PROBLEM SET 1.2. KINETICS AND DIFFUSION

ANSWER KEY

1. A. The empirical formula of glucose is $C_6H_{12}O_6$. What is its molecular weight?

$C_6H_{12}O_6$ should have a molecular weight of (6 x 12) + (12 x 1) + (6 x 16) = 72 + 12 + 96 =

180 g mol^{-1}

B. Isotonic glucose is 5% glucose w:v. How much glucose would we need to make 100 mL of isotonic glucose?

5 g% means 5 g per dL; so we need **5 g** of glucose to make up this 100mL 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?

Amount of ATP = C x V = 0.1 mol L^{-1} x 0.25 L = 0.025 mol

0.025 mol x 605.2 g mol^{-1} = **15.13 g**

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 below.

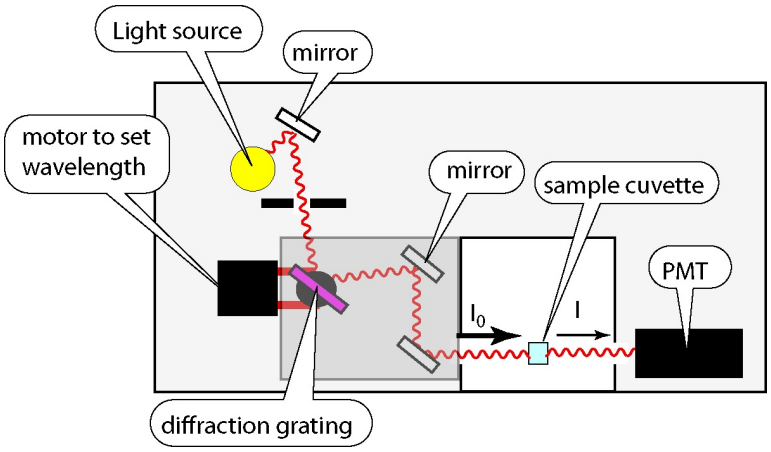


Fig. 1.PS2.1. Light path in a single-beam spectrophotometer. The view is from above. Light from a source is collimited (making a narrow beam) and passed through a monochromator that selects a narrow band of wavelength of light to be passed through the sample. A photomultiplier tube (PMT) detects the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

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 pathlength. The molar extinction coefficient is defined for a pathlength 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?

Dilution gives $C_1 \times V_1 = C_2 \times V_2$; $0.025\ mL \times 0.1\ M = 100\ mL \times C_{ATP}$

$C_{ATP} = 0.025\ mL \times 0.1\ M / 100\ mL = 25 \times 10^{-6}\ M$

$A = \varepsilon C l = 15.4 \times 10^3\ M^{-1} \times 25 \times 10^{-6}\ M =$ **0.385**

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?

10 mL = 0.010 L; $0.010\ L \times 10 \times 10^{-3}\ mol\ L^{-1} = 0.1 \times 10^{-3}$ mol;

$1 \times 10^{-4}\ mol \times 493.54\ g\ mol^{-1} = 0.04935\ g =$ **49.35 mg**

B. You make a dilution of the 10 mM ryanodine stock by pipetting 10 µL of the stock solution to a 10 mL volumetric flask and adding water to the mark. You measure the absorbance as a function of wavelength (against a water blank) and find a peak at 271 nm with an absorbance of 0.179. What is ε_{271} for ryanodine? (See 1.PS2 problem #2 for a discussion of the Beer-Lampert Law and a definition of the molar extinction coefficient).

Dilution gives $C_1 \times V_1 = C_2 \times V_2$; $0.01\ mL \times 10\ mM = 10\ mL \times C_{RY}$

$C_{RY} = 0.01\ mL \times 10\ mM / 10\ mL = 0.01\ mM = 10 \times 10^{-6}\ M$

$A = \varepsilon C l$; $0.179 = \varepsilon \times 10 \times 10^{-6}\ M$; $\varepsilon = 0.179 / 10 \times 10^{-6}\ M =$ **$1.79 \times 10^4\ M^{-1}$**

4. A. Magnesium chloride has a formula of $MgCl_2 \cdot 6H_2O$. What is its formula weight?

Mg has an atomic weight of 24.31 g mol^{-1}; Cl has an atomic weight of 35.45 g mol^{-1}. H_2O has a formula weight of 18 g mol^{-1}. Thus the formula weight of $MgCl_2 \cdot 6 H_2O$ is $24.31 + 2 \times 35.45 + 6 \times 18 =$ **203.21 g mol^{-1}**.

