

**Use of Ion-molecule Reactions in Inductively
Coupled Plasma Mass Spectrometry (ICP-MS) to
Improve Selenium Analysis Accuracy and
Detection**

Brandon Henderson

Abstract

ICP-MS measurement of selenium, an essential trace element and potential cancer preventative, is complicated by argon-containing molecular ions at each of the selenium isotopes. SeH^+ complicates measurement of tracers based on isotope ratios when methane is used in a dynamic reaction cell to remove the argon-containing molecular ions. Nitrogen and oxygen were tested as alternative reaction gases. Methane, nitrogen and oxygen all reacted with ArAr^+ , Ar_xH^+ , Ar_2H_2^+ and ArCl^+ . Methane, nitrogen and oxygen reduced the ArAr^+ signals by 7, 5, and 2.6 orders of magnitude, respectively, with minimal loss of Se^+ signal (less than an order of magnitude). The Ar_2H^+ and Ar_2H_2^+ also react rapidly with each of the reaction gases; however, these reaction rates could not be accurately determined due to the low background equivalent concentration that occurs as a result of their signal. The ArCl^+ signal can be reduced by up to 5 orders of magnitude by ion-molecule reaction with methane and up to 4 orders of magnitude with nitrogen. The SeH^+ signals increase to about 7% of the corresponding Se^+ signal when methane is used as the reaction gas. The SeH^+ signals are less than 1% of the corresponding Se^+ signal when N_2 or O_2 reaction gases are used. Isotope ratio precision and accuracy were also assessed in order to determine how small the concentration of a ^{77}Se spike concentration could be accurately measured in the presence of naturally occurring Se in blood plasma and other human biological samples.

Introduction

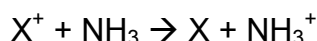
The essential trace mineral, selenium, is indispensable for the function of the human body (Cafferky¹). Selenium deficiency causes various adverse health effects (Bendahl²) and in recent years the element has gained increasing interest owing to its possible cancer protective effects (Cafferky¹).

Inductively coupled plasma mass spectrometry (ICP-MS) can accurately measure elemental chemical concentrations as low as 1 part per trillion if there is no other ion at the same mass/charge (m/z) ratio as the elemental ion of interest (Holler³). Furthermore, stable isotopes can be used as tracers and then small changes in isotope ratios can be measured by ICP-MS to follow the Se pathways through the body without requiring high tracer concentrations (Larsen¹).

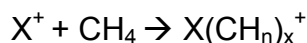
Unfortunately, there are molecular ions with the same m/z as each of the Se isotopes. These include $^{36}\text{Ar}^{38}\text{Ar}^+$, $^{38}\text{Ar}^{38}\text{Ar}^+$, $^{38}\text{Ar}_2\text{H}^+$, $^{38}\text{Ar}^{40}\text{Ar}^+$, $^{40}\text{Ar}^{40}\text{Ar}^+$ and $^{40}\text{Ar}_2\text{H}_2^+$ at the same m/z as $^{74}\text{Se}^+$, $^{76}\text{Se}^+$, $^{77}\text{Se}^+$, $^{78}\text{Se}^+$, $^{80}\text{Se}^+$ and $^{82}\text{Se}^+$, respectively. In addition, $^{40}\text{Ar}^{37}\text{Cl}^+$ and $^{76}\text{SeH}^+$ occur at the same m/z as $^{77}\text{Se}^+$ and $^{77}\text{SeH}^+$ occurs at the same m/z as $^{78}\text{Se}^+$.

The purpose of the conducted research is to find a reaction gas to produce ion-molecule reactions that remove ArAr^+ while providing sufficient Se sensitivity without formation of SeH^+ . Methane was used as a comparison to previously reported data and then nitrogen and oxygen were explored as alternatives

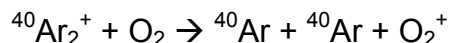
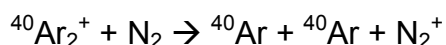
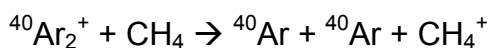
Ion molecule reactions have proven to provide a means to reduce spectral overlaps in Inductively Coupled Mass Spectrometry (Tanner⁵). Ion molecule reactions allow the analysis of ions with overlaps by either reacting with the overlap itself and removing the overlap from its interfering mass or by reacting with the analyte of interest and moving it to another mass where it can be analyzed. Removal of overlaps via charge transfer occurs in the manner:



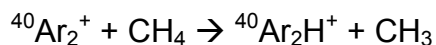
In this case, X is the overlap ion and CH₄ is the reaction gas. Another way of removing spectral overlaps is to move the overlap ion to a different mass. This occurs in this manner:



In the reaction of methane, nitrogen, and oxygen the removal of the background overlaps occurs by the following charge transfer reactions: (Larsen⁶, Anicich⁷, Tanner⁵)



In addition to this, it is believed that to a small degree, the reaction gas methane also reacts with the background overlaps in the form of a hydrogen proton transfer (Larsen⁶)



High resolution ICP instruments are an alternative way to analyze isotopes with overlapping spectra; however, the resolving power necessary to separate many isotopes from their overlaps is still not powerful enough to resolve between $^{80}\text{Se}^+$ and the argon dimer at $^{40}\text{Ar}_2^+$ (Simpson⁸).

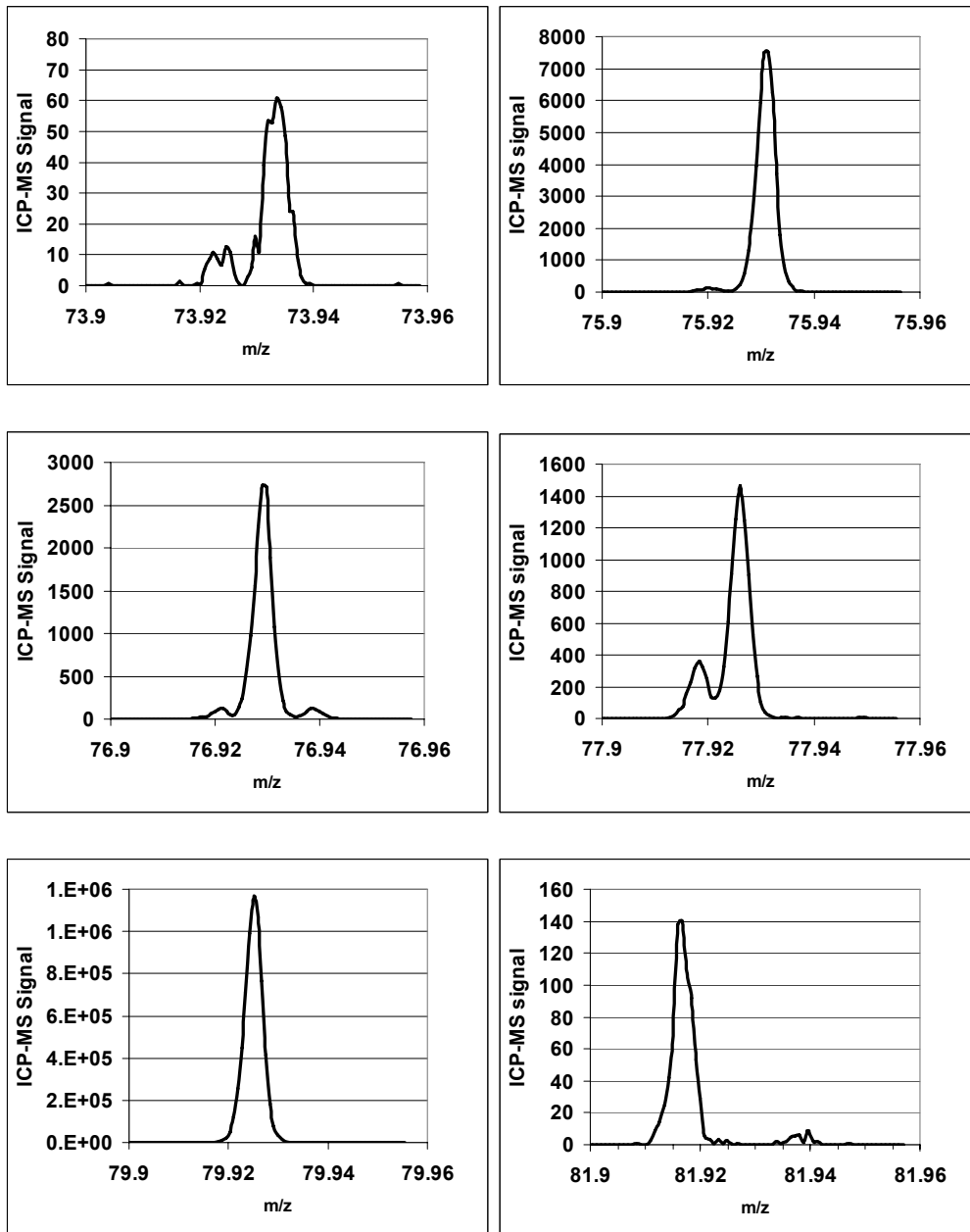


Figure 1. Shows high spectra of selenium isotopes from a high resolution ICP-MS.

