Micelles

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Micelles

Abstract

This module introduces micelles and the concept of critical micelle concentration (CMC). Micelles have important applications in biotechnology. Micelles are sometimes used to deliver drugs to targeted organs or tissues in the body. Micelles are also sometimes used to deliver genes into plant cells for agricultural applications. This module explores micelles as a demonstration of the intersection between nanotechnology and biotechnology. The module includes a laboratory activity that uses a surfactant and dye to allow students to experiment with micelle formation.

Outcomes

After completing this module, students will have

- explored the phenomenon of self-assembly on the nanoscale.
- learned about the concept of CMC and its practical relevance in drug delivery.

Prerequisites

- High School biology and chemistry
- Operational knowledge of spectrophotometers

Science Concepts

- Chemistry: hydrophobic and hydrophilic materials; polar nature of the water molecule
- Biology: micelles in cell membranes and other biological systems

Nanoscience Concepts

- Self-assembly
- Role of close range forces at the nanoscale
- Applications of micelles in medicine and biotechnology

Background Information

What is a micelle?

To understand micelles, it is important to understand that some compounds are **hydrophobic,** meaning they do not "like" water, that is, they do not dissolve in water or readily interact with it. Other compounds are **hydrophilic,** meaning that they "like" water: they easily mix with water and will dissolve in it. **Surfactants** are molecules that have a hydrophilic "head" and a hydrophobic hydrocarbon chain, or "tail," as shown in Figure 1. Therefore, part of an individual surfactant molecule can readily mix with water and part is more likely to mix with oily substances. Common examples of surfactants are soaps and detergents.

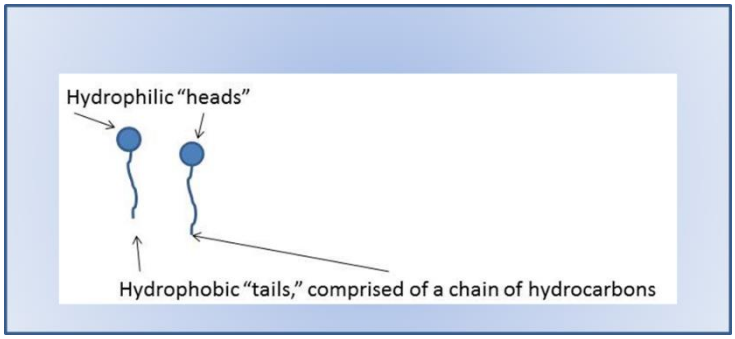
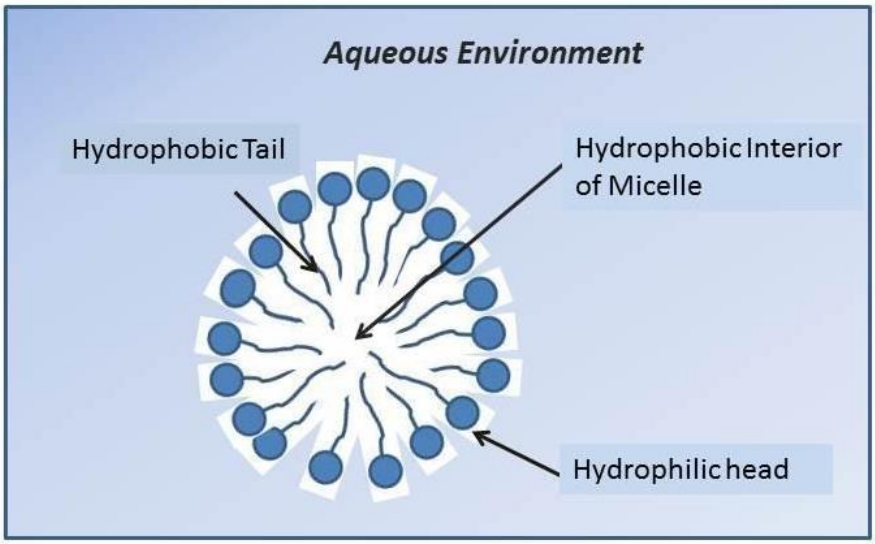


Figure 1. Structure of a surfactant molecule.

