Chapter 20 Molecular Mass Spectrometry

Problems: 1, 2, 4, 7, 10, 11, 15, 16

Note may have to go over sections of Chapter 11, Atomic Mass Spectrometry

20A Molecular Mass Spectra

Figure 20-1 Typical Mass Spectrum
Relative abundance vs m/z

m/z mass/charge

This example ethyl benzene should have a molecular mass of 106
see 106
see 91
see lots of others

Why?

How is sample analyzed
start with ethyl benzene in vapor phase
hit with a stream of electrons
that knocks off one of the molecules stating electrons

\[ \text{C}_6\text{H}_5\text{CH}_2\text{CH}_3 + e^- \rightarrow \text{C}_6\text{H}_5\text{CH}_2\text{CH}_3^\cdot + 2e^- \]

So now have molecular ion \( M^\cdot \)
the dot indicates that is a radical ion
The collision between electron in molecule leaves it in an excited state
Often fragments into pieces as gets rid of the energy
In this case one fragment is to lose a CH\(_3\)
Thus one common ion is the \( \text{C}_6\text{H}_5\text{CH}_2^\cdot \) that has a MW of 91

Other smaller positively changes ions are also formed

All positive ions are attracted to a negatively charges lens that gets ions moving into the mass spectrometer

The mass spectrometer then sorts ions by mass/charge ratio (m/z)
And displays the relative amounts found at any particular m/z

Note the largest peak is called the base peak
arbitrarily assigned a value of 100
all smaller peaks scaled from that
(Done automatically by computer running the instrument)
20B Ion Sources

Starting point then, is creation of gaseous ions
Appearance of mass spectrum (relative sizes of all the peaks) depends on method used to get ions
Table 20-1 different methods used to get gaseous ions
Note 2 major categories - Gas phase sources & desorption sources
In gas phase sources start with molecule already in gas
In desorption sample is in liquid or solid and must be converted to gas
Commercial mass spec have different units to plug in and out for different methods

Our mass spec hooked to GC, so already in gas phase
Have 2 sources Electron Impact (EI) and Chemical Ionization (CI)

Gas-phase sources usually stable compounds, BP <500°C
usually less than 1000 MW
Desorption sources up to 10,000MW

Class sources as hard or soft
Hard give molecular ion enough E that it tends to fragment
Rupture bonds lots fo fragments with m/z ratios < molecular ion
Soft sources - little fragmentation
Compare same compound - different sources figure 20-2
That is why we have both EI and CI, EI is hard, CI is soft

29B-1 EI source
(This is what we normally have hooked to the mass spec)
Historically this was the first kind of source used

Heat sample to make a vapor
Ionize by passing through beam of electrons
See figure 20-3

Electrons emitted from a heated Tungsten or rhenium filament
Standard V is 70V between filament and anode

Electrons at 90° to path of molecular vapor

Electrons don’t actually hit the molecules
As electrons make a near miss, electrons in molecule are repelled

\[ M + e^- \rightarrow M^+ + 2e^- \]
Actually not very efficient
1 molecule in a million gets ionized

Direct + ions in right direction using a slit with a -5V charge relative to + repeller on other side of E source

Additional slits, lenses, etc to focus beam and accelerate into mass analyzer

**EI Spectra**

Electron source V must be at least 50 V to get ions

Usually set at a standard V for reproducibility

Libraries made under specific conditions

Electron impact doesn’t do much to molecules speed

But does put into a high E state that fragments during relaxation

Lower mass ions called *daughter ions*

Table 20-2

How a compound can fragment

How it can recombine into larger ions!

Fragmentation very complex

Can’t always assign every peak

But pattern can be used to identify compound

(Like in IR can’t assign every peak but spectrum is fingerprint of molecule)

Note that most of the time the **base peak is not the molecule ion**, but is smaller

Sometimes can see the molecular ion, sometimes not

**Isotope peaks**

**Figure 20-4a**

Something simple CH\textsubscript{2}Cl\textsubscript{2}

CH\textsubscript{2}Cl\textsuperscript{-} parent ion

CH\textsubscript{2}Cl\textsubscript{2-} molecular ion

See other ions of slightly higher mass (+1 or +2?)

Where did these come from?

