spectrometry-quadrupole mass spectrometry (ESI-IMS-QMS). Chemical standards were classified in two classes according to their mobility response to the introduction of contamination into the buffer gas: standards to determine contamination in the ion mobility spectrometer (mobility standards) and standards to calibrate the mobility instrument (instrument standards). An instrument standard should be insensitive to contamination in the buffer gas and solvent composition. In contrast, a mobility standard should be sensitive to the presence of neutrals in the buffer gas to detect contamination in the drift tube. DTBP was corroborated as a better instrument standard than 2,4-lutidine in IMS because its K_0 value is not only independent of temperature, moisture, and electric field,[26] but was also less affected by contamination in the buffer gas. Reduced mobilities of tetraalkylammonium ions are not only independent of field, temperature, and composition of the ESI solvent,[25] but were also independent of contamination in the buffer gas. Therefore, DTBP and tetraalkylammonium ions are not good mobility standards, but they are excellent instrument standards. The mobilities of TBA and TPA ions were the most stable of the compounds tested when doping the buffer gas with polar contamination, and could be used as instrumental standards for electrospray ionization methods. However, a drawback of tetraalkylammonium ions as chemical standards for IMS is that these salts can be only used in solution, which would hinder their use with sources that require vapors, such as radioactive sources.

Acknowledgments

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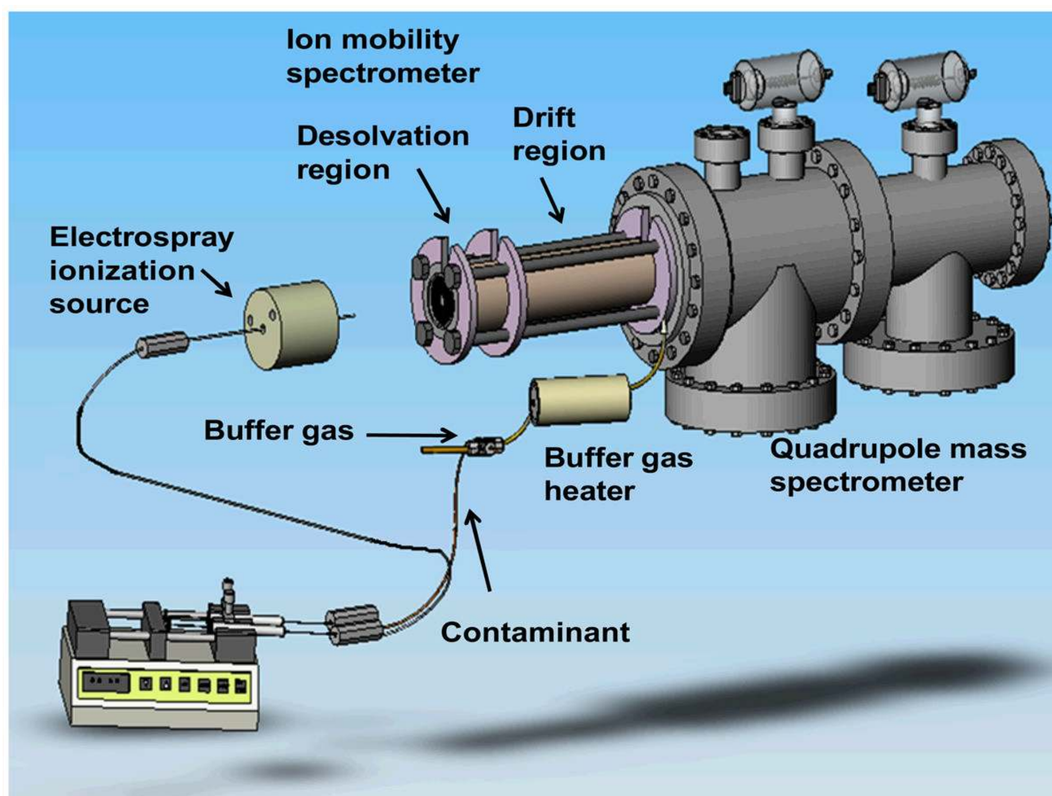

Figure 1. Instrument

Sketch of the electrospray ionization-atmospheric pressure ion mobility-mass spectrometer including setup for the injection of contaminants and heating of the buffer gas.

