No Organic crib available
October 18, 2014
Written by Professor Uyeda
Ph# 45268

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No Physical crib available
October 18, 2014
Written by Professor Yang
Ph# 63346

**Analytical Chemistry Cume**
**October 2014**

NOTE: Be careful to write and draw legibly. Illegible answers will not be graded.

(1) Describe (< 100 words) the goal of the first Michelson experiment (10 points).
Given the word limit in the prompt, complete answers gave some detail as to the experimental as well as conceptual goals. For instance:

In the late 1800s, the scientific community believed that light was propagated through a medium referred to as the 'luminiferous ether'. Michelson sought to establish whether the ether moved relative to the surface of the earth, by measuring the speed of light traveling along two orthogonal axes, using a device that became known as a Michelson interferometer. If the motion of the ether was more closely aligned with one axis than the other, the light should complete its trip on that axis more slowly, leading to interference between the two beams when they converged at the detector. Interference was a unique way of measuring what would be an extremely small difference between the times it took for light to travel each path.

(2) Derive the relationship $d = D\frac{v}{V+v}$ (10 points).

Not graded due to a typo. Denominator should have been V-v.
Remaining answers were scaled to 100.

(3) Draw a labeled diagram of the instrument showing the locations of the source, mirrors, and detector, quantities D, T, $T_1$, $T_0$, and the relevant beam path lengths, picking the most reasonable orientation of the two arms (10 points).

Figure is shown in Michelson manuscript. 2 points for drawing schematic, 1 point each for clearly and correctly labeling all relevant quantities, including direction of motion of the ether along one arm, which sets the directions for T, $T_1$, and $T_0$. Note that it is necessary to distinguish in the labeling *what* the quantities D and T are.

(4) Draw **a labeled diagram** showing the error Michelson makes in his first paper that leads to the second paper (10 points).

In considering the difference in the time it takes light to travel to and from a point, Michelson incorrectly assumes that if a beam of light travels perpendicular to the motion of the ether, that it would not be affected by the motion of the ether. The situation is analogous to driving a car along a path with and against the wind vs driving the car across the wind; the car will still experience drag from the wind when driving in the cross direction. Taking this effect into account decreases the travel time difference by a factor of two, putting it under the detection limit of the experiment.

Acceptable answers began from either the labeled diagram from (3) or some version of Figure 1 from the Michaelson-Morely paper. Diagram should show direction of earth's motion, and make an explanation connected with the detection limit.

(5) **Use your answer from (4)** as the basis for **calculating** a new value for $\tau$ (10 points).

Use a geometric argument based on your diagram in 4. If the original distance between mirrors is D, the time it takes light to travel that distance is D/V. In that amount of time, earth moves v(D/V). Thus the new effective distance to the mirror perpendicular to the ether flow can be calculated based on Pythagorus' theorem using D and Dv/V as the lengths of the two sides. New values can then be plugged into the equation for tau: $\tau = (T+T_1) - 2T_0$
The new value of $T_0 = D_{new}/V$

Simplifications of the new distance between mirrors based on the Michelson-Morley paper were accepted, but answer needed to include the diagrammatic argument and actual calculations, rather than just copying large amounts of text from the manuscript.

(6) Describe (< 50 words) how the Michelson-Morley interferometer improves the experimental apparatus to compensate for the problem described above including the magnitude of the key parameter that changes (10 points).

The new apparatus increases the path length by a factor of 10, which should increase the difference in distance by a factor of 10, to 0.2 x the wavelength of yellow light. This difference would be well above the detection limit of the experiment.

(7) Describe (< 50 words each) two additional sources of experimental error in the original Michelson experiment, and how the Michelson-Morley experiment addressed them (10 points).

Two additional issues were: 1) that the instrument was extremely sensitive to environmental vibrations, overcome by mounting the entire experiment on a stone slab. 2) that it was extremely difficult to rotate the apparatus without distorting the measurement, which was solved by floating the experimental apparatus on a pool of liquid mercury and making the measurements with the apparatus in constant motion, rather than stopping and starting.

(8) Calculate (using information provided in the Michelson paper) the anticipated change in path length with a 2 $^{0}$C temperature change (10 points).