Same chemical formula, different isotopic compositions

CH\textsubscript{2}Cl\textsuperscript{-}

\[ ^{12}\text{C} ^{1}\text{H} _{2} ^{35}\text{Cl} _{2} = 84 \]

\[ ^{13}\text{C} ^{1}\text{H} _{2} ^{35}\text{Cl} _{2} = 85 \]

\[ ^{12}\text{C} ^{1}\text{H} _{2} ^{36}\text{Cl} _{2} ^{37}\text{Cl} = 86 \]

\[ ^{13}\text{C} ^{1}\text{H} _{2} ^{35}\text{Cl} _{2} ^{37}\text{Cl} = 87 \]

\[ ^{12}\text{C} ^{1}\text{H} _{2} ^{37}\text{Cl} _{2} = 88 \]
Common isotopes listed table 20-3
F, P, I, Na essentially single isotopes
Isotope peaks sometimes useful in structure determination

Collision Product Peaks
Collisions to make higher molecular weight ions possible
Under ordinary conditions the only collision product you see is
collision of an H⁺ with the parent molecule
So MH⁺ with a +1 molecular weight can be observed
Height of (M+1)⁺ peak is concentration dependent
So if change pressure or concentration but don’t mess with
electronics and get changes of M+1 peak, then know what it is

Advantages and disadvantages of EI sources
Advantages
Easy to use
Gives (comparatively) large number of ions
Lots of fragmentation - use pattern to identify compound

Disadvantages
Lots of fragmentation - sometimes can find the molecular ion
Sample must be a stable gas
(Sometime get thermal decomposition before ionization)
(Ways to avoid this problem as well)
Limited to MW in $10^3$ range

20B-2 CI
Usually an interchangeable unit with CI
(It is that way on our machine)
Ions formed by colliding molecule with ions of an excess reagent gas
Can have both + and - CI, but + most common
2nd most common source

Same set up as before with 2 changes
Put whole thing in a vacuum chamber
Keep about a 1 torr of reagent gas in chamber
(Analyzer still at about $10^{-5}$ torr)

By filling analyzer with reagent gas have about $10^3$ to $10^4$ more gas
molecules in chamber than target molecules

With this higher conc, it is the reagent gas that gets ionized
Typical reagent gas is Methane ($\text{CH}_4$) (That is what we use)
Get several ions
\[ \text{CH}_4^+, \text{CH}_3^+ \text{ and CH}_2^+ \]
First 2 are 90%
Additional collision fragments:
\[ \text{CH}_4^+ + \text{CH}_4 \rightarrow \text{CH}_3^+ + \text{CH}_3 \]
\[ \text{CH}_3^+ + \text{CH}_4 \rightarrow \text{C}_2\text{H}_5^+ + \text{H}_2 \]

These last two are most reactive with the target molecule
\[ \text{CH}_5^+ + \text{MH} \rightarrow \text{MH}_2^+ + \text{CH}_4 \text{ (proton transfer)} \]
\[ \text{C}_2\text{H}_5^+ + \text{MH} \rightarrow \text{MH}_2^+ + \text{C}_2\text{H}_4 \text{ (proton transfer)} \]
\[ \text{C}_2\text{H}_5^+ + \text{MH} \rightarrow \text{M}^+ + \text{C}_2\text{H}_6 \text{ (hydride transfer)} \]

First 2 give you \((\text{M}+1)^+\) ions
Last gives you \((\text{M}-1)^+\) ion
Sometimes see an \((\text{M}+29)^+\) ion when \(\text{C}_2\text{H}_5^+\) sticks

But, bottom line, get the molecular ion +/- 1
Little addition fragmentation

Other gases used
Propane, isobutane, ammonia
Each is a little different
Figure 20-2 is a comparison of CI and EI
CI much cleaner

20B-3 Field ionization Sources and spectra - Skip

20B-4 Desorption Source
While can't do here, big in Biochem, can do through BRIN at USD so will look at briefly

How do you handle nonvolatile or thermally unstable compounds
Or how do you handle a Protein or DNA with MW in the 10,000 to 100,000

Field Desorption - skip

Matrix-Assisted Laser Desorption (MALDI)
Great for biopolymers in 1,000-100,000's range
First developed independently in 1988 by Germans and Japanese
Analogy - how do you get an elephant to fly? You blast the ground out from under him.

Mix sample in aqueous or alcohol solution
Solution contains a large excess of radiation absorbing 'matrix'
material (Matrix materials listed table 20-4)

Dry onto a metal target

Put into machine under vacuum
Blast tiny spots on surface with a laser pulse
Everything in spot is instantly sublimed
spectrum taken then another pulse is fired and another sample is analyzed
(Spots just about visible to eye)
Laser tuned to absorption of matrix

Typical output figure 20-7
Main peak M+
See also M++, M+++ 
2M++, 3M++, 2M+ (dimers and trimers)