Figure 2. Structure of a micelle.

When added to water, a group of surfactant molecules can form a **micelle** by self-assembling into a ball-like structure, such that their hydrophobic tails orient into the center of the ball (away from the water) and the hydrophilic heads orient outwards into the surrounding water. This configuration is stable because the hydrophobic tails that do not "like" water are protected from it inside the sphere, as shown in Figure 2. Micelles are small, often in the 10-30 nm range, so micelle formation is an example of how molecules spontaneously associate to form a nanoparticle.



Critical micelle concentration, or CMC, is the concentration at which detergents, soaps, or other surfactants spontaneously assemble into micelles when they are placed in water. At concentrations below the CMC the surfactant molecules do not form micelles but rather exist in the water as individual molecules. There are many surfactants that you can buy and each of them has its own CMC.

Why are micelles important? The reason is because micelles can facilitate the dissolving of a compound that otherwise would be insoluble in water. A compound that is hydrophobic can be packaged inside of a micelle. The internal environment of the micelle is hydrophobic, an environment that is compatible with the hydrophobic compound. The exterior of the micelle is hydrophilic and is easily dispersed in an aqueous environment. Thus, packaging a hydrophobic compound inside a micelle allows it to be suspended in a watery medium.

Medical applications of micelles

One of the major hurdles in developing an effective drug is to ensure that it reaches its site of action in the body. For example, a drug that is intended to kill cancer cells must get to the cancer cells in order to be effective. Consider, for example, a drug that is taken orally. This route of delivery poses many problems for drug developers. First, the human digestive system, or gut, acts to break down and degrade molecules that are ingested. If the gut could not break down molecules, then food could not be digested and used for energy or as building blocks for growth and repair. The same mechanisms that digest food also break down ingested drugs. If a drug is destroyed in the gut it will not reach its site of action and it will not help a patient. Even if a drug can survive in the gut without being destroyed, it still needs to be able to cross the membranes of the cells lining the gut in order to get into the body. If a drug cannot cross cell membranes, it will pass through the gut and be excreted, again without helping the patient.

One approach to overcome these problems with an ingested drug is to surround the drug agent with a shell. The shell serves two purposes. It protects the drug from being destroyed by the enzymes in the digestive system that break down food. It also facilitates the passage of the drug into the cells that line the gut. Micelles are one type of shell or package that may be used for drug delivery. Scientists are working to create micelles that are highly effective as systems to deliver drugs to their targets in the body. They are experimenting with different types of micelle-forming compounds to find the best ones. These experiments involve various important drugs such as Medicelle, an anti-cancer agent; Flucide, a drug to treat influenza; and Basulin, a long-acting insulin (4). Genexol-PM is an anticancer drug that has been approved for the treatment of breast cancer and is delivered in micelles. Genexol-PM interferes with normal mitosis and so prevents cancer cells from dividing. The use of micelles increases the ability of this drug to mix with water, and allows delivery at higher doses than would be achievable without micelles (5).

The concept of the critical micelle concentration was mentioned earlier. Why is the CMC important? It turns out that the CMC is a critical aspect of a drug delivery system. This is because micelles containing a drug will be diluted when they enter the gut or the bloodstream. Below the CMC, the micelle-forming agent will disassemble and will fail to maintain a shell around the drug. It is therefore important that the CMC be as low as possible so that the micelles do not fall apart when they are diluted in the body. Scientists therefore carefully analyze the CMC of different micelle-forming compounds as they search for the best ones for drug delivery.

Learning Activity: Micelles

Laboratory Overview

The activity described here demonstrates the formation of micelles at a certain concentration of added surfactant. The surfactant (a detergent known as SDBS) is added to beakers of water at six different concentrations, along with a strip of paper that contains an oily (hydrophobic) dye known as PAN. Like all surfactants, SDBS is made up of molecules having hydrophilic and hydrophobic parts. If the concentration of detergent reaches a certain level (the critical micelle concentration, or CMC), it will begin forming micelles and the hydrophobic dye will begin to collect inside them. These dye-containing micelles form little colored bodies that are visible to the eye and may be counted by a lab spectrophotometer. The first beaker is a negative control to which no detergent is added. This negative control is necessary to be sure that the dye absorbed onto the strip of filter paper does not spontaneously dissolve in water. The other five beakers have progressively higher concentrations of detergent.

