

## **Experiment 5: Swelling and solute transport properties of hydrogels.**

### **Aim:**

(a) To study the response of polyacrylamide hydrogel networks (synthesized in experiment 4) to temperature and ionic strength/ composition of the aqueous phase by measuring swelling or shrinking relative to the volume as synthesized.

(b) To study the diffusion of colored molecules of known size through the gels.

### **Materials and Apparatus:**

Buffer solution: Phosphate buffer saline (PBS), PH 7.4

Vitamin B12 (1%) -- diameter 12 Å.

Cytochrome C -- diameter 18 Å

Hemoglobin -- diameter 40 Å.

Larger diameter petri dish - bottom or cover

60 mm X 15 mm plastic petri dishes -- bottom and covers, 3 sets

Saran wrap

Surgical blades

Scintillation vials, 6

Ice bath

Hot water bath

### **Brief Background:**

#### Swelling of networks in solvents:

A network is crosslinked. Hence, when a solvent enters it, it does not dissolve it but instead swells it. Thus, the total volume increases to that of the network and the solvent. The degree to which the volume increases depends on a) the degree of cross-linking and b) the degree to which the polymer "likes" the solvent.

In a dry non-crystalline (amorphous) polymer that has been cross-linked into a network, the chains are in an entangled and relaxed conformation between two network junctions. However, when the solvent enters the polymer, the solvent molecules move the network junctions away from each other (as the polymer swells in the solvent). As the network junctions move away, the chains attached to these junctions experience a stress that is counteracted by the tendency of the polymer chain to return back to the relaxed state (where the entropy is higher). At some stage, an equilibrium is reached wherein the polymer refuses to accept anymore of the solvent. Clearly, this equilibrium depends on how

long the chains between network junctions are and the degree to which the polymer chains "like" (energetically) to have solvent molecules around them.

Hence, given a polymer (same chemistry) and solvent, the equilibrium degree of swelling will be smaller when the degree of cross-linking is higher. Why? Higher the degree of cross-linking ----> the larger the number of cross-links -----> smaller the chain between network junctions -----> smaller the extent to which these chains will extend as volume increases -----> smaller the degree of swelling.

The degree of swelling is thus used as a common measure of the degree of cross-linking. The degree of swelling is measured as follows:

- 1) Measure the dry weight and/ or volume of the polymer before swelling.
- 2) Swell the polymer in a solvent till equilibrium swelling.
- 3) Measure the weight of the swollen polymer and / or volume of the polymer after equilibrium swelling.
- 4) The degree of swelling is measured using the following two swell ratios:

$$\text{Mass swell ratio} = \frac{\text{Mass of swollen polymer}}{\text{Mass of dry polymer}} \quad (1)$$

$$\text{Volume swell ratio} = \frac{\text{Volume of swollen polymer}}{\text{Volume of the dry polymer}} \quad (2)$$

5) Exercise 1: If  $\rho_s$  and  $\rho_p$  are the densities of the solvent and the polymer respectively, how are the mass and volume swell ratios related?

6) Exercise 2: Consider the case when the initial polymer that you had was not completely cross-linked but had some free chains. (Do you understand the difference?) Here, the initial dry weight is the weight of the network and the free chains. When this sample is swollen in a solvent, the free chains are slowly extracted from the swollen polymer by dissolution while the network remains swollen in the solvent. The weight of this swollen mass is that of the network (without the free chains) and the solvent. Now if the swollen mass is dried thoroughly to remove the solvent, what remains is the network. This we can weigh again to get the weight of the network. In this case, the following two parameters are measured:

$$\text{Mass swell ratio} = \frac{\text{Mass of swollen network}}{\text{Mass of dried network after swelling}} \quad (3)$$

$$\text{Percent extracted} = \frac{\text{Swollen mass} - \text{mass of dried network}}{\text{Initial mass of polymer sample}} \quad (4)$$

Think about the difference in definition between eq. (1) and (3) and the physical significance of eq. (4).

#### Polyacrylamide electrophoresis hydrogels:

The gel you produced in experiment 4 is the commonly used polyacrylamide electrophoretic gel. It is used widely by biologists and biochemists in the electrophoretic separation of proteins, DNA and RNA. They are usually "cast" (i.e., synthesized from solution) by the experimenter just before the electrophoretic separation. These are called "sieving gels" because they delay the movement of the biological species (protein, DNA, etc.) under the imposed voltage gradient to a degree increasing with the size of the species. For the biologist the sieving "size" hole is of paramount importance. To get large sieving sizes, for large solutes, low acrylamide concentration (high water content) and low bisacrylamide/acrylamide ratios are used. (why ?)

