

Using Different Drift Gases To Change Separation Factors (α) in Ion Mobility Spectrometry

G. Reid Asbury and Herbert H. Hill, Jr.*

Department of Chemistry, Washington State University, Pullman, Washington 99164-4630

The use of different drift gases to alter separation factors (α) in ion mobility spectrometry has been demonstrated. The mobility of a series of low molecular weight compounds and three small peptides was determined in four different drift gases. The drift gases chosen were helium, argon, nitrogen, and carbon dioxide. These drift gases provide a range of polarizabilities and molecular weights. In all instances, the compounds showed the greatest mobility in helium and the lowest mobility in carbon dioxide; however the percentage change of mobility for each compound was different, effectively changing the α value. The α value changes were primarily due to differences in drift gas polarizability but were also influenced by the mass of the drift gas. In addition, gas-phase ion radii were calculated in each of the different drift gases. These radii were then plotted against drift gas polarizability producing linear plots with r^2 values greater than 0.99. The intercept of these plots provides the gas-phase radius of an ion in a nonpolarizing environment, whereas the slope is indicative of the magnitude of the ion's mobility change related to polarizability. It therefore, should be possible to separate any two compounds that have different slopes with the appropriate drift gas.

Ion mobility spectrometry (IMS) has been used extensively for more than 20 years to analyze trace organic vapors, especially for compounds such as explosives, drugs, and chemical warfare agents.¹ These analyses were accomplished primarily by using radioactive ionization sources such as ⁶³Ni, which only allowed analysis of volatile compounds. With the development of electrospray ionization² for IMS, high molecular weight and nonvolatile compounds could easily be analyzed by IMS.

The development of electrospray ionization ion mobility spectrometry greatly expanded the range of compounds that could be analyzed by IMS; however, the utility of the technique for mixtures was still somewhat limited by the lack of sufficient resolving power. IMS traditionally has been considered a low-resolution technique with resolving powers generally no higher than 30 as defined by

$$R = t_d/w_h \quad (1)$$

where t_d is the ion drift time and w_h is the width of the ion pulse at half-height measured at the detector. More recently, high-resolution IMS systems have been developed,³ allowing the technique to be used as a stand-alone separation device without the use of chromatographic separations prior to ion mobility detection. The use of electrospray IMS as a separation and detection device has recently been demonstrated for explosives,⁴ chemical warfare degradation products,⁵ and biological mixtures.⁶

Ion mobility separates compounds on the basis of their different gas-phase ion mobilities, commonly in nitrogen or air, giving mobility constants (K), defined as

$$K = v/E \quad (2)$$

where v is the velocity of the ion and E is the electric field in the drift region of the spectrometer. Mobility constants are usually calculated by measuring the time an ion travels down the drift tube using a rearranged form of eq 2 given as

$$K = L^2/Vt_d \quad (3)$$

where L is the distance the ion drifts, V is the total voltage drop the ion experiences, and t_d is the time it takes the ion to travel down the drift tube. Regardless of the resolving power of an instrument, it is clear that it would be impossible to separate two compounds with identical K values. The ratio of these K values can be used to determine a separation factor (α) defined here as

$$\alpha = K_1/K_2 \quad (4)$$

where K_1 is the mobility constant of the faster drifting compound and K_2 is the mobility of the slower drifting compound. Therefore,

- (3) Brokenshire, J. L. FACSS Meeting, Anaheim, CA, October 1991. Leonhardt, J. W.; Rohrbeck, W.; Bensch, H. Fourth International IMS Workshop, Cambridge, U.K., 1995. Dugourd, P.; Hudgins, R. R.; Clemmer, D. E.; Jarrold, M. F. *Rev. Sci. Instrum.* **1997**, *119*, 2249. Wu, C.; Siems, W. F.; Asbury, G. R.; Hill, H. H. *Anal. Chem.* **1998**, *70*, 4929–4938. Srebalus, C. A.; Li, J.; Marshall, W. S.; Clemmer, D. E. *Anal. Chem.* **1999**, *71*, 3918–3927.
- (4) Asbury, G. R.; Klasmeier, J.; Hill, H. H. *Talanta*, **1999**, *50* (6), 1291–1298.
- (5) Asbury, G. R.; Wu, C.; Siems, W. F.; Hill, H. H. *Anal. Chim. Acta*, in press.
- (6) Henderson, S. C.; Valentine, S. J.; Counterterm, A. E.; Clemmer, D. E. *Anal. Chem.* **1999**, *71*, 291–301.