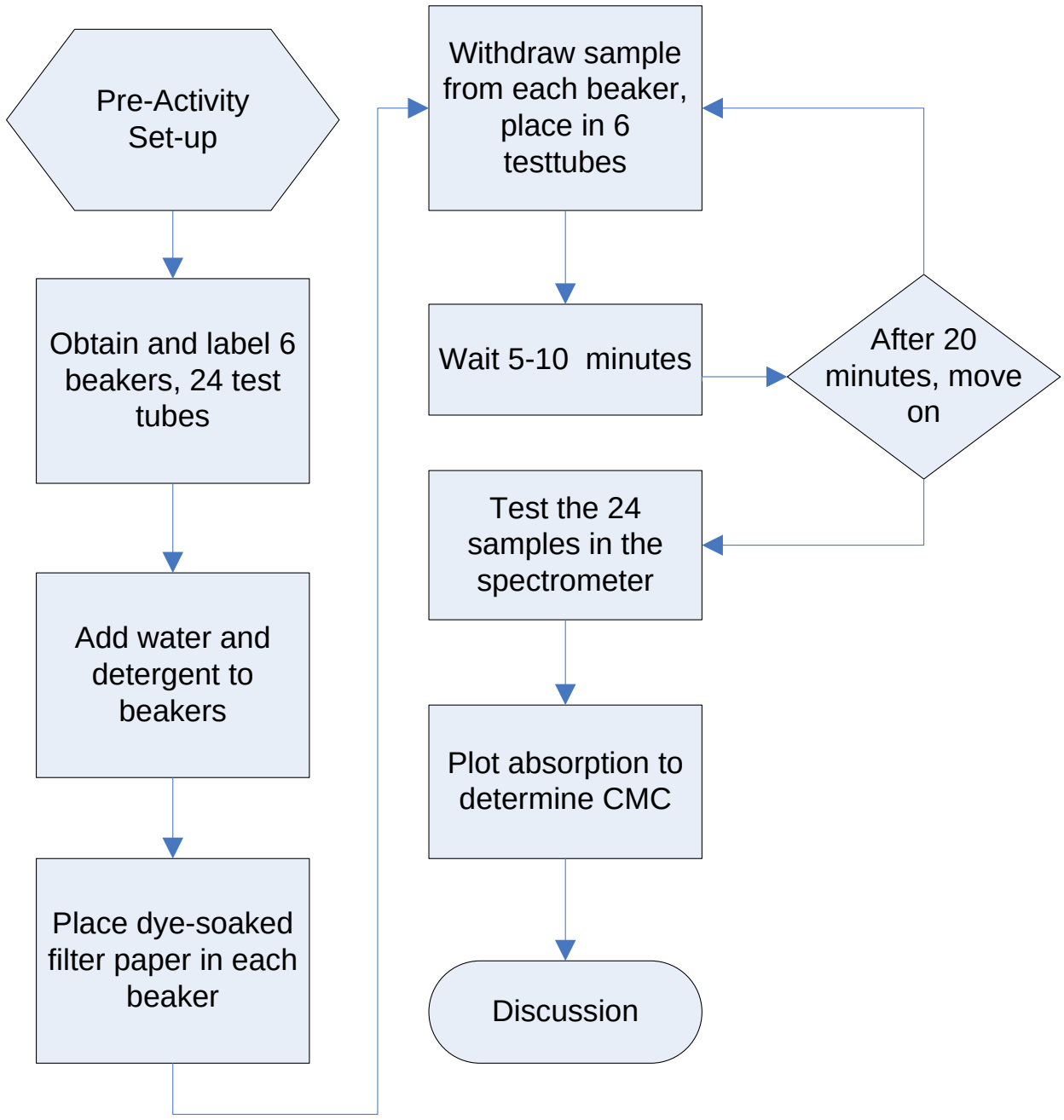
The dye in our experiment will represent a drug we might wish to encapsulate within micelles formed by SDBS detergent molecules. Once the simulated drug is encapsulated within the micelles a yellow color should begin to appear in the water, this is because the dye is now emulsified (i.e. suspended) within the dye-containing micelles. The detergent molecules, much like the molecules that make up cellular membranes, are amphipathic (i.e. containing both hydrophilic and hydrophobic parts) and will form the micelles as seen in Figure 1.