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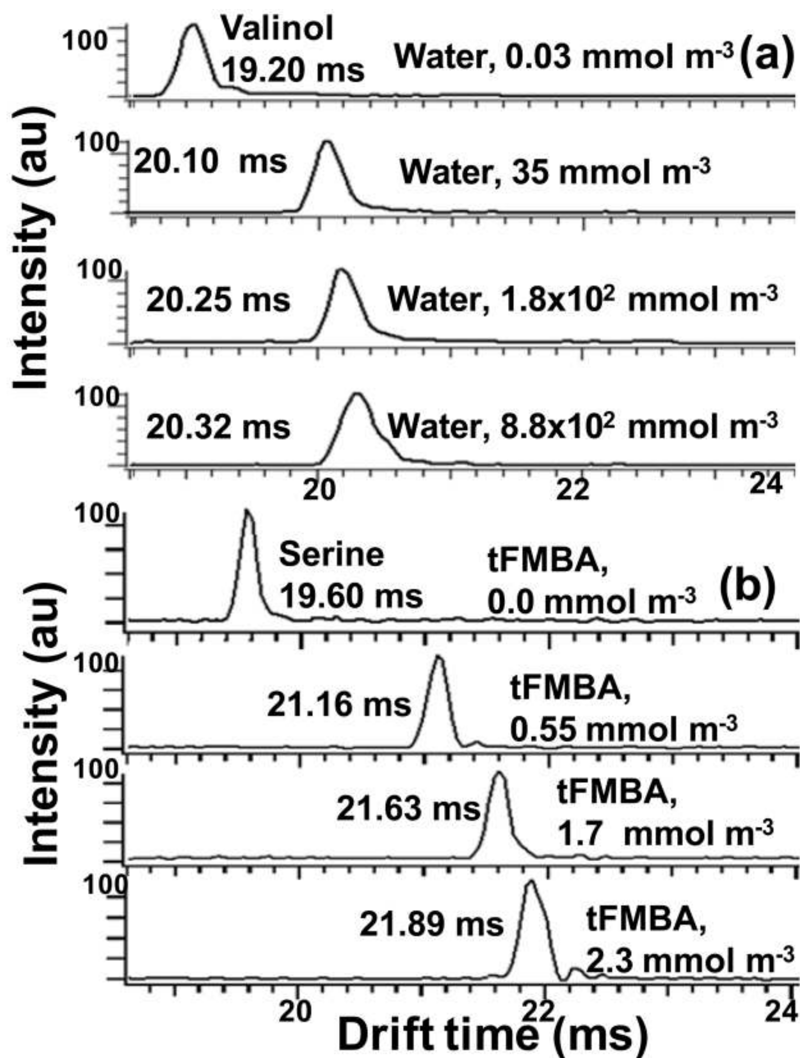

Figure 2. Changes in mobility when contaminants were introduced into the buffer gas

SIM-IMS spectra illustrating the reduction in mobility of the response ions in 100-μM solutions of analytes when contaminants were introduced into the buffer gas at 150°C. (a) Spectrum of valinol when water contaminant was introduced into the buffer gas. (b) Spectrum of serine when tFMBA contaminant was introduced into the buffer gas.

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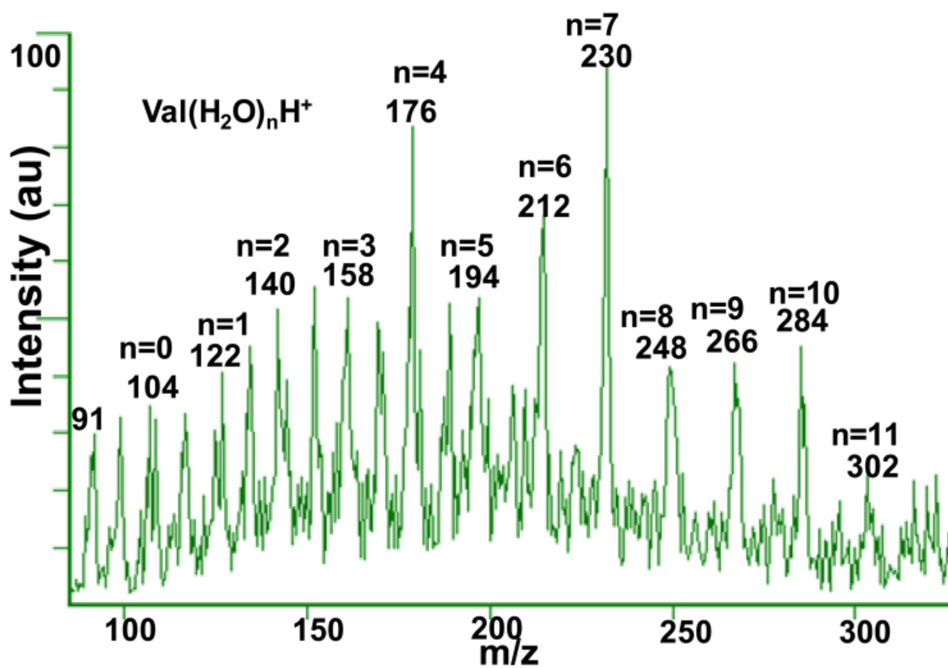

