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Speciation of Chromium(VI) and Chromium(III) Using Pneumatically Assisted Electrospray Mass Spectrometry

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Mass spectra can be obtained from aqueous solutions containing both Cr^{3+} and $Cr_2O_7^{2-}$ as negative ions under the same spray conditions. An excess of HCl is used so that Cr^{3+} sprays as an anionic chloro complex. Moderately energetic collision conditions produce CrO_3^- from Cr^{VI} and $CrOCl_2^-$ from Cr^{III}. Detection limits are 100 and 60 ppb for Cr^{III} and Cr^{VI}, respectively. Reasonable calibration curves are provided by plotting the ratio of the analyte signal to that for $^{37}Cl^-$.

Keywords: *Chromium determination; speciation; electrospray; ion spray; mass spectrometry*

Speciation information is needed as the toxicity and biological role of a particular element can vary greatly depending on the chemical form. At low levels, Cr^{III} is essential for living organisms. It is an essential nutrient and is believed to help activate insulin.[1] On the other hand, Cr^{VI} can cross cell membranes and cause skin lesions, lung cancer and other forms of cancer.[2] Chromium has many industrial applications such as dyeing, tanning and use in the steel industry. Accurate determination of each species rather than just the total chromium level is important in determining toxicity.

In the late 1960s, Dole and co-workers[3,4] described the formation of gas-phase ions when a liquid was sprayed out of a capillary held at high voltage. Since that time, the efforts of Fenn and co-workers[5–8] have led to the development of electrospray mass spectrometry (ES-MS). Pneumatically assisted electrospray, also referred to as ion spray, was first reported by Bruins *et al.*[9] Ion spray can be considered as a concentric pneumatic nebulizer combined with electrospray. These two terms are often used interchangeably. Since the development of electrospray and ion spray, many applications have been discovered. Electrospray has found widespread use for the determination of the relative molecular mass of large biological molecules.[10–12] This technique has also been used for the determination of many inorganic species, from ion clusters to bare metal ions.[13–21]

Using electrospray mass spectrometry, it is easy to distinguish Cr^{3+} from $Cr_2O_7^{2-}$ using separate spray conditions.[21] A positive voltage is applied to the electrospray needle to spray Cr^{3+}, while a negative voltage produces anions from $Cr_2O_7^{2-}$. This procedure requires separate runs to observe both cations and anions. The present work describes a possible way to distinguish Cr^{III} and Cr^{VI} with the same spray conditions. The cation Cr^{3+} is sprayed as a negative chloro complex. Horlick and co-workers refer to this as the 'intermediate' or 'counter ion' mode because collisions during the ion extraction process occur at moderate kinetic energies such that anions remain associated with the metal cation.[20] This concept could become a general procedure for distinguishing cations and oxoanions of the same element under the same spray conditions.

EXPERIMENTAL

Standard solutions were prepared by diluting aliquots from 1000 ppm aqueous standards (ICP emission standards from Aldrich Chemicals) with a 50% methanol–50% water solvent. The methanol–water solution was prepared using HPLC grade methanol (Fisher Scientific) and water de-ionized to a resistivity of 18 MΩ cm(with a Barnsted Nanopure-II system (Newton, MA, USA). ULTREX II ultra pure grade HCl (J. T. Baker) was used to bring the final concentration (v/v) of solutions up to 1% HCl. Approximately 500 µl of solution were drawn into a 1 ml syringe (Hamilton). Solutions were transported from syringe to ion source through a 100 µm id fused silica capillary (Polymicro Technologies) using a syringe pump (74900 Series, Cole-Parmer Instruments).

A Perkin-Elmer SCIEX API 1 mass spectrometer was used (Fig. 1). Typical conditions used for the instrument are summarized in Table 1. Voltages were optimized on a daily basis to maximize the signal for the species of interest. The 'best' voltages varied slightly (±5 V) from day to day; typical values are listed in Table 1. Peak hopping data were collected using a 100 ms dwell time. Spectral scans were collected by adding ten consecutive scans together using a 10 ms dwell time.

