Controlled Release Drug Delivery from Hydrogels

Student's Guide

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1 Objective

The aim of this project will be to design a drug delivery system capable of delivering a drug over an extended period of time at a known rate to a local area. The method of using implantable materials to treat tissues locally is known as controlled release. You will be using gelatin as a controlled release material and food dyes as a model drug. The gel concentration will be varied to show the effect of increased cross-linking on the rate drug release. The drug release of each gel concentration will be considered for three conditions–no enzyme, low enzyme concentration (1 mg/ml), and high enzyme concentration (5 mg/ml). An enzyme is a catalyst for reactions involving biological molecules. Finally, you will use your data to design an optimal drug delivery strategy for treating a brain tumor.

2 Background

Controlled release drug delivery is a new way to treat illnesses. The term controlled release refers to the ability of a drug delivery system to release a drug over an extended period of time at a controlled rate. Over the last 20 years, it has become more popular as a way to treat diseases such as cancer and diabetes. It generally involves implanting an engineered polymer directly into the organ or system that is affected by a disease. Since the polymer is implanted directly into the tissues affected by disease, the side effects are often small compared to systemic drug delivery (i.e. taking a pill or getting a shot). Brain diseases are

particularly good candidates for controlled release techniques because of a physiological feature known as the blood-brain barrier. The blood-brain barrier refers to a tight sheath of cells that surround the blood vessels in your brain. These cells make sure that only specific types of molecules get into the brain. More specifically, only small (molecular weight less than 1000 Da), water insoluble molecules can get into the brain. Consequently, the types of drugs that are developed for brain disease must fit this criteria, which is unfortunate because many promising drugs are water soluble or large. The use of controlled release techniques has led to tremendous breakthroughs in treating people brain diseases.

In this activity we will be using a special type of polymer called a hydrogel. Briefly, a polymer is any molecule made of repeating units, called monomers, that is bonded to itself many times. The most well known types of polymers are plastics. Polymers can take on lots of shapes such as rods, loose spaghetti like molecules (i.e. proteins), helical coils (i.e. DNA), and meshes (Figure 1).

A mesh consists of linear molecules connected to each other by bonds called cross-links. The degree of cross-linking is partly determined by the concentration of the polymer. At low concentrations a polymer will be a loose mesh. As you increase concentration, and consequently cross-linking, the mesh will become tighter (Figure 2). Hydrogels are a special type of mesh like polymer that have the ability to absorb large amounts of water. In fact, most hydrogels are over 90% water by weight. The most well known hydrogel is gelatin, or more commonly known as Jell-O®. Gelatin is a processed version of collagen which accounts for 1/3 of the protein in your body[1].

Proteins are polymers of molecules called amino acids. There are 22 amino acids and they make up the hundreds of millions of proteins in biological systems. Enzymes are a special type of protein in your body that have very specialized roles. One type of enzyme, called a protease, breaks down other proteins. They work by cutting the bonds between

[1] Collagen makes up the majority of your skin and is a constituent in connective tissue and bones. The collagen in gelatin comes from processing animal carcasses.

