A-level Biology example for required practical 4

**Investigation into the effect of a named variable on the permeability of cell-surface membranes:**

**The effect of alcohol concentration on the leakage of pigment from beetroot cells**

**Student sheet**

## Introduction

Beetroot contains high concentrations of betalin. This is a purple pigment found inside the vacuoles of the cells. The pigment cannot move across undamaged plasma membranes. You will investigate the effect of alcohol concentration on the leakage of pigment leaking through beetroot plasma membranes.

In **Part 1** of the investigation, you will produce a set of standards and use them to produce a calibration curve. In **Part 2** you will use these standards to compare the colour of the solutions obtained when beetroot discs have been soaked in different concentrations of alcohol.

## Method

You are provided with:

- stock solution of beetroot extract
- five concentrations of alcohol labelled 100%, 80%, 60%, 40%, 20%
- discs cut from a beetroot and rinsed thoroughly in water
- forceps
- graduated pipettes or syringes
- boiling tubes
- bungs to fit some of the test tubes
- thermometer
- large beaker to use as a water bath
- stopwatch
- test-tube rack
- small beakers
- permanent marker pen
- water.

You should read these instructions carefully before you start work.

### Part 1: Making the colour standards

1. Use the extract and water to prepare a series of six boiling tubes containing 5cm$^3$ of different concentrations of extract. The concentrations should be equally spaced and cover a range from pure water (0%) to pure extract (100%). These will be your colour standards.
2. Label these standards 0, 2, 4, 6, 8, 10.
3. Complete **Table 1** to show the concentration of extract in each tube.
4. Complete **Table 1** to show how you made the colour standards in **Part 1** of the investigation.

**Table 1**

| Label of tube | Volume of beetroot extract/$cm^3$ | Volume of water/$cm^3$ | Concentration of extract/% |
|---|---|---|---|
| 0 | | | 0 |
| 2 | | | |
| 4 | | | |
| 6 | | | |
| 8 | | | |
| 10 | | | 100 |

**Part 2: The investigation**

5. Set up a water bath at 30°C.
6. With a second set of boiling tubes add 2$cm^3$ of 100% alcohol to a test tube and put a bung in the tube.
7. Label the tube with the alcohol concentration.
8. Repeat steps 6 and 7 with alcohol concentrations of 80%, 60%, 40% and 20%.
9. Put the tubes of alcohol in the water bath until the temperature of the alcohol reaches 30°C.
10. Blot 10 discs of beetroot with a paper towel to remove excess water.
11. Using the forceps, gently put two discs of beetroot in each of the five tubes. Replace the bungs as soon as possible after doing so.
12. Leave the tubes in the water bath for 5 minutes. Shake the tubes gently once every minute. Then remove the tubes from the water bath.
13. Immediately pour each solution into a separate clean boiling tube, being careful to label the tubes appropriately. Throw the beetroot discs away.
14. Compare each of your solutions with the colour standards you made in **Part 1.** Note which standard has the same colour as each of your solutions. If the colour of the solution falls between two of the values you can use the intermediate number. For example, if the colour value is between 2 and 4, record the colour value 3.
15. Record your results in a suitable table.