A section of filter paper impregnated with dye is placed in each of the beakers. In beakers where the concentration of detergent is below the CMC, students may see a bit of color from the dye entering the beaker, but not much. In beakers with concentrations of detergent above the CMC a yellow color should be seen; the color change is due, in part, to the concentration of suspended micelles which have encapsulated the dye. Our eyes can detect the color but are not very good at quantifying how much color is present in each beaker. The amount of color in each beaker is best determined using a spectrophotometer.

Spectrophotometers work by passing light of a certain wavelength, selected by the user, through a small sample and measuring the degree to which that light is absorbed. If the appropriate wavelength of light is selected, this absorption is a very sensitive measure of quantity of dye present in a sample vial. As the surfactant level increases and reaches the CMC, much more of the hydrophobic dye will be suspended by the micelles, resulting in sharp increase in light absorption recorded by the spectrophotometer.

One of the factors that influence the results in this system is how long students wait after adding the detergent and filters to the beakers. It takes some time for the color to develop. We suggest 0 minutes, 5 minutes, 10 minutes, and 20 minutes. Students remove a small sample of water from each of their six beakers at each of the four time points. These samples are for testing later with a spectrophotometer. After testing each sample with a spectrophotometer, students graph and analyze their data.

Activity Flow Chart



Activity Set-up (prior to doing the activity).

At least one day before the experiment, cut filter paper into uniform strips, approximately 1.5 x 0.5 inches. The exact size is not as important as making them as uniform as possible. Eight filter strips will be needed for each experiment.

1. Prepare the dye solution, 1-(2-pyridylazo)-2-naphthol (i.e. PAN), Aldrich 101036. Dissolve about 0.25 g in 10 mL acetone. If the acetone evaporates, replace with more acetone. Better results may be obtained by filtering the resulting dye solution to avoid particles. This quantity will be enough for 20 students.
2. Saturate the filter paper strips with dye by placing them in the dye solution. Remove the strips and allow the strips to completely dry. (Overnight is best.)
3. Prepare the stock SDBS detergent solution by dissolving 2.44 g of SDBS in 100 mL purified water. This quantity will be enough for 20 students.

Instructor Notes

1. **Other systems**. We tried adapting the laboratory activity by using methyl red and turmeric instead of 1-(2-pyridylazo)-2-naphthol and sodium dodecyl sulfate instead of sodium dodecylbenzenesulfonate. Unfortunately, these substitutions did not work in our hands. Possibly, enterprising students will be able to find an inexpensive dye/detergent combination that works.

2. **Controls.** Students should be prepared for some ambiguity in the results of the lab activity. Point out that even in the negative control, some color may appear and concentrations of detergent below the CMC will promote some dissolution of the dye. Point out the importance of controls in all experiments. The other concentrations can be done as a variation to this experiment. All results shown include the various concentrations.

3. **Spectrophotometer.** It is possible to do the activity without a spectrophotometer, relying on visual observations of color. However, this activity works much better when a spectrophotometer is available.

4. **Further experimentation.** The activity as provided here is intended as a starting point for experimentation. This system is easily modified to allow students to experiment with various factors that influence the CMC. Students can also try to more precisely determine the CMC than is done in this initial activity. Thus, students might want to:

- Vary the amount of detergent added to each beaker to more precisely identify the CMC.
- Use different surfactants, such as common household detergent and sodium dodecyl sulfate.
- Raise or lower the temperature at which the experiment is performed.
- Add electrolytes (e.g.; sodium phosphates and sodium carbonate)

Note that commercial laundry detergents often include electrolytes to decrease the CMC and improve the cleaning action of their product (1). It may work to add 0.01 M sodium carbonate solution to the beaker (1).

