Analytical Cume Question and Answers

Two dimensional gas chromatography coupled with time-of-flight (TOF) mass spectrometry is theoretically capable of seeing 60,000 compounds in 30 minutes. This new technique is becoming popular in the analysis of petroleum samples, metabolite analysis, and environmental monitoring where it is an advantage to examine and identify huge numbers of volatile organic compounds within a short time.

1. How is two dimensional gas chromatography (2-D GC) achieved with this instrument?

Answer. One of the major issues is the transfer of analytes between the two columns. By far the most widely used method is by thermal modulation TM. The principle in thermal modulation is to 1) rapidly cool the interface between the two columns for short period of time to concentration a portion of an analyte peak from the first dimension and 2) then rapidly heat that portion of the column to "inject" the concentrated fraction into the second dimension. While that portion of the column is being heated a section behind it is being cool to trap the next fraction of analyte. Both the cold and hot zones are moved a short distance along the column in a peristaltic movement. TM used to be achieved with a cold, groved mechanical device that fit around the column and moved for a short distance along the column. Now it is generally done with compressed CO2. It is also important that the second column is contained in a second oven operated at a higher temperature than the first. Elution from columns is achieved by temperature programming both ovens.

2. Discuss the stationary phases generally used in 2-D GC and the order in which columns containing them are coupled.

Answer. 2-D GC is most generally achieved by coupling a long column coated with a non-polar liquid phase with a short column coated with a polar liquid phase. The non-polar stationary phase is generally a poly(dimethylsiloxane) of high molecular weight to give it a very low vapor pressure at temperatures up to 350 oC. The polar stationary phase is generally also a polysiloxane, but with some nitrile or phenyl group in the silane monomer. Older polar phases such as polyoxyethylene or Apeizon L are even used. The first column separates analytes nearly in the order of their solution vapor pressure or boiling point. The polar column separates mixtures of analytes in a fraction on the basis of their polarity. Analyte polarity generally arises from functional groups in the molecule.

3. What are typical separation times in the first and second dimensions of GC?

Answer. The separation time in the first dimension is in the range of 30 min to an hr. Based on the fact that a high resolution GC column can produce 500 peak this means peak widths in the first dimension are 4-8 sec. Separations in the second dimension are generally achieved within 1-4 sec. Peak capacity on the short second column is generally no more than 10-20 peaks. This means that peaks in the second dimension are often in the range of 100 msec wide. Separations in the TOF mass spectrometer are achieved in roughly a usec. Peak capacity in the TOF is often 10 but could be up to 100. Thus separations in the three dimensions vary by roughly a thousand fold in total separation time between each dimension. Using peak capacities of 500, 10, and 10, respectively in the three separation dimensions it is seen that the peak capacity of the system easily exceeds 50,000 in 30-60 min.

4. Why is TOF preferred over other types of mass spectrometry?

Answer. TOF is 10-00 times faster that most other mass spectrometers. In light of the fact that peaks in the second dimension can be 100 msec wide and you would like to acquire 10 or more mass spectra from across the width of the GC peak, mass spectra must be acquired in a msec time frame. This is most easily done with the TOF.

5. What advantage does GC/GC-MS have over multidimensional MS as seen in the analysis of petroleum samples?

Answer. The major advantage of GC/GC is that one readily gains a picture of analyte polarity for large numbers of analytes. This is more difficult to achieve with MS/MS. Although hydroxyl groups are often seen in mass spectra by an M+-18 peak, other polar functional groups are not as easily identified. With petroleum samples two dimensional plots using retention time in the first dimension on the X axis and the second dimension on the Y axis give a series of nearly straight lines in which the slope is a function of increasing relative polarity. In other words, polar functional groups on small molecule give larger slopes than those on large molecules. It is also very easy to recognize a homologous series with multiple polar functional groups.

Answers to Biochemistry Cumulative Exam Questions

1. An average globular protein is stabilized by hundreds of weak bonds. However, the ΔG of denaturation of an average globular protein is only ~15 kcal/mole, because although hundreds of weak bonds must be broken during unfolding of the native protein, nearly an identical number of weak bonds are formed in the denatured state. Thus, the net difference in weak bonds between a native and denatured protein is generally less than 15. With the average ΔG of formation of a weak bond being between 1 and 5 kcal/mole, a difference of only a few weak bonds between a native and denatured protein can account for the small free energy of denaturation. Energetically, the most important weak bond in the stabilization of a native protein is the hydrophobic bond. The reason for this is that protein H-bonding groups can form as easily with water as with other H-bonding sites in the protein. Further, salt bridges or ion pair interactions are generally on the protein surface where hydration forces can weaken them. Van der Waals interactions also undoubtedly contribute to native protein stability, but many also form in the denatured state and the magnitude of their ΔG formation is small.

