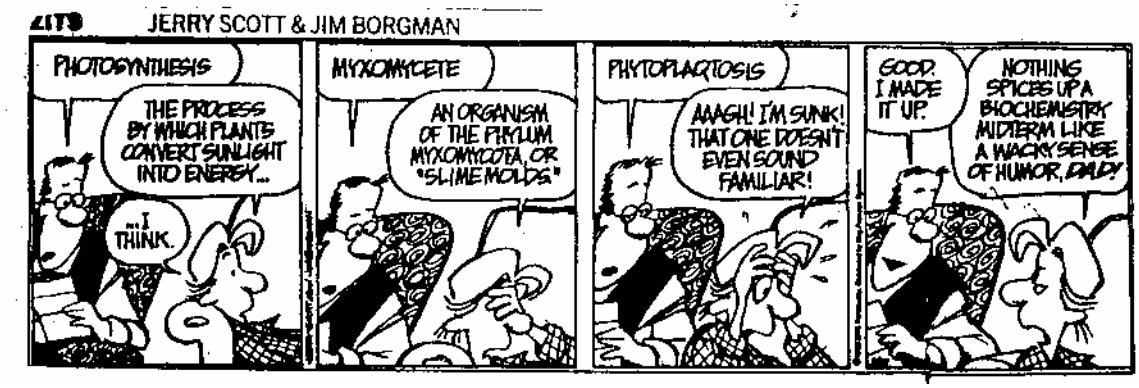
CHEM-342 Introduction to Biochemistry Name _____
Midterm Examination - Individual Part
Wednesday, 23 March 2005
H. B. White - Instructor

Important - Please read this before you turn the page.

- Write your name on each page
- This part of the midterm examination is worth 80 points distributed among four 15-point and two 10-point questions.
- You may refer to your notes, course reader, handouts, or graded homework assignments Textbooks, reference books, and wireless laptop computers cannot be used.
- This examination will assess your learning, problem-solving skills, and ability to communicate clearly. It is intended to be challenging even to the best students in the class.
- Writing reflects how you think. Better quality answers will receive higher marks. Therefore organize your thoughts before you write and draw. Among the "right answers" I will read for the following questions, some will be better than others because they
  - show greater depth of understanding,
  - avoid extraneous or inaccurate information,
  - provide a more logical structure,
  - use appropriate examples,
  - include appropriate illustrations, and
  - choose words with precision.
- Strive to write not that you may be understood, but rather that you cannot possibly be misunderstood. Stream of consciousness answers are rarely well organized or clearly presented.

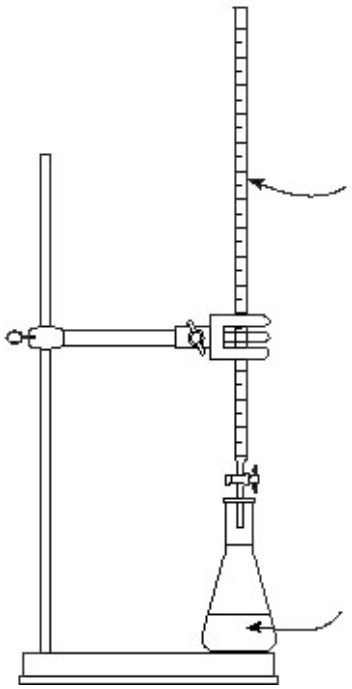


1. (15 Points) Zinoffsky determined the iron content of horse hemoglobin titrimetrically. First he incinerated in air a known weight of dry crystalline hemoglobin. This converted the constituent elements to their oxides, most of which were volatile, e.g. CO_2, H_2O, NO_2, and SO_2. Nonvolatile ferric oxide remaining in the ashes was dissolved in acid, and the iron converted to Fe^{2+} with Zn as indicated (Eq. 1).