* Corresponding author: (tel) (509) 335-5648; (e-mail) hhhill@wsu.edu.

(1) Eiceman, G. A.; Karpas, Z. *Ion Mobility Spectrometry*; CRC Press: Boca Raton, FL, 1994.

(2) Wittmer, D.; Chen, Y. H.; Luckenbill, B. K.; Hill, H. H. *Anal. Chem.* **1994**, *66*, 2348–2355.

as in chromatography, an α value of 1 indicates that two compounds cannot be separated with the current selectivity of the instrument.

Recently, the possibility of changing α values in IMS was shown by operating at very high electric fields.⁷ This instrument, a high-field asymmetric waveform ion mobility spectrometer (FAIMS), filters ions at atmospheric pressure by taking advantage of the difference in the mobility constant of an ion in low and high electric field conditions. At these electric fields, the velocity of an ion is no longer proportional to the electric field and therefore the calculated K value at low electric fields may differ from the value at high electric fields. A second method of altering the α value is to change the polarizability of the drift gas. This approach was theoretically investigated and reviewed almost 25 years ago,⁸ however, the use of different drift gases to alter α values in practice has seen very little research. Several important papers have been published over the past 25 years using drift gases other than nitrogen and air,^{9–16} primarily to study gas-phase reactions in different drift gases or the feasibility of using different drift gases such as CO₂.¹⁶ More recently, the use of helium as a drift gas has found wide acceptance for measurements of collision cross sections^{17–20} of biomolecules in the gas phase, presumably because helium is not very polarizable and hence does not complicate theoretical calculations.

In this paper, we explore the feasibility of using different drift gases to alter α values in IMS. Four drift gases were used: helium, argon, nitrogen, and carbon dioxide. In each of the drift gases, mobility measurements were made for a series of low molecular weight compounds and three small peptides. Several factors related to drift gas in addition to α value changes were investigated including (1) change in mobility, (2) change in ion size, (3) mass effects, (4) change in sensitivity, and (5) resolving power.

EXPERIMENTAL SECTION

Chemicals and Solvents. The seven small compounds used in this study were purchased from Aldrich Chemical Co. (Milwaukee, WI) and included aniline, fluoroaniline, chloroaniline, bromoaniline, iodoaniline, 4-aminobenzonitrile, and hexylamine. The three peptides were purchased from Sigma Chemical Co. (St. Louis, MO). All compounds were used without further purification. The compounds were prepared in a 50:50 water/methanol mixture at a concentration of approximately 100 ppm. All solvents were

HPLC grade and were purchased from J. T. Baker (Phillipsburgh, NJ).

Instrumentation. The instrument used in this study was constructed at Washington State University and consisted of an electrospray ionization source and a high-resolution ion mobility spectrometer interfaced to a quadrupole mass spectrometer. A detailed description of this instrument and the electrospray source can be found in refs 2 and 3. All compounds were sprayed in the positive mode with a spray voltage of +4000 V with respect to the target screen of the spectrometer. A continuous flow of solvent through the ESI source was provided by a dual-piston syringe pump (Brownlee Labs, Santa Clara, CA) at a flow rate of 5 μ L/min. Samples were injected via a six-port injector (Valco Industries, Houston, TX) with an external injection loop.

The ion mobility spectrometer was operated in the positive mode with an electric field strength of 230 V/cm and a total drift voltage of +3000 V. The drift tube was operated at 250 °C at atmospheric pressure. A counterflow of preheated drift gas (helium, argon, nitrogen, or carbon dioxide) was introduced at the end of the drift region at a flow rate of 1200 mL/min.

The ion mobility spectrometer was interfaced to a C50-Q quadrupole mass spectrometer (ABB Extrel, Pittsburgh, PA). The ions entered the MS via a 40- μ m pinhole leak which served as the barrier between the atmospheric pressure of the IMS tube and the vacuum of the mass spectrometer.

All mobility data were collected by replacing the stock preamplifier with a Keithley 427 amplifier (Keithley Instruments, Cleveland, OH) and sending the amplified signal to the data acquisition system, constructed at WSU. A detailed description of the IMS data control and data acquisition system was reported previously.² All data shown were the average of 256 individual spectra. The mobility spectra were all collected in the mass-selected mode.

