Supporting Information

Using Soapnut Extract as a Natural Surfactant in Green Chemistry Education: A Laboratory Experiment Aligning with UN SDG 12 for General Chemistry Courses

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Expt.1 Extraction of Saponin

Place the soapnut powder (0.1 g) in 20 mL of distilled water in a beaker. Gently heat the mixture to a temperature between 60°C to 70°C while stirring. This moderate heat helps in extracting the active compounds from the soapnut powder into the water. After heating for 5 minutes, remove the mixture from heat. Allow the solution to cool down to room temperature. Filter the mixture to remove any remaining particulate matter or impurities. The filtrate obtained after the filtration is now the extracted solution of soapnut in distilled water.

Expt. 2.1 Foam Test

Dilute 5ml of the soapnut extract with water (5 mL) and shake vigorously for 2 minutes. Record the observation. Measure and record the height of the foam.

Prepare the soapnut extract as described in Expt.1. Take 5 mL of the soapnut extract in a clean test tube. Add 5 mL of water. Cap the test tube securely and shake it vigorously for 2 minutes. After shaking for 2 minutes, carefully observe the appearance of the solution. Note the formation of foam and its characteristics such as stability, and texture. Using a ruler measure the height of the foam column formed above the liquid surface. Record the height of the foam.

Expt. 2.2 Surface Tension Demonstration

Get a clean beaker and fill it with water. Take a piece of tissue paper that is slightly larger than the paperclip and place it on the surface of the water in the beaker. Carefully put the paperclip on top of the tissue paper. After a short while, the tissue paper will sink, and the paperclip will remain floating on the surface of the water. Add a few drops of previously prepared saponin solution to the water and observe what occurs. Take note of the experiment's results.

See the link for the video demonstration:

https://drive.google.com/drive/folders/13ROZ-LmH4rarZK8LPZxMKTw9h_JCwUga?usp=sharing

Expt. 2.3 Emulsification

Vigorously shake 2 ml of saponin extract and 2 ml oil in a test tube. Emulsion stability was determined as the time required to separate the saponin solution from the emulsion.

Take a clean test tube and add 2 mL of soapnut extract into it. Add 2 mL of oil (e.g., vegetable oil). Cap the test tube securely and shake it vigorously. The vigorous shaking is essential to thoroughly mix the saponin extract and oil and create an emulsion. After shaking, observe the test tube to confirm the formation of an emulsion. Repeat the experiment with water and oil. Observe the emulsions in both test tubes over time and note the duration it remains stable before phase separation occurs.

Expt. 3.1 Test for Triterpenoid

Mix 1 mL of saponin extract with 9 mL of water in a clean test tube. Transfer 0.33 mL of the diluted saponin solution into a separate clean test tube. Add 0.33 mL of vanillin solution (8% w/v in ethanol) to the test tube containing the diluted saponin solution. Add 3.33 mL of sulfuric acid solution (72% v/v in water) to the test tube containing the mixture of saponin extract and vanillin solution. Place the test tube in a water bath and heat to 60°C. Allow the mixture to react for 15 minutes at 60°C. Remove the test tube from the water bath and allow it to cool to room temperature. Observe and note the color of the solution. A positive test for triterpenoids is indicated by the development of a pink to red color in the solution after the addition of sulfuric acid. The intensity of the color change correlates with the concentration of triterpenoids present in the saponin extract.

Expt. 3.2 Test for Reducing Sugars

Take 1 mL soapnut extract and mix it with 1 mL of Benedict's reagent. Gently boil the mixture and observe any color changes.

In another test tube, combine 1 mL of soapnut extract with 1 mL of 10% sulfuric acid solution. Gently boil the mixture for 3-5 minutes. Neutralize the acidic solution by adding sodium hydroxide solution until it becomes alkaline. Add 1 mL of Benedict's reagent to the neutralized solution. Gently boil the mixture again and observe any color changes.

Expt. 4.1 Reverse micellar extraction of Chromium (VI)

Adjust the pH of a 2.0 mL of 1 x 10^{-4} M potassium dichromate solution to 2 using HCl. Prepare soap nut extract by heating 0.1g of crushed soapnut with 20 mL of 1-butanol at 60°-70 ^{0}C C for 5 minutes and filter the solution after cooling. Mix 2.0 mL of soap nut extract with dichromate solution, shake thoroughly, and allow the layers to separate.

Take 2.0 mL of a 1 x 10^{-4} M potassium dichromate solution. Adjust the pH to 2 using hydrochloric acid (HCl).

Heat 0.1 g of crushed soapnut with 20 mL of 1-butanol at 60-70°C for 5 minutes. Allow the solution to cool, then filter to obtain the soapnut extract.

Mix 2.0 mL of the soapnut extract in butanol with the pH-adjusted potassium dichromate solution. Shake the mixture thoroughly to ensure complete mixing. Allow the layers to separate.

Experiment 4.2 Reverse micellar extraction of Methylene Blue Dye molecules

Prepare a solution containing 2 mL of Methylene Blue dye in water at a concentration of 1 x 10^{-5} M. Add 2 mL of soapnut extract in butanol to the Methylene Blue solution. Shake the mixture thoroughly to ensure complete mixing of the two phases. Allow the mixture to settle to facilitate the separation of the layers.

