Encapsulation & Controlled Release: Macrocapsules

Center for Nanotechnology Education

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Encapsulation & Controlled Release

Abstract

This module introduces the process of forming macrocapsules and the concept of self-assembly. Forming capsules at the macro, micro, and nano levels have very important applications in drug delivery, cosmetics, food technology, and cleaning up environmental toxins. The module includes a laboratory activity that uses organic compounds, nanoparticles, and dye to allow students to study the process of encapsulation and controlled release.

Outcomes

- Understand the roles of nanoparticles and self-assembly in the process of microencapsulation
- Learn about the actual and theoretical applications of micro- and nanocapsules in medicine and other technology

Prerequisites

- High school chemistry

Science Concepts

- Diffusion
- Forces and interactions: Van der Waals, hydrogen bonding, electrostatic interaction
- Spectrophotometry
- Encapsulation

Nanoscience Concepts

- Nanoparticles
- Self-assembly

Background Information

What is encapsulation? The phenomena of encapsulation occurs naturally and is also the center of focus for many fields of engineering (pharmaceutical, agriculture, food, printing, cosmetics, textile and defense (8)). An example of natural encapsulation is the formation of liposomes via a process called self-assembly. A liposome is a small, spherical *vesicle* made up of at least one lipid bilayer (Figure 1) with diameters ranging from tens of nanometers to tens of micrometers. The interior of the liposome (the "core") is lined with hydrophilic (water-loving) molecules, allowing it to contain a variety of aqueous or hydrophilic substances depending on the surrounding environment, the size of the liposome, and

natural processes such as diffusion and osmosis. The outer membrane of the liposome is also hydrophilic, allowing to be suspended in water, saline solution, or other aqueous media.

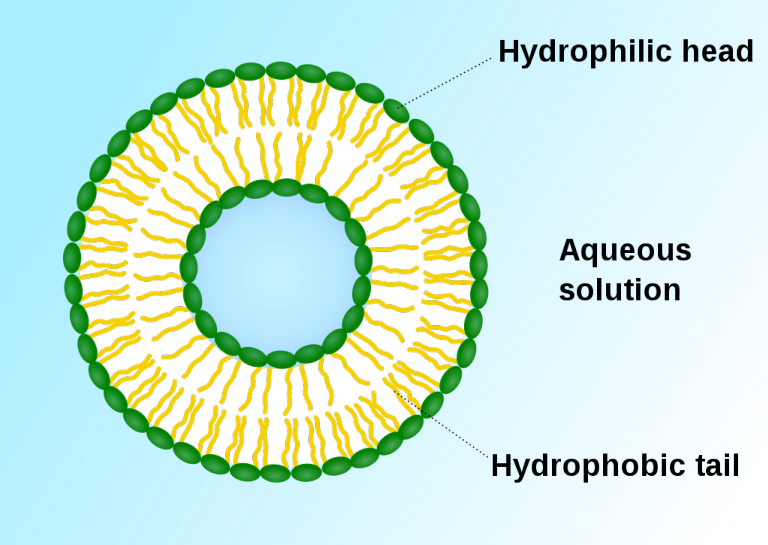


Figure 1: Schema of a liposome showing phospholipid bilayer surrounding an aqueous interior and excluding an aqueous exterior environment (https://en.wikipedia.org/wiki/Liposome)

Lipid bilayers are formed via a process known as self-assembly. Self-assembly is a natural phenomenon where various physical, chemical, electrical, and other environmental forces act upon a disordered system to form an organized structure. One example of self-assembly is the formation of a snowflake, where the intricate hexagonal structure arises as water molecules condense from the vapor phase. Due to the bonding angle of H2O, the resulted assembly of water molecules has a hexagonal form. Another example of self-assembly is life itself, specifically the proteins that make up cells. Proteins assemble from amino acids in a wide variety of structures, depending on the distribution of positively- and negatively charged groups on the amino acids. The specific shape of the resulting protein governs its role in the life process within the cell.