RESULTS AND DISCUSSION

Selection of Collision Conditions

The polarity of the voltage applied to the electrospray needle determines whether positive or negative ions are observed. The collision conditions then determine the extent of fragmentation observed. In this experiment, the difference between the orifice plate voltage (V_{OR}, see Fig. 1) and the voltage on the RF-only rods (V_{RF}) has the largest effect on the chromium species

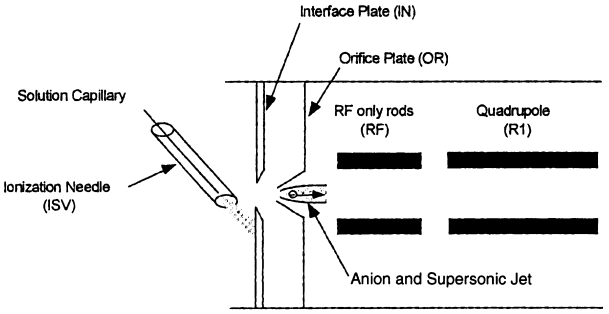

Fig. 1 Schematic diagram of electrospray mass spectrometer including ion source, interface assembly and some ion optics. Representation is not drawn to scale.

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Table 1 Typical operating conditions

Sample flow rate	17 μl min^{-1}
Nebulizer gas	Nitrogen
Nebulizer gas pressure	280 kPa
Curtain gas	Nitrogen, ultra pure carrier grade
Curtain gas back pressure	550 kPa
Curtain gas temperature	60 °C
Ionization needle voltage (V_{ISV})	−3200 V
Interface plate voltage (V_{IN})	−500 V
Orifice plate voltage (V_{OR})	−125 V
RF only quadrupole voltage (V_{RF})	−60 V
Mass analyzer quadrupole voltage (V_{R1})	−59 V
CEM detector voltage	+2500 V
Operating pressure of quadrupole chamber	5.333×10^{-3} Pa

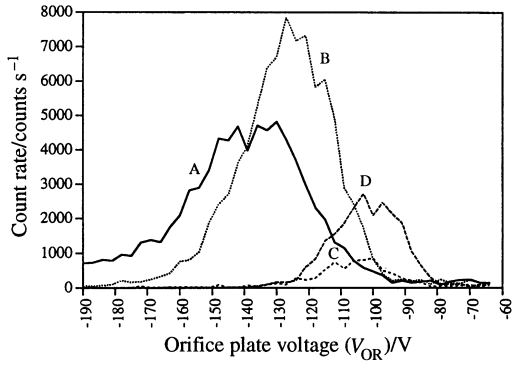

Fig. 2 A plot of signals from the major Cr species, A $^{52}CrO_2{}^{35}Cl^-$, $m/z=119$; B $^{52}CrO^{35}Cl_2{}^-$, $m/z=138$; C $^{52}CrO^{35}Cl_2{}^- \cdot H_2O$, $m/z=156$; D $^{52}CrO^{35}Cl_2{}^- \cdot 2H_2O$, $m/z=174$, observed from a 50 ppm Cr^{III} solution while varying the voltage on the orifice plate.

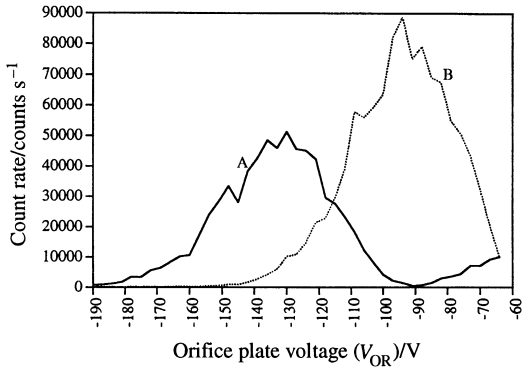

Fig. 3 A plot of signals from the major Cr species, A $^{52}CrO_3{}^-$, $m/z=100$; B $^{52}CrO_3{}^{35}Cl^-$, $m/z=135$, observed from a 10 ppm Cr^{VI} solution while varying the voltage on the orifice plate.