$\Delta$ path length = (2 $^{0}$C)(1000 mm)(0.000019/$^{0}$C)(2) = 7.6 x $10^{-5}$ m
Remember the path involves going both to the mirror and back.
This is important in calculations in modern FTIR spectrometers as well.

(9) Describe (< 30 words each) four key differences between the original Michelson interferometer and the interferometer used in a modern IR spectrometer (5 points each, total 20):

1. in the source and its output
2. in the beam paths
3. in the detector
4. in how the data are processed and interpreted

1) The Michelson experiment uses a visible source to create interference fringes that are read out by eye ($\lambda$ < 800 nm). A modern IR spectrometer operates at longer (IR!) wavelengths (typically >1000 nm) and uses a more time-stable source since intensity is an important part of the measurement.

2) Michelson: motion of the ether would lead to differences in the speed of light, resulting in beam interference. Modern: a moving mirror creates a path difference leading to interference; the beam also passes through a sample at which radiation is absorbed.

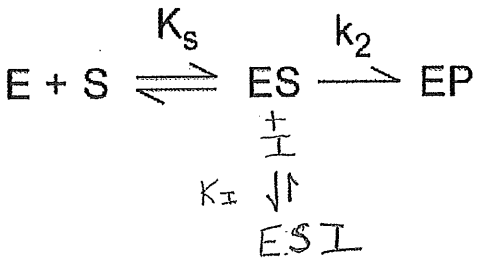
3) Michelson: Shifts in the interference pattern were detected by eye. Modern: photons are detected either thermally or electronically, using for example a thermoelectric or a photoconductive detector such as an MCT detector. It's important that the detector be able to measure low-energy IR photons.

4) Michelson: a linear shift in the visible interference fringes is measured by eye against a ruler, and tabulated. Modern: The time-varying number of photons reaching the detector (an interferogram) undergoes Fourier transformation to assess absorption by the sample in the beam path.

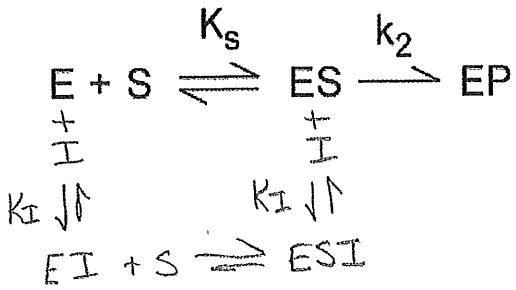
***QUESTION 1 (25 POINTS TOTAL) Basic Enzyme Inhibition Mechanisms***
The following questions (A-C) are all based on the different types of enzyme inhibition.

***Part A. (10 Points)***
Fill out the remainder of each the kinetic schemes below for each of the following types of enzyme inhibition. Make sure you indicate the proper kinetic rate constants and dissociation constants in your schemes.

**Uncompetitive (Pure)**

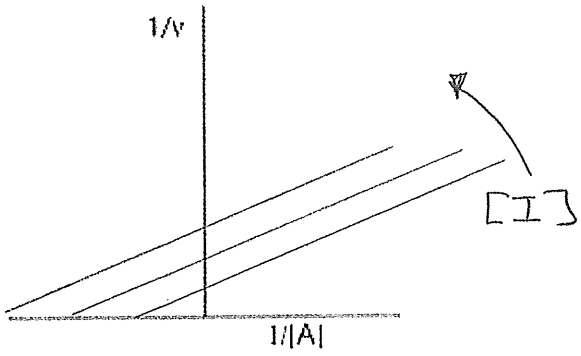



**Non-competitive (Pure)**

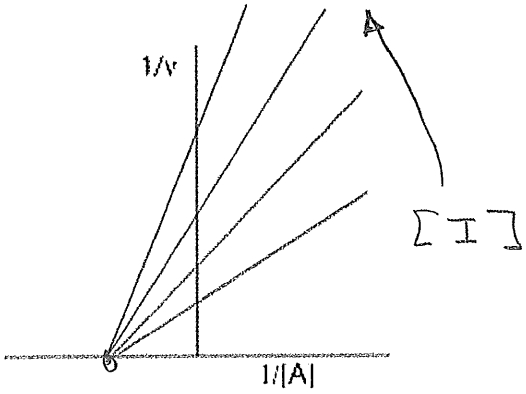



***Part B. (10 Points)***
Fill in three lines for each of the plots below at fixed **[I]**, varying **[A]**. Be sure to include an ARROW indicating the direction of increasing **[I].**

