CHEM-342 Introduction to Biochemistry   Name _____
Midterm Examination - Individual Part (75%)
Wednesday, 24 March 2010
H. B. White – Instructor

Average = 69.5 ± 16.5; Range = 30-100; N=27

Important - Please read this before you turn the page.

There are 8 pages to this examination including this page.

Write your name on every page.

This individual part of the midterm examination is worth 100 points.

Answer questions 1-8. Then answer any two of the remaining questions 9 - 12.

The examination is closed book until 8:15 AM. Thereafter you may refer to your notes, course reader, handouts, or graded homework assignments. Textbooks and reference books cannot be used. The examination ends promptly at 9AM.

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, some will be better than others because they:

- show greater depth of understanding,
- provide a more logical structure,
- use appropriate examples,
- include appropriate illustrations,
- avoid extraneous or inaccurate information, 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. Also, **USE YOUR OWN WORDS**, transcription of words from your notes does not show me that you understand.
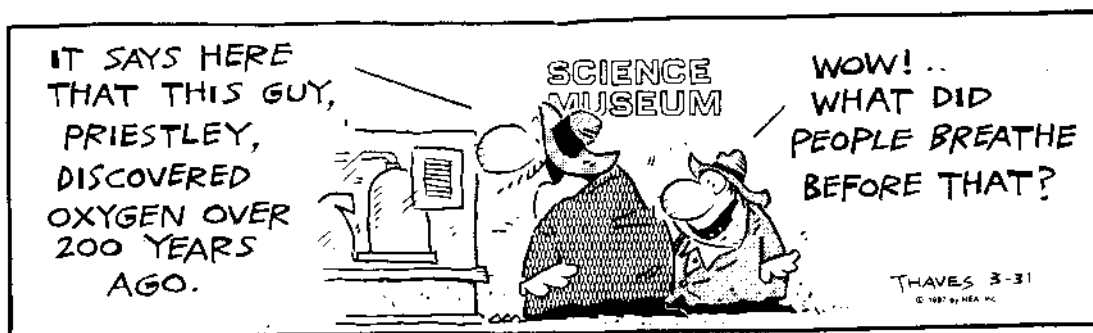
**Answer Questions 1 – 8 and then two of the remaining questions 9-12.**

1. (5 points) Translating from Section 1 of Stokes' article into modern language, Stokes says that, based on the observed spectra, hemoglobin is stable in solutions of sodium carbonate but is immediately denatured by acids and more slowly denatured by sodium hydroxide. This means that hemoglobin is more stable in basic solutions than in acidic solutions.
Explain why solutions of Na_2CO_3 are basic?

2. (10 points) Consider the following experiment in which a solution containing *both* oxyhemoglobin and indigo are titrated with a reducing agent such as dithionite, $S_2O_4^=$, and the concentration of oxidized indigo and oxyhemoglobin are simultaneously monitored with a spectrophotometer as a function of added reducing agent. As in the demonstration in class, the profile for oxidized indigo would be as in line D. The profile for oxyhemogobin would follow line C, not A or B.
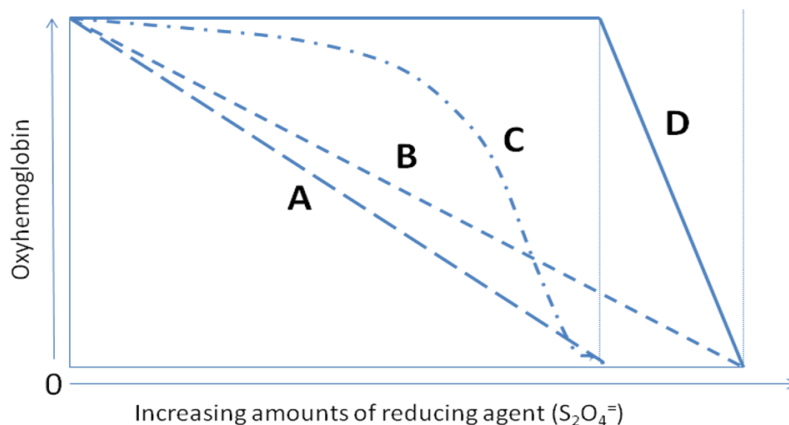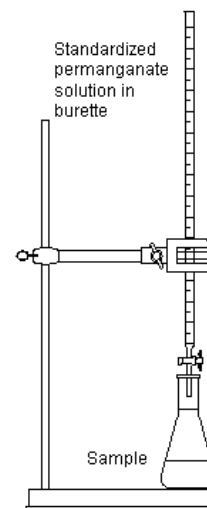

Clearly explain what is happening chemically in this titration and explain the shape of line C.

