Chemistry 2710 Spring 2004 Final Examination

Total marks: 108

Time: 3 hours

Aids allowed: Calculator. One $8\frac{1}{2} \times 11$-inch piece of paper containing any information you need. No other printed materials (e.g. periodic tables, calculator manuals) are allowed.

Instructions: Answer all questions in the booklets provided. In sections 2 and 3, you have some choice. Clearly indicate which questions from these sections you are answering. **Do not** answer more than the required number of questions. **Extra answers will not be marked.** In the event that you answer extra questions, I will arbitrarily choose which answer(s) to mark and ignore the others. If you start a problem and decide that you do not want it marked, just cross out your work. Don't let me decide for you.

Graphs should *either* be sketched in your exam booklet (if you are using a graphing calculator) or drawn on the graph paper provided. If you are hand-drawing graphs, make sure to put your name and the question number on the graph paper. If you decide to use a graphing calculator, provide a clearly labeled and reasonably accurate sketch of any graphs used in answering a question.

Clarity may be considered in evaluating your answers. Make sure to explain your reasoning (in a few words) for any mathematical derivation or calculation presented.

Useful data:

$k_B = 1.380\,650\,3 \times 10^{-23}\,\mathrm{J/K}$

$h = 6.626\,068\,8 \times 10^{-34}\,\mathrm{J/Hz}$

$R = 8.314\,472\,\mathrm{J\,K^{-1}mol^{-1}}$

To convert degrees Celsius to Kelvin, add 273.15.

1 Answer all questions in this section.

Value of this section: 78

1. Explain briefly how the generation of shock waves in temperature-jump experiments is avoided for reactions in aqueous medium. [4 marks]

2. List two advantages of laser flash photolysis over the conventional UV-lamp method. [4 marks]

3. (a) Using the Michaelis-Menten rate law, derive a condition under which an enzyme-catalyzed reaction will display zero-order kinetics. [4 marks]

   Note: The "derivation" here is pretty simple.

   (b) Suppose that an enzyme preparation with $v_{\max} = 35\,\mu\text{mol}\,\text{L}^{-1}\text{s}^{-1}$ and $K_M = 18\,\mu\text{mol/L}$ is operating in the zero-order regime. How long would it take for this enzyme to make $1\,\mu\text{mol/L}$ of its product assuming that the stoichiometry of the reaction is $\text{S} \rightarrow \text{P}$? [4 marks]

4. Flash photolysis of iodide ions in aqueous solution produces the radical I_2^-. This radical absorbs strongly at 400 nm, and so can be detected by simple spectrophotometric methods. The absorbance is directly proportional to the concentration of the radical. I_2^- disappears rapidly from solution. Either of the following two elementary reactions could be responsible for the decay of this radical:

$$\text{I}^-_{2(\text{aq})} + \text{I}^-_{2(\text{aq})} \rightarrow \text{I}^-_{3(\text{aq})} + \text{I}^-_{(\text{aq})}$$

$$\text{I}^-_{2(\text{aq})} + \text{H}_2\text{O}_{(\text{l})} \rightarrow \text{I}_{2(\text{aq})} + \text{H}_{(\text{aq})} + \text{OH}^-_{(\text{aq})}$$

   (a) Explain why the second of these two reactions would behave as a first-order reaction, even though it is formally a second-order process. [4 marks]

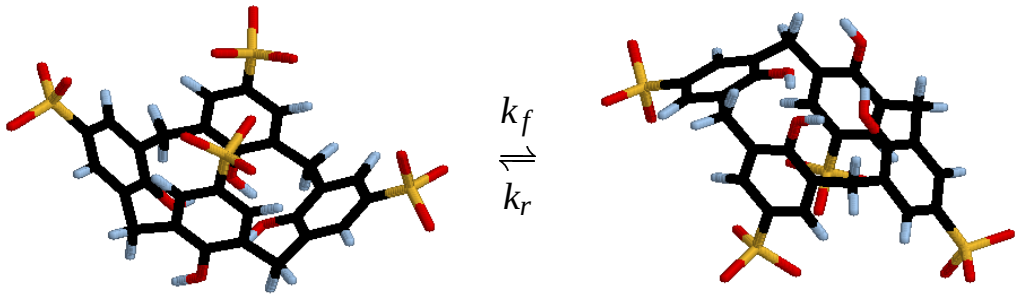
   (b) The following absorbance vs time data have been obtained:

t (μs)	50	100	150	200	250	300	350	400
A_{400}	0.277	0.189	0.156	0.122	0.102	0.087	0.083	0.065

   Determine which of the two elementary reactions is the correct decay mode for I_2^-. [13 marks]

   (c) Predict the absorbance at $t = 1\,\text{ms}$. [4 marks]

5. Calix[n]arenes are cyclic oligomers of phenols which are being studied as catalysts (by analogy to enzymes) and as building blocks for advanced materials. In solution, *p*-sulfonatocalix[4]arene undergoes the following inversion process:



(The picture is much more convincing on a graphical workstation. Imagine each of the rings "rotating" in such a way that the whole molecule turns itself inside-out.)