B. You desire to make 1 L of 0.1 $MgCl_2$ solution. How much $MgCl_2 \cdot 6H_2O$ should you weigh out?

Amount = volume x concentration = 1 L x 0.1 M = 0.1 mol; 0.1 mol x 203.21 g mol^{-1} = **20.32 g**

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?

Dilution gives $C_1 \times V_1 = C_2 \times V_2$; $25 \times 10^{-3}\ L \times 25 \times 10^{-3}\ M = X\ mL \times 0.1\ M$;

X = 6250 x 10^{-6} L = **6.25 mL**

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?

Amount = Volume x concentration; = 14 L x 143 x 10^{-3} mol L^{-1} = 2002 x 10^{-3} mol = ; **2.002 mol.**

We convert this to grams by multiplying by the gram atomic weight of Na = 22.99 g mol^{-1}:
2.002 mol x 22.99 g mol^{-1} = **46.03 g**

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

1.5 L x 50 x 10^{-3} mol L^{-1} = 0.075 mol = **1.72 g** Na^+ lost in the sweat
0.03 L x 600 x 10^{-3} mol L^{-1} = 0.018 mol = **0.41 g** Na^+ lost in the urine
Total loss = **2.13 g Na**

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.

Final ECF = 14 L - 1.53 = 12.47 L; Final Na^+ = 2.002 mol - 0.075 - 0.018 = 1.909 mol
Final [Na^+] = 1.909 mol/ 12.47 L = **0.153 M**

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 x Plasma concentration of creatinine. 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.

At steady state, the rate of excretion of creatinine has to match its rate of production. The rate of production is given at 2 g/day. The urinary excretion is given as GFR x $P_{Creatinine}$. The GFR is given as 120 mL min^{-1}. Thus we have

2 g day^{-1} = 120 mL min^{-1} x 60 min hr^{-1} x 24 hr day^{-1} x $P_{Creatinine}$;

$P_{Creatinine}$ = 2 g day^{-1} /172800 mL day^{-1} = 1.157 x 10^{-5} g mL^{-1} = **1.157 mg%**

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 you blood glucose rise if all the glucose in the soda was absorbed and none of it was metabolized?

The 35 g of glucose would be added to a volume of 14 L. Thus the increase in blood glucose, if it were all absorbed and none of it were metabolized, would be

35 g / 14 L = 2.5 g L^{-1} which is 2.5 g L^{-1} x 0.1 L dL^{-1} = 0.25 g dL^{-1} = **250 mg%**

B. Given that post-prandial (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 one hour.

If the blood glucose increases only 40 mg%, then the amount of glucose that wasn't absorbed would be 40 mg dL^{-1} x 10 dL L^{-1} x 14 L = 5600 mg = 5.6 g. So the amount of glucose which would be taken up by peripheral tissues would be 100 g - 5.6 g = **94.4 g in 1 hour.**

8. The association reaction for Ca^{2+} and EGTA (a chemical that binds Ca^{2+}) is written as

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

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 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?

The associaton constant for the reaction is given as

K_A = [Ca•EGTA] / [Ca^{2+}] [EGTA] = $2.52 \times 10^6\ M^{-1}$

In this case we are given that [Ca^{2+}] = 4×10^{-7} M and that the total [EGTA]$_{tot}$ = 400×10^{-6}M. The question asks for the total [Ca^{2+}] in the mixture, which consists of the free [Ca^{2+}] plus [Ca•EGTA]. Therefore, we need to solve for [Ca•EGTA]. We let [Ca•EGTA] = X ; then the free [EGTA] at equilibrium is 400×10^{-6} - X . We write

$2.52 \times 10^6\ M^{-1} = X / 4 \times 10^{-7}\ M \times (4 \times 10^{-4} - X)$_

$10.08 \times 10^{-1}\ (4 \times 10^{-4} - X) = X$

$4 \times 10^{-4} - 1.008\,X = X$

$4 \times 10^{-4} = (1 + 1.008)\,X \quad X = 4 \times 10^{-4} / 2.008 = 1.992 \times 10^{-4}$ M

So the total [Ca] = 1.992×10^{-4} M + 4×10^{-7}M = **1.996×10^{-4}M**

9. 2,4-dinotrophenyl acetate decomposes in alkaline solution with a pseudo-first order rate constant of 11.7 s^{-1} at 25°. 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 s?