Calculations. Collision cross sections (Ω) were calculated using a rearranged form of the equation governing gas-phase ion transportation⁸ shown below:

$$t_d = \frac{16N(\mu kT)^{1/2} L^2 \Omega}{3(2\pi) Vq} \quad (5)$$

where N is the number density of the gas, μ is the reduced mass, k is the Boltzmann constant, T is the temperature in the drift region, q is the charge on the ion, and K is the mobility of the ion.

The radii of the drift gas molecules were estimated using a hard-sphere model based on the viscosity of the gas given by

$$r^2 = \frac{1}{4} \left(\frac{5}{16\pi^{1/2}} \frac{(MRT)^{1/2}}{N_A \eta} \right) \quad (6)$$

where d is the diameter of the molecule, M is the mass of the molecule, R is the gas constant, T is the temperature, N_A is Avogadro's constant, and η is the viscosity of the gas. Using the result of eq 6 and rearranging the relation $\Omega = \pi(r_{\text{ion}} + r_{\text{gas}})^2$, the radii of the ions can be calculated using

$$r_{\text{ion}} = \sqrt{\Omega/\pi} - r_{\text{gas}} \quad (7)$$

- (7) Purvis, R. W.; Guevremont, R. *Anal. Chem.* **1999**, 71, 2346–2357.
- (8) Revercomb, H. E.; Mason, E. A. *Anal. Chem.* **1975**, 47, 970–983.
- (9) Ellis, H. W.; Pai, R. Y.; Gatland, I. R.; McDaniel, E. W.; Wernlund, R.; Cohen, M. J. *J. Chem. Phys.* **1976**, 64, 3935–3941.
- (10) Sennhauser, E. S.; Armstrong, D. A. *Can. J. Chem.* **1978**, 56, 2337–2341.
- (11) Carr, T. W. *Anal. Chem.* **1979**, 51, 705–711.
- (12) Sennhauser, E. S.; Armstrong, D. A. *Can. J. Chem.* **1980**, 58, 231–237.
- (13) Berant, Z.; Karpas, Z.; Shahal, O. *J. Phys. Chem.* **1989**, 93, 7529–7532.
- (14) Karpas, Z.; Berant, Z. *J. Phys. Chem.* **1989**, 93, 3021–3025.
- (15) Yamashita, T.; et al. *Nucl. Instrum. Methods Phys. Res.* **1989**, A283, 709–715.
- (16) Perkins, M. D.; Chelf, R. D.; Eisele, F. L.; McDaniel, E. W. *J. Chem. Phys.* **1983**, 79, 5207–5208.
- (17) Rokushika, S.; Hatano, H.; Hill, H. H. *Anal. Chem.* **1986**, 58, 361–365.
- (18) von Helden, G.; Wyttenbach, T.; Bowers, M. T. *Science* **1995**, 267, 1483.
- (19) Clemmer, D. E.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1995**, 117, 10141.
- (20) Valentine, S. J.; Anderson, J. G.; Ellington, A. D.; Clemmer, D. E. *J. Phys. Chem B* **1997**, 101, 3891–3900.
- (21) Valentine, S. J.; Counterman, A. E.; Clemmer, D. E. *J. Am. Soc. Mass Spectrom.* **1997**, 8, 954–961.

Table 1. Molecular Weight, Polarizability, and Calculated Radius of Drift Gases

drift gas	MW	polarizability ^a (10 ⁻²⁴ cm ³)	calcd radius ^b (Å)
helium	4	0.205	1.03
argon	40	1.641	1.67
nitrogen	28	1.740	1.73
carbon dioxide	44	2.911	2.02

^a As tabulated in the *CRC Handbook of Chemistry and Physics*, 70th ed.; Lide, D. R., Ed.; CRC Press: Boca Raton, FL, 1989. ^b Calculated using eq 7.

The radii, the polarizability, and the molecular weight of each drift gas are summarized in Table 1.

The diffusion-limited peak width is calculated using

$$w_h^2 = t_g^2 + \left(\frac{16kt \ln 2}{VeZ} \right) t_d^2 \quad (8)$$

where t_g is the duration of the gate pulse, t_d is the drift time of the ion, k is Boltzman's constant, T is the temperature in kelvin, V is the potential across the drift region, e is the elementary charge, and z is the number of charges on the ion.

