

Experiment 4: Synthesis of hydrogels of acrylamide and bisacrylamide in water.

Aim: (a) To synthesize polyacrylamide electrophoresis hydrogels by copolymerization of acrylamide and bisacrylamide in water by free radical polymerization using a redox pair of initiators.

(b) To observe the time to reach "gel point" as a function of the concentration of monomers and ratio of monomer concentration.

(c) To prepare "worms" and Petri dish gel slabs for experiment #5.

Materials and Apparatus:

Buffer solution: Phosphate buffer saline (PBS), PH 7.4

Master solution \square : 30 wt % acrylamide and 0.8 wt % bisacrylamide solution

(mol ratio = 80:1 or wt. ratio = 37.5:1)

Master solution \square : 40 wt % acrylamide and 2 wt % bisacrylamide solution

(mol ratio = 40:1 or wt ratio = 19:1)

AMPS (ammonium persulphate)

TEMED (tetramethyl ethylene diamine)

250 ml Erlenmeyer flask

Large beakers

Glass wool insulation

25 ml plastic test tube

Capped test tubes (~5 " long)

Glass beads, 3 mm

Parafilm

12 " section of "FEP" 1/4" diameter tube

10 ml syringe

Septum

Thermocouple set

Stop watch or eq.

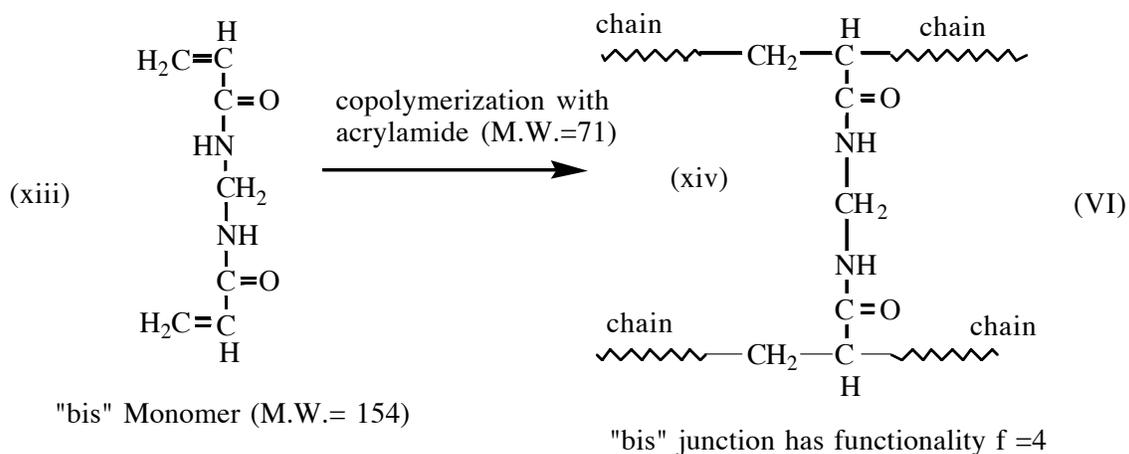
Weighing machine

Helium tank with reducing valve (multiple outlets) for sparging

Brief Background:

Copolymerization of acrylamide and bisacrylamide:

This experiment is like experiment 1, in that a water solution of acrylamide (labeled as (iii) in experiment #1), is initiated to undergo radical polymerization by the same redox initiator pair used in that experiment. However, a tetrafunctional molecule, N, N' methylene bisacrylamide "BIS" (labeled (xiii) in equation below) is added in a mol ratio to acrylamide of about 1/50 to 1/100. The copolymerization of bis results in the bridging junction shown in (xiv) into which four chains of polyacrylamide come together in this irreversible covalent connection.



The result is that the initial aqueous solution of acrylamide and "bis" following initiation undergoes an exponential increase in viscosity until a network filling the reaction vessel has been created, at which point the system "gels", i.e., viscosity is infinite.

Gel point:

At the gel point the polymer transforms suddenly from a viscous liquid to an elastic gel (network). Prior to the gel point, all of the polymer is soluble in suitable solvents, and it is fusible also. Beyond the gel point, it is no longer fusible to a liquid nor is it entirely soluble in solvents. We shall get an estimate of the gel point from the point at which the viscosity of the solution increases to infinity (i.e. a solid-like substance).

Hydrogels:

A gel is a network i.e., chains are cross-linked to each other (unlike in experiment #1 where there were several independent chains in the polymer solution that you prepared). A gel that is hydrophilic (water-loving) is called a hydrogel. So when dry hydrogels are put into water, they absorb water and hence swell in volume (but the gel does not dissolve;

why?). The equilibrium degree of swelling depends on the degree of cross-linking and the hydrophilicity (extent to which it loves water) of the gel. Can you name some common uses of hydrogels? (Are soft contact lenses hydrogels; if so how do they work? How could a hydrogel be used in controlled drug delivery? Some super-absorbing gels are used to slowly supply water to seeds in semi-arid, desert like areas; do you know how they work?)

Precautions:

Use vinyl gloves. No special precautions.

