12 Answers to Study Guide Questions

Note: The answers/solutions you find in this section are dr. tj's answers. As such, there could be errors or alternatives. Consequently, if your response/solution is different from any of the following, don't worry. Just ask your instructor! ☺ dr. tj

2 Spectrophotometry and the Analysis of Riboflavin

1. Since each compound absorbs some of the total absorbance is from each compound $A_{total} = \varepsilon_1 l_1 c_1 + \varepsilon_2 l_2 c_2$ + etc.
2. Absorbance of the solution versus wavelength (nm). This type of analysis generates a fingerprint that can be used to identify the solute.
3. $\varepsilon = A/cl = 0.750/(6 \times 10^{-4}M \times 1.2cm) = 1042M^{-1}cm^{-1}$
4. $c = A/\varepsilon l = 0.650/(13{,}000M^{-1}cm^{-1} \times 1\ cm) = 5 \times 10^{-5}M$
5. Absorbance at a single wavelength versus solute concentration. The curve can be used to determine the unknown concentration of the particular solute in the same solvent.
6. $M_2 = M_1V_1/V_2 = (5.31 \times 10^{-5}M)(5mL)/25mL = 1.06 \times 10^{-5}M$
7. A *quantitative statement* is one based on actual numerical data whereas a *qualitative statement* is the result of a relative comparison of results or information.
8. According to the regression analysis, the slope of the generated line is 13,207. Since the slope is equal to the quantity εl, $\varepsilon = 13{,}207M^{-1}/0.7cm = 18{,}867\ M^{-1}cm^{-1}$

3 The Preparation of Buffers at Desired pH

1. $[H^+]_{tomato\ juice} = 10^{-4}M$; $[H^+]_{NaOH} = 10^{-12}M$; $[H^+]_{tomato\ juice}/[H^+]_{NaOH} = 10^{-4}/10^{-12} = 10^8$
2. 10 mL phosphoric acid, 9.29 mL NaOH.
3. It must first be rinsed with deionized water and blotted dry.
4. Weak acid = KH_2PO_4; conjugate base = K_2HPO_4
5. Effective buffer range would be pH 9.5 – 11.5
6. Preparing 100mL of 25mM acetate buffer at pH 5.50 from stock solutions of 250mM acetic acid and 250mM NaOH

	NaOH	Acetic acid
Decimal fraction	0.8460451	0.1539549
Molarity	0.021151128	0.003848872
Moles	0.002115113	0.000384887
Total moles		0.002500000
Liters	0.008460451	0.01
mL	8.46	10.0
mL dH_2O to volume		

7. 25g NaCl/100g solution = 25g NaCl + 75g H_2O
8. 1.5g NaBr/100mL solution = 1.5g NaBr + H_2O to 100mL
9. 65mL acetone/100mL solution = 65mL acetone +H_2O to 100mL
10. mass = Molecular weight*concentration*volume = g/mol*mol/L*L

    = 180g/mol*1.2*10^{-7}M*0.5L = 0.0000108g = 1.08*10^{-5}g

11. pH = pKa + log [base]/[acid]
    3.80 – 4.76 = log [base]/[acid]
    -0.96 = log [base]/[acid]
    $10^{-0.96}$ = [base]/[acid]
    0.10964782 = [base]/[acid]
    decimal fraction base = 0.098813171; % base = 0.098813171*100 = ~9.88%
    decimal fraction acid = 0.901186829; % acid = 0.90118629*100 = ~ 90.12%

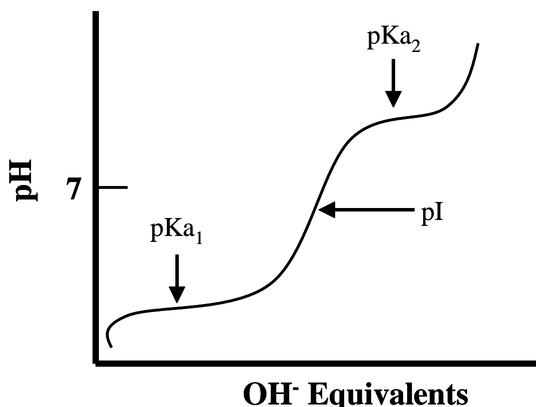
12. ? µmols = 100pmols * $\frac{1 \text{ mole}}{10^{12} \text{ pmols}}$ * $\frac{10^6 \text{ µmols}}{1 \text{ mol}}$ = 1 x 10^{-4}

