Drug Delivery with Alginate

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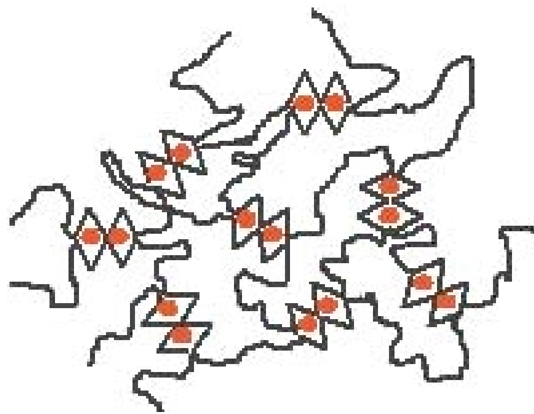
Objectives

- Define a hydrogel.
- Define the chemical structure and ionic crosslinking of alginate to form hydrogels.
- Discuss the role of hydrogels in drug delivery.
- Determine how diffusion rate of tartrazine varies with hydrogel surface area.
- Compare the release rate of a protein, bovine serum albumin (BSA), to that of the dye to understand the effect of drug molecular weight.



Introduction

In this laboratory, hydrogels will be formed by ionic crosslinking of alginate with divalent calcium ions. Model drugs will be incorporated into the hydrogels and the release rate will be measured as a function of hydrogel surface area and drug molecular weight.

Hydrogels

Hydrogels are very important materials. In fact, in June 2007, the Society for Biomaterials declared hydrogels "Biomaterial of the Month". Hydrogels are three-dimensional, water-swollen structures composed of mainly hydrophilic polymers. These materials are for the most part insoluble due to the presence of crosslinks. The crosslinks provide the network structure and physical integrity. Hydrogels are superabsorbent (they can contain over 99% water) natural or synthetic polymers. Hydrogels possess also a degree of flexibility very similar to natural tissue, due to their significant water content.

Common uses for hydrogel are:

- Scaffolds in tissue engineering. When used as scaffolds, hydrogels may contain human cells in order to repair tissue.
- Hydrogels that are responsive to specific molecules, such as glucose, can be used as biosensors.
- In disposable diapers where they "capture" urine, or in sanitary towels
- Contact lenses (silicone hydrogels, polyacrylamides)
- Medical electrodes using hydrogels composed of cross linked polymers (polyethylene oxide, polyAMPS and polyvinylpyrrolidone)
- Breast implants
- Dressings for healing of burn or other hard-to-heal wounds. Wound GEL are excellent for helping to create or maintain environment.
- Sustained-release delivery systems

Alginate

In this activity, we are going to work with a material called alginate, a polymer that is very important bioengineering. Alginate is used in a lot of foods, so you have probably already eaten alginate, and didn't even realize it! The structure of alginate is shown in Figure 1:

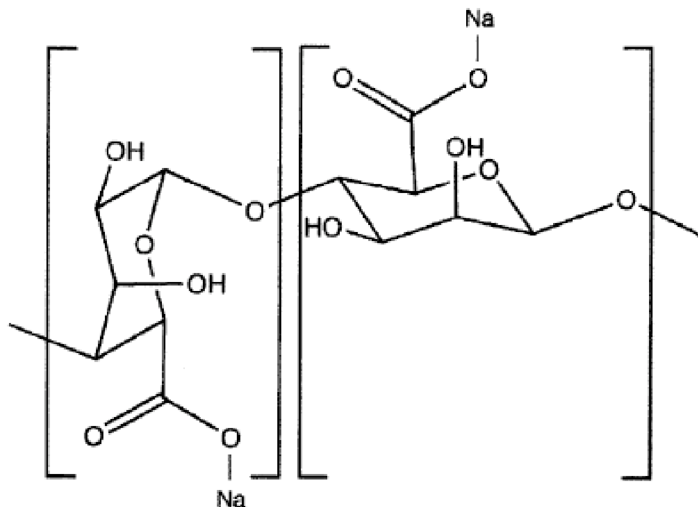


Figure 1. Chemical structure of alginate.

