Multilayer Encapsulation of Biological Media for Controlled Release Coatings

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Abstract:
There are many biological applications that use polymers to encapsulate biological media for their controlled release within the human body. This laboratory exercise provides analogy of the controlled release mechanism within multilayered polymeric coatings. Such types of coatings are used in a variety of biological applications to release different agents/drugs. Typical examples include: drug eluting stents, oral tablets, and tissue scaffolds. Students use household materials such as candle wax, vinegar and food color to investigate how different biological agents are released.

Keywords
Controlled release coatings, biological media, encapsulation, drug delivery.

Module Objective:
The objective of this module is to provide a basic understanding of how a polymer based multilayered coating method can be used to control the release of biological agents for different applications.

Type of module/mode of presentation: Laboratory

Time required: Approximately 40 minutes

Pre-requisite knowledge: General biology.

Target grade level: High school students in advanced biology program (11^{th} or 12^{th} grade) or in one of the units in the International Baccalaureate Program.

Equipment and Materials:
1. Food dye (yellow, blue, and red) to mimic biomedia
2. White candle wax, emulating biodegradable polymer
3. White distilled vinegar 5% or less acetic acid (this emulates enzymes in human body)
4. Baking soda (emulates biomedia, i.e. drug or nutrient)
5. Hot plate
6. Small container top (approximately: 1" inner diameter, 1/2" deep)

7. Six 200 mL glass beakers (heat resistant)
8. Three microscope glass slides labeled slide1, slide 2 and slide 3
9. Heat resistant gloves
10. Safety glasses
11. Beaker Tongs

Curriculum overview and notes for the instructor:
The release rate of a biological agent such as a drug can be controlled by encapsulating it within a polymer material. The use of multilayer biopolymer encapsulation technique can result in the controlled release of different drugs within a single encapsulation system. Controlled release coatings have been used in a variety of biological applications including cardiovascular (circulatory system comprising of the heart and blood vessels) drug eluting stents, oral tablets, and biological constructs (tissue scaffolds).

Stents are metallic mesh tubes used to enlarge the narrowing of blood vessels (arteries) to treat the coronary artery heart disease. Stents that are coated with medicines for slow and continuous release are called drug eluting stents. Medical doctors and researchers have designed drug eluting stents that use polymers to encapsulate different drugs on cardiovascular stents. A multi-layered drug coated stent was found to be effective in preventing thrombosis (clotting of blood due to platelets sticking to the inner surface of the stent) and restenosis (narrowing of a blood vessel, leading to restricted blood flow) at the implant site. To prevent restenosis, a polymeric coating containing a drug was first placed on a stent. The next layer comprised of a hydrophobic (non-wetting) drug loaded polymer that suppresses blood coagulation (i.e. thrombosis) and provides a surface to reduce the friction between stent and implantation site. This stent released the required dosages of the drugs over an extended amount of time (Byun, 2004). Another example where polymeric multi-layering technique has been used is for producing biological constructs. Different biological factors including nutrients and cells have been placed within polymeric multilayer structures for building tissues (Benjamin J. Lawrence, 2009).

An experiment is designed for high school students to introduce concepts of including biomaterials within multiple polymer layers. To ensure that the experiment is inexpensive to replicate basic materials that could be purchased at any grocery store are used. The following materials and their analogies to real-world multilayered biomedia controlled release structures are presented. Candle wax is used to emulate biodegradable polymers such as poly (lactic-co-glycolic acid) (PLGA). Baking soda and food coloring are used to mimic biomedia or drugs (e.g. cellular nutrients or Tylenol). Similarly, vinegar is used to imitate enzymes that are responsible for decomposing biodegradable polymers. A hot plate is also used to simulate the human body's heat which in tandem with the vinegar assists in dissolving the polymers.

Safety Precautions and Procedures:

1. **General Safety**

- Always wear safety glasses when performing any lab procedure contained in this document.

- Lab procedures should be performed under adult supervision.
- Read all safety precautions before performing any procedure.

