Problem Set

2.1 Surface Tension, Membrane Surface Tension, Membrane Structure, Microscopic Resolution, and Cell Fractionation

1. Consider a soap film stretched over a wire frame, one end of which is movable. Experimentally, one observes that there is a force exerted on the movable member as indicated in Figure 2.PS1.1. Clearly, this force depends on the dimensions of the wire frame. Therefore, we express the force per unit length as γ. Write an expression for the work performed in expanding the film a distance dx. Rewrite this in terms of the area increment, dA, by which the film is expanded.
2. Consider a soap film again in the form of a bubble as shown in Figure 2.PS1.2. The surface tension can be thought of as either the force per unit length or the energy per unit area. The minimal energy form for a soap film is the minimum area for a given volume. This is the sphere. So, in the absence of other effects, including gravity, the soap bubble should be a sphere.
   A. What is the total surface energy of the sphere? Remember that the variable we have been using for surface tension is γ.
   B. If the radius were to decrease by dr, what would be the *change* in the surface energy?
   C. Since shrinking decreases the surface energy, at equilibrium the tendency to shrink must be balanced by a pressure difference across the film, ΔP. At equilibrium, the work against this pressure for an increment in radius dr is exactly equal to the decrease in surface energy. That is, at equilibrium the free energy change is zero. Otherwise, the bubble would not be stable and it would shrink. What is the work that must be done against this pressure difference? *Hint*: Pressure is force per unit area, so the total force must be the area times the pressure. Work is force times distance.
   D. Equate the pressure–volume work in part C to the surface energy decrease in part B. From this equation, derive an expression for ΔP in terms of γ and r. This result is a famous equation, the Law of Laplace, which finds application in respiratory physiology and cardiovascular physiology.
3. When heart cells are exposed to a hypotonic medium, they swell and measurements show that their volume has increased. Measurements of their membrane capacitance, however, do not change. How can this happen?
4. Liposomes form structures 100 nm across their outside diameter. The average density of the lipids used to form the liposomes is 0.89 g cm^{-3}. Assume that the thickness of the bilayer is 8 nm.
   A. What is the volume of the lipid shell? What is its mass?
   B. What is the ratio of the outer surface area to the inner surface area of the liposomes?
   C. What is the enclosed volume of the liposome?

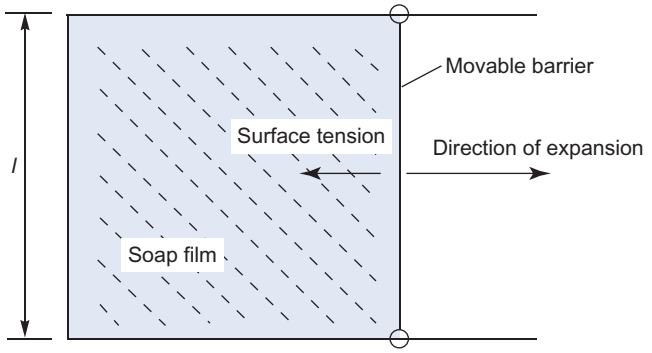

FIGURE 2.PS1.1 Soap film on a wire frame. The soap film exerts a force per unit length on the movable barrier. This force is the surface tension. To expand the film, we must do work.

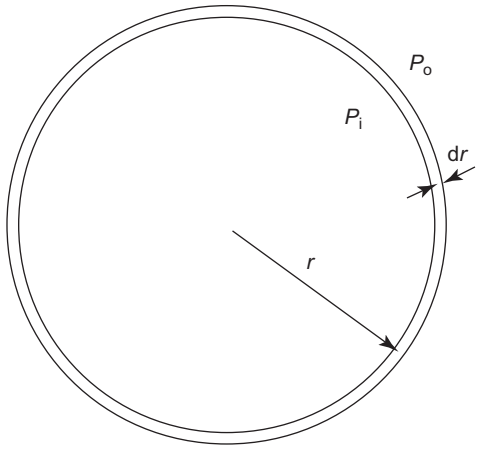

FIGURE 2.PS1.2 A soap bubble of radius r. Because the surface tension results in an inwardly directed force, the bubble will tend to collapse unless there is a pressure difference across the membrane that prevents its collapse.

