Analytical Cume

1. Using elementary relationships connecting velocity and time, energy and mass, etc, derive mass analysis equations for three types of mass analyzers.

2. Consider each of these mass analysis equations and comment on the effects of operating parameters on mass range, mass resolution and mass measurement accuracy.

3. For one type of analyzer, comment in detail on the effects of initial conditions on mass analyzer performance and describe steps used to improve performance.

1. \[ eV = \frac{1}{2}mv^2 \quad V = \text{accel voltage}, e = \text{charge}, v = \text{velocity} \]
   \[ \Rightarrow v = \left(\frac{2ev}{m}\right)^{\frac{1}{2}} \]
   \[ B = \frac{mv}{r} \quad B = \text{field strength} \]
   \[ \Rightarrow B = \frac{m^2}{r} \quad (i) \]
   \[ m/e = \frac{Br}{v} = \frac{Br}{(2ev)^{\frac{1}{2}}} \]
   \[ \Rightarrow m/e = \frac{B^2r^2}{2V} \]

2. \[ f = \frac{1}{T} = \frac{v}{2\pi r} \quad f = \text{cyclotron frequency} \]
   from (i) above,
   \[ f = \frac{v}{2\pi r} = \frac{Be}{2\pi rm} = \frac{Be}{2\pi m} \]
   \[ \Rightarrow m/e = \frac{B}{2\pi f} = \frac{B}{\omega} \]

3. \[ t = \frac{d}{v} = \frac{dm^2}{(2ev)^{\frac{1}{2}}} \]
   \[ \Rightarrow m/e = \frac{2Vt^2}{d^2} \]
1. Sector:
\[ \frac{\text{mass range incr. w. } B, r \text{ and dec. w. } V}{m} = \frac{2SB}{B} + 2\frac{sr}{r} - \frac{SV}{V} \]
- incr. as \( B \) decreases (stable field)
- decr. as \( B \) increases (collimated beam)
- \( V \) decreases (narrow range KE's)

Mass accuracy depends on some factors.

2. ICR:
\[ \frac{\text{mass range incr. as } B \text{ increases } s \text{ as } r \text{ decr.}}{m} = \frac{SB}{B} - \frac{Sw}{w} \]
- incr. as \( B \) increases (field stability)
- decr. as \( B \) increases (\( Sw \) is normally negligible except for space charge effects)

Mass acc. - same factors

3. TOF:
\[ \frac{\text{mass range incr. w. time, with accl.}}{m} = \frac{SV}{V} + 2\frac{st}{t} - 2\frac{sd}{d} \]
- Strong dependence of range KE's (\( SV \))
- Range times and range distances

Details of anyone - e.g. in TOF:
- Focuss for position - velocity by
- Focuss for angle - velocity by
  - (McIver - McTearson)
  - (sector field)
1. a. In general

\[ \frac{d(P)}{dt} = k \cdot (A)^a \cdot (B)^b \]

In this particular case, and at low substrate concentration, \( S \ll K_m \),

The reaction is:

\[ \frac{d(P)}{dt} = k \cdot (E)_{total} \cdot (S) \]

It is first order with respect to enzyme, first order with respect to substrate and second order, overall. Incidentally, the rate constant \( k = \frac{k_{cat}}{K_m} \) and this has the dimensions of a second order rate constant \( (M^{-1} \cdot s^{-1}) \).

As the substrate concentration is greatly increased so that \( S \gg K_m \) the enzyme is completely saturated with substrate. Thus adding more substrate does not increase the rate and \( \frac{d(P)}{dt} = k \cdot (E)_{total} \) where \( k = k_{cat} \) whose units are \( s^{-1} \), the units of a first order rate constant. The reaction is first order in enzyme, first order overall.

b. The rate changes due to the depletion of reactants. However, the enzyme, which serves as a catalyst, does not become depleted – only the substrate does.

At low substrate concentration, the time course is first order with a half-life of

\[ t_{1/2} = \frac{1}{k_{cat} \cdot (E)_{total}} \] where \( (E)_{total} \) is a constant. At high substrate concentration,

\[ \frac{d(P)}{dt} = k \cdot (E)_{total} = k_{cat} \cdot (E)_{total} \]
2. a. Ribonucleic acids, RNA, occasionally show enzymatic activity. Until recently, most known enzymatic activity was restricted to the processing of RNA into mature RNA primarily by splicing out introns.

b. The RNA enzymes are called ribozymes.

c. The recent X-ray structural analysis of ribosomes provided convincing evidence that rRNA (not the proteins) is the principal catalytic agent of ribosomal protein synthesis. A slogan “ribosome is a ribozyme” was coined.

3. a. Ser. Serine proteinases (proteases) trypsin, chymotrypsin, thrombin, as well as subtilisin and serine carboxypeptidases (plant) are examples.

b. Thr. Thr proteosomes. The NH₂ terminal residue is the catalytic residue in all β subunits of proteosomes. In Tetrehyrena there is only one kind of β subunits. In eukaryotes there are several kinds with different specificities.

c. Cys. Cysteine proteinases. The plant enzymes papain (from papaya) and bromelain from pineapple are examples. Most proteinases in eukaryotic lysosomes (cell organelles) are cysteine proteinases. Most of these are called cathepsins and are distinguished from one another by a capital letter such as cathepsin L. Note that this nomenclature was introduced too early. Cathepsin G is a serine proteinase.

