Cumulative Examination: Analytical

August 2010

Solutions

Question 1.

Thermal noise: fluctuations in current flow through a resistor.

\[ i_{\text{noise}} = \sqrt{\frac{4k_B T \Delta f}{R}} \text{ where } \Delta f \text{ is the bandwidth} \]

\[ v_{\text{noise}} = \sqrt{4kTR\Delta f} \]

Shot noise: Random passage of charge at a junction

\[ i_{\text{noise}} = \sqrt{2e\Delta f} \]

Environmental noise: radio, power source, etc. interference at particular frequencies.

1/f noise: origin is not clear. It increases with 1/f.

a. Reduces thermal noise
b. 1/f noise high.
c. Amplification at single frequency avoids shot and thermal (Johnson) noise and gives fixed gain. Chop signal and use phase sensitive detector.
d. Measure encoded signal all time; avoid usual dispersion by wavelength etc. Encoding can be chopping at particular frequency.
e. Fundamental limitation is random noise, drift. Also S/N improves with no. of measurements; so time becomes a factor.

Question 2.

a. Principal Component Analysis (PCA). The data is converted into a matrix which is solved to summarize original data in terms of a set of synthetic variables that are orthogonal each other and can be ordered in terms of importance. Most variation is included in PC1, etc.
b. Set of data on a group of samples which is used to discover the underlying sources of variance. Once this is done, the data from a validation set can be tested against the outcomes of the training set to establish degree of similarity w.r.t particular variables. The training set is used to make a predictive model for data which have particular characteristics in common.

Question 3.

a.

b. Low energy means poor conversion into electrons, can’t use photoelectron effect.

c. Higher energy – 1 β-ray = 1 KeV e⁻ => 10⁷ secondary e’s.

Question 4.

a. Phosphorescence, chemical reactions (photochemistry), Rayleigh scattering, Raman scattering.

For organics Jablonski diagram S₀→Sₙ→...S₁→S₀

Sₙ > 10%

Quantum dots are stable to photo bleaching.
b. By examining X-ray fluorescence.
   Use an energy dispersive instrument – measure height of signal as proportional to
   Atomic number. Individual counts.

c. **Photo ionization mass spectrometry** \( m + h \nu \rightarrow m^+ + e^- \)
   (measure \( m^+ \))

**Photoelectron spectroscopy** \( m + h \nu \rightarrow m^+ + e^- \)
   (measure \( e^- \))

Combined in PIPECO experiment. Coincidence measurement of ion and photoelectron.

**Question 5.**

Standard addition, Internal standard (including externally labeled standards), External standard.

a. \( \sigma^2 \) (variance) decreases with \( 1/N \).

b. Qualitatively decreases with amount of analyte.

c. LOD: \( S/N = 3 \)
   LOQ: \( S/N = 10 \)
Multivariate statistics

(From Wikipedia)

Multivariate statistics is a form of statistics encompassing the simultaneous observation and analysis of more than one statistical variable. The application of multivariate statistics is multivariate analysis. Methods of bivariate statistics, for example ANOVA and correlation, are special cases of multivariate statistics in which two variables are involved.

There are many different models, each with its own type of analysis:

1. Multivariate analysis of variance (MANOVA) methods extend analysis of variance methods to cover cases where there is more than one dependent variable to be analyzed simultaneously.

2. Principal components analysis (PCA) finds a set of synthetic variables that summarize the original set. It rotates the axes of variation to give a new set of ordered orthogonal axes that summarize decreasing proportions of the variation.

3. Factor analysis is similar to PCA but attempts to determine a smaller set of synthetic variables that could explain the original set.

4. Canonical correlation analysis finds linear relationships among two sets of variables; it is the generalized (i.e. canonical) version of correlation.

5. Redundancy analysis is similar to canonical correlation analysis but deriving a minimal set of synthetic variables from one set of (independent) variables that explains as much variance as possible in the other (dependent) set. It is a multivariate analogue of regression.