Entire process not well understood
Only a few matrix material that work well

Electrospray Ionization
ESI/MS
Also good for biopolymers in 100,000 range

figure 20-8
Use ambient pressure and T
solution pumped through a needle
(Note this will be good for HPLC/MS)
Needle several 1000 V above cylindrical electrode around needle
End up with fine droplets that have - charges on them
Droplets pass through a desolvating capillary
Droplets shrink, charge density on droplet increases, desorption of ions into gas occurs
Very little fragmentation occurs
most molecules multiply charged so m/z ratio is lower so can be analyzed on quadrupole instrument that is designed for 1,500 MW with m/Z of 1

Example spectra 20-9

Adjacent peaks differ by 1 charge
average charge about proportional to MW
charge determined from peak distribution
put al together can get MW of parent, even though don’t see M/z of the true molecular ion
**Fast Atom Bombardment (FAB)** - skip

**20C Mass Spectrometers**

**20C-1 General Description of Instrument Components**

Basic Block diagram figure 20-10
- Inlet system - get tiny sample < 1 µmole
  - Also get it in gas form
- Ion Source - turn gas molecules into gas ions
  - May be combined with inlet system
- Mass analyzer - separate ions by m/z
  - Detector transducer to change beam of ions into an electrical signal
    - Transducers covered in 11B-1 I don’t think I will get into here

Whole thing under vacuum of $10^{-4}$ to $10^{-8}$ torr (ours $10^{-5}$)
- Any vacuum leak will kill signal because molecules from the atmosphere will interact with and annihilate your gas ions

**20C-2 Sample Inlet Systems**

get your sample into the machine without killing the vacuum

**Batch Inlet systems** Figure 20-11a
- get sample into gas externally, then leak some of that gas into the vacuum
  - This system works up to bp's of 500° C
    - For gases, measure volume between 2 valves
      - Then expand into the reservoir
    - For liquids inject a µl or two into the reservoir
      - Then pull a vacuum until in the $10^{-4}$ to $10^{-5}$ torr
    - If sample needs T>150, keep it all heated
      - About the best you can do is to heat to 350°C
      - And this will work for samples with BP up to 500

Finally open a pin hole into the ionizer

**Direct Probe Inlet** Figure 20-11b
- for solids or nonvolatile sample
  - sample introduced in a holder via an airlock so don’t lose vacuum
    - need as little as a few ng of material
  - sample in a holder with a few mm of ionization source and spectrometer slit
  - combination of low pressure and proximity of ionization source often make it so you don’t have to heat the sample to get volatile ions so decomposition doesn’t occur
Inlet for a GC system described in chapter 27

20C-3 Mass Analyzers
several different devices for determining m/z ratio. The ones I want to cover are back in previous chapters. We will skip the others

Go back to section 11B-2 and 11B-3 for quadrupole and TOF spectrometers

11B-2 Quadrupole Mass Analyzer
(This is what we have on the GC)
Most common
more compact, less expensive, more rugged than other finds
High scan rate, get entire spectrum in .1 sec! (That is why good for GC!)

Figure 11-4
Heart is 4 parallel cylindrical rods
   Opposite sides attached to variable DC source so up/down is +
with respect to right/left (or vive-versa)
   Additional AC signal in radio frequency range on top of DC
      Here 2 sets of rods are 180 out of phase with each other

Ions come from ions source with a 5-10 V acceleration

As move down the tube the AC signal between 1 set of rods makes ion want to oscillate between rods
AC signal between other rod makes want to oscillated between these rods too
Net - spirals down between the rods

At any specific set of AC and DC values only ions with 1 m/z ratio make it out the end
All other ions spiral out and collide with metal rods
Can easily resolve ions that differ by one mass unit
So scan consists of varying voltages, to let different ions through at different times and keeping track of how much comes through at a particular time
   Sometime called a mass filter, because just filters 1 particular mass to hit the detector at a time

Ion Trajectories
Let’s see if we can understand how the spiral works
   Lets start with the pair of rods in Up/down (Figure 11-5)
Start with the AC component
When both +, + ions repelled, so will converge toward center
When both -, + ions attracted to will try to move out

Whether or oscillation hit the rod depends on
  Size of +/-
  Frequency of +/-
  Mass of ion
    More mass, moves slower, can oscillate as wide
  Ion heavy or oscillation so fast it can’t respond
    Ions stays in course down the middle
  Ion light or oscillation slow enough that can follow
    Will oscillate bigger & bigger till it hits the rod

Now put overall bias on these rods that is +
  This tend to drive ions toward middle

Net effect, acts as a high pass filter
  Only high masses make it through

Now lets start with other rods that are side/side
  Same general idea, but these rods are - overall so overall bias is to pull ions out toward the rod
  So slow moving ions respond to this bias and hit the walls
  While the fast moving once respond to the AC and just oscillate

Net effect low pass filter, only low masses get through

Overall tune the 2 so only 1 mass right in middle gets through

Now how do you scan?