(Equation 1) $2Fe^{3+} + Zn \rightarrow 2Fe^{2+} + Zn^{2+}$

Aliquots of the resulting solution were titrated with a standardized solution of potassium permanganate as shown in the unbalanced reaction (Eq. 2)

(Equation 2) $\_\_ Fe^{2+} + \_\_ MnO_4^- + \_\_H^+ \rightarrow \_\_ Fe^{3+} + \_\_ Mn^{2+} + \_\_ H_2O$



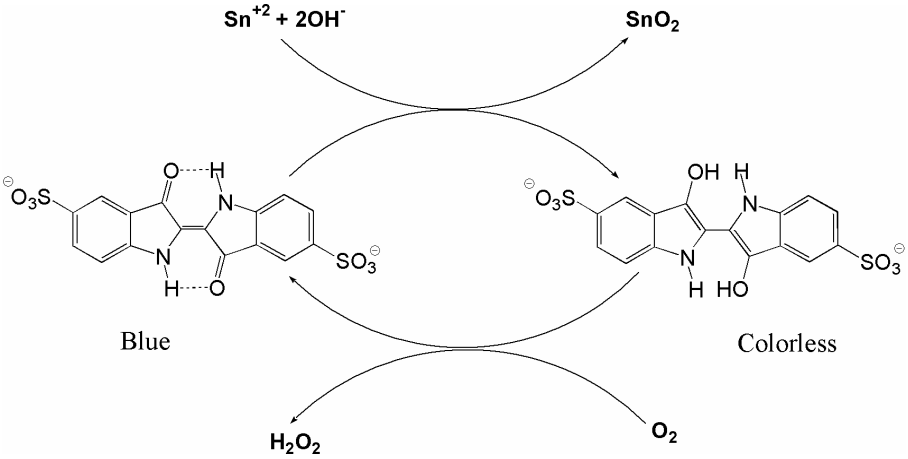
4a. (5 Points) Supply the missing coefficients to balance Equation 2 above.

4b. (2 Points) In this reaction, what is being oxidized? What is being reduced?

4c. (3 Points) Please label the diagram at the left indicating which solution is in the burette and which is in the flask.

4d. (5 Points) Every titration has an end point that indicates when a stoichiometric reaction is complete. Specifically, how would Zinoffsky detect the end point of his titration?

2. (15 Points) Stokes did not know the chemical structures of either indigo or hemoglobin; however, by observing spectroscopic changes, he found the similarities between the redox reactions of indigo carmine (shown below) and those of hemoglobin to be so great that he emphasized that similarity with italics in Section 8 of his article.



"We may infer from the facts above mentioned that *the colouring matter of blood,* like indigo, *is capable of existing in two states of oxidation, distinguishable by a difference of colour and a fundamental difference in the action on the spectrum. It may be made* to *pass from the more to the less oxidized state by the action of suitable reducing agents, and* recov*ers its oxygen by absorption from the air."*

We now know that Stokes' analogy is flawed in ways he could not have known.
How are the reactions of indigo similar to those of hemoglobin and in what respects are they different? Please ***illustrate*** your answer with appropriate representations of hemoglobin reactions.

3. (15 Points) Considering that J. J. Thomson did not discover the electron until 1897, a third of a century after Stokes published his article, it is no surprise that Stokes' understanding of oxidation and reduction was less refined than ours. However, he is remembered for his observations on the chemistry of hemoglobin and his insights to the role of hemoglobin as an oxygen carrier in the body.

   In Section 9 of his 1864 paper, he observes a change in color of blood and concludes, "It thus appears that scarlet cruorine is capable of being reduced by certain substances derived from the blood…" Later, in Section 19, he concludes, "…carbonic acid acts *as if it were* a reducing agent. We are led to regard the change of colour not as a *direct* effect of the *presence of carbonic acid*, but a consequence of the *removal of oxygen*." Both of these conclusions have physiological relevance. Please explain.

