Physics 200
Problem Set #4 Electrophoresis, Light Scattering, & Fluorescence

1. a. In what direction (ie. toward anode: +, cathode: -, or stationary) will the following proteins migrate in an electric field at the pH indicated?

Protein	isoelectric point
egg albumin	4.6
β- lactoglobulin	5.2
chymotrypsinogen	9.5
serum albumin	4.9
hemoglobin	6.8
urease	5.0
myoglobin	7.0

-egg albumin at pH 5.0

-β-lactoglobulin at pH 5.0, at pH 7.0

- chymotrypsinogen at pH 5.0, at 9.5, at 11

b. Electrophoresis at what pH would be most effective in separating the following protein mixtures?

-serum albumin + hemoglobin

-myoglobin + chymotrypsinogen

- egg albumin + serum albumin + urease

2. An invertebrate hemoglobin is found, under native conditions, to have a sedimentation coefficient of about 4.4 S and a diffusion coefficient of about 6×10^{-7} cm^2/s (both at 20°C in water). The parameter $\bar{v}$ is estimated to be 0.73 cm^3/g. The following data are found from SDS-gel electrophoresis:

- After treatment with β-mercaptoethanol, the protein migrates as a doublet. The bands have traveled 10.0 and 10.6 cm.
- In the absence of β-mercaptoethanol treatment, only the 10.0 cm band of the doublet is seen, but there is a new band at 5.6 cm.
- A series of standard proteins on the same gel migrates as shown below.

Protein	M	d (cm)
Phosphorylase	94,000	0.5
Bovine albumin	67,000	1.1
Ovalbumin	43,000	3.9
Carbonic anhydrase	30,000	6.6
Trypsin inhibitor	20,100	9.3
α-Lactalbumin	14,400	11.7

Describe the subunit structure of this protein in as much detail as you can from the data. (Don't expect it to behave like human hemoglobin; invertebrate hemoglobins are often quite different from mammalian types.)

3. A combination ultracentrifuge-electrophoresis apparatus, in principle, could be constructed by placing electrodes at the top and bottom of a centrifuge cell.
    a. Derive an equation for the velocity of motion of a particle of mass m and charge ze in such a combined field.
    b. If we imagine a sucrose gradient experiment in such an apparatus, it is clear that the sedimenting band could be stopped at any point by turning on an appropriate electrical

field. For normal adult hemoglobin (with an effective charge of +10 e, $D_{20,w} = 6.0 \times 10^{-7}$ cm^2/s, $s_{20,w} = 4.3 \times 10^{-13}$ sec), calculate the potential gradient (or electric field in V/cm) needed to keep a band stationary at 6.5 cm from the center of rotation in a rotor turning at 60,000 rpm at 20°C. Note that in cgs units e $= 4.8 \times 10^{-10}$ and to convert from voltage cgs units to Volts you need to multiply by 300)

c. Will the immobilized boundary be stable with time?

4. The data given below describe light scattering measurements on a small globular protein. Use these to calculate the molecular weight and the second virial coefficient. Other data you will need are $\lambda = 436$ nm and $dn/dc = 0.196$ cm^3/g.

C (mg/ml)	(KC/R) x 10^5
0.5	5.50
1.0	5.60
1.5	5.79
2.0	5.86
2.5	6.05

5. If a large molecule exhibits rotational diffusion as well as translational diffusion, the dynamic light scattering autocorrelation decay is described by

$$[g^{(2)}(\tau) - 1]^{1/2} = g^{(1)}(\tau) = e^{-D_T q^2 \tau}(A_o + A_1 e^{-6D_R \tau})$$

where D_T and D_R are translational and rotational diffusion coefficients and q = $(4\pi n/\lambda)$ sin($\theta/2$). Devise a way to obtain both of the diffusion coefficients from a set of such data.

6. The following data describe the steady-state fluorescence polarization of a protein that has been labeled with a dye. The dye has a fluorescent lifetime of 7.0 ns. Sucrose has been added to solutions at 20°C to increase the viscosity, η. Using Equation D8.14, calculate $\bar{A}_o$ and V_h from a plot of $\bar{A}$ vs. T/η, using the following data:

T/η (10^4 cgs units)	$\bar{A}$
0.30	0.292
0.82	0.269
1.49	0.247
2.10	0.227
2.51	0.217
2.92	0.206