The decay of DNPA obeys the reaction

d[DNPA]/dt = - 11.7 s^{-1} [DNPA]

This equation can be solved by separation of variables and integration:

$\int d[DNPA]/[DNPA] = \int -11.7\ s^{-1}\ dt$

$\ln [DNPA] = -11.7\ s^{-1}\ t + C$

Taking the exponent (to e) of both sides gives

$[DNPA] = [DNPA]_0\ e^{-11.7\ t}$

Here the constant of integration is evaluated by the boundary conditions, which give that at t=0 $[DNPA] = [DNPA]_0$

We can evaluate [DNPA] at 15 s simply by inserting $[DNPA]_0 = 1 \times 10^{-3}$ M and t= 15 s. We find

$[DNPA] = 1 \times 10^{-3}\ M\ e^{-11.7 \times 15} =$ **6.04×10^{-80} M**

This value for the concentration, although a true solution to the equation, is absurd. The concentration is actually a discrete variable, on the scale of 1 L, in increments of 1.6×10^{-24} M ($1/N_0$). This concentration is effectively zero.

B. At what time is the concentration reduced to 0.5 mM (that is, what is the half-life of the reaction)?

The half life of the reaction is the time at which $[DNPA] = 1/2\ [DNPA]_0$. This occurs when

$1/2\ [DNPA]_0 = [DNPA]_0\ e^{-11.7\ t1/2}$

Here the $[DNPA]_0$ cancels; taking the ln of both sides we have

$-\ln 2 = -11.7\ s^{-1}\ t_{1/2}$

$t_{1/2} = \ln 2 / 11.7\ s^{-1} = 0.0592$ s or **59 ms.**

C. After 5 min of reaction, what is the concentration of DNPA.

We don't even have to do this calculation to realize the concentration is zero. The concentration here, as a solution to the equation, will be physically meaningless.

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

The Arrhenius plot is obtained by plotting the ln of the ATPase activity against the reciprocal of the absolute temperature. The results is shown below.

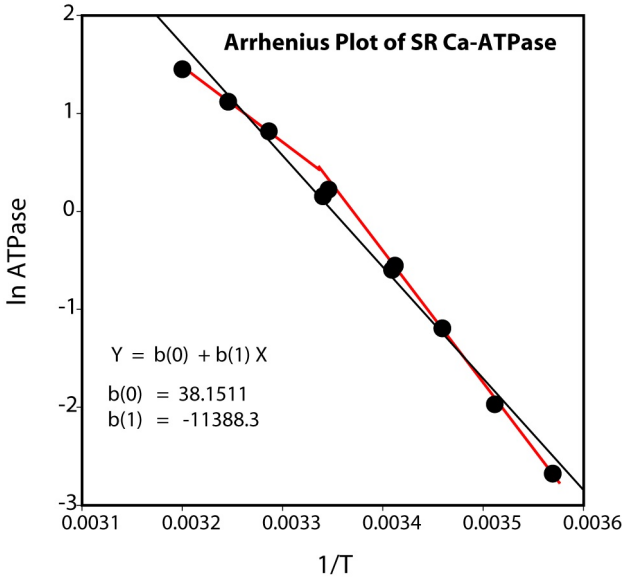


Fig. 1.PS2.PROBLEM 10 ANSWER. The ln of ATPase activity is plotted against the inverse of the absolute temperature. This is the Arrhenius Plot.

The slope of the Arrhenius Plot is - E_a/ R = -11388 °K Since R = 8.319 j mol^{-1} $°K^{-1}$, we calculate that E_a = **94.74 kj mol^{-1}, which is also 22.63 kcal mol^{-1}.** However, the graph is not a single line. It really is composed of two lines, or perhaps is a curve. The origin of this curve is not definite. It may be due to prior equilibrium or to a shift in the rate-limiting step of the enzyme with temperature.

11. Superoxide reduces cytochrome C in the reaction

$$\text{Cyt C}\bullet Fe^{3+} + O_{2^-} \Rightarrow \text{Cyt C}\bullet Fe^{2+} + O_2$$

Where Cyt C•Fe^{3+} is the oxidized form and Cyt C•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 $\varepsilon_{OX} = 0.89 \times 10^4\ M^{-1}$ (Massey, V. The microestimation of succinate and the extinction coefficient of cytochrome C. Biochim. Biophys. Acta 34:255-256 (1959)). See 1.PS2 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.