6. Correspondence analysis (CA), or reciprocal averaging, finds (like PCA) a set of synthetic variables that summarize the original set. The underlying model assumes chi-squared dissimilarities among records (cases). There is also canonical correspondence analysis (CCA) for summarizing the joint variation in two sets of variables.

7. Multidimensional scaling covers various algorithms to determine a set of synthetic variables that best represent the pair wise distances between records. The original method is principal coordinates analysis (based on PCA).

8. Hotelling’s T-square is a generalization of Student’s t statistic that is used in multivariate hypothesis testing.
9. Discriminant function or canonical variate analysis attempts to establish whether a set of variables can be used to distinguish between two or more groups.

10. Linear discriminant analysis (LDA) computes a linear predictor from two sets of normally distributed data to allow for classification of new observations.

11. Clustering systems assign objects into groups (called clusters) so that objects from the same cluster are more similar to each other than objects from different clusters.

12. Recursive partitioning creates a decision tree that strives to correctly classify members of the population based on a dichotomous dependent variable.

Biochemistry Cumulative Exam

Title: Enzymes

August 27, 2010

1. Absorption of food in the stomach and intestine depends on the ability of molecules to penetrate the cell membranes and pass into the bloodstream. Because hydrophobic molecules are more likely to be absorbed than hydrophilic or charged molecules, the absorption of the orally available drugs depends on their pK_a values and the pH of the digestive systems. Aspirin has a carboxylic group (pKa = 4). Calculate the percentage of the protonated form of aspirin available for absorption in the stomach (pH = 2) and in the intestine (pH = 6).

(20 points)

Stomach pH = 2

\[
pH = pK_a + \log \frac{[RCOO^-]}{[RCOOH]}
\]

\[
2 = 4 + \log \frac{[RCOO^-]}{[RCOOH]}
\]

\[
\frac{[RCOO^-]}{[RCOOH]} = \frac{1}{100}
\]

The percentage of the uncharged species (RCOOH) is equal to the amount of RCOOH divided by the total of RCOO^- and RCOOH, times 100%

\[
\frac{[RCOOH]}{[RCOOH]} + \frac{[RCOO^-]}{[RCOOH]} \times 100 = \frac{100}{100} + 1 \times 100 = 99%
\]

Therefore nearly all aspirin will be in the form available for absorption.

Intestine pH = 6

\[
pH = pK_a + \log \frac{[RCOO^-]}{[RCOOH]}
\]

\[
6 = 4 + \log \frac{[RCOO^-]}{[RCOOH]}
\]

\[
\frac{[RCOO^-]}{[RCOOH]} = \frac{100}{1}
\]

\[
\frac{[RCOO^-]}{[RCOOH]} + \frac{[RCOO^-]}{[RCOOH]} \times 100 = \frac{1}{100} + 1 \times 100 = 0.99\% (\sim 1\%).
\]

Only 1% will be absorbed at pH = 6 in the intestine.

2. (A) The covalent catalytic mechanism of an enzyme depends on a single active site Cys whose pKa is 8. A mutation in nearby residue alters the microenvironment and changes it to 10. Would the mutation cause the reaction rate to increase or decrease? Explain. (10 points)
The active form of the enzyme contains the thiolate ion. The increased pKₐ would increase the nucleophilicity of the thiolate, and thereby increase the rate of reaction catalyzed by the active form of the enzyme. However, at physiological pH there will be less of the active form of the enzyme and therefore the overall rate would decrease.