Uncompetitive (Pure)




Non-competitive (Pure)

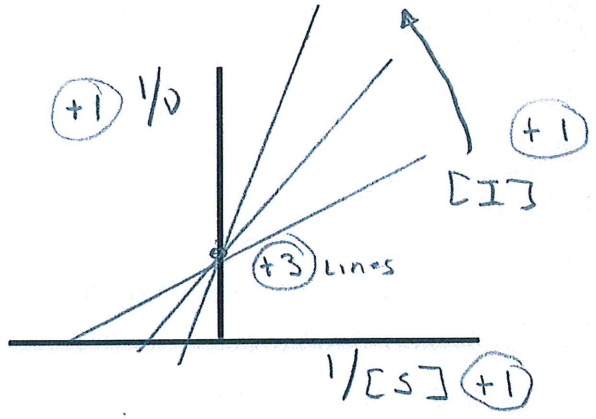



***Part C. (5 Points)***
One of the following equations describes ***Competitive Inhibition***. Please CIRCLE the correct one. Remember that $k_{cat}$ is directly proportional to $V_{max}$.

$$v = \frac{k_{cat}[S]}{K_m + [S](1 + [I]/K_i)} \qquad \boxed{v = \frac{k_{cat}[S]}{K_m(1 + [I]/K_i) + [S]}} \qquad v = \frac{k_{cat}[S]}{(K_m + [S])(1 + [I]/K_i)}$$

***Q2 Basic Enzyme Inhibition Mechanisms-Continued*** **(20 Points)**
You have recently purified an enzyme drug target and you have developed a new assay to rapidly measure the $K_I$ and $IC_{50}$ values of inhibitors synthesized by the chemistry department. The chemistry department gives you two new compounds, X-114 and X-225 for determination of the inhibitor mechanism. You perform a full mechanistic experiment on both compounds (varying both [S] ad [I] and measuring the rate) and you plot your data as 1/rate versus 1/[S] at variable inhibitor concentrations [I]. You find that the patterns in your plots are consistent with **Pure-Competitive inhibition**.

**Part A (5 Points).** Show the expected pattern in the data by drawing the Double-Reciprocal plot described above. Label your axis properly.




**Part B (5 Points).** From the double reciprocal plots for each compound, you determine that the $K_I$ value for compound X-114 is 5,000 nM and that the $K_I$ value for compound X-215 is 5 nM. Which compound binds to the enzyme most strongly and why?

X-215 binds to the enzyme tighter because the $K_I$ value is lower

**Part C (5 Points).** What is the difference in the binding free energy (ΔΔG) in Kcal/mole between these compounds at room temperature (22 °C or 295 °K)? The ideal gas constant R = 1.985 cal $mol^{-1}$ $°K^{-1}$

$\Delta G = -RT \ln K_{eq} = -RT \ln(1/K_D)$ $K_D = K_I$

$\Delta\Delta G = -\left(1.985 \frac{cal}{mol \cdot °K} * 295°K\right)\left(\ln(1/5\times10^{-9}M) - \ln(1/5000\times10^{-9}M)\right)$

$\Delta\Delta G = -4,044$ cal/Mol·K or $\boxed{-4.04 \frac{kcal}{mol \cdot °K}}$

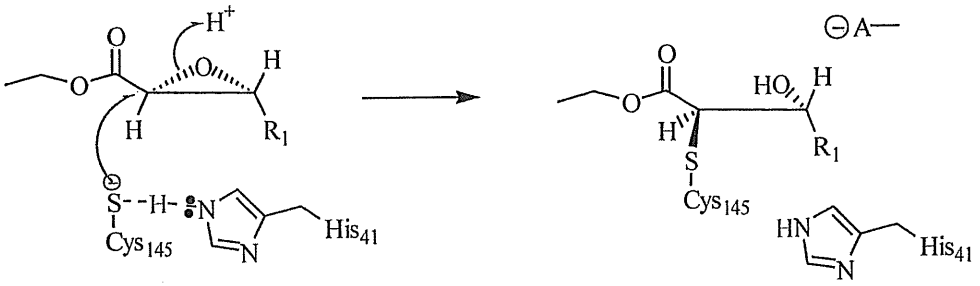
**Part D (5 Points).** If the difference in binding energy between these two compounds can be ascribed to a single type of bond, what type of bond(s) would it be and why?