Note that theoretically, for ionic detergents, such as we use here, the CMC should be reduced by increasing the ionic strength but should be relatively unaffected by temperature. In contrast, when using non-ionic detergents, the CMC should be relatively unaffected by ionic strength but should increase significantly with higher temperature.

Sodium dodecyl sulfate (SDS) is a detergent commonly found in biology labs. Do not confuse this detergent with the one that is used in this laboratory, sodium dodecyl benzene sulfonate (SDBS). The CMC of sodium dodecyl sulfate, when expressed as a weight/volume percent, is reported to be 0.23. When the CMC of sodium dodecyl sulfate is expressed in terms of millimolarity, it is reported to be 7-10 mM. The molecular weight of sodium dodecyl sulfate is 288.5 (2). According to one reference (6) sodium dodecyl sulfate will not form micelles at temperatures below 25 degrees if no other compounds are added.

5. **Calculating the concentration of detergent in each beaker in units of molarity.**

The stock solution contains **2.44 g SDBS/100 mL** water, the same concentration as **24.4 g/L**

The molecular weight of this detergent is **348.48** so we know that, by definition, 1 M SDBS = **348.48/1 L.** Setting up a proportion, we can calculate the molarity of our stock. **In words: if one molar SDBS contains 348.48 grams in a liter, then what molarity is 24.4 g in one liter?**

$$\frac{348.48\ \text{g}}{1\ \text{molar}} = \frac{24.4\ \text{g}}{?}$$

$$? \approx 0.07\ \text{molar}$$

Now that if we know the concentration in the stock solution we can use the $\mathbf{C_1V_1 = C_2V_2}$ equation to calculate the concentration in each beaker. In this equation:

$\mathbf{C_1}$ *is the concentration in the stock solution (0.07 M)*

$\mathbf{V_1}$ *is the amount of the stock in the beaker, which varies in each beaker*

$\mathbf{C_2}$ *is what we want to know, the concentration of detergent in each beaker*

$\mathbf{V_2}$ *is the total amount of volume in the beaker; in the protocol as given, it is 25 mL*

For example, in a beaker to which 0.25 mL of detergent stock solution has been added, the concentration of detergent in molarity is calculated thus:

$$\mathbf{C_1\ V_1 = C_2\ V_2}$$

$$\mathbf{(0.07\ M)(0.25\ mL) = (?)\ (25\ mL)}$$

$$\mathbf{? = 0.0007\ M}$$

Substitute into the $\mathbf{C_1V_1 = C_2V_2}$ equation to calculate the concentrations in each of the beakers. For the protocol as written:

Amount Detergent Stock	Concentration in molarity
0 mL SDBS	0
0.25 mL SDBS	0.0007
0.75 mL SDBS	0.0021
0.85 mL SDBS	0.0024
1.0 mL SDBS	0.0028
1.5 mL SDBS	0.0042

8. **Sample data from one of our experiments**. Figure 3 shows absorbance versus concentration for ten concentrations of detergent. Eight of these concentrations are below the CMC. These results were recorded 60 minutes after adding filters to the beakers, but 20 minutes should be sufficient to see the CMC, as shown in Figure 4.

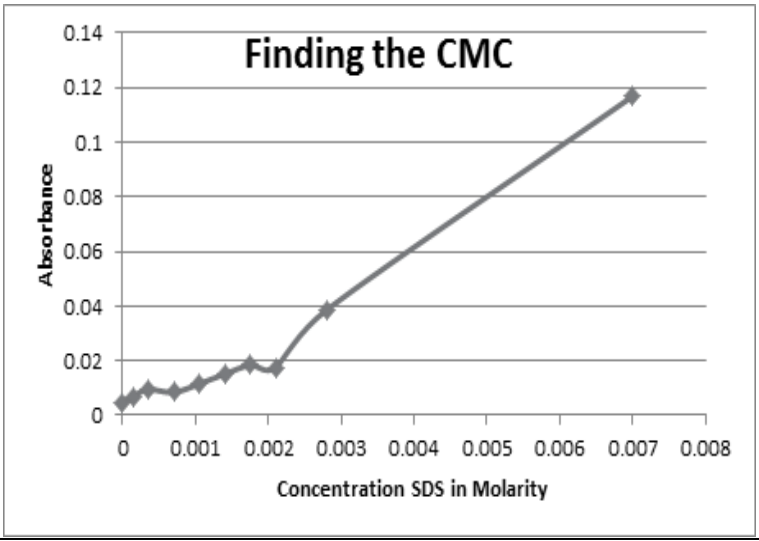


Figure 3. Finding the CMC. The CMC is the concentration at which the absorbance abruptly increases. At concentrations above the CMC the absorbance is expected to increase linearly with increasing detergent concentration. Based on this graph, the CMC occurs is slightly over 0.002 M.

