Alleviation of Polyatomic Ion Interferences for Determination of Chlorine Isotope Ratios by Inductively Coupled Plasma Mass Spectrometry

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A simple variation in sample preparation and introduction allows the measurement of chlorine isotope ratios by inductively coupled plasma mass spectrometry (ICP/MS). Dissolution of the sample in D_2O rather than H_2O attenuates the major polyatomic ion $^{36}ArH^+$ and frees m/z 37 for determination of $^{37}Cl^+$. The isotope ratio $^{35}Cl/^{37}Cl$ in a 50 mg/L solution of Cl as LiCl is determined with a relative standard deviation of 0.21%. Sample memory is low, as the ^{35}Cl signal decays to less than 1% of its original value after ~2 min of cleanout with D_2O. The detection limit for Cl using this procedure is approximately 20 μg/L. (*J Am Soc Mass Spectrom* 1990, 1, 284–287)

Inductively coupled plasma mass spectrometry (ICP/MS) is a fast method for the determination of elemental concentrations and stable isotope ratios [1–6]. Determination of isotope ratios for the intracellular electrolytes magnesium, potassium, and chlorine is important for monitoring physiological function [7–10]. The determination of Mg is straightforward [11], but K requires major modification of plasma operating conditions [12] or the use of a completely different plasma [13] or flame [14]. Determination of Cl is difficult for two reasons. First, the degree of ionization of Cl is only ~0.1–1% in the plasma [15], so the sensitivity is lower than for other elements. Second, the major polyatomic ion $^{36}ArH^+$ overlaps with $^{37}Cl^+$. Chlorine can be detected as the negative ion Cl^- by ICP/MS, but the high background degrades detection limits [16, 17]. A helium-supported ICP was studied with ICP/MS for the detection of Cl in gaseous organic samples [18]. However, the introduction of water often destabilizes helium plasmas and causes poor or erratic performance. Recently, a helium microwave-induced plasma (MIP) coupled to a mass spectrometer demonstrated a Cl detection limit of 39 μg/L for aqueous samples, but an isotope ratio was not reported [19]. Bromine is more readily measured and has been proposed as a substitute for Cl in physiological experiments [20], but a direct determination of Cl is also desirable.

This paper describes a simple procedure that allows the measurement of Cl isotope ratios by using Cl^+. The sample is dissolved in 99.9% D_2O, so little nebulized water is injected into the plasma. Because most of the hydrogen comes from the injected water, the problematic polyatomic ion is now $^{36}ArD^+$ at m/z 38. The $^{36}ArH^+$ background decreases, which facilitates determination of ^{37}Cl.

Experimental

Instrumentation

The Sciex ELAN Model 250 (Perkin-Elmer, Thornhill, Ontario) was used. The cryopump and mass spectrometer electronics were cooled with an HX-150 recirculating water chiller (Neslab Instruments, Portsmouth, NH). The water temperature was set at 10°C. The nebulizer gas flow rate was regulated by a mass flow controller from Matheson Scientific (East Rutherford, NJ). Samples were introduced to the nebulizer at a rate of 0.5 mL/min by a Rainin minipuls 2 peristaltic pump (Rainin Instrument Co., Woburn, MA). The continuous flow ultrasonic nebulizer was operated at a forward power of 40–45 W [21, 22]. The wet aerosol was desolvated with a heating chamber (200 °C) and a condenser (0 °C) [22] and was transported to the plasma through tygon tubing (~1 m long).

Chemicals and Sample Preparation

The 99.9% D_2O was obtained from Cambridge Isotope Laboratories (Woburn, MA). Lithium chloride from Fisher Scientific (Fair Lawn, NJ) was used as the source of Cl. This compound was chosen because the light Li^+ ion should cause less suppression of Cl^+ signal than a heavier counterion like K^+ or Na^+ [23, 24]. High purity deionized water (resistance ~18 MΩ) was

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Table 1. Operating conditions