Se ⁺ Isotope	1 ppm selenium standard	Background	Background Equivalent Concentration (ppb)
74	2500	60	25
76	28000	7484	267
77	23475	2700	115
78	73492	1560	21
80	1169696	1166976	1000
82	27616	140	5

Table 1. Shows the background equivalent concentration of the ArAr⁺ signals in ICP-SF-MS

The above data detail the analysis of a 5 ppb Solution of selenium with a high resolution mass spectrometer. The data were obtained from a Thermo Finnigan Element 2 Inductively Coupled Plasma Sector Field Mass Spectrometer (ICP-SF-MS). For the isotopes at m/z 74, 76, 77, 78 there are observed to be two peaks. The first is from the 5 ppb selenium standard while the second is from the ArAr⁺ background signal. From the above data, it shows that the ICP-SF-MS is able to resolve Selenium isotopes at m/z 74, 76, 77, and 78 from their corresponding ArAr⁺ overlaps at a resolving power of 11,000. The peak of ⁷⁸Se⁺ appears at a m/z of 77.910 while the peak of ⁴⁰Ar³⁸Ar⁺ is at 77. Here, the instrument is able to resolve down to a m/z difference of 0.005. However, from m/z 82, the ArAr⁺ overlap lies directly over the selenium signal and cannot be resolved. For m/z 80, the ArAr⁺ signal is very large (greater than 1 million

counts) and also covers the selenium signal. For $^{80}\text{Se}^+$, the peak appears at 79.905 and the peak of $^{40}\text{Ar}^{40}\text{Ar}^+$ is at 79.915. Here, the ArAr^+ signal is too large to be resolved from the $^{80}\text{Se}^+$ signal. Thus, ICP-SF-MS is suitable for analyzing $^{74}\text{Se}^+$, $^{76}\text{Se}^+$, $^{77}\text{Se}^+$, and $^{78}\text{Se}^+$; but is unable to measure $^{80}\text{Se}^+$ and $^{82}\text{Se}^+$

It has been determined in past research that the complications of the argon dimer on selenium analysis can be overcome by the use of the reactive gas methane and the use of a dynamic reaction cell (Larsen, et al^{4,6}). However, this method is plagued by the formation of hydrides with all of the isotopes of selenium. The formation of SeH^+ complicates the analysis of all isotopes of selenium, especially the isotopes at m/z 77 and 78 where SeH^+ signal occurs at the same m/z. It was determined experimentally that SeH^+ formed at $9.6 \pm 0.5\%$ (Larsen⁶).

A dynamic reaction cell (DRC) with the reaction gases: methane, Nitrogen, and Oxygen, was used to effectively remove the overlaps that interfered with the isotope of selenium.

Isotope	$^{74}\text{Se}^+$	$^{76}\text{Se}^+$	$^{77}\text{Se}^+$	$^{78}\text{Se}^+$	$^{80}\text{Se}^+$	$^{82}\text{Se}^+$
Background Overlap	$^{36}\text{Ar}^{38}\text{Ar}^+$, $^{37}\text{Cl}_2^+$	$^{38}\text{Ar}_2^+$	$^{38}\text{Ar}_2\text{H}^+$, $^{40}\text{Ar}^{37}\text{Cl}^+$	$^{40}\text{Ar}^{38}\text{Ar}^+$	$^{40}\text{Ar}_2^+$	$^{40}\text{Ar}_2\text{H}_2^+$
SeH+ Overlap			$^{76}\text{SeH}^+$	$^{77}\text{SeH}^+$		

Table 2. A listing of all selenium isotopes and their spectral overlaps. [Burton⁹]

The above table lists the isotopes of selenium with the accompanying background overlaps. In addition to ArAr^+ background overlaps the above table lists potential chloride overlaps as well as selenium hydride overlaps. The

naturally occurring background equivalent concentrations of the ArAr^+ species are listed above in table 1. The chloride overlaps do not occur naturally; however, do occur in the presence of chloride analytes. This may occur in practical situations due to the fact that many elemental standards are stabilized in acidic conditions (HCl or HNO_3). Therefore, in the presence of HCl , these chloride interferences may occur. As stated before, SeH^+ occurs at approximately 9.6% of the corresponding selenium signal. Therefore, the background equivalent SeH^+ signal depends on the concentration of the selenium standard that is being analyzed via ICP-MS.

Experimental Setup

For all experiments, a Perkin Elmer SCIEX ELAN 6100^{plus} ICP-DRC-MS (Concord, Ontario, Canada) was used. Liquid samples were introduced into a quartz concentric nebulizer (TQ-30-A3, Meinhard) which was then connected to a quartz cyclonic spray chamber (WE025221, Perkin Elmer SCIEX). The sample flow rate to the nebulizer was maintained and controlled by a peristaltic pump (Rabbitt Plus, Rainin, Woburn, MA).

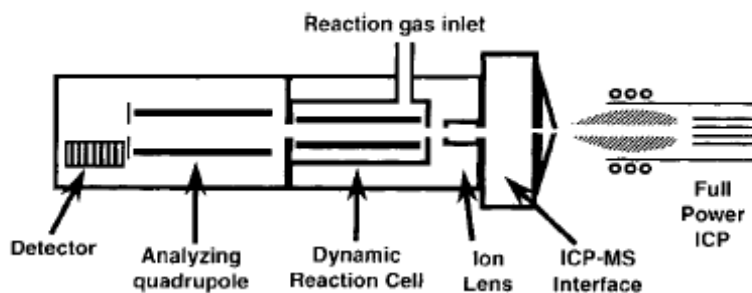


Figure 2. Diagram of the ICP-DRC-MS (Larsen⁴)

The above figure depicts a diagram of the ICP-DRC-MS. The instrument is identical to typical ICP-MS instruments except for the dynamic reaction cell that is housed behind the ion lens and in front of the analyzing quadrupole.

<u>ICP Settings</u>	
Nebulizer Gas Flow	(optimized daily) Typically 0.8-1.10
Auxiliary Gas Flow	1.20 l/min
ICP RF Power	1200 W
Plasma Gas Flow Rate	15 l/min
Quadrupole Rod Offset	0
<u>DRC Settings</u>	
Cell Rod Offset	-14.0 V
Cell Path Voltage	-15.0 V
Rpa	0.00
RF Voltage	200 V
Rpq	0.55 typically
Axial Field Voltage	340 V

Table 3. Instrument parameters.

The above voltages list settings that controlled the analyzing quadrupole and the DRC quadrupole. The RPq is a rejection parameter that was typically set to 0.55. Each day the instrument was used, daily optimizations took place. The lens voltage, nebulizer gas flow rate, and x-y alignment of ICP were optimized for maximum sensitivity with a daily performance solution that contained 1 part per billion (ppb) of magnesium, cerium, indium, barium, and uranium. The instrument was also optimized for a maximum of 3% cerium oxide ion to cerium

ion signals. Following December 12, 2005, an external mass flow controller (Brooks Instrument Division, Model 5805 C. Hatfield, PA, USA) was utilized to control the nebulizer gas flow. Prior to this, the nebulizer gas flow was controlled and maintained by an internal mass flow controller which was set within the ELAN 6100 computer software.

Gas cylinders were attached to the mass spectrometer (methane, 99.997% pure; nitrogen, 99.987% pure; oxygen, 99.999% pure (Praxair, Danbury, CT). Both nitrogen and methane were connected through the instrument's channel A, which contained a getter that prevents water and oxygen from reaching the reaction cell. Oxygen was run through the instrument's channel B, which lacked a getter. Gas flow rates were controlled by mass flow controllers that are part of the instrument.

Sample Preparation

All samples were prepared in a Microzone, class 10 laminar flow hood (model V4Pv99C365). All samples were diluted to desired concentrations with deionized water (18 mΩ) from a Millipore Milli-RO Plus water purification system connected to a Milli-Q water deionizer. Sample solutions were made by diluting Peak Performance(CPI International, Santa Rosa, California) single element 1000 ppm standards. Samples that contained acids were all acidified using double distilled ultra-pure acids.

Results and Discussion

Removal of Overlaps

Spectral overlaps are very common with Selenium and all of its isotopes (see Table 1) due to the fact that the ICP-MS plasma is argon, which occurs at masses of 36, 38, and 40. With argon occurring at these masses, the argon dimers ($^{40}\text{Ar}_2^+$, $^{38}\text{Ar}^{40}\text{Ar}^+$, etc) also occur at the same masses as the isotopes of selenium (74, 76, 77, 78, 80, and 82).