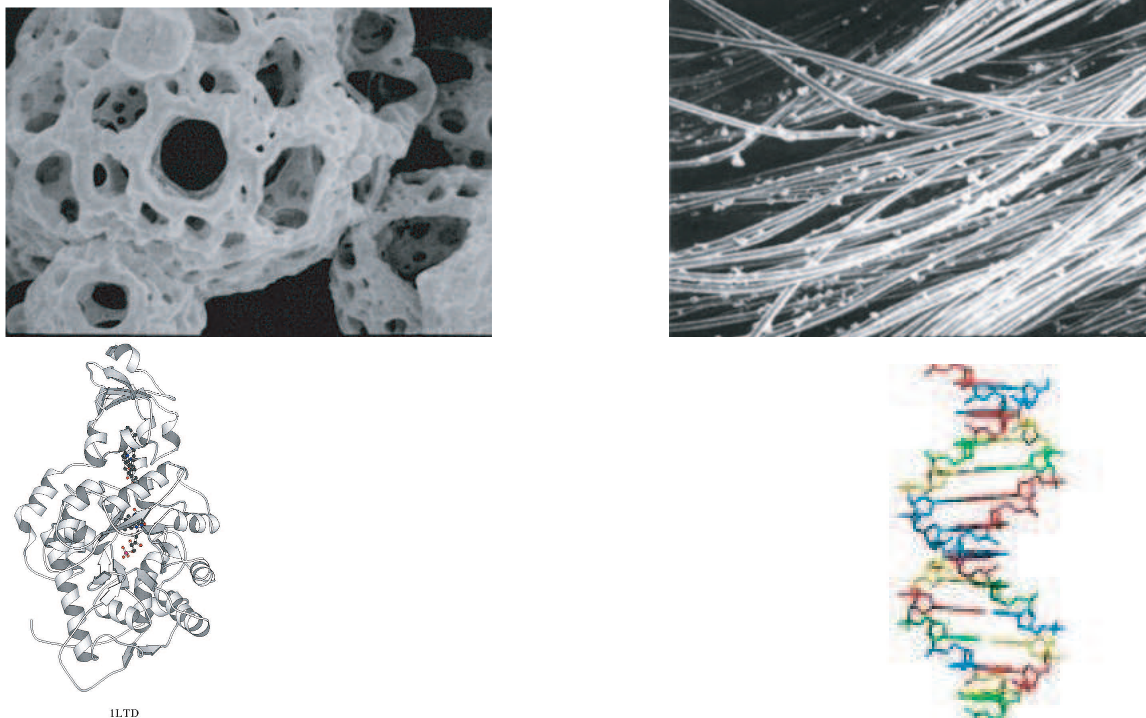


Figure 1: Examples of the structures of polymers used for drug delivery. Top left: Aerosol particle for drug delivery to lungs. Top right: Degradable polymer fibers for tissue engineering(Langer and Peppas, 2003). Lower left: Characteristic protein structure. Lower right: DNA double helix

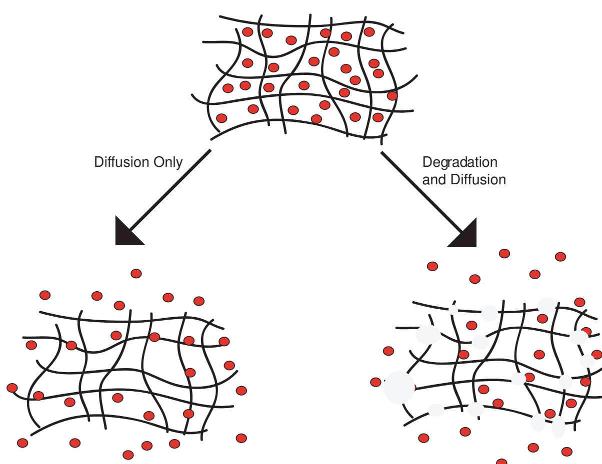
amino acids in proteins. In this activity meat tenderizer will break bonds in the Jell-O®, and make it a looser mesh. The enzyme in meat tenderizer is called bromelain and is found in pineapples. Bromelain is the reason you cannot put pineapples in your Jell-O©fruit salad.

There are two ways that a drug can be released from a polymer implant: 1) Diffusion through the implant and into the surrounding tissue. 2) Degradation of the implant by enzymes, water, or acidic/basic conditions coupled with diffusion (Figure 3). Some systems are designed not to degrade and release is controlled only by diffusion. However, these systems may require an extra surgery to remove the implant. Biodegradable systems only require one initial surgery and are ultimately digested by the body.

In this activity we will be simulating controlled release drug delivery using gelatin hydrogels. The gels will be loaded with a known con-

Figure 2: The effect of concentration on the hydrogel mesh. Left: Low concentration. Right: High concentration

Figure 3: Mechanisms of Drug Release



centration of a food dye which acts as the drug molecule. Following gelation, 5 mm^3 cubes of gel will be cut and placed into three different aqueous solutions. The first solution is plain water, which simulates a non-degradable polymer matrix. Here release is due to diffusion through the implant only. The other two solutions will contain a low (1 mg/ml) and high concentration (5 mg/ml) of meat tenderizer. In these second two cases release will be due to both diffusion and degradation.

To quantify the release of the dye molecules from the gels we will use spectrophotometry. Spectrophotometry is a method of characterizing solution concentration by measuring the amount of light that is transmitted through a sample. In a clear sample, like a test tube of water, all of the light will be transmitted. In a darker sample, like

water with dye in it, some of the light will be absorbed. The amount of light absorbed can be correlated to the concentration of the dye.

3 Formulating a Hypothesis

Before you start any scientific investigation it is important to formulate a hypothesis. There are several types of hypotheses. One of the most common is to predict what you think will happen.
In your own words describe what the purpose of this activity is?

How do you think gel concentration, or the degree of cross-linking, will affect the release profile?

4 List of materials

Below is a list of materials you will need for this activity. Make sure you have everything you need before starting the lab.

- Two packets of gelatin (≈15 g)
- 100 mL food dye solution
- Meat tenderizer (≈1 g)
- Graduated cylinders (10 mL and 100 mL)
- Small beaker (50-200 mL)
- Stir rod or stir bar
- Hot plate

- Four-inch petri dish with top
- Razor blade or scalpel
- Parafilm
- Ten 10 mL test tubes that fit in spectrophotometer

5 Experimental Procedure

The procedure for this activity will take place over two class periods. The first period will involve making gels of varying concentration with different molecules, in our case food dyes, and making the appropriate enzyme solutions. In the second period the gels will be placed in the enzyme solutions and release will be measured by spectrophotometry. For best results we are going to take data at 1 hour, 24 hours, and 48 hours.

