December 17, 2002 name__**master**_______section__

# **Final Examination** — 115:413 Experimental Biochemistry – Annotated Answers

The exam was in two versions, differing only in the placement and letter of the correct answer in the multiple choice questions, and in some of the numbers in the problems, so that even if this does not give the absolute answers to problems in the other version, it shows how they were done. **The correct answers are given in Times bold face; further commentary is in Arial Rounded typeface.**

Part A - multiple choice; answer each question by circling the letter of the correct answer.

1. Statistical treatments of plotted data seek to minimize
   a. the sum of deviations of actual points from the best line through the points.
   **b. the sum of squares of deviations of actual points from the best line through them.**
   c. the intercept of the best line through the points.
   d. the variation of slope of lines from the origin through the points.
   **The sum of deviations of course would be zero; the squares of deviations are all positive, minimizing the sum of squares gives the best fit of the line to the data.**
2. In pipetting with a micropipetter, you should
   **a. push the plunger down to the first stop when sucking up, beyond it when delivering.**
   b. deliver the sample into the solution to which you are adding it.
   c. hold the pipetter horizontal as much as possible.
   d. suck up into the tip as quickly as possible.
3. The $pK_1$ of $H_2CO_3$ is 6.1, the $pK_2$ is 10.6, and the $pK_a$ of $NH_4^+$ is 9.3. The pH of 1 M $NH_4HCO_3$ is approximately
   a. 10.0 b. 9.3 **c. 7.7** d. 6.1
   **This is an isoelectric point question, the pH will be half way between the $pK_a$ of $HCO_3^-$ picking up a proton and $NH_4^+$ losing one.**
4. To take 50 ml of water to pH 12.0 will require how much 0.5 M NaOH?
   a. 0.5 ml **b. 1.0 ml** c. 2.5 ml d. 5.0 ml
   **A pH = 12 is equivalent to 0.01 M $OH^-$; 50 ml of it would contain 0.01x50 = 0.5 mmole $OH^-$, which would be provided by 1.0 ml 0.5 M $OH^-$.**
5. Which of the following is **not** an advantage of the Coomassie Blue method?
   a. speed b. sensitivity c. lack of interference **d. low background**
   **The reagent of course has high absorbance, not low background – the one drawback of the method.**
6. For a protein standard curve you wish samples (0.25 ml) to contain a maximum of 50 µg of protein. You have a stock solution of ovalbumin, 10 mg/ml. How much should you dilute this to make a working solution, samples of which you will use for the standard curve?
   a. 1:10 b. 1:25 **c. 1:50** d. 1:100
   **If 0.25 ml is to contain 50 µg = 0.05 mg, its concentration is 0.05 mg/0.25 ml = 0.2 mg/ml, which is a 1:50 dilution from 10 mg/ml.**
7. Solutions of a protein appear much higher in concentration when measured by the Coomassie Blue method than when measured by the Lowry method, and have very little UV absorbance. This suggests that the protein contains
   a. a lot of tyrosine and tryptophan residues.
   b. a lot of leucine and isoleucine, very few polar residues.
   c. a lot of proline and hydroxyproline residues, very few charged side chains.
   **d. very few tyrosine and tryptophan residues, a lot of arginine.**
8. In which protein determination method would polyglycine give a higher absorbance per mg than polytryptophan?
   **a. biuret** b. Lowry c. ultraviolet absorbance d. Coomassie Blue
   **Because glycine has a much lower mol. wt. (75 Da) than tryptophan (204 Da), there will be more glycine residues in a mg of polyglycine than tryptophan residues in a mg of polytryptophan, therefore more peptide bonds and more reaction with the biuret reagent. This was mentioned in lecture.**
9. In polarimetry, the symbols + and – indicate
   **a. whether the solution rotates the plane of polarization to the right or left of zero.**

b. whether the optical rotation is greater or less than zero.
c. whether the sugar is of the D or the L series (related to D- or L-glyceraldehyde).
d. whether or not the sugar is a reducing sugar.

