**Determining glucose concentration STUDENT**

**Introduction**

A quantitative test tells us not only whether a particular substance is present, but at what concentration. Reducing sugars such as glucose reduce the Cu2+ in Benedict’s reagent to Cu1+ causing a precipitate to form. This results in a colour change that can be used as a simple qualitative or semi-quantitative test. By measuring the concentration of Cu2+ remaining in solution using a colorimeter we can transform this into a fully quantitative test.

**Aim**

* To determine the concentration of glucose in a solution of unknown concentration.

**Intended class time**

* 60 minutes

**Equipment**

* Benedict’s reagent
* Distilled water
* 1% glucose solution
* Unknown glucose solution
* 5 x 50 cm3 beakers
* 7 boiling tubes
* 6 x 5 cm3 syringes
* Water bath set at 100˚C
* 14 dropping pipettes
* Centrifuge
* 8 centrifuge tubes
* 8 clean cuvettes
* Colorimeter

**Method**

1. Collect 10 cm3 of 1% glucose solution in a 50 cm3 beaker.
2. Using a clean syringe for each transfer and mixing thoroughly at each stage, make a dilution series of glucose in distilled water to give the following concentrations: 1.00%, 0.50%, 0.25%, 0.13%, 0.06%. Label these as you create them.
3. Label the 7 boiling tubes: 1.00%, 0.50%, 0.25%, 0.13%, 0.06%, 0.00% and UNKNOWN.
4. Next add 5 cm3 of the corresponding glucose concentration to each tube. Put 5 cm3 distilled water in the 0.00% tube and 5 cm3 of the Unknown glucose solution in the UNKNOWN tube.
5. Then add 2 cm3 Benedict’s reagent to each tube.
6. Place all 7 boiling tubes into the water bath and incubate for 15 minutes.
7. Remove the boiling tubes from the water bath and stand them in a rack to cool.
8. Pipette 2 cm3 of each solution into a labelled 2 cm3 centrifuge tube. It does not matter if any precipitate in the boiling tube has started to settle out.
9. Create an eighth centrifuge tube with 2 cm3 water to act as a balance tube. Then centrifuge all 8 centrifuge tubes for 2 minutes ensuring that every tube has a balancing partner opposite it in the rotor head.
10. Set the colorimeter to ‘red light’ and use distilled water in a cuvette to set 100% transmission.
11. Carefully pipette the supernatant from each centrifuge tube in turn into a clean cuvette, measure the transmission in the colorimeter and record the results in a suitable table.
12. With the results from 1.00%, 0.50%, 0.25%, 0.13%, 0.06% and 0.00% create a calibration curve.
13. Use your calibration curve to find the glucose concentration of the Unknown glucose solution and record this.

**Extension questions**

1. Explain why the transmission of red light increases as the glucose concentration in the sample rises.
2. Why is it necessary to centrifuge the sample before taking colorimeter readings?
3. If the reading for your unknown glucose solution did not fall within the range of your calibration curve (i.e. suggests a concentration higher than 1%) what could you do to obtain an accurate value for its concentration?

**To submit**

For this piece of work to count towards Practical Activity Group 5 of the GCE Biology Practical Endorsement, you need to have your table of results, your calibration curve and a value for the concentration of the unknown solution. You also need to have considered the above questions as the answers to these will aid you in preparation for your written examinations.