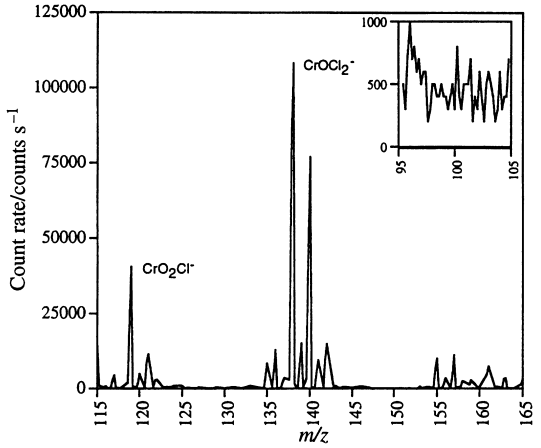

Fig. 4 Mass spectrum of a 50 ppm Cr^{III} solution. The inset is the region from $m/z=95$ to 105.

detected. Ions from the spray flow through the 200 μm diameter orifice in the orifice plate, along with nitrogen from the curtain gas. As the ions are accelerated through the potential difference ($V_{OR}-V_{RF}$), they collide with nitrogen molecules in the supersonic jet that forms behind the orifice plate. The larger the voltage difference ($V_{OR}-V_{RF}$), the more energetic the collision conditions, which produce more extensive fragmentation. This voltage difference is essentially the collision energy for collision-induced dissociation in the laboratory frame of reference.

In the present work, the voltage difference ($V_{OR}-V_{RF}$) was varied by changing V_{OR} only. The ion kinetic energy inside the mass filter is determined by the difference ($V_{RF}-V_{R1}$); this difference is kept constant so the resolution and peak shapes do not change greatly as V_{OR} is altered.

Fig. 2, a plot of signal *versus* V_{OR}, was generated from a 50 ppm Cr^{III} solution. The plots shown in Fig. 2 are for various chromium oxochloro species identified in the caption. The ions selected contain ^{52}Cr and ^{35}Cl and are the most abundant ones from the various isotope patterns. The main ions under soft conditions ($V_{OR} \approx 90$ V, $V_{OR}-V_{RF} \approx 30$ V) still have chromium as Cr^{III} and intact oxygen ligands. As can be seen in Fig. 2, using a V_{OR} value of −125 V (*i.e.*, $V_{OR}-V_{RF} \approx$ 65 V) yields a maximum signal for $CrOCl_2{}^-$ ($m/z=138$). Using −125 V on V_{OR} minimizes the abundance of $(CrOCl_2 \cdot nH_2O)^-$ ions.

The onset voltage for observation of the hydrated ions is $V_{OR} \approx -80$ V. The onset voltages for the analyte species CrO_2Cl^- and $CrOCl_2{}^-$ are approximately 15 V more negative. This 15 V difference represents the additional kinetic energy necessary to create CrO_2Cl^- and $CrOCl_2{}^-$ from whatever anion is formed originally. Presumably, this precursor ion is $(CrCl_4 \cdot 2H_2O)^-$, or something similar, but such ions could not be observed even under the 'softest' extraction conditions.

Fig. 3 shows a plot of signal *versus* orifice voltage (V_{OR}), from a 10 ppm Cr^{VI} solution. At a $V_{OR}-V_{RF}$ of between 10 and 50 V, the chromium oxochloro species CrO_3Cl^- ($m/z=135$) is observed. At slightly more harsh collision conditions, the ion $CrO_3{}^-$ ($m/z=100$) is observed. Using conditions that are favorable for detecting $CrOCl_2{}^-$ ($m/z=138$) from the Cr^{III} solution (−125 V on V_{OR}, $V_{OR}-V_{RF} \approx 65$ V), both $CrO_3{}^-$ and CrO_3Cl^- are observed from Cr^{VI}, with $CrO_3{}^-$ being the dominant species.

Thus, a voltage difference of 65 V between V_{OR} and V_{RF} produces stable chromium oxo or oxochloro species from both Cr^{III} and Cr^{VI} solutions. These conditions were used to obtain all the following results unless otherwise indicated. It should be noted in Fig. 3 that the signal at $m/z=100$, between −60 V and −90 V, is not due to Cr. Scans under these conditions reveal no Cr isotope pattern in this region. Scans do reveal species present at both $m/z=98$ and $m/z=100$, in a 3 to 1 intensity ratio. This would seem to indicate that this species contains chlorine and is probably due to a background ion.