2. Assume the density of an average globular protein is 1.3 g/cc.

$$40{,}000\text{g/mole} \times \frac{1cc}{1.3g} \times \frac{1mole}{6.02x10^{23}\,molecules} \times \frac{(10^7\,nm)^3}{1cc} = \text{Volume}$$

$V = 51nm^3 = 4/3\pi\, r^3$

$r = 2.3nm$

diameter = 2r = 4.6nm

3. <u>α-helicol structure</u>

C=0 of each amino acid is H-bonded to the N-H of the amino acid four residues downstream in the sequence.

There is a 1.5Å rise along the helical axis for each amino acid

All side chains extend radially outward

There is an amino acid every 100° around the helical axis or 3.6 residues per complete turn

The screw sense is right-handed or clockwise

<u>β-sheet structure</u>

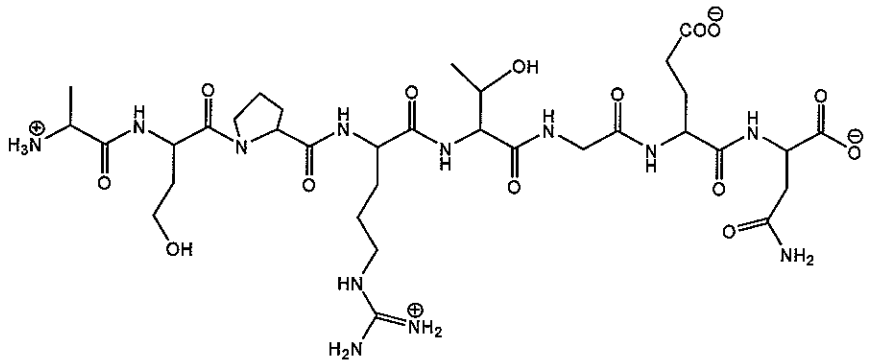
The axial distance between contiguous amino acids along the same strand is ~ 3.5Å

The sheet is stabilized by H bonds connecting peptide carbonyl and amide NH residues in adjacent strands

Side chains alternate pointing vertically above and below the sheet

Adjacent strands in a sheet can run either parallel or anti-parallel to each other

4.



5. a) List all ionicable amino acids starting from most acidic to most basic terminal COOH

Asp - 5
Glu - 4
His - 5
Cys - 2
Tyr - 2
Lys - 6
Arg - 5
Terminal - NH_3^+

b) Start with low pH form and titrate mentally with NaOH until you achieve an equal number of positive and negative charges. In the above case, that will occur after both cysteins but no tyrosines or lysines have been titrated to their high pH forms. Thus, the protein will be neutral at the mid-point between the two pka's.

$$P^I = \frac{pkacys + pkaTyr}{2} = \frac{8.3 + 10}{\alpha} = 9.15$$

Inorganic Cumulative Exam
November 12, 2005

KEY

1. a. $\chi_N = 3.04$ (Pauling scale)

$\chi_H = 2.20$

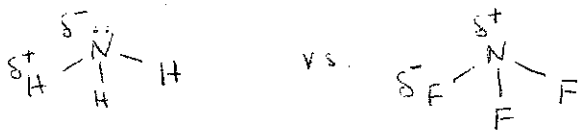
$\chi_F = 3.98$

$\Delta\chi(N-H) = 0.84$

$\Delta\chi(N-F) = 0.94$

However, $\mu(NH_3) = 1.47\,D$

$\mu(NF_3) = 0.24\,D$



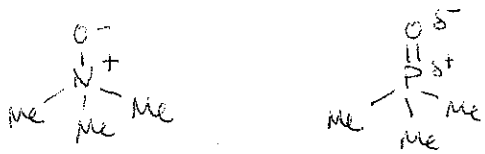
(more covalent also)

b. $\mu(NH_3) = 1.47\,D$ $\Delta\chi(N-H) = 0.84$

$\mu(PH_3) = 0.374\,D$ $\Delta\chi(P-H) = 2.20 - 2.19 = 0.01$

PH_3 has very little ionic character. Thus, much lower charge density on each atom.

c. Me_3NO greater μ than Me_3PO.



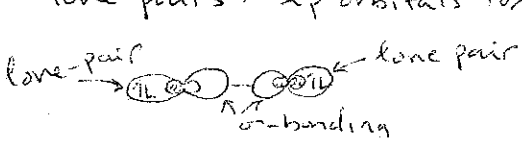
P=O is a multiple bond with high covalent character.

