Coacervates

Lab III-4

EQUIPMENT AND MATERIALS

You'll need the following items to complete this lab session. (The standard kit for this book, available from *www.thehomescientist.com*, includes the items listed in the first group.)

MATERIALS FROM KIT

- Goggles
- Beaker, 50 mL or 100 mL
- Beaker, 250 mL
- Centrifuge tube, 15 mL
- Centrifuge tubes, 50 mL
- Coverslips
- Gelatin
- Graduated cylinder, 10 mL
- Graduated cylinder, 100 mL
- Gum arabic
- Hydrochloric acid
- pH test paper
- Pipettes
- Reaction plate, 96-well
- Slides (flat)
- Stain, methylene blue
- Stain, Sudan III
- Test tubes
- Thermometer
- Spatula
- Stirring rod
- Stopper (to fit test tube)

MATERIALS YOU PROVIDE

- Gloves
- Balance (optional)
- Microscope
- Microwave oven
- Water, distilled

BACKGROUND

Scientists believe that life originated from simple molecules, a process called *abiogenesis*, when those simple molecules combined into more complex molecules that eventually became complex enough to be self-replicating. We will never know for certain exactly how this occurred. Even if (or when) scientists eventually create life in the lab, the most they will be able to assert is that their method is *a* way that life on Earth may have originated, not that it is *the* way.

One of the pioneers in proposing possible mechanisms for abiogenesis was a Russian scientist named Alexander Oparin, who studied *coacervates*, which are colloidal droplets of hydrophobic molecules in an aqueous medium. These droplets are typically about the size of a cell—1 to 100 micrometers (μm) in diameter—and at first glance under a microscope resemble a cluster of cells. Coacervates form spontaneously in mixtures of proteins and carbohydrates at specific pH values.

Coacervates are not alive, but they present a simulacrum of life as they form what appear to be cell membranes, ingest materials from their environment, grow, and reproduce. Although most current abiogeneticists assign higher importance to information transfer (e.g., DNA and RNA), coacervates may have had a key role in forming the nonliving precursors to the first living cells.

In this lab session, we'll prepare and observe a coacervate, using gelatin as the protein and gum arabic as the carbohydrate.

The gelatin is best prepared immediately before use. Allow it to cool to room temperature before proceeding. If necessary, you can prepare the gelatin the day before and store it in the refrigerator. When you remove it from the refrigerator, immerse the container in a bath of hot water to remelt the gelatin.

If you don't have gelatin and/or gum arabic, or if you'd like to prepare a coacervate using other types of protein and carbohydrate, you can substitute a level teaspoon of table sugar dissolved in about 400 mL of water as the carbohydrate and/or about 10 mL of uncooked egg white diluted to about 125 mL with water as the protein. Always add the carbohydrate solution to the protein solution when making coacervates.

PROCEDURE III-4-1: PREPARE AND OBSERVE A COACERVATE

If you have not done so before the session, prepare 1% gelatin and gum arabic solutions as follows:

1. Transfer about 40 mL of distilled water to a labeled 50 mL centrifuge tube, and heat it in the microwave until it reaches about 60 °C. (**Careful: HOT!**)

2. Transfer about 10 mL of cold distilled water to a small beaker, and gradually stir in 0.5 g (one rounded spatula spoon) of gelatin powder. Stir thoroughly, making sure there are no lumps and the powder is completely dispersed.

3. Pour the gelatin slurry slowly and with constant stirring into the hot water in the 50 mL centrifuge tube. Stir until all the gelatin has dissolved and the solution is clear. If necessary, you can reheat the contents of the tube in the microwave and continue stirring until the gelatin solution is clear. Allow the tube to cool to room temperature.

4. Repeat steps 1 through 3 with a second tube, substituting 0.5 g (one rounded spatula spoon) of gum arabic powder.

If you have not done so before the session, prepare a dilute solution of hydrochloric acid as follows:

1. If you have not already done so, put on your goggles, gloves, and protective clothing.

2. Label a 15 mL centrifuge tube "dilute HCl" and transfer about 12.5 mL of distilled water to it.

3. Using a plastic pipette, draw up 0.25 mL of 6 M hydrochloric acid and transfer the acid to the water in the centrifuge tube. (The graduation lines on the pipette stem are at 0.25, 0.50, 0.75, and 1.00 mL.)

4. Replace the cap on the tube, invert the tube several times to mix the solution, and set it aside for now.

To make the coacervate, proceed as follows:

1. If you have not already done so, put on your goggles, gloves, and protective clothing.

2. Transfer 5 mL of the room-temperature gelatin solution to a test tube, followed by 3 mL of the gum arabic solution. Stopper the tube and invert it *gently* several times to mix the solutions. Do not shake or otherwise strongly agitate the contents of the tube, which will hinder the formation of the coacervate.

3. Observe the contents of the test tube against a strong light. Record the appearance of the contents, particularly with regard to clarity, in your lab notebook as "Trial 1."

4. Use the stirring rod to transfer one drop of the liquid to a small piece of pH paper. Compare the color of the paper against the scale provided with the pH paper to determine the approximate pH of the liquid. Record this value in your lab notebook.

5. Label a microscope slide "Trial 1." Transfer one drop of the liquid to the slide, put a coverslip in place, and observe the slide at low magnification. If anything interesting appears, center the object(s) and observe them at higher magnification. Record your observations in your lab notebook. Retain the slide for comparison with later trials.

6. Use a plastic pipette to transfer 0.25 mL of the solution to well A1 of the reaction plate.

If you're unable to find any coacervates, don't despair. The formation of coacervates is strongly dependent on pH. In subsequent trials, we'll adjust the pH of the liquid by adding dilute hydrochloric acid until coacervates begin to appear.

7. For trial 2, transfer three drops of the dilute hydrochloric acid to the test tube. Stopper the tube and invert it gently several times to mix the solutions. Repeat steps 3 through 6, using well A2 of the reaction plate to store 0.25 mL of the solution from trial 2.

8. Repeat step 7 for additional trials until the pH of the liquid in the test tube reaches 3 or lower. After each addition of hydrochloric acid to the liquid, transfer a 0.25 mL sample to the corresponding well in the reaction plate.

Once you have determined experimentally the pH level that is optimum for the formation of coacervates, the next step is to observe the effect of two biostains on the coacervates to determine if coacervates, like living cells, have selectively permeable membranes. The first, methylene blue, is a hydrophilic (water-loving) stain. The second, Sudan III, is a lipophilic (fat-loving) stain.

9. Place one drop of the optimum-pH coacervate liquid on a microscope slide, add one drop of methylene blue stain, and position a coverslip over the specimen. Observe the slide at low, medium, and high magnifications. Note the effect of the stain on the parts of the coacervate and record your observation in your lab notebook.

10. Repeat step 9, using Sudan III stain.

REVIEW QUESTIONS

Q1: What visual evidence suggests that coacervates are forming?

Q2: Based on your observation of the coacervate slides, what pH level is optimum for the formation of coacervates?

Q3: What correlation did you observe between the clarity of the liquid and the population of coacervates?

Q4: In what ways do coacervates resemble cells?

Q5: What do you conclude from your experiments with the methylene blue and Sudan III stains?