Mass Spectrometry Lecture # 1

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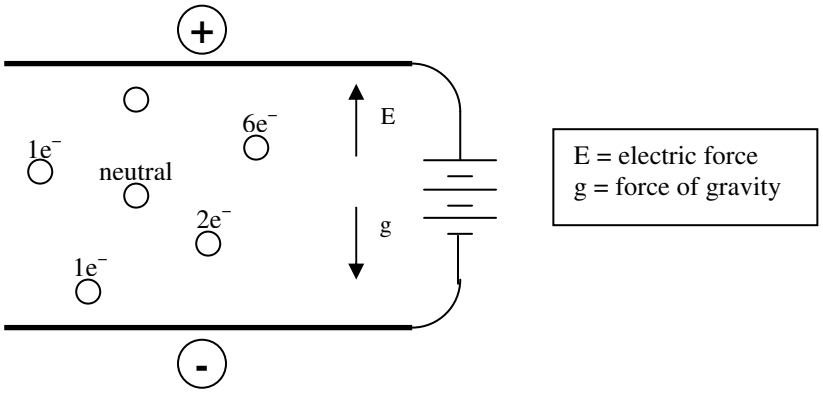
Millikan Oil Drop Experiment – The First MS Experiment

- wanted to determine the charge on the electron
- used an atomizer to create a fine mist of oil drops
- applied a voltage across the atomizer so that some of the drops picked up one or more negative charges (in the next lecture Dr. Gehring corrected this: the ionization was done with X-rays)
- charged oil drops were allowed to pass between two metallic plates connected to a variable voltage power source:



Step 1:

- voltage turned on (positive on top), negatively charged drops rise towards the top plate
- the force on the drops is determined by:
  Force = voltage * charge on the electron * number of electrons ($F = V * e^- * \#e^-$)
- we assume Millikan could figure out which drops had just one charge
- by measuring the velocity of the oil drops and, knowing the viscosity of air, you can figure out the force on the drops

*Note: Dr. Gehring got this slightly wrong. In fact, F = E*q (electric field * charge). So it should be $F = E * e^- * \#e^-$. What he gave us is the equation for electric potential energy of the oil drops. I'll substitute E where he wrote V.*
E = V / (distance between plates)

Step 2:

- the mass of the oil drops in unknown
- turn off voltage, allow drops to fall by gravity, measure velocity to determine F_g

Step 3:

- adjust voltage so the electric and gravitational forces balance, drops remain stationary:

$$\vec{F}_g = gm = E \cdot e^-$$

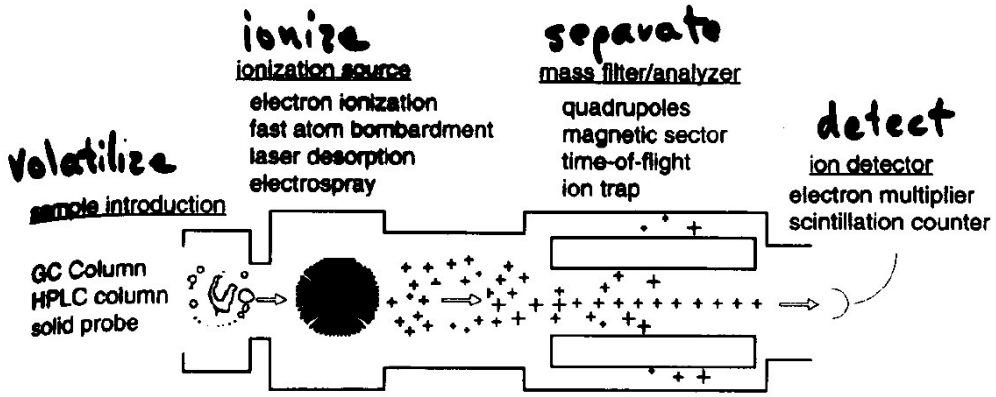
$$\frac{m}{ze^-} = \frac{E}{g}$$, where z = the number of e^-