RESULTS AND DISCUSSION

Mobility of Ions in Different Drift Gases. Mobility spectra were obtained in each drift gas for all 10 compounds. In all cases, the ions had the greatest velocity (shortest drift time) in helium and the lowest velocity in carbon dioxide, with nitrogen and argon being intermediate, respectively. Figure 1 shows the mobility spectra of aniline in the four different drift gases. As can be seen from the figure, the drift time of aniline was only 3.5 ms in helium but was almost 25 ms in carbon dioxide. This is expected, considering that carbon dioxide has the largest calculated radius and that it is the most massive of all of the drift gases used. In fact, the ions drifted much as would be expected by looking at eq 5, where the drift time is determined in large part by the mass of the drift gas.

Radii of Ions in Different Drift Gases. By measuring the time it takes the ions to drift a fixed length, mobility constants (K) can be calculated using eq 3. From these K values it is possible to calculate the collision cross section (Ω) for each ion using eq 5. Knowing the cross section and the radius of the drift gas leads to a simple calculation to determine the radii of the ions by eq 7. For each of the 10 compounds, the radii were determined and are displayed in Table 2. Since these calculations account for the mass of the drift gas and the radius of the drift gas, it would be expected that the ions would have the same radius in all drift gases if there are no other parameters that affect the calculated ion radius. As the table shows, there is a significant difference in the calculated ion radii in the four drift gases. In all cases, the calculated radii increases in the following order: helium, argon, nitrogen, and carbon dioxide. This order is slightly different from that observed for the mobility of the ions. Since the ions were mass selected (only the protonated molecules were allowed to pass) via a quadrupole mass filter, it is believed that the ions produced were the same in each drift gas, thus not accounting

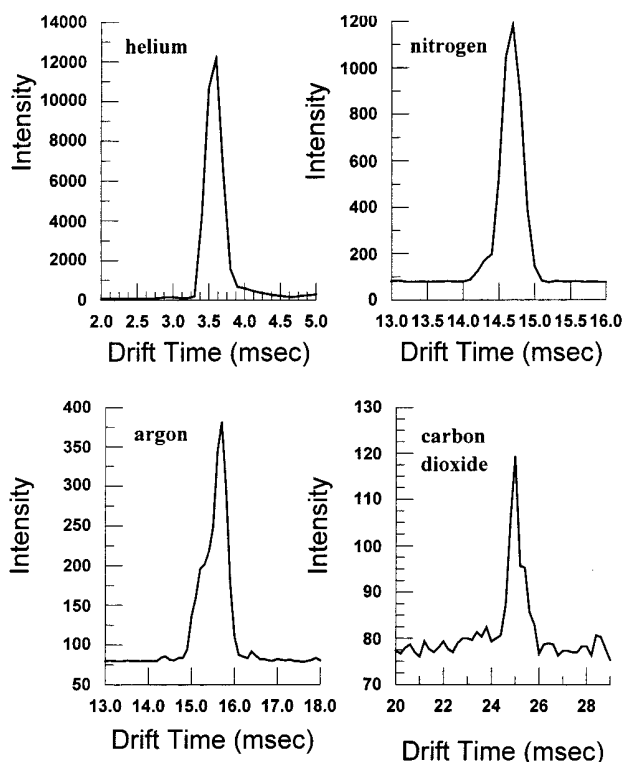


Figure 1. Ion mobility spectra of aniline in each of the four drift gases.

Table 2. Radii of Ions Measured in Different Drift Gases^a

compound	MW (amu)	charge	radius (Å)			
			He	Ar	N ₂	CO ₂
iodoaniline	220	1	3.02	3.81	3.83	4.50
bromoaniline	172	1	2.65	3.94	4.04	4.92
chloroaniline	128	1	2.60	3.85	3.96	4.87
fluoroaniline	112	1	2.43	3.71	3.82	4.75
aminobenzonitrile	119	1	2.60	3.65	3.77	4.52
aniline	94	1	2.38	3.61	3.72	4.53
hexylamine	102	1	2.80	3.92	4.04	4.89
Gly-Arg-Gly-Asp	404	1	4.74	5.45	5.57	6.05
Gly-Pro-Arg-Pro	426	1	4.92	5.61	5.77	6.10
Gly-Gly-Tyr-Arg	452	1	5.06	5.70	5.88	6.25

