Name_____

Micelles and Emulsifiers for Drug Delivery Lab

Pre-Lab Assignment

- This pre-lab assignment is worth 5 points.
- This part of the pre-lab assignment is due *at the beginning* of the lab period, and must be done individually *before you come to lab!*

I. Background Preparation

- **Read this experiment thoughtfully**

Mentally note any procedural questions and plan how you and your partner will complete all experiments efficiently during the three-hour lab period.

II. Safety Hazards/Precautions

1. Complete the following table. Refer to the Safety Data Sheets (SDS) provided by your instructor. You can also search for a SDS by typing in the chemical name into the search box on the Sigma-Aldrich website: http://www.sigmaaldrich.com/united-states.html. After selecting the correct material, click on the SDS link to view.

Materials	GHS Pictograms (Circle all that apply)	Hazard Statements (Check and list all that apply)
1-(2-Pyridylazo)-2-naphthol		☐ Corrosive ☐ Toxic _____ ☐ Flammable ☐ Reactive _____ ☐ Irritant ☐ Other? _____
acetone		☐ Corrosive ☐ Toxic _____ ☐ Flammable ☐ Reactive _____ ☐ Irritant ☐ Other? _____

sodium dodecylbenzene sulfonate		☐ Corrosive ☐ Toxic _____ ☐ Flammable ☐ Reactive _____ ☐ Irritant ☐ Other? _____
Waste Disposal	Identify (briefly) how you will dispose of waste materials from this experiment.	

2. **Workplace/Personal Cleanup Notes** (indicate what you will do to clean up yourself and your lab space before you leave the lab):

III. Pre-Lab Questions

1. What is a micelle? What are some unique properties they have?

2. What are some natural-occurring micelles?

3. What is the difference between a micelle and an emulsifier?

4. What happens when you are treated with a drug? (Ingesting Tylenol per os, Flu vaccination subcutaneous, Nasal spray, Nitroglycerin sublingual for Angina) When would using micelles be a better approach for drug delivery?

5. Why is it important to deliver drugs to specific areas of the body?

6. What are some challenges to site-specific drug delivery? Why can crossing the cell membrane be a challenge for drugs?

7. What could be some drawbacks of introducing drug delivery vehicles treated with nanotechnology?

Introduction

Nanomedicine is the application of nanoparticles in medicine.[1] Nanoparticles are substances with particle size in the range of 1-100 nm in at least one dimension. A nanometer is one billionth of a meter (10^{-9} m). Nanoparticles of a substance have an increased surface area that makes them behave differently from the macroscopic scale. Nanomedicine draws inspiration from nano-sized vesicles, lipids, proteins and other biomolecules naturally occurring in our body. Nanoparticles have found use as carriers for drugs owing to their tunable chemical reactivity and ability to be constructed into spheres, cages, rods, wires, etc. As of 2008, 24 nanoparticle-based therapeutic products have been approved for clinical use, with total sales exceeding $5.4 billion. Some examples of nanomicelle-based therapeutics include Liposomal doxorubicin (for treating metastatic breast cancer), Albumin-bound paclitaxel (for metastatic breast cancer), Liposomal morphine (for postsurgical analgesia).[2]

A polymeric nanomicelle is a type of nano-sized organic compound that is composed of surfactant molecules that contain a hydrophilic shell and a hydrophobic core component. These types of molecules that contain both a hydrophobic and hydrophilic components is said to be amphiphilic. Polymer micelles, with its core-shell structure is able to encapsulate or envelop a fat-soluble (hydrophobic) drug in its core. On reaching a target organ the micelle undergoes bio-degradation to deliver the drug to the target organ. The polymeric nanomicelles have advantages such as high stability in dilute solutions and low critical micelle concentration (CMC).[1, 3, 4]

Vitamins and fats are also hydrophobic compounds required for normal metabolism. Vitamins are not synthesized in our body and they need to be acquired from our diet. While Vitamin C and many of the B Vitamins are water soluble, Vitamins D, E, A, and K are fat-soluble. This is a great example of how different chemicals (vitamins) will migrate and be stored in different parts of the body depending on their solubility profile. Micelles allow for delivery of water insoluble chemicals like Vitamins D, E, A, and K to locations they would otherwise be unable to reach.

To model the action of nano-micelles, we will be use milk (fats and fat-soluble vitamins) with food coloring. We will drop dish soap (natural micelles) on to the milk and observe what happens to the food coloring.[5, 6]

In the second part of the experiment, we will use Oil-Red O, red dye, as a model for a hydrophobic drug a surfactant such as dodecylbenzenesulfonate (SDS) as a model for the nanomicelle. We will observe how the surfactant SDS is able to self assemble into a micelle and “encapsulate” or “trap” the Oil-Red O dye inside it. We will use UV-Vis spectrometer to measure the absorbance of the solution of the SDS and Oil-Red O.[7]

Materials

Magic Milk Activity

- Petri dish or similar container
- Whole milk or Half-and-Half; high fat content is desired
- Liquid food coloring (red, blue, yellow, green)
- Liquid dish detergent
- Cotton swabs or toothpicks

Critical Micelle Concentration (CMC) Lab

- Stock Solutions for many batches:
    1. 0.070 M sodium dodecylbenzenesulfonate (SDS), Aldrich 289957. Dissolve 2.44 g in 100 mL.
    2. 1-(2-pyridylazo)-2-naphthol, Aldrich 101036. Dissolve about 0.25 g in 10 mL acetone. If the acetone evaporates, replace with more acetone. If you cannot find this solute as listed, use an alternative non-polar dye (like Oil Red O) that will dissolve in acetone.
- Pasteur pipet (with ~25 drops/mL)
- Filter paper
- Magnetic stirrer
- Cuvettes and visible absorbance spectrophotometer