13. ? fL = 1 mL * $\frac{1 \text{ L}}{10^3 \text{ mL}}$ * $\frac{10^{15} \text{fL}}{\text{L}}$ = 1 x 10^{12}

14. Based on the H-H equation, if pH is 2 units below the pKa,
    -2 = log [A^-] / [HA]
    10^{-2} = [A^-] / [HA]
    0.01 = [A^-] / [HA]
    ∴ the ration of [A^-] / [HA] is 1:100.

4 Titration Curve of an Amino Acid

1. Glycine titration curve



2. The precise structure depends on the class of amino acid

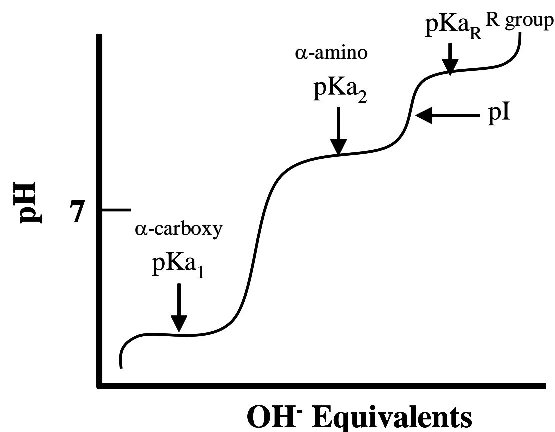
	Simple AA	Acidic AA	Basic AA
Acid Titration ↑		$H_3N^+{-}CH(RH){-}COOH$	
	$H_3N^+{-}CH(R){-}COOH$	$H_3N^+{-}CH(RH){-}COO^-$	$H_3N^+{-}CH(RH^+){-}COOH$
Physiological	$H_3N^+{-}CH(R){-}COO^-$	$H_3N^+{-}CH(R^-){-}COO^-$	$H_3N^+{-}CH(RH^+){-}COO^-$
Base Titration ↓	$H_2N{-}CH(R){-}COO^-$	$H_2N{-}CH(R^-){-}COO^-$	$H_2N{-}CH(RH^+){-}COO^-$
			$H_2N{-}CH(R){-}COO^-$

3. Stirrer must be turned off because magnet interferes with pH meter needle.

4.

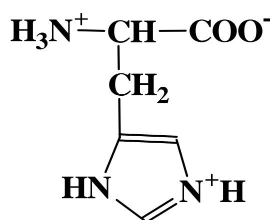
A. Triprotic

B. – D.

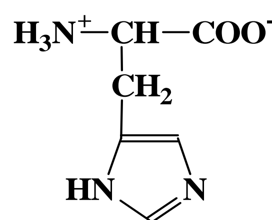


5. pI $_{\text{acidic AA}}$ = $(pKa_1 + pKa_R)/2 = (1.99 + 3.90)/2 = 2.94$
6. at pKa_2
7. Y is neutral at physiological pH.
8. It will not migrate at its pI.
9. A. Although H is a "basic" amino acid, its ionization pattern follows that of an acidic amino acid because the dissociable proton of the imidazole group (pKa_R) is lower than that of the α-amino group (pKa_2). The ionization pattern, thus, is $+2 \xrightarrow{pKa_1} +1 \xrightarrow{pKa_R} 0 \xrightarrow{pKa_2} -1$. Since pH 7.4 is greater than pKa_R but less than pKa_2, the predominant ionic form is the zwitterion.

   B.



