NEATH PORT TALBOT COLLEGE
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School of Maths & Science
Science Practical

An Introduction To Colorimetry

◆ Aim

To practice using a colorimeter and preparing a calibration curve with food colouring.

◆ Introduction

A colorimeter is an instrument that measures the amount of light which either passes through, or is absorbed by a coloured solution. (For further details see coursework booklet).

Determination of the concentration of a substance requires the use of a calibration curve. This is prepared by plotting a graph of the absorbance of a range of standard solutions against their concentrations. The concentration of the test solution is then found by reading a value from the calibration curve. If the absorbance of the test solution is off the scale, or higher than the maximum value on your calibration curve, an appropriate dilution of the test solution must be made.

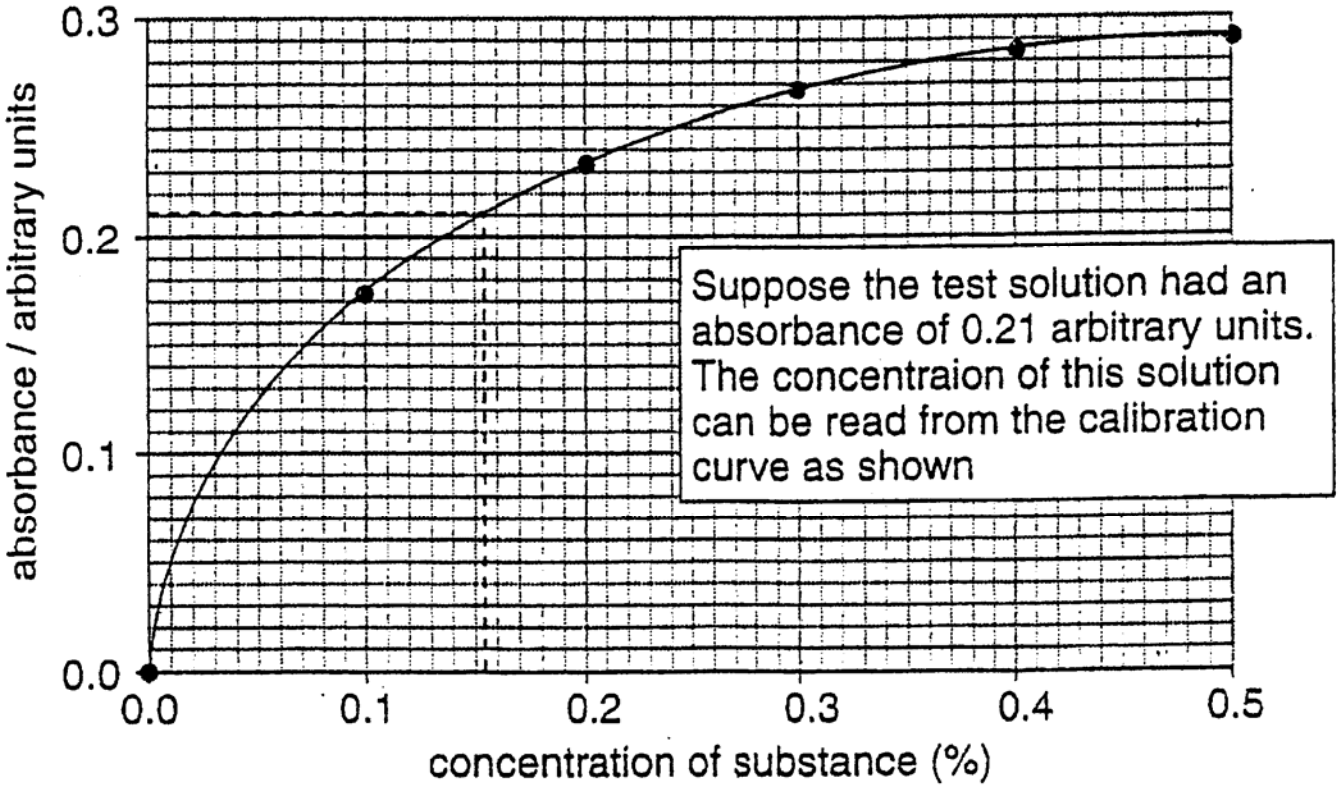
An example of a calibration curve is shown overleaf.

◆ 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.
- 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.