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D. Using the answers to A and C, what is the overall density of the liposomes? Assume that the liposome is filled with water with density = 1 g cm^{-3}.
E. How many liposomes can be derived from 100 mg of lipids?
F. A drug is soluble to 5 mM. If the liposomes were formed in a solution of this drug, and therefore the enclosed volume included the drug to this concentration, how many moles of drug would be contained in the liposomes derived from 100 mg of lipids?

5. Isolated cardiac sarcoplasmic reticulum (SR) vesicles have an average outside diameter of about 150 nm. The membrane itself is about 10 nm thick. The enclosed volume can be estimated by measuring the efflux of passively loaded tracer materials such as mannitol, and the result gives 5 μL mg^{-1} SR protein.
   A. How many vesicles are there per mg of SR protein?
   B. What is the surface area of the vesicles per mg of SR protein?
   C. If the SR Ca-ATPase Ca^{2+} uptake activity is 4 μmol min^{-1} mg^{-1}, what is the uptake activity per unit surface area?
6. The method of measuring the surface tension of a liquid–air interface is the **drop weight method**. In this method, drops are allowed to form at the end of a tube of known radius, and a number of them are collected and then weighed so that the weight per drop can be determined accurately. The weight per drop is given by Tate's Law (1864):

[2.PS1.1] $$W = 2\pi r\gamma$$

where r is the radius of the tube. This equation uses the idea that the surface tension is the force per unit length and that the maximum force that can be used to support the weight of the forming drop is the circumference of the tube times its surface tension. In practice, the weight of the drop is less than that given by Tate's Law because some of the liquid supported by the tube remains after the drop falls. More detailed analysis makes use of a correction factor such that

[2.PS1.2] $$W' = 2\pi r\gamma f$$

where W' is the actual weight per drop and f is the correction factor. It turns out that the correction factor f varies with $rV^{-1/3}$, where V is the volume of the drop. Approximate values of the correction factor are given in Table 2.PS1.1:
   A. Using a tip with an outside diameter of 0.40 cm and an inside diameter of 0.20 cm, 20 drops of an organic liquid weighed 0.80 g. The density of the liquid was 0.95 g cm^{-3}. This liquid wet the tip. (*Hint*: This goes to determine whether you use the inside or the outside diameter!) Use the appropriate correction factor from Table 2.PS1.1, and the drop weight, to calculate the surface tension of the organic liquid.
   B. Using a tip with and outside diameter of 0.21 cm and an inside diameter of 0.18 cm, 20 drops of a liquid had a mass of 0.766 g. The density of the liquid was 1.00 g cm^{-3}. This liquid wet the tip. (*Hint*: This goes to determine whether you use the inside or the outside diameter!) Use the appropriate correction factor from Table 2.PS1.1, and the drop weight, to calculate the surface tension of the organic liquid.
7. The surface tension of pure water is 72.0 dyne cm^{-1}. 1 dyne cm^{-1} is equivalent to 1 mN m^{-1}, which is the SI unit for surface tension. When dipalmitoyl lecithin is spread at 50 $Å^2$ mol^{-1}, the surface pressure is 11 mN m^{-1}. What is the surface tension when dipalmitoyl lecithin is spread on the surface?
8. The tension in a biological membrane can be measured in a variety of ways. One way is called the **pipette aspiration technique**. In this technique, a specially manufactured micropipette is attached to a vesicle by light suction. These pipettes typically have a open diameter of 1–2 μm and have a square end. Application of increasing suction draws the vesicle into the pipette, forming a cylindrical part within the pipette and a spherical part outside of it (see Figure 2.PS1.3).
   A. Assume that the Law of Laplace holds for both the spherical part of the vesicle outside the pipette and the hemisphere within the pipette. Write the two equations relating P_v, P_o to D_v and T, the tension in the vesicle, and P_v, P_p to D_p. There is only one tension in the membrane, which is the same everywhere.
   B. Defining $\Delta P = P_o - P_p$, use the answer in part A to solve for ΔP in terms of T, D_v, and D_p:

TABLE 2.PS1.1 Correction Factors for the Drop Weight Method

$rV^{-1/3}$	f
0.3	0.7256
0.4	0.6828
0.5	0.6515
0.6	0.6250
0.7	0.6093
0.8	0.6000
0.9	0.5998
1.0	0.6098
1.1	0.6280
1.2	0.6535
1.3	0.640
1.4	0.603
1.5	0.567
1.6	0.535

Source: Data from A.W. Adamson, Physical Chemistry of Surfaces, *Interscience, New York, NY, 1967.*

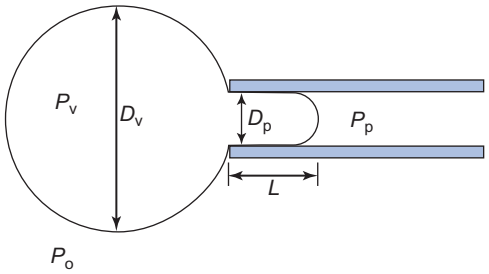

FIGURE 2.PS1.3 Pipette aspiration technique. A vesicle obtained from a "bleb" on a cell when the cell is exposed to hypotonic medium is excised and attached to a micropipette by application of suction. Increasing the suction draws the vesicle into the pipette a distance L and reduces the diameter of the remaining vesicle. By LaPlace's law, this increases the tension in the membrane. Continual decreases in P_p eventually causes the vesicle to rupture. The critical tension for rupture can be determined in this way.

TABLE 2.PS1.2 Data for the Dilatation of Sarcolemma Vesicles Using the Micropipette Aspiration Technique

Tension (mN m^{-1})	$\Delta A/A$	Tension (mN m^{-1})	$\Delta A/A$
1.4	0.0027	9.1	0.0178
2.6	0.0058	10.5	0.0212
3.9	0.0078	11.9	0.0248
5.1	0.0112	13.1	0.0261
6.6	0.0127	14.5	0.0296
7.9	0.0160		

J.A. Nichol and O.F. Hutter, Tensile strength and dilatational elasticity of giant sarcolemmal vesicles shed from rabbit muscle, ***J Physiol****. 493:187–198 (1996)*

C. Solve part B to express T in terms of ΔP, D_p, and D_v

D. A giant sarcolemmal vesicle was obtained from a rabbit muscle and subjected to the pipette aspiration technique (J.A. Nichol and O.F. Hutter, Tensile strength and dilatational elasticity of giant sarcolemmal vesicles shed from rabbit muscle, **J Physiol**. 493:187–198 (1996)). The pipette diameter (D_p) was 19 μm and the vesicle diameter was 66 μm. At a pipette suction of 8 cm H_2O, what is the tension in the membrane?

E. What is the total area of the membrane in terms of D_v and D_p and the length L?

F. When suction pressure is increased, L increases and D_v decreases. The increased tension stretches the membrane, causing a dilatation. The elastic area expansion modulus is *defined* as

$$K = \frac{T}{\frac{\Delta A}{A}}$$

where ΔA is the expansion of the membrane area due to dilatation and T is the tension. Considering your answer for part E, write an expression for the increase in area attributable to membrane dilatation, ΔA, in terms of an initial condition with D_{vi} and L_i and a final condition with D_{vf} and L_f.

G. In practice, the increase in the length of the projection is not long enough to cause an easily measurable difference in D_{vf} compared to D_{vi}. Assuming that the volume of the vesicle plus projection remains constant, express D_{vf} in terms of D_{vi}, ΔL, and D_p.

9. Using the micropipette aspiration technique described in Problem #8, the following data were obtained for the tension and area dilatation for sarcolemma vesicles obtained from rabbit skeletal muscle (see Table 2.PS1.2):
The vesicle ruptured at the last point recorded.
Calculate the elastic area expansion modulus (see Problem 2.PS1 problem #8 for a definition of the elastic area expansion modulus).