"Gradient" gels are routinely formed by filling the gel cavity with a solution in which the bisacrylamide/acrylamide ratio decreases as the cavity is filled. Within minutes the layered solution gels, leaving the most porous gel at the top (where the biological species are introduced) and the "tightest" gel at the bottom.

#### Permeability of molecules in a gel:

In diffusion, the distance,  $x$ , a solute travels is proportional to the square root of time,  $t$ ,

$$x \sim \sqrt{Dt} \quad (5)$$

where  $D$  is the diffusivity of the solute. Clearly,  $D$  depends on the size of the solute and of the "sieving size hole" in the material it is diffusing through. If  $C_0$  is the concentration at the boundary of the semi-infinite slab and  $C(x,t)$  is the concentration at location  $x$  at time  $t$ , then the concentration profile looks like

$$C(x,t) = C_0 \left( 1 - \operatorname{erf} \left[ \frac{x}{2\sqrt{Dt}} \right] \right)$$

$\operatorname{erf}()$  is the error function:

$$\operatorname{erf}(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-u^2} du$$

Values of the error function may be looked up in a table or computed using software like Maple or Mathematica. If  $L(t)$  is the location at time  $t$  of the advancing front where the solute concentration is just visible, say  $C(x,t) \sim 2\%$  of  $C_0$ , you should be able to show that the distance  $L$  traveled by the solute in the hydrogel is given approximately by:

$$L \approx 4\sqrt{Dt} \quad (7)$$

**Precautions:**

No special precautions.

**Procedure:**

A. Swelling experiments:

1. Cut the worms into six approximately equal (maybe 2") lengths, for investigation of swelling/shrinking behavior. Label with permanent marker 6 scintillation vials: 1,2,3, etc. You will thus need 18 vials (3 recipes X 6 vials).
2. Weight each vial without its cap and record. Introduce one 2" worm piece into a vial, record new weight, and thus record weight of the piece.
3. Fill 3 of the vials containing the worm pieces with pure water, and 3 of the vials with PBS buffer solution. In pairs (pure water, buffer) hold two at about 0 C in an ice bath, hold two at room temperature (measure and record) and hold two at 100 C (in boiling water) for at least 30 minutes.
4. At intervals of one hour, one by one suddenly empty the vial of all the water or buffer in it, draining as much as possible any free liquid. With the worm back in the vial, reweigh vial and worm, and determine the gain (or loss) of weight of the worm. After taking each measurement, refill the vial with its original swelling fluid (water or buffer solution).

B. Circular slab diffusion experiment:

1. You should have set aside 9 circular gel slabs from Experiment 4 – three from each recipe that you made.
2. For each gel slab, Gently remove the bottom of the 60 X 15 mm set. A spherical hole will result from the removal of the bead glued onto the dish cover.
3. For each set of three slabs for a given recipe you will do the following: Into one hole introduce a small volume of 1% vitamin B12 (mol. wt. 1000, diameter 1.2 nm) in PBS, in another a small volume of 1% cytochrome C (mol. wt. 12000, diameter 1.8 nm) in PBS and

in the third, a small volume of 1% hemoglobin (mol. wt. 44000, diameter 5.0 nm) in PBS. (Only one solute per hole: 9 samples involving 3 recipes and three solutes)

6. Cover with Saran wrap to prevent evaporation. Be careful not to allow the solute to penetrate to the bottom of the dish, where excess water will carry it everywhere, and be sure to prevent Saran wrap or other items to accidentally spread the solute across the surface of the gel. Examine the gels after 2 days to determine the average radial distance that the solute has diffused. Estimate the diffusion coefficient for each solute using eq. 7.

Note: The gels may be transported to your living quarters to facilitate periodic investigation.

### **Observations and Calculations:**

1. Answer all equations and exercises in the theoretical background section in your lab notebook.
2. Tabulate weight measurements for the worms.
3. Calculate mass swell ratio in all cases.
4. What is the best way to plot the data for the swelling experiments? Do it so. (The best way: Ask the questions "what do you want to learn about?", "what do you want to educate somebody about?")
5. Tabulate data of time vs. distance for diffusion experiments.
6. Plot the data to illustrate the concept being demonstrated here. (what is the concept?: effect of recipe variables and effect of size of diffusing molecule)
7. Report doesn't need tabulated raw data. Just plots and final tables are needed.
8. Show how eqn (7) is obtained.

### **Discussion:**

1. Discuss the effect of synthesis variables, temperature and bathing solution on the swell ratio.
2. Discuss the effect of molecule size and synthesis variables on diffusion in gels.