Forces that play crucial roles in self-assembly and the formation of liposomes include Brownian motion (random motion of particles suspended in a fluid as a result of colliding with quick moving atoms or molecules) and electrostatic forces that drive the hydrophobic effect (the tendency of hydrophobic molecules to aggregate in an aqueous (hydrophilic) environment, which explains why a drop of oil in a pot of water remains as one drop even after turbulent mixing).

Microencapsulation is a process that takes advantage of self-assembly. This process involves the formation of small (micron-sized) capsules that contain some active ingredient (food ingredients, enzymes, drugs, etc.) intended to be released under defined conditions. The process may be extended to nanoscale capsules in nanoencapsulation.

Why is encapsulation important? Encapsulating a substance at the micro or nano scale is important wherever it's necessary to entrap a substance within a shell, permanently or temporally, in order to extend its life and stability and to control its release under specific environmental conditions.

The manufacture and application of microcapsules has been ongoing for decades. Microcapsules are used in everyday products including adhesives, e-paper/e-ink, food additives, pesticides, perfumes, textiles, and medicines. The manufacture and application of nanocapsules show huge potential in areas such as food technology, cosmetics, and drug delivery.

One common example of micro- or nano-encapsulation is a drug that must be released only in the small intestine. In order to prevent the drug from being digested in the stomach prior to making it to the intestine, the drug agent is microencapsulated in a shell that is insoluble in the low pH environment of the stomach ($pH \leq 3$) and will only dissolve in the somewhat higher ($pH = 6$) pH of the small intestine. Other examples of microencapsulated products include chemical catalysts that are designed to be released in a reactor at certain temperatures and dyes that release from small capsules when mechanically broken – this is how carbonless forms work.

What types of microcapsules can be made? There are two general types of microcapsules: core-shell and matrix. A core-shell capsule is like an egg, with a solid continuous shell that encloses the liquid or solid active ingredient as the core. Release of the active ingredient is accomplished by breaking, melting, or digesting the shell, resulting in fast one-time release.

A matrix capsule is more like a sponge: it has a porous structure that holds the active ingredient by capillary action. The release of the capsule fill occurs by diffusion to the external environment, and tends to be more continuous. Matrix capsules may be subsequently coated with a shell if a different release profile is desired. A common material that is used in forming matrix capsules is an organic polymer known as alginic acid (derived from brown seaweeds). When calcium ions are present chains of alginic acid are cross-linked together forming a gel.

In this lab activity students will create and characterize capsules on a larger scale (macrocapsules), using naturally occurring organic polymers such as chitosan and sodium alginate and a food coloring dye as the capsule fill. If the lab is equipped with a spectrophotometer which measures the absorption of light by a liquid sample, students can also measure the release of the capsule fill versus time. They can also study the structure of the capsules if they have access to a light microscope, a fluorescence microscope, and/or a scanning electron microscope (SEM).

Current and Future Applications

Many products on the market currently take advantage of encapsulation. Fragrance companies mail out scratch and sniff advertising cards to customers with a special coating containing microcapsules that, when friction is applied, release the perfume scent. This same approach has been used to introduce consumers to the smell of natural gas so they can identify leaks in pipes at home (2). Cosmetics also utilize encapsulation. Sunscreens are manufactured with nanocapsules that aid in reflecting UV radiation, and lipsticks have nanocapsules with gold nanoparticles that intensify the color red (4). There is much ongoing research into how nanocapsules can be incorporated into medicine. Some cancer medicines contain nanocapsules that allow for slower release of chemotherapy agents into the body, thus leading to improved absorption, longer residence time, higher dispersion within the body, control over release conditions, improved organ targeting, decreased toxicity, and decreased irritation (1). However, the application of nanocapsules in diagnosing and treating diseases is still in various stages of the drug development process (1).