The linear regression of the line gives a slope of 0.01472 Absorbance units per minute. We convert this to mole L^{-1} min^{-1} by dividing by the difference in extinction coefficient between the oxidized and reduced form. Thus $\varepsilon_{RED} - \varepsilon_{OX} = 2.99 \times 10^4\ M^{-1} - 0.89 \times 10^4\ M^{-1} = 2.10 \times 10^4\ M^{-1}$ and the rate is $0.01472\ min^{-1} / 2.10 \times 10^4\ M^{-1}$ = **7.01×10^{-7} mol L^{-1} min^{-1}** which is equivalent to **0.701×10^{-9} mol mL^{-1} min^{-1}**

B. The xanthine oxidase was added in 75µL of 6.5 mg XO per mL into a 3 mL reaction mixture. Calculate the specific activity of cytochrome C reduction (mols of cytochrome C reduced per min per mg of XO protein.)

The concentration of xanthine oxidase is $0.075\ mL \times 6.5\ mg\ mL^{-1} / 3\ mL =$ $0.1625\ mg\ mL^{-1}$. We get the specific activity by dividing the rate of cyt C reduction by the concentration of XO:

$0.701 \times 10^{-9}\ mol\ mL^{-1}\ min^{-1} / 0.1625\ mg\ mL^{-1}$ = **4.31×10^{-9} mol min^{-1} mg^{-1}**

12. You suspect you are anemic and your physician orders some tests. He finds that your hemoglobin is 13g %. The molecular weight of hemoglobin is 66,500 g mol^{-1}.

A. What is the concentration of hemoglobin in molar in your blood?

The [Hb] is 13 g% which is 13 g dL^{-1} x 10 dL L^{-1} = 130 g L^{-1}. The molecular weight of Hb is 66,500 g mol^{-1} so the [Hb] in molar is

[Hb] = 130 g L^{-1} / 66,500 g mol^{-1} = **1.95×10^{-3} M**

B. Each hemoglobin binds 4 oxygen molecules. If the hemoglobin is saturated with oxygen, what is the concentration of O_2 bound to Hb, in molar?

The oxygen concentration ought to be 4 times the [Hb] since each Hb molecule binds 4 oxygens. Thus it would be **7.82×10^{-3} M**

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.

We can convert this to units of volume %. One dL of blood would contain 7.82×10^{-4} mol (part C x 1L /10 dL). So PV = nRT where P = 1 atm, V is the unknown, n = 7.82×10^{-4} mole dL^{-1}, R = 0.082 L atm mol^{-1} $°K^{-1}$ and T = 273.1 °K. The volume per cent of gas at STPD is thus 0.082 L atm mol^{-1} $°K^{-1}$ x 273.1 °K x 7.82×10^{-4} mol dL^{-1} = 175.1×10^{-4} L dL^{-1} = **17.51 mL dL^{-1}**

13. The rate of ATP hydrolysis by ATPases can be followed by the coupled enzyme assay shown below:

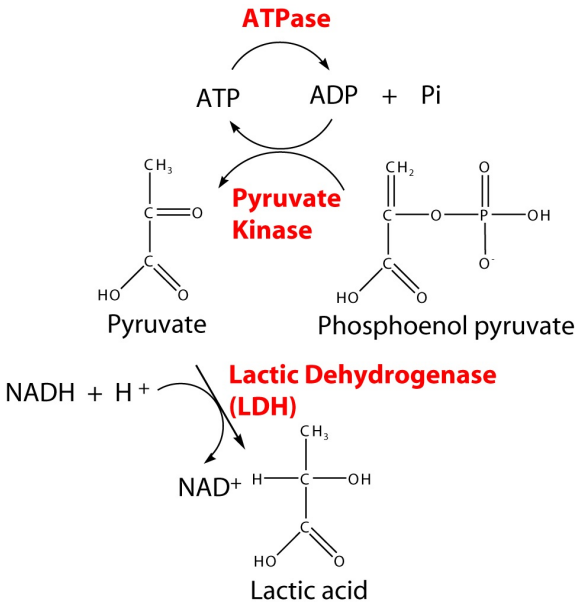


Fig. 1.PS2.2 ATP hydrolysis of pyruvate kinase converts phosphoenolpyruvate to pyruvic acid. This is coupled by lactic dehydrogenase to the conversion of pyruvic acid to lactic acid and conversion of NADH to NAD^+. The progress of the reaction can be followed spectrophotometrically by the change in absorbance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

The progress of the reaction can be followed by A_{340}. The extinction coefficient of NAD^+ at 340 nm is negligible. The exctinction coeffcient of NADH at 340 nm is $6.2 \times 10^3\ M^{-1}$. See 1.PS2.2 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}?