This value is most consistent with a hydrogen bond

To strong for VDW and to weak for covalent.

***Q3 Enzyme Inhibition via Covalent Modification (25 POINTS TOTAL)***

A chemistry graduate student is working on the design of covalent modifying agents that target the active cysteine residue of a 3 chymotrypsin-like protease (3CLpro) from the MERS virus that causes pneumonia-like symptoms. The compounds are based upon an expoxide scaffold, and the proposed reaction mechanism for inactivation is shown below:




The student synthesized two compounds (Compound **1** and Compound **2**) and then performed a series of kinetic experiments that were designed to elucidate the mechanism of inactivation by these epoxide-based compounds. The % remaining enzyme activity was measured as a function of time and at a series of different inhibitor concentrations. The data for Compound **1** were obtained over an inhibitor concentration range from 0 to 160 μM and these data are shown in Figure 1. The resulting rate constants for inactivation at each concentration ($k_{inact,obs}$) of Compound **1** are shown in Table 1.

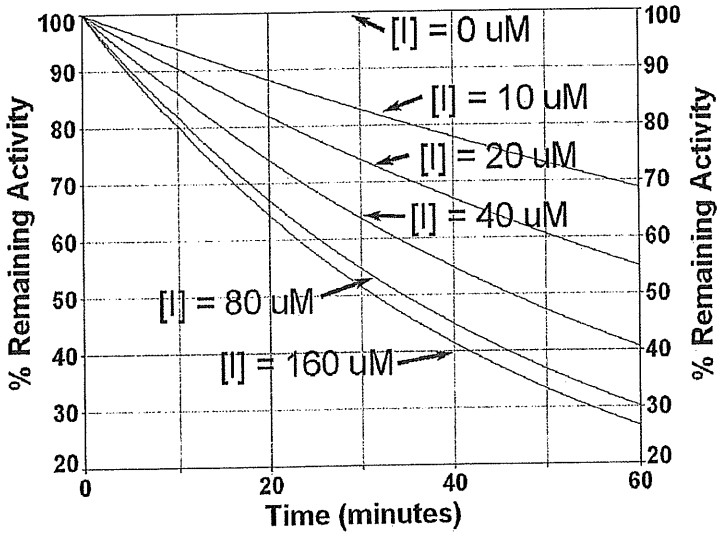



**Figure 1.** Compound **1** inactivation of 3CLpro

**Table 1** Compound **1** rate constants

| [Compound 1] (μM) | $k_{inact,obs}$ ($min^{-1}$) |
|---|---|
| 0 | 0 |
| 10 | 0.00625 |
| 20 | 0.01 |
| 40 | 0.015 |
| 80 | 0.02 |

The student next determined the kinetics of inactivation of 3CLpro by Compound **2** over a concentration range of 1 to160 µM. These data are shown in Figure 2 with the resulting rate constants for inactivation ($k_{inact,obs}$) given in Table 2.