- m/z is always the quantity measured in an MS experiment, which means you are not directly measuring mass
- mass and charge are both unknown, but this isn't a problem because the charge can only take on certain discrete values

Modern Mass Spectrometry

- essentially the reverse of Millikan's experiment
- if we know the number of charges on the molecule (and e^- from Millikan) we can figure out the mass

The Steps of MS:



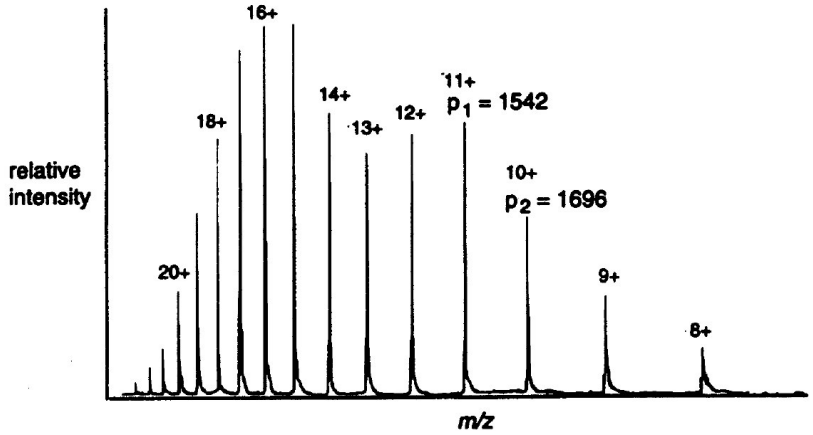
- in Millikan's experiment:
  The sample was Volatilized with the atomizer
  X-rays were used for Ionization
  The electric plates allowed for Separation
  Detection was done with a microscope and a bright light

- a mass spectrum is a plot of # of ions in a mixture against m/z ratio

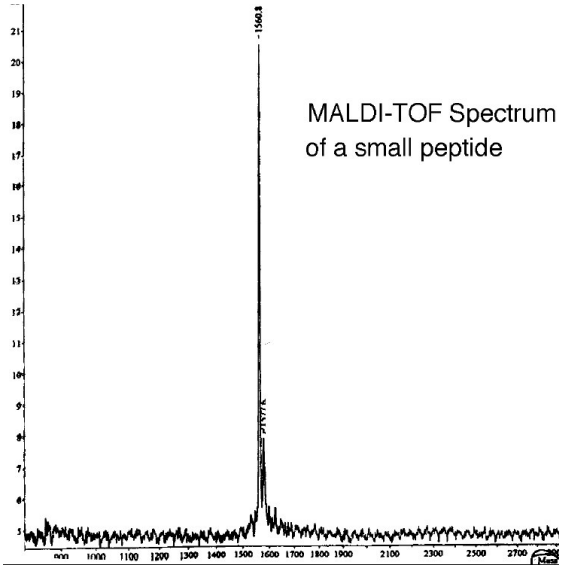
- Millikan used negatively charged particles, we will look at using both –ve and +ve charged ions (you usually use +ve for proteins)
- in addition to adding charge, you can remove electrons or protons

The Effect of Charge

- electrospray MS is most similar to Millikan's experiment:
- spray protein in small drops, drops dry down to individual protein molecules
- by doing this in acidic solution the protein molecules will pick up varying numbers of protons
- if you take a sample with only **one molecular species** (ex. myoglobin) and plot m/z you get different peaks for the proteins molecules with different amounts of charge:



- there are other types of MS that yield only singly ionized species
- MALDI-TOF (Matrix Assisted Desorption/Ionization – Time Of Flight) is an example
- this gives a spectrum (of a peptide this time) with only one peak at 1600 m/z:



- in this case the charge is 1, so the mass is 1600 Da

The Effect of Isotopes

- in a sample of a pure protein or peptide there are actually many species present
- not all molecules in the sample have the same molecular weight, because not all carbons are ^{12}C