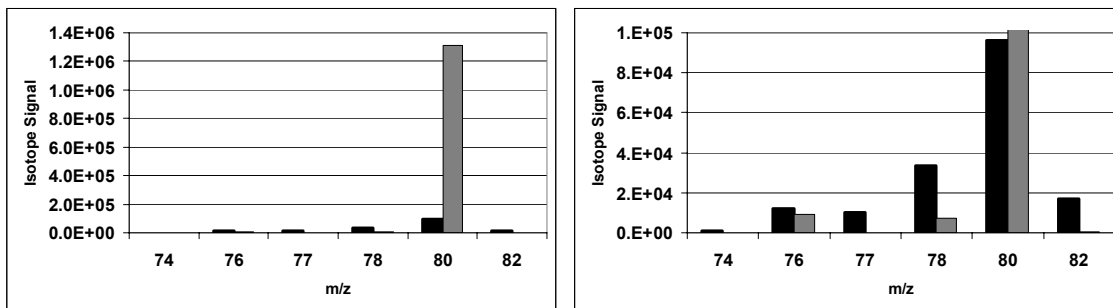


Figure 3. Background equivalent concentrations of argon dimer. The gray bars represent ArAr^+ signal while the black bars show signal from a 100 ppb selenium standard.

Se ⁺ Isotope	100 ppb Se	Background	Background Equivalent Concentration (ppb)
74	1136	47	4
76	11948	9263	74
77	10435	91	1
78	33485	7255	21
80	96121	1314244	1299
82	16883	598	3

Table 4. Similar to table 1, Shows the background equivalent concentration of the ArAr^+ signals in ICP-QMS.

The figures above show the unreacted argon dimer signals compared to the signal of a 100 ppb selenium standard in the presence of Nitrogen gas at a flow of 1.0 ml/min. The first plot is scaled so that the full $^{40}\text{Ar}_2^+$ can be observed, where the second plot on the right is scaled down to view the other argon-containing background signals that occur at the same m/z as the remaining selenium isotopes. The accompanying table also lists the background equivalent concentrations of the argon dimer.

From this data it is clearly evident that the most abundant isotope of Selenium, mass 80, is impossible to analyze in a conventional ICP-MS due to $^{40}\text{Ar}_2^+$ even at concentrations as high as 1 part per million (ppm). In an ICP-MS coupled with a dynamic reaction cell, the overlaps that inhibit the analysis of selenium can be dramatically reduced.

At this point, it is important to note that due to the nature of certain selenium experiments, simply measuring the signal are difficult even with the aid of a dynamic reaction cell. An example of this can be seen in selenium tracer experiments. In these experiments, 1 ppb or less of a selenium isotope is introduced into the human system, which has a typical concentration of 160 ppb selenium in blood plasma. Unfortunately the instrument does not have the required precision and is unable to differentiate between a 160 ppb sample and a 161 ppb sample. To overcome this small problem, the isotope signals are simply calculated as a ratio. Due to the fact that ^{77}Se is commonly chosen as the selenium spike, it is also common to ratio the selenium isotopes to ^{77}Se .

Methane

As stated before, methane is proven to be effective in removing the overlaps of argon dimer (Larsen^{4,6}).

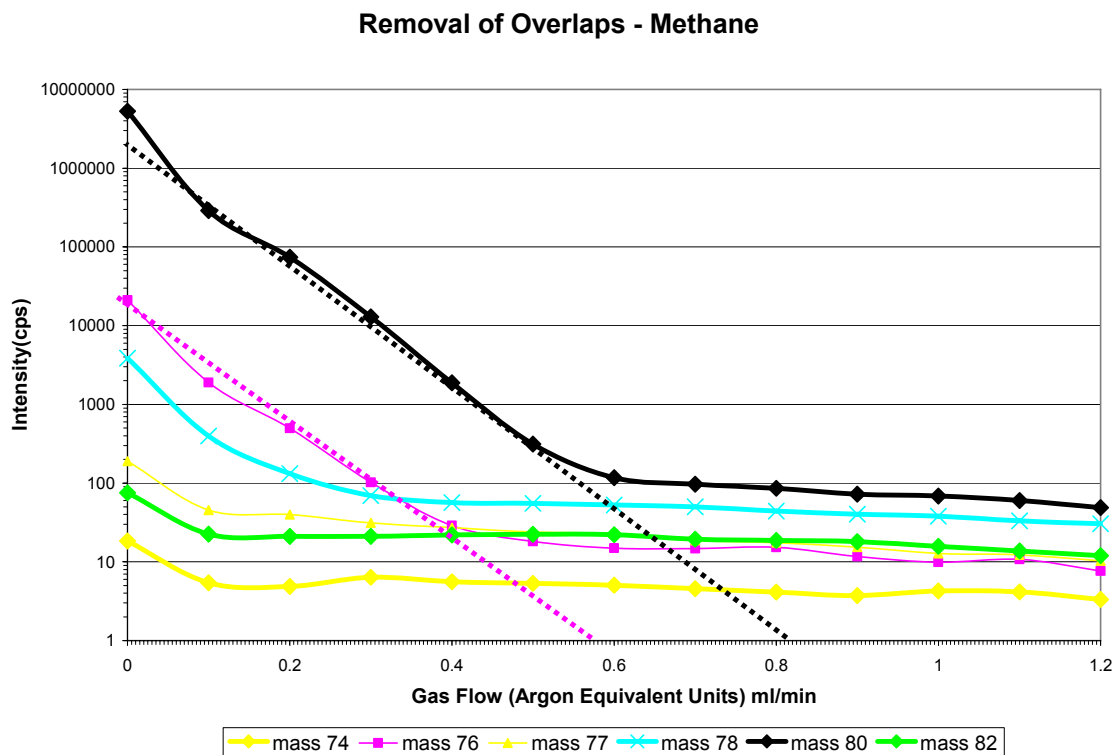


Figure 4. Illustrates the reaction of ArAr^+ with methane.

Figure 1 illustrates the reaction of the argon dimers at m/z 74, 76, 77, 78, 80, and 82. The data were compiled using an automated program within the instrument software called a cell gas optimization, where the software is set to scan for signals first in the vented mode (no reaction gas) and then by increasing the gas flow of methane to 1 ml/min. For all of the cell gas optimizations, the gas flow was increased in increments of 0.1 ml/min from 0 ml/min (no gas) to 1 ml/min.

From the predominant dimer, $^{40}\text{Ar}_2^+$, located at mass 80, an approximate relative reaction rate of $7.53 \text{ decades/ml}\cdot\text{min}^{-1}$ was calculated. This reaction rate is considered to be only relative, based on the fact that it is calculated as a function of signal loss and gas flow of methane instead of pressure. The relative reaction rate was calculated through the observation of the slope of the loss of signal. The points used were at 0.1, 0.2, 0.3, 0.4, and 0.5 ml/min Ar equivalent flow rate for the signal that was obtained from mass 80. These points were used because they were the closest to a linear relationship. Figure 1 also contains extrapolated lines with slope equal to the estimated relative rate of reaction. For masses 80 and 82, the relative rates of reactions seem to be equal upon comparison of the extrapolated lines, as expected. Unfortunately, the signals at the other m/z units are too low to produce a good enough slope to compare to the signal behavior at m/z 80 and m/z 82. However, due to the fact that both 80 and 82 behave similarly, it is believed that all of the isotopes react at the same rate. The fluctuation of signal at less than 10 counts per second is attributed to the fact that the overlaps have been removed and the signal is due to background counts.

An important factor of the reactions between argon dimer and the reaction gas methane is that while the background overlaps are being reacted away, the

selenium does not react with the gas and is unaffected.