Expt.4.3 Modelling micellar extraction of water Insoluble pollutants

Prepare a solution containing 2,4-dinitrophenylhydrazine in 2 mL of ethyl acetate at a concentration of 1 x 10^{-4} M. Combine 2 mL of soapnut extract in water with the prepared 2,4-dinitrophenylhydrazine solution in ethyl acetate. Shake the mixture thoroughly to ensure thorough mixing of the components. Allow the mixture to stand and observe the separation of the layers.

Expt. 5 Strawberry DNA extraction

Gently heat (60-70°C) soapnut powder (0.1 g) in distilled water (20 mL) for 5 minutes. Filter the mixture while still hot and allow it to cool down.

Place a strawberry in a resealable plastic bag. Seal the bag and mash the strawberries thoroughly for two minutes. This process breaks the cell walls, releasing the cellular contents, including the DNA.

Open the bag and add 10ml of the soapnut extract to the mashed strawberries. Reseal the bag and continue mashing for an additional two minutes. The soapnut extract contains natural surfactants that help to further break down cell membranes and release the DNA into the solution.

Place a coffee filter over a clean cup or beaker. Carefully pour the strawberry mixture into the filter, allowing the liquid to pass through into the cup. Gently squeeze the filter to extract as much liquid as possible. This step separates the solid strawberry debris from the liquid containing the DNA.

Slowly add 10ml of isopropanol to the filtered strawberry liquid. Hold the cup at an angle and pour the isopropanol down the side so it forms a layer on top of the strawberry solution. After a few seconds, you will see white, stringy DNA precipitating out of the solution and floating to the top.

Use a wooden stick to gently lift and collect the strawberry DNA from the top layer.

See the link for the video demonstration:
https://drive.google.com/drive/folders/1VzXhjJJbecFEbuPAwv-u8LGgFsIwClkW?usp=sharing

Pre-Lab Assignment

1. Which of the following is among the 12 Principles of Green Chemistry?

   A. uses renewable raw materials

   B. eliminate waste

   C. avoid the use of toxic and hazardous reagents and solvents

   D. use catalysts, not stoichiometric reagents

   E. all of the above

2. How does the use of soapnut extract as a surfactant align with the 12 Principles of Green Chemistry? Consider aspects such as the use of renewable resources, reduction of toxicity, and minimization of waste.

3. Identify the Sustainable Development Goals (SDGs) that are relevant to the use of natural surfactants in the laboratory. Explain how the experiment addresses these goals.

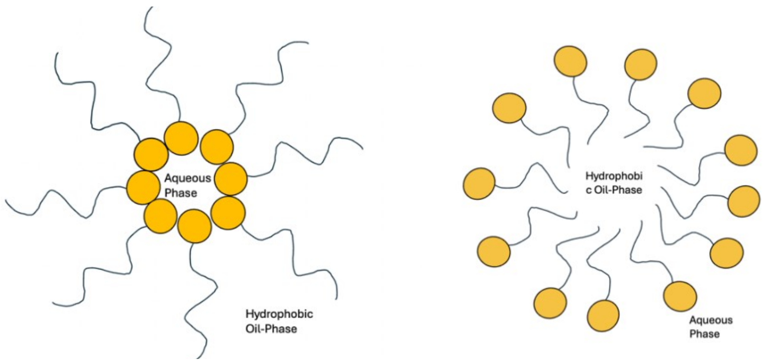
4. Discuss why the selected SDGs are important in the context of this laboratory activity. Consider the broader implications of using natural surfactants versus synthetic ones, including aspects like environmental impact and sustainability.

5. Explain the role of saponin as a surfactant or emulsifier. How does its function compare to that of synthetic surfactants in terms of environmental impact and sustainability?

6. Fill in the blanks with the correct terms:

   "A" corresponds to _______________ micelle

   "B" corresponds to _____________________ micelle



A B

7. What is the difference between reducing and non-reducing sugars?

8. Research the following substances and complete the table below. Consider their environmental and health impacts, sources of pollution, and existing regulatory limits. How do these factors influence the choice of surfactants in the laboratory?

	Environmental Impacts	Health Impacts	Source of Pollution	Regulatory Limits
Cr (VI)				
Methylene Blue Dye				
Pesticides				

Student Feedback Survey

	1	2	3	4	5
How would you rate your understanding of green chemistry principles before participating in these laboratory activities?	23	6	1	0	0
How would you rate your understanding of green chemistry principles after participating in these laboratory activities?	0	0	4	20	6
How important do you think sustainability is in the field of chemistry?	0	0	2	10	18
Has your participation in these laboratory activities changed your perception of the importance of sustainability in chemistry?	0	0	2	20	8
How likely are you to apply sustainable practices in your future academic or professional work in chemistry based on what you learned?	0	0	5	12	13
How engaging did you find the laboratory activities in relation to learning about sustainability?	0	0	2	20	8

How well do you feel the laboratory activities explained the concept of surfactant chemistry and its relevance to green chemistry?		2	3	15	10