The decrease in [NADH] is given as (0.65 - 0.455)/ $6.2 \times 10^3\ M^{-1}$ = 0.0315×10^{-3} M = 0.0315×10^{-6} mol mL^{-1}, but this decrease occurs in 2 min. So the rate of decrease is 0.0157×10^{-6} mol mL^{-1} min^{-1}. The Ca-ATPase activity is obtained by dividing this rate of decrease in NADH by the amount of protein, which was 0.22 mg mL^{-1}. So we have

0.0157×10^{-6} mol min^{-1} mL^{-1} / 0.22 mg mL^{-1} = **0.0715×10^{-6} mol 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

Calculations of n! And $\sqrt{2\pi n}\, e^{n\ln - n}$ are given in Table 1.PS2.2 below:

n	n!	Stirling's Formula
1	1	0.92
2	2	1.92
3	6	5.84
4	24	23.51
5	120	118.02
6	720	710.08
7	5040	4980.4
8	40320	39902.4
9	362880	359536
10	3628800	3598694

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.

This equation can be shown to obey Fick's Second Law of Diffusion by taking ∂ /dt and $\partial^2/\partial x^2$ and showing their equivalence. When taking the derivatives, we carefully apply the chain rule:

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

$$= C_0 \sqrt{\frac{1}{4\pi Dt}}\; e^{\frac{-x^2}{4Dt}} \left[-\frac{1}{2t} + \frac{x^2}{4Dt^2} \right]$$

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

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

$$\frac{\partial^2 C(x,t)}{\partial x^2} = C_0 \sqrt{\frac{1}{4\pi Dt}} e^{\frac{-x^2}{4Dt}} \left[-\frac{1}{2Dt} + \frac{x^2}{4D^2t^2} \right]$$

A comparison of the last lines in these two derivatives gives us Fick's Second Law of Diffusion:

$$\frac{\partial C(x,t)}{\partial t} = D \frac{\partial^2 C(x,t)}{\partial x^2}$$

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^2s^{-1}.

The time for one-dimensional diffusion is related to the distance by

$$\overline{x^2} = 2\,D\,t$$

where x is the distance, D is the diffusion coefficient and t is the time of diffusion. In the problem, $D = 0.4 \times 10^{-5}$ cm^2s^{-1} and $x = 20 \times 10^{-4}$ cm. Inserting these values, we calculate

$t = (20 \times 10^{-4} \text{ cm})^2 / (2 \times 0.4 \times 10^{-5} \text{ cm}^2\text{s}^{-1})$ = **0.5s**

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?

Table 1.PS2.1 Diffusion coefficients and M_r for a variety of proteins

Protein	Molecular Weight	D x 10^7 (cm^2s^{-1})
milk lipase	6,600	14.5
Metallothionein	9,700	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

The Stokes-Einstein equation states that the diffusion coefficient is given by

$$D = \frac{kT}{6\,\pi\,\eta\,a}$$

where D is the diffusion coefficient, k is Boltzmann's constant, T is the absolute temperature, π is the geometric ratio, η is the viscosity and a is the radius of the molecule, assuming it is a sphere. As the molecular size increases, a increases and D should decrease. All other variables are unchanged among the different proteins. What we are searching for is the relationship between D and a. If the molecules are spherical, and their densities are constant, then molecular weight should be proportional to the volume occupied. Thus we have

$$Volume = \frac{4}{3}\pi a^3 = \frac{Mr}{N_0}\bar{v}$$

where Mr is the molecular weight (in g mol^{-1}), N_0 is Avogadro's number (molecules mol^{-1}) and v is the partial specific volume equal to the inverse of the density, in units of cm^3 g^{-1}. According to this relationship, we could re-write a as