Figure 3. Valinol-water cluster formation upon introduction of water contaminant into the buffer gas

Mass spectrum of 100-μM valinol (Val) at 150°C and a water concentration of 8.8×10^2 mmol m^{-3} in the buffer gas. The formation of valinol-water clusters with up to 11 molecules of water decreased the mobility of valinol when water was injected into the buffer gas.

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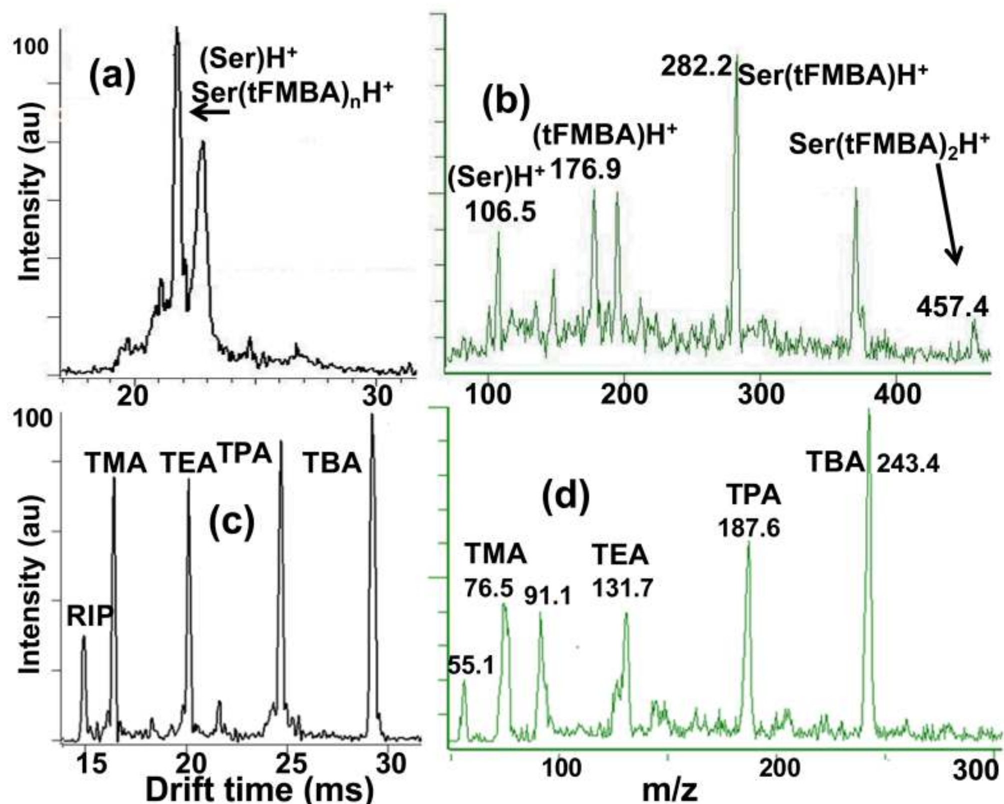

Figure 4. Clustering of tetraalkylammonium ions and serine with contaminants

Concentration was 8.8×10^2 mmol m^{-3} for water (c and d) and 2.3 mmol m^{-3} for tFMBA (a and b) in the buffer gas. (a) (IMS spectrum) Broad peaks indicate clustering of serine with tFMBA; the peak at ~23 ms might be the sodium adduct of tFMBA at m/z 199 (b) mass spectrum showing extensive clustering of serine when tFMBA was introduced into the buffer gas; cluster formation was due to the small size and absence of steric hindrance on the amino acid structure; (c) (IMS spectrum) well-defined peaks denote the absence of fragmentation, adduction, or clustering of tetraalkylammonium ions in the drift region; (d) (mass spectrum) water clusters were not seen for TEA, TPA, and TBA ions, which indicates the large steric hindrance of tetraalkylammonium ions. Buffer gas temperature was 150°C.