The alginate we are going to use today contains atoms of the element sodium, therefore it is called “sodium alginate”. When we dissolve the sodium alginate in water, the sodium atoms leave the molecules, making the oxygen it was bonded to it free to attach to something else.

Now let’s suppose we make a calcium chloride solution. What happens when dissolve a salt in water? It is dissociates into ions. So, if you dissolve calcium chloride in water (CaCl2), it will break into calcium (2+ ions) and chloride (-1 ions). Now lets investigate what happens when you place alginate in this calcium-containing solution.

The calcium ion will bond, or attach to the free oxygen atom. However, calcium has a 2+ charge, means that it can attach to two 1- charges (Figure 2). This means that one calcium ion can attach to two oxygen atoms at the same time. When the single calcium ion bonds to two oxygen atoms that are on different alginate chains, it ends up linking the two alginate chains together, called a crosslink. Here is a cartoon of what crosslinked polymer chains look like:

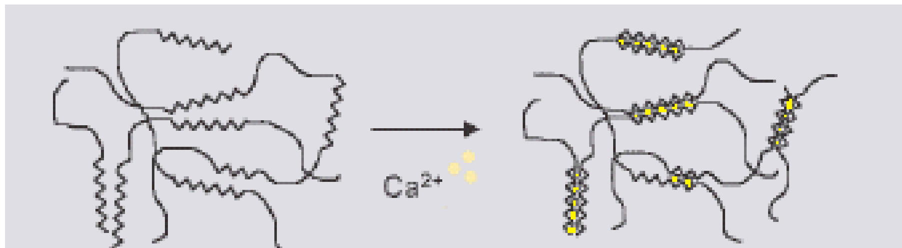


Figure 2.

After the polymer chains encounter a certain amount of calcium ions, the polymer chains become completely insoluble in water, and precipitate out of solution. As a result, you can visually detect a solid alginate in the liquid.

Drug Delivery

Controlled drug delivery is a field dedicated to the release of therapeutically active agents into the body. Drug delivery is an important part of the pharmaceutical aspect of chemical engineering. Although it is the chemists and pharmacists job to combine the chemicals necessary to treat an illness, it is the chemical engineers who are able to design the drug-delivery system to deliver a predetermined amount of drug for a decided length of time to a particular location in the human body. The engineers are able to bring things to the pharmaceutical industry that the chemists and pharmacists cannot. Chemical engineers are able to combine their knowledge of the physical and chemical properties, chemical reactions, mass transfer rates, polymer materials, and system models that are not taught in the other disciplines, and are therefore a vital role in the pharmaceutical industry.
Controlled drug delivery is the method of administering an optimal dosage of drug to a human in order to cure or control the present condition as quickly and conveniently as possible.1 The goal of controlled drug delivery is to dispense the drug at a predetermined rate, either constant or in

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[1] Robinson, Joseph R. (1978). *Sustained and Controlled Release Drug Delivery Systems*. Informa Health Care.

intervals, to the optimum target area for absorption in order to control the drug concentration in these regions. This is different from prolonged or sustained release systems which only delay the absorption of the drug into the body at an undetermined rate for an unknown period of time with no specific target region. Controlled drug delivery systems are more advanced than sustained release systems because they are able to maintain effective drug levels at a target location. This not only minimizes negative side effects due to drugs passing through organs that do not require treatment, but similarly lowers the amount of drug needed because of direct application. The drug is able to target specific cell types of the body with the use of carriers and excipients of different chemical complexes.2 If a commercial drug promises eight hours of pain relief, it means that the drug will be delivered to the body at a constant rate for eight hours. This is of importance to pharmaceutical companies because they are constantly looking for ways to increase the time drugs are delivered to the body (i.e. longer pain relief) and keep the amount of the drug released constant over time.