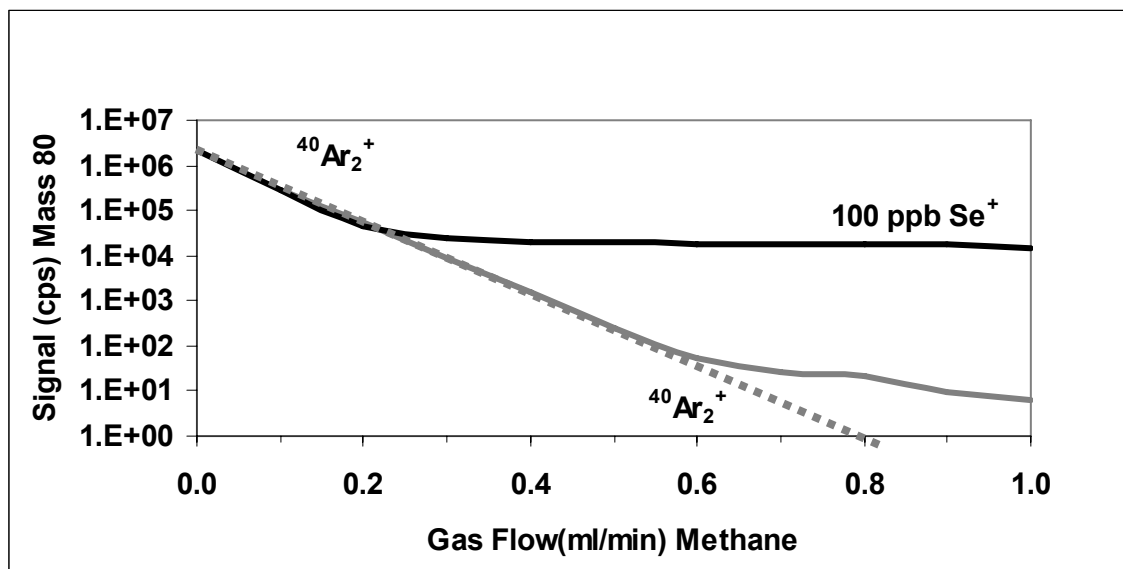


Figure 5. Shows the removal of Argon Dimer and Stable Se signal. The Solid line shows the signal of a 100 ppb Se⁺ standard at mass 80 where the faded line is a blank of deionized water used to observe the mass 80 (⁴⁰Ar₂⁺) background overlap. The dotted line is an extrapolation of the continued reaction after background level signal has been achieved. The change in slope in the blank sample after a flow rate of 0.6 is most likely due to a trace amount of selenium in the sample.

In this experiment, a 100 part per billion (ppb) Se standard was used in addition to a blank of de-ionized water. With no methane (vented mode) both solutions produce signals that are dominated by Ar₂⁺. The water blank was used to obtain data involving the Argon dimer signal at mass 80 while the 100 ppb Se standard was used to collect data for ⁸⁰Se signal. With methane, the estimated detection limit was calculated to be 34 part per trillion (ppt).

The mass spectra in the figures below show the pattern in signal intensities at m/z 74, 76, 77, 78, 80, and 82 with an overlay of the natural abundance of the

isotopes at a methane gas flow of 1.0 ml/min. Figure 7 was obtained from recent data while figure 8 was obtained by Larsen in previous research. Each show how the isotope ratios deviate from the natural abundances when methane is used as a reaction gas.

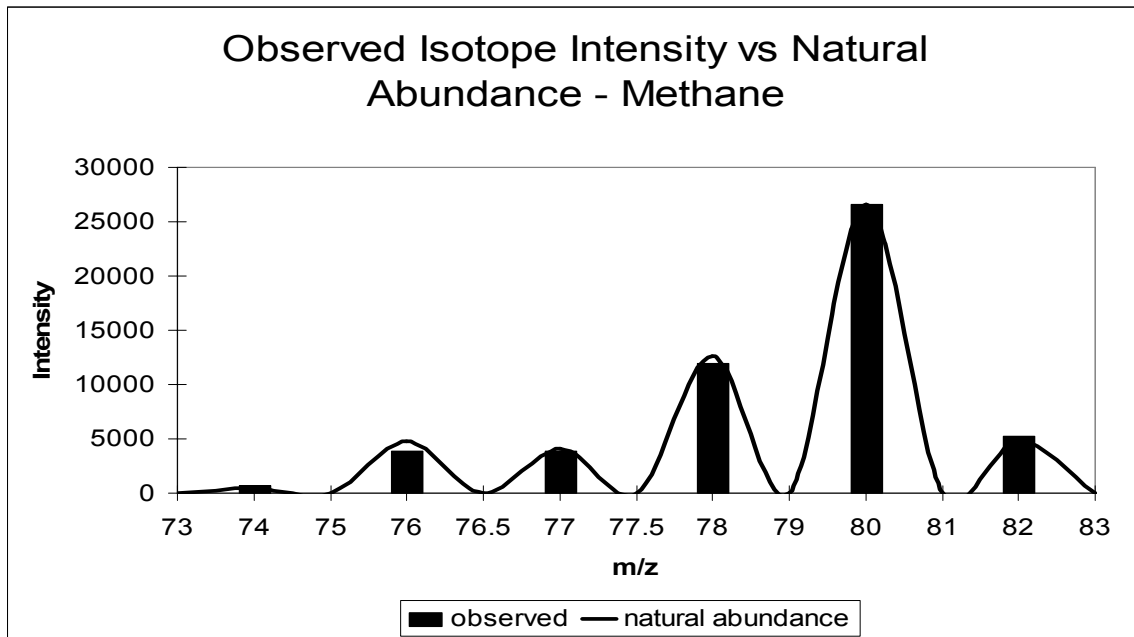


Figure 6. A comparison of obtained Selenium isotope ratios

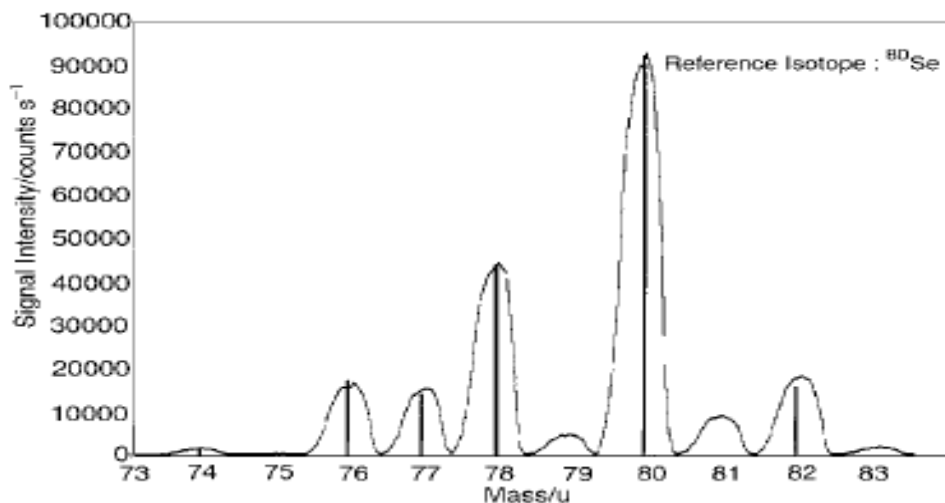


Figure 7. Past data from Larsen's study (Larsen⁶)

The isotope ratios in the above plots were normalized at m/z 80 from a 100 ppb selenium solution at 1.0 ml/min (argon equivalent) methane. The natural abundance was calculated by using the signal at mass 80 as a baseline and then calculating the other isotopes from the known abundance ratios. These plots show how well isotopic measurements can be obtained but cannot fully describe the improvement over quadrupole mass spectrometry (ICP-QMS) without a dynamic reaction/collision cell. Table 3 illustrates the acquired isotope ratio measurements of a 100 ppb selenium standard without the use of a reaction gas and also with the use of methane at a flow of 1.0 ml/min. The uncertainties of the measurements are reported as RSD% and the theoretical RSD % was calculated based on the counting statistic limits. The counting statistics limit was first calculated by dividing the square root of the total signal counts by the signal counts:

$$C.S. = (\text{Total Counts})^{1/2}/(\text{Total Counts})$$

Here is an example of how this was calculated. In this study, the dwell time for each m/z unit was 500 milliseconds (ms). The instrument was set for 50 sweeps and a single reading. Therefore, the total integration time was 25,000 milliseconds or simply 25 seconds. To calculate the total counts, the signal for each isotope in counts per second is multiplied by the total integration time. For example, $^{80}\text{Se}^+$ may be shown to have a signal of 5000 cps at a methane gas flow of 1.0 ml/min. 5000 is then multiplied by 25 seconds.

Following this, the individual counting statistic values for each isotope were used in the following equation upon calculating the isotope ratio:

$$\text{RSD}\% = [(\text{CS}_x)^2 + (\text{CS}_{77})^2]^{1/2}$$

CS_x represents the counting statistics of the isotopes at m/z 74, 76, 78, 80, and 82 and CS_{77} represents the counting statistics at m/z 77.

Se Isotope Ratio	74/77	76/77	78/77	80/77	82/77
Natural Abundance	0.118	1.18	3.11	6.54	1.21
No Reaction Gas	0.110 +/- 8.25%	6.36 +/- 123%	2.63 +/- 97.0%	1510 +/- 127%	0.600 +/- 79.8%
Methane	0.093 +/- 0.54%	1.07 +/- 0.11%	3.07 +/- 0.16%	6.59 +/- 0.11%	1.42 +/- 0.12%
Theoretical RSD %	0.32%	0.13%	0.11%	0.10%	0.12%

Table 6. Isotope ratios without Reactive gas and with methane. Uncertainties are reported as

RSD %

The RSD % that is reported in the above table was calculated with the following formula:

$$s_{\Gamma} = s / \bar{x}$$

Where s marks the standard deviation of six separate replicate measurements and x bar represents the average. The obtained value was then multiplied by 100 to become a percentage.