^a Uses the hard-sphere model with no correction for long-range effects.

for the difference in calculated radii. The difference in calculated ion radius for a specific species can be attributed to the difference in polarizability of the drift gases. In fact, plots of calculated ion radius versus drift gas polarizability produced linear plots, as shown in Figure 2, for iodoaniline and chloroaniline. The linear regression data for all of the compounds was tabulated and shown in Table 3. The slopes of the curves for the 10 compounds exhibited a wide range of values from a low of 0.452 to a high of 0.875; the higher the value, the greater the effect of drift gas polarizability on calculated ion radius. In other words, compounds that have steep slopes will have smaller average velocities relative to those with less steep slopes, as the ions move from a low-polarizability gas to a high-polarizability gas. This change can

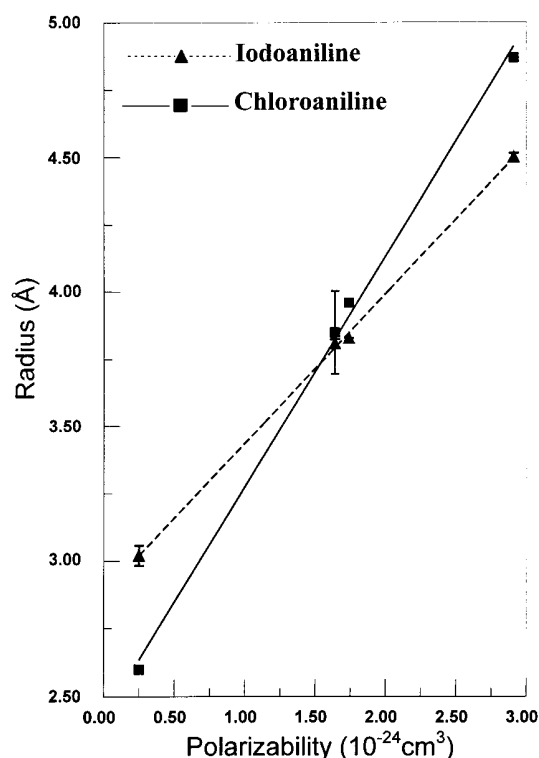


Figure 2. Calculated ion radii as a function of drift gas polarizability for iodoaniline and chloroaniline. The slope of the chloroaniline curve is significantly steeper than that of the iodoaniline, indicating that chloroaniline is affected to a greater extent by the polarizability of the drift gas than iodoaniline.

Table 3. Linear Regression Data for the Size of the Ion vs the Polarizability of the Drift Gas

compound	MW (amu)	charge	slope	intercept	r^2
iodoaniline	220	1	0.556	2.881	0.9994
bromoaniline	172	1	0.857	2.486	0.9953
chloroaniline	128	1	0.856	2.420	0.9977
fluoroaniline	112	1	0.875	2.247	0.9976
aminobenzonitrile	119	1	0.723	2.450	0.9967
aniline	94	1	0.812	2.232	0.9936
hexylamine	102	1	0.788	2.624	0.9985
Gly-Arg-Gly-Asp	404	1	0.495	4.643	0.9929
Gly-Pro-Arg-Pro	426	1	0.449	4.865	0.9679
Gly-Gly-Tyr-Arg	452	1	0.452	4.984	0.9778

then be used to alter α values in IMS as defined by eq 4. Figure 2 further demonstrates the effect. In drift gases with low polarizabilities, iodoaniline has a significantly larger calculated radius than does chloroaniline; however, the reverse is true in gases with high polarizabilities. The curves cross at approximately the polarizability of argon. The less massive compounds tend to have steeper slopes, indicating that the radius change is predominantly a factor of charge density of the ion. This is not completely true as in the case of aniline and fluoroaniline. Despite being more massive, fluoroaniline shows a steeper slope than aniline. In addition to the slope, Table 3 also provides r^2 values. The r^2 values for all of the small compounds are greater than 0.99, indicating a linear relationship between the calculated radius and the drift gas polarizability. The final data shown in Table 3 are the y-intercepts for each of the ions. These values are the extrapolated ion radii in a nonpolarizing gas. This may show great utility for comparing