2. Beaker

- In this experiment beakers should not be heated to more than 75°C; however, as a safety precaution ensure that all beakers are fire resistant glassware (e.g. borosilicate glassware).
- Before using beakers check them for cracks or chips. If cracks or chips are present discard the beaker.
- Never place an empty beaker directly on a hot plate. First place material to be heated into break (i.e. wax or vinegar) then place beaker on the hot plate.
- Use heat resistance gloves (e.g. silicone gloves) when handling heated beaker.

3. Vinegar

- Use white vinegar that contains 5% or less of acetic acid. Vinegars of 11% acetic acid and greater may cause burns.
- Avoid direct inhalation of vinegar. Inhalation of vapors can cause irritation to respiratory tract. If vapors are extensively inhaled remove individual to fresh air.
- Vinegar should not be placed on skin or in eyes. If vinegar gets on eyes or skin flush thoroughly with water. If irritation persists after flushing contact a physician.
- Do not ingest vinegar. If swallowed, water should be consumed to dilute. Do not induce vomiting. Contact a physician.

4. **Hot Plate**

- Hot plates should be turned off after usage. It can be hard to recognize if hot plates are off or on so, others should be notified once the hot plate is turned off. Doing this will inform others that the hot plate may still be hot.
- Do not store volatile or flammable materials in the vicinity of a hot plate.
- Do not use metal containers on the hot plate since they pose a risk of electric shock.
- Check for corrosion of thermostats, which can create a spark hazard.
- Place hot plate's thermostat on 65°C. If the hot plate is not equipped with a digital thermostat set temperature between low and medium.
- It is important that the surface of the hot plate must be larger than the actual object that is being heated up.
- When removing the heated beaker from the hot plate, use heat resistant gloves and tongs to keep hands from being burned. Also use protective equipment when pouring liquids.

5. Baking soda

- Inhalation of baking soda can cause irritation to respiratory tract. Avoid direct inhalation of baking soda. If baking soda is directly inhaled remove individual to fresh air.
- Baking soda should not be placed in eyes. If baking soda gets on eyes flush thoroughly with water. If irritation persists after flushing contact a physician.

Module Procedure:

This experiment demonstrates the release kinetics for multilayers of wax and food color which is analogous to multilayered polymer encapsulation release.

1. Obtain six 200 mL beakers (heat resistant glass) and label them in the following manner: label the 1st beaker as "Baking Soda," label the 2nd beaker as "Wax/Baking Soda," label the 3rd beaker as "Wax," label the 4th beaker as "Slide 1," label the 5th beaker as "Slide 2," label the 6th beaker as "Slide 3."
2. Obtain approximately 44g of wax. Place 22g of wax in beaker labeled "Wax" and 22g of wax in the beaker labeled "Wax/Baking Soda". **Safety Precaution: Ensure beakers are composed of heat resistant glass**
3. Pour 120mL of vinegar in all of the following beakers: "Slide 1" beaker," "Slide 2" beaker, and "Slide 3" beaker.
4. Place all beakers on a hot plate for 7 minutes with plate setting at 73°C.
5. After melting the wax, decrease the temperature to 65°C (wait 2 min. to allow temp to drop) and place 2.93g of baking soda in "Wax/Baking Soda" beaker. Note: do not remove beakers from hot plate.
6. Obtain glass slide 1. Dip Slide in "Wax" beaker 4 times. Note: Allow 10 seconds for wax to dry between each dip (Figure 2). **Safety Precaution: When dipping slide in wax use heat resistance gloves.**
7. After wax has dried, obtain microscope slide and on waxed end place one drop of blue dye (food coloring) in the center of the wax attachment (Figure 3a).
8. Place approximately 0.1g of baking soda around food color. Mix baking soda and food color (Figure 3b).
9. Quickly dip slide in "Wax/Baking Soda" beaker 1 time. Allow 20 seconds for wax to dry.
10. After wax has dried approximately 15 seconds, place one drop of red dye (food coloring) in the center of the wax attachment (i.e. directly on top of previous blue dye location). Allow 20 seconds for red dye to dry (Figure 4).
11. Repeat steps 5-9 for slides 2 & 3; however, for step 8 quickly dip slide in the "Wax/ Baking Soda" beaker for 2 and 4 times respectively (refer to data collection section). This ensures that 2 and 4 layers are deposited on slides 2 & 3 based on the number times these slides are dipped in the “Wax/Baking Soda” beaker.
12. Completed slides should resemble Figure 5.
13. Place one slide in each of the 3 beakers in a vertical position with the colored waxed end closest to the hot plate (Figure 6).
14. Release should start to occur within 8 minutes for slide 1.
15. Observe dye release and record for slides 1, 2 and 3 in data collection section.