10. Colorimetric methods for carbohydrates in strong acid depend on
a. dehydration of the carbohydrates to furfurals.
b. depolymerization of polysaccharides by acid.
c. reaction of furfurals with other molecules to produce color.
**d. all of the above.**

11. The glycosidic oxygen in a sugar (through which glycosides are formed) may be identified as the one which in the open-chain version of the structure
a. is at the top. **b. is a carbonyl.** c. is next to the bottom. d. is at the bottom.
**This becomes a hydroxyl when another hydroxyl attacks to form the ring. It is more reactive than other hydroxyls.**

12. In the Nelson-Somogyi method, the product actually measured is
a. a furfural complex b. $Cu^+$
**c. reduced arsenomolybdate** d. a carboxylic acid
**The initial product is $Cu^+$. This then reduces the arsenomolybdate to generate the intense blue color.**

13. A carbohydrate shows as much color per mg in the orcinol reaction as L-arabinose, but half as much per mg in the Nelson-Somogyi reaction. Its calculated $[\alpha]_D$ is +27°, but after hydrolysis it shows essentially no optical rotation. Relevant $[\alpha]_D$ are: L-arabinose, +104°; D-xylose, +18.1°; D-mannose, +14.1°. A possible structure is
a. L-xylosyl-(1→4)- D-mannose. b. L-xylosyl-(1→4)- L-arabinose.
c. L-xylosyl-(1→1)- D-xylose. **d. L-xylosyl-(1→5)- D-xylose.**
**Too tricky for almost everyone. As much color per mg as L-arabinose indicates that all sugars are pentoses; half as much color per mg in the Nelson-Somogyi reaction indicates it is a reducing disaccharide (1→5, not 1→1); that the optical rotation after hydrolysis is 0 indicates one L and one D of the same sugar, their optical rotation canceling out perfectly.**

14. In our purification of D-amino acid oxidase, the largest amount of protein is removed by
**a. acidification + heating** b. $(NH_4)_2SO_4$ precipitation
c. DEAE-Sepharose chromatography d. phenyl-Sepharose chromatography
**Remember digging all that sludge out of the bottles!**

15. Phenyl-Sepharose chromatography is an example of
a. ion exchange chromatography b. gel filtration
**c. hydrophobic chromatography** d. hydroxylapatite chromatography

16. We use crotonate, not benzoate, during the DEAE-Sepharose chromatography because
**a. benzoate absorbs strongly at 280 nm.** b. crotonate keeps FAD on the enzyme better.
c. crotonate buffers better at pH 6.8. d. crotonate interferes less with the assay.

17. An advantage of $(NH_4)_2SO_4$ for protein precipitation is that
**a. very high concentrations can be made** b. its ions associate with proteins
c. it doesn't shift the pH of neutral solutions d. all of the above
**As is stated in the manual, $(NH_4)_2SO_4$ ions do not associate with proteins, and this is good. It does shift the pH, down to ≈ 5.2.**

18. To determine the µmoles pyruvate formed in an assay tube,
a. multiply the $A_{560}$ by the slope of the standard curve (A/µmole).
**b. divide the $A_{560}$ by the slope of the standard curve.**
c. divide the $A_{560}$ by the molar extinction coefficient.
d. divide the $A_{560}$ by the ml dilute enzyme used.
**The slope of the standard curve has the units A/µmole; thus dividing $A_{560}$ by the slope gives µmoles.**

19. The peroxidase assay would be inhibited by presence of
a. $(NH_4)_2SO_4$ b. L-alanine c. oxygen **d. catalase**
**Catalase would destroy the peroxide which is measured in this assay.**

20. In the polarographic assay, the curvature of the chart trace is due to
a. loss of activity of the enzyme
b. accumulation of pyruvate
**c. $[O_2]$ decreasing below the $K_m$**
d. bubble formation
**As the reaction proceeds, the $[O_2]$ decreases, and because it is already below the $K_m$, the reaction rate decreases.**

21. In the spectrum of the purified enzyme, a peak at 405 nm is due to
a. FAD bound to the D-amino acid oxidase
**b. denatured hemoglobin**
c. free FAD
d. benzoate **This was mentioned often.**

22. In high speed centrifugation, it is important to
a. fill the bottles no more than 3/4 full.
b. balance bottles to achieve equal mass.
c. fasten the lid to both rotor and shaft.
d. wash out the rotor if solution has spilled.
**e. all of the above.**

