

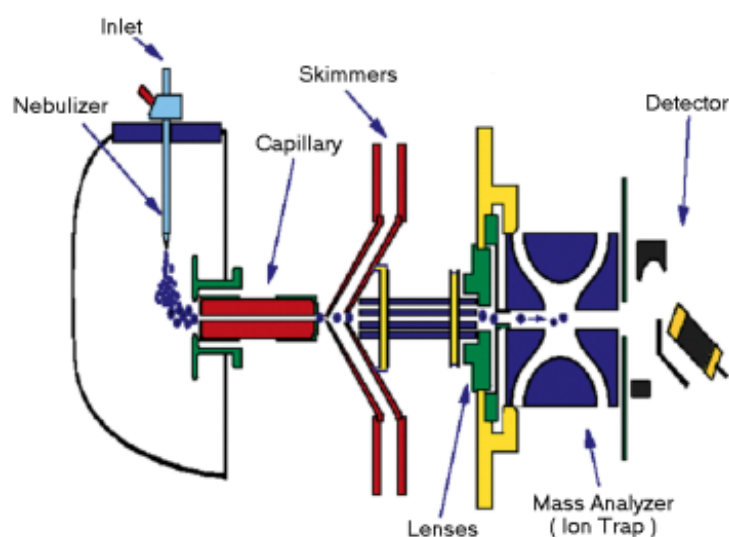
Electrospray Mass Spectrometry of *Tris*-acetylacetonatochromium(III)

Purpose:

Verify the synthesis of the synthesis product, $\text{Cr}(\text{acac})_3$. The presence of any urea containing by-products will also be determined.

Theory

Mass spectrometry, MS, is the ideal tool for characterization of the results of synthesis experiments. However, mass spectrometers operate under high vacuum. A special source is needed to convert solid or solution samples into an ion beam under this high vacuum. Until the advent of electrospray MS, the mass spectroscopy of inorganic complexes has required very expensive ion sources, operating with only solid samples¹. Electrospray MS allows the rapid characterization of components in solution.



An electrospray source produces a fine mist of a solution by spraying the solution from a small tip in an electric field. A nebulizer, which uses a rapid flow of nitrogen gas over the tip can also be used to aid the formation of the mist. The source uses a stream of hot, dry nitrogen to dry the mist droplets. As the droplet size decreases, the ions begin to repel each other until a Coulomb explosion fragments the droplets and produces gas phase ions. The electric field of the source then pulls the ions into a small capillary.

The capillary and two skimmers restrict the flow of air into the low vacuum region of the MS. Ion optics carry the ions to the mass analyzer where the m/z ratio is determined. Our MS uses an ion trap for the mass analyzer.

Electrospray ion sources are soft ionization sources, that is they produce mostly protonated molecular ions, MH^+ . The proton transfer can occur in your solution or in the droplets produced by the electrospray source.



The mass of the MH^+ ion is one greater than the molecular weight, $M+1$, because of the extra hydrogen ion that gives the ion its charge. Little fragmentation of the MH^+ ion occurs, giving very simple mass spectra. This soft ionization should be contrasted with electron impact ionization that is most often used with GC/MS, as you did a few weeks ago. Electron impact ionization is violent and produces many fragment ions for each compound. Therefore electron impact mass spectra contain many peaks from many fragment ions. The simplicity of electrospray ionization mass spectra is very useful when analyzing complex mixtures, without separation.

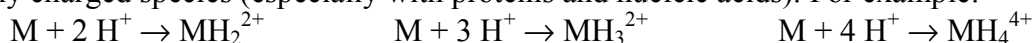
Electrospray ionization is particularly well suited to biological samples for analysis of proteins and nucleic acids. One of the newest areas of biology, proteomics, is based in large part on electrospray MS. MS has become a routine tool in all the molecular life sciences. In this experiment, however, we focus on the mass spectrometry of simple inorganic complexes. For the biologists, however, it should be noted that the majority of enzymes are also metal complexes. For examples, hemoglobin is an iron complex and many enzymes have zinc finger domains.

Mass Spectrometry of Inorganic Complexes

In this experiment, we will use MS to verify the synthesis of the desired product, $\text{Cr}(\text{acac})_3$. We will also be able to use MS to judge the purity of the product. Urea can also form complexes. If the synthesis is done carefully, no urea complexes will form. How successful were you? We expect to find a peak for $\text{Cr}(\text{acac})_3\text{H}^+$. Transition metal complexes also show a large peak for an ion that is formed in the source by the loss of one ligand¹, which is $\text{Cr}(\text{acac})_2^+$ in our case.

For many compounds, ions are formed by acid-base reaction with the H^+ ion, as shown in Eq. 1. However, metal complexes can already be charged, so reaction with H^+ is not necessary to form an ion. So for example, $\text{Cr}(\text{acac})_3$, is neutral, so to form an ion you would need to protonate to give $\text{Cr}(\text{acac})_3\text{H}^+$. On the other hand $\text{Cr}(\text{acac})_2^+$, and $\text{Cr}(\text{acac})_2(\text{urea})^+$ are already positively charged, so an additional H^+ is not necessary.

