AQA Biology A-Level

Required Practical 11

Production of a dilution series of a glucose solution and use of colorimetric techniques to produce a calibration curve with which to identify the concentration of glucose in an unknown 'urine' sample.

A **quantitative Benedict's test** can be used to find an unknown concentration of glucose in a sample by creating a **calibration curve**, using known concentrations. This is because glucose is a **reducing sugar**, hence will reduce **Cu2+ ions** in the Benedict's solution to **insoluble copper (I) oxide**. This causes a colour change from **blue to brick red**. The extent of this colour change depends on the concentration of glucose in the sample, so the concentration can be found by measuring the **absorbance** in a **colorimeter**.

Equipment list

- 10 mmol dm^{-3} glucose solution
- Distilled water
- "Urine" samples of unknown glucose concentration
- Benedict's solution
- Boiling tubes
- Boiling tube rack
- Water bath
- Colorimeter
- Cuvettes

Method

1. Create a **dilution series** of glucose using distilled water. Use six boiling tubes to make concentrations ranging from **0 to 10 mmol dm^{-3}**.
2. Place 2cm^3 of each of the **unknown samples** in separate boiling tubes.
3. Add 2cm^3 of **Benedict's solution** to all boiling tubes.
4. Place boiling tubes in a **water bath at 90°C** for **four minutes**.
5. Use tongs to take the boiling tubes out of the water bath. Leave to cool.
6. **Zero the colorimeter** using a cuvette with **distilled water**. The colorimeter should be set to the **red filter**.
7. Place known samples into cuvette and measure the **absorbance** of each using the colorimeter.
8. Make a **calibration curve**, plotting the **absorbance** against the **known glucose concentrations**.

9. Measure the **absorbance of the unknown samples** using the colorimeter. Use the calibration curve to determine glucose concentrations.

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Benedict's solution	Irritant to skin and eyes	Wear eye protection	Wash eyes/skin with cold water	Low
Broken glass	Cuts from sharp object	Take care when handling glassware; keep away from edge of desk	Elevate cuts; apply pressure; do not remove glass from wound; seek medical assistance	Low
Hot liquids	Scalding	Handle with care; use tongs to remove boiling tubes from water bath; wear eye protection; keep away from edge of desk	Run burn under cold water; seek medical assistance	Low

Graph

For calibration curve:

- Plot **absorbance** against **known glucose concentrations.**
- Draw **line of best fit.**

Conclusion

You will usually be asked to determine which of the unknown samples of "urine" is most likely to come from a person at a **high risk of diabetes**:

- **A high concentration of glucose in a sample indicates that the person is at high risk of diabetes** (the normal range is 0-0.8 mmol dm^{-3}). This is because the **lack of insulin** produced by **β islets of Langerhans**, along with the **sensitivity to insulin of liver cells**, leads to a **higher** than usual concentration of glucose in the blood.
- The blood is filtered in the **glomerulus**. A high concentration of glucose in the blood leads to a high concentration of glucose in the glomerular filtrate. Consequently, some is **not able to be reabsorbed** in the proximal convoluted tubule and remains in the filtrate and thus in the urine.