NEATH PORT TALBOT COLLEGE
COLEG CASTELL NEDD PORT TALBOT

School of Maths & Science
Science Practical

In this practical you will be assessed on skills C and D

The Effect Of Temperature On The Release Of Red Pigment From Beetroot

◆ Aim

To investigate the effect of temperature on the release of red pigment from beetroot

◆ Introduction

Beetroot contains an intensely red, water-soluble pigment in the cell vacuoles. If the plasma membrane is damaged, the pigment may escape in sufficient quantities to be detected in the aqueous medium around the tissue.

◆ Safety

Control Measures

- The wearing of **safety glasses** and a **laboratory coat** at all times will be sufficient to take account of most hazards and significant risks.
- Take care when using sharp dissection equipment, and when it is placed on the bench besides you.
- When cutting do **not** hold the beetroot in your hand but place it on a white tile.
- Always use a pipette filler when filling graduated pipettes. **NEVER** suck up liquids by mouth.
- All waste is to be placed in the labelled container immediately after use.
- You are reminded of the need of good laboratory practice in order to maintain a safe working environment.

◆ Materials

Beetroot; distilled water

◆ Apparatus

Test tubes; 100 cm^3 beaker; white tile; cork borer (size No. 6); knife; ruler; forceps; graduated pipette; pipette filler; paper towels, water baths at 20^0C, 40^0C, 50^0C, 60^0C and 80^0C; thermometers; stop watch; colorimeter

◆ Procedure

1. Electric water baths are set up at 20^0C, 40^0C, 50^0C, 60^0C and 80^0C.

2. Obtain cylinders of beetroot using a cork borer (size No. 6). Cut off and discard 2 mm from the ends of cylinder. Each cylinder should be 3 cm long. Wash the cylinders in a beaker of distilled water. Place cylinders on paper towel to remove excess water.

3. Take 15 test tubes. Set up test tubes at each temperature (20^0C, 40^0C, 50^0C, 60^0C and 80^0C).

4. Using a graduated pipette add 10 cm^3 of distilled water to each test tube.

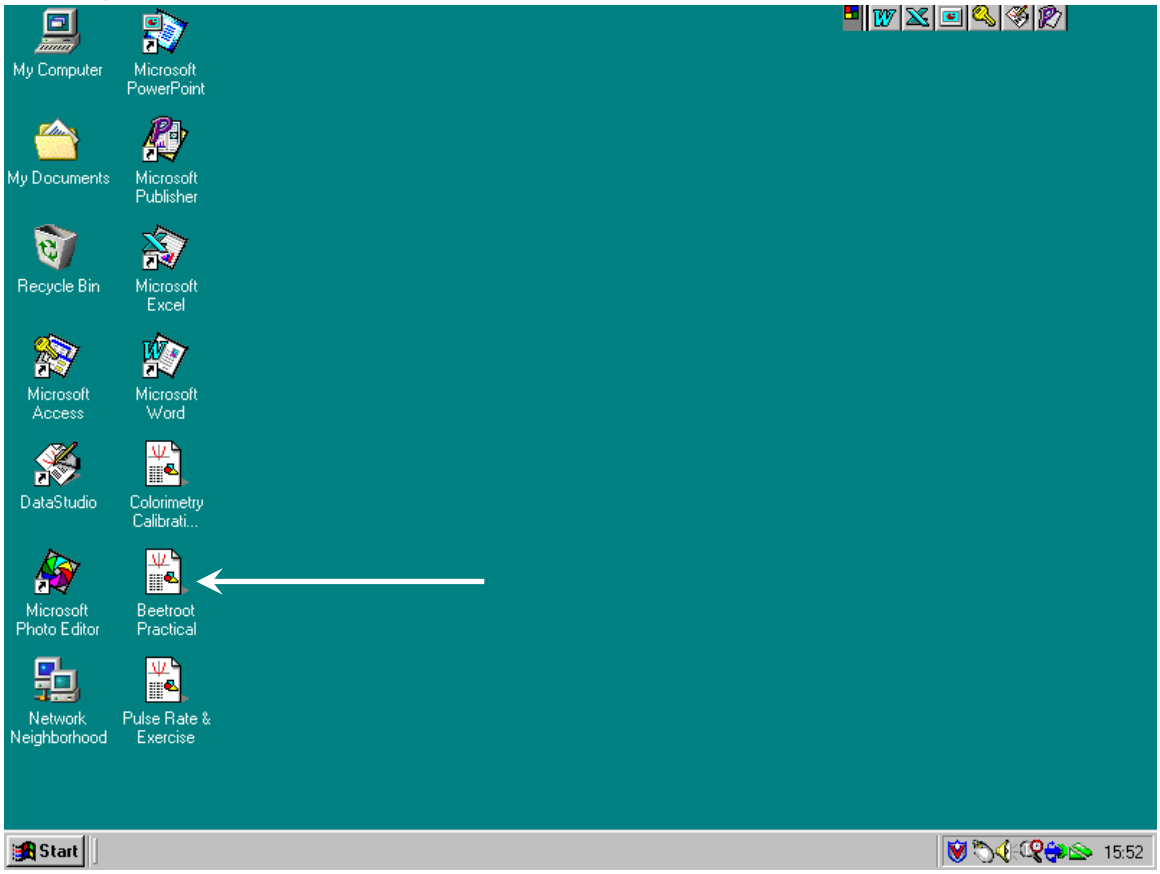
5. Place 3 test tubes at each temperature and allow to equilibrate for 5 minutes.