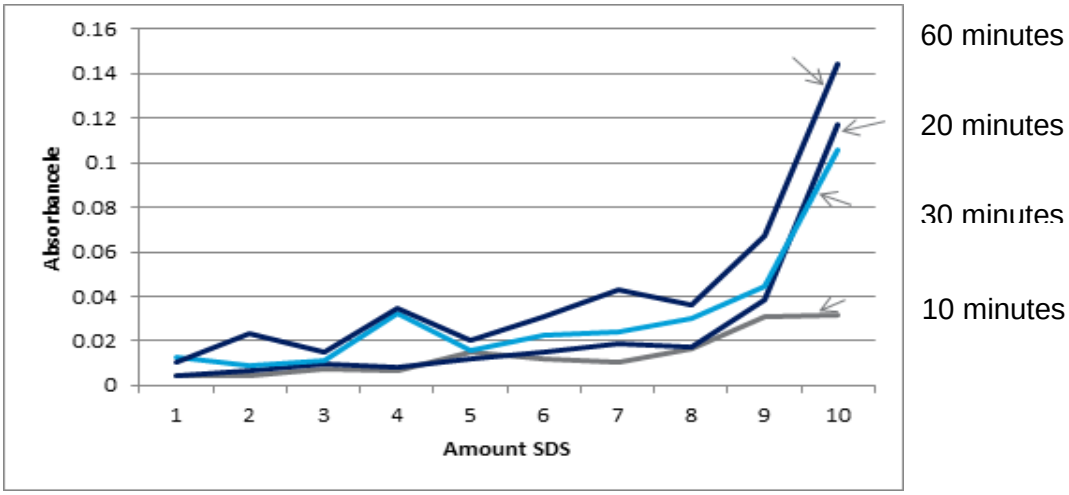


Figure 4. Effect of time elapsed on color formation. The four lines on this graph represent four different time points after putting the filter into the beaker with water and SDBS. The purpose of this experiment was to determine how long it is necessary to wait before taking spectrophotometer readings. The X axis is sample number; increasing sample numbers had increasing amounts of SDBS added. The graph indicates that 10 minutes is probably too little time to see the color pattern develop in the samples; there is no sharp inflection in the line. By 20 minutes there is a clear inflection point at sample 9. Therefore, twenty minutes is a sufficient wait time; 15 minutes might work just as well but was not tested.

Micelles

Materials and Equipment

- Dye solution: 1-(2-pyridylazo)-2-naphthol (i.e. PAN).
- Surfactant solution: sodium dodecylbenzenesulfonate (SDBS)
- Distilled or deionized water
- Strips of filter paper, cut into strips
- Two beakers 50 ml
- Eight test tubes
- Test tube rack
- Disposable plastic pipettes
- Spectrophotometer (optional)

Procedure

1. Obtain two 50 mL beakers. Label them with tape showing the concentration of surfactant to be added, for example:

- 0 mL SDBS - control
- 1.0 mL SDBS

2. Pour 25 mL of water into each of the beakers.
3. Obtain 8 small test tubes and place them in a test tube rack. Label them with the SDBS concentration (0 or 1.0 ml) and time point (1 through 4)—see the diagram in Table 1 as an example. (There are two concentrations of surfactant and four time points, so you need eight test tubes.)
4. Add the required volume of stock SDBS solution to each beaker, in the amount specified by the label on the beaker (#1 above).