ICP torch	Ames Laboratory design [25]; outer tube extended 30 mm from inner tubes
Forward power	1.25 kW
Argon flow rates	
Outer gas	13 L/min
Auxiliary gas	0.2 L/min
Aerosol gas	1.59 L/min
Sampling position	17 mm from load coil, on center
Sampler	Nickel, 1.1 mm diam orifice
Skimmer	Nickel, 0.9 mm diam orifice
Ion lens settings	
Bessel box barrel	+ 5.4 V
Bessel box plate	− 11.0 V
Einzel 1 and 3	− 19.8 V
Einzel 2	− 130 V
Bessel box stop	− 4.9 V
Electron multiplier voltage	− 3200 V
Operating pressures	
Interface	1 torr
Quadrupole chamber	3×10^{-5} torr
Data acquisition	Low resolution setting; measurements per peak spaced 0.1 m/z unit about peak top
$^{35}Cl/^{37}Cl$ ratio and background measurements	Multielement monitoring mode, three measurements per peak, dwell time 1 ms at each position, total measurement time 1.0 s
Cleanout study	Multielement monitoring mode, three measurements per peak, dwell time 20 ms, total measurement time 0.5 s
Spectra	Sequential monitoring mode, 10 measurements per peak, total measurement time 1.0 s

obtained from a Barnstead Nanopure-II system (Barnstead Co., Newton, MA). A 50 mg/L chlorine solution was prepared in D_2O. The LiCl used was dried in an oven at 250°C and stored in a desiccator. An accurately weighed amount of LiCl was then dissolved directly in 50.0 mL of D_2O.

Instrumental Conditions

General instrumental and sampling conditions are given in Table 1. Isotope ratios were measured in a peak-hopping mode, and spectra were obtained by scanning, as described in Table 1. Before each experiment, the sample introduction system was kept as free from water as possible in the following ways. The spray chamber of the nebulizer and the desolvation system were dried with a heat gun. In addition, the waste U-tube leading from the spray chamber and the condenser was filled with D_2O. Operating conditions for the instrument were adjusted to maximize the signal from $^{36}Ar^+$ while nebulizing D_2O. This procedure was employed because nebulization of aqueous standard solutions during optimization of operating condi-

tions would have contaminated the sample introduction device with water.

Results and Discussion

Mass Spectra

The background mass spectrum obtained during nebulization of D_2O is shown in Figure 1. The major background ions in the given mass range are $^{16}O_2^+$, $^{36}Ar^+$ and both $^{38}Ar^+$ and $^{36}ArD^+$ at m/z 38. The injection of D_2O yields a peak at m/z 34 that is from DO_2^+.

The magnitude of the background at m/z 35 and m/z 37 is shown in Table 2. Most of the background at m/z 37 is probably from residual $^{36}ArH^+$. Hydrogen, water, or hydrocarbons in the argon and water impurity in the D_2O are the likely sources of hydrogen. Numerous polyatomic ions contribute to the background at m/z 35; these are probably $^{16}O^{17}OD^+$, $^{17}O^{17}OH^+$, $^{18}O^{16}OH^+$, and $^{17}O^{18}O^+$.

A spectrum obtained during nebulization of the LiCl sample (50 mg/L in D_2O) is shown in Figure 2. Both Cl isotopes (m/z 35 and 37) were clearly observed. The background peaks at m/z 36 and m/z 38 are suppressed by approximately 40% relative to the count rates seen from the D_2O blank solution. The DO_2^+ peak at m/z 34 is of about the same intensity in Figures 1 and 2.

Deionized water was then introduced into the ICP. The count rate at m/z 37 was monitored in the multielement mode until a steady value was reached. The nebulizer and desolvation system became saturated with water in approximately 30 min. The background mass spectrum from deionized water was then obtained (Figure 3). A substantial peak from $^{36}ArH^+$ is readily apparent at m/z 37. In addition, the DO_2^+ peak at m/z 34 has been replaced by HO_2^+ at m/z 33. The count rates given in Table 2 show that injection of D_2O rather than H_2O reduces the background at m/z 37 by almost a factor of 10. The background at m/z 35 is still modest when D_2O is nebulized; an increase of a factor of 2 is indicated. Thus, m/z 35 remains fairly clear.

Isotope Ratio Measurements

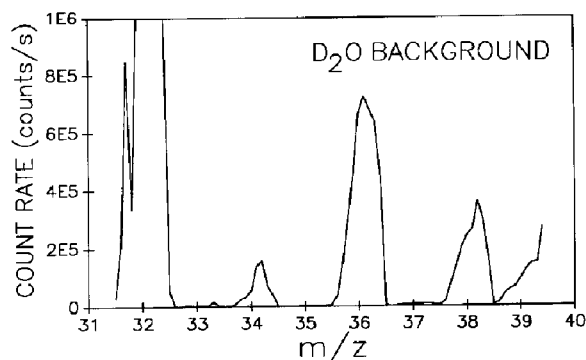Isotope ratio and sensitivity data for the 50 mg/L solution of Cl in D_2O are shown in Table 3. These data


Figure 1. Background spectrum of 99.9% D_2O.