6. To each test tube add a cylinder of beetroot and leave for 5 minutes.

7. Remove test tubes from water baths and place the cylinder of beetroot in 10 cm^3 of **fresh** distilled water in a labelled test tube. Leave at room temperature for 15 minutes. The water will contain any pigment that has escaped from the tissue.

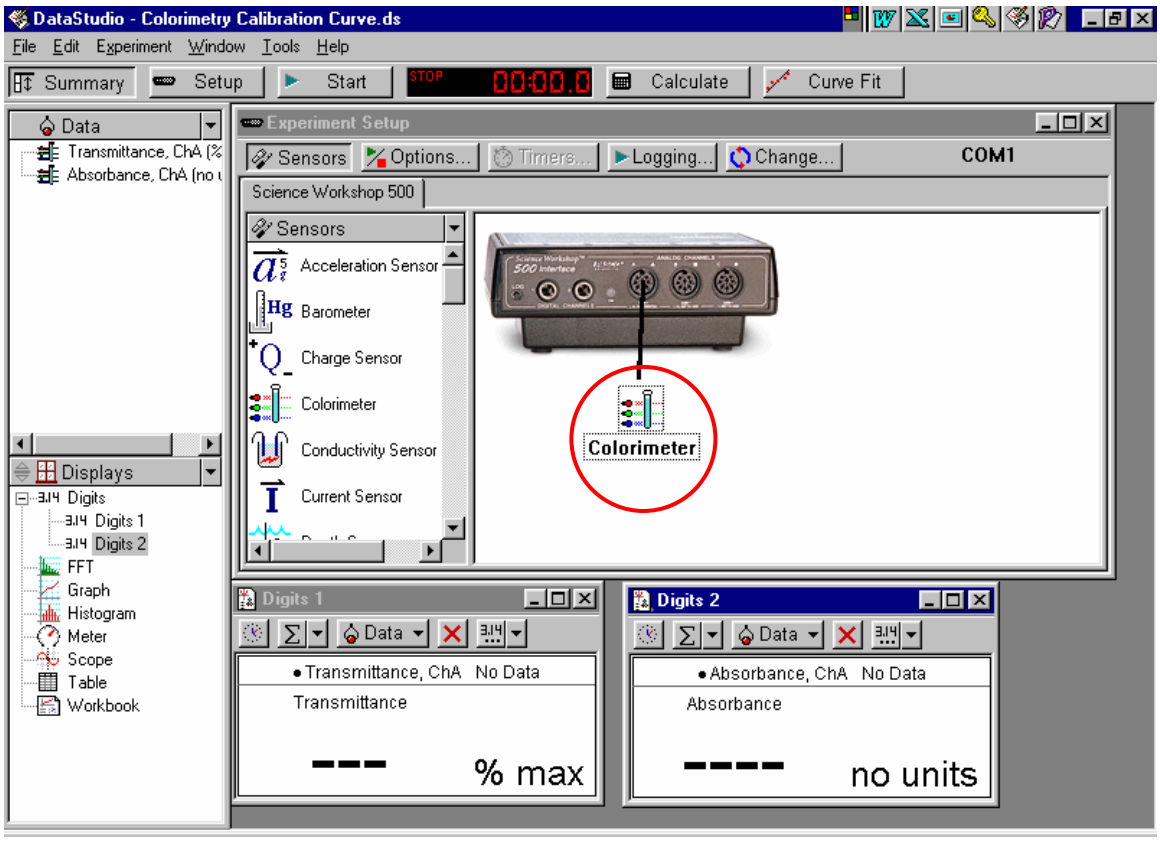
8. Decant the distilled water **only** into a labelled test tube. To measure the absorbance of the samples you will use a Data Logging computer package.