- when you weigh a sample you're looking at large (molar, millimolar, etc.) quantities
- you get an average mass based on the average composition of ^{12}C / ^{13}C
- when you determine the molecular mass of a sample by looking at individual particles it makes a big difference if a ^{13}C is substituted for a ^{12}C

- there are many non-radioactive isotopes which are present in the environment:

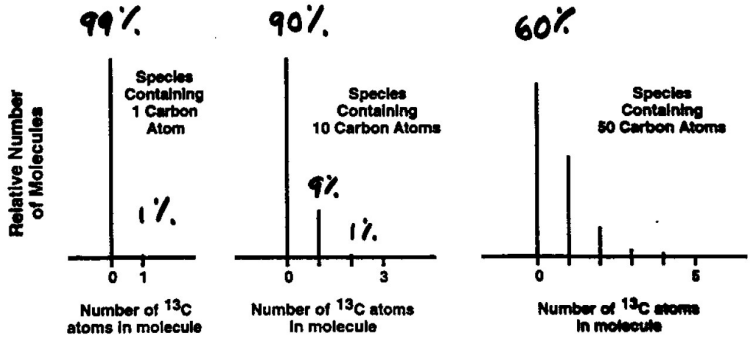
^{12}C	^{13}C
99%	1%

^{1}H	^{2}H
99.986%	0.014%

^{14}N	^{15}N
99.6%	0.4%

- C is most important in biomolecular MS because of the high abundance of ^{13}C

Amount of ^{13}C In Compounds



- for a 1 carbon molecule (ex. ethanol): 99% will have mass 32 Da, 1% will have mass 33 Da (extra mass from ^{13}C)
- for a 10 carbon hydrocarbon ($C_{10}H_{22}$): 90% will have mass 142 Da (all ^{12}C)
  the probability that all 10 C are $^{12}C = (0.99)^{10} = 0.904$
- how many will have mass 152 (all ^{13}C)?
  $(0.01)^{10} = 1*10^{-20}$
  this peak will definitely not show up
- as can be seen in the graphs above, there are species with masses in between these two extremes
- with 10C you see two peaks (probably won't see peak for 2 ^{13}Cs)
- with 60C you see several peaks

Nomenclature for Isotopes

nominal mass = the mass of an ion with a given empirical formula calculated using the integer mass of the most abundant isotope of each element

isotope averaged mass = the mass of an ion calculated using the average weight (averaged over all isotopes) for each element

monoisotopic mass = the mass of an ion for calculated using the exact mass of the most abundant isotope of each element

- nominal mass not very useful except as rough estimate (does not correspond to a peak)
- for small peptides you will see a monoisotopic peak (all ^{12}C, ^{14}N, ^{1}H)
- as number of C goes up, monoisotopic peak gets smaller because chance of having a molecule with no ^{13}C gets really small
- isotope averaged mass doesn't necessarily correspond to a peak, if you approximate the peaks as normal distribution (bell curve) the isotope averaged mass will be at the center of the bell curve

Other Isotopes

^{79}Br	^{81}Br
50%	50%

^{35}Cl	^{37}Cl
75%	25%

- high abundance, but not present in proteins
- numbers of the different isotopes in molecules follow the same rules as for C (see slide 7 of http://www.mcgnmr.ca/kalle/404/MS/MS_lecture_2.pdf)
- the effect of isotopes of S can be seen in protein MS, but there's not that much S in proteins so it's not as important as C

- what is the distance between two peaks that correspond to two molecules where 1 ^{13}C has been substituted for a ^{12}C?
  It depends on the charge: 1 m/z unit if the charge is 1, 0.5 m/z units if the charge is 2, etc.

Molecular Formula Determination

- when MS gets very accurate (4 digits after the decimal) it's possible to tell the difference between 1 ^{16}O and 16 ^{1}H's
- for small molecules you can determine the elemental composition, can't do this for proteins
- essentially you figure out what combination of elements would give the exact mass measured, which is why you need such high accuracy