Fall '10 **CH370** Name ______________________

Hackert **Exam II** UTID ________________

$k = \ln(2)/\tau_{1/2}$; $R = 8.314 \times 10^7$ g-cm^2 /(sec^2-mol-K); RCF = $(1.119 \times 10^{-5})(\text{rpm})^2(r)$;

$\rho_{water} = 1.00$ g/cm^3; $s = M(1 - \nu'\rho)/N^o f$; $(1/c_r)(dc_r/dr) = M\omega^2 r(1 - \nu'\rho)/RT$; $A = A_o \exp(-kt)$;

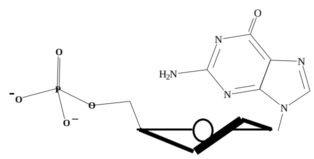
$k = 1.38 \times 10^{-23}$ J/K; $h = 6.63 \times 10^{-34}$ J-sec; $KC/R_\theta = 1/(M^*P(\theta)) + 2A_2C$

$D = RT/N^o f$; $a(K)(\Delta c) = \lambda(\Delta J)$; $\eta = 0.01$ g/(cm-sec) ; $N^o = 6.02 \times 10^{23}$

(Note: Set up equations and show work to get full or partial credit on all calculations.)

1. DNA methods (T/F, circle your choice)

(1) a) T F Automated DNA sequencers rely on P-32 radioactive labels to identify the fragments.
(1) b) T F Capillary electrophoresis coupled with SDS gels are used in modern DNA sequencers
(1) c) T F Most commercial microarrays employ short sequences of bound m-RNA.
(1) d) T F In a typical application to compare "normal" cells vs. "diseased" cells where a green-dye marker is used for the "normal" cell sample and a red-dye marker for the "diseased" samples, a blue spot would indicate approximately equal expression in both cell lines.



(2) What is the sugar pucker illustrated? __________________

(1) What is the conformation of the nucleoside? _________________

2. **Radioactivity:** Consider an isotope of "Te" with 77 neutrons that undergoes beta (+) decay with a half life of 22.4 days.

(3) a) Identify the daughter isotope product produced by this decay? _________

39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn

b) What is the rate constant for this decay (half life of 22.4 days)?

(3)

c) You have discovered a radioactive sample of calcium ^{60}Ca with a decay constant of 2.1×10^{-4}/yr in an ancient tomb that was labeled as having 1000 disintegration per minute. You measure the activity and find that it is now 167 disintegrations per minute. Estimate the age of the radioactive sample since it was last calibrated based on this data. Ans: ___________________________, show work below.

(5)

3) Briefly (**in three sentences or less**), explain why aromatic solvents and chromophores are used when making the "cocktail" in counting beta radioactivity by the method of LSC (liquid scintillation counting)?

(5)

4. **SDS-DISC-PAGE** gels utilize glycine buffers.

i) What is the approximate pH in the "stacking" gel? ______________

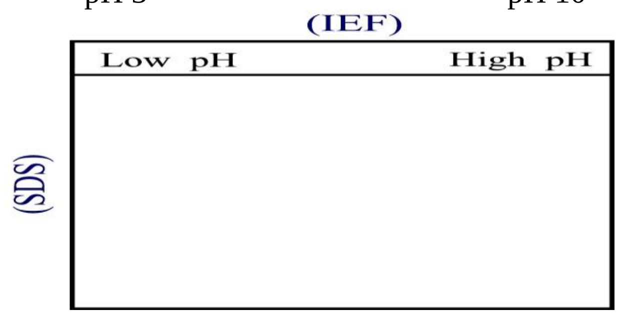
(2)

ii) Why this pH?

(3)

5. You performed a **2D IEF-SDS PAGE** experiment on peptides P1, P2, P3 and P4 with masses of 30kDa, 45kDa, 60kDa, and 120kDa (Note: P3 is a trimer, the others are monomers), and with pI's of 4, 6, 7 and 9, respectively. On the figure below **sketch the expected outcome** of this experiment by **drawing and labeling circles** to represent the predicted results (locations) for the four peptides on this gel.

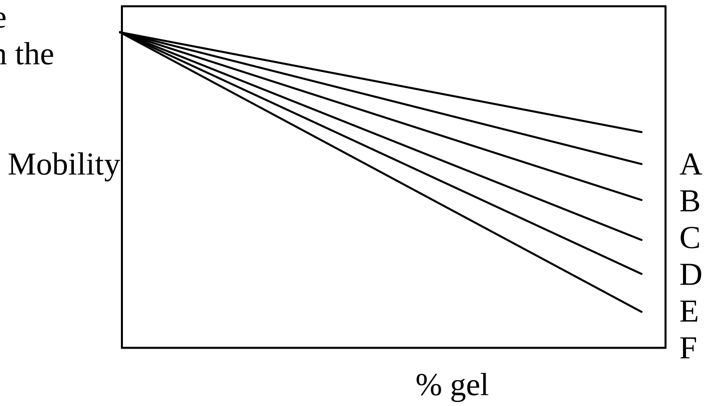
pH 3 pH 10



(4)

6. What can you conclude about the nature (size, shape, charge) of the molecules (A thru F) whose electrophoretic mobilities are illustrated in the diagram on the right?

(4)



7. In one sentence briefly identify the role of each of the following compounds in performing SDS-PAGE electrophoresis.

i) Coomassie Blue:

(4)

ii) N-N'- methyl-bis-acrylamide:

8. Sketch the expectedSclieren pattern for a centrifuge cell that is 0 mg/mL protein in the "top" third of the cell, 0.2 mg/mL in the middle one-third, and 0.4 mg/mL in the "bottom" third.