$[I]_0$ =

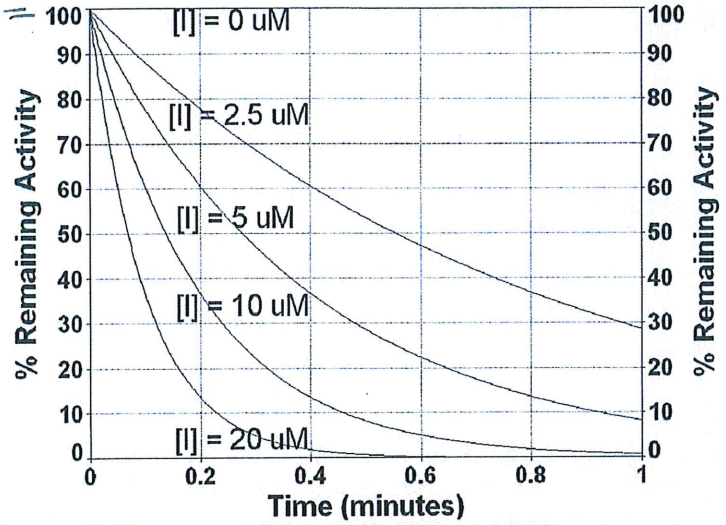



**Figure 1.** Compound **2** inactivation of 3CLpro.

**Table 2.** Compound **2** rate constants

| [Inhibitor] (µM) | $k_{inact,obs}$ ($min^{-1}$) |
|---|---|
| 0 | 0 |
| 2.5 | 1.25 |
| 5 | 2.5 |
| 10 | 5.0 |
| 20 | 10 |

***PART A. (5 points)***

Describe how you would treat or analyze the data in Figures 1 and 2 for each of the inhibitor concentrations to arrive at the observed, apparent rate constants for inactivation, $k_{inact,obs}$, for each of the inhibitor concentrations. In other words, what equation(s) would you use to produce the curves in Figures 1 and 2?

These kinetics are simple first-order rates of decay. You can fit the curves to the following equation.

(*) percent Remaining Activity $= e^{-Kinact/time}$

(*) $[I]_T = [I_0]e^{-Kinact \cdot t}$

$\frac{[I]_T}{[I]_0}$ = Percent Remaining Activity

You could put it in Natural Log form + put on log graph to get slope

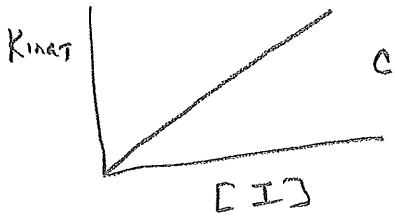
(*) $\ln\left(\frac{[I]_T}{[I]_0}\right) = -K_{inact.} \cdot t$

(*) Any of these = 5pts

***PART B. (10 points)***
From your analysis of the data in Tables 1 and 2 and Figures 1 and 2, what are the kinetic mechanisms for inactivation for each of these inhibitors? Are there any differences?

Compound 1 shows saturation with inhibitor


Compound 1




Compound 2

Compound 2 does Not show saturation → Linear response means first-order.

$$E + I \underset{}{\overset{K_I}{\rightleftharpoons}} EI \xrightarrow{K_{INACT,MAX}} E\text{-}I^*$$

Compound 1 produces EI complex before reaction

$$E + I \xrightarrow{} EI^*$$

Compound 2 reacts upon collision and has no central complex

***PART C. (10 points)***
From the data presented in Table 1 and Figure 1, and from your proposed mechanism in Part B, what kinetic parameters should you be able to determine from the $k_{inact,obs}$ data and what are they for each inhibitor?

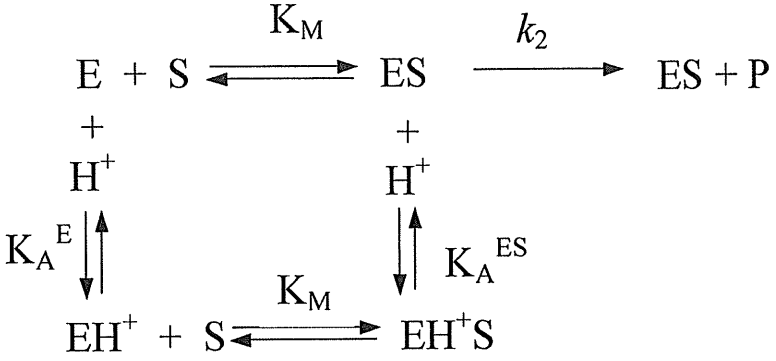
For Compound 1 you can determine a $K_I$ value from the concentration that gives ½ $K_{INACT,MAX}$ and you get $K_{INACT,MAX} \cong 0.02 min^{-1}$. $K_I$ or $IC_{50} = 20 \mu M$

