

Physics 200

Problem Set #4 Electrophoresis, Light Scattering, Fluorescence & Chromatography

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 anode
- β -lactoglobulin at pH 5.0, at pH 7.0 cathode, anode
- chymotrypsinogen at pH 5.0, at 9.5, at 11 cathode, stationary, anode

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

- serum albumin + hemoglobin ~5.85
- myoglobin + chymotrypsinogen ~8.25
- egg albumin + serum albumin + urease ~4.9

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 $6 \times 10^{-7} \text{ cm}^2/\text{s}$ (both at 20°C in water). The parameter \bar{v} is estimated to be $0.73 \text{ cm}^3/\text{g}$. 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.

<i>Protein</i>	<i>M</i>	<i>d (cm)</i>
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.)

The *s* and *D* values give a "native" molecular weight of about 66,000 Da. In the absence of BME, the gel data show two components – one of about 32,000 Da and the other about 18,000 Da. To make the complete molecule, one of the former and two of the latter are needed (sum = 68,000). The 32,000 Da subunit is an S-S bridged dimer of two 16,000 Da units, as shown by SDS gels in the presence of BME.

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.

$\omega^2 r m (1 - \nu \rho) - fu - QE = 0$ (from adding on the electric force to the sedimentation balance of forces) since a terminal velocity is reached.

Then $u_{\text{terminal}} = \omega^2 r s - QE/f$.

- 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 $+10e$, $D_{20,w} = 6.0 \times 10^{-7} \text{ cm}^2/\text{s}$, $s_{20,w} = 4.3 \times 10^{-13} \text{ sec}$), 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 $60,000 \text{ rpm}$ 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)

To hold $u_{\text{terminal}} = 0$ we then require

$$QE = \omega^2 r s f \quad \text{or} \quad \text{with } V = \text{voltage} = Ed, \text{ we require}$$

$$V = Ed = \omega^2 r s f d / Q$$

Given $d = 1 \text{ cm}$ (so E in V/cm), $\omega = 60,000 \times (2\pi/60) \text{ rad/s}$, $r = 6.5 \text{ cm}$, s from above, and $f = kT/D$, we have

$$V = 1.6 \times 10^{-3} \text{ (in cgs units)} = 480 \text{ mV}$$

- c. Will the immobilized boundary be stable with time? Diffusion still acts, but in a density gradient the boundary is fairly 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 may need are $\lambda = 436 \text{ nm}$ and $dn/dc = 0.196 \text{ cm}^3/\text{g}$.

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

A plot of KC/R vs c gives an intercept $(1/M) = (5.35 \pm 0.03) \times 10^{-5}$ and a slope of $(2B) = (2.7 \pm 0.2) \times 10^{-3} \text{ ml/mole}$. So we have $M = 18,700 \pm 10^5 \text{ Da}$ and $B = (1.4 \pm 0.1) \times 10^{-3} \text{ ml/g-Da}$.

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

$$[g^{(2)}(\tau) - 1]^{1/2} = g^{(1)}(\tau) = e^{-D_T q^2 \tau} (A_0 + A_1 e^{-6D_R \tau})$$

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.

Treating $g^{(1)}(\tau)$ as a function of scattering angle (through q) as well, note that the D_T term depends on q but the D_R term does not. The best way to separate these is to take the natural log of both sides to get

$$\ln(g^{(1)}(\tau)) = -D_T q^2 \tau + \ln(A_0 + A_1 e^{-6D_R \tau})$$

Then a plot of this \ln vs q^2 at a fixed value of τ (arbitrary choice, but can do several different τ values to get more reliable values) results in a line with a slope that gives D_T and an intercept can be used to get D_R .

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

A plot of $1/\bar{A}$ vs T/η gives an intercept of 3.26 ± 0.01 and a slope of 0.541 ± 0.007 (but this needs to be corrected for the 10^4 factor for the abscissa) so we have $A_o = 1/\text{intercept} = 0.31 \pm 0.09$ and

$$V_h = (1/A_o) (\tau_F k / \text{slope}) = (1/3.26)(7 \times 10^{-9} * 1.38 \times 10^{-16}) / (0.541/10^4) = 5.5 \times 10^{-20} \text{ cm}^3.$$

Note: this corresponds to an equivalent sphere radius of 2.4 nm.

7. Suppose an enzyme is dissociated into 4 identical subunits and that you want to test for the enzymatic activity of individual subunits, but you must first be sure there are no tetramers remaining in the sample. What chromatographic technique would you choose (and why) to free the monomers from any residual tetramers? **Gel filtration chromatography**

8. A procedure has been devised for purifying a particular enzyme using gel chromatography. In an effort to increase the amount of material to be handled, you use a sample whose concentration of protein is ten times that normally used with this procedure. The enzyme activity now elutes principally in the void volume. Explain what happened. **Probably aggregated at high concentration**

In general, to increase the amount of material to be handled, should the diameter of the column be increased? Should the length be increased? Or should both be increased?

Increasing the diameter alone should work