1.2 Problem Set
Kinetics and Diffusion

1. A. The empirical formula of glucose is $C_6H_{12}O_6$. What is its molecular weight?
   B. Isotonic glucose is 5% (w/v) glucose. How much glucose would we need to make 100 mL of isotonic glucose?
2. A. You need to make 250 mL of a stock solution of 0.1 M Na_2 ATP. Its formula weight is 605.2 g mol^{-1}. How much Na_2 ATP should you weigh out?
   B. Your advisor is skeptical of your abilities. He wants you to check out the 0.1 M ATP solution and tells you to do it spectrophotometrically. Spectrophotometry relies on the different abilities of chemicals to absorb light of specific wavelengths. A diagram of a spectrophotometer is shown in Figure 1.PS2.1.

   At particular wavelengths, chemicals absorb light according to their chemical structure and their concentration. The law governing the absorption of light is the Beer–Lambert Law:

$$A = \varepsilon C d$$

   where A is the absorbance; ε is a constant that depends on the chemical and typically varies with the wavelength of light—it is the **molar extinction coefficient** and is in units of M^{-1}; C is the concentration of the chemical (in M); and d is the path length. The molar extinction coefficient is defined for a path length of 1 cm. The absorbance is defined as

$$A = \log(I_0/I)$$

   where I_0 is the incident light intensity and I is the transmitted light intensity. Your advisor tells you that $\varepsilon_{259} = 15.4 \times 10^3\ M^{-1}$; this is the molar extinction coefficient of ATP at a wavelength of incident light of 259 nm. He tells you to make a dilution of the stock by taking 25 μL of the stock solution and diluting it to 100 mL. What absorbance do you expect of the final diluted solution, if you made it up correctly, at $\lambda = 259$ nm?
3. A. The molecular weight of ryanodine is 493.54 g mol^{-1}. You want to make 10 mL of a 10-mM stock solution. How much ryanodine should you weigh out?
   B. You make a dilution of the 10-mM ryanodine stock by pipetting 10 μL of the stock solution into a 10-mL volumetric flask and adding water to the mark. You measure the absorbance as a function of wavelength (against a water blank, using a standard 1 cm path length optical cell) and find a peak at 271 nm with an absorbance of 0.179. What is ε_{271} for ryanodine? (See Problem #2 for a discussion of the Beer–Lambert Law and a definition of the molar extinction coefficient.)
4. A. Magnesium chloride has a formula of $MgCl_2 \cdot 6H_2O$. What is its formula weight?
   B. You desire to make 1 L of 0.1 $MgCl_2$ solution. How much $MgCl_2 \cdot 6H_2O$ should you weigh?
   C. You need to make 25 mL of a 25-mM solution of $MgCl_2$. How much of the 0.1 M stock solution do you add to the 25 mL volumetric flask?
5. The extracellular fluid volume varies with the size of the person. Suppose in an individual we determine that the ECF is 14 L. The average $[Na^+]$ in the ECF is about 143 mM.
   A. What is the total amount of Na^+ in the ECF, in moles? in grams?
   B. Suppose this person works out and sweats 1.5 L with an average $[Na^+]$ of 50 mM. During this time the urine output is 30 mL

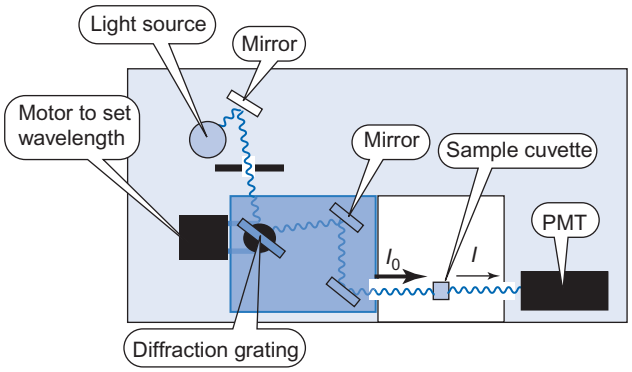

FIGURE 1.PS2.1 Light path in a single beam spectrophotometer. The view is from above. Light from a source is collimated (making a narrow beam) and passed through a monochromator that selects a narrow band of wavelength of light to be passed through the sample. A photomultiplier tube (PMT) detects the light and measures its intensity. Comparison of this intensity, I, to the intensity when the sample is missing, I_0, allows the calculation of the absorbance. Absorbance is recorded with time or as a function of wavelength.