Table 2. Background count rates

Blank solution	Background Count Rates			
	m/z 35		m/z 37	
	Mean		Mean	
	(counts/s)[a]	RSD (%)[b]	(counts/s)[a]	RSD (%)[b]
D_2O	3,900	2.6	5,900	2.8
H_2O	2,100	2.3	57,300	3.4

[a] Measurement time was 1.0 s.
[b] Relative standard deviation of 20 measurements.

were obtained by using a very short dwell time (1 ms) so more measurements could be taken in the 1.0-s measurement time. Rapid measurements may help average out fluctuations in the intensity of the ion beam [26, 27]. However, the acquisition of 20 ratio measurements takes approximately 12 min, partly because of the 4-ms computer overhead between hops. Nonetheless, if samples containing Cl are sufficiently concentrated (10–100 mg/L), then the Cl^+ signal is high enough for the remaining background to be unimportant.

In this case, the precision for the ratio $^{35}Cl/^{37}Cl$ was 0.21% RSD, which was comparable to that expected from counting statistics based on the total counts accumulated for each peak. Similar precision was obtained when the experiment was repeated on a different day. The close agreement between the experimental precision and that expected from counting statistics is unusual. For ion count rates in the range of 10^5–10^6 counts/s, the experimental precision for isotope ratios measured with this particular instrument (and others like it) is generally worse than the precision expected from counting statistics by a factor of 2–4 [5, 6, 10, 12, 27, 28, 30]. The fast peak hopping (dwell time 1 ms) employed in the present work may help improve precision, as indicated in other, preliminary measurements with silver and zinc [27]. The RSD in the isotope ratio (0.21%) was much better than the RSD of 1.8% for the count rates for either $^{35}Cl^+$ or $^{37}Cl^+$. These latter RSDs are typical of the short-term precision in the signal achievable by present ICP/MS devices at these count rates. They are well above the counting statistics values of 0.1–0.2% (not shown in Table 3).
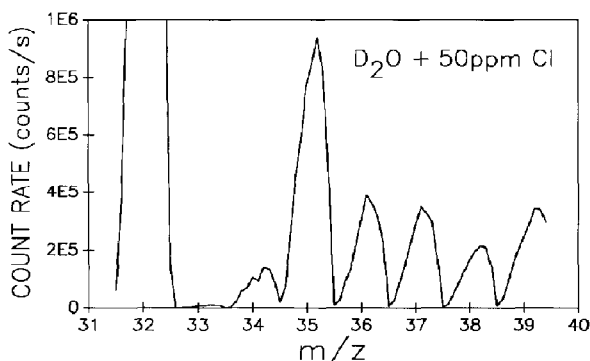
Figure 2. Spectrum of 50 mg/L Cl (as LiCl) in D_2O.

Table 3. Chlorine isotope ratio measurements

| ^{35}Cl count rate (counts/s) | | ^{37}Cl count rate (counts/s) | | Determined $^{35}Cl/^{37}Cl$ | | RSD (%) for $^{35}Cl/^{37}Cl$ |
Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)	counting stats
793,000	1.8	277,200	1.8	2.861	0.21	0.22

Mean and RSD of 20 measurements are reported. Measurement time was 1.0 s. Count rates are corrected for D_2O background. Accepted natural ratio = 3.127 [29].

The determined value for the $^{35}Cl/^{37}Cl$ ratio in Table 3 is about 9% below the accepted natural abundance ratio. Mass bias effects for other isotopes 2 m/z units apart can cause determined ratios to deviate from accepted values by 2–10% [2, 4, 30, 31]. The somewhat low value for the $^{35}Cl^+/^{37}Cl^+$ ratio may also have been caused by loss of gain in the detector during measurement of $^{35}Cl^+$, which was quite possible at count rates approaching 10^6 count/s [32, 33]. Plasma operating conditions and ion lens voltages are known to influence isotope ratio values [12], but no experiments with these parameters were performed in this study because of the limited amount of D_2O available.