10. For light of wavelength 5000 Å (=500 nm), calculate the theoretical maximum resolution of an optical microscope.

11. Typically the energy of the electron beam in an electron microscope is known, because the voltage through which the electrons are accelerated is known. One electron volt is the energy gained by an electron when it is accelerated across a potential of 1 V. One electron has a charge of 1.602×10^{-19} C. So the electron volt is 1.6×10^{-19} V C = 1.6×10^{-19} J (1 J = 1 V C = 1 N m). The rest mass of the electron is 9.109×10^{-31} kg.
    A. Using this information, and assuming that all of the energy are converted to kinetic energy, calculate the momentum of a 150-keV electron (the denominator in Eqn (1.2.A1.2)). (*Hint*: Kinetic energy $E = p^2/2m$.)
    B. Calculate the wavelength of an electron having a kinetic energy of 150 keV.
    C. Using the result of (B), calculate the theoretical resolving power of an electron microscope using a 150-keV electron beam.

12. In a Sorvall T-865 fixed angle rotor, the distance to the axis of rotation is 3.84 cm at the top of the tube and 9.10 cm at the bottom of the tube. Calculate the RCF at 20,000 rpm at the top and bottom of the tube.

13. We are centrifuging a collection of particles with diameter 150 nm and average density of 1.10 g cm^{-3} through a water solution with density 1.0 g cm^{-3} at 20,000 rpm. The viscosity of the water is 1×10^{-3} Pa s, where Pa is the pascal = 1 N m^{-2}.
    A. At a distance of 8.5 cm from the axis of rotation, what is the net force on a particle?
    B. From Stoke's equation, calculate the frictional coefficient.
    C. What is the particle's terminal velocity?
    D. What direction is the net force?
    E. What causes this net force?

14. In eukaryotic cells (cells with a nucleus), ribosomes have two major subunits, a 60s and a 40s. If we assume both are spheres and have the same average density, what are their relative sizes?
15. Intestinal cells make a calcium-binding protein called calbindin. Calbindin has a molecular weight of about 9000 Da. Its synthesis requires the active form of vitamin D, $1,25(OH)_2$ cholecalciferol, to turn on the gene for the protein. When $1,25(OH)_2$ cholecalciferol is given to vitamin-D-deficient people, it takes about 45 minutes for the intestinal cells to make the first complete calbindin.
    A. Identify the major steps that could account for the 45-minute lag in appearance of calbindin.
    B. Eukaryotic cells (cells with a nucleus) attach amino acids to new proteins at the rate of about 2 per second. Is the synthesis of the protein the major part of the lag? (*Hint*: The average molecular weight of an amino acid is about 100 Da. You can estimate how many amino acids are in the protein from this information—you could look it up because its sequence is known, but we are just doing a "back of the envelope" calculation here.)
16. We have a double-stranded DNA segment of 1000 base pairs. Its nucleotide composition is randomly distributed among A, T, C, and G. Assume that each hydrogen bond in the double strand has an energy of 4 kcal mol^{-1} (1 J = 0.239 cal).
    A. How many hydrogen bonds are there in the segment? (*Hint*: Consult Figure 2.2.3 for the numbers of hydrogen bonds for each base pair.)
    B. What is the total energy necessary to pry apart the two strands, assuming that hydrogen bonding is the only force keeping them together? (It is not.)
    C. Assume that hydrogen bonds break when they are stretched 0.2 nm. How much force is necessary to break one?
    D. If all the hydrogen bonds in our DNA segment were to be ruptured all at once, how much force would be necessary?
17. The molecular weight of the protein myosin is 525,000 g mol^{-1}. Its sedimentation coefficient is 6.4S (this S is the Svedberg, not seconds) in water with a density of 1.0 g cm^{-3}, and its partial specific volume is 0.73 cm^3 g^{-1}. Calculate the frictional coefficient, β. This is sometimes called f.
    A. From the molecular weight and the specific volume, calculate the radius myosin would have if it were spherical.
    B. From the radius calculated in (B), determine the frictional coefficient from Stoke's equation. This is called f_0. The viscosity of water at 25 °C is 1×10^{-3} Pa s, where Pa is the pascal = 1 N m^{-2}.
    C. If the protein were spherical we would expect $f = f_0$. Is myosin spherical?
18. Consider the device shown in Figure 2.PS1.4 that consists of two reservoirs, S_1 and S_2, that initially contain the limiting concentrations C_1 and C_2, respectively, where $C_1 < C_2$. A pump removes fluid from S_1 at rate R_1 and places it in reservoir S_2. Therefore, as soon as the pumping starts the concentration in S_2 begins to change. A magnetic stir bar rapidly mixes reservoir S_2, and a second pump withdraws fluid from S_2 at rate R_2 and places it in a centrifuge tube. The total volume of $S_1 = S_2$ and both are one-half of the capacity of the tube, so that when all of the solutions are pumped into the tube, the tube is filled. Show that for $R_2 = 2 \times R_1$, the gradient is linear in volume from C_2 at the bottom of the tube to C_1 at the top.
19. The SR is a specialized endoplasmic reticulum in skeletal, cardiac, and smooth muscle cells. It contains a Ca-ATPase pump that actively pumps Ca^{2+} ions from the cytosol to an enclosed compartment within the SR, its lumen. The activity of the SR can be estimated by the rate of oxalate-supported Ca^{2+} uptake. This activity is useful because it can also be measured in homogenates of the tissue. Evidence suggests that the