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with an average $[Na^+]$ of 600 mM. How much Na^+ is lost during the workout?

C. If the person does not drink fluids at all during the workout, what will be the $[Na^+]$ in the plasma at the end of the workout? Assume that all of the fluid in the sweat and urine originated from the ECF.

6. The body normally produces about 2 g of creatinine per day. The amount varies with individuals and is approximately proportional to the muscle mass. It is excreted through the kidneys according to urinary excretion of creatinine = GFR × plasma concentration of creatinine, where GFR is an abbreviation for "glomerular filtration rate." If the GFR is 120 mL min^{-1}, what is the plasma concentration of creatinine at steady state? *Hint*: Assume the body is at steady state with respect to creatinine.
7. Just before noon, your plasma glucose concentration was 100 mg dL^{-1}. This plasma glucose is approximately evenly distributed among 3.5 L of plasma and 10.5 L of interstitial fluid that comprises your 14 L of ECF. Glucose is readily distributed in both compartments. You drink a can of soda that contains 35 g of glucose.
   A. How much would your blood glucose rise if all the glucose in the soda was absorbed and none of it was metabolized?
   B. Given that postprandial (after eating) *increases* in blood glucose amount to maybe 40 mg dL^{-1}, depending on the meal, over a period of an hour, give a crude estimate of the rate of glucose uptake by the peripheral tissues. Assume that the meal contains 100 g of carbohydrates and all of it is absorbed in 1 hour.
8. The association reaction for Ca^{2+} and EGTA (a chemical that binds Ca^{2+}) is written as

$$Ca^{2+} + EGTA \rightleftarrows Ca \cdot EGTA$$

Under defined and particular conditions of temperature and ionic mixture, the association constant was determined to be $K_A = 2.52 \times 10^6\ M^{-1}$. In a chemical mixture, 400 μM total EGTA was included and the free $[Ca^{2+}]$ determined by a Ca^{2+}-selective electrode was found to be 4×10^{-7} M. Assuming that there are no other binding agents for Ca^{2+}, what is the total $[Ca^{2+}]$ in the mixture?

9. 2,4-Dinitrophenyl acetate decomposes in alkaline solution with a pseudo-first-order rate constant of 11.7 s^{-1} at 25°C. It is a "pseudo"-first-order rate constant because it depends on the pH.
   A. If the initial concentration of DNPA is 1 mM, what is its concentration after 15 seconds?
   B. At what time is the concentration reduced to 0.5 mM (i.e., what is the half-life of the reaction)?
   C. After 5 minutes of reaction, what is the concentration of DNPA?
10. The following data were obtained for the rate of the Mg, Ca-ATPase activity of vesicles of cardiac sarcoplasmic reticulum as a function of temperature. What can you tell about the activation energy?

Temperature (°C)	ATPase Rate (μmol min^{-1} mg^{-1})
6.9	0.068
11.5	0.138
15.8	0.300
19.8	0.568
20.2	0.585
25.6	1.236
26.1	1.154
31.0	2.238
34.8	3.030
39.2	4.220

