Sample Questions – Biochemistry 3380G

February 2013

1. When operating a digital micropipette
   a) always operate the plunger (or piston) with your forefinger.
   b) never lay the pipette down with liquid in the tip.
   c) it is acceptable practice to rotate the control mechanism up to 10% beyond the upper limit.
   d) put the tip on gently to avoid damaging the micropipette.
   e) the tip should not touch the walls of the container into which you are dispensing liquid

2. You are required to make 750 mL of 300 mM NaCl. How much solid NaCl would you need to weigh out to make this solution? The atomic weights of sodium and chlorine are 22.99 and 35.45, respectively.

3. You are required to make 250 mL of 2.5 mM EDTA. The available stock solution is 50 mM. How would you make the 2.5 mM solution?

4. Define absorbance (A) in terms of incident light intensity (I_o) and transmitted light intensity (I).

5. Spectophotometers are calibrated in percent transmittance (%T) and absorbance (A). What absorbance corresponds to 100% T?

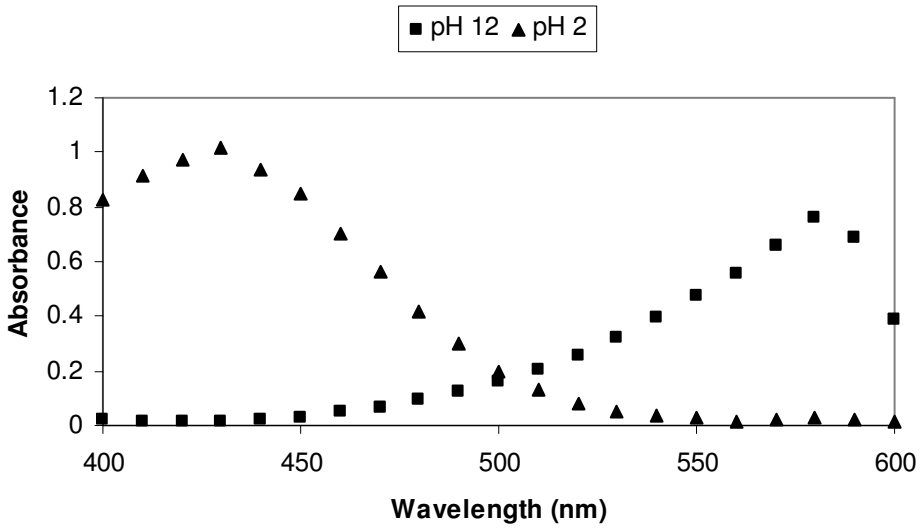
6. Do you think that a solution of denatured hemoglobin would absorb light in the visible region of the spectrum? Why or why not?

7. Write the Henderson-Hasselbalch equation for the generic acid HA.

8. The pK_a of acetic acid is 4.76. Over what pH range could you use it as a buffer?

9. From the titration curve of the amino acid glutamate, three pK_as were identified ($pK_1 = 2.19$, $pK_R = 4.25$, $pK_2 = 9.67$, where the pK_a of the side chain group is designated pK_R). Draw the structures of the two main ionic forms of glutamate at pH 2.19.

10. Suppose an indicator has absorption spectra at pH 2 and pH 12 as shown in the figure below. If you wanted to determine the pKa of this indicator by measuring the absorbance change as a function of pH, and the sensitivity of your measurement was of extreme importance, what wavelength would you choose?



11. Suppose a sample contains two polypeptides, one with a mass of 2000 and the other with a mass of 3000. If this sample is analyzed by MALDI-TOF, and both peptides become singly charged, which polypeptide will reach the detector first?
    a) The polypeptide with m/z of 2000.
    b) The polypeptide with m/z of 3000.
    c) The polypeptides will reach the detector at the same time.

12. Suppose you want to use trypsin to digest a protein in solution for analysis by mass spectrometry. Which of the following mass ratios of substrate protein : trypsin would be most appropriate?
    a) 400:1
    b) 20:1
    c) 1:2
    d) 1:20
    e) 1:400

13. Using mass spectrometric data to search a database for an unknown protein from an unknown source, you have received output from ProFound as shown in the table below. How confident would you be that the first-ranked protein is the correct one? On what do you base your evaluation of the data?

Rank	Expectation	Protein information	%	pI	kDa
1	3.4×10^{-14}	gi|50419271|ref|XP_458159.1| hypothetical protein DEHA0C11968g [Debaryomyces hansenii CBS767]	26	6.1	112.07
2	0.92	gi|15678324|ref|NP_275439.1| hypothetical protein MTH296 [Methanothermobacter thermautotrophicus str. Delta H]	13	10.3	47.83
3	1.0	gi|6898|emb|CAA33463.1| twitchin [Caenorhabditis elegans]	3	5.4	668.45

14. The double reciprocal plot of Lineweaver and Burk plots 1/v versus 1/[S]. What is the slope of the line generated by this plot?

15. For an enzyme-catalyzed reaction, the values for V_{max} and K_M can be quickly estimated by inspecting values of v at increasing [S]. Estimate values for V_{max} and K_M for the enzyme-catalyzed reaction for which the following data were obtained:

[S] (M)	Velocity (μM/min)
2.5×10^{-6}	28
4.0×10^{-6}	40
1.0×10^{-5}	70
2.0×10^{-5}	95
4.0×10^{-5}	112
1.0×10^{-4}	128
2.0×10^{-3}	139
1.0×10^{-2}	140

16. Sketch an Arrhenius plot of log *v* versus 1/T measured at four different temperatures (it is not necessary to use specific numbers). Label the axes and slope.

17. Describe the relationship between protein molecular weight and distance migrated during SDS-PAGE. How exact is this relationship?

18. Give three properties of a hexa-histidine tag that make it useful for protein purification.

19. In the protein purification lab, why did you digest the DNA in your cell extract?

20. In one sentence, describe the role of bromophenol blue in SDS-PAGE sample buffer.

21. What chemical used in SDS-PAGE is hazardous, and what is the nature of the hazard?