23. The mobility of proteins in SDS polyacrylamide gel electrophoresis is determined by
a. charge density
b. molecular shape
**c. molecular weight**
d. all of the above
**Shape is lost due to denaturation by dodecylsulfate, and charge density is constant due to association of dodecylsulfate$^-$ with the peptide bonds. This leaves only molecular weight.**

24. The key property of the stacking gel is
a. low (4%) acrylamide concentration
b. presence of a zwitterionic buffer
**c. pH several units lower than $pK_a$ of glycine**
d. all of the above
**The pH reduces the mobility of the glycine to below that of the protein, because few glycine molecules are anions at any given moment. This is the necessary condition for stacking (concentration of the protein into a narrow band).**

25. The key ingredient for forming a gel, rather than a viscous solution, by polymerization of acrylamide is
a. ammonium persulfate
b. tetramethylethylenediamine
c. triethanolamine
**d. methylenebisacrylamide**
**This is what cross-links chains into a gel (as stated in the manual).**

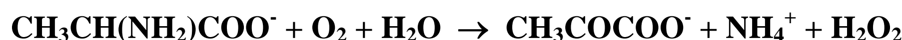
**26.** What is the function of the 'blocking agent' in which the nitrocellulose is soaked?
a. to keep transferred proteins on the membrane.
b. to block any proteins other than D-amino acid oxidase from reacting with antibody.
**c. to block all other sites on the membrane so that they do not bind antibody directly.**
d. to inhibit the growth of bacteria.
**Otherwise antibody would bind all over the membrane, and there would be dye everywhere.**

27. What is the function of the 'second antibody' (goat anti-rabbit IgG alkaline phosphatase conjugate) in western blot visualization?
a. to react with D-amino acid oxidase.
**b. to react with rabbit antibody which has bound to D-amino acid oxidase.**
c. to catalyze hydrolysis of 5-chloro-4-bromoindole-3-phosphate.
d. to reduce Nitro Blue Tetrazolium, which precipitates where the antibody has bound.
**Allen pointed out that catalyzing hydrolysis could also be the answer, if one focused on the alkaline phosphatase conjugated to the antibody; but the main reference was to 'second antibody', which binds to the bound rabbit antibody.**

28. Isoelectric focusing separates proteins on the basis of
a. charge density.
b. size.
c. SDS binding.
**d. isoelectric point.**
**This should have been obvious, and usually was.**

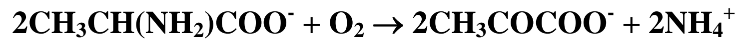
## Part B – Short Answers.

1. (3 pts) Write the balanced chemical reaction for the reaction catalyzed by D-amino acid oxidase.



$$CH_3CH(NH_2)COO^- + O_2 + H_2O \rightarrow CH_3COCOO^- + NH_4^+ + H_2O_2$$

1a (3 pts EXTRA CREDIT) Write the balanced chemical reaction for the net reaction catalyzed by D-amino acid oxidase + catalase.

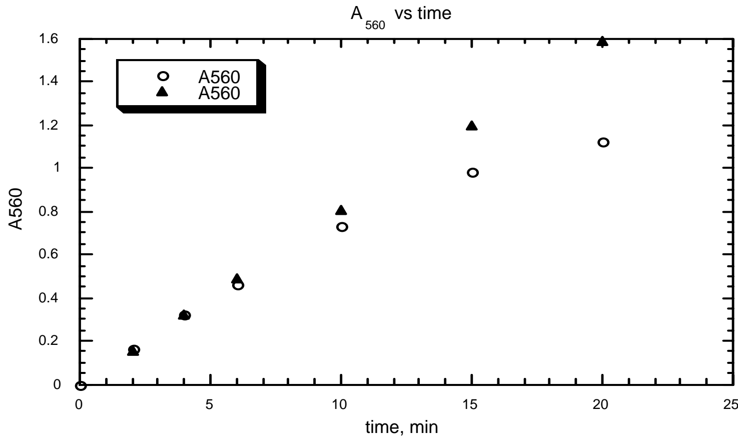


$$2CH_3CH(NH_2)COO^- + O_2 \rightarrow 2CH_3COCOO^- + 2NH_4^+$$

**Both of these were gone over in lecture, as well as being on prior exams.**

2. (5 pts) In the graph below, the circles represent $A_{560}$ data obtained using FAD without catalase, incubated without shaking. The triangles represent data obtained using FAD with catalase and shaking the tubes during incubation. Explain the difference between the results under the two conditions.