11. Superoxide reduces cytochrome C in the reaction

$$Cyt\ C \cdot Fe^{3+} + O_2^- \Rightarrow Cyt\ C \cdot Fe^{2+} + O_2$$

where Cyt $C \cdot Fe^{3+}$ is the oxidized form and Cyt $C \cdot Fe^{2+}$ is the reduced form of cytochrome C. The reaction can be followed spectrophotometrically at 550 nm. The extinction coefficient for the reduced form of cytochrome C is $\varepsilon_{RED} = 2.99 \times 10^4\ M^{-1}$ and the extinction coefficient for the oxidized form $\varepsilon_{OX} = 0.89 \times 10^4\ M^{-1}$ (V. Massey, The microestimation of succinate and the extinction coefficient of cytochrome C. *Biochimica et Biophysica Acta*, **34**:255–256, 1959). See Problem #2 for a discussion of extinction coefficients and spectrophotometry. When xanthine oxidase converts xanthine to uric acid, it produces superoxide that can be measured using cytochrome C reduction. The following data were obtained for A_{550}:

Time (min)	A_{550}
0	0.1326
1	0.1478
2	0.1637
3	0.1791
4	0.1941
5	0.2073
6	0.2202

   A. Calculate the rate of cytochrome C reduction.
   B. The xanthine oxidase was added in 75 μL of 6.5 mg XO per mL into a 3-mL reaction mixture. Calculate the specific activity of cytochrome C reduction (moles of cytochrome C reduced per min per mg of XO protein).
12. You suspect you are anemic and your physician orders some tests. He finds that your hemoglobin is 13 g%. The molecular weight of hemoglobin is 66,500 g mol^{-1}.
   A. What is the concentration of hemoglobin in molar in your blood?
   B. Each hemoglobin binds four oxygen molecules. If the hemoglobin is saturated with oxygen, what is the concentration of O_2 bound to Hb, in molar?

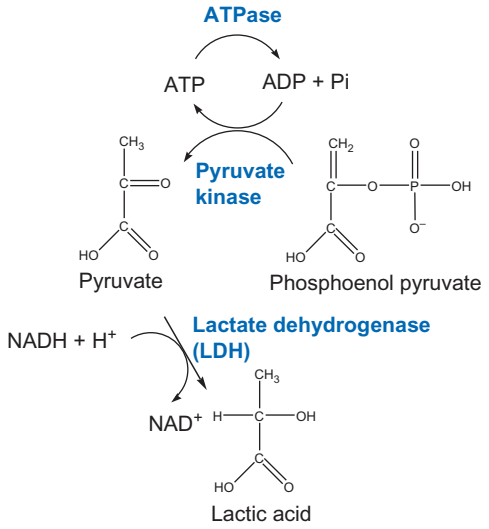

FIGURE 1.PS2.2 ATP hydrolysis by pyruvate kinase converts phosphoenolpyruvate to pyruvic acid. This is coupled by lactate dehydrogenase to the conversion of pyruvic acid to lactic acid and conversion of NADH to NAD^+. The progress of the reaction can be followed spectrophotometrically by the change in absorbance of NADH.

C. Convert the answer in B to volume using the ideal gas equation, $PV = nRT$, where T is the absolute temperature, $R = 0.082$ L atm mol^{-1} K^{-1}, V is the volume that we seek, and $P = 1$ atm. The conditions for volume of gas are usually STPD—standard temperature and pressure, dry. The standard temperature is 0°C and pressure is 1 atm.

13. The rate of ATP hydrolysis by ATPases can be followed by the coupled enzyme assay shown in Figure 1.PS2.2. The progress of the reaction can be followed by A_{340}. The extinction coefficient of NAD^+ at 340 nm is negligible. The extinction coefficient of NADH at 340 nm is 6.2×10^3 M^{-1}. See Problem #2 for a discussion of extinction coefficients and spectrophotometry. In one reaction, the concentration of Ca-ATPase was 0.22 mg mL^{-1} and A_{340} was 0.65 at $t = 0$ min and 0.455 at $t = 2.0$ min. What is the activity of the Ca-ATPase in units of μmol min^{-1} mg^{-1}?
14. Show by representative calculations that Stirling's formula

$$n! = \sqrt{2\pi n}\, e^{n \ln (n'-n)}$$

is a good approximation for $n!$ Use $n = 1, 2, 3, 4, 5$.
15. Show that the equation