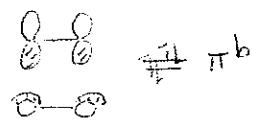
Me_3NO is purchased as the dihydrate. $Me_3\overset{+}{N}OH\ [OH^-]$ – ionic.

d. $:C\equiv O:$ (δ⁻ on C, δ⁺ on O)

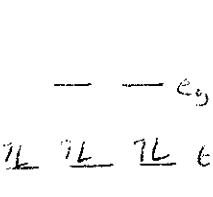
Small μ because the molecule is covalent with significant π-bonding between C and O. Thus, formal charges are as shown.

sp on C & O. One sp forms σ bond and the other two accom. lone pairs. 2p orbitals for 2 π bonds.

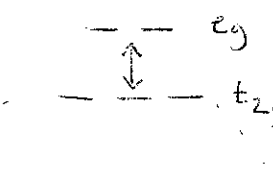




2. a. CrI_6^{3-} d^3 $t_{2g}^3 e_g^0$



b. $Cr(CO)_6$ d^6 $t_{2g}^6 e_g^0$



c.

for NH_3 - σ only

π orbitals - filled ligand centered

For I^- - π donor raises t_{2g} close to e_g in energy

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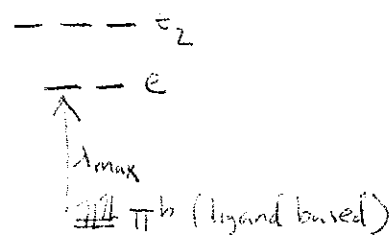
3. MnO_4^- $\lambda_{max} \sim 520$ nm

ReO_4^- $\lambda_{max} \sim 240$ nm

ReS_4^- $\lambda_{max} \sim 480$ nm

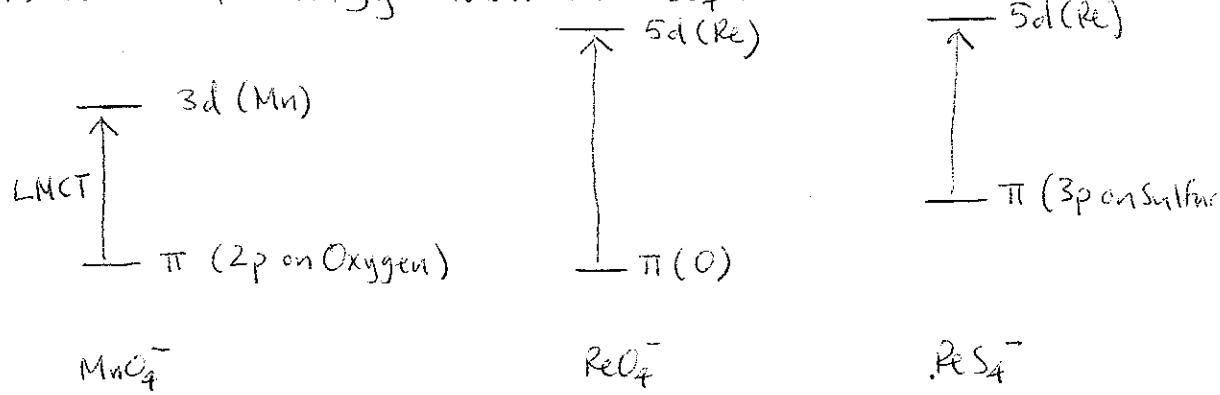
This is due to LMCT (ligand to metal charge transfer)



The energy gap increases for ReO_4^- because 5d orbitals are higher in energy than 3d orbitals in Mn. Thus, ReO_4^- absorbs in the UV.

3. Continued:

As for ReS_4^-, the π orbitals from sulfur (3p) are higher in energy than 2p of oxygen. Thus, the absorbance is lower in energy than for ReO_4^-.



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4. d^5 $\underset{m_\ell}{\underset{+2}{\uparrow}\ \underset{+1}{\uparrow}\ \underset{0}{\uparrow}\ \underset{-1}{\uparrow}\ \underset{-2}{\uparrow}}$ ^6S

d^7 $\underset{m_\ell}{\underset{+2}{\uparrow\downarrow}\ \underset{+1}{\uparrow\downarrow}\ \underset{0}{\uparrow}\ \underset{-1}{\uparrow}\ \underset{-2}{\uparrow}}$ ^4F

