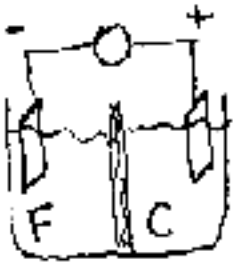
ferredox $F(Fe^{+3}) + e^- \rightarrow F(Fe^{+2})$ -0.432 V

cytochrome $C(Fe^{+3}) + e^- \rightarrow C(Fe^{+2})$ $+0.254$ V

Second Midterm Chem 130A Name_____

1. Proteins interact with metal ions in different ways to alter their oxidation/reduction activity. For example the formal reaction $Fe^{+3} + e^- \rightarrow Fe^{+2}$ is quite different when the iron is in an iron-sulfer cluster (as in spinach ferredoxin), or in a heme group (as in cytochrome-c). The standard reduction potentials of these systems are given in Table 4~~5~~ 4, pg. ~~346~~ 141.

A) If an electrochemical cell is made up at 20°C such that one half cell has a Pt electrode 50μM total ferredoxin in solution adjusted so that the concentrations of the oxidized and reduced forms are equal, and the other has a Pt electrode with cytochrome-c, also 50 μM total, half oxidized half reduced, both in pH 7 buffer. What will the cell potential be when these are connected?



$$\mathcal{E} = \mathcal{E}^\circ - \frac{0.0591}{n} \log Q$$

since equal. conc. Fe^{+3}, Fe^{+2} on each side $Q = 1$ so $\mathcal{E} = \mathcal{E}^\circ$

$C(Fe^{+3}) + e^- \rightarrow C(Fe^{+2})$ $+0.254$

$F(Fe^{+2}) \rightarrow F(Fe^{+3}) + e^-$ $+0.432$

$+0.686$ V

B) If the two electrodes are simply connected with a wire, allowing current to flow freely between the two half cells, what will be the concentrations of oxidized and reduced protein in each cell when current flow stops?

cell voltage will be 0 V when current stops

so $\mathcal{E}^\circ = \frac{0.0591}{1} \log Q$ $\frac{0.686}{0.0591} = \log Q = 11.61$

goes far toward products

$$Q = \frac{[F(Fe^{+3})][C(Fe^{+2})]}{[F(Fe^{+2})][C(Fe^{+3})]}$$

$[F(Fe^{+3})] + [F(Fe^{+2})] = 50\mu M$

$[C(Fe^{+3})] + [C(Fe^{+2})] = 50\mu M$

try $[F(Fe^{+2})] = [C(Fe^{+2})] = 50\mu M$

$[Fe(Fe^{+2})] = [C(Fe^{+3})] = X$ small

$$Q = \frac{(5 \times 10^{-5})^2}{X^2}$$ $X = 8 \times 10^{-11}$ M

Name_____

2. When sucrose (m.w. 342.3 g/mol) is dissolved in water up to a mole fraction $X_{suc} = 0.0671$, the measured vapor pressure of water above the solution at 0°C is 4.148 mm Hg. The vapor pressure of pure water at this temperature is 4.579 mm Hg, and the enthalpy of fusion for water is 6.007 kJ/mol, H_2O 1 gram/cm^3 and 18 grams/mol.

A) Calculate the activity coefficient of the water in the sucrose solution at 0°C.

$$a_{H_2O} = \frac{p_A}{p_A^\circ} = \frac{4.148}{4.579} = 0.9059 \qquad X_{H_2O} + X_{suc} = 1$$

$$a_{H_2O} = \gamma_{H_2O} \cdot X_{H_2O} \qquad X_{H_2O} = 0.9329$$

$$\Delta o \quad \gamma_{H_2O} = \frac{0.9059}{0.9329} = 0.9711$$

B) Calculate the freezing point of the real sucrose solution described above.

$$\ln a_{H_2O} = \frac{\Delta \bar{H}_{fus}}{R}\left(\frac{1}{T_0} - \frac{1}{T_f}\right)$$

T_0 = f.p. pure H_2O = 273 K

T_f = unknown

$$\frac{8.3145 \text{ J/K/mol}}{6.007 \times 10^3 \text{ J/mol}} \cdot \ln(0.9711) = \left(\frac{1}{273} - \frac{1}{T_f}\right) = -4.059 \times 10$$

T_f = 270.0 K.

Name_____

3. A sample weighing 1.00 gram containing a mixture of a single protein and some NaCl (salt, m.w. 58.4 g/mol) was dissolved to give 10.0 mls of solution at 20°C. This was placed in an osmometer opposite a solution of pure water using a membrane which was permeable to water, Na^+ and Cl^- but not protein. The resulting osmotic pressure was determined to be 0.00403 atm. When the same measurement was done with a membrane which was permeable only to water (not protein, Na^+ or Cl^-), the osmotic pressure was determined to be 0.880 atm.

A) Calculate the molecular weight of the protein.