Clearly, there is a drastic improvement in both accuracy and precision with the use of methane as a reaction gas. For example, the RSD % found for ratio of 80/77 is reduced from 127% to only 0.11%. The isotope ratio of this value (1510) is much greater than the natural abundance (6.54) due to the presence of $^{40}\text{Ar}_2^+$ at the m/z 80. Not only are the isotope calculations more precise, they are also closer to the natural abundances. In addition to this, the RSD of the measurements with methane is in close agreement to the theoretical RSD.

Product Ions

Although methane reacts with the argon dimer, it was also discovered that methane reacts with the selenium isotopes (to far less an extent) to create several types of product ions.

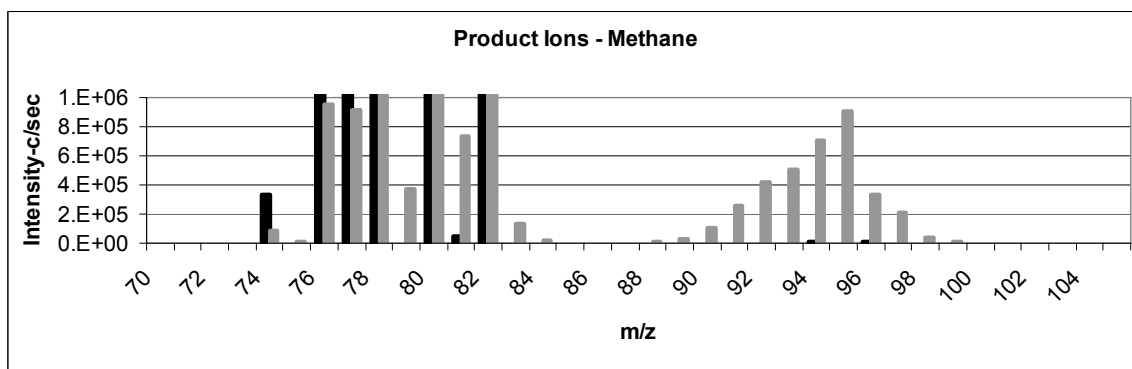


Figure 8a. Displays the product ion formation that results from methane

The above plot displays the product ions that are formed from the reaction of methane gas with selenium isotopes (m/z 90-100). The plot above has been scaled down in order to observe the product ions. The signal for ArAr⁺ is significant and exceeds 1 x 10⁷ counts and is not shown to its full extent. Below, the same plot is given in log signal in order to compare all of the ArAr⁺ signal to the resulting product ions. The black bars represent the signal from a 10 ppm solution of selenium in the vented mode with no methane present while the gray bars represent the m/z signals when methane is introduced at 1.0 ml/min. Note that the vented selenium isotope signal is much greater than what is shown on this plot, where it has been scaled to clearly see the product ion pattern.

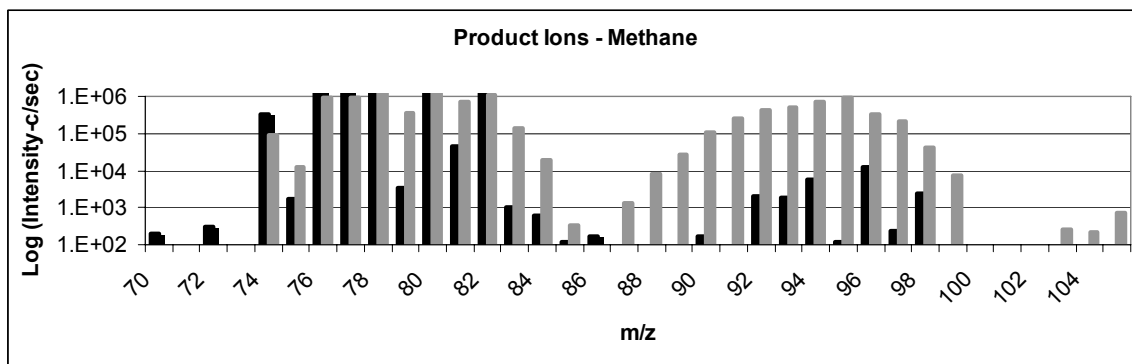
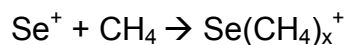
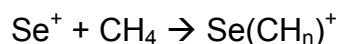


Figure 8b. The same plot as 8a, only in a log scale.

Figure 8a and 8b show the spectra resulting from the reaction between the reaction gas, methane, and the isotopes of selenium. The signal at masses 90, 92, 93, 94, 96, and 98 as well as 106, 108, 109, 110, 112, and 114 provide evidence that methane forms cluster products with selenium



In addition to this, the existing signal at masses 87, 88, 89, 91, 95, and 97 show that condensation products are also forming:



Due to the formation of product ions from the reaction of selenium and methane, the signal that results from unreacted selenium analyte ions is reduced by 30% when the methane gas flow is set to 1.0 mL/min

Selenium Hydride Formation

Another complication that has arisen with the use of Methane as a reaction gas is the formation of selenium hydrides with the isotopes of selenium and possibly hydrides of the argon dimer. In a reported study, Larsen determined that SeH^+ forms at approximately $9.6 \pm 0.5\%$ with methane set to 1.0 ml/min (Larsen⁶). The following table details the data that has been collected regarding the formation of SeH^+ in the presence of methane gas at a flow rate of 1.0 ml/min.

	$^{78}\text{SeH}^+$	$^{80}\text{SeH}^+$	$^{82}\text{SeH}^+$
replicate 1	6.99%	6.69%	5.93%
replicate 2	7.22%	6.83%	5.95%
replicate 3	7.26%	6.85%	6.06%
replicate 4	7.39%	6.84%	6.04%
replicate 5	7.55%	6.84%	6.29%
average	7.28%	6.81%	6.05%
Uncertainty	$\pm 0.029\%$	$\pm 0.010\%$	$\pm 0.024\%$

Table 7. SeH^+ formation with uncertainties.

The formation of $^{78}\text{SeH}^+$, $^{80}\text{SeH}^+$, and $^{82}\text{SeH}^+$ were calculated from the ratio of m/z 79/78, 81/80, and 83/82. The uncertainty that is reported above was

calculated as a percent relative standard deviation (RSD %). From this data, it can be estimated that SeH^+ occurs at a background equivalent concentration of 7 ppb at a methane gas flow of 1.0 mL/min . The estimated calculation for m/z 75/74 may be higher due to possible formation of Ar_2H^+ and was therefore omitted. One theory on the formation of SeH^+ is that it is possibly formed from impurities found in the reaction gas, methane. From previous studies, Larsen determined that SeH^+ does not form through a reaction between Se^+ and CH_4 . In this study, Larsen compared data that was obtained by using deuterated methane (CD_4). The analysis showed no formation of hydrides at two m/z units higher than selenium isotopes.

Nitrogen as Reaction Gas

In addition to methane, nitrogen was also used as a reaction gas in the DRC in an attempt to remove the argon-containing interfering overlaps similar to methane and without the complications of product ions or SeH^+ formation.

