

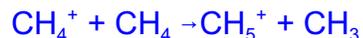
## Chem 434 Hour Exam III

All questions worth 14 points, you may skip 1 question

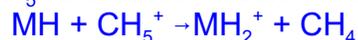
1. The most commonly used gas in chemical ionization is  $\text{CH}_4$ . The most common molecular ions formed in chemical ionization with this gas are  $\text{MH}_2^+$  and  $\text{M}^+$ . Give the chemical reactions and explain the process that takes you from the ionization of  $\text{CH}_4$  gas to the  $\text{MH}_2^+$  and  $\text{M}^+$  molecular ions.

Most common ions made are  $\text{CH}_4^+$  and  $\text{CH}_3^+$

These react with un-ionized methane to produce:



The  $\text{CH}_5^+$  then tries to transfer a proton to the molecule of interest



The  $\text{C}_2\text{H}_5^+$  either does a proton transfer to the molecule of interest, or it removes a hydride from the molecule of interest



2. How does Matrix-Assisted Laser Desorption work?

The molecule you are interested in is dissolved in a large excess of a matrix that contains a material that can absorb specific wavelengths of light. This solution is then deposited on a metallic probe and dried to the probe's surface. This probe is then placed in the sample chamber of the MALDI/TOF spectrometer, and a laser tuned to the absorbance of the matrix is pulsed at the probe's surface. The light of the laser sublimates the material of the matrix, so both the matrix and the compound of interest are turned into a gas by the light. In this light pulse the material of interest also loses one or more electrons to become positively charged. The charged molecule of interest is accelerated into the time of flight analyzer by an electronic potential.

3. We talked about two different types of mass spectrometers, quadrupole mass spec, like with have on our machine that is used to determine the molar mass of molecules of  $< \sim 500$ , and Time of Flight spectrometers like those used in Matrix Assisted Laser Desorption spectrometers used to get the molar masses of proteins  $> 1000$ . Compare and contrast how these machines get ions and find the masses of those ions.

In our quadrupole machine either electron impact or chemical ionization is used to feed a *continuous* stream of ions into the quad mass filter. In the quad filter are four parallel metal bars. One opposing pair is at a positive DC potential relative to the other pair. An AC signal is then superimposed on top of the DC offset. At any particular AC and DC setting, only ions of one charge/mass ratio can traverse the quad filter from ion entrance to mass detector. By sweeping through a range of AC and DC in under a second, the amount of each ions of any mass between 0 and  $\sim 2000$  can be scanned and the entire mass spectrum determined.

In a time of flight spectrometer a laser pulse is used to liberate a single burst of the molecule of interest from a matrix. This molecular ion is then accelerated by a constant potential into a vacuum chamber. The time in it takes for the ions to traverse the vacuum chamber is carefully measured, and this time is directly related to the charge/mass ratio of the ion.

4. When an electron beam hits an object in a scanning electron microscope it can penetrate to different levels, and produce different kinds of interactions with the matter it is striking. Describe these different interactions and the resulting emissions

As electrons penetrate the surface of an object then reflect off, we see various interactions. Interactions at the uppermost  $\frac{1}{2}$  nm (first 1-5 layers of atoms) of the surface give rise to Auger interactions and represent the true surface of the material. Below that we see (in order) secondary electrons, back scattered electrons, characteristic X-rays and continuum X-rays.

The Back Scattered Electrons (BSE) represent elastic collisions between the electron beam and the material and are detected at an acute angle to the incident beam (nearly straight up). The back scattered beam may have a diameter of several times larger than the incident beam and this is the major limitation to the resolution of the electron microscope.

The secondary electrons come from inelastic interactions between the electron beam and the material being examined. This signal is much weaker, between  $\frac{1}{2}$  and  $\frac{1}{5}$  the intensity of the BSE electrons. These electrons are one that have been ejected from the conduction bands material, and are detected with a transducer that is closer to  $90^\circ$  from the incident beam.

The characteristic X-rays are the one that are normally detected with the EDS or EDX detector. These are X-rays that are emitted by the material as inner core electrons are ejected by the electron beam and electrons from higher orbitals release energy to drop down to fill in the missing electrons. The precise wavelengths (or energies) of these emissions can be used to identify different elements in the material.