(a) How are k_f and k_r related? Explain briefly. [3 marks]

(b) The value of k_f can be measured directly using an NMR technique not discussed in class. The following measurements have been obtained in a 50:50 (by weight) mixture of D_2O and deuterated acetone:[1]

T (K)	265	270	290	300	310
k_f (s^{-1})	169.1	320	2.37×10^3	5.2×10^3	1.124×10^4

Calculate the activation energy and preexponential factor of this reaction. [11 marks]

Note: It is not necessary to show a graph for this question.

(c) Calculate the entropy of activation at 25°C. Provide a molecular interpretation of the result. (Is the entropy of activation large? Small? Why?) [7 marks]

6. The signal transduction cascade of the bacterial chemotactic system involves the formation of a complex between CheY and phosphorylated CheA (P-CheA). The phosphate group is subsequently transferred to CheY:

$$\mathrm{CheY} + \mathrm{P-CheA} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \mathrm{CheY:P-CheA} \overset{k_{\mathrm{cat}}}{\longrightarrow} \mathrm{P-CheY} \cdot \mathrm{CheA}.$$

Stewart and Van Bruggen have studied the association and dissociation kinetics of CheY and CheA,[2] i.e. the first step of the mechanism. It turns out that phosphorylation and binding are independent events. As is often done in molecular biology, they therefore isolated the domain (part of the protein) of CheA responsible for binding in order to avoid complicating factors due to the catalytic transfer of the phosphate group. This domain is called P2, and they studied two variants which they called $P2_S$ and $P2_L$ (small and large). They then carried

[1] Y. Israëli and C. Detellier, Phys. Chem. Chem. Phys. **6**, 1253 (2004).
[2] R. C. Stewart and R. Van Bruggen, J. Mol. Biol. **336**, 287 (2004).

out a set of experiments to determine the equilibrium constant and forward and reverse rate constants for binding of the P2 domain to CheY:

$$\mathrm{CheY} + \mathrm{P2} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \mathrm{CheY \cdot P2}.$$

(a) At 4°C and an ionic strength of 0.13 mol/L (close to physiological ionic strengths) with the $\mathrm{P2_S}$ domain, the phenomenological equilibrium constant for the binding reaction was found to be $(1.59 \pm 0.25) \times 10^6\,\mathrm{L/mol}$. In their kinetics experiments, they found

$$\begin{aligned} k_a &= (2.8 \pm 0.8) \times 10^7\,\mathrm{L\,mol^{-1}s^{-1}}, \\ \text{and } k_d &= 24 \pm 2\,\mathrm{s^{-1}}. \end{aligned}$$

Are the rate constants consistent with the equilibrium constant measurement? Based on the uncertainties in the rate constants, we can calculate that the uncertainty in the calculated equilibrium constant will be about 30%. [4 marks]

(b) The following data were obtained at 4°C to quantify the effect of ionic strength on the association of $\mathrm{P2_S}$ with CheY:

I (mol/L)	$10^{-6}k_a$ ($\mathrm{L\,mol^{-1}s^{-1}}$)
0.03	63
0.08	45
0.13	28
0.23	19
0.53	12
1.03	10
2.03	7.1

i. The Brønsted-Bjerrum equation which we studied in class can't be used to study this data set. Why not? [2 marks]
Note: It has nothing to do with the charges on the proteins, which are unknown. Proteins have multiple ionizable groups and so their ionization state in solution can't easily be guessed at.

ii. These data may be treatable using a slightly more sophisticated form of Debye-Hückel-Brønsted (DHB) theory, which gives the equation

$$\ln k = \ln k_0 + \frac{2.214 \times 10^{-10}}{(\varepsilon T)^{3/2}} Z_A Z_B \frac{\sqrt{I}}{1+\sqrt{I}}.$$

The permittivity of water at 4°C is $7.660 \times 10^{-10}\,\mathrm{C^2\,N^{-1}m^{-2}}$.
Propose and implement a graphical method for determining whether or not the data are consistent with DHB theory. If so, what can be said about the charges on the two proteins? If not, explain in detail what features of your graph led you to your conclusion. [14 marks]

2 Answer *one* question from this section.

Value of this section: 10 marks

1. Suppose that a reaction $A + B \to C$ has a rate law $v = kab$. Does this mean that the reaction is necessarily elementary?