(4) "top" "bottom"

9. Briefly (**in three sentences or less**) describe a typical isopycnic centrifugations run in terms of the solute, solvent and purpose of the run.

(4)

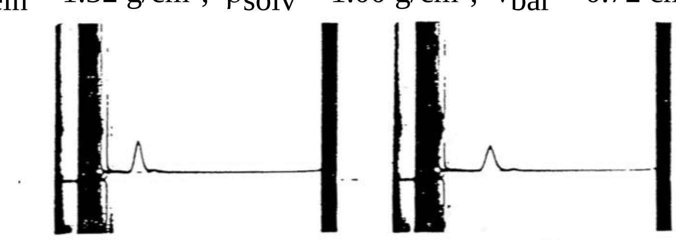
10. Assume the following: $\rho_{protein}$ = 1.32 g/cm^3; ρ_{solv} = 1.00 g/cm^3; v_{bar} = 0.72 cm^3/g; T = 20^{o}C and that the Texas Rangers win their first World Series. Calculate the diffusion coefficient for this protein if it was determined by sedimentation equilibrium to have a molecular weight of 74,000 and by sedimentation velocity to have a sedimentation coefficient of 7.4 S.

Ans: ___________________________, show work below.

(8)

11. Estimate the sedimentation coefficient of the protein from the two data points below from a sedimentation velocity run. The results show that the protein moved from r_1 = 6.40cm to r_2 = 7.60 cm over a time interval of 2.0 hr at a rotor speed of 30,000 rpm:

(Assume: $\rho_{protein}$ = 1.32 g/cm^3; ρ_{solv} = 1.00 g/cm^3; v_{bar} = 0.72 cm^3/g; T = 20^{o}C)



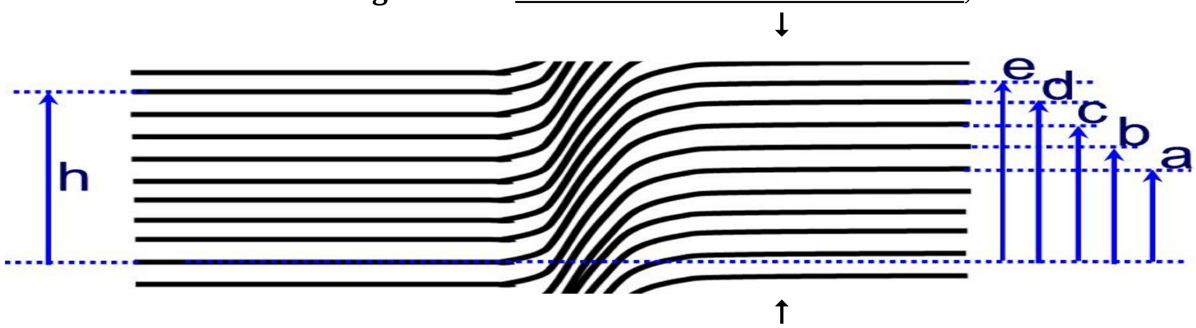
(8)

Place answer in Svedberg units here ______________________________, show work below.

12. **Concentration by Interterence Optics** :

Consider a centrifuge double sector cell with protein solution on the sample side and dilute buffer on the reference side that has a hairline crack between the two sectors so that the buffer can layer over the protein solution once the centrifuge is turned on. For this experiment, the T = 20^{o}C, the rotor speed ω = 4250 rpm, the radius to the sample optics section is 6.66 cm, the cell path length to be 12.00 mm, λ = 546 nm, and (dn/dc = 0.186 (g/cm^3)$^{-1}$. Using interference optics to monitor the run, the results shown below are obtained where a = 4.54 mm, b = 5.61 mm, c= 6.65 mm, d = 7.72 mm, e = 8.79 mm and h = 8.46 mm.

a) Calculate the concentration of the protein sample at the arrow (↓) in **mg/mL** from the data given above. Place answer in **mg/mL** here ____________________________, show work below.



(8)

13. In a **sedimentation equilibrium** experiment, a protein is centrifuged at 7200 rpm. The temperature of the system is maintained constant at 20 ^{o}C and the density of the colloid is 1.25 kg/L and it is known that 1.00 g of sediment displaces 0.742 mL of water and that the density of the dilute solvent is 1.00 g/mL. Using absorption optics we determine that the concentration at r_1 (6.66 cm) to be 0.091 mg/mL and the concentration at r_2 = 7.72 cm to be 0.819 mg/mL. Calculate the molecular weight of this protein. Ans: __________________________, show work below.

(8)

14. **Light Scattering**:

a) What is the relationship between the intensity of scattered light as a function of the wavelength and distance from source (I is proportional to λ, or $1/\lambda$, etc., similarly with "r"):

(2) i) Wavelength: I is proportional to ________ ; ii) Distance: I is proportional to ________

b) Identify the kinds of information that can be obtained from "static" light scattering vs. that from "dynamic" light scattering experiments (**2-3 sentence limit each**).

i) Static -

(3)

ii) Dynamic -

(3)

b) Why are both light scattering (LS) and refractive index increment (RI) measurements required to measure and "absolute" molecular weight using LS methods?

(3)

15. **CD:**

a) In one sentence what is actually measured when obtaining a CD spectrum?

(3)

b) Briefly, **in one to three sentences**, state why CD is so useful to study or monitor protein folding, include what properties of a protein are responsible for the usefulness of a CD measurement..

(4)

c) What range of wavelengths is normally used to investigate the secondary structure of proteins?

(2) ____

(Please **sign your name** on the **back** of this exam **near the top** in a manner that you can recognize for returning.)