$$C(x,t) = C_0 \sqrt{\frac{1}{4\pi Dt}}\, e^{\frac{-X^2}{4Dt}}$$

obeys Fick's Second Law of Diffusion.
16. The intestinal enterocytes form a covering over the intestinal lining which, to the first approximation, can be considered to be a plane. Assuming no binding or sequestration within the cell, what is the estimated time of diffusion of Ca^{2+} across the intestinal enterocyte? The length of the enterocyte is 20 μm and assume that the effective diffusion coefficient of Ca^{2+} is about 0.4×10^{-5} cm^2 s^{-1}.
17. Table 1.PS2.1 lists the diffusion coefficients and the molecular weight of a variety of proteins. What relationship can you deduce between the size and the diffusion coefficients of these soluble proteins? (*Hint*: regress ln D against ln M_r). Is the relationship you found consistent with the Stokes–Einstein equation?
18. The free diffusion coefficient of oxygen in aqueous solutions is about 1.5×10^{-5} cm^2 s^{-1}. If the diffusion distance between air and blood is 0.5 μm, about how long is the diffusion time?
19. Suppose a soluble protein has a molecular weight of 45 kDa and a density of 1.06 g cm^{-3}. Suppose further that the viscosity of the cytoplasm has a viscosity of 0.005 Pa s (about five times that of water—there is debate about the viscosity of cytoplasm with numbers varying from 0.001 to over 0.1 Pa s).
    A. Estimate the diffusion coefficient for the protein in the cytoplasm at 37°C.
    B. If the proteins were synthesized in the cell body, or soma, of a neuron in the spinal cord, about how long would it take to diffuse to the axon terminal 75 cm away?
20. Diffusion coefficients in cytoplasm have been estimated by a technique of photobleaching recovery. In this technique, an area of the cytoplasm is irradiated with light to photobleach a fluorescent probe. Recovery of fluorescence in the region is achieved by diffusion of unbleached probes from adjacent areas of the cytoplasm. The translational diffusion coefficient can be estimated from the half-time of fluorescent recovery. (D. Axelrod et al., Mobility measurements by analysis of fluorescence photobleaching recovery kinetics.

TABLE 1.PS2.1 Diffusion Coefficients and M_r for a Variety of Proteins

Protein	Molecular Weight	$D \times 10^7$ (cm^2 s^{-1})
Milk lipase	6600	14.5
Metallothionein	9700	12.4
Cytochrome C	12,000	12.9
Ribonuclease	12,600	13.1
Myoglobin	16,890	11.3
Chymotrypsinogen	23,200	9.5
Carbonic anhydrase	30,600	10.0
Peroxidase II	44,050	6.8
Albumin	68,500	6.1
Lactoperoxidase	92,620	6.0
Aldolase	149,100	4.6

Biophysical Journal **16**:1055–1069, 1976.) This technique was applied to estimate the relative viscosity of cytoplasm and nucleoplasm by microinjecting fluorescein isothiocyanate-labeled dextrans of varying molecular sizes and measuring the fluorescence photobleaching recovery (I. Lang et al., Molecular mobility and nucleoplasmic flux in hepatoma cells. *Journal of Cell Biology* **102**:1183–1190, 1986). These authors obtained the following data:

Probe	Molecular Weight (kD)	Equivalent Radius (nm)	*D* in Dilute Solution	D in Cyto-plasm	D in Nucleo-plasm
			D is in units of 10^{-6} cm^2 s^{-1}		
FD20	17.5	3.30	0.651	0.080	–
FD40	41.0	4.64	0.463	0.044	0.069
FD70	62.0	5.51	0.390	0.029	0.056
FD150	156.9	9.07	0.237	0.015	0.036

A. Plot D against $1/a$, where a is the molecular radius, for each of the solutions. From the Stokes–Einstein relation, you would expect the resulting curves to pass through the origin of zero diffusion coefficient with infinite radius. Do the curves extrapolate back in this way? Why or why not?
B. Regardless of the intercept, the slope of the plot from part A ought to be related to the viscosity of the medium. Use the slopes to estimate the relative viscosity of the dilute solution, cytoplasm, and nucleoplasm.