For Compound 2 you can only calculate a second order rate constant for inactivation which is the slope of the line. $K_{INACT,OBS} = K_{INACT}[I]$ or $K_{INACT} = \frac{K_{INACT,OBS}}{[I]}$

$$K_{INACT} = \frac{1.25 min^{-1}}{2.5 \mu M} = 0.5 min^{-1} \mu M^{-1}$$

**Q4 Protons as Enzyme Inhibitors (30 POINTS TOTAL)**

The enzyme *Pleasepassmease* has recently been purified from the tortured brains of chemistry students. The reaction is a single-substrate, single-product reaction and the $K_M$ and $V_{Max}$ values are easily measured by monitoring the breakdown of caffeine as a function of time. A biochemistry division graduate student wanted to determine which amino acid(s) are present within the active site of the enzyme. The general reaction scheme they **first** thought possible is shown below in **Scheme 1**:




**Scheme 1**

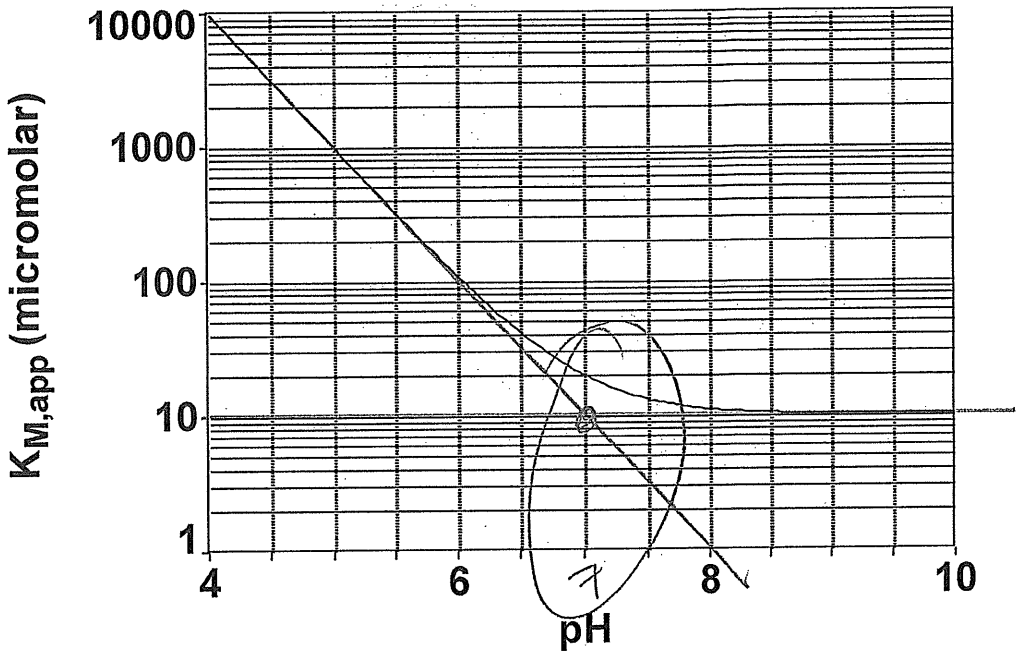
In order to test this model, the student measured the kinetic parameters ($K_M$ and $V_{Max}$) of the reaction as a *function* of pH. They then plotted each parameter as a function of pH and observed the following results:

- A plot of $V_{Max,app.}$ as a function of pH showed that **$V_{Max,app}$ values DO NOT vary with pH**. The $V_{Max}$ value was constant at 100 $sec^{-1}$.
- A plot of $K_{M,app}$ as a function of pH showed that the **$K_{M,app}$ values DO VARY with pH.** The plot that the student obtained for $K_{M,app}$ versus pH is shown on the next page in Figure 1.

The intelligent student noticed that ***only a single ionization constant ($K_A$)*** was necessary to describe the data. Thus, the student fit the data to an equation to obtain the kinetic parameters **$K_M$ and $pK_A$**.

From the above information and from the plot of $K_{M,app}$ versus pH, answer the questions on the following pages.