All mass spectrometers determine the m/z ratio. Electrospray ionization often produces multiply charged species (especially with proteins and nucleic acids). For example:



The m/z ratio for these multiply charged ions would be at $(\text{M}+2)/2$, $(\text{M}+3)/3$, and $(\text{M}+4)/4$. For example, a protein with a M of 10,000, would show MH^+ at 10001, MH_2^{2+} at 5001, and MH_3^{3+} at 3334 m/z . The metal in inorganic complexes can also provide multiple charges. For example, $\text{Cr}(\text{acac})_2^+$.

In your earlier mass spectrometry experiment on methylchloride and methylbromide, you used the isotope peaks to calculate the average molar mass of Cl and Br. Isotope peaks can also be very useful for transition metal complexes. The isotopes of Cr are listed in Table 1. Many metals have characteristic isotope ratios, so observing the isotope peaks in MS confirms the presence of the metal in the complex. In other words, metal complexes with one Cr should have an isotope pattern similar to Figure 1.

Table 1. Isotopes of Cr.

Isotope	Atomic mass (m_a/u)	Natural abundance (atom %)
^{50}Cr	49.9460464 (17)	4.345 (13)
^{52}Cr	51.9405098 (17)	83.789 (18)
^{53}Cr	52.9406513 (17)	9.501 (17)
^{54}Cr	53.9388825 (17)	2.365 (7)

*uncertainties are given in the ()

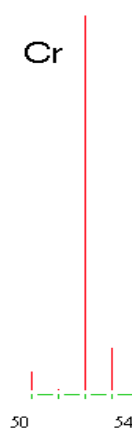


Figure 1. Cr isotope peaks

Mass Spectrometry Procedure

1. Mass spectrometry is very sensitive, so we must be careful to keep the solution concentration of your sample very small (concentrations in the 1×10^{-7} M range are a good starting point). If your solution concentration is too large it will take a long time to clean your sample out of the system before the next sample can be run. Using a small spatula, place a tiny amount of your sample in a plastic centrifuge tube. Use a sample size of around the size of this dot: [•]. Add about 1.5 mL of isopropanol. Sonicate your sample tube, using an ultrasonic bath, to make sure the sample has dissolved; about 30 sec. should be sufficient. Look at your tube carefully to ensure that the entire sample has dissolved. If your sample solution looks purple at all you have too much sample and should start over again.
2. Fill a 1-mL plastic syringe with sample. Hold the syringe vertically and expel the bubble.
3. The *CH141Cr.m* method should already be started when you get to the MS. Select the *Tune* tab, Figure 2, and the *Expert* mode. Verify that the capillary voltage is 2200V. Switch to the *Smart* mode and verify the source settings as in Figure 3. Check the MS scan range and Rolling Averaging as shown in Figure 4. The 10 rolling averages will give good signal-to-noise plots without averaging for too long a time.

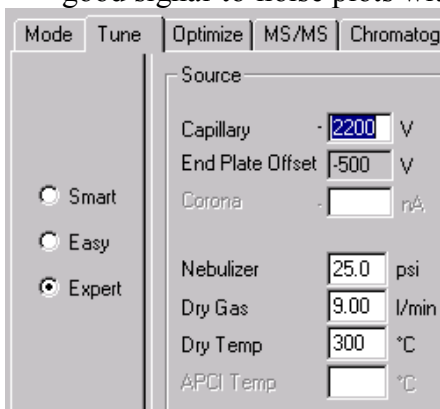


Figure 2. The capillary voltage.

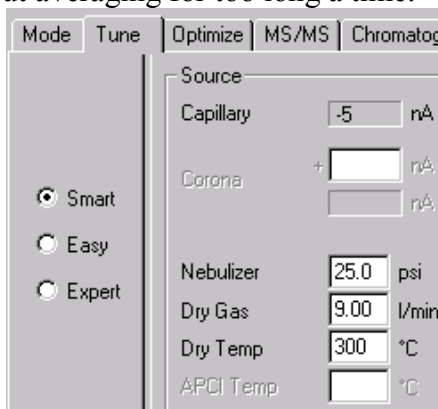


Figure 3. Source settings

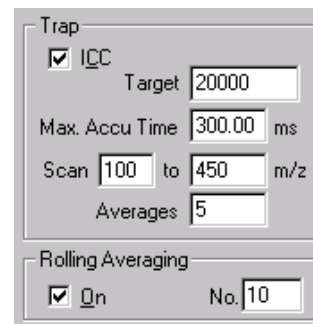


Figure 4. Rolling Averages

4. The sensitivity of the instrument can be optimized for different mass ranges and different types of samples. The settings in Figure 5 have been chosen for good sensitivity for the 350 m/z peak.

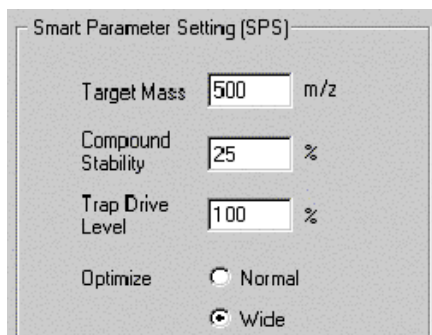


Figure 5. Ion optics settings with good response around 350 m/z for this complex.

