Core practical 5: Investigate the effect of temperature on membrane permeability

Objectives

- Know how the effect of temperature on membranes can be determined
- Be able to recognise quantitative variables that should be controlled in an investigation

Safety

- Water baths at temperatures above 50 °C may scald. Take care when removing lids to allow steam to escape away from the face or body.
- Take care with sharp items such as the cork borer and knife.

Specification links

- Practical techniques 1, 2, 3, 8
- CPAC 1a, 2a–2d, 4a, 4b, 5b

Procedure

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 membranes.

1. Prepare eight water baths pre-set to a range of temperatures between 0 °C and 70 °C.
2. Use a syringe to add 10 cm^3 of distilled water to eight test tubes. Label each test tube with a temperature from the pre-set range.
3. Place each tube in the water bath set to the corresponding temperature for 5 minutes.
4. Check the temperature of each bath is correct using a thermometer. It is unlikely to be exactly the desired temperature. Record the actual temperature and use this in your table and graph.
5. Cut eight beetroot cylinders using a cork borer. Using a knife, ruler and white tile, trim them all to the same length (1 cm is sufficient). Wash the cylinders thoroughly with water until the water runs clear and pat dry gently with a paper towel.
6. Add one beetroot cylinder to each of the eight tubes and leave in the water bath for 15 minutes.
7. Shake the tubes once. Working quickly, use forceps to remove the cylinders carefully from each tube. Discard the cylinders, keeping the supernatant liquid. It may be easier to decant the liquid into clean test tubes.
8. Set the colorimeter to a blue/green filter and percentage transmission. Zero the colorimeter using a blank cuvette filled with distilled water.
9. Transfer liquid from each test tube in turn into a colorimeter cuvette, place into the colorimeter and read the percentage transmission reading, recording your results in a suitable table.
10. Plot a graph of transmission against temperature.

Notes on procedure

- Students should have measured absorbance using a colorimeter previously in Core Practical 1 (see Practical 2). This beetroot investigation suggests the use of transmission to give further experience with the colorimeter, but absorbance could be used instead.
- Students can work in pairs. To save time and space in water baths a smaller number of temperatures could be designated to each pair, with students combining results as a class to provide a complete set with repeats at each temperature.
- Warn students that although beetroot juice is harmless, it will stain skin and clothes badly.
- At the lowest temperatures, condensation will develop on cuvettes and will cause low transmission readings unless wiped off.

Answers to questions

1. The variables controlled during the experiment are:
   - the volume of bathing water in each tube (10 cm^3)
   - the surface area and volume of the beetroot cylinders (dependent on size of cork borer; 1 or 2 cm in length)
   - the equilibration time (5 minutes)
   - the soaking time for the cylinders (15 minutes)
   - the volume of coloured liquid in the cuvettes (e.g. 4 cm^3)
   - the colorimeter filter/wavelength used (blue/green)
   - the part of the beetroot the core was taken from (e.g. the centre)
   - the age, variety and storage time of the beetroot (the same beetroot or beetroots from the same batch may have been used).
2. The temperature must be equilibrated to ensure the tubes contain water at the correct temperature before starting the experiment. This allows confidence that the effect of the correct temperature is being assessed.
3. The cylinders are washed and dried to remove excess surface pigment from the cut cells at the edge. This excess pigment would distort the transmission readings, giving inaccurate results.
4. The percentage transmission decreases as the temperature rises. Initially there is little increase, but at around 40–60 °C the percentage transmission decreases sharply. Students should use values from their own graphs. At higher temperatures the rate of decrease usually levels out.
5. At lower temperatures the tonoplast and plasmalemma are intact and betalain molecules are too large to pass through these membranes easily, meaning light transmission remains high (note that if cells freeze, damage to membranes may cause pigment release). The higher the temperature, the greater the kinetic energy and the faster the movement and diffusion of pigment molecules. Greater kinetic energy also causes phospholipids of the membrane to become more fluid and bonds between the fatty acid tails can begin to separate so that some pigment molecules can pass through. Therefore more pigment passes through the membrane, decreasing the amount of light that can pass through the liquid (percentage transmission).

   The point of sudden increase in percentage transmission occurs when proteins in the membrane begin to lose their tertiary structure. At higher temperatures, the protein molecules in the membrane become completely denatured and the membrane develops gaps through which the pigment can flood out.