Figure 1: An example of a calibration curve for a colorimeter



◆ Materials

Food dye
Distilled water

◆ Apparatus

Test tubes
Colorimeter
Tissues
Graduated pipettes and pipette filler
Cuvettes

◆ Procedure

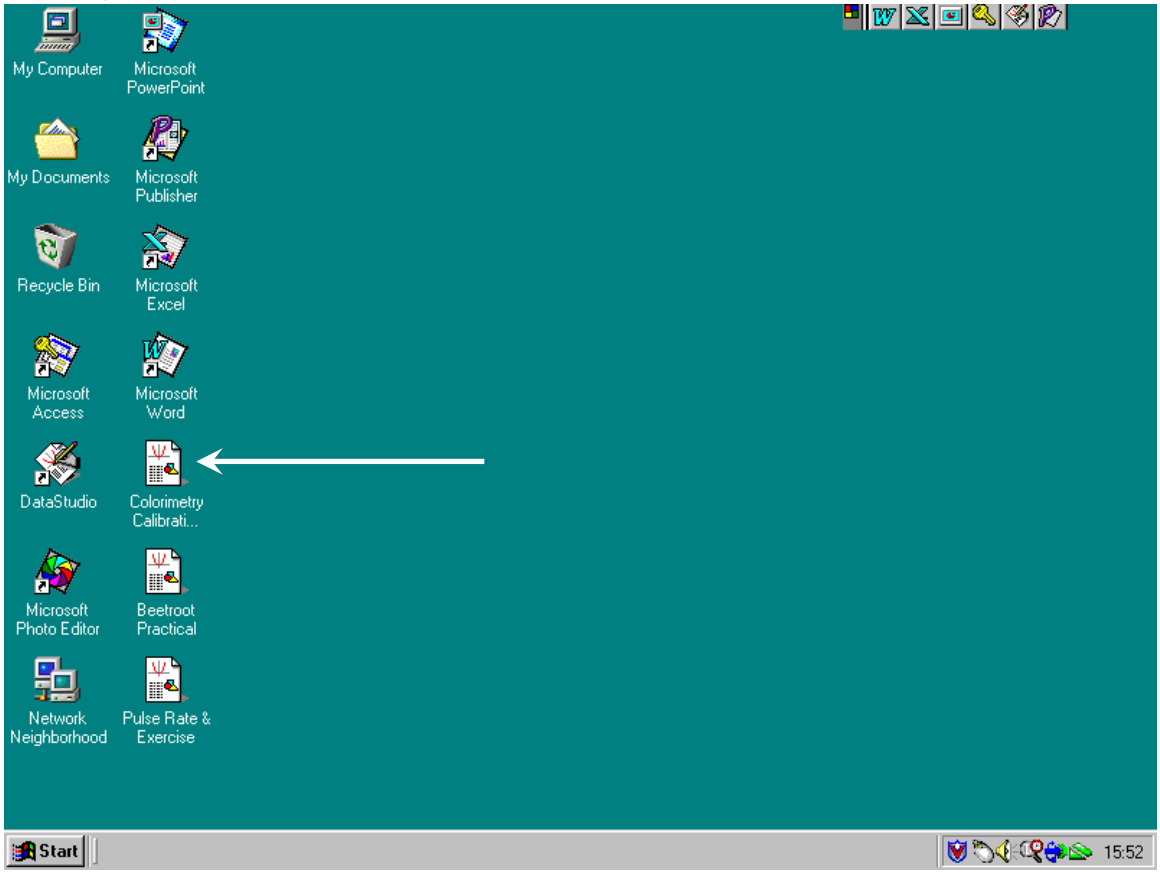
Prepare a range of standard solutions, including a blank (distilled water) for your calibration curve as shown in Table 1

Table 1: Dilutions of a food dye for a colorimetric investigation

Volume of food dye / cm^3 (stock solution)	**Volume of distilled water / cm^3**	**Concentration of solution / %**
10.0	0	100 **(undiluted)**
8.0	2.0	80
6.0	4.0	60
4.0	6.0	40
2.0	8.0	20
0.0	10.0	0 **(blank)**