5.1 Solution Calculations

You will make 31 mL of 5%, 10%, and 15% gelatin with a food dye solution. The first step is to calculate how much gelatin we need. Fill in the blanks below.

$$5\% \Rightarrow 31mL \times 0.05 = 1.5g$$
$$10\% \Rightarrow 31mL \times \qquad = \qquad g$$
$$15\% \Rightarrow 31mL \times \qquad = \qquad g$$

The second solution is the meat tenderizer. Calculate the appropriate amount of meat tenderizer for 100 ml of 1 mg/ml and 5 mg/ml solutions. Fill in the blanks below.

$$1mg/ml \Rightarrow \frac{1mg}{ml\ water} \times \frac{1g}{1000mg} \times 100ml\ water = 0.1g\ meat\ tenderizer$$
$$5mg/ml \Rightarrow \frac{\quad mg}{ml\ water} \times \frac{1g}{1000mg} \times 100ml\ water = \qquad g\ meat\ tenderizer$$

5.2 Making Hydrogels

1. Pour 31 mL of the food dye solution into a beaker.
2. Place on a hot plate and bring it to a boil.
3. Place a stir bar in the beaker and begin stirring or stir by hand with a stir rod.
4. Slowly add the gelatin.
5. Continue stirring until all of the gelatin is dissolved.
6. Pour the hot gelatin solution into the plastic dish and cover.
7. Place the plastic dish in the refrigerator and allow to gel overnight.
8. Repeat steps 1-7 for each gelatin concentration.

5.3 Making Enzyme Solutions

1. Weigh out the amount of meat tenderizer you calculated in the **Solution Calculations**
2. Pour 100 ml of water into a beaker
3. Add the meat tenderizer to the water and stir until dissolved

5.4 Controlled Release Experiment in Test Tubes

1. Select and clean 9 test tubes. Make sure to remove any fingerprints with alcohol.
2. In 3 test tubes add 5 ml of plain water
3. In 3 test tubes add 5 ml of 1 mg/ml meat tenderizer solution
4. In 3 test tubes add 5 ml of 5 mg/ml meat tenderizer solution
5. Cut 5 mm X 5 mm X 5 mm cubes of each of the gelatin concentrations

6. For each gelatin concentration place 1 cube into each of the solutions.
7. Cover the top of the test tube with a piece of parafilm.
8. Fill out the table below so that you can keep track of your solutions.

Table 1: Test tube contents.

Test Tube	Gel concentration (%)	Solution (mg/mL)
1		
2		
3		
4		
5		
6		
7		
8		
9		

5.5 Calibrating the Spectrophotometer

1. Turn the spectrophotometer on and allow it to warm up for at least 5 minutes.
2. Turn top right knob to the appropriate wavelength (see Table 1 below).
3. Adjust the bottom left knob so that the transmission reads 0%.
4. Place a test tube of water only into the spectrophotometer.
5. Adjust the bottom right knob so the transmission reads 100%.

Table 2: Absorption Maxima for McCormick Food Coloring

dye	wavelength (nm)
red	500
blue	610
green	625
yellow	430

5.6 Collecting Data with the Spectrophotometer

1. After you have calibrated the spectrophotometer, you are ready to take data.
2. Take a test tube and turn it upside down 3 times. This ensures that the contents are well mixed.
3. Place the test tube in the spectrophotometer and close the lid.
4. Record the Absorbance/Transmission in Table 3 in the next section.

Repeat this procedure for each test tube at each time point.

6 Data and Analysis

Table 3: Absorbance/Transmission data for each test tube.

time (hrs)	1	2	3	4	5	6	7	8	9

6.1 Drug Release Profile

Using Excel, enter in the data from Table 3. Normalize the data by dividing each data point by the maximum absorbance for each test tube. Graph the normalized data (call it fractional release) versus time for each test tube. You should have a total of nine curves. Fit a logarithmic trendline to each curve and display the equation on the graph.

7 Results and Conclusions

1. Did you observe any times of more rapid rise in concentration? If so, when? What might explain this?

2. How does the release profile change with gelatin concentration?

3. How does the release profile change with enzyme concentration?

4. The food dye used in this activity is a relatively small molecule. How do you think the release profile would look if the drug was a much larger molecule?

5. The experiment was performed at room temperature (70 °F). How do you think the release profile would change at body temperature (98 °F)? Why?

6. What other variables do you think could influence the release?

References

R. Langer and N. A. Peppas. Advances in biomaterials, drug delivery, and bionanotechnology. *AIChE Journal*, 49(12):2990–3006, 2003.

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