$w_{NaCl} + w_p = 1.0 \times 10^2$ g/L

$\Pi = \frac{w}{M} RT$ protein only membrane $\frac{w_p}{M_p} = \frac{\Pi}{RT} = \frac{0.00403 \text{ atm}}{0.08205 \cdot 293 \frac{\text{L atm}}{\text{mol K}}}$

$= 1.68 \times 10^{-4} \frac{\text{mol}}{\text{liter}}$

H_2O only membrane

$\Pi = \left(\frac{w_{Na}}{M_{Na}} + \frac{w_{Cl}}{M_{Cl}} + \frac{w_p}{M_p}\right) RT$ $\frac{0.880 - 0.00403}{0.08205 \cdot 293} = \frac{2 w_{NaCl}}{58.4}$ so $w_{NaCl} = 1.0$ g/L

B) What percentage error in the molecular weight would there have been if the protein had been assumed to be pure (no salt), and only the membrane permeable to just water had been used?

then assume

$\frac{0.880}{RT} = \frac{w_p}{M_p} = 3.660 \times 10^{-2}$ M

$w_p = 100$ g/L M_p then $= 2.7 \times 10^3$ g/mol

$w_p = (100 - 1.064)$ g/L

$= 98.94$ g/L

$\frac{w_p}{M_p} = 1.68 \times 10^{-4}$

so $M_p = 59{,}000$ g/mol

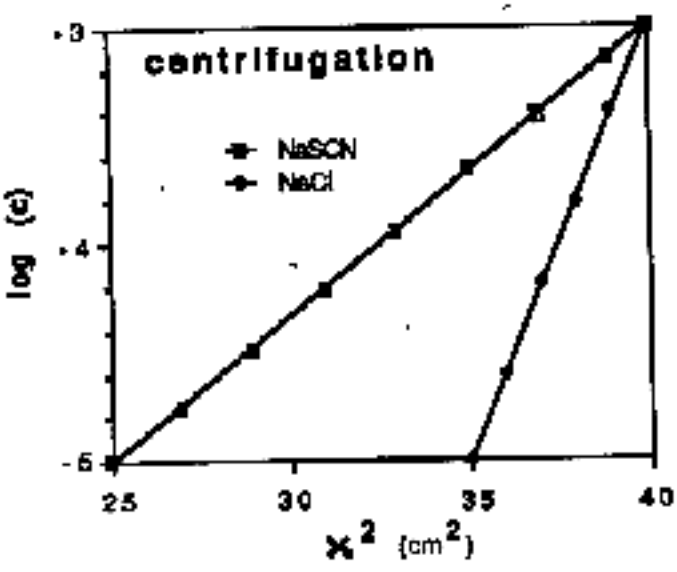
$= 5.9 \times 10^4$

95% error

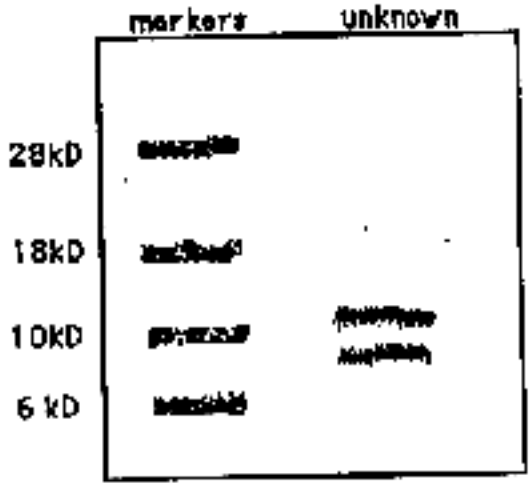
% error $= \frac{M_p(\text{true}) - M_p(\text{apparent})}{M_p(\text{true})}$

Name_____

4. A protein involved in light harvesting for photosynthesis in bacteria was investigated using ultracentrifugation and SDS gel electrophoresis. Two centrifuge experiments were done, the first in 1 M NaCl solution, and the second in 1 M NaSCN. The samples were spun until an equilibrium concentration gradient was established (spinning at 20°C at 21,380 rpm; v_2 for the protein was found to be 0.709 cm^3/gram, ρ for the salt solutions was 1.20 g/cm^3 (ignore any possible effects of density gradients due to the salt in the solutions). The concentration profile was determined, and used to generate the graph below to the left. On the right is a drawing of the denaturing SDS gel.



SDS gel electrophoresis



A) Using the centrifuge data what is the effective molecular weight of the protein in each of the salt solutions? (be careful with units)

$$M_{NaSCN} = \frac{2RT}{\omega^2(1-\bar{v}_2\rho)} \cdot \frac{d\ln c}{d(x^2)} = \frac{2 \cdot 8.1345\times10^7 \frac{g\,cm^2}{mol\,K} \cdot 293K}{\left(\frac{2\times3.14\times21380}{60}\right)^2(1-0.709\times1.20)} \cdot \frac{2.3\times2}{15}$$

$\ln c = 2.303 \log c$

$= 20\,kD$

M_{NaCl} is 3 times higher. $= 60\,kD$

Name_____

B) Using the centrifuge data together with the electrophoresis data can you explain the observations? (give a concise explanation of what each observation indicates and how the observations fit together).

from the SDS gel there are two different components ≈ 8 kD and ≈ 12 kD

neither matches the NaSCN solution, but the sum does - so this protein must be a heterodimer (one of each).

$\alpha\beta$

The NaCl data clearly show that the assembly is 3 x larger - logically this would be $(\alpha\beta)_3$