Mass Spectra

Fig. 4 shows a mass spectrum of a 50 ppm Cr^{III} solution obtained under the optimum conditions ($V_{OR}-V_{RF}=65$ V) described above. The most abundant chromium oxochloro species are observed at $m/z=138$, 140 and 142. This set of peaks is attributed to $CrOCl_2{}^-$ and appears in the appropriate isotope ratios, 9 : 6 : 1, for an ion with two chlorine atoms. Less intense peaks can also be observed at $m/z=119$ and 121; the

3 : 1 isotope pattern shows they have one chlorine atom. These peaks are likely to be CrO_2Cl^-. The region at $m/z = 100$, shown in the inset of Fig. 4, does not contain ions from Cr^{3+}.

A mass spectrum of Cr^{VI} at 10 ppm is shown in Fig. 5. Chromium species can be observed in the $m/z = 100$ and $m/z = 135$ regions. The intensities of the peaks around $m/z = 100$ agree very well with the known isotope ratio of chromium. The dominant peak at $m/z = 100$ is assigned to $^{52}CrO_3^-$. The set of peaks at $m/z = 135$ and 137 are probably CrO_3Cl^-. The peak observed at $m/z = 113$ in Fig. 5 and another fragment peak at $m/z = 69$ (not shown) are due to contamination of the instrument with trifluoroacetate (TFA^-). The TFA contamination can be seen throughout this study and is a result of other experiments on electrospray of organic acids. Cleaning procedures have as yet been unable to remove the TFA completely from the instrument. TFA does not interfere with the determination of chromium but does appear in these scans. However, this observation does illustrate one possible problem: organic ions that remain intact can cause memory or spectral interferences.

During this work, when an aqueous Cr^{VI} solution was allowed to stand, a small amount of Cr^{VI} was reduced to Cr^{III}. The characteristic peaks for Cr^{III} can then be seen from the Cr^{VI} solution. This is a common problem with inorganic speciation. When species are sampled, care should be taken to avoid changing the species present in the initial sample. Sampling and sample preparation work can alter the speciation information and lead to inaccurate measurements.

Fig. 6 shows a mass spectrum of a mixture containing 50 ppm Cr^{III} and 10 ppm Cr^{VI}. The dominant peaks observed

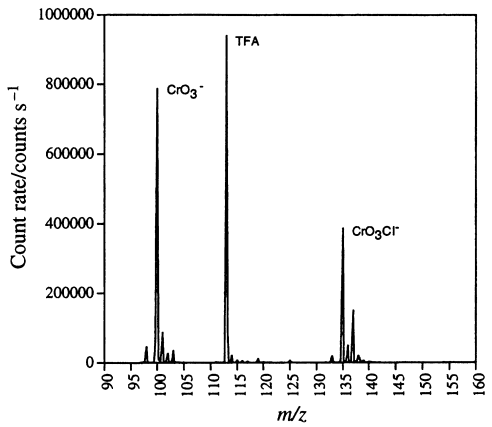

Fig. 5 Mass spectrum of a 10 ppm Cr^{VI} solution.

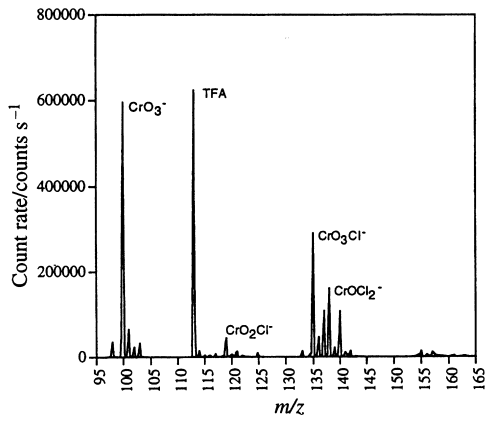

Fig. 6 Mass spectrum of a mixture containing 50 ppm Cr^{III} and 10 ppm Cr^{VI}.

are $m/z = 100$, CrO_3^- from Cr^{VI}, and $m/z = 138$, $CrOCl_2^-$ from Cr^{III}. In addition to the peaks associated with the isotope patterns from the previously mentioned species, CrO_2Cl^- from Cr^{III} and CrO_3Cl^- from Cr^{VI} are also observed.