Removal of Overlaps

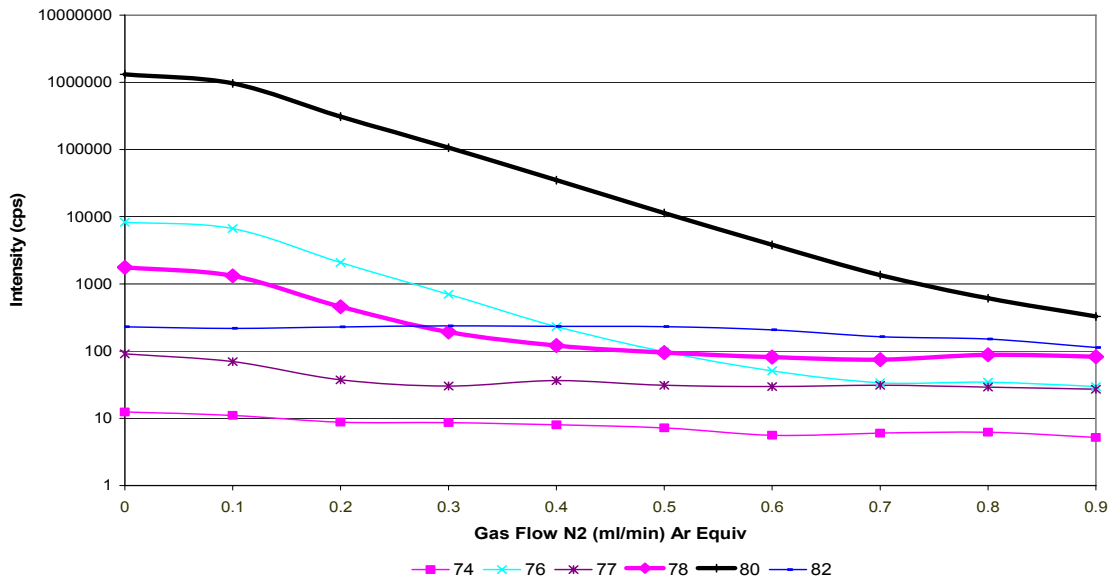


Figure 9. Details the removal of ArAr^+ interfering overlaps with varied gas flow of Nitrogen

Figure 4 details the reaction of nitrogen with the argon dimer ions at m/z of 74, 76, 77, 78, 80, and 82. A straight line was fit to the log of the data for masses 76 and 80. The approximate reaction rate for Nitrogen with Argon dimer has been calculated to be $5.07 (\pm 0.30)$ decades/ml*min. The obtained relative reaction rate for both 76 and 80 are similar and lead to the conclusion that the relative reaction rate is the same for all ArAr^+ isotopes, as would be expected. As seen from figure 4, the data at all points does not follow a straight line, the slope changes. This is due to the fact that the instrument is initially set to a q of 0.25 when it is in the vented mode (gas flow is 0 ml/min) and then when the gas increases, the q value is changed to 0.55. For this reason, select gas flows were used in the calculation. The calculations were made with three different q values, 0.25, 0.55, and 0.75

**Reaction Rate (decade/mL*min⁻¹) By
Mass and q value**

Mass	q=0.25	q=0.55	q=0.75
76	-5.48	-4.77	-4.81
80	-5.37	-4.77	-5.07

Table 8. Reaction rates with Nitrogen

Although the relative reaction rate for N₂ (5.07 decades/ml*min⁻¹ on average) with ArAr⁺ was determined to be slower than the reaction between methane and ArAr⁺ (7.53 decades/ml*min⁻¹), nitrogen allows for the accurate determination of isotope ratios and provides isotope ratios with nearly identical precision. The following table shows the obtained isotope ratios with nitrogen in comparison to methane and the natural abundance. The uncertainties (RSD%) for both methane and nitrogen are also given.

Se Isotope Ratio	74/77	76/77	78/77	80/77	82/77
Natural Abundance	0.118	1.18	3.11	6.54	1.21
Methane	0.093 +/- 0.54%	1.07 +/- 0.09%	3.07 +/- 0.16%	6.59 +/- 0.1%	1.42 +/- 0.2%
Nitrogen	0.100 +/- 0.3%	1.17 +/- 0.09%	3.26 +/- 0.18%	7.29 +/- 0.1%	1.38 +/- 0.3%

Table 9. Isotope ratios and uncertainties obtained with methane and nitrogen

In addition to this, it was determined that nitrogen, unlike methane, forms no major product ions.

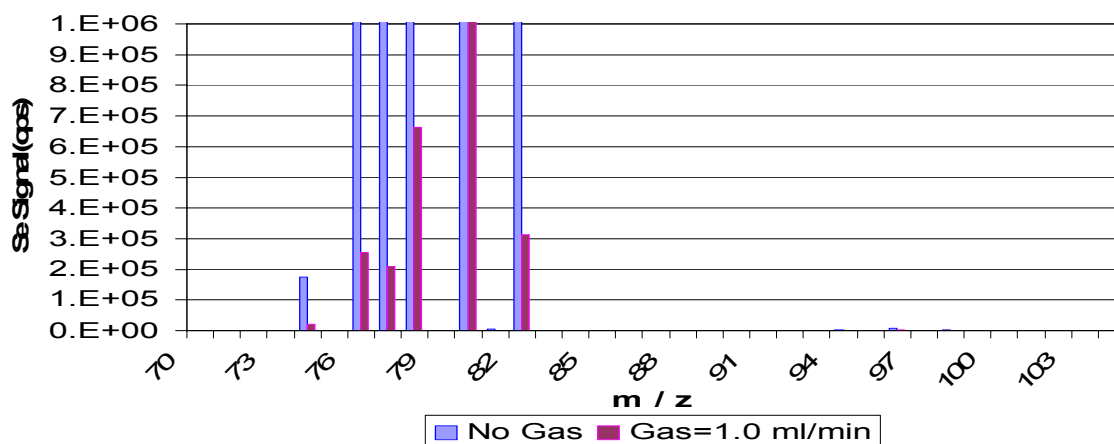


Figure 10. Shows the lack of product ion formation when N₂ is used in the reaction cell.

In addition to this, Nitrogen differs from methane in the fact that SeH⁺ formation is not a problem. Although SeH⁺ was found to form with Methane at 7%, data from nitrogen shows that SeH⁺ is created at less than 0.1 % at a gas flow rate of 0.5 mL/min or greater.

Gas Flow(ml/min)	0.0	0.1	0.15	0.2	0.25	0.5	0.75	1.0
⁷⁴ SeH %	0.22	0.58	0.43	0.34	0.28	0.08	0.10	0.07
⁷⁸ SeH %	0.01	0.05	0.07	0.07	0.06	0.03	0.03	0.02
⁸⁰ SeH %	0.02	0.02	0.01	0.01	0.01	0.02	0.01	0.02
⁸² SeH %	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02

Table 10. Percent formation of SeH⁺ as a function of gas flow rate

As shown in the above table, the signal one mass unit higher than each Se isotope is usually less than 1% of the Se⁺ isotope signal when the N₂ flow rate is 0.5 Ar equiv mL/min or higher. It is important to note here that the increased level of ⁷⁴SeH⁺ formation is possibly due to an arsenic contamination.

Oxygen as a Reaction Gas

Oxygen has been shown in previous research to be able to reduce many background overlaps that complicate the analysis of trace metal elements in ICP-MS (Olesik¹⁰) and has therefore been explored as a means of reducing ArAr⁺ background overlaps.

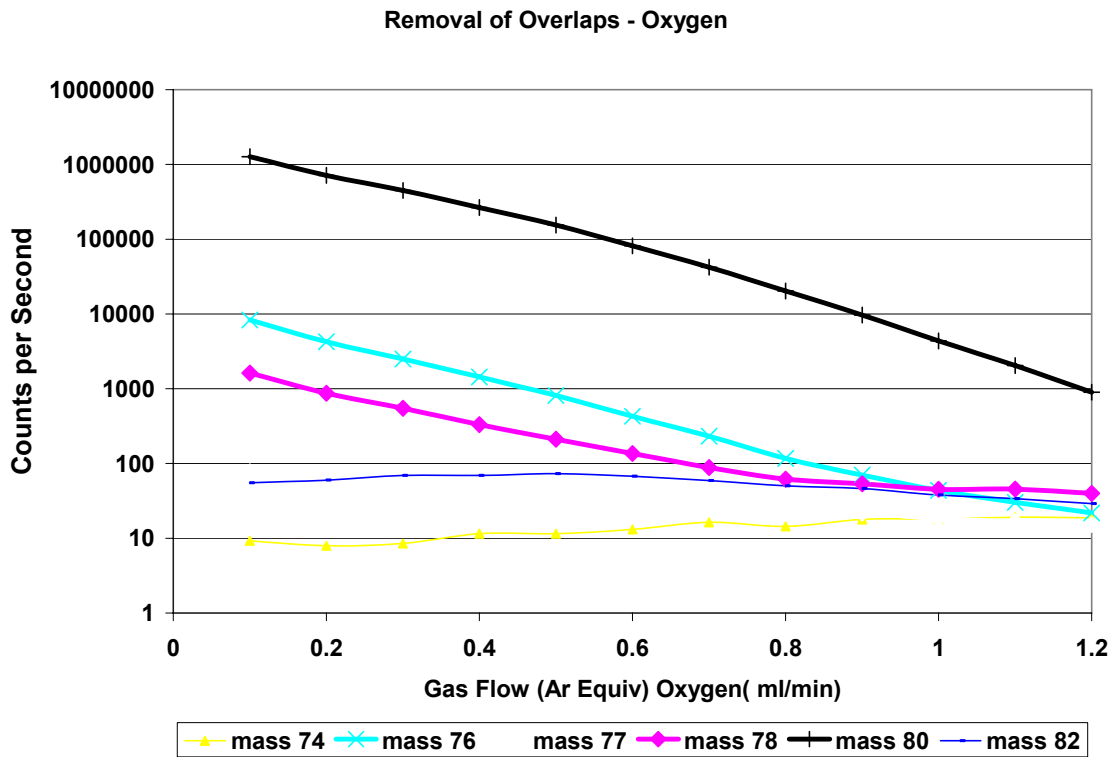


Figure 11. Shows the removal of Argon overlap ions with the use of Oxygen as a reaction gas. A blank solution of deionized water was used to observe the overlapping ions at masses 74, 76, 78, 80, and 82. Oxygen gas was then introduced into the cell using a computer generated cell gas optimization ranging from 0 ml/min to 1.2 ml/min (in argon equivalent units).