TABLE 1: SAMPLES

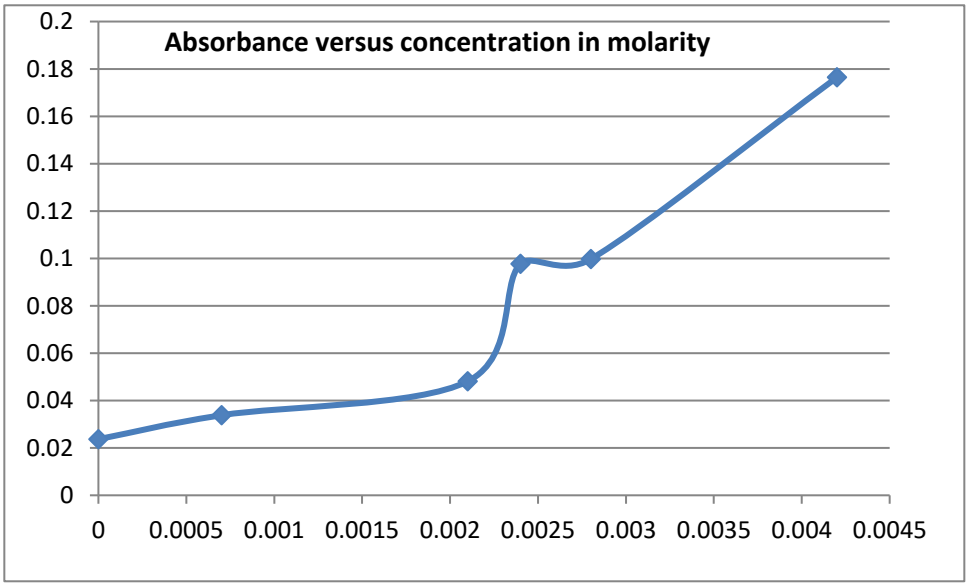
Concentration of SDBS	**Time** **Point 1** (0 min)	**Time** **Point 2** (5 min)	**Time** **Point 3** (10 min)	**Time** **Point 4** (20 min)
0 mL SDBS				
1.0 mL SDBS				

5. Place a filter paper in each beaker and record the time.
6. Swirl each beaker.
7. Using a pipette, remove about 1 mL from each of the beakers and place it into the corresponding test tube; this is time = 0 min. You now have a sample in 2 test tubes.
8. Observe and record the color of the water in each beaker at time = 0 min.
9. Wait until 5 minutes have elapsed since placing the filters into the beakers. Swirl each beaker. Using a pipette, remove about 1 mL from each of the beakers and place it into the corresponding test tube; this is time = 5 min. You now have sample in 4 test tubes.
10. Observe and record the color of the water in each beaker at time = 5 min.
11. Wait until 10 minutes have elapsed since placing the filters into the beakers. Swirl each beaker. Using a pipette, remove about 1 mL from each of the six beakers and place it into the corresponding test tube; this is time = 10 min. You now have sample in 6 test tubes.
12. Observe and record the color of the water in each beaker at time = 10 min.
13. Wait until 20 minutes have elapsed since placing the filters into the beakers. Swirl each beaker. Using a pipette, remove about 1 mL from each of the six beakers and place it into the corresponding test tube; this is time = 20 min. You now have sample in all 8 test tubes.
14. Observe and record the color of the water in each beaker at time = 20 min.

15. Using a spectrophotometer if available, measure the absorbance at 470 nm of all 8 samples. Use distilled or deionized water as the blank. Record the values in a table, similar to Table 1.
16. There are numerous ways to graph your data and your instructor may suggest a particular method. One way to graph all the data is to place absorbance on the Y-axis and concentration on the X-axis. You can then plot four lines on the same graph: a line for time point 1, a line for time point 2, a line for time point 3, and a line for time point 4. Based on the results you can select one time point that seems best for analyzing the CMC.
17. If a spectrophotometer is not available, estimate the CMC by eye. To do this, carefully observe the color in each beaker. This will be easiest to do at the longest time point; e.g., 20-60 minutes. Look for a point in which the color in the beakers becomes abruptly more intense. The CMC is between the concentration of detergent in the less colored beaker and the more colored beaker.