**Scanning**
  Equations governing this are complicated differential, beyond the scope of your instructor and this text
  Depends on
    M/z
    DC voltage
    AC voltage
    AC frequency
    Distance between rods

In the end major factor is ration of AC to DC
  Resolution is maximum when AC is just less than 6 times DC
To do scan ramp up AC and DC simultaneously
This lets successively large ions through

Commercial quads up to 3000-4000m/z
(I think ours is only up to 1500?)

11B-3 Time-of-flight
Usually coupled with a MALDI source
starts with a pulse of + ions
pulse frequency up to 50 kHz
Time of pulse .25μsec
Ions accelerated by electric field of 1000-10000V
(Field is turned on just after ionization pulse, and stays on just long enough to get ions moving
Ions then drift through an evacuated ‘drift tube’

All ions enter with sem KE because same acceleration V
while same KE, larger masses move slower
so small molecules get to the far end first
    Larger ions take longer
Flight time 1-30μsec

Count how long it takes, and figure mass from there

Need extremely fast electronics
resolution and reproducibility not nearly as good as other types
but simple to use, extremely rugged, essentially no upper limit on range

20C-4 Fourier Transform Instruments - Skip since we don’t have one

20C-5 Computerized Mass Spectrometers
Almost all mass spectrometers are computerized
    A simple 500 MW compounds may have 100 or more different ions
    for each ion ant to record height m/z
    So lots of data
    + lots of instrumental parameters need to be monitored and this is most easily done with a computer

Figure 20-20 example block diagram of a computer control

20D Applications of Molecular Mss Spectrometry
Lots and lots
Table 20-5 just the start

20D-1 Identification of Pure Compounds
Mass spectrum of pure compound has several useful parameters:
- MW of compound
- Molecular formula of compound
- Fragmentation pattern gives clues to functional groups
- Fragmentation pattern match can be used to identify the compound

**Molecular Weights of Mass Spectra**
For compounds or methods that give molecular ions, get MW of compound (or M+1 or M-1) directly. This gives molecular weight to high precision.

Have to be careful with EI, since often are looking at fragments rather than entire molecule.

**Molecular Formula's and exact molecular weights**
Certain mass spectrometers can determine molecular masses of a few ppm.

With this kind of resolution can distinguish purine (C₉H₄N₄ 120.044) from benzamidine (C₇H₈N₂ 120.069) from ethyltoluene (C₉H₁₂ 120.096) from acetophenone (C₈H₈O 120.07).

So can use highly precise mass to differentiate between compounds.
See Table 20-6 different formulas for nominally identical MW's.

**Molecular Formulas from Isotopic ratios**
Low resolution instruments only differentiate mass +/-1.
Look at ratio of M and M+1 peaks or M and M+2 for isotopic.
These isotopic ratios can also be used to narrow down a formula.
Again look at table 20-6.

**Structural information in Fragmentation Patterns**
Some general rules to predict fragmentation pattern:
(Rules not give in this text)
Usually can't identify each and every peak, but patterns used to pick up structural features.
Figure 20-22 peaks differ by 14, typical of straight chain hydrocarbons.

Alcohols usually have an (M-18)^+ peak due to loss of water.
Also usually a Mass 31 fragment from CH₂OH^+ ion.

See other texts.

**Identification by spectral comparison**
Trying to identify unknown from fragmentation pattern
1. Fragmentation pattern for each compound must be unique
2. Experimental conditions are reproducible

#1 can't differentiate stereo or geometric isomers, also some closely related compounds

#1 gets better with more peaks to look at therefore EI with large number of fragments is best

#2 is not always that good either. Pattern can depend on E of electron beam, distance between sample / source/ slit and general geometry of spectrometer. Particularly different between different kinds of instruments quad vs ion trap vs Maldi

Often can get a close match but not an exact match. If that is the case then put the actual compound on your machine and compare fragmentation patterns of samples obtained on exactly the same machine

**Computerized Library Searches**
Often Computer has built in library to compare to
That is what we have on our machine
Computer set up to find best match

**20D-2 Coupling Mass Spec with other techniques**
Can run a mass spec as a detector on HPLC, GC, or Capillary electrophoresis. In the case of the GC your sample is already in the gas phase, so about all you have to do is to split off a small sample to the mass detector and you are ready to go. In HPLC and Electrophoresis you have to get rid of a large amount of solvent first and this is more difficult

Tandem Mass Spec is another interesting new development
Here the ions from one mass spectrometer are subjected to conditions that further fragment them, and then they are passed to a second mass spectrometer for additional analysis

Used in Biochem to sequence proteins!

**20E Quantitative Applications of Mass Spec**
Can be done, won't cover here because fairly vague