**In the unshaken assay tubes, oxygen is being depleted by the enzymatic reaction faster than it dissolves in from the atmosphere. Since the reaction is occurring at $[O_2] < Km$, the rate of the reaction decreases. With shaking and in presence of catalase, oxygen is restored fast enough to keep the reaction occurring at its initial velocity.**



## Part C. Problems.

1. The following rather involved pH problem has been broken into sections to assist you in proceeding to the final answer – if I just asked for the final answer, I expect most would be daunted. Try to do as many parts as you can.

a. (4 pts) What is the molarity of free acetic acid ($CH_3COOH$) in 10 M acetate buffer pH 4.0?

$$4.0 = 4.76 + \log\frac{10-x}{x} \quad -0.76 = \log\frac{10-x}{x} \quad 0.174 = \frac{10-x}{x} \quad 0.174x = 10 - x \quad 1.174x = 10 \quad x = 8.52\text{ M}$$

**Far too many people put 10 into the numerator instead of 10-x. Always remember that when a buffer concentration is stated as 10 M acetate, 10 M is the *sum* of the concentrations of the acidic and basic forms. If [acidic] is x, then [basic] is 10-x.**

What is the molarity of free acetic acid in 10 M acetate buffer pH 5.3? The difference between these may be used later in the problem as the effective acetic acid concentration available to adjust pH to 5.3, since appreciable acetic acid must remain to fulfill the Henderson-Hasselbalch equation at this pH. (Calculation same as the first part, different pH.)

$$5.3 = 4.76 + \log\frac{10-x}{x} \quad 0.54 = \log\frac{10-x}{x} \quad 3.467 = \frac{10-x}{x} \quad 3.467x = 10 - x \quad 4.467x = 10 \quad x = 2.238\text{ M}$$

**The difference = <u>6.282</u> M. Same calculation as the preceding, with different numbers.**

b. (2 pts) If the molecular weight of Na benzoate is 144, and the molecular weight of Ammediol (2-amino-2-methyl-propane-1,3-diol) is 105, what are their respective concentrations in 0.1% Na benzoate – 0.25% Ammediol?

$$[\text{Na benzoate}] = \frac{1.0\,g/L}{144\,g/mole} = 6.9\text{ mM} \qquad [\text{Ammediol}] = \frac{2.5\,g/L}{105\,g/mole} = \underline{23.8\text{ mM}}$$

**I was shocked at how few could do this. I should have explicitly said *molar* concentration, but that should been obvious. But then, it should have been obvious that to go from a weight concentration (0.1% = 1 g/L) to a molar concentration, you *divide* by the molecular weight (g/mole), the units will then come out moles/L. Many people multiplied – and got a ridiculous answer.**

c. (2 pts) If the $pK_a$ of benzoic acid is 5.0, what will be the concentrations of benzoic acid and benzoate if the pH of 0.1% Na benzoate is adjusted to 5.3?

$$5.3 = 5.0 + \log\frac{6.9-x}{x} \quad 0.3 = \log\frac{6.9-x}{x} \quad 2 = \frac{6.9-x}{x} \quad 2x = 6.9 - x \quad x = 6.9/3 = \underline{2.3\text{ mM}} \quad [Bz^-] = \underline{4.6\text{ mM}}$$

**The calculation is similar to part a, using the total concentration derived in part b.**

d. (2 pts) Therefore, how many millimoles of acid will have to be added to titrate 400 ml 0.1% Na benzoate – 0.25% Ammediol to pH 5.3? (The Ammediol will be fully protonated at this pH.)

**2.3 mmoles/L benzoate + 23.8 mmoles/L Ammediol = 26.1 mmoles total/L must be acidified, x 0.4 L = 10.44 mmoles acid must be added.**
**The tricky part here is that you have to titrate *all* the Ammediol (originally all in the basic form) as well as 1/3 of the benzoate. But few got this far.**

e. (2 pts) Using your results from a and d, how many ml 10 M acetate buffer pH 4.0 will have to be added to titrate 400 ml 0.1% Na benzoate – 0.25% Ammediol to pH 5.3? (You actually added more, because you were also titrating proteins and other cellular constituents.)