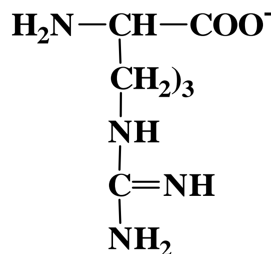
Weak acid



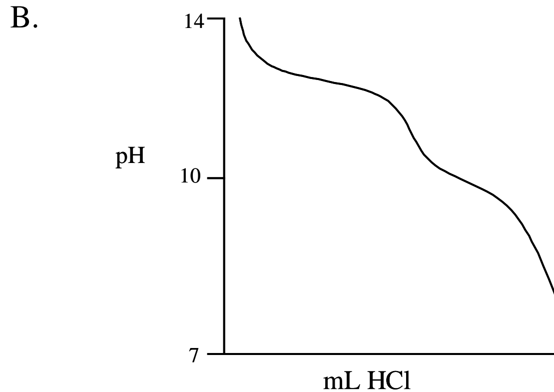
Conjugate base

   C. When the pH is one unit below pKa_R,
   $-1 = \log [A^-] / [HA]$
   $10^{-1} = [A^-] / [HA]$
   $0.1 = [A^-] / [HA]$
   $\therefore$ the ratio is 1:10 $[A^-] / [HA]$

10. Buffer regions are generally defined as pKa ± 1 pH unit. Such being the case, lysine's pKa_2 and pKa_R buffer regions overlap because they are ~1 pH unit apart.
11. A.



B.



5 Quantitative Determination of Proteins

1. The blank should contain all of the assay components *except* the substance that is being measured.
2. 10μg/0.1mL = 100μg/mL
3. Examples are urea and guanidine˙HCl (substances often used to denature proteins), Tris buffer (common buffer used at pH 8), EDTA (a chelating agent. protease inhibitor)
4. The Lowry reagent has phosphomolybdotungstate in place of alkaline copper sulfate. As such, it is a more sensitive reagent unless the protein being measured has a paucity of W (and to a lesser degree Y) residues. When this is the case, the Lowry is not a good reagent to use.
5. It is more advantageous to use the Lowry when there is the possibility that the protein concentration is very low because the Lowry is much more sensitive than the Bio-Rad (0.005 – 0.3mg protein/mL [Lowry] versus 0.2 - 1.4mg/mL [Bio-Rad]).
6. Yes, the biuret contains copper sulfate.
7. (A) $c = A/(\varepsilon l) = 0.358/(18{,}550 M^{-1}cm^{-1} * 1cm) = 1.93 \times 10^{-5} M$
   (B) $moles = (1.93 \times 10^{-5} M) \times 0.25L = 4.82 \times 10^{-6}$ moles
   (C) $g/mole = 2.58 \times 10^{-3} g / 4.82 \times 10^{-6} mole = 534.7$

6 Isolation and Characterization of Casein and Globulin

1. Covalently to –OH-containing residues, 1°ly via S, but also via T, Y
2. Contains all of the EAAs.
3. Dissolve them both in dilute salt. Add an equal volume of saturated A.S. to the protein-containing solution. Incubate on ice for 20 minutes. Centrifuge. Transfer the supernatant to a clean tube. The pellet contains the albumin; the supernatant, the globulin.

4. Salting-in is the process by which dilute salt solution is used to dissolve a protein. Salting-out is the process by which concentrated salt solution is used to precipitate a protein from solution.
5. C
6. Solubility decreases
7. Positive; negative
8. B
9. D
10. Can use isoelectric precipitation to separate the two proteins because their respective pIs are different. Could also use ammonium sulfate precipitation because globulin comes out only when the solution is completely saturated with ammonium sulfate.

7 Electrophoresis

1. The migration of charged molecules in an electric field
2. Its charge to size ratio
3. When they are of similar size
4. Acrylamide concentration
5. 1 %(w/v) = 1g solute / 100mL solvent; 1g/100mL = x g/40mL; x = 0.4g ∴ Dissolve 0.4g agarose in 40mL buffer.
6. (A). Pattern A
   (B). Q = pI – pH; Cytochrome c: 9.6 – 8.3 = 1.3 (goes toward negative pole); the remaining proteins are ordered (from well to positive pole): myoglobin (-0.7), carbonic anhydrase (-1.8), and β-lactoglobulin B (-3.2)
7. Under physiological conditions, most have a net negative charge.
8. For this type of analysis, capillary electrophoresis could be done. Additionally, PAGE could be performed if the gel were silver stained rather than stained with a Coomasie-based dye. This is because silver staining very easily detects nanogram quantities of protein.
9. Denaturants disrupt noncovalent interactions, whereas degradative agents must break covalent bonds between the building blocks of a macromolecule. SDS does not break the covalent peptide bonds.
10. 2ng is well below the detection limit for Coomasie. If it is necessary to run an agarose gel, a significantly larger quantity of protein must be loaded. Alternatively, PAGE stained with silver or capillary electrophoresis could be performed.