The CrO_3^- ion that is formed from Cr^{VI} actually contains Cr in the oxidation state +5. No $Cr_2O_7^{2-}$ or CrO_4^{2-} were detected, even under the softest ion extraction conditions. As pointed out by Stewart and Horlick,[21] $Cr_2O_7^{2-}$ is readily reduced by methanol, which explains why intact ions from chromate or dichromate were not observed. Nevertheless, the CrO_3^- ion is characteristic of Cr^{VI} in the original sample, *i.e.*, before the methanol was added to enhance the electrospray process.

Calibration Curves and Detection Limits

Calibration curves and detection limits were determined for both Cr^{III} and Cr^{VI} solutions. Calibration curves for Cr^{III} and Cr^{VI} are shown in Figs. 7 and 8, respectively. These plots were generated by taking the ratio of the analyte signal, $m/z = 138$ for Cr^{III} or $m/z = 100$ for Cr^{VI}, to the signal at $m/z = 37$ due to $^{37}Cl^-$ from the aqueous HCl solvent. As described by Agnes and Horlick, it is often advantageous in ESMS to ratio the analyte signal of interest to another signal to compensate for

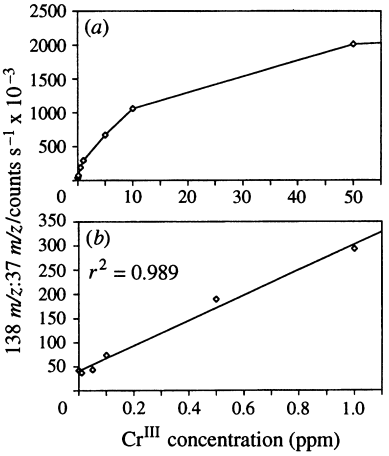

Fig. 7 Calibration curves for Cr^{III} solutions: (*a*) wide range concentration plot, including non-linear region; and (*b*) a plot of the lower concentration region of (*a*).

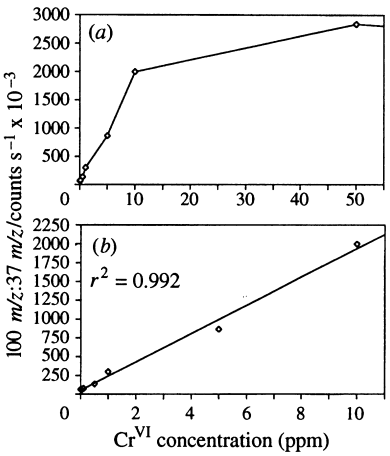

Fig. 8 Calibration curves for Cr^{VI} solutions: (*a*) wide range concentration plot, including non-linear region; and (*b*) a plot of the lower concentration region of (*a*).

Table 2 Detection limits

Species	Relative/ppb	Absolute/pg
Cr^{III}	100	3
Cr^{VI}	60	1.5

the variation of electrospray signal with the total ionic composition of the sample.[20] Fairly linear calibration curves (correlation coefficients of 0.989 for Cr^{III} and 0.992 for Cr^{VI}, respectively) were observed at low analyte concentrations, *i.e.*, up to 1 ppm for Cr^{III} and 10 ppm for Cr^{VI}. The calibration curves roll over at higher analyte concentrations. Detection limits are shown in Table 2. These values represent the solution concentration necessary to produce a net signal equivalent to three times the standard deviation of background during single-ion monitoring for the dwell time used (0.1 s). The detection limits are 60–100 ppb (relative) or 1.5–3 pg (absolute).

CONCLUSION

The concept described in this paper could become a general procedure for distinguishing cations and oxoanions of a particular element under the same spray conditions, in cases where both forms remain stable in the same solution. Difficulties include high background, mediocre detection limits and probable spectral interferences from either organic or inorganic anions. These last problems could be alleviated to a large extent by the use of tandem mass spectrometry, which should eliminate much of the background.

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