The reaction rate of oxygen with argon dimer was approximated in an identical method to what was described earlier. For oxygen, the data were

collected at the same q value, 0.55, and were averaged between the calculated reaction rates for three masses: 76, 78, and 80, assuming that all the masses react at the same rate. The relative reaction rate for oxygen was calculated to be 2.62 (+/- 0.23)decades/ml*min. Of the three reaction gases, oxygen reacts much slower with ArAr⁺ than both nitrogen (5.07 decades/ml*min) and methane (7.53 decades/ml*min).

Gas Flows	Mass 76	Log Data	Gas Flows	Mass 78	Log Data	Gas Flows	Mass 80	Log Data
0.1	8336.19	3.92	0.0	2827.00	3.45	0.1	1272548.70	6.10
0.2	4265.64	3.63	0.1	1624.49	3.21	0.2	714919.94	5.85
0.3	2493.14	3.40	0.2	873.59	2.94	0.3	449857.66	5.65
0.4	1437.07	3.16	0.3	548.29	2.74	0.4	265740.82	5.42
0.5	813.70	2.91				0.5	154214.55	5.19
0.6	429.37	2.63				0.6	81484.21	4.91
0.7	233.00	2.37				0.7	42349.88	4.63
0.8	117.52	2.07				0.8	20286.51	4.31
						0.9	9700.77	3.99
						1.0	4358.79	3.64
						1.1	2045.75	3.31
						1.2	898.55	2.95
	<u>Obtained Rate</u>	-2.60 decades ml*min ⁻¹		<u>Obtained Rate</u>	-2.41 decades ml*min ⁻¹		<u>Obtained Rate</u>	-2.86

<u>Average Reaction Rate</u>		<u>St Dev</u>
-2.62 decades ml*min ⁻¹		0.23

Table 11. Data used to calculate the reaction rate for Oxygen.

Although oxygen reacts the slowest the of reaction gases, it is similar to Nitrogen in the fact that SeH⁺ formation is not a problem.

Gas Flow (mL/min)	⁷⁸ SeH ⁺	⁸⁰ SeH ⁺	⁸² SeH ⁺
0	0.76%	0.17%	0.89%
0.1	0.17%	0.10%	0.95%
0.2	0.19%	0.10%	0.98%
0.3	0.15%	0.10%	0.80%
0.4	0.13%	0.11%	0.54%
0.5	0.09%	0.07%	0.28%
0.6	0.08%	0.04%	0.34%
0.7	0.05%	0.03%	0.16%
0.8	0.05%	0.03%	0.11%
0.9	0.05%	0.00%	0.09%
1	0.03%	0.01%	0.04%

Table 12. Percent formation of SeH⁺ as a function of N₂ flow rate.

The data in the above table were collected in a method identical to the determination of SeH⁺ formation in nitrogen that was explained earlier. Like nitrogen, the formation of SeH⁺ in oxygen is less than one percent and is therefore negligible. However, this also proposes another question. The above data shows the formation of SeH⁺ to vary with each isotope. This suggests that the reaction responsible for the formation of SeH⁺ may be dependent upon the isotope in question. This hypothesis cannot be determined at this time and will require future analysis to find the correct solution. In addition to this, the above data shows that the formation of SeH⁺ decreases with the increase of oxygen gas flow. Once again, this cannot be explained at this time and requires additional research.

Calculation of Isotope Ratios

As stated earlier, in this study, it is very important to achieve accurate isotope ratios for selenium. Data were collected to study how the isotope ratios

compared between methane, nitrogen, and oxygen. For each gas, intensities were collected for the six isotopes of selenium. Next, the isotope intensities 74, 76, 78, 80, and 82 were calculated as a ratio to the isotope intensity of mass 77. The isotope ratios were normalized to m/z 77 due to the fact that this particular isotope is used in the spiking of samples in human nutrition experiments and acts as a tracer. The values that were obtained were compared to known values of the selenium isotope ratios that were obtained by calculating the ratios of the natural abundances of the isotopes 74, 76, 78, 80, and 82 to the natural abundance of 77.

Se Isotope Ratio	74/77	76/77	78/77	80/77	82/77
Natural Abundance	0.118	1.18	3.11	6.54	1.21
No Reaction Gas	0.110 +/- 8.25%	6.36 +/- 123%	2.63 +/- 97.0%	1510 +/- 127%	0.60 +/- 79.8%
Methane	0.093 +/- 0.54%	1.07 +/- 0.09%	3.07 +/- 0.16%	6.59 +/- 0.1%	1.42 +/- 0.2%
Oxygen	0.099 +/- 0.51%	1.16 +/- 0.34%	3.27 +/- 0.15%	7.43 +/- 0.4%	1.41 +/- 0.4%
Nitrogen	0.100 +/- 0.3%	1.17 +/- 0.09%	3.26 +/- 0.18%	7.29 +/- 0.1%	1.38 +/- 0.3%

Table 13. Shows a comparison of obtained isotope ratios for methane, nitrogen, and oxygen along with uncertainties (RSD %).

The ion signals from six selenium isotopes were measured. Isotope ratios were calculated for ion signals from $^{74}\text{Se}^+$, $^{76}\text{Se}^+$, $^{78}\text{Se}^+$, $^{80}\text{Se}^+$, and $^{82}\text{Se}^+$ to $^{77}\text{Se}^+$ with reaction gas flow rates of 1.0 ml/min (argon equivalent units). The row that lists data as “no reaction gas” shows data that was collected without the use of methane, nitrogen, or oxygen and is similar to the results that would be obtained in a conventional quadrupole ICP-MS without a reaction cell. The data shows that the obtained isotope ratios in this setting deviate significantly from the natural abundances. In addition to this, with uncertainties greater than 100%, the observed precision is atrocious. It is clearly evident that the utilization of a reaction gas provides a dramatic improvement in accuracy and precision of the isotope ratio measurements (< 1% uncertainty).

The data above shows that each gas obtains similar data and is not sufficient by itself to determine whether a particular gas yields more accurate sets of isotope ratio data. However, this data does offer something useful. From this, it appears that the isotope ratios follow a trend where the lower masses are consistently lower than the known values and the higher masses are consistently greater than the known values. This occurrence can be explained by a common problem that is found in mass spectrometry instrumentation.

Mass Bias

Mass bias is characteristic of mass spectrometry and especially ICP-MS. Mass bias is simply an instrumental mass discrimination where it is preferable for

heavier mass ions to pass through the mass spectrometer (Anrén¹¹). The generally accepted theory for this phenomenon can be explained in part by space-charge theory where columbic forces cause the displacement of lighter positively charged ions.³

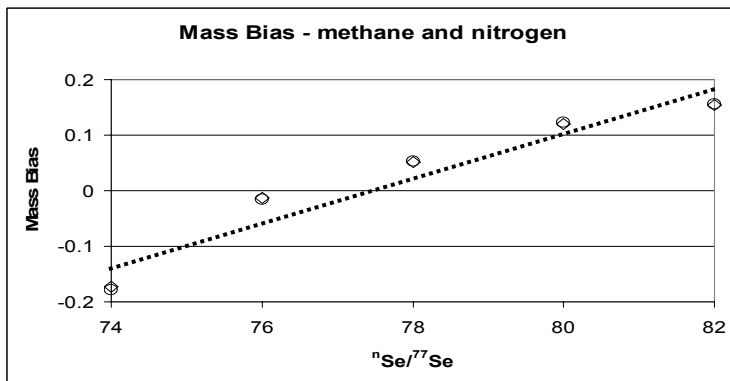
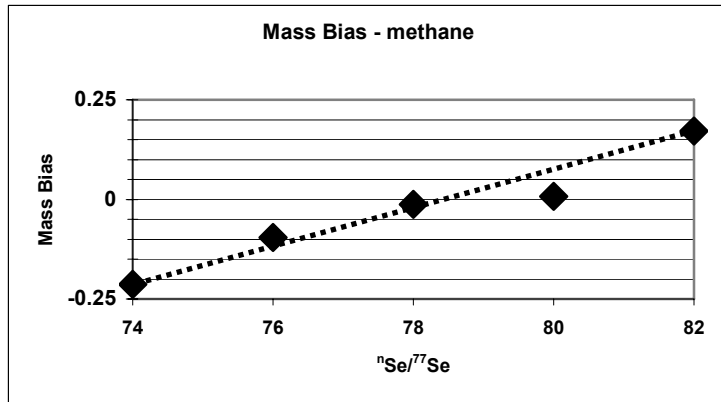


Figure 13 (a) and (b)

The above figures show the calculated mass bias of methane, nitrogen, and oxygen. The artificial mass bias was calculated by the formula:

$$\text{Mass Bias} = \frac{(^n\text{Se}_{\text{observed}} / ^{77}\text{Se}_{\text{signal}})}{(^n\text{Se}_{\text{natural}} / ^{77}\text{Se}_{\text{natural}})} - 1$$

The behavior of the Se⁺ isotope ratios in the presence of methane follows a linear pattern with the exception of mass 80, which is lower than what is expected. As of this moment, there is no clear explanation for this phenomena. Nitrogen and Oxygen are very close in behavior but behave less like a linear pattern when compared to Methane due to the outlying points at masses 74 and 82. From the data collected here, it is shown that the instrument displays a mass bias of 3% for each mass unit and is relatively the same for each of the reaction gases. This error is easily corrected for upon normalizing to a standard.