Procedure:

1. In preparation for the viscosity test, set aside a screw cap plastic test tube with a 3mm glass bead inside it. Also set aside a second 25 ml plastic test tube with glass wool for calorimetry. Finally, set up tubing with plugs and nine petri dishes with covers in preparation for steps 7 and 8 (see detailed description under each of these steps below).
2. Synthesize the following gels (one at a time) using the recipes given below for your team. Master Solution \square has acrylamide at the low bis cross-linker content and Master Solution \square has acrylamide with the high bis cross-linker content. The buffer solution is used for dilutions to maintain the appropriate pH to avoid conversion of acrylamide to acid groups, other side reactions. Mix p ml of the buffer solution and q ml of master solution to produce a total of 200 ml of x % acrylamide solution. Split the 200 ml into two approximately equal volumes of 100 ml each, which will be sparged with helium to remove dissolved oxygen. Use 250 ml Erlenmeyer flasks.
3. After sparging, add y grams of AMPS to one flask and z grams of TEMED to the other flask (these are our redox initiators). Swirl each flask to dissolve the added redox initiator.

Table: Recipes

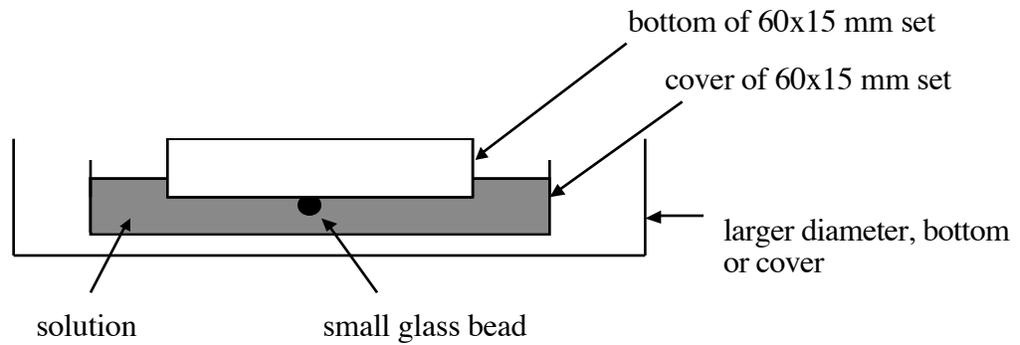
Variation	x=5% gel	x=7.5% gel	x=10% gel
Team A,C,D	----- Use master solution \square -----		
	p=83 ml	p=75 ml	p=67 ml
	q=16.6 ml	q=25 ml	q=33 ml
	y=0.1 g	y=0.1 g	y=0.1 g
	z=0.05 ml	z=0.05 ml	z=0.05 ml
Team B,E,F,G	----- Use master solution \square -----		

p=87.5 ml
q=12.5 ml
y=0.1 g
z=0.05 ml

p=81.2 ml
q=18.8 ml
y=0.1 g
z=0.05 ml

p=75 ml
q=25 ml
y=0.1 g
z=0.05 ml

4. Add the contents of one flask into the other. Simultaneously, start the stop watch (This shall be time = 0 for both temperature and viscosity measurements). Mix the solution by swirling. At this point you are ready to immediately fill all of your containers as described below in steps 5-8.
5. Pour some of this solution to completely fill (to overflowing) the test tube set aside in step 1. Screw the cap onto the filled tube to exclude any bubbles of air. Every minute, invert the tube and estimate the time it takes for the glass bead to fall from the top to bottom. Also record the time when the bead gets stuck.
6. Set up the 25 ml plastic tube with some of the remaining solution, surrounded by glass fiber (glass "wool") as insulation. Inset a thermocouple probe through the parafilm or a stopper, which excludes air. Allow the remaining solution to polymerize. Record the temperature of the contents of the flask every minute.
7. Preparation of "worms" for the diffusion Experiment 5: Place a septum over the end of a 12" section of "FEP" 1/4" diameter tube. Inject the mixed AMPS and TEMED solution via a 10ml syringe and needle until it just starts to appear at the other end. You will make three of these- one for each of the three recipes (5%, 7.5%, 10%). Remove the syringe needle. Let the tube and contents rest for at least 45 minutes. Then apply gentle pressure to force the "worm" out of the FEP tube into a petri dish. Leave the worms for the swelling study next time.
8. Preparation of circular slabs for the diffusion Experiment 5:
 - a. Set aside 9 sets of the following: (bottom + cover) of 60 X 15 mm plastic petri dishes, and one bottom or cover of any larger diameter petri dish to take overflow. Cement a 6 mm glass bead onto the outer surface of the 60 X 15 mm petri dish bottom. You will be preparing 3 slabs for each of three recipes (3 X 20 ml volume solution required).
 - b. In the case of each set, place the inverted cover of the 60 X 15 mm set on the larger diameter dish. Fill it (for all three sets) nearly to overflowing (about 21 ml) with one of the solutions. Thus one of each of the three recipes is used in each case. Immediately float the bottom on the solution, so that it forms a covering surface.
 - c. Set aside for 45 minutes or longer (not critical). The solutions will have gelled.



Schematic diagram of placement of various petri dish bottoms and covers.

Observations and Calculations:

1. Tabulate data and analyse the data similar to what you did in experiment 1.
2. Be sure to identify the gel point and study the trends.
3. Address one or more of the questions asked in the introduction of this text in your lab write-up introductory section.

Discussion:

1. Similar discussions as in experiment #1 without repetition.