(B) A 12-mer polypeptide was digested with proteases, and following cleaved products were isolated. What is the sequence of the original peptide? Which proteases were used? (10 marks)

ISPKEF
NQRGY
NQR
EFK
GYISPKEFK

NQRGYISPKEFK

Trypsin (cuts after K and R)
Chymotrypsin (cuts after F and Y)

3. (c) Draw the Lineweaver Burk plot for an uncompetitive inhibitor? How does it affect \( V_{\text{max}} \) and \( K_m \)? (20 marks)

\[
\begin{align*}
E + S & \rightleftharpoons ES \\
& \rightarrow E + P
\end{align*}
\]

\[
\frac{1}{v_i} \frac{1}{[I]} \\
\frac{1}{[S]}
\]

\( V_{\text{max}} \) decreased by conversion of some E to ESI
\( K_m \) (\( K_{\text{mapp}} \)) is also decreased

4. (a) Why do allosteric enzymes show sigmoidal curves? (10 marks)

Due to cooperativity
(b) Define the following terms:
(a) Lyases

A lyase is an enzyme that catalyzes the breaking of various chemical bonds by means other than hydrolysis and oxidation, often forming a new double bond or a new ring structure.

(b) Pseudo-first order reaction

A second-order reaction carried out with a large excess of one reactant, with the consequence that the rate depends only on the concentration of the other reactant.

For example: A and B react to produce P:

If the initial concentration of the reactant A is much larger than the concentration of B, the concentration of A will not change appreciably during the course of the reaction. The concentration of the reactant in excess will remain almost constant. Thus, the rate only depends on the concentration of B. This will be a pseudo-first order reaction.

6. The enzyme catalase catalyzes the decomposition of H₂O₂.

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

The turnover number for catalase is \(4 \times 10^7\) per second. Km of catalase for its substrate is 0.11M.
(a) In an experiment using 3 nanomole/L of catalase, what is the Vmax.

\[ V_{\text{max}} = k_{\text{cat}} [E]_{\text{Total}} \]

\[ = \left(4 \times 10^7\right) \times \left(3 \times 10^{-6}\right) = 120 \text{ mmol/ml sec} \]

(b) What is the catalytic efficiency of catalase?

\[ K_{\text{cat}}/K_m = 3.63 \times 10^8 /\text{M sec} \]
-Answer Key-
Inorganic Chemistry Cumulative Exam
Some Basic Inorganic Chemistry
Purdue University
August 28, 2010

Question 1:
It’s a $d$-$d$ transition. The octahedral compound $[\text{Ti(OH}_2\text{)}_6]^{3+}$ has Ti in the +3 oxidation state, with one $d$ electron. This one $d$ electron moves between the $e_g$ and $t_{2g}$ set of orbitals in the octahedral field splitting scheme.

Question 2:
The Cl$^-$ and I$^-$ ligands each have a different effect upon splitting of the crystal field. Cl$^-$ is a stronger field ligand than I$^-$, thereby creating a larger energy gap and a different color of light to be absorbed.

Question 3:
Although TiBr$_4$ has no $d$ electrons, and hence no $d$-$d$ transitions to bring about color, there are bromide-to-titanium charger-transfer bands. These charge-transfer bands are in the visible region, thus giving this compound color.

Question 4:
Ethylenediaminetetraacetic acid. Consisting of 4 carboxylate and 2 amine ligands, EDTA is a very strong chelator of metals. EDTA is used often when a generic strong metal chelator is needed.

Question 5:
Paramagnetic metal ions have unpaired electrons and diamagnetic metal centers do not.

Question 6:
The chelate effect states that a ligand with multiple donor atoms will bind to a metal center with higher affinity than monodentate ligands. For example, ethylenediamine ($\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$) will bind to a metal center with a significantly higher affinity than two ammonias. The driving force for the chelate effect comes from increased entropy in the system. For example:

$$\text{ethylenediamine ("en") + M(OH}_2\text{)}_2 \rightarrow \text{M(en) + 2 H}_2\text{O}$$

increases entropy by starting with two molecules and yielding three. Whereas:

$$2 \text{NH}_3 + \text{M(OH}_2\text{)}_2 \rightarrow \text{M(en) + 2 H}_2\text{O}$$

begins with 3 molecules and ends with 3 molecules.
a) $\text{Ph}_2\text{C} = \text{O} = \text{O} - \text{CPh}_3 \rightarrow 2 \text{Ph} - \text{C} = \text{O}$