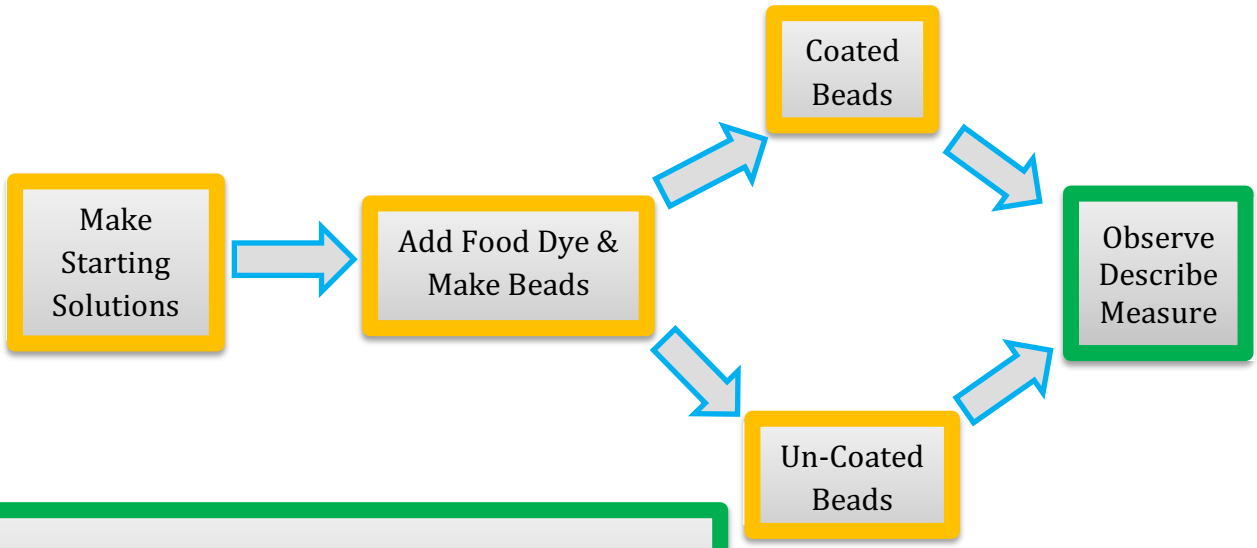
Future applications of nanoencapsulation lie in many areas, including in food technology and drug delivery. Many food companies are designing ways to improve the preservation of food, deliver food additives such as flavor and color, and increase efficiency, storage, and safety of production and handling (3). Nanocapsules are also being studied for targeted insulin delivery, improved bone health and development, immunotherapy, chemotherapy, diagnostics, and gene therapy (1).

Learning Activity:

Making and testing macrocapsules

Discussion/Student Inquiry

- Diffusion
- Forces: Van der Waals, Hydrogen bonding, electrostatic interactions
- Hydrophilic/Hydrophobic interaction
- Self-assembly
- Encapsulation



Optional

- Measure dye release over time
- Study effects of temp./salt/pH on dye release

Discussion/Student Inquiry

- Dye release in coated v. un-uncoated macrocapsules
- Environmental effects on dye release
- Application to medicine/environment/agriculture/food/cosmetics
- Potential challenges/hurdles/concerns

Encapsulation & Controlled Release

Description

In this lab exercise, you will explore the process of encapsulation and self-assembly techniques. To help visualize the processes of encapsulation and controlled release, you will form matrix macrocapsules (a few mm in diameter) encapsulating a dye, and use a natural compound to coat the capsule. You will then measure the rate of dye release between the coated and uncoated matrix capsule.

Objectives

- Encapsulate a food dye using a common encapsulation method
- Learn some of the chemistry behind encapsulation
- Measure the release of the capsule fill under controlled conditions

Materials and Equipment

- Alginic acid sodium salt
- Low molecular weight chitosan
- Citric acid monohydrate
- Calcium chloride
- Food dye concentrate
- Vinyl gloves
- Magnetic stirrer and stir bar
- 1ml disposable droppers
- Spectrophotometer (optional)
- Cuvettes (optional, for use with spectrophotometer)

Procedure

Step 1. Make the starting solutions.
NOTE: alginate and chitosan are water soluble natural organic compounds, but require special handling to avoid lumping and very long dissolving times. For alginate: stir the water vigorously using a magnetic stirrer, and add the powdered alginic acid SLOWLY to the vortex so that all the powder is wetted by the water. Do not dump the powder in too fast, or you will get a sticky clump that will be difficult to dissolve. Continue stirring the suspended powder for 25-30 minutes until the powder is completely dissolved, resulting in a clear solution. For chitosan: stir the water vigorously, add the chitosan powder to the vortex slowly, and stir for 10 minutes.