$$a = \left(\frac{3\bar{v}}{4\pi N_0}\right)^{\frac{1}{3}} Mr^{\frac{1}{3}}$$

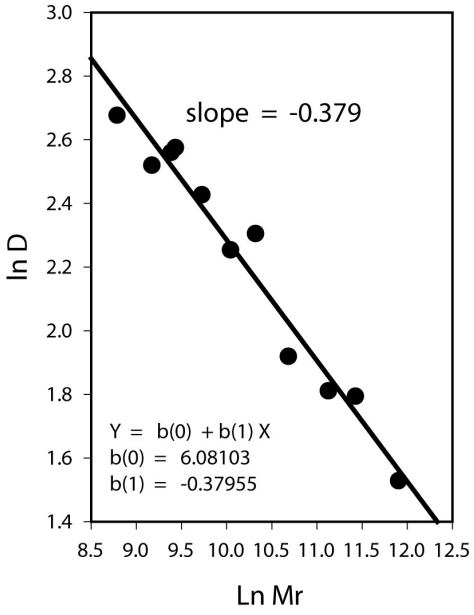
We can insert this back into the Stokes=Einstein equation to get

$$D = \frac{kT}{6\pi\eta}\left(\frac{4\pi N_0}{3\bar{v}}\right)^{0.333} Mr^{-0.333}$$

All of the terms to the left of the Mr term are constants. Thus we would expect the diffusion coefficient to be inversely proportional to the cube root of the molecular weight. We can determine the empirical relationship by plotting ln D against ln Mr. In this case we expect

$$\ln D = constant - 0.333 \ln Mr$$

ln D vs. ln Mr



The plot of ln D against ln Mr for the data supplied is shown at the left. The slope of the line is -0.379. Thus the results are not an exact fit to the Stokes-Einstein relation, but they seem to be a reasonably good fit. This may be in part because these proteins are not spherical.

Fig. 1.PS2. PROBLEM 17 ANSWER. As suggested by the Stokes-Einstein equation, the natural log of the diffusion coefficient was plotted against the natural log of the molecular weight.

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?

The time is estimated from $\Delta t = x^2 / 2D = (0.5 \times 10^{-4}\ cm)^2 / 2 \times 1.5 \times 10^{-5}\ cm^2 s^{-1} =$

$0.25 \times 10^{-8}\ cm^2 / 3 \times 10^{-5}\ cm^2\ s^{-1} = 0.083 \times 10^{-3}\ s =$ **0.083 ms**

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 .1 Pa s).

A. Estimate the diffusion coefficient for the protein in the cytoplasm at 37°C

Here we use the Stokes-Einstein equation: $D = kT / 6\pi\eta r$ We are given the viscosity (η = 0.05 Pa s) and the temperature (T = 310 °K) and k = 1.38×10^{-23} joule °K^{-1} ; we estimate r from the density

and the molecular weight. The molar volume = N_o V = N_o 4/3 πr^3 = 45,000 g / 1.06 g cm^{-3} = 42543 cm^3; we calculate r to be
r = 25.6 x 10^{-8}cm = 2.56 x 10^{-9} m .

Plugging this into the Stokes-Einstein equation, we have

D = 1.38 x 10^{-23} joules $^oK^{-1}$ x 310 ^oK / 6π x 0.005 N m^{-2} s x 2.56 x 10^{-9} m = 1.77 x 10^{-11} m^2s^{-1}

= **1.77 x 10^{-7} cm^2 s^{-1}**

B. If the protein 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?

Here t = x^2 / 2D = (75 cm)^2 / 1.77 x 10^{-7} cm^2 s^{-1} = **3.18 x 10^{10} s** = 5.3 x 10^8 min

20. Diffusion coefficients in cytoplasm has 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. (Axelrod, D., et al., Mobility measurements by analysis of fluorescence photobleaching recovery kinetics. Biophys. J. 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 (Lang, I., et al., Molecular mobility and nucleoplasmic flux in hepatoma cells. J. Cell Biol. 102:1183-1190 (1986)). These authors obtained the following data:

Probe	Molecular Weight	Equivalent radius	D in Dilute solution	D in cytoplasm	D in nucleoplasm
	(kD)	(nm)	D is in units of 10^{-6} cm^2s^{-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?