8 Measuring the Activity of the Enzyme Catalase

1. C
2. Its turnover number under optimal assay conditions is much too high for accurate measurements of enzyme activity to be made using our laboratory equipment. Performing the experiment at 0^oC sufficiently slows down the enzyme enough for our measurements.
3. B
4. C
5. 187.5 (Tube1$_{H_2O_2}$) – 131.25 (Tube2$_{H_2O_2}$) = B

9 Kinetic Properties of Acid Phosphatase- Determining K_M & V_{max}

1. Hydrolases catalyze bond breaking by adding water across the susceptible bond.
2. Mg is an essential cofactor; KOH quenches the reaction and converts the product to an easily quantifiable chromophore.
3. 1U converts 1 micromole substrate to product / minute ∴ 23 units = C
4. c = 0.68/188,00M-1cm-1 = 3.62 x 10^{-6}M
   [3.62 x 10^{-6}mol/L(7.5mL/1000mL/L)(10^6μmols/mol)]/5min
   = 0.02715μmol/5min = 0.00543μmol/min = 0.00543U
   0.00543U/0.2mL = 0.0271U/mL ≡ activity
5. When 1/[S] = 0. $1/v_0$ = 25h/mole ∴ Vmax = $(25h/mol)^{-1}$ = 0.04mol/h
   When $1/v_0$ = 0, -1/[S] = -1.3 x 10^2L/mol ∴ K_M = $-(-1.3 \times 10^2 L/mol)^{-1}$ = 7.69 x 10^{-3}mol/L
6. a. K_M; b. Vmax

10 Restriction Analysis of Genomic DNA

1. A virus that infects *E. coli* bacteria
2. REs are proteins that hydrolyze phosphodiester linkages found in double-stranded DNA.
3. The Type II REs hydrolyze phosphodiester bonds found in specific DNA sequences.
4. Nucleic acids migrate towards the anode because the have a net negative charge. Recall that anions are called anions because they move toward the anode, and I'm sure you remember from G-chem that anions are negatively charged.
5. (A) Xylene cyanol and bromophenol blue
   (B) Bromophenol blue

6. It serves as a negative control so that you will be able to identify when a restriction does not cleave your DNA.
7. C

11 The Polymerase Chain Reaction

1. Mechanized *in vitro* DNA replication
2. The "amplification sequence" in PCR is a set of repeated steps that allow the target DNA sequences to be copied by the polymerase. The sequence is *denaturation*, *annealing*, and *replication*.
3. It is not possible to have automated PCR without a heat-stable DNA polymerase because the high temperatures that are required to denature the double-stranded template would denature the normal cell DNA polymerase.
4. *Buffer* – maintain near-constant pH
   dNTP mix – a mixture containing the four deoxyribonucleotides that are required for
   incorporation into the new (copied) strand
   $MgCl_2$ – salt to supply the Mg^{2+} cofactor for the DNA polymerase
   Primers – required to give the polymerase a place to start copying the sequence
   pBluescript – the vector in which the insert is cloned; serves as a positive control for the
   primers; is a DNA template
   DNA construct – represents the inserted DNA cloned into pBluescript; is a DNA template
   Taq polymerase – the heat-stable DNA polymerase required to copy the template
   water – required to bring all samples to same component concentrations and same volume
5. pBluescript: $2\mu L(0.5pmol/\mu L)(10^{-12}mole/pmol)(635g/mol\ bp)(2961bp) = 1.88 \times 10^{-6}g =$
   $1.88\mu g$
   Primer: $1\mu L(10pmol/\mu L)(10^{-12}mole/pmol)(635g/mol\ bp)(bp/2bases)(23bases) = 7.30 \times 10^{-8}g$
   $= 0.073\mu g$
6. The agarose concentration is higher because the sizes of the DNA fragments being resolved are smaller in this experiment.