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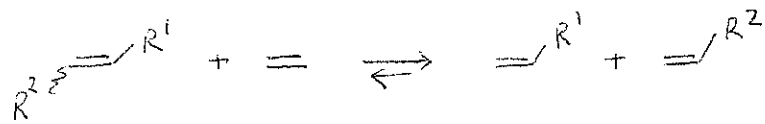
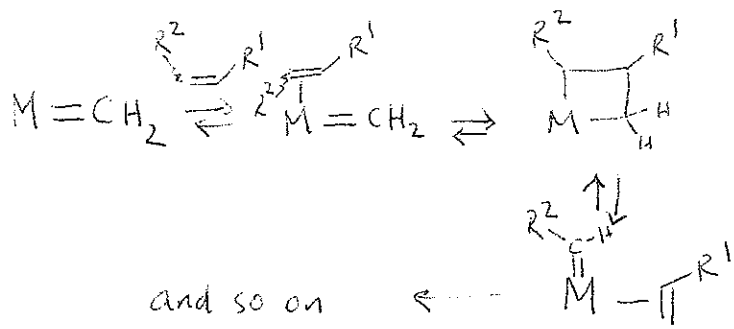
5. Olefin Metathesis:



Mechanism:



and so on until equil. is reached (above equation)

5.

Continued:

Chauvin: Proposed the mechanism thirty years ago and provided which metals are appropriate for this reaction.

Schrock: Developed Mo-based single site catalysts for this reaction. His catalyst was the first well characterized $M=C^{R}_{R}$ (metal alkylidene) catalyst.

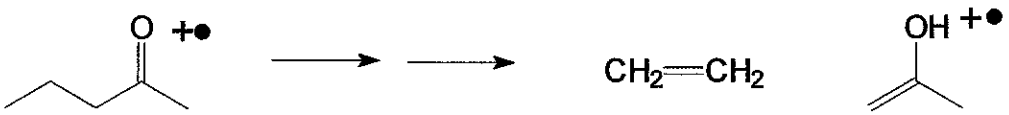
Grubbs: Developed Ru-based catalyst that is tolerant of air/moisture & other functional groups. This allowed olefin metathesis use in Organic synthesis.

Both Schrock & Grubbs provided key support for Chauvin's initial mechanism.

THE END

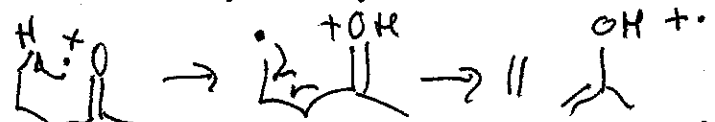
Organic cumulative examination **Hilkka I. Kenttämaa**

Consider the only named unimolecular dissociation reaction in gas-phase ion chemistry and mass spectrometry, the McLafferty rearrangement, shown below for the radical cation of 2-pentanone:



1) Show two likely mechanisms (include arrows) for this reaction, a stepwise one and a concerted one (remember to indicate the formal charge and radical sites). Explain why you think that these mechanisms are thermodynamically and kinetically feasible. Compare them, and point out which one (if either) is likely to be more favorable thermodynamically and which one kinetically. 30 pts.

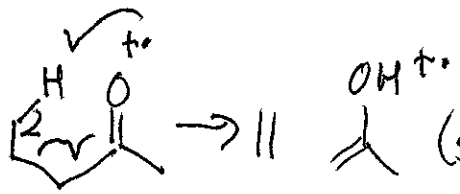
Stepwise:



(10 pts) m+• (5 pts)

Both yield stable ionic and neutral products. Both TS's involve a 6-membered ring which is kinetically favorable. They are equally favorable thermodynamically (same products), stepwise is likely to be kinetically favored due to less tight TS.

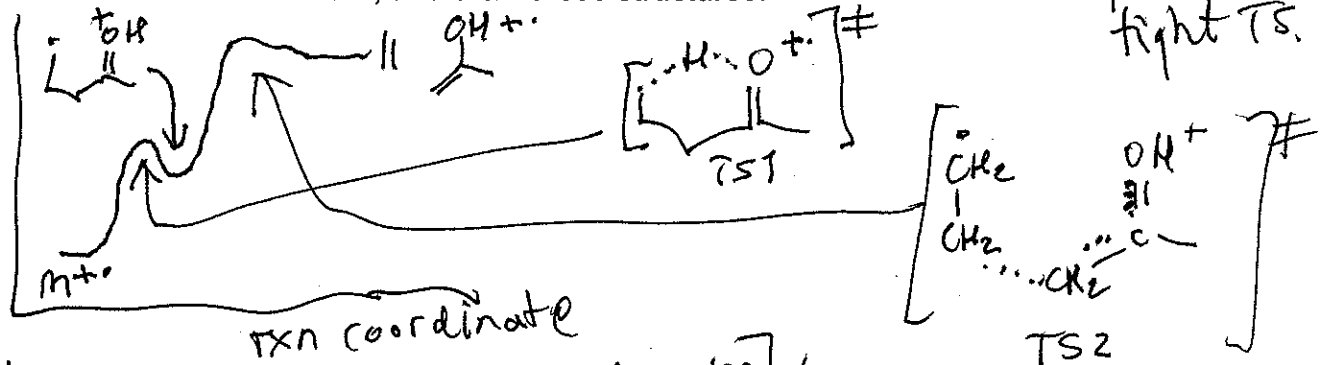
concerted:



(10 ptr.) (5 pts.)

