CHEM 322 **Name**_____
Fall 2012 **Due In Class Wednesday, Nov. 14**

Complete the following on separate paper. Show your work and **clearly identify your answers**.

1. Why is an internal standard often used in quantitative analysis by ICPMS?

   An internal standard is often used in preparing calibration curves for ICPMS in order to compensate for random error from instrument drift and noise, torch instabilities, and some matrix effects. The internal standard chosen should be an element that is absent from the sample, but that has an atomic mass and ionization potential close to that of the analyte.

2. What types of interferences are encountered in atomic mass spectrometry?

   Isobaric interferences are encountered when the isotopes of two elements have the same mass. A second type of spectroscopic interference occurs from molecular species that have the same mass as that of an analyte ion. A third type of interference is from matrix species that combine with the analyte and reduce the analyte signal as a result.

3. Describe the interface between the ICP torch and the mass spectrometer in ICPMS.

   The interface consists of a water-cooled metal cone with a tiny orifice in its center. The region behind the cone is maintained at a pressure of about 1 torr by pumping. The hot gases from the ICP impinge on the cone, and a fraction of these gases pass through the orifice where they are cooled by expansion. A fraction of the cooled gas then passes through a second orifice into a region that is maintained at the pressure of the mass spectrometer. Here, the positive analyte ions are separated from electrons and negative ions by a suitable field and are accelerated into the mass spectrometer itself.

4. Why do double-focusing mass spectrometers give narrower peaks and higher resolution than single-focusing instruments?

   The resolution of a single focusing mass spectrometer is limited by the initial kinetic energy spread of the sample molecules. This spread is minimized in a double focusing instrument by accelerating the sample through an electrostatic analyzer, which limits the range of kinetic energies of ions being introduced into the magnetic sector analyzer. Signicantly narrower peaks are the result.

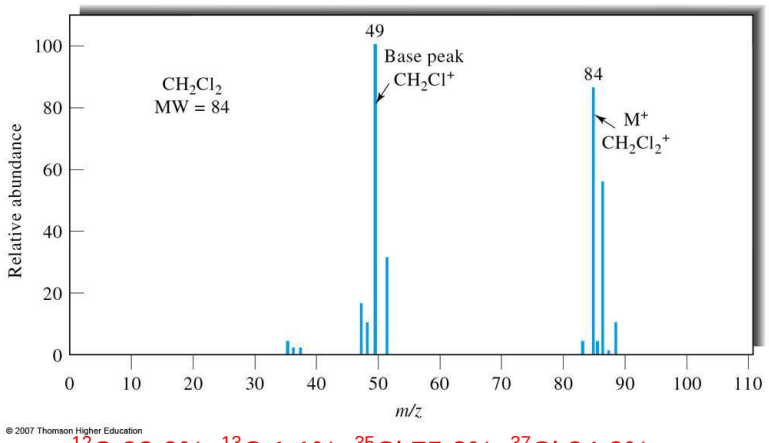
5. Describe the similarities and differences between quadrupole ion trap and Fourier transform ICR mass spectrometers.

   A quadrupole ion trap is similar to a linear quadrupole filter except it as a spherical 3-dimensional configuration. By a combination of fields, ions are temporarily stored within the trap. They are then released sequentially by increasing the radio frequency voltage applied to the ring electrode. The ejected ions then strike a detector. A plot of detector signal vs. the radio frequency voltage, related to the m/z value, is the mass spectrum. In an FT ICR instrument, ions are trapped in a cell by an electric trapping voltage and a magnetic field. Each ion assumes a circular motion in a plane perpendicular to the direction of the field. The cyclotron frequency depends on the inverse of the m/z value. In modern instruments a radio frequency pulse that increases linearly in frequency is employed. A time domain image current is generated after termination of the pulse. Fourier transformation of the time decay signal yields the mass spectrum.

6. How does a quadrupole mass analyzer function to allow only a narrow range of m/z to transmit?

   As they move through the mass filter, ions are subject to both AC and DC potentials. Depending on their size, ions may be influenced differently by the RF and DC components. For example, heavy ions are least influenced by the RF component, while light ions are most influenced by the RF. The balance between DC and RF determines whether an ion will have a stable path. Spectra can be scanned by systematically adjusting either the RF or DC voltages.

7. Consider the peak at m/z 84 below in the spectrum for methylene chloride. Identify the ions that result in the four peaks of m/z greater than 84 in the mass spectrum and justify why the peaks have the relative sizes that they have.