The detection limit (i.e., the solution concentration necessary to yield a net signal equivalent to three times the standard deviation of the background) was 20 $\mu g/L$ for Cl using $^{35}Cl^+$. This value is comparable to other detection limits reported for Cl as Cl^- from an argon ICP [16, 17] or Cl^+ from a helium MIP [19]. Typically, we observe ion count rates of $\sim 10^6$ counts/(s·mg·L) for a metal ion of medium mass ($m/z \sim 23$–60) that would be expected to be nearly 100% ionized in the ICP. Comparison of this value to the $^{35}Cl^+$ count rate in Table 3 shows that Cl is of the order of 1% ionized, as expected [15].

Sample Cleanout

Figure 4 shows a cleanout curve for the 50-mg/L sample in D_2O. The original signal for $^{35}Cl^+$ ($\sim 800,000$ counts/s) decays to approximately 4500 counts/s after ~ 2 min. This level corresponds to about 0.5% of the original signal and is only several hundred counts per second above the D_2O background at m/z 35 in Table 2. Elements such as osmium, boron, and mercury that have forms that are volatile at the temperature of the heating chamber (200 °C) can suffer from long memory or loss with the desolvation system employed [22], but apparently this was not a problem for Cl in the present work.

This rapid cleanout of $^{35}Cl^+$ is necessary owing to the expense of D_2O, and two simple measures greatly reduce sample memory. A fresh length of tygon tubing was installed between the desolvator and the plasma, and the nebulizer spray chamber was soaked in concentrated H_2SO_4 for 24 h. The former provided an aerosol conduit free from long-term sample memory, whereas the latter facilitated smooth drainage of condensed droplets from the walls of the spray chamber.
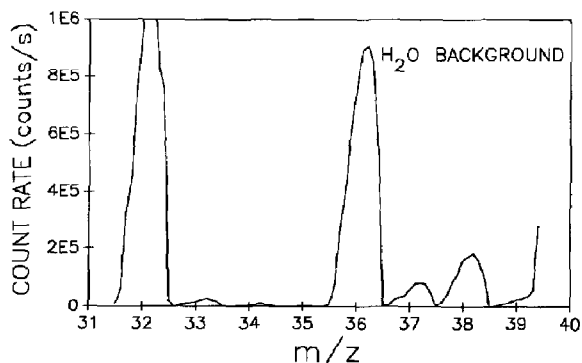
Figure 3. Spectrum of deionized H_2O.

Conclusions

Sample dissolution in D_2O can significantly reduce the $^{36}ArH^+$ interference and allow the measurement of the $^{35}Cl/^{37}Cl$ ratio. The precision of 0.2% RSD is at least as good as that obtained in ICP/MS for other elements and approaches the 0.1% level desirable for widespread application studies [10]. A substantial amount of Cl (0.1–1 mg) is required, but most biological samples contain high concentrations of this element. For example, the normal Cl content of human blood is ~100 mmol/L [34], so a sample volume of only 30 μL is necessary to provide 0.1 mg of Cl. As in most present isotope tracer studies with ICP/MS, sample cleanup would likely be desirable to remove the biological matrix (proteins, etc.) and interfering metal ions such as Na^+ and K^+ [23, 24]. A blood sample could be centrifuged and the resulting serum transferred to a cation-exchange column in the Li^+ form. The collected solution would be evaporated to dryness and reconstituted in D_2O. Such refinements in sample preparation should lead to a useful protocol for fast measurement of Cl isotope ratios by ICP/MS.

Acknowledgment
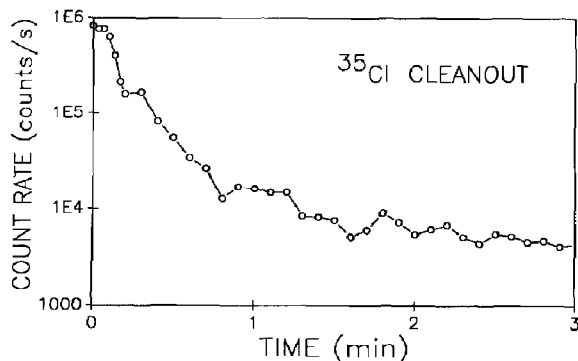
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Figure 4. Cleanout curve for $^{35}Cl^+$.