Discussion Questions

1. People sometimes say, “Like dissolves like.” How does this saying relate to the information in this module?
2. Draw a micelle as you understand it.
3. Explain in your own words how a micelle can affect the interaction of a hydrophobic compound (like oil) with water.
4. If you swallow a therapeutic drug that is hydrophobic and is not in any way protectively packaged (for example by a capsule or micelle), what might happen to the drug in your gut? Is this drug likely to be effective?
5. What does CMC mean?
6. The graph below was obtained in a CMC experiment. What do you think is the approximate CMC based on this graph?



Answers to questions:

1. People sometimes say, “like dissolves like.” How does this saying relate to the information in this module?

This phrase usually refers to that fact that water dissolves aqueous-based substances and oil-based substances dissolve in oil. You may have observed this with paints. Oil paints cannot be cleaned up with water because they do not dissolve in it or mix with it, but water-based paints are easily cleaned with water.

2. Draw a micelle as you understand it.

Figure 2 on page 3 represents a micelle, but other representations are possible. The important points are that the micelle is roughly a sphere with a water-loving shell surrounding a hydrophobic interior.

3. Explain in your own words how a micelle can affect the interaction of a hydrophobic compound with water.

The hydrophobic compound normally will not dissolve in or interact with water. When surrounded by a micelle, however, the hydrophobic compound is protected from water. The surface of the micelle is hydrophilic, and the micelle will be stably suspended in the water, thus causing the hydrophobic compound to exist in the water environment.

4. If you swallow a therapeutic drug that is hydrophobic and is not in any way protectively packaged (for example by a capsule or micelle), what might happen to the drug in your gut? Is this drug likely to be effective?

A hydrophobic drug may be degraded by gut enzymes, depending on its particular chemistry. If the drug is not degraded it is likely to pass through the gut and be excreted without entering the body and exerting any therapeutic effect.

5. What is the “CMC” for a detergent?

It is the minimum concentration at which the detergent when present in water will spontaneously form micelles.

6. This graph was obtained in a CMC experiment. What do you think is the approximate CMC based on this graph?

The CMC is roughly 0.0022 M. (It is conventional to read all the digits of which we are sure and one more that is estimated.)Observe that the graph is not “perfect,” there is a slight plateau between the fourth and fifth points. This is not surprising because our experimental system introduces some inaccuracies. For example, different filter papers may absorb different amounts of dye. Nonetheless, the there is an abrupt change in absorbance that likley represents the CMC.

Contributors

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- Kyle Forgette – Biology and Nanobiotechnology Instructor, Dakota County Technical College
- Dr. James Marti – Senior Scientist – University of Minnesota Nano Center

Multimedia Resources

Articles

1. Determining the Critical Micelle Concentration of Aqueous Surfactant Solutions Using a Novel Colorimetric Method. Kenneth G. Furton and Arnold Norelus. Journal of Chemical Education. *J. Chem. Educ.*, 1993, 70 (3), p 254.
2. For more in-depth information about micelles and drug delivery:
3. *Polymeric Micelles in Anticancer Therapy: Targeting, Imaging, and Triggered Release*. Chris Oerlemans, Wouter Bult, Mariska Bos, Gert Storm, J. Frank W. Nissen, and Wim E. Hennink. *Pharm Res* 2010 December; 27(12):2569-2589.
4. ***Polymeric Micelles for Oral Drug Delivery: Why and How.*** Mira F. Francis, Mariana Cristea, and Françoise M. Winnik. *Pure Appl. Chem.* 2004*;* 76 (7–8): 1321–1335.

Video

How Soap and Detergents Work. www.youtube.com/watch?v=kpRbnLZX_dI

Web Sites

1. This activity was adapted from an activity by Jonathan Breitzer, Ming-Fong Lye, and George Lisensky for MRSEC Education at the University of Wisconsin-Madison. http://education.mrsec.wisc.edu/279.htm.
2. http://openwetware.org/wiki/Critical_micelle_concentration_%28CMC%29. This website reports the CMC for several commonly available detergents.
3. http://www.cancer.gov/drugdictionary?CdrID=434427. This is the website of the National Cancer Institute featuring a micelle-based cancer drug.
4. http://www.wisegeek.com/what-is-critical-micelle-concentration.htm. This is a brief explanation of CMC.