When you see the screen



Double click on the ***"Beetroot Practical"*** icon.

You will see this screen



Read the following instructions carefully:

◆ Calibrate the colorimeter

1. Fill a clean cuvette with distilled water and cap the cuvette.

   *Ensure that the cuvette is clean and avoid touching the optical surfaces. Use the **same** cuvette for all readings, rinsing between each solution. Wipe the outside of the cuvette with a tissue to make sure it is dry before placing in the colorimeter.*

2. On the **colorimeter** press the **Select** button and the **Start/Stop** button at the **same** time.

3. The colorimeter LCD display will show "**Insert reference then push SELECT**".

4. Place the capped cuvette inside the colorimeter. Make sure that the clear sides of the cuvette (without ridges) are lined up with the light path in the colorimeter. Close the lid on the colorimeter.

5. On the **colorimeter**, press the **Select** button.

6. The colorimeter LCD reads
   "Please wait" then "CAL done, push SELECT or START".

◆ Calibrate the Software

1. Leave the cuvette with distilled water inside the colorimeter.

2. On the **computer screen** in the **Experiment Setup window** double-click the **colorimeter icon** attached to the interface (circled on image on previous page).

3. The **sensor properties window** will open. Click **"calibration" tab**. The **sensor setup window** will open.

4. On the **colorimeter** press the **SELECT** button until **"Blue 460nm"** appears on the LCD.

5. On the **colorimeter** press the **Start/Stop** button. The LCD shows
   "Blue 460nm 100.0% T RUN".

6. On the **computer screen** check the **voltage "Current Reading".** When the voltage stabilises click the **"Take Reading"** button under **"High Point".**

7. On the **colorimeter** press the **Start/Stop** button, the LCD changes from **RUN** to **STOP**.

8. On the **computer screen** click "**OK**" in the **sensor properties window** to return to the **Experiment Setup window**. The software will now be calibrated for the colorimeter.

Recording Absorbance Readings at Varying Temperatures

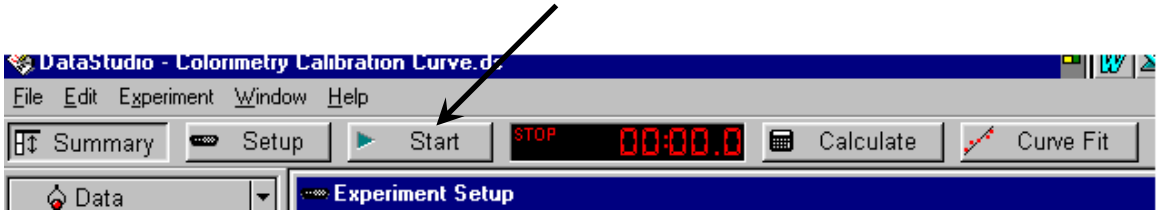
1. You need to construct an appropriate table to record your results — see **SKILL D in COURSEWORK BOOKLET.**

2. Fill cuvette with sample for 20°C and cap cuvette.

3. Place cuvette inside **colorimeter** and close lid.

4. Press **Start/Stop** button to start **colorimeter**, the LCD shows **"Blue 460nm % transmittance RUN".**

5. On the **computer screen** click the **Start** button which will change to **Stop** button.



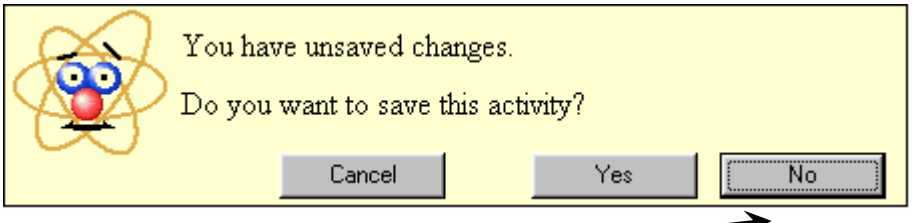
6. Click the **Stop** button to record the **absorbance** value, enter this value in your table.

7. Press **Stop** on the **colorimeter** to STOP reading.

8. Remove cuvette from colorimeter and **repeat stages 2 -7** for the other temperatures.

9. To exit the program, click the **close** button in the far right corner.

10. You will see this window on screen:



11. Click **NO**