Relative abundances: ^{12}C 98.9%, ^{13}C 1.1%: ^{35}Cl 75.8%, ^{37}Cl 24.2%.
m/*z* = 84 due to $^{35}Cl_2{}^{12}CH_2{}^+$
m/*z* = 85 due to $^{35}Cl_2{}^{13}CH_2{}^+$ Size = 2(1.1/98.9) times as large as 84 peak
m/*z* = 86 due to $^{37}Cl^{35}Cl^{12}CH_2{}^+$ Size = 2(24.2/75.8) times as large as 84 peak
m/*z* = 87 due to $^{37}Cl^{35}Cl^{13}CH_2{}^+$ Size = 2(24.2/75.8) x(1.1/98.9) times as large as 84 peak
m/*z* = 88 due to $^{37}Cl_2{}^{12}CH_2{}^+$ Size = (24.2/75.8) x(24.2/75.8) times as large as 84 peak

8. Explain the steps in an MS-MS experiment. Why are MS^n experiments valuable?

In tandem in space instruments, two independent mass analyzers are used in two different regions in space. This is a rather straight-forward way to do tandem ms and some conventional mass spectrometers can be converted to tandem instruments. The advantages are that it is relatively easy to take all the different types of spectra (product ion, precursor ion, neutral loss, multidimensional. The disadvantages are that the efficiency can be very low and thus the sensitivity can be low. Tandem in time instruments form the ions in a certain spatial region and then at a later time expel the unwanted ions and leave the selected ions to be dissociated and mass analyzed in the same spatial region. The efficiency can be fairly high and the process can be repeated many times. It is, however, only straight forward to take product ion spectra. Both approaches require quite expensive instrumentation.

9. What resolution is necessary to differentiate between:
   a. $C_4H_6O_2^+$ (m/z = 86.0367) and $C_5H_{10}O^+$ (m/z = 86.0731)
   b. A substitution of a methyl group for a proton on a protein with m/z of 11,500 Da
   c. $ArCl^+$ and As^+

Resolution = *m*/Δ*m*
(a) *m* = (86.0367+86.0731)/2 = 86.0549
m/Δ*m* = 86.0549/(86.0731 - 86.0367)= **2364**
(b) *m*/Δ*m* = 11,500/(15.04 - 1.01) = **820**
(c) *m* = (75.401 + 74.921)/2 = 75.161
m/Δ*m* = (75.161)/(75.401 - 74.921) = **157**

10. How does the addition of a reflectron help to improve resolution in TOF-MS? Why don't all TOF-MS instruments have a reflectron?

The time of flight mass spectrometer separates ions on the basis of velocity by monitoring the time it takes for them to drift through a field-free flight tube. In order for the mass separation to be accurate, ions must be introduced into the flight tube in a small pulse and ions of the same m/z must have the same kinetic energy to that they have the same velocity. In practice, ions are produced with a small spread in KE leading to a small spread in velocity. The reflectron serves to minimize the impact of this spread in velocities on the resolution of the measurement. It does so by presenting an increasing electric field in such a way that slower moving ions penetrate less deeply into the reflectron and faster moving ions penetrate more deeply before being redirected toward the detector. As a result of this slight difference in flight distance, the packet of ions ends up being more focused once it reaches the detector.

11. Suggest a reasonable combination of ionization source, mass analyzer and detector for the following applications:
    a. The determination of heavy metal contamination in drinking water.
    Reasonable combination would be ICP source, quadrupole, and electron multiplier
    b. The identification of a bacterium with a mass of 30 kDa.
    Reasonable combination would be MALDI source, TOF, and electron multiplier
    c. Molar mass and structural identification of a newly developed steroid-based antibiotic.
    Reasonable combination would be EI/CI source, quadrupole, and electron multiplier

12. In terms of utility, the mating of LC with MS holds tremendous promise. Why is it difficult to interface and LC with a MS? How has this problem been addressed to lead to commercial LC-MS instruments?

The key challenge in interfacing LC and MS is the very different conditions at which each instrument operates. In traditional HPLC, eluent exits the column at mL/min flow rates, resulting in a large amount of material exiting the LC in a short time. If all of this material were introduced into the MS, it would be impossible to maintain the high vacuum conditions required to provide a large mean free path for the ions produced in the MS.

One of major developments in the coupling of MS to LC is the electrospray ionization source. In electrospray, the sample solution eluting from the LC flows through a needle which is subject to a large electric field. As solution leaves the needle, it obtains a charge. Electrostatic repulsion causes the charged stream to break into smaller charged droplets, which continue to "explode" until solvent is essentially evaporated and ionized analyte remains. When coupled with a sampler and skimmer, these ions can be extracted into the MS.

13. MALDI is a very common ionization source for TOF mass analyzers, why is this the case? How could an ionization source like electrospray be paired with a TOF? What benefits would electrospray ionization provide compared to MALDI?

MALDI: The analyte is dispersed in a MALDI matrix (a molecule that readily sublimes when it absorbs energetic photons) and deposited onto a target. The target is irradiated with a laser pulse, resulting in absorption and sublimation of the matrix (including the analyte) and ionization. The result is the formation of intact molecular ions, some of which may be multiply charged. Benefits: Soft ionization source. Good for molar mass determination. Challenges: Pulsed source, need mass analyzer that can handle pulsed introduction. Need appropriate matrix.

Electrospray: The sample solution flows through a needle which is subject to a large electric field. As solution leaves the needle, it obtains a charge. Electrostatic repulsion causes the charged stream to break into smaller charged droplets, which continue to "explode" until solvent is essentially evaporated and ionized analyte remains. This is a more energetic ionization source, capable of producing multiply charged ions and fragments. Benefits: Continuous source. No additional sample handling steps. Challenges: May lead to complex spectra. Need to remove some sample to produce lower pressure for mass analyzer.