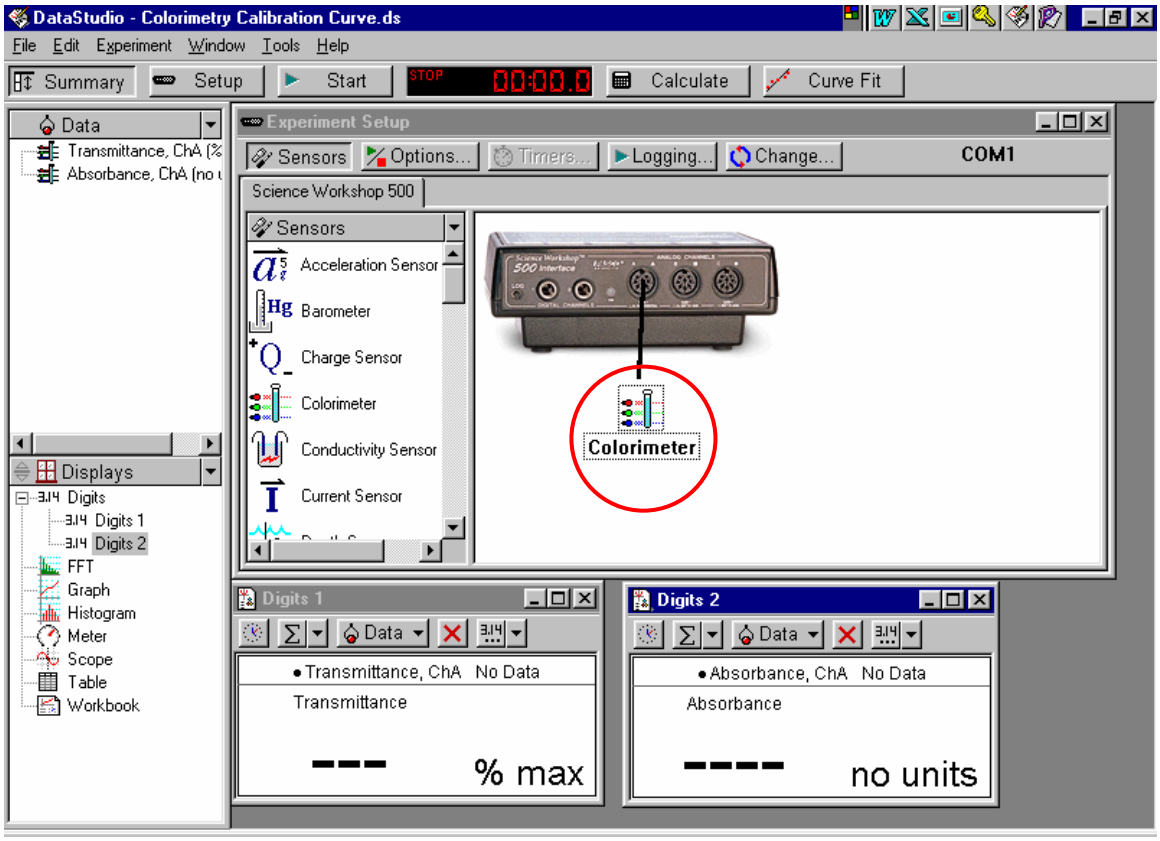
To measure the absorbance of the samples you will use a Data Logging computer package.

When you see the screen



Double click on the ***"Colorimetry Calibration"*** 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 Concentrations

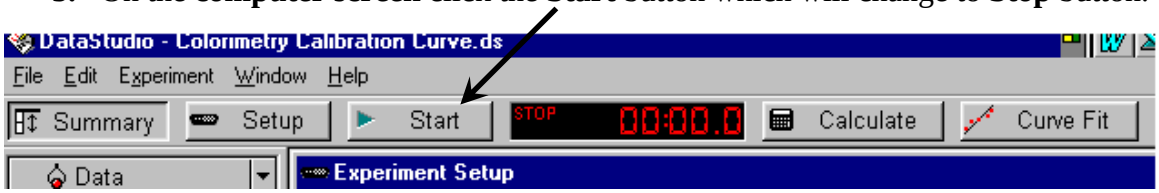
1. You need to construct an appropriate table to record your results — see **SKILL D in COURSEWORK BOOKLET.**

2. Fill cuvette with sample for 0% solution 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.

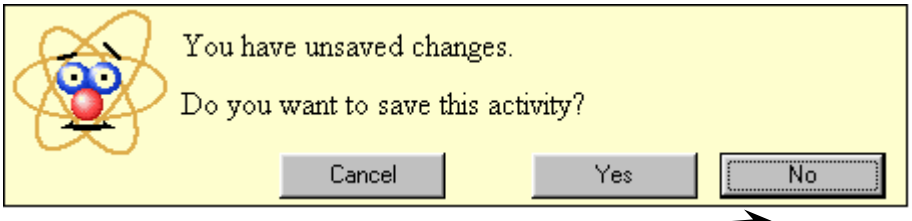
7. Press **Stop** on the **colorimeter** to STOP reading.

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

9. Rinse the cuvette and fill with an **unknown solution**, cap the cuvette. **Repeat stages 3 – 7.**

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

11. You will see this window on screen:



12. Click **NO.**

- Plot a calibration curve for your standard solutions, as shown in Figure 1.
- Read off the concentration of the unknown solution from the calibration curve.