Figure 1: Household materials for demonstrating multilayer encapsulation of biomedia

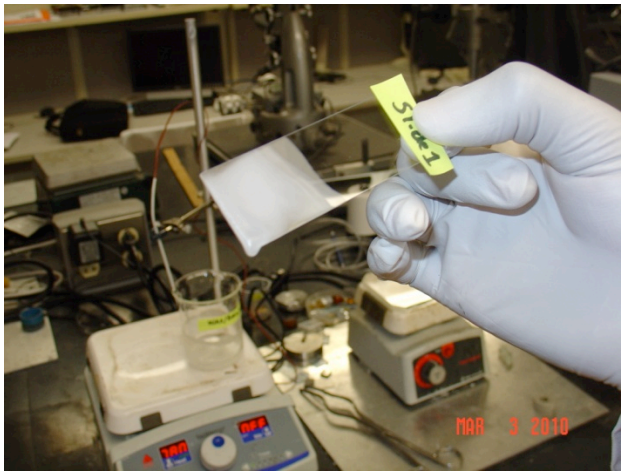
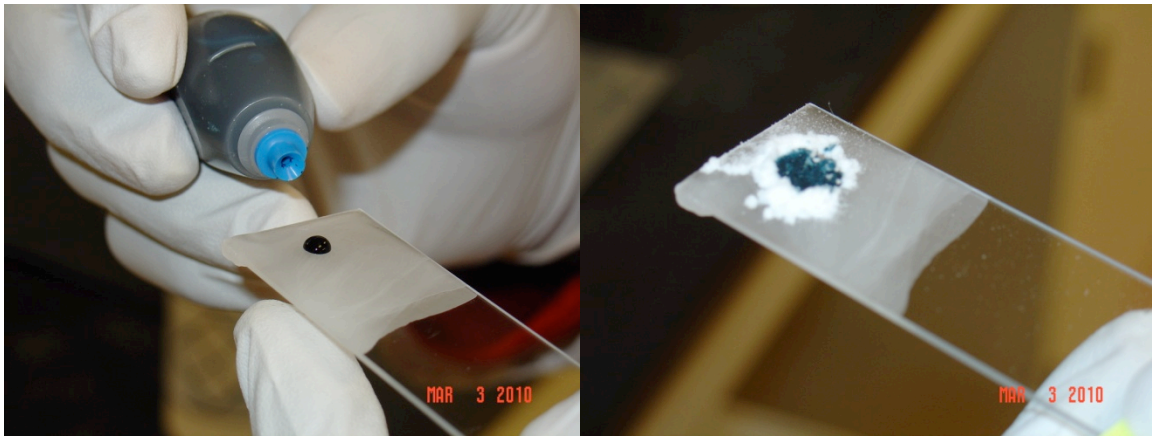


Figure 2: Glass slide (1) dipped 4 times in a wax beaker



(a) **(b)**

Figure 3: (a) Place one drop of blue dye (food color) in the center of the wax attachment, (b) Mix baking soda and food color.

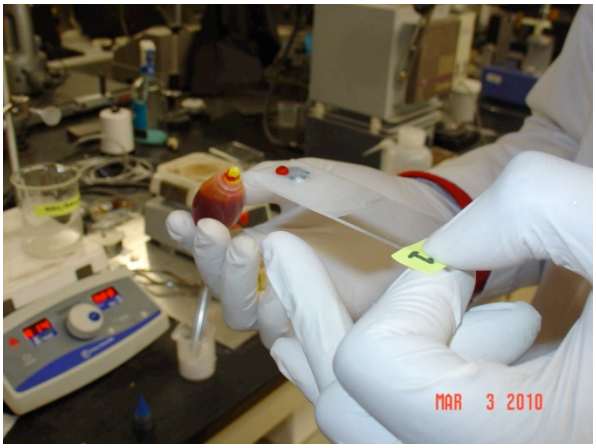


Figure 4: Place one drop of red dye (food color) in the center of the wax attachment, directly on top of previous blue dye location.

