

Spring '05
Hackert

CH370 / CH387D
Exam II

Name _____
UTID _____

$$R = 8.314 \times 10^7 \text{ g-cm}^2 / (\text{sec}^2 \cdot \text{mol} \cdot \text{K}); \quad RCF = (1.119 \times 10^{-5})(\text{rpm})^2(r); \quad \eta = 0.01 \text{ g/(cm-sec)}$$
$$\rho_{\text{water}} = 1.00 \text{ g/cm}^3; \quad s = M(1 - v'\rho)/N^0f; \quad (1/c_r)(dc_r/dr) = M\omega^2r(1 - v'\rho)/RT;$$
$$k = 1.38 \times 10^{-23} \text{ J/K}; \quad h = 6.63 \times 10^{-34} \text{ J-sec}; \quad A = A_0 \exp(-kt); \quad k = \ln(2)/\tau_{1/2}$$

1. A solution has a %T of 42%. What is the absorbance?

(3)

An extinction coefficient is given as an $E^{0.1\%}$ extinction coefficient for $\lambda = 440 \text{ nm}$ with a magnitude of 3.55. Calculate the concentration in mg/mL of a solution that measures an absorbance of 0.50 at 440 nm.

(5)

2. Briefly explain the advantages of using an expression vector that incorporates an added "His₆-tag" at the N-terminus of your desired protein. Include in your explanation the type of column you would use and the methods required for eluting your protein during the purification procedures.

(5)

3. In what order would the following proteins be eluted from a DiEthylAminoEthyl (DEAE)-Sephadex column using first a buffer wash at pH 7.2 followed by an increasing salt gradient?

Protein:	Lactate DH	Cytochrome c	Tyrpsin
pI :	10.5	7.2	2.5

(4)

(Off first) _____ (Off Last)

4. Consider a FRET experiment where the measured efficiency of energy transfer between chromophores BevoOrange and AggieMaroon is only 8%. If $R_0 = 26.7 \text{ \AA}$ for these two chromophores, calculate the expected separation between the Orange and Maroon.

(6)

5. Explain the roles of each of the following agents used in running an SDS-PAGE.

a) Bromophenol blue

(8)

b) Glycine buffer

c) Sodium dodecyl sulfate

d) Dithiothreitol

6. You performed a 2D IEF-SDS PAGE experiment on peptides P1, P2, P3, and P4 and obtained the following results. Match each spot with its peptide number:

A = ___; B = ___; C = ___; D = ___.

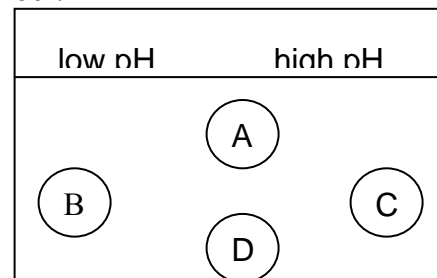
(4)

P1 = AGWDPLEFD

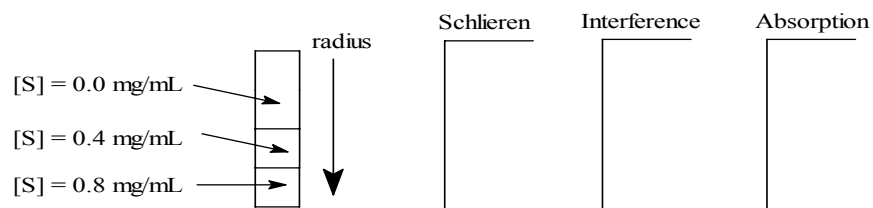
P2 = DDGAKR

P3 = IKLRGAKPV

P4 = FAGRRALVEDPIW



7. Consider the following low-speed, diffusion run looking at an ultracentrifuge sample cell with three liquids layered over each other as shown on the left. On the right sketch the appearance expected for Schlieren, Interference and Absorption optics results expected as a function of “r” or position in the cell.



(6)

8. Consider a G-200 “gel filtration” column that is 100 cm in length and 2.0 cm in diameter. It is packed with spherical beads that are approximately 0.2 mm in radius. The experimentally calibrated V_0 for this column is 33% of V_{tot} . Calculate the expected elution volume for the protein Seniorase that has a reported “partition coefficient” value “ z ” = 0.33. Recall that $z = (V_e - V_0)/(V_{tot} - V_0)$.

(6)

9. Consider an element “X” with 50 neutrons and 50 protons.

(1) What is the element? _____ Identify the product of each of the following events:

(2) If such an isotope were found, predict its preferred decay mode(s)? Why?

(3) $X \rightarrow$ _____ + alpha ; $X \rightarrow$ _____ + positron ; $X \rightarrow$ _____ + gamma

A radioisotope has a half-life of 1.54 days. If a patient is given 5 milliCuries on Monday at 8:00 am, how much radioactivity remains by noon on Wednesday?

(5)

In which of the following techniques is radioactivity measured in photomultiplier tubes by the intensity of the light that fluors emit when excited by solvent interacting with radioactive decay particles? _____

(2) A) Film B) Geiger Counter C) Scintillation counter D) Phosphor Imager

10. You have sequenced the gene of your favorite protein know it is composed of subunits containing 457 amino acid residues for a subunit molecular weight of 50,627. Your protein comes off in the void volume of a G-150 column and you suspect the protein is either a dodecamer (12 subunits) or a decamer (10 subunits). To determine the oligomeric nature of your protein you are scheduled to do a **sedimentation equilibrium** run over the weekend. The temperature of the system will be maintained constant at 20 °C. The density of your protein is estimated by its amino acid composition to be 1.36 g/mL and its \bar{v} -bar estimated to be 0.735 cm³/g. To get the best data from the experiment, you would like to have a 5x concentration variation between the concentration at r_1 (7.00 cm) and the concentration at $r_2 = 7.50$ cm. Assuming your protein to be the dodecamer, **calculate the rotor speed in rpm** that you should run your sedimentation equilibrium experiment to achieve the **5x difference in concentration between r_1 and r_2** .

(8)

11. What is the “Hydrodynamic Radius” of a macromolecule, and how can this be measured?

(6)

Write an equation to calculate the R_{min} for a macromolecule in solution given only the molecular weight and \bar{v} -bar for the molecule?

(3)

What are the expected magnitude and units for the frictional coefficient of a 730 kDa protein with a partial specific volume of 0.72 cm³/g and a frictional coefficient ratio $f/f_{min} = 6.5$?

(5) _____

12. An RNA molecule is said to be a 16S particle. Briefly explain what “16S” means, and what is involved experimentally in collecting the data and the analysis needed to determine a sedimentation coefficient.

(6)

13. Contrast the kinds of information that can be obtained from “static” light scattering versus “dynamic” light scattering experiments.

(6)

14. What is actually measured when obtaining a CD spectrum?

(2)

c) What range of wavelengths is normally used to investigate the secondary structure of proteins by CD spectroscopy? _____ ;

(4) Wavelength range for monitoring the secondary structure for DNA? _____