d. Asp. Aspartyl proteinases. Two Asp residues, one from each of the two subunits comprise the active site. The genes for such enzymes are clearly products of elongation by gene duplication. Examples: pepsin and gastricsin in the stomach. Many viral enzymes are aspartylproteinases. The HIV enzyme is a noncovalent homodimer.
4. \[
\frac{d(P)}{dt} = \frac{d(S)}{dt} = \frac{kcat (S)_c (E)_c}{K_m + \frac{(S)}{K_m} + \frac{(I)}{K_I}}
\]

Substituting given values we get

\[
\frac{d(P)}{dt} = \frac{100 S^{-1} x (1.00 \times 10^{-2} M)(1.00 \times 10^{-8} M)}{1 + (1.00 \times 10^{-2} M) + (1.00 \times 10^{-3} M) + (1.00 \times 10^{-4} M)} = \frac{1.00 \times 10^{-5} Ms^{-1}}{1 + 10} = 8.3 \times 10^{-8} Ms^{-1}
\]

5. a. Proteolytic enzymes that are used to digest food are quite dangerous outside of the digestive tract. Thus synthesis of the inactive precursors, which are then secreted into the digestive tract and activated there, gets rid of this danger. Proteolytic enzymes of the blood clotting cascade are circulating in the blood as zymogens. Only small portion of these zymogens is activated to produce a local blood clot and to avoid massive clotting.

b. The peptides are removed from the NH$_2$ terminus of zymogens. Thus the active enzyme is never synthesized as protein synthesis is always from the NH$_2$ terminus to the COOH terminus. If the COOH terminal peptide were to be removed, the active enzyme would be transiently present during the synthesis.
1. (20 pts.) Humulones are a class of α-acids found in hops. When hops are boiled, the insoluble humulones are isomerized to the soluble isohumulones, which give beer its pleasant bitterness.

Provide a mechanism for this transformation.
(from the Autumn 2003 issue of *Chemistry*)

There is more than one plausible mechanism for this rearrangement. The most straightforward approach to ring contraction involves an acyloin (α-hydroxyketone) rearrangement, as illustrated below:

It is also plausible to proceed through an acyclic intermediate involving an enediol and ketene:
2. (30 pts.) An enantioselective version of the three-component Mannich reaction which uses L-proline as a catalyst has recently been developed (see example reaction below). The reaction proceeds with high levels of syn diastereoselectivity.

\[
\begin{align*}
\text{CHO} + \text{OCH}_3 \text{NH}_2 + \text{CHO} \text{Br} & \rightarrow \text{CHO} + \text{OCH}_3 \text{NH}_2 + \text{CHO} \text{Br} \\
& \text{L-proline (30 mol\%)} \\
& \text{DMF, 4 \degree C} \\
& \text{81\% yield} \\
& \text{91\% ee}
\end{align*}
\]

a) Provide a reaction mechanism which explains the stereoselectivity of this reaction.

b) What is the product ratio of (2R, 3R) to (2S, 3S) enantiomer? 4.5 : 95.5

(from J. Org. Chem. 2003, 68, 9624-34)
(30 pts.) In their quest for diversity-oriented synthesis, Schreiber and coworkers have recently introduced the concept of "sigma elements"—functional groups which are pre-designed to increase architectural complexity from a common skeletal backbone. This involves generating reactive intermediates capable of undergoing a variety of context-dependent transformations. An example of this is presented below:

\[ X1 \]
\[ \text{RO}(\text{CH}_2)_6 \]
\[ \text{OH} \]
\[ \text{O} \]
\[ \text{NHPh} \]
\[ X2 \]
\[ \text{RO}(\text{CH}_2)_6 \]
\[ \text{OH} \]
\[ \text{N} \]
\[ \text{Bn} \]
\[ X3 \]
\[ \text{RO}(\text{CH}_2)_6 \]
\[ \text{OH} \]
\[ \text{O} \]
\[ \text{Bn} \]
\[ a, b \]
\[ Y1 \]
\[ \text{RO}(\text{CH}_2)_6 \]
\[ \text{OH} \]
\[ \text{O} \]
\[ \text{NHPh} \]
\[ Y2 \]
\[ \text{RO}(\text{CH}_2)_4 \]
\[ \text{CH}_3 \]
\[ \text{Bn} \]
\[ Y3 \]
\[ \text{RO}(\text{CH}_2)_6 \]
\[ \text{CH}_3 \]
\[ \text{Bn} \]

a) N-bromosuccinimide, NaHCO\textsubscript{3}, THF/H\textsubscript{2}O, rt. b) PPTS (mild protic acid), CH\textsubscript{2}Cl\textsubscript{2}, 45 °C.

All of the transformations above go through a common structural intermediate. Provide mechanisms for each reaction, making clear use of this intermediate.

(from Science 2003, 302, 613-18)
4. (20 pts.) Boc-protected serine methyl ester was treated with excess vinyl magnesium bromide to yield two products with equal molecular weight, A and B. Adding copper salts had a profound effect on the ratio of A to B.

\[
\begin{align*}
\text{HO} & \text{C} & \text{O}_2 \text{C} & \text{H}_3 \\
\text{NH} & \text{Boc} & \text{CO}_2 \text{CH}_3 & \text{MgBr}
\end{align*}
\xrightarrow{\text{THF}, -45 ^\circ C}
\begin{align*}
\text{A} + \text{B} & \\
\text{1.0 equiv} & \text{3-5 equiv} & 0 \text{ mol\% CuCN:} & 50 : 50 \\
& & 30 \text{ mol\% CuCN:} & 92 : 8
\end{align*}
\]

a) What are A and B? Provide a mechanistic rationale.

b) Why does the ratio change when copper salts are added?

CuCN promotes the formation of organocuprates, which add to enones in 1,4-fashion.

(from Org. Lett. 2003, 5, 4887-90)