Table 4. Separation Factors^a (α) for Aniline with the Other Compounds in Each Drift Gas

compound	helium	argon	nitrogen	carbon dioxide
iodoaniline	1.21	1.19	1.12	1.10
bromoaniline	1.15	1.22	1.18	1.21
chloroaniline	1.11	1.14	1.13	1.16
fluoroaniline	1.03	1.07	1.06	1.10
aminobenzonitrile	1.11	1.05	1.04	1.04
hexylamine	1.22	1.14	1.13	1.12
Gly-Arg-Gly-Asp	2.52	2.08	1.98	1.75
Gly-Pro-Arg-Pro	2.66	2.19	2.09	1.77
Gly-Gly-Tyr-Arg	2.78	2.24	2.10	1.86

^a Calculated using eq 4.

computer-simulated models of gas-phase ion structures to IMS data, without the added complexity that the polarizability of the drift gas adds.

Changes in Separation Factors (α) in Ion Mobility Spectrometry. The previous section discussed and demonstrated the changes in calculated radii for compounds measured in different drift gases. The extent of the drift gas effects was different for each ion. Therefore, changing the drift gas changes the α values in IMS. This can be thought of analogously to changes in the mobile phase in chromatography to change the retention of one compound relative to another. Table 4 shows the α values of aniline with the other nine compounds in the four drift gases. Aniline was used because it was the fastest drifting compound in all of the drift gases and, therefore, would always be in the numerator of eq 4 when compared to any of the other compounds. The table shows that the α values change significantly for all of the compounds when the drift gas is changed. This is further demonstrated by observing the spectra in Figure 3. This figure shows the mobility spectra of iodoaniline and chloroaniline in the four drift gases. In the helium spectrum, chloroaniline drifts faster than iodoaniline. The two peaks are fairly well resolved. The argon spectrum is very similar to the helium spectrum; however, the nitrogen spectrum shows the two compounds having nearly identical drift times. In the most polarizing drift gas, carbon dioxide, the compounds are once again resolved, but are in the reverse order compared to the helium spectrum. If these changes in drift time are strictly a function of polarizability, it would seem that the argon and nitrogen spectra should appear more similar than the helium and argon spectra. This, however, is clearly not the case. The reason for this can be seen by close examination of eq 5. The equation shows that the drift time is related not only to the cross section but also to the reduced mass and the charge. The charge term is not important as the two ions are +1 in all of the spectra. The reduced mass term is the primary reason for the argon spectra appearing not to follow the expected trend. The reduced mass term for the argon/chloroaniline system is significantly smaller than that of the argon/iodoaniline system. This is simply because iodoaniline is significantly more massive than chloroaniline. This mass dependence is only important in cases where the mass of the drift gas is of the same magnitude as the ions drifting through it. In cases where the ions are much more massive than the drift gas, the reduced mass becomes effectively the mass of the drift gas and, therefore, would not contribute to the separation of two very massive ions. Ion mobility spectrometry

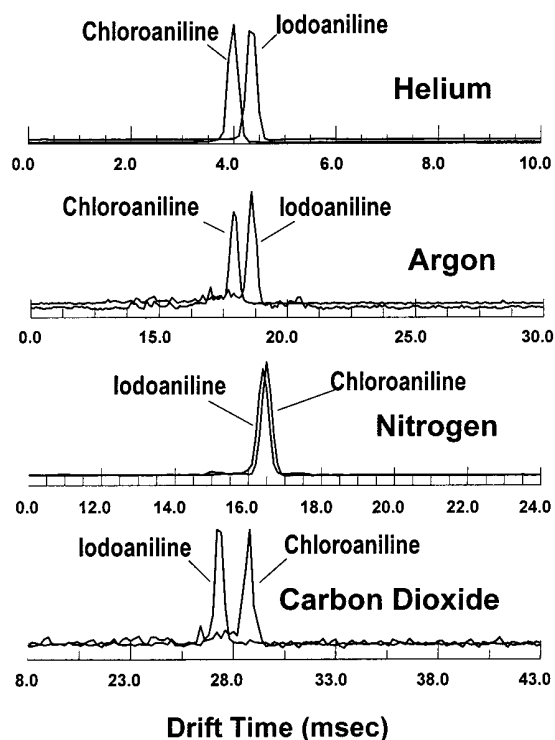


Figure 3. Ion mobility spectra of chloroaniline and iodoaniline in each of the four drift gases. As the polarizability increases, the velocity of the chloroaniline ion decreases relative to the iodoaniline ion. This change allows one to use drift gas polarizability to change separation factors (α) in ion mobility spectrometry.