3. Zinoffsky determined the iron content of his samples by titrating with a standardized solution of permanganate according to the following equation:

$$5\ Fe^{2+}\ +\ MnO_4^-\ +\ 8\ H^+ \rightarrow\ 5\ Fe^{3+}\ +\ Mn^{2+} +\ 4\ H_2O$$
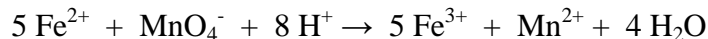
A. (5 points) How did Zinoffsky determine the end point of this reaction?

B. (10 points) Table 4 of Zinoffsky's article reports the data for calculating the percent iron in the crystalline hemoglobin he purified. Two lines from Prep III are provided below. The first set of data is from the titrimetric determination and the second is from the gravimetric determination. **Set up** the equations with numbers that yield the 0.334% Fe that Zinoffsky calculates for each analysis. (You do not need a calculator to do this for full credit.)

Crystal weight	Calc. dry weight	KMnO$_4$	KMnO$_4$ titer	Fe$_2$O$_3$	Fe
grams	grams	cc	mgFe/cc	grams	%
20.5787	19.8790	69.60	0.955	-	0.3343
24.6854	23.6116	-	-	0.1127	0.3341

4. (10 points) Zinoffsky did not have to determine the sulfur content of hemoglobin to estimate its minimum molecular weight to be ~16,700 Da. If instead of finding 2 sulfur atoms per iron, he had found 2.5, what empirical weight would he have calculated for hemoglobin? Show or describe your reasoning.

5. (5 points) What is the argument that a crystal is a pure substance when the solute in the mother liquor has an identical elemental composition to that of the crystal grown in it.

6. (10 Points) Herrick's patient had jaundice, a condition caused by the accumulation of bilirubin, a breakdown product of heme. The structures of bilirubin and heme are shown below. Circle all of the atoms in heme that are lost and all of the atoms in bilirubin that are gained in this metabolic transformation.
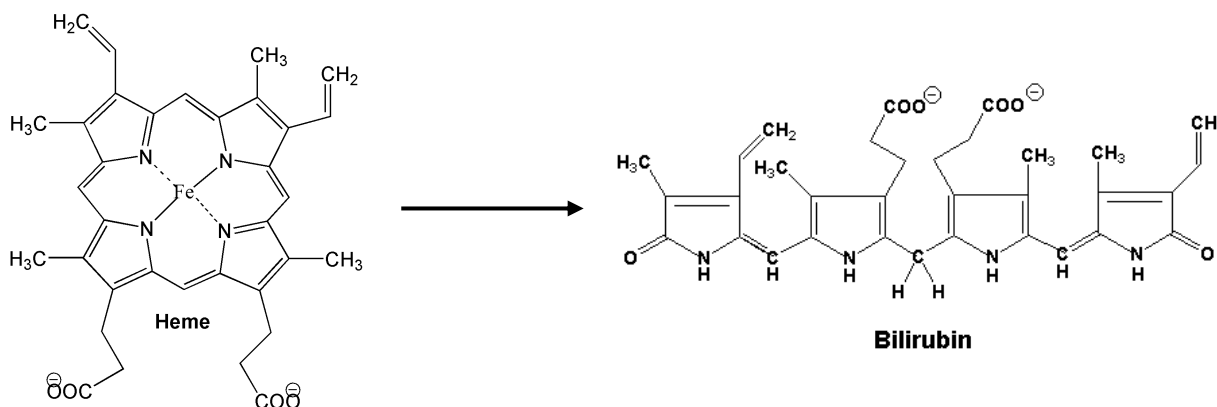
7. (10 points) The title of the article by Svedberg and Fåhraeus does not mention hemoglobin. Rather, it focuses on a method and on proteins in general. Given that there are thousands of proteins, make a convincing case that the authors used good judgment in selecting hemoglobin as the specific example to illustrate their method.