**Figure 3**




**PART A (10 POINTS).** The original model in Scheme 1 needs to be "UPDATED" to explain the actual data obtained by the student. It is your job to propose a new model based on the data. Therefore, **draw a new kinetic mechanism scheme below** that models the data presented in Figure 3. I have started it for you:

$$E + S \underset{}{\overset{K_M}{\rightleftharpoons}} ES \xrightarrow{k_2} E + \text{Products}$$

$$E + H \quad (K_I)$$

$K_I$ =

Protons compete with substrate for free enzyme

**PART B (5 POINTS).** From the plot in Figure 1, what is the approximate $pK_A$ for the amino acid group ? What amino acid(s) can have this $pK_A$?

The pKa is ~7. This could be a histidine or cysteine residue with an altered pKa value from what we normally observe in solution.

**PART C (15 POINTS).** Derive the ***rapid-equilibrium rate equation*** for your model in **SCHEME 2**. The final equation should include terms for $H^+$, $V_{max}$, $K_M$, and $K_A$. Place your final rate equation in terms of pH and $pK_A$ instead of $H^+$ and $K_A$ in the box below.

① $\text{rate} = k_2[ES]$ ② $V_{max} = k_2[E]_T$

③ $[E]_T = [E]_F + [ES] + [EH]$ ④ $pH = -\log[H]$

⑤ $pKa = -\log K_I$

⑥ $K_I = 10^{-pKa}$

⑦ $[H] = 10^{-pH}$

⑧ $K_M = \dfrac{[E]_F[S]_F}{[ES]}$ ⑨ $K_I = \dfrac{[E][H]}{[EH]}$

$[E]_F = \dfrac{K_M[ES]}{[S]}$ $[EH] = \dfrac{[E][H]}{K_I} = \dfrac{K_M[ES][H]}{K_I[S]}$

$[E]_T = \dfrac{K_M[ES]}{[S]} + [ES] + \dfrac{K_M[ES][H]}{K_I[S]}$

$[E]_T = [ES]\left(\dfrac{K_M}{[S]} + \dfrac{K_M[H]}{K_I[S]} + 1\right)$ $[ES] = \dfrac{[E]_T}{\left(\dfrac{K_M}{[S]} + \dfrac{K_M[H]}{K_I[S]} + 1\right)}$

$\text{rate} = \dfrac{k_2[E]_T}{\dfrac{K_M}{[S]} + \dfrac{K_M[H]}{K_I[S]} + 1}$

multiply by $[S]$

$V_{max} = k_2[E_T]$

$\text{rate} = \dfrac{V_{max}[S]}{K_M + \dfrac{K_M[H]}{K_I} + [S]} = \dfrac{V_{max}[S]}{K_M\left(1 + \dfrac{[H]}{K_I}\right) + [S]}$

$$\text{rate} = \frac{V_{max}[S]}{K_M\left(1 + \dfrac{10^{-pH}}{10^{-pKa}}\right) + [S]}$$

substitute equations from above.

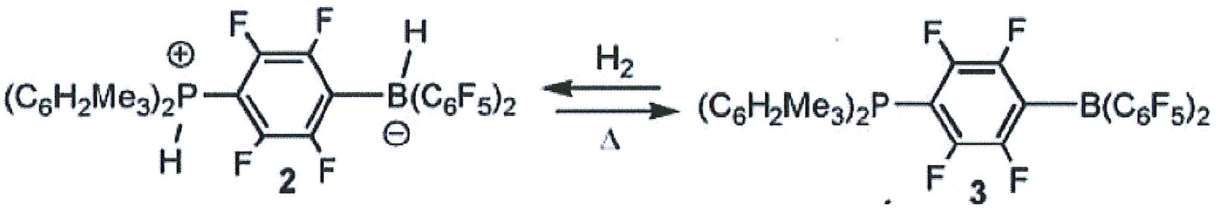
$10^{pKa-pH}$

**Inorganic Chemistry Cumulative Exam** (October 18, 2014)

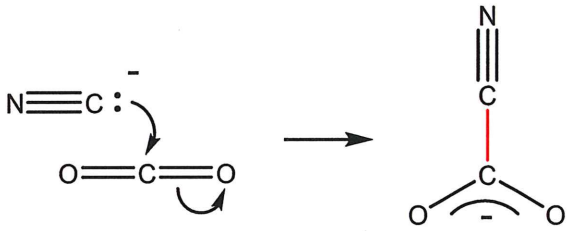
Purdue University

1 (30) Frustrated Lewis Pair (FLP) has attracted intense interest from inorganic / organometallic chemists in recent years. (**A**) Please define FLP and give a clearly drawn structural example. (**B**) Provide an example of small molecule activation by FLP. (hand waving or random guesses will receive **ZERO** point)

In a FLP (e.g. **3** below), Lewis acid and Lewis base functions were incorporated into the same molecule and sterically precluded from quenching each other. Activation of $H_2$ by **3** is also shown below.