Isotope Ratio	74/77	76/77	78/77	80/77	82/77
Natural	0.118	1.18	3.11	6.54	1.21
Obtained	0.100	1.17	3.26	7.29	1.38
% Deviation	15.3%	0.8%	4.8%	11.5%	14.0%
Corrected	0.110	1.19	3.16	6.60	1.18
% Deviation	6.8%	0.8%	1.6%	0.9%	2.5%

Table 14. Shows the isotope ratios before and after the mass bias correction.

The above table shows the difference in the isotope ratios before and after the correction is applied. Before the correction is applied, there is a 15.3%, 0.8%, 4.8%, 11.5%, and 14.0% error when the obtained isotope ratios are compared to the natural abundance for the isotope ratios of m/z: 75/74, 79/78, 81/80, and 83/82. When the correction is applied, the error is reduced to 6.8%, 0.8%, 1.6%, 0.9%, and 2.5%. The figures below illustrate the difference in the observed selenium isotope signals once the mass bias correction is applied.

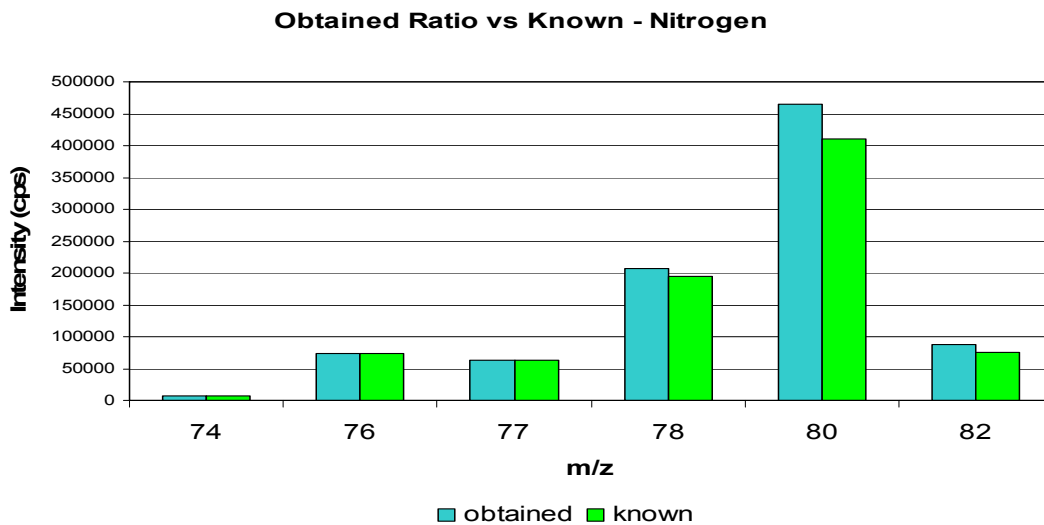


Figure 14. Shows the isotope signals prior to mass bias correction compared to the natural abundances

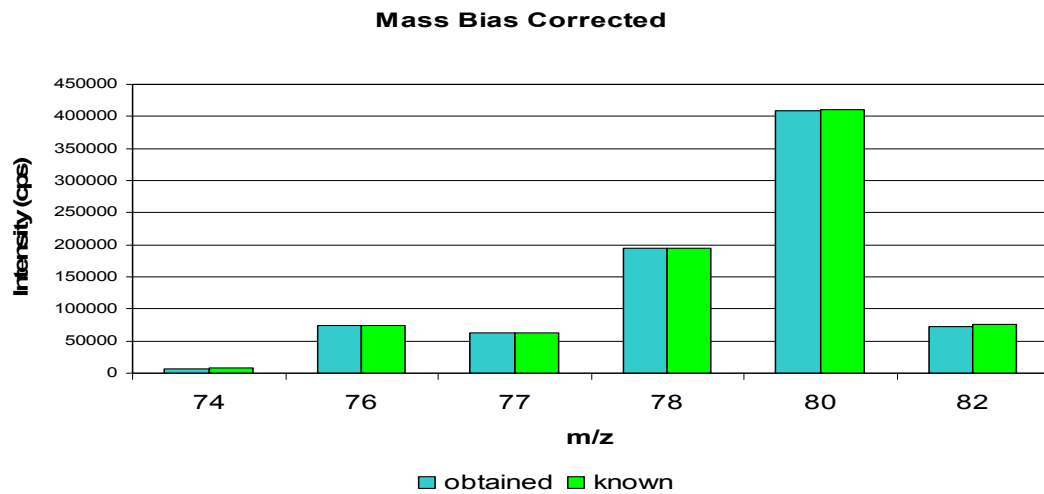


Figure 15. Shows isotope signals after the mass bias correction compared to the natural abundances

Precision of Measurements

After determining how accurate the isotope ratios can be with the different reaction gases, the next step was to determine how precise the measurements were. To accomplish this, the Relative Standard Deviation (RSD) of the measured isotope ratios was compared to the theoretical limits, or theoretical RSD. For this comparison, the theoretical RSD was calculated by determining the counting statistics limit, in this case, this is calculated by the ratio of the square root of the total counts divided by the total counts:

$$\text{C.S.L} = (\text{Total Counts})^{1/2}/(\text{Total Counts})$$

The counting statistics were calculated for each isotope selenium. From this, the theoretical RSD % was calculated by calculating the uncertainty of the individual counting statistics limit of each isotope that has been calculated as a ratio to ^{77}Se :

$$\text{Theoretical RSD \%} = [(\text{CSL}_x)^2 + (\text{CSL}_{77})^2]^{1/2}$$

Here, x designates all of the isotopes that are calculated as a ratio to $^{77}\text{Se}^+$ ($^{74}\text{Se}^+$, $^{76}\text{Se}^+$, $^{78}\text{Se}^+$, $^{80}\text{Se}^+$, $^{82}\text{Se}^+$).

	74/77	76/77	78/77	80/77	82/77
RSD (%) CH₄	0.77%	0.12%	0.23%	0.34%	0.33%
RSD (%) O₂	0.57%	0.36%	0.17%	0.43%	0.38%
RSD (%) N₂	0.30%	0.15%	0.14%	0.17%	0.16%
Theoretical RSD %	0.28%	0.11%	0.10%	0.09%	0.11%

Table 15. RSD of reaction gases compared to theoretical RSD

The three reaction gases, methane, oxygen, and nitrogen exhibit similar RSD %. In addition to this, the obtained RSD % is very close to the calculated theoretical RSD % and show very good precision. Compared to previous experimental data

of Larsen, the obtained RSD % are slightly lower than what was seen in the previous study of methane.

	74/77	76/77	78/77	80/77	82/77
RSD (%)	0.98	0.3	0.16	0.32	0.44
Theoretical RSD %	0.77	0.23	0.14	0.24	0.22

Table 16. RSD % and Theoretical RSD % obtained by Larsen with methane (Larsen⁶)

Conclusions And Future Work

From the data that was collected, it appears that Nitrogen is the best alternative as a reaction gas. Nitrogen succeeds in the removal of all argon containing molecular ion overlaps and also is not plagued by formation of SeH^+ . In addition to this, Nitrogen is more effective than Oxygen in the removal of argon containing overlaps.

Future work will involve using lower purity Methane as a reaction gas to determine if impurities in the reaction gas are responsible for the formation of SeH^+ .

As stated earlier, the formation of SeH^+ seems to be dependent upon the isotope in question. The reason for this is not clear, therefore this phenomena must be studied further.

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