   If your answer is *yes*, derive an integrated rate law for the reaction assuming that $a_0 \neq b_0$.

   If your answer is *no*, give a two-step mechanism which has the above rate law for appropriate values of the rate constants. Give the relationship between the elementary rate constants appearing in your mechanism and the empirical rate constant k.

   [10 marks]

2. Suppose that a reaction $A \to 2P$ has an empirical rate law

$$v = \frac{k_1 a}{1 + k_2 p}.$$

   Obtain an integrated rate law for this reaction. [10 marks]

   Hints: Start by relating a and p using stoichiometry. Working from the rate equation for a gives an integral which can be broken into pieces, each of which is easy to handle.

3 Answer *one* question from this section.

Value of this section: 20 marks

1. Carry out a complete scaling analysis to determine the range of validity of the steady-state approximation for the mechanism

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P.$$

   [20 marks]

2. In bacteria, phosphoglucose isomerase catalyzes the transformation of fructose-6-phosphate into glucose-6-phosphate (or vice versa), allowing the cell to select between two different fermentative pathways according to the prevailing conditions. This selection requires a metabolic switch, i.e. a means of turning the activity of phosphoglucose isomerase on or off. This can be accomplished if appropriate metabolites (intermediates in biochemical pathways) inhibit the enzyme. Richter and coworkers have studied the inhibition of phosphoglucose isomerase from *Oenococcus oeni* by metabolites of the two fermentative pathways.[3]

[3] H. Richter, A. A. De Graaf, I. Hamann and G. Unden, Arch. Microbiol. **180**, 465 (2003). I would like to thank Professor Unden for making his raw data available.

In particular, for the conversion of fructose-6-phosphate (F6P) to glucose-6-phosphate in the presence of varying concentrations of erythrose-4-phosphate (E4P), the following data were obtained:

[F6P] (mmol/L)	v (μmol g^{-1}min^{-1})				
0.2	132	74	75	30	18
0.5	283	205	183	97	43
1	392	257		155	92
1.5	471	398	325	243	132
2	525	434	373	296	169
5	560	551	435	374	250
7	580	581	396	380	280
10	640	591	448	439	336
[E4P] (μmol/L)	0	1.5	3	4.5	6

These data were generated from a cell extract rather than a purified enzyme, so the rates were normalized by the dry weight of the preparation, hence the units given above.

Determine whether these data exhibit competitive or uncompetitive kinetics, or neither. If the type is competitive or uncompetitive, determine the values of K_S, K_I and $v_{\max}$. Otherwise, explain exactly how you reached your negative conclusion. [20 marks]

3. **Note:** This question requires moderately sophisticated use of your graphing calculator. Don't attempt it unless you are very comfortable with this tool.

   It is possible to carry out enzymatic reactions in CSTRs if you can find a way to keep the enzyme inside the reactor. One simple solution to this problem is to place size-selective membranes over the inlet and outlet of the reactor. Since enzymes are large molecules, if the substrate and product aren't too large, we can keep the enzyme inside the reactor but allow the substrate and product to get out.

   Under typical operating conditions, the Michaelis-Menten equation can be used to describe the rate of reaction. Suppose that the overall reaction catalyzed by the enzyme is S $\rightarrow$ P.

   (a) Derive an equation for the steady-state concentration of S in the CSTR. Derive an equation for the steady-state concentration of P in terms of [S]. [10 marks]

   (b) The reactor has a working volume of 2.5 L. The feed stock contains 400 μmol/L of S. Suppose that $K_M = 120\,\mu$mol/L and that, for the amount of enzyme in the reactor, $v_{\max} = 18\,\mu\text{mol}\,\text{L}^{-1}\text{s}^{-1}$. S costs \$1200/mol and P is worth \$25 000/mol.[4] Write down an equation for the rate at which profits are generated. Using your graphing calculator, find the flow rate which maximizes the profit. [10 marks]

HAVE A GOOD SUMMER!

[4] If these numbers seem outlandish, consider the fact that a mole is a very large amount of chemical. For a typical medium-sized organic molecule with a molar mass of perhaps a few hundred g/mol, \$25 000/mol is only about \$100/g, which isn't all that expensive for specialty chemicals or drugs which might be made on this scale.