**10.44 mmoles acid/6.282 mmoles/ml = <u>1.66 ml.</u> Simple, if you got this far.**

2. You are given 15 ml of a solution of pure carbohydrate, and told its $[\alpha]_D$ is +27°.

a. (3 pts) You determine the optical rotation in a 2 dm cell to be +3.6°. What is the concentration of the stock solution in g/100 ml? In mg/ml?

**Optical rotation = $[\alpha]_D \cdot c \cdot l/100$   c = $\frac{100 \cdot OR}{[\alpha]_D \cdot l} = \frac{100 \cdot 3.6}{27 \cdot 2}$ = <u>6.67 g/100 ml</u> = 66.7 mg/ml**

**I just remember one form of the equation, and rearrange to solve for whatever is asked for. You have to remember that c is in g/100 ml and l in decimeters.**

b.(1 pt) You dilute this stock 1:100, and then dilute 3 ml of the dilute solution to 20 ml. What is the concentration, in mg/ml?

**A 1:100 dilution of 66.7 mg/ml = 0.667 mg/ml. $\frac{0.667 \cdot 3}{20} = \frac{2}{20}$ = <u>0.1 mg/ml</u>**

**In the other version the stock was 80 mg/ml, but in both the dilution gave 0.1 mg/ml.**

c. (3 pts) You carry out the Nelson-Somogyi reaction on this second diluted solution and a glucose standard, 0.1 mg/ml. The results are:

| sugar | ml | | | | |
|---|---|---|---|---|---|
| | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 |
| | | | $A_{660}$ | | |
| glucose | 0.21 | 0.41 | 0.83 | 1.27 | 1.68 |
| unknown | | 0.151 | 0.298 | 0.456 | 0.664 |

What is the apparent concentration(mg/ml) of the dilute unknown sugar?

**The slope of the standard curve is 4.2 $A_{660}$/mg. The slope of the plot for the unknown sugar is 1.66 $A_{660}$/ml. $\frac{1.66 A_{660}/ml}{42 A_{660}/mg}$ = 0.0395 mg/ml, slightly over 1/3 of the value calculated by dilution of the stock solution. Quite a few got this, which did not depend on parts a and b.**

d. (3 pts)What is a likely reason that the result from c does not agree with that from b?

**The sugar is a trisaccharide, with molecular weight just below 3x that of the monosaccharide glucose. Therefore its molarity, and concentration as reducing sugar, is about 1/3 that of an equal weight of glucose. (EXTRA CREDIT: because an equivalent of water is eliminated in formation of each glycosidic bond, the trisaccharide has molecular weight 36 Da less than 3x the mol. wt. of glucose, hence the molar concentration is slightly more than 1/3 that of 0.1 mg/ml glucose.)**

**This required getting a correct value of 0.1 mg/ml in b, realizing that the value from c was about 1/3 of this – not exactly, see EXTRA CREDIT – and explaining the discrepancy as due to the molarity of the sugar being ≈1/3 that of an equal weight of glucose, the molecular weight ≈3x greater, the sugar thus a trisaccharide. A few got as far as suggesting a disaccharide. "Experimental error" is not an acceptable answer on an exam – you should assume that experiments and calculations have been done correctly.**

3. a) The following rates of enzymatic oxidation of samples of 0.04 M DL-methionine are observed in the pyruvate assay (assay volume 1.0 ml, slope of standard curve 3.0 A/μmole):

| ml DL-met/$PP_i$ | 0.015 | 0.03 | 0.06 | 0.1 | 0.2 | 0.3 | 0.5 |
|---|---|---|---|---|---|---|---|
| [D-met], mM: | **0.3** | **0.6** | **1.2** | **2** | **4** | **6** | **10** |
| $A_{560}$ | 0.156 | 0.264 | 0.403 | 0.51 | 0.638 | 0.695 | 0.750 |