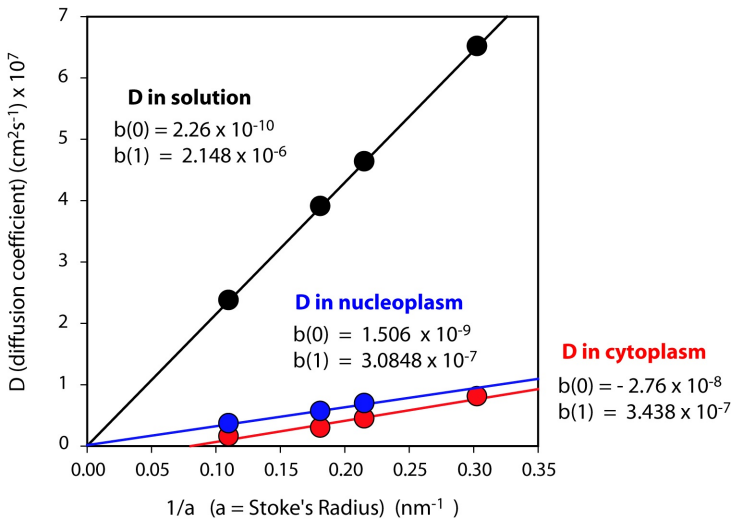


Fig. 1.PS2. PROBLEM 20 ANSWER. The slope of the curve of D against 1/a, where a is the Stoke's radius, is linear for all three situations: solution, cytoplasm and nucleoplasm. The curve extrapolates back to zero for the solution and nucleoplasm, and not quite to zero for the cytoplasm.

The curves for the diffusion coefficient determined in solution extrapolate to the origin, which corresponds to a zero diffusion coefficient for an infinitely large solute. This is also the case for the nucleoplasm, but is NOT the case for the cytoplasm. The error, however, is not large, and confidence in the results is not high. A possible cause for deviation from the origin intercept is disobedience to the Stokes-Einstein equation. This could be due to diffusional obstacles in the cytoplasm that artificially lower the diffusion coefficient for larger solutes.

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.

According to the Stokes-Einstein equation,

$D = kT / 6\pi\eta a$ the slope of the line should be $b(1) = kT/ 6\pi\eta$. Therefore we can calculate

$\eta = kT/ 6\pi\, b(1)$

Using $k = 8.314$ joules $mol^{-1}\ {}^{\circ}K^{-1} / 6.02 \times 10^{23}\ mol^{-1} = 1.381 \times 10^{-23}$ joules ${}^{\circ}K^{-1}$, $T = 310\,{}^{\circ}K$, we calculate that

$\eta = 2.271 \times 10^{-22}$ joule / $b(1)$

The slope of the plots given in part A are:

solution: $b(1) = 2.148 \times 10^{-6}\ cm^2 s^{-1}\ nm = 2.148 \times 10^{-6}\ cm^2\ s^{-1}\ nm \times 10^{-4}\ m^2\ cm^{-2} \times 10^{-9}\ m\ nm^{-1}$

$= 2.148 \times 10^{-19}\ m^3\ s^{-1}$

Inserting this into the equation for $\eta = 2.271 \times 10^{-22}$ joule / $2.148 \times 10^{-19}\ m^3\ s^{-1}$ gives

$\eta = 1.05 \times 10^{-3}\ N\ m^{-2}\ s$ = **0.00105 Pa s for the solution**

This is close to that of water, 8.9×10^{-4} Pa s.

For the cytoplasm, the slope is 3.438×10^{-7} cm^2 s^{-1} nm $\times 10^{-4}$ m^2 cm^{-2} $\times 10^{-9}$ m nm^{-1}

$= 3.438 \times 10^{-20}$ m^3 s^{-1}

Solving for the viscosity, we obtain $\eta = 2.271 \times 10^{-22}$ joule / 3.438×10^{-20} m^3 s^{-1} giving

$\eta = 0.661 \times 10^{-2}$ N m^{-2} s = **0.00661 Pa s for the cytoplasm**

which is about 5 times that of water.

For the nucleoplasm, the slope is 3.085×10^{-7} cm^2 s^{-1} nm $\times 10^{-4}$ m^2 cm^{-2} $\times 10^{-9}$ m nm^{-1}

$= 3.085 \times 10^{-20}$ m^3 s^{-1}

Solving for the viscosity, we obtain $\eta = 2.271 \times 10^{-22}$ joule / 3.085×10^{-20} m^3 s^{-1} giving

$\eta = 0.736 \times 10^{-2}$ N m^{-2} s = **0.00736 Pa s for the nucleoplasm**

which is about 7 times that of water.