b) $2 \text{Ph}_2\text{COPh} \rightarrow 2 \text{Ph} - \text{C} = \text{O}$

ii) Loss of $\text{Ph}^+$ is less likely because the $\text{sp}^2$ phenyl radical is less stable than $\text{Cl}_3^+$ and thus more difficult to form. (Note: Mercuric $\text{Cl}_3^+$ or Ph$^+$ are really all equally stable, and it is an issue of relative stability.)
C) If the hydrogen atom transfer product is less than 5% (≤ 5%), then the rate for the Wieland reaction must be >200 times that for hydrogen atom transfer with alcohol.

The rate of HAT is given by:

\[
\text{rate} = k_{\text{HAT}} [\text{RADICAL}] [\text{alcohol}]
\]

\[
\text{rate}_{\text{Wieland}} = k_{\text{Wieland}} [\text{RADICAL}]
\]

Since \( \text{rate}_{\text{Wieland}} > 200 \times \text{rate}_{\text{HAT}} \),

\[
k_{\text{HAT}} [\text{RADICAL}] < \frac{\text{rate}_{\text{Wieland}}}{200} \cdot \frac{[\text{alcohol}]}{[\text{RADICAL}]} = 1.3 \ M
\]

Using \( k_{\text{HAT}} = 1.8 \times 10^{-6} \) M s\(^{-1}\) and \( [\text{alcohol}] = 13 \ M \),

\[
k_{\text{Wieland}} = (200)(1.8 \times 10^{-6})(13) = 4.7 \times 10^{-4} \text{ s}^{-1}
\]

Given that the specified LFP value is \( > 6 \times 10^{-6} \) s\(^{-1}\),

that is also greater than \( 4.7 \times 10^{-4} \), so they **concur**.
d) LFP is a photochemical process and the reaction probably occurs in an excited electronic state.

e(i) The reaction of

$$\text{Ph}_3\text{CCl} \xrightarrow{\text{Zn}} \text{Ph}_3\text{CO}$$

(or mark as

\[
\text{Ph}_3\text{C} - \text{C} - \text{Ph}_3
\]

It does \textit{NOT} give \text{Ph}_3\text{C} - \text{C} - \text{Ph}_3

That's what Gomberg was trying to make.

ii) Gomberg's paper is considered the first report of transient carbon radical \( \text{Ph}_3\text{C}^\bullet \). It also contains the famous statement "I will continue this work in the future at wish to reserve the field to myself."
The planar structure is unstable for two reasons:

1) The C-C-C bond angles are a little bit off (108° instead of 109.5°) this is minor.
2) All the C-H bonds are eclipsed. That's very bad.

MP2: Möller-Plesset 2nd order
B3LYP: Becke 3(4)ran
cc-pVTZ: correlation-consistent polarized valence triple zeta
2c) i) Coupled Cluster with Single/double excitations

ii) Normally one would want triples added (CCSD(T)) to get accurate energies. However, for a simple closed-shell molecule it is probably OK to use CCSD. I would worry about the H-bonded structure, but it should be OK.

2d) Eyring's definition of TS is "the point of minimum flux" on the PES. A more practical answer might be a "saddle point on the free energy surface" which is often estimated by considering the electronic energy surface. So "a single imaginary frequency" or "negative force constant" is pretty close.
3) The proportion of B at temperature \( T \) is given by

\[ \frac{p_B}{p_A} = e^\frac{-\Delta E}{kT} \]

Since \( e^\frac{-\Delta E}{kT} \approx \frac{I_B}{I_A} \)

\[ \ln \left( \frac{I_B}{I_A} \right) = \ln \frac{dp_B}{dp_A} - \frac{\Delta E}{kT} \]

\[ = \ln \frac{dp_B}{dp_A} + \Delta E \left( \frac{C}{kT} \right) \]

\[ y = mx + b \]

slope