**Remember that 0.04 M = 40 mM DL-methionine = 20 mM D-methionine. 0.1 ml of this diluted to 1 ml would be 2 mM; and once you have one number at the right level, the others follow easily.**

a. (4 pts) Calculate D- methionine concentration (above), and either [S]/v (Woolf plot) or 1/[S] and 1/v (Lineweaver-Burk plot) and plot on the graph below. It is simplest to plot v as $A_{560}$ and calculate μmoles/min etc. only for $V_{max}$. Label the axes clearly

| 1/[S] | **3.33** | **1.67** | **0.83** | **0.5** | **0.25** | **0.167** | **0.1** |
|---|---|---|---|---|---|---|---|
| 1/v | **6.41** | **3.79** | **2.48** | **1.96** | **1.567** | **1.439** | **1.333** |
| [S]/v | **1.92** | **2.27** | **2.98** | **3.92** | **6.27** | **8.63** | **13.33** |

**I should have said *use* v as $A_{560}$; I meant you to plot either the Lineweaver-Burk form (1/v vs. 1/[S]) or the Woolf form ([S]/v vs. [S]). Next time I will be explicit in asking for one of these – too many people plotted v vs. [S], which cannot give an accurate $K_m$ and $V_{max}$. This was given 2 pts out of 3 (1 pt for correct [D-methionine]; 0.5 pt for concentrations of DL-met.)**b. (4 pts) Determine $V_{max}$ (in $A_{560}$) and $K_m$ (mM) from the data above.

**By construction, $V_{max}$ = 0.85 $A_{560}$, $K_m$ = 1.333 mM. In the Lineweaver-Burk plot, the intercept should come out 1.11 $A_{560}^{-1}$, the slope = $K_m$/ $V_{max}$ = 1.57. In the other version the $V_{max}$was the same, the $K_m$ was 1.133 mM, the slope 1.333. Do not commit the sin of saying $V_{max}$ = $K_m$/2!**

c. (1 pt) If the enzyme used was 0.1 ml of a 1:100 dilution, what is the $V_{max}$ in μmoles/min· **stock** enzyme? ***Now* convert $A_{560}$ to μmoles, by dividing by the slope of the standard curve! Divide by 10 min, and by the 0,1 ml used, and multiply by 100.**

$$\frac{0.85\, A_{560}\ in\, 10\min}{3\, A_{560}/\mu mole}$$ **= 0.283 μmole in 10 min = 0.0283 μmole/min, x** $\frac{100}{0.1}$ **= <u>28.3 μmole/min·ml stock</u>**

d. (1 pt) If the stock enzyme had a protein concentration of 0.8 mg/ml, and the molecular weight (per subunit) is 39,000, what is the turnover number?

$$\frac{28.3\,\mu mole}{\min\cdot ml\, stock}\cdot\frac{ml}{0.8\, mg}\cdot\frac{39\, mg}{\mu mole\ enzyme}$$ **= <u>1380 min$^{-1}$ = 23 sec$^{-1}$</u> Set it up so that the units cancel.**

5. (4 points) Observed $R_m$s and molecular weights of the standard proteins for molecular weight determination by SDS gel electrophoresis are as follows:

| Protein | $R_m$ | mol. wt. | Protein | $R_m$ | mol. wt. |
|---|---|---|---|---|---|
| lysozyme | 0.85 | 14,300 | ovalbumin | 0.375 | 45,000 |
| trypsin inhibitor | 0.71 | 20,000 | albumin, bovine serum | 0.22 | 66,000 |
| carbonic anhydrase | 0.56 | 29,000 | phosphorylase b | 0.075 | 93,000 |

An unknown protein has an $R_m$ of 0.35 on the same gel. Calculate its molecular weight (use one of the graphs below, or fit the standard molecular weights to an appropriate equation) .

**By construction, $R_m$ = 4.983 – 0.985(log mol. wt.), log mol. wt. =** $\frac{4.983 - R_m}{0.985}$**. For $R_m$ = 0.35, log mol. wt. = 4.703, mol. wt. = <u>50,530</u>. In the other version the plot was the same, the $R_m$ was 0.50, corresponding to a mol. wt. of 35,585.**

**Most people got this, more or less (for some reason almost everyone was slightly low). You had to know to plot *log* mol. wt. on the right (linear) graph, preferably scaling the x axis only from 4.15 to 4.95; or you could plot the molecular weights directly on the left (semilog) graph, scaling the x axis from 10,000 to 200,000.**