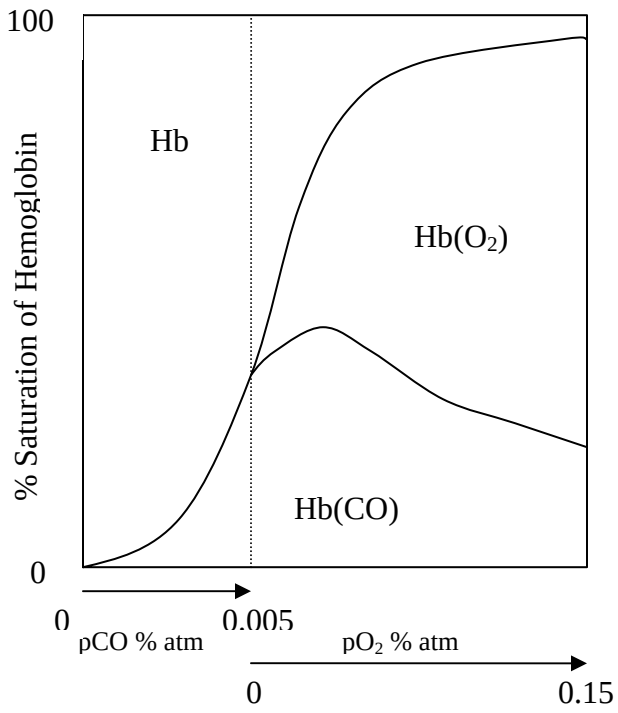
4. (15 Points) Dynamic equilibria pervade biochemical systems. Rapid reversible proton dissociation from acids, substrate-product binding and release from enzymes, and oxygen binding to hemoglobin are examples. These ideas also pervade chemical systems as in phase equilibria and solubility.

   Hemoglobin was one of the first proteins crystallized and one of the first proteins to have its three-dimensional structure determined by X-ray crystallography. Today methods of protein crystallization by vapor diffusion are small scale and very controlled relative to the way Zinoffsky crystallized horse hemoglobin.

   The vapor diffusion procedure is as follows: a small drop (microliters) of a purified and concentrated, but not supersaturated, protein sample dissolved in some nonvolatile solute (e.g. polyethylene glycol in a buffer), is placed on see-through lid ("hanging drop") of a sealed chamber containing a much larger volume of a more concentrated, protein-free solution of the same solute. The sample is kept in a refrigerator and examined periodically through the lid with a microscope. If the conditions are right, crystals will form in a few days as the system comes to equilibrium. Based on the information provided.

   A. Draw a picture of the crystallization set up.
   B. Using words and illustrations as needed, describe conceptually what happens in the hanging drop over time and why this is a gentle and controlled way to obtain protein crystals.

1. (10 Points each) Questions relating to the Jigsaw articles: Answer **only 2** of the following 4 questions for a total of 20 points. For full credit, answers must provide logical explanations based on factual information. Illustrations to support your text are strongly encouraged.



5a. The figure at the left depicts parts of different figures in Douglas et al. (1912). Before the dotted vertical line represents binding of CO to hemoglobin at increasing pressures of CO. Beyond the dotted line represents the binding of CO and O_2 as a function of O_2 pressure at a constant CO partial pressure of 0.005 atm.

What was unexpected about these data?

How does one explain this phenomenon?

5b. Adair (1925) published his result that hemoglobin had a molecular weight four times that estimated by Zinoffsky before Svedberg and Fåhraeus (1926) published the same conclusion. Yet, it was Svedberg, not Adair, who received the Nobel Prize for the work. Explain.

5c. Conant (1923) prepared ferrohemoglobin by bubbling nitrogen through a solution of oxyhemoglobin. He also generated ferrohemoglobin from ferrihemoglobin with dithionite. What is the color of each of these hemoglobin forms? Using diagrams and/or equations, portray both methods of generating ferrohemoglobin.

5d. Figure 2 in Diggs et al. (1933) shows the incidence of sickle cell trait in African Americans as a function of age. The low values found among newborn infants is now explained by the presence of fetal hemoglobin. Generate at least three reasonable hypotheses to explain the general decline in the frequency of sickle cell trait beyond one year?