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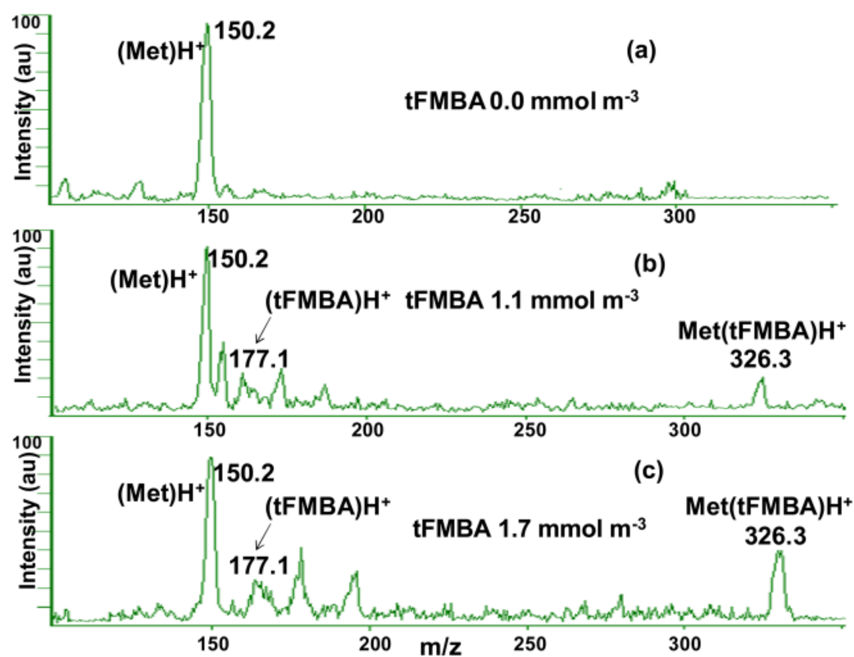

Figure 5. Methionine-tFMBA clusters

The mass spectra show that as tFMBA concentration increased from a) 0.0 to c) 1.7 mmol m^{-3} in the buffer gas, the ratio of the intensities of the analyte-contaminant peak to the protonated peak of methionine, Met(tFMBA)H^+:(Met)H^+, increased, which indicates increasing clustering of methionine with tFMBA.

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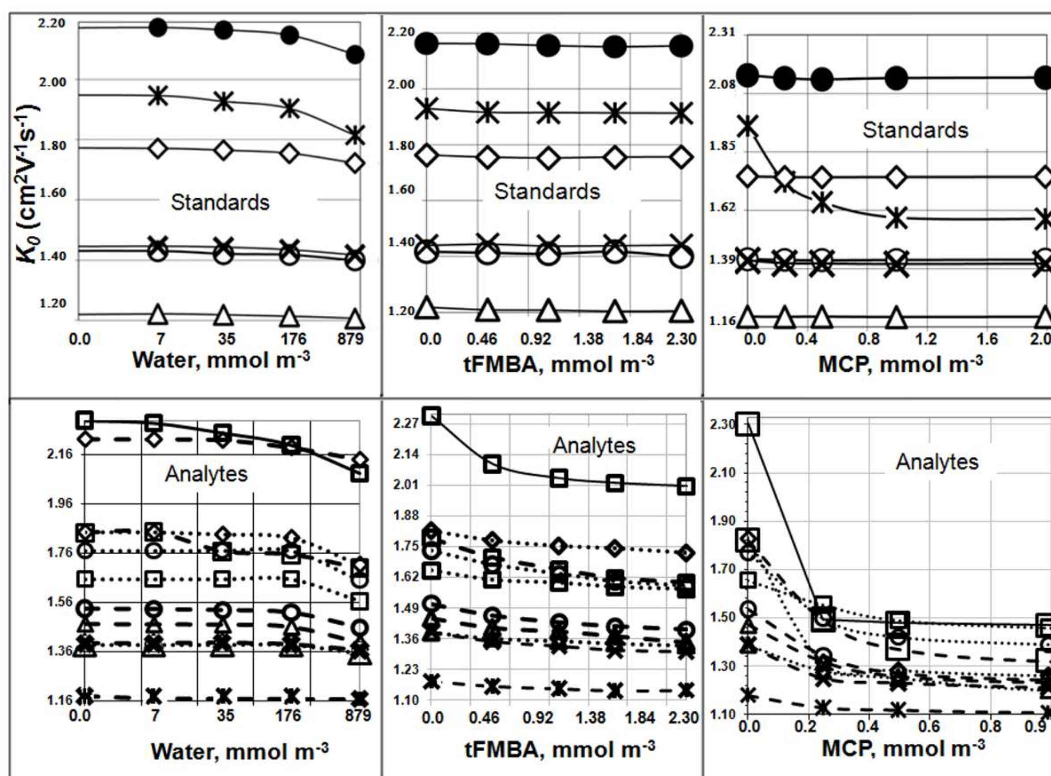