Finally the continuum X-rays represent an overall energy background that must be subtracted from all other measurements to identify significant 'peaks' of energy.

5. Here are several equations from chapter 26 'An Introduction to Chromatographic Separations'. For each equation: Tell what we are calculating and why it is important, and what each of the parameters in the equation are.

$\bar{v} = \frac{L}{t_R}$   $\bar{v}$  is the average linear rate of solute migration. It is used to characterize the length of time a material takes to travel through a column. L is the length of the column and  $t_R$  is the retention time, or the time it takes from injection to elution from the column.

$H = \frac{LW}{4t_R}$  H is the plate height, and is a measure of column efficiency. The smaller the plate height the more efficient the column. In this equation the column height is calculated from the empirical data of an actual elution profile. L is the length of the column, W is the width of the of a triangle extrapolated from the Gaussian shape of a peak and  $t_R$  is again the retention time of the peak.

$N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2$  N is the number of plates in a column and is another measure of column efficiency. Here the larger the N value the better the column. In this calculation N is derived from empirical data of actual chromatographic data.  $t_R$  is the retention time and  $W_{1/2}$  is the width of the peak at  $\frac{1}{2}$  height

$N = 16R_s^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + k'_B}{k'_b} \right)^2$  N is again the number of plates, but in this calculation it is derived from a theoretical calculation based on the  $R_s$  the resolution required in a separation,  $\alpha$ , the selectivity factor of one peak over the other in the column, and  $k'_b$  the retention factor of the column for component b. This equation is used to see how the number of plates depends on selectivity and retention.

6. What is the van Deemter equation, what do the three major terms in the equation correspond to, and why does it always show a minimum in H as a function of flow rate.

The van Deemter equation is :  $H = A + B/u + Cu$ , where H is plate height,  $u$  is the linear flow rate of a column, and A, B and C are parameters that describe how the plate height varies with flow. A, called the multipath flow, represents a term in which plate height does not vary with flow rate, and represents a minimum broadening that occurs because there are different paths that a solute can take as it passes through a column. B is the Longitudinal Diffusion term and represents a term in which column efficiency is inversely proportional to flow rate. Essentially as the flow rate decreases the material has more of a chance diffuse and this broadens the peak and ruins efficiency. The C term is the Mass-transfer term. Here the column efficiency decreases linearly with flow rate because as the flow rate increases the solute has a less time to attempt to come to equilibrium between the stationary phase and the mobile phase, so the peaks broaden out and again efficiency is ruined.

7. What are the different kinds of columns used in gas chromatography, both currently and historically. If you give an abbreviation for they type of column be sure you explain what it means.

Packed column - A tube into which a material like diatomaceous earth is pack and coated with some stationary phase. Not used currently because not a very efficient column type.

Open Tubular columns - A column which is has the stationary phase adhered to the wall of the column in some way and an opening through the middle of the tube through which a carrier gas can pass. Most modern columns are of this type because they have high efficiency and high flow rates. There are several types of open tubular columns such as:

SCOT - Support Coated Open Tubular - a material to support the stationary phase is placed on the wall of the column

WCOT- Wall Coated Open Tubular- the stationary phase is applied directly to the walls of the column

FCOT - Fused Silica Open Tubular - Fused silica is used for the column so it is thinner, it is also coated with a polyimide coating to make it flexible, like the WCOT the stationary phase is applied directly to the column walls, but this type of column has replaced the WCOT because it is stronger, more flexible and less reactive.

PLOT - Porous Layers Open Tubular. The column walls are coated with a porous material that serves to slow passage through the column based on molecular mass. Can differentiate between materials like  $C_2H_2$  and  $C_2H_4$ .

8. Compare and contrast 'normal' phase and 'reverse' phase chromatography.

Historically 'normal' phase chromatography evolved first, and in this chromatography a polar stationary phase is used to bind moderately polar compounds and then a non-polar mobile phase is used to elute the most non-polar components from a sample first.

'Reverse' phase chromatography appeared later. In this chromatography a non-polar mobile phase is used to bind moderately non-polar components, and then a polar solvent is used to elute the most polar components of the sample first.

Intriguingly both types of chromatography start with a polar silica based stationary phase. However in 'reverse' phase chromatography the polar silica is chemically changed to a nonpolar substance by covalently attaching various long chain alkanes.