   Eventually, the change in transmission levels out as the concentration of pigment is the same inside and outside the cells.

Sample data

Temperature/°C	Transmission (%)					
	Student 1	Student 2	Student 3	Student 4	Student 5	Mean
0	6	5	6	0	9	5
10	0	14	3	3	5	5
20	3	8	4	4	5	5
30	18	4	4	4	6	7
40	18	9	4	8	7	9
50	32	26	45	60	18	36
60	60	65	75	80	65	69
70	75	50	80	75	70	70

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All the maths you need

- Recognise and use expressions in decimal and standard form.
- Use an appropriate number of significant figures.
- Find arithmetic means.
- Understand measures of dispersion, including standard deviation and range.
- Plot two variables from experimental or other data.

Equipment

- water baths pre-set at required temperatures
- thermometer
- distilled water
- syringe
- large beetroot
- cork borer size no. 4 or 5
- ruler
- white tile
- knife
- 10 cm^3 syringe
- pipette
- test tubes
- colorimeter
- nine cuvettes
- labels or pens for labelling
- forceps
- crushed ice

Diagram

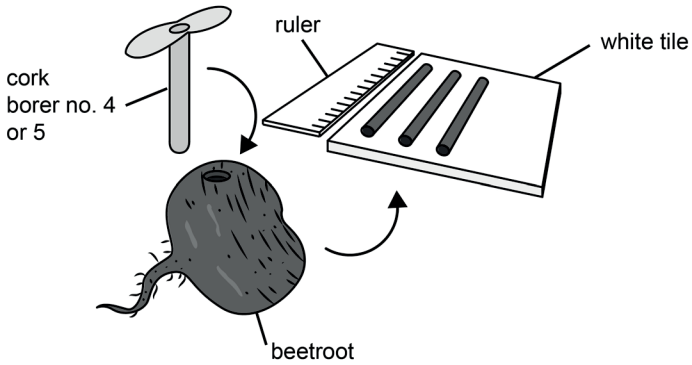

fig A Making beetroot cores to standardise surface area and volume.

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10. Plot a graph of transmission against temperature.

Analysis of results

1. Using pooled class results if necessary, record results in a table showing repeats and a mean for each temperature.
2. Highlight the maximum and minimum values at each temperature and decide if any values are anomalies (results that show a substantial deviation from the general pattern of results). If possible, the tests that produce such results should be repeated and a new mean should be calculated.
3. Plot a graph of mean percentage transmission against temperature. Do not forget to use the actual temperature in each water bath. Add error bars of the maximum and minimum to show the range of transmission values at each temperature.

Learning tips

- Make sure that you draw tables and graphs correctly. The independent variable should always go in the left hand column of a results table and on the horizontal axis of your graphs. Numbers in tables, including calculations, should only be reported to the limits of the least accurate measurement. Data on graphs should be scaled so that they always occupy more than half of the available space.

Questions

1. List the variables that were controlled during the experiment and state how they were controlled. This could be done using a table.
2. Suggest why the tubes were placed in the water baths for 5 minutes before the cylinders were added.
3. Why were the beetroot cylinders washed with distilled water and dried before the experiment began?
4. Use the trend line of your graph to describe the effect of temperature on the percentage transmission between 0 °C and 70 °C.
5. Explain your results in detail in terms of what is happening to the beetroot membrane.

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Equipment per student/group	Notes on equipment
water baths	Pre-set at 0 °C, 10 °C, 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C. These can be shared.
thermometer	These can be placed in shared water baths.
distilled water	
syringe	One per group
large beetroot	One per student. Beetroot must be fresh, not cooked. Fresh beetroot is generally available between July and March in the UK. If it is not available, red cabbage discs are a possible alternative but are not as effective.
cork borer size no. 4 or 5	Beetroot cores could be produced for students in advance to save a lot of time (and beetroots). Eight cores per group are needed.
ruler	One per group
white tile	One per group
knife	One per group
10 cm^3 syringe	One per group
pipette	One per group
test tubes	Eight per group, or 16 per group if liquid is to be decanted
colorimeter	Set to a blue/green filter
cuvettes	Nine per group
labels or pens for labelling	
forceps	One pair per group
crushed ice	Add salt to the ice to lower the temperature if needed.

Notes