Figure 6. Change in K_0 values for test compounds upon addition of contaminants into the buffer gas

MCP: methyl 2-chloropropionate; tFMBA: α-trifluoromethyl benzyl alcohol. Standards: 2,4-lutidine (–✳–), DTBP (–○–), TBA ions (–△–), TEA ions (–◇–), TMA ions (–●–), TPA ions (–**x**–); Analytes: ethanolamine (–□–), methionine (··□··), phenylalanine (--○--), reactant ion peaks (--◇--), serine (--□--), threonine (··●··), tribenzylamine (--✳--), tributylamine (--**x**--), tryptophan (··△··), tyrosine (--△--), and valinol (··◇··).

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Table 1

ESI-APIMS operating conditions summary

Parameter	Settings
Reaction region length	7.5 cm
Drift tube length	25.0 cm
ESI voltage	15.6 kV
Voltage at first ring	12.12 kV
ESI flow	3 μl min^{-1}
Voltage at the gate	10.80 ± 0.01 kV
Gate closure potential	±40 V
Gate pulse width	0.1 ms
Scan time	35 ms
Buffer gas	Nitrogen
Buffer gas temperature	150 ± 2°C
Buffer gas flow	930 ml min^{-1}
Contaminant flow rate	0.03 to 1250 μl hr^{-1}

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Table 2

%ΔK_0 values when organic contaminants and moisture were introduced into the buffer gas

Percent reduction in mobility, %ΔK_0, for selected compounds at a concentration of 0.93 mmol m^{-3} (35 ppmv) of MCP, 2.3 mmol m^{-3} of α-trifluoromethyl benzyl alcohol (86 ppmv), or 8.8×10^2 mmol m^{-3} (3.3×10^4 ppmv) of water in the buffer gas. %ΔK_0 is defined as the percentage difference between K_0 in nitrogen buffer gas and K_0 when a contaminant is introduced into the buffer gas at a given concentration. %ΔK_0 values for water at 2.3 mmol m^{-3} (86 ppmv) in the buffer gas were 0.0 indicating a smaller influence of moisture in ion mobilities than that of tFMBA or MCP.

Compound	Molecular weight, g mol^{-1}	Methyl 2-chloropropionate	α-trifluoromethyl benzyl alcohol	Water
RIP's				−3.8
2,4-lutidine	107.1	−19	−2.0	−3.8
DTBP	191.3	−0.9	−1.3	−2.1
TBA ions	242.5	−0.1	0.0	−0.9
TEA ions	130.3	−0.1	0.0	−2.9
TMA ions	74.2	−0.4	0.0	−4.0
TPA ions	186.4	−0.1	0.0	−1.8
Methionine	149.2	−14	−4.6	−5.5
Phenylalanine	165.2	−21	−7.3	−5.1
Serine	105.1	−29	−10.6	−7.9
Threonine	119.1	−23	−8.6	−6.6
Tyrosine	181.2	−16	−7.0	−5.1
Tryptophan	204.2	−10	−4.0	−2.2
Tribenzylamine	287.4	−6.1	−3.1	−1.0
Tributylamine	185.3	−15	−6.2	−2.0
Valinol	103.2	−36	−5.1	−7.1
Ethanolamine	61.1	−31	−13	−9.2