8. Select one of the "jigsaw" articles and describe *in your own words*, the

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A. (5 points) Hypothesis or purpose motivating the work.

B. (5 points) Most notable result(s) or observation(s)

C. (5 points) Major significant conclusions.

Answer only 2 of the following 4 questions.

9. *"It cannot be claimed that the model is hereby shown to be correct, since any model, is <u>ipso facto</u>, an oversimplification, and therefore, 'correct model' is a contradiction in terms. However, it seems probable that the model, like a good cartoon, captures important features of the reality it is meant to portray..."*   Norbert Muller

    A. (5 points) Below is a copy of a model of hemoglobin. What "important features of reality" is it meant to portray? In what ways is the model "an over simplification?"

    B. (5 points) Based on your knowledge of hemoglobin make two lists, one that identifies the structural, chemical, and dynamic aspects of hemoglobin portrayed in the model and another that identifies aspects that are not represented. Your list may be as long as you like.
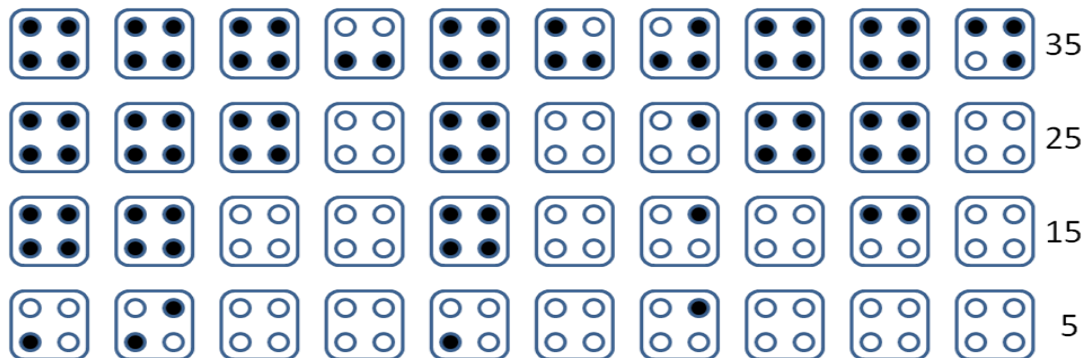

Figure from P. J. Josephy (1992) *Biochem. Educ* **20.**, 91-93.

10. The electrochemical titrations done by Conant obey the Nernst equation as follows:

$$E = E_o + \frac{RT}{F} \ln \frac{[metHb]}{[Hb]}$$

Because oxygen would interfere with Conant's electrochemical titrations, he removed the oxygen from his electrode solutions containing hemoglobin by passing nitrogen through the solution.

A. (3 points) Why/How would oxygen interfere with Conant's titrations?

B. (7 points) Draw a diagram and describe how nitrogen bubbled through a solution containing dissolved oxygen removes oxygen from the solution.

11. (10 points) Svedberg derived the following equation: $\frac{dc}{c} = -\frac{\omega^2 M(1-V\rho)xdx}{RT}$. It describes how a number of different factors determine the experimentally observable concentration gradient of a protein in a centrifugal field. The collected terms, $\omega^2 x$ and $M(1\text{-}V\rho)$, have particular meaning. Explain.

12. (10 points) Although they didn't know it, Diggs and coworkers estimated the genetic frequency of the sickle gene in the US black population. At that time, they and others did not appreciate the genetic relationship between sickle cell anemia and sickle cell trait. Had they known that, they could have applied the Hardy-Weinberg Law to their data and estimated the frequency of sickle cell anemia from the frequency of sickle cell trait. The Hardy-Weinberg Equation predicts the frequency of different genotypes based on allele frequencies and has the form $(p + q)^2 = p^2 + 2pq + q^2$, for two alleles. This relationship can be displayed visually as a Punnett Square in which each side has length $p + q$, and the areas within the square correspond to each term in the expanded equation. Using the reasonable values for the frequencies of the sickle cell allele and the normal allele, fill in and label the diagram below. Identify the area that would represent people with sickle cell anemia. Estimate the percentage of the US black population that would suffer from sickle cell anemia.