Role of Hydrogels in Drug Delivery

Because of their wide range of properties, hydrogels have been considered in drug delivery applications for over 30 years. One approach for managing drug release rates is to embed the drug within a three dimensional hydrogel and allow it to diffuse out of the hydrogel network over time (Figure 3). The release rate of a drug can be tailored by changing the structure of the hydrogel. For instance, the release rate of a drug can by tailored simply by varying the surface area of the hydrogel. A higher surface area will provide more "space" through which drug molecules can diffuse out of the gel and into the surrounding environment.

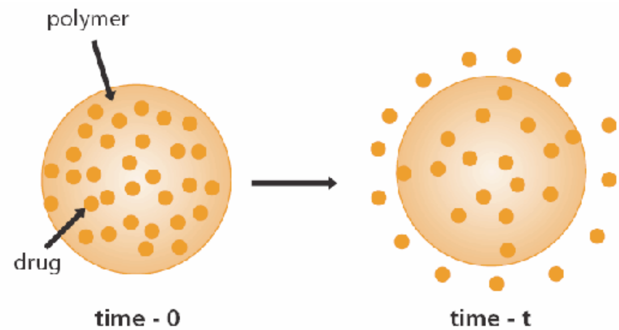

Figure 3. Solute diffusing through a spherical hydrogel.

Another important concept designing controlled release systems with hydrogels is related to how permeable the hydrogel structure is to drug. In other words, the rate of release is dependent on how easily the drug molecule can diffuse through the polymer network. Remember that hydrogels are made up of crosslinked polymer chains. This means that the polymer chains are connected at different points (Figure 4). In the case of alginate our hydrogels, the chains are connected by calcium ions. There are "spaces" between crosslinks (designated by the symbol ζ in Figure 4) that allow for drug diffusion. Smaller molecules can diffuse through these spaces more easily, resulting in increased drug delivery rates.

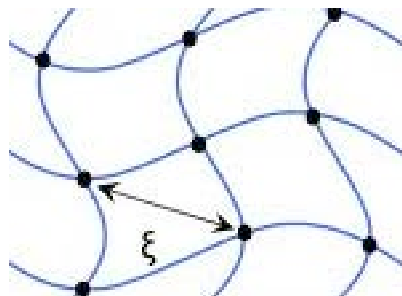

Figure 4. Schematic of a crosslinked polymer

Laboratory Procedure

Materials:

- 20 mL tartrazine (model drug) solution (0.5 mg/mL), in small beaker, provided by instructor
- 0.2 g sodium alginate powder, provided by instructor
- Calcium chloride solution (2 weigh boats), provided by instructor
- Disposable syringe (without needle)
- Magnetic stir rod
- Magnetic stir plate
- Plastic tube with cap for hydrogel storage
- Filtration set-up, provided by instructor
- Tweezers

Procedure:

1. Add the alginate powder to the tartrazine solution in the small beaker.
2. Add the stir bar to the beaker and stir vigorously until all the alginate "chunks" are gone. Solution should be a uniform yellow color when done stirring.
3. Load exactly 3 mL of the alginate solution into the disposable syringe by immersing the tip of the syringe in the alginate solution and pulling up on the plunger.
4. Dispense the alginate solution into the calcium chloride solution by continuously pushing down on the plunger. Dispense all the alginate solution in one place, instantaneously forming an alginate "blob" in the calcium chloride solution. You will note that solidification occurs instantaneously.
5. Retrieve your alginate hydrogel immediately using the tweezers and place in the capped container. Place in freezer, LABELED with your group names.
6. Load the disposable syringe again with exactly 3 mL of alginate solution. Slowly dispense the alginate into another weigh boat containing calcium chloride solution. This time, push on the plunger very gently, generating several "beads" of alginate solution that solidify instantaneously.