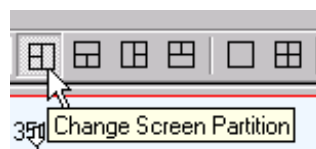


Figure 6. Normal screen arrangement with the *stick* or *histogram* style spectrum in the upper left.

- Click on "Operate" if the system is in "Standby" mode. Click on the window arrangement icon shown in Figure 6, if not already chosen. The spectrum on the left is a "histogram" style spectrum, similar to the display on the GC/MS. The spectrum on the right is a "profile" spectrum that shows the full resolution of the mass spectrometer.
- Attach your syringe to the plastic connector on the blunt-tipped syringe needle. Insert the needle in the stainless steel injection port. Place the syringe in the v-groove of the syringe pump. Lift the syringe hold-fast over the syringe. Depress the brass button on the pusher plate with your thumb and move the pusher plate against the syringe barrel. Inject sample by pushing on the pusher plate until you can see the spray in the spray chamber window.
- Press the Run button on the syringe pump. The arrow on the LCD screen should be blinking.
- You should see continuous mass spectra being plotted. Pull down the File menu and choose Print.
- Expand the profile spectrum to show the isotope peaks around 250 m/z. To expand the plot move the mouse cursor until the cursor changes to a "↔", see Figure 6. Then use the right mouse button to control the scale expansion and the left mouse button to control the scale offset. When you have expanded the scale sufficiently, you will see the isotope peaks clearly as shown diagrammatically in Figure 1.
- Print the expanded spectrum.
- If you wish you can increase the "histogram" spectrum plot size by clicking on the window arrangement icon shown in Figure 7. Then print the new windows.
- Please return the window arrangement to the default as shown in Figure 6.
- Remove the syringe from the injection port.
- Place the wash syringe containing isopropanol into the injection port. By hand inject about 5 mL of pure isopropanol to wash out the source for the next sample (in other words, you will need to refill the syringe at least 5 times). As you inject isopropanol by hand observe the spectrum. Note the intensity of the peak at 250 m/z. Repeatedly inject isopropanol until this 250 m/z peak is much smaller than the other noise peaks in the spectrum.
- If the next student isn't waiting, place the MS in standby mode.



Figure 6. Mouse based scaling: use the right mouse button.

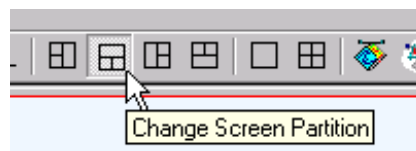


Figure 7. Bigger "stick" spectrum.

Discussion

- A Web applet is available to help you identify the peaks in your mass spectrum: "Metal Complex Finder," the link for which is listed on the course home-page². Use this applet to determine the identity of any ions you find between 120 and 370 m/z. A good set-up for this experiment is shown in Figure 8.

	Metal	charge	coordination
Metal 1:	Cr	3	6
Metal 2:		0	0

	Ligand	charge	protonation	coordination
Ligand 1:	acac	-1	1	2
Ligand 2:		0	0	0
Accessory Ligand 1:	urea	0 *	0 *	1
Accessory Ligand 2:		0 *	0 *	0

Figure 8. Metal Complex Ion Finder

The ions that you may find above 370 m/z are most likely plasticizers that have contaminated your reagents or from the syringe rubber tip. Since the lightest isotope of Cr is not the most abundant, start your search with the options:

Charge State: Using: and maximum metal atoms:

For a few peaks you may need to try a charge state of 2. You should identify at least 4 peaks, but try to find 5-6.

- View your expanded plot around 250 m/z. Does the isotope cluster match the expected isotope pattern? You can check elemental isotope patterns at <http://www.webelements.com/>.
- On the basis of your peak assignments discuss the purity of your sample. Include a copy of your mass spectrum in your report.
- OPTIONAL:** Make an Excel spreadsheet to calculate the masses of ions made from different combinations of acac⁻, H⁺, and urea with Cr³⁺. Remember that the total charge must always be +1. So for example, Cr(acac)₃ is neutral, so to form an ion you would need to protonate to give Cr(acac)₃H⁺. On the other hand Cr(acac)₂⁺, and Cr(acac)₂(urea)⁺ are already positively charged, so an additional H⁺ is not necessary. Use the spreadsheet to determine the identity of any ions you find between 120 and 370 m/z.

References

- J. L. Pierce, K. L. Busch, R. G. Cooks, and R. A. Walton, Desorption Ionization Mass Spectrometry: Secondary Ion and Laser Desorption Mass Spectra of Transition-Metal Complexes of β -Diketones, *Inorg. Chem.*, **1982**, 2597-2602.
- <http://www.webelements.com/webelements/elements/text/Cr/isot.html>
- <http://www.colby.edu/chemistry/NMR/scripts/complexfind.html>