a. Enough alginate for the entire class may be prepared by dissolving 1.0 g of the alginic acid-sodium salt in 200 mL of DI water to produce a 0.5 wt% solution.

b. Prepare a 2.5 wt% calcium chloride bath by dissolving 2.5 g of $CaCl_2$ in 100 mL of DI water.

c. Prepare a chitosan solution by dissolving 0.2 g of chitosan in 50 mL of DI water containing 2 g of citric acid under vigorous stirring.

Step 2. Form the macrocapsules.
a. Add a drop of the food dye concentrate to 5 mL of the alginate solution and mix with a stir rod or dropper

b. Fill a dropper with this dye-loaded alginate solution and add it drop-wise into the calcium chloride bath to make ~50 beads. Hold the dropper about 4-5 mm above the surface of the $CaCl_2$ bath. The alginate will react with calcium ions to form a gel-like bead. The beads formed should be spherical; dropping from too high above the bath will form flat shapes when they splat into the bath.

c. Drain the bath and recover the alginate beads. Rinse the recovered beads in water. Divide your beads into two portions.

d. To one portion of the rinsed alginate beads add 30 mL of DI water. Label these macrocapsules MC 1. Obtain images and determine capsule diameter.

e. To the second portion of the alginate beads, add 5 mL of the chitosan-citric acid solution. This will place a chitosan coating around the bead. After 5 min, pour off the chitosan solution while retaining the beads and add 30mL of DI water. Label these macrocapsules MC 2. Obtain images and determine capsule diameter.

(OPTIONAL) *Step 3. Measure the dye release rates of the coated and uncoated alginate beads. This requires a lab spectrometer that can measure light absorption and transmission through a small sample of liquid.*

a Transfer some of each type of the suspended macrocapsules into a spectrophotometer cuvette.

b Place the cuvette into the well on the spectrophotometer. Perform a light absorption measurement and record the amount of absorption due to the dye diffusing out of the capsules. This initial absorption measurement is the baseline. (If your instrument allows, record the absorption at two different wavelengths.)

c Repeat this measurement at 10 minute intervals for about 30-40 minutes. Changes in the absorption are due to the dye releasing from the macrocapsules. Record the amount of absorption for each type of macrocapsule in Tables 1 and 2. Plot absorption vs time for each type of capsule.

Table 1: Light absorbance (a measure of the dye release) of uncoated alginate macrocapsules (MC1) over time.

Time (minutes)	Absorbance
0	
10	
20	
30	
40	

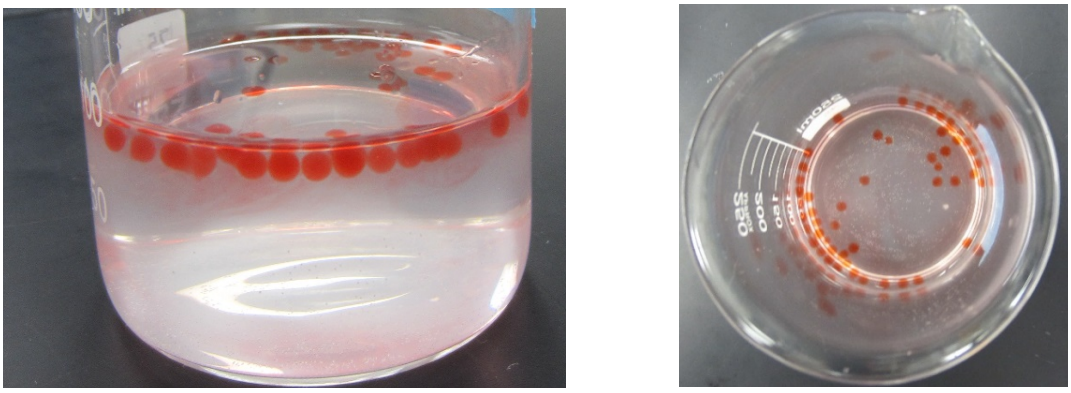
Table 2: Light absorbance (a measure of the dye release) of chitosan-coated alginate macrocapsules (MC2) over time.