7. When all of the alginate solution has been dispensed, quickly take the weigh boat to the filtration set up to collect the beads. The beads should be placed in another plastic container, capped, and frozen. Make sure to LABEL container with your group names.

Materials:

- Small beakers (2)
- Tweezers
- DI water
- Disposable pipettes (2)
- Stir plate and magnetic stir rod (2)
- Spectrophotometer
- Disposable curvets
- 20 mL of 0.5 mg/mL tartrazine stock solution (provided by instructor)
- 7 glass test tubes, labeled 1-7
- Test tube rack
- 1 mL automatic pipettes

Procedure:

1. Retrieve your hydrogels from the freezer and allow them to thaw on the counter.

2. Generate a standards curve. Refer to Table 1. Place in the indicated volume of 0.5 mg/mL tartrazine stock solution into test tubes 1-7.

3. Referring to Table 1 again, place the indicated volume of DI water into test tubes 1-7.

4. Transfer the contents of test tube 1 into a cuvet. Zero the spectrophotometer with this sample.
5. Read and record the absorbance values for test tubes 2-6 in Table 1. This data will comprise your calibration curve.

Table 1

Column A	Column B	Column C	Column D	Column E
Test Tube	Stock solution volume (mL)	DI water volume (mL)	Final concentration (mg/mL)	Absorbance value
7	5	-	0.5	
6	5	1	0.42	
5	4	2	0.33	
4	3	3	0.25	
3	1.5	4.5	0.13	
2	0.5	5.5	0.04	
1	-	5	0	-

1. Fill the two beakers with exactly 100 mL of deonized water and place each on a stir plate.

2. Transfer your alginate samples into each beaker.

3. Place magnetic stir rods into each beaker and begin stirring on low.

4. Use a dropper to fill the UV cuvet about ¾ full (approximately 3 mL) and read the absorbance at 427 nm using the spectrophotometer. Record the absorbance value in Table 2.

5. Continue to pipette and analyze 1 mL samples from the buffer solution every 10-15 minutes for 60 minutes. Record the results in Table 2.

6. After reading the absorbance values, make sure to place all of the release medium back into the corresponding beaker.

Table 2

Time	Absorbance for hydrogel beads	Absorbance for large hydrogel	Time	Absorbance for hydrogel beads	Absorbance for large hydrogel

Data Analysis

1. Make a Beer's Law plot of concentration (x-axis) vs. absorbance (y-axis) from your calibration data. Determine the slope, intercept, and R2 value.

2. Note how to determine the total amount of model drug, tartrazine, loaded into the hydrogels. Remember that both alginate samples were formed by crosslinking a total of 3 mL of 0.5 mg/mL tartrazine solution. Therefore, each sample of alginate hydrogels has a total of 1.5 mg tartrzine loaded initially.

3. For each time point, calculate the concentration of tartrazine in the release media using your calibration curve (part 1). Enter this data into Table 3, Column B and E.

4. For each time point in Table 2, calculate the fractional release of tartrazine using Equation 1.

$$fractional\ release = \frac{mg\ tartrazine\ released,\ time\ t}{total\ mg\ loaded}$$ **Equation 1**

5. Enter the fractional release data into Columns C and F.

6. Make of plot of time (x-axis) versus fractional release (y-axis).

Table 3

Column A	Column B	Column C	Column D	Column E	Column F
Time	Concentration of Tartrazine in Release Medium	Fractional Release of Tartrazine (Equation 1)	Time	Concentration of Tartrazine in Release Medium	Fractional Release of Tartrazine (Equation 1)

Materials:

- 3 mL bovine serum albumin (BSA) solution (0.5 mg/mL), in small vial, provided by instructor.
- 30 mg sodium alginate powder, provided by instructor.
- Calcium chloride solution in a weigh boat, provided by instructor.
- Disposable syringe (without needle)
- Magnetic stir rod
- Magnetic stir plate
- Plastic tube with cap for hydrogel storage.
- Filtration set-up, provided by instructor.
- Tweezers

Procedure:

1. Add the alginate powder to the BSA solution in the small vial.

2. Add the stir bar to the beaker and stir vigorously until all the alginate “chunks” are gone. Solution should be a uniform yellow color when done stirring.