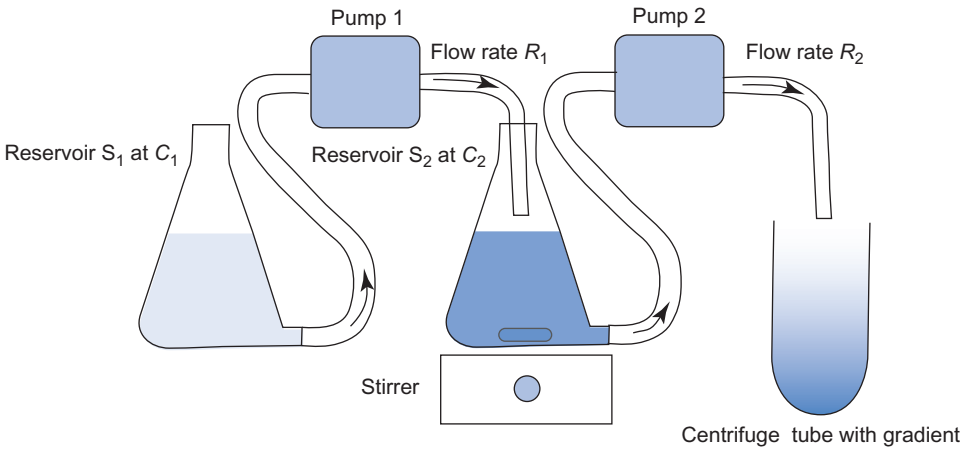

FIGURE 2.PS1.4 One way to make a gradient. Two reservoirs have the limiting concentrations C_1 and C_2. Pump 1 removes fluid from reservoir S_1 at rate R_1 and places it in reservoir S_2, initially at C_2 but then becomes diluted with fluid from S_1. Pump 2 removes fluid from S_2 at rate R_2 and places it in a centrifuge tube. The gradient that is formed depends on the values of R_1 and R_2 and the volumes of the reservoirs. Both S_1 and S_2 begin with identical volumes equal to one-half of the volume delivered to the centrifuge tube.

oxalate-supported Ca^{2+} uptake rate is only due to the SR and other organelles—the surface membrane or mitochondria—do not contribute to it. The left ventricles from a set of dogs were removed under general anesthesia, weighed and homogenized in 3 volumes of buffer (3 mL of buffer for every g wet weight of heart) and homogenate protein, volume and oxalate-supported Ca^{2+} uptake rate were measured. The homogenate was subjected to differential and then sucrose-gradient centrifugation to isolate membrane vesicles of the SR. The following was obtained from an average of 10 preparations: heart homogenate volume: 4.1 mL g^{-1} wet weight of heart, heart homogenate protein: 46.1 mg mL^{-1}, heart homogenate oxalate-supported Ca^{2+} uptake rate: 119 nmol min^{-1} (mg homogenate protein)$^{-1}$ isolated SR oxalate-supported Ca^{2+} uptake rate: 3.44 μmol min^{-1} (mg SR protein)$^{-1}$.

A. Calculate the homogenate protein per g wet weight of heart tissue.
B. Calculate the total homogenate Ca^{2+} uptake rate per g wet weight of tissue.
C. Assuming that the SR is 100% pure, how much SR is there, in mg of SR protein, per g of wet weight of heart tissue? (*Hint*: think about the units in the calculation.)