Time (minutes)	Absorbance
0	
10	
20	
30	
40	

Analysis

1) Using an Excel spreadsheet, plot the absorbance data versus time. For each type of capsule (MC1 and MC2), determine the rate of dye release with time, using a linear regression.

Results (for illustration—your results may vary)



a) b)

Figure 2: Approx. 50 spherical dye-loaded alginate beads formed in calcium chloride bath (lateral view (a), bird's eye view (b)).

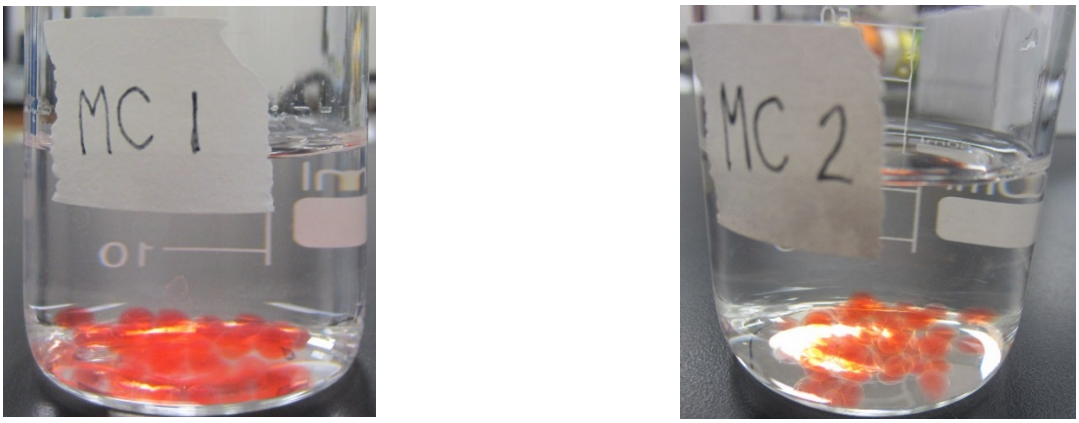


Figure 3: a) Approx. 25 uncoated alginate beads in 30mL DI water (MC1); b) Approx. 25 chitosan-coated alginate beads in 30mL DI water (MC2).

Discussion Questions

- What trends did you observe in dye release rates between chitosan-coated and uncoated alginate beads under 'normal' conditions? Answers may vary.

- How did varying temperature, salinity, or pH of the surrounding macrocapsules influence dye release? Answers may vary.

- Discuss why alginate and chitosan are suitable polymers for encapsulation. Alginates form a gel with porosity that allows a fill to be added and controllably released. Both materials are natural, organic, biocompatible, biodegradable, and have low toxicity, making them good for food and drug applications.

- How might you be able to apply your new knowledge of encapsulating food dye and studying its release to drug delivery the medical field/agriculture/food production/cosmetics? Answers may vary.

Going further (requires additional research)

- What other materials might be suitable as encapsulating agents for drugs or other materials destined for medical or environmental purposes? Poly(amino acids), hyaluronic acid, albumin, dextran, gelatin

- Are there any drugs on the market today approved by the Food and Drug Administration (FDA) that are effective based on the concept of encapsulation and controlled release?

- What might be some problems or challenges researchers face when developing new drugs that can be released under very specific environments in the body (ex: pH, salinity, osmotic pressure, presence/absence of enzymes, etc.)?

Contributors

- Based on a lab activity developed by Dr. James Marti, University of Minnesota, and adapted from "Self-Assembly and Nanotechnology" by Hitesh G. Bagaria, et al, J. Chem. Educ. 2011, 88, 609-614.
- Adapted and edited by Kyle Forgette, Biology & Nanoscience Instructor, Dakota County Technical College

Resources

Videos

- Microencapsulation: www.youtube.com/watch?v=BS7fJU9nafE
- Encapsulation and controlled release technology: www.youtube.com/watch?v=d3VmumaiBtI

Articles

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