Experimental Procedure

(i) Magic Milk Activity [1st hour]

1. Pour enough milk into the container to completely cover the bottom and allow it to settle.
2. Add 3 drop of each color of the food coloring to the milk. ***Make sure that drops are separated and not in center of dish***.
3. Make a prediction on the results sheet. What will happen when the tip of a clean cotton swab touches the center of the milk?
4. Observe dish contents after you have touched the clean cotton swab to the center of the milk. **Record observations on the results sheet.**
5. Grab another cotton swab and place liquid detergent on the tip.
6. Make a prediction on the results sheet. What will happen when the tip of the cotton swap with liquid detergent touches the center of the milk?
7. Place the tip of the cotton swap (with its liquid detergent) into the center of the milk. Hold the tip in place for 10 seconds. Then, remove cotton swab tip.
8. After motion of food coloring has stopped, add more liquid detergent to cotton swap tip and place in center of milk again.
    - Observe. **Record observations on the results sheet.** Remove cotton swab from center of milk.
9. Repeat addition of cotton swab tip with liquid detergent to different points in the milk, but in an area other than the center.
    - Observe. **Record observations on the results sheet**. What do you think is happening?

(ii) Critical Micelle Concentration (CMC) Lab [2nd – 3rd hour]

1. **[2nd hour]** Obtain your dye Oil Red O in acetone solution, using a 50 mL beaker. For 1-(2-pyridylazo)-2-naphthol dye solution procedure, see CMC reference website.
2. Handle filter paper with forceps. Add a strip of filter paper to the dissolved dye that is sitting in acetone.
3. Let filter paper sit for approximately 1 minute or until the dye has completely absorbed into the filter paper.
4. Remove filter paper from dye and acetone solution. Place filter paper in 500 mL beaker. Add stir bar to beaker. Add 100 mL water to beaker.
5. Place beaker with contents on stir plate.
6. Remove 3 mL of solution (enough to fill cuvette in spectrophotometer) from the beaker with pipet and place in a test tube.

7. Before turning on stir plate, add 1 mL SDS solution to the beaker.
8. Turn on stir plate to speed 5 (or medium speed).
9. After 2 minutes, remove 3 mL of solution from the beaker and place in a second test tube. Add 1 mL SDS solution more into beaker.
10. Repeat step 9 by removing 3 mL of solution from the beaker and placing into another test tube. Add an additional 1 mL SDS solution into beaker.
11. After about 45 minutes (or when the color of the beaker solution has significantly changed), end test tube collection.
12. **[3rd hour]** Transfer test tubes to cuvettes and take absorbance readings on spectrophotometer. **Record in the data table on the report sheet the absorbance at the wavelength max absorption (λmax).**

References

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2. Zhang, L.; Gu, F. X.; Chan, J. M.; Wang, A. Z.; Langer, R. S.; Farokhzad, O. C. Nanoparticles in Medicine: Therapeutic Applications and Developments. *Clinical Pharmacology & Therapeutics.* **2008,** *83* (5), 761-769.
3. Furton, K. G.; Norelus, A. Determining the critical micelle concentration of aqueous surfact solutions: Using a novel colorimetric method. *Journal of Chemical Education,* **1993,** *70* (3), 254.
4. Wang, J.; Mongayt, D.; Trochilin, V. P. Polymeric Micelles for Delivery of Poorly Soluble Drugs: Preparation and Anticancer Activity In Vitro of Paclitaxel Incorporated into Mixed Micelles Based on Poly(ethylene Glycol)-Lipid Conjugate and Positively Charged Lipids. *Journal of Drug Targeting.* **2005,** *13* (1), 73-80.
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6. Matter, S.; Hurlbut, L. Magic Milk Activiy. *Accessed August 20, 2015.* http://www.nipissingu.ca/education/jeffs/4284winter/pdfs/magicmilk.pdf
7. An education video on Ultraviolet/Visible Spectrometry from the Royal Society of Chemistry. *Accessed August 20, 2015.* http://www.youtube.com/watch?v=039avevqndU]

Acknowledgements

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Name:_____

Partner's Name:_____

Micelles and Emulsifiers for Drug Delivery Lab Report Sheet

Data and Results

Magic Milk Activity

1. For Step 3: What will happen when the tip of a clean cotton swab touches the center of the milk?

2. In Step 4: What actually happened?

3. For Step 6: What will happen when the tip of the cotton swab with liquid detergent touches the center of the milk?

4. In Steps 8 and 9: What actually happened

Critical Micelle Concentration

Data Tables

Tube #	Volume of SDS added (mL)	Initial Volume of solution (mL)	Volume Removed (mL)	Final Volume (mL)	Concentration of SDS (M)	Abs @ λ_{max}
1	0	100	3	97	0	
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

Data Analysis

1. Using the concentrations of the stock of SDS given by your instructor calculate the concentration of SDS in each test tube. Assume the amount of SDS in solution is cumulative and the amount removed in each aliquot is negligible. **Hint: use M1V1 = M2V2.**

2. Plot Abs at λ_{max} versus the concentration of SDS. **Note: Either graph paper or a spreadsheet program like Excel can be used for this part**.

3. Fit a line to the data from test tubes #7 - #15. **You can use a linear regression function if the graph is done in a spread sheet (i.e. Excel).** If not, use a ruler to draw your best fit line.

4. What is the equation of the line (y= mx + b)?

5. The x-intercept of this line represents the critical micelle concentration. What is your critical micelle concentration?

6. The literature value for the critical micelle concentration of SDS is 8.14×10^{-3} M. What is your percent error?

7. Why was the data fitted only to test tubes #7 - #15?

8. Why do you think knowing the critical micelle concentration is important when using micelles for drug delivery?