3. Load the 3 mL of the alginate solution into the disposable syringe by immersing the tip of the syringe in the alginate solution and pulling up on the plunger.

4. Dispense the alginate solution into the calcium chloride solution by continuously pushing down on the plunger. Dispense all the alginate solution in one place, instantaneously forming an alginate “blob” in the calcium chloride solution. You will note that solidification occurs instantaneously.

5. Retrieve your alginate hydrogel immediately using the tweezers and place in the capped container. Place in freezer, LABELED with your group names.

Materials:

- Small beaker
- Tweezers
- DI water
- Automatic 100 microliter and 250 microliter pipets
- Stir plate and magnetic stir rod
- Microplate spectrophotometer
- BCA Assay working reagent, provided by instructor
- 6 microcentrifuge tubes
- 96-well plate

Procedure:

1. Retrieve your hydrogels from the freezer and allow to thaw on the counter.

2. Fill the two beakers with exactly 100 mL of deonized water and place each on a stir plate.

3. Transfer you alginate samples into each beaker.

4. Place magnetic stir rods into each beaker and begin stirring on low.

5. Use an automatic pipet to remove 25 microliter samples of release media every 10-15 minutes for 60 minutes. Place each sample in the microcentrifuge tube and LABEL it with the time point and group names.

6. Collect all the samples until the end of the test, at which point they will be analyzed.

7. After all the samples have been collected, you will add 200 microliters of Bicinchoninic Acid (BCA) assay reagent (provided by your instructor) to each microcentrifuge tube. This reagent will turn a purple color in the presence of proteins such as BSA. Comparing the intensity of the purple color for our samples with a calibration curve will tell us the amount of BSA that was released at each time point.

8. Incubate the samples at 37°C for 30 minutes.

9. Set a 250 microliter automatic pipet to 225 microliters. Pipette the contents of each microcentrifuge tube into a well of the 96 well plate. Since you cannot label the wells, you will need to keep track of which sample you put into each well.

10. With assistance from the instructor, read the absorbance of each sample at 562 nm. Your data will automatically print.

Data Analysis

1. A Beer's Law plot of concentration (x-axis) vs. absorbance (y-axis) has been done for you.

2. Note how to determine the total amount of model drug, BSA, loaded into the hydrogels. Remember that the alginate hydrogel was formed by crosslinking a total of 3 mL of 0.5 mg/mL BSA solution. Therefore, the alginate hydrogel has a total of 1.5 mg BSA loaded initially.

3. For each time point, calculate the concentration of BSA in the release media using your calibration curve. Enter this data into Table 4, Column B.

4. For each time point, calculate the fractional release of tartrazine using Equation 2.

$$fractional\ release = \frac{mg\ BSA\ released,\ time\ t}{total\ mg\ loaded} \qquad \textbf{Equation 2}$$

5. Enter the fractional release data into Columns C.

6. Make of plot of time (x-axis) versus fractional release (y-axis).

Table 4

Column A	Column B	Column C
Time	Concentration of BSA in Release Medium	Fractional Release of BSA (Equation 2)

Discussion

1. In both of the experiments you performed, you may notice that the amount of model drug released initially very high, but the release rate tends to slow down at later time points. Why is this? Think back to our lesson on diffusion, and how concentration gradients are a driving force for the flow of solutes.

2. How did the release rate of tartrazine change with increasing surface area of the alginate hydrogels? Is this what you would expect? Why?

3. Tartrazine is a small molecule, approximately 534 g/mol in molecular weight. On the other hand, BSA is a large protein, weighing almost 70,000 g/mol. How did release rate of BSA from the large alginate hydrogel compare to that of the tartrazine? Is this the result that you expected? Why?