

### Objective

To study the effect of alcohol on membrane permeability.

### Background

Beetroots are root vegetables that appear red because the vacuoles in their cells contain a water soluble red pigment called **betalain**. These pigment molecules are too large to pass through cell membranes. The effect of alcohol on membranes can be investigated

### Materials

- Eye protection
- Distilled water
- Syringe
- Beetroot
- Cork borer
- Ruler
- White tile
- Knife
- 10 cm<sup>3</sup> syringe
- Pipette
- Test tubes
- Labels or pens for labelling
- Forceps
- Ethanol (at 10%, 20%, 30% 40% concentrations)

### Procedure

1. Cut seven uniform cylinders of beet using a cork borer with a 5-mm inside diameter.
2. Trim each cylinder to exactly 15 mm in length. All the cylinders must be the same size.
3. Place these cylinders of beet tissue in a beaker and rinse them with tap water for 2 min to wash betacyanin from the injured cells on the surface. Be sure that all of the cylinders are the same size. Discard the coloured rinse-water.
4. Place one of the seven beet sections into each of five dry test tubes. Do not crush, stab, or otherwise damage the cylinders when moving them to the test tubes.
5. Label the tubes 1–6 and write the organic-solvent treatment on each tube as listed in observation table.
6. Add 10.0 mL of the appropriate solvent (see table) to each of the tubes.

### Procedure (cont)

7. Keep all test tubes at room temperature for 20 min and shake them occasionally. Then remove and discard the beet sections and measure the extent of membrane damage.
8. Quantify the relative colour of each solution between 0 (colourless) and 10 (darkest red). If colour standards are available in the lab, use them to determine relative values for the colours of your samples.
9. Draw a labelled diagram / paste photo of experimental set up.

### Observation Table

Test Tube No.	Content in the tube/ Treatment	Volume of Ethanol (ml)	Volume of Water (ml)		Colour Intensity
1	Distilled Water	0	10	Keep All the	Colourless
2	20% Ethanol	2	8	Test Tubes	-----
3	40% Ethanol	4	6	at room	-----
4	60% Ethanol	6	4	temper- ature	-----
5	80% Ethanol	8	2	for 20 min	-----
6	100% Ethanol	10	0	and shake occasi- onally. them	-----

### Result

The solvent stress damages the membranes, and betacyanin will leak through the tonoplast and plasma membrane. This leakage from the stressed beet will colour the surrounding water red. Thus, maximum membrane damage results in the maximum intensity of colour.



By UmeshJagtap

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