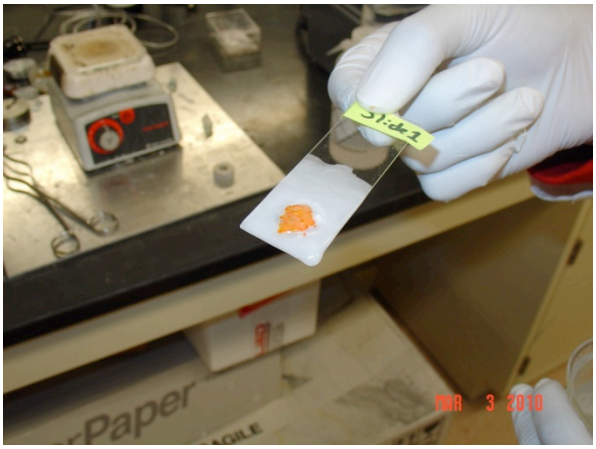


Figure 5: Slide with multilayer controlled release coating

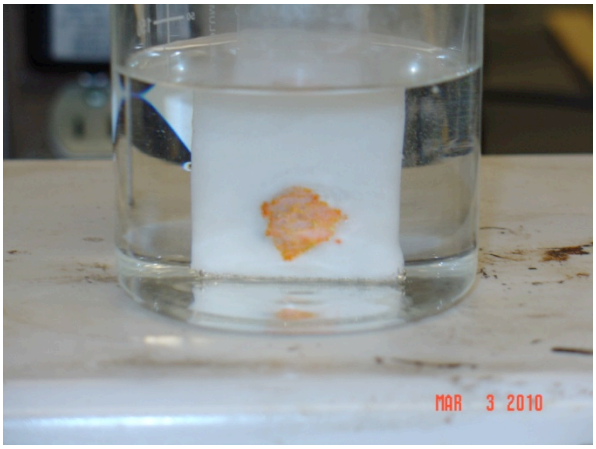


Figure 6: Slide with multilayer colored coating in vinegar beaker (Prior to release)

Data Collection:

Students should observe and record the different amount of release times with respect to the number of wax layers. Students will find that as wax layer numbers increase the time for initial release of dye also increases. Also, time intervals for release of different colors should be recorded. Students can also alter the number of drops of the food dye to obtain different color intensities. Further, when both the dyes are released and mix together at the end of the experiment different gradients of color can be observed. An experimental design table as show in Table 1 can be developed based on the variables of interest.

Table 1. Illustrative experimental design table for controlled release coatings

Comments:

Students are encouraged to further investigate multilayered controlled release characteristic by adjusting experimental variables such as hot plate temperature, baking soda amount, number of layers, and dye amount.

The following input variables can be manipulated for the experiments:

1. Hot plate temperature, catalyzes the release kinetics
2. Baking soda amount, catalyzes the release kinetics
3. Number of wax layers, influences time to release
4. Dye amount, influences color intensity

Output Measures:

1. Time to release
2. Color intensities
3. Gradients of colors after mixing of two or more colors

Acknowledgements:

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References:

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2. Byun, Youngro; and Yoon, Jung Han, (2004) US Patent: 06702850 Cl. 623-1.44., "Mediplex Corporation Korea Multi-coated drug-eluting stent for antithrombosis and antirestenosis."

Biographies

Ardarion Richardson received his Master of Science and Bachelor of Science in Industrial & Systems Engineering from North Carolina Agricultural and Technical State University. His research focuses on controlled release mechanisms for regenerative tissue engineering (Biomanufacturing). Currently, he is working at Cummins Inc. in the Manufacturing Development Program (MDP) at Columbus, Indiana.

Salil Desai is an Associate Professor of Industrial & Systems Engineering and Joint Faculty of Bioengineering at North Carolina A&T State University. Dr. Desai's expertise is in the area of micro/nano and bio fabrication, multiscale & multiphysics modeling, drug delivery and tissue engineering. His other research interests include Product Design, Manufacturing Systems and Statistical Optimization.