Table 5. Comparison of Sensitivity and Resolving Power Achieved for Each Drift Gas

drift gas	relative peak area (av) ^a	resolving power (av) ^b	ratio of peak width and diffusion width ^c
helium	8.94 ± 0.56	15.7 ± 1.9	1.243 ± 0.095
argon	0.507 ± 0.045	47.2 ± 8.6	1.255 ± 0.214
nitrogen	1	45.0 ± 4.9	1.239 ± 0.138
carbon dioxide	0.128 ± 0.011	58.7 ± 8.5	1.165 ± 0.189

^a Relative to the sensitivity using nitrogen as drift gas. Taken using peak areas and includes the average for all seven compounds. ^b Average resolving power for all seven compounds. ^c Average ratio of the measured peak width and the theoretical diffusion-limited peak width for each of the seven compounds.

separates low molecular weight ions on the basis of their mass and their cross section, whereas it separates high molecular weight compounds primarily on the basis of their cross section. It should be noted that the number of charges on the ion also influences the separation in ion mobility spectrometry.

Sensitivity and Resolving Power in Different Drift Gases.

The effect of drift gas on resolving power and response was also explored. Table 5 gives a summary of the relative response and the resolving power achieved for each drift gas. The relative sensitivity was obtained by determining the peak area for the seven small compounds in each drift gas and then dividing the area by that achieved for our standard drift gas, nitrogen. Helium was by far the most sensitive, giving nearly 9 times the peak area than was seen in nitrogen. Argon and carbon dioxide were both

considerably less sensitive than nitrogen, achieving relative sensitivities of 0.507 and 0.128, respectively. These relative sensitivities appear to directly follow the amount of time the ions spend in the spectrometer. This sensitivity loss may be related to reactions of ions with neutrals in the drift tube and less efficient electrospray in different drift gases. The resolving power observed for each drift gas is also shown in Table 5. In this case, the longer the ions drifted, the better the resolving power achieved. This was certainly related to the contribution of the initial pulse width to the overall width of the peak at the detector. For very fast moving compounds, the initial pulse width (0.2 ms) was a very significant component to the overall peak width, whereas for slower drifting compounds, the diffusion of the ion pulse becomes the dominant band-spreading mechanism. A better comparison is shown in the third column of Table 5. This column shows the ratio of the achieved peak width with the width expected by diffusion only as calculated from eq 8. This calculation removes the effect of the initial pulse width and shows how narrow the peaks are in comparison to theoretical minimum peak width. A value of 1 indicates the spectrometer is achieving the maximum possible resolving power. In this case, the drift gases all performed almost identically, with each being within experimental error of the others. This probably indicates that there is no fundamental advantage in terms of resolving power for the different gases. The only difference is seen because of initial pulse width considerations.

CONCLUSIONS

Using different drift gases in ion mobility spectrometry has a number of significant effects on experimental outcome. Ions primarily travel faster in less massive drift gases than in more massive drift gases. The polarizability of the drift gas also affects the drift time of ions in IMS. This polarizability effect changes the calculated ion radii in a linear fashion but does not affect all ions equally. This difference can be exploited to change the separation factor (α) in IMS. In theory, all ions that show different slopes in their polarizability versus ion radii curves could be separated with IMS with the appropriate drift gas. For very small ions, an additional mass effect can be used to change α values in IMS. This is only true when the drifting ions have masses of the same magnitude as the drift gas. When different drift gases are used in an IMS instrument, two other factors need to be considered, sensitivity and resolving power. As ions drift faster their peak areas increase, but the resolving power decreases considerably because of increased peak width contributions from the initial pulse width.

ACKNOWLEDGMENT

The authors thank the U.S. Army for Grant DAAG559810107 for partial funding of this work. Additional support was provided by the Society of Analytical Chemistry of Pittsburgh for an ACS summer fellowship awarded to G.R.A.

Received for review August 6, 1999. Accepted November 22, 1999.

AC9908952