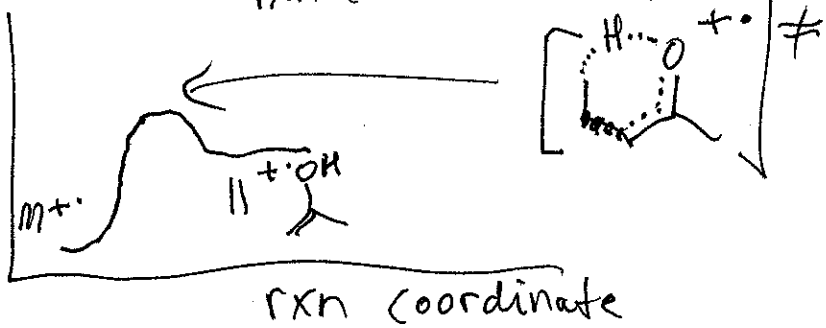
20 pts. 2) Sketch a potential energy surface for each mechanism, and explain where the mechanisms differ. Point out what structure corresponds to each energy minimum and transition state, and draw these structures.

Stepwise:



(8 pts.)

Concerted



(8 pts.)

Difference: Stepwise → intermediate (4 pts.)

20 pts. 3) Devise an experiment that might allow you to distinguish the above two mechanisms either in the gas phase or in solution. Explain what sort of data you expect, and how this data could allow you to distinguish the mechanisms.

- trap the intermediate in stepwise mechanism by radical reactions; use MS or NMR to analyse products
- calculate $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for each reaction, and study their E (or T) dependences
- label the transferred H with D and study D-isotope effects (should be larger for stepwise mechanism)

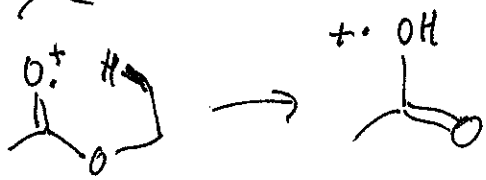
10 pts. 4) Give a guess as to which mechanism is most likely, and justify your answer. Either:

Stepwise: less ordered TS → kinetically favorable

Concerted: TS may be of lower energy → possibly making this pathway kinetically favorable

20 pts. 5) Show the products that McLafferty rearrangement should yield (if any) for the radical cations of acetone, ethyl acetate, ethyl propionate, and 1-pentene.

none (5 pts.)



(5 pts.)

(5 pts.)

(5 pts.)

P. Chem Exam Nov. 12 2005

I.

1) $\frac{3}{2}RT$

2) $\Delta S = R \ln (V/V_0)$

3) $\Delta S = R \ln (T/T_0)^C$, $C = C_V/R = 3/2$

4) $\Delta \mu = -T\Delta S = -RT \ln 2 \cong -1.7$ kJ/mol ($\cong 2.9 \times 10^{-21}$ J/molecule)

5) $\Delta A = \cancel{\Delta U}^{0} - T\Delta S = -1.7$ kJ/mol

II.

1) question was not worded correctly, should have been const. S, P, N_i or T, V, N_i etc. (all answers are accepted)

2) $Y = U \underbrace{-TS}_{-\left(\frac{\partial U}{\partial S}\right)_{V,N_i}} \underbrace{+PV}_{-\left(\frac{\partial U}{\partial V}\right)_{S,N_i}} \underbrace{-\mu_1 N_1}_{-\left(\frac{\partial U}{\partial N_1}\right)_{S,V,N_i \neq N_1}} = G - \mu_1 N_1$

3) $\mu_{solid} = \mu_{solution}$

4) $2\mu_A = \mu_B$

5) $\left(\frac{\partial P}{\partial T}\right)_V = \frac{-(\partial V/\partial T)_P}{(\partial V/\partial P)_T} = \frac{-\frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_P}{\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T} = \frac{-\alpha_P}{-K_T} = \frac{\alpha_P}{K_T}$