2 (15) Formation of cyanoformate (adduct of carbon dioxide and cyanide) is a common way to store cyanide in bacteria. Draw the structure of cyanoformate with a concise justification, and explain why it has a minimum stability.




The C-C bond formed is relatively weak and bond energy barely compensates the reorganization energy required to bend $CO_2$ from its linear geometry.

3 (15) LiBr has a density of 3.464 g/cm$^3$ and the NaCl type crystal structure. Calculate the interionic (Li ··· Br) distance (in Å).

$a = 2\ D$(Li-Br); $Z = 4$;

$d = 3.464$ g/cm$^3$ $= (86.84 * 4)/(a^3 \times 6.022 \times 10^{23})$ ➔ $a = 5.502 \times 10^{-8}$ cm

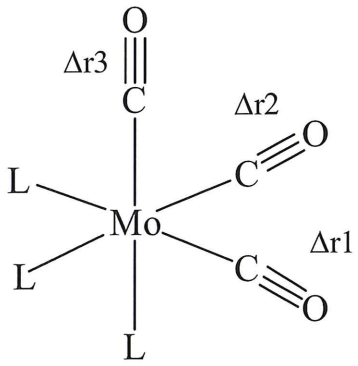
$D$(Li-Br) = 2.751 Å

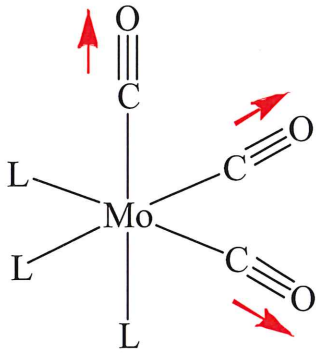
4 (10) Year 2014 has been designated as the International Year of Crystallography. Among notable contributors to X-ray Crystallography are Kathleen Lonsdale, Rosalind Franklin and Dorothy Hodgkin. Pick one and provide a concise description (<100 words) of her contribution.

| | |
|---|---|
| Kathleen Lonsdale | hexamethylbenzene structure proving aromaticity |
| Rosalind Franklin | *B*-DNA X-ray diffraction pattern that led to Watson-Crick model |
| Dorothy Hodgkin | structure determination of penicillin and vitamin $B_{12}$ |

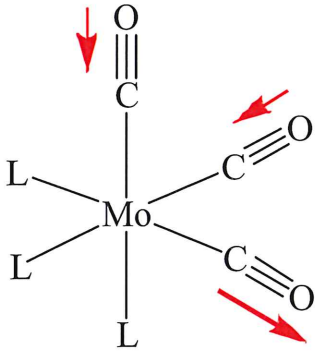
5 (30) Determine the number of IR active CO stretch modes for *fac*-$Mo(CO)_3(NCEt)_3$. Potentially useful character tables are provided below.

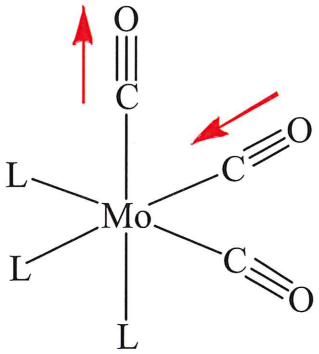
| $C_{3v}$ | E | $2C_3$ (z) | $3\sigma_v$ | |
|---|---|---|---|---|
| $A_1$ | +1 | +1 | +1 | z |
| $A_2$ | +1 | +1 | -1 | $R_z$ |
| E | +2 | -1 | 0 | $(x, y)$ $(R_x, R_y)$ |
| $\Gamma_{\Delta r}$ | 3 | 0 | 1 | $= A_1 + E$ |




$A_1$




$E$