Saptasense Protocol

Dextran Hydrogel Characterization

Background:

This protocol describes the four characterization assays Team Saptasense performed on the dextran hydrogels. A simple water absorption assay was used to determine the water holding capacity of the various gels. Similarly, we performed the aforementioned absorption assay using buffers of various pHs to determine if the pH of the environment affects the swelling capacity of the hydrogel. A diffusion test using food dye was performed to help determine the pore sizes of the hydrogels. Finally, a seed germination assay was conducted to confirm that seed growth is viable using the created hydrogels.

PROTOCOL: Dextran Isolation

Part I: Absorption Assay

Materials

Deionized (DI) H_2O

Instruments

500 mL beaker

Scale

Methods

- Create three 20% (w/v) dextran hydrogels (see "Creating Dextran Hydrogels" protocol) of varying %wt MBAm crosslinker. Collect the dry mass of the gels.
- Measure 350 mL of DI water in a 500 mL beaker. Submerge the gels in the water so that they sit at the bottom of the beaker.
- Lightly cover the beaker with aluminum foil. Leave the gels submerged overnight (12 hrs) in water at 25°C.
- After 12 hrs, remove the gels from the water bath. Remove any excess water from the sides of the dish and top of the gel.
- Weigh the swelled gel. This is the swelled mass of the gel. Calculate the grams of H_2O absorbed by the gel.
- Assay can be repeated for 10% (w/v) and 15% (w/v) dextran gels with varying %wt MBAm concentrations to determine how %wt MBAm crosslinker affects the mass of H_2O absorbed by the gels.

Part II: pH Swelling Assay

Materials
Tris buffer pH 2.5
Tris buffer pH 5.5
Tris buffer pH 8.5
DI H_2O

Instruments
4 250 mL beakers
Scale

Methods

- Create four 20% (w/v) dextran hydrogels using 10% (w/w) MBAm crosslinker. Collect the dry masses of each gel.
- Fill each beaker with 100 mL of the four experimental swelling solutions of varying pH. Place one gel in each beaker, ensuring the gel is completely submerged.
- Cover the beaker lightly with foil. Leave the gels submerged in the solution for 1 hour.
- After 1 hour, remove the gel. Tap dry any excess solution. Weigh the gel and record its mass. Resubmerge the gel in the solution.
- Repeat Step 4 again after 1 hour (2 hours of total swelling time). Repeat again after another hour (3 hours of total swelling time). Use the 3 hr final wet mass of the gel for further calculations.
- Calculate the swelling ratio of the gels. The swelling ratio is defined as the following:

$$Swelling\ Ratio\ = \frac{Mwet - Mdry}{Mdry}$$ where M = mass of gel

Part III: Diffusion Assay

Materials
Food dye

Instruments
Stopwatch

Methods

- Cut a small square of swelled gel (see Part 1: Absorption Assay to make gels that have absorbed DI water). The gel should have a height of 7 mm.
- Add about 15 µL of food dye to the top of the gel's surface. Begin the stopwatch and monitor as the dye moves through to the bottom of the gel. Stop timing once the dye front reaches the bottom and record this time.

Part IV: Germination Assay

Materials

Chia seeds
Petri dishes (60 mm diameter)
DI H_2O
Paper towel

Methods

- Add 9 g of hydrogel to a petri dish and orient it so that the bottom of the petri dish is covered by the gel. Create the following controls in two additional petri dishes: a.) paper towel lining the bottom of the dish; b.) no lining
- Add 10 chia seeds to each petri dish. Ensure that the seeds are physically separated.
- Add 10 mL of DI $H_2O$to each petri dish. Cover each dish lightly with parafilm or foil and poke small holes through it. Leave the dishes at room temperature
- Add an additional 5 mL of DI H_2O to each dish per day. Monitor the seeds over the course of 3-5 days to observe germination of the seeds.