Finding out the concentration of glucose in urine using a colorimeter

Specification references
- 3.1.2
- AT b
- AT c

Learning objectives
After completing the practical you should be able to:
- produce a dilution series of a glucose solution
- use a colorimeter to measure the absorbance of known and unknown solutions
- draw a calibration curve graph
- use your calibration curve to identify the correct glucose concentration of your urine solution.

Background
Around 3 million people in the UK are living with diabetes and this number is increasing every year. One of the symptoms of diabetes is finding glucose in the patient’s urine. You are going to be given a sample of urine and asked to find its concentration of glucose. In order to do this, you will need to produce a serial dilution of a known glucose solution, carry out the test for reducing sugars, and make a calibration curve graph of the known concentrations of glucose. This can then be used to identify the concentration of glucose in your patient’s urine. Do they have diabetes?

Safety
- Wear eye protection.
- Use a test tube holder when removing hot test tubes from the water bath.

Equipment and materials
- 0.32 M glucose solution
- distilled water
- Benedict’s quantitative solution
- 2 × 10 cm³ measuring cylinders
- 5 × 5 cm³ syringes
- 7 × 2 cm³ syringes
- 1 × 1 cm³ syringe
- colorimeter
- test tube holder
- urine sample
- 13 × test tubes
- 5 × test tube bungs
- test tube rack
- 8 × dropping pipettes
- permanent marker pen
- 8 × cuvettes
- water bath set to 95 °C
Method

Carrying out the serial dilution

1. Label six test tubes with the permanent marker pen as 0.32 M, 0.16 M, 0.08 M, 0.04 M, 0.02 M, and 0.01 M, and place into a test tube rack.

2. Using the 10 cm³ measuring cylinder, measure out 10 cm³ of the 0.32 M glucose solution. It is important to be as accurate as possible, so use one of the dropping pipettes to make sure the meniscus of the solution is exactly on the 10 cm³ line of the measuring cylinder (see Figure 1).

3. Add the 10 cm³ of 0.32 M glucose solution to the test tube labelled 0.32 M.

4. Using the other 10 cm³ measuring cylinder and a dropping pipette, add 5 cm³ of distilled water to each of the other five test tubes.

5. Using a 5 cm³ syringe, take out 5 cm³ of the solution in the 0.32 M test tube and add it to the test tube labelled 0.16 M.

6. Invert the test tube three times. You do this by putting a test tube bung into the test tube and turning the test tube upside down.

7. Using a new 5 cm³ syringe, take out 5 cm³ of the solution in the 0.16 M test tube and add it to the test tube labelled 0.08 M. Invert the test tube three times.

8. Repeat step 7 for the 0.04 M, 0.02 M, and 0.01 M test tubes (see Figure 2).
**Carrying out the test for reducing sugars**

1. Take 2 cm\(^3\) from the solution in the 0.32 M test tube using a 2 cm\(^3\) syringe, and add it to a new test tube. Label this as 0.32 M B. Using a 1 cm\(^3\) syringe, add 1 cm\(^3\) of Benedict’s quantitative solution.

2. Do the same with the solutions in the 0.16 M, 0.08 M, 0.04 M, 0.02 M, and 0.01 M test tubes, labelling them as 0.16 M B, 0.08 M B, 0.04 M B, 0.02 M B, and 0.01 M B.

3. Add these six test tubes to a water bath set to 100 °C for 5 minutes.

4. Remove the test tubes and place back into a test tube rack. Allow the precipitate to settle to the bottom of the test tube.

**Measuring the absorbance with a colorimeter**

1. Fill a cuvette almost to the top with the solution from the 0.32 M B test tube. Do the same with the solutions from the 0.16 M B, 0.08 M B, 0.04 M B, 0.02 M B, and 0.01 M B test tubes.

2. Fill another cuvette almost to the top with distilled water. This will be your blank.

3. Make sure the colorimeter is set to 0.0 and the filter is set to 680 nm.

4. Place the blank cuvette into the colorimeter and press the reading button. This should give an absorbance of 0.0.

5. Place the 0.32 M cuvette into the colorimeter and press the reading button. Record the absorbance. Do the same with the other cuvettes.

**Drawing a calibration curve graph**

1. Plot the absorbance of each concentration of glucose (y-axis) against its concentration (x-axis). Join the points together using a sharp pencil and a ruler, or draw a line or curve of best fit.

**Finding the concentration of glucose in a urine sample**

1. Take 2 cm\(^3\) of the urine sample and add to a test tube. Label this as U.

2. Add 1 cm\(^3\) of Benedict’s quantitative solution to the test tube.

3. Place the test tube in a water bath set to 100 °C for 5 minutes.

4. Remove the test tube and place it into the test tube rack. Allow the precipitate to settle to the bottom of the test tube.

5. Fill a cuvette almost to the top with the solution from the test tube.

6. Use the blank cuvette and repeat steps 3 and 4 from ‘Measuring the absorbance with a colorimeter’.

7. Place the U cuvette into the colorimeter and press the reading button. Record the absorbance.

8. On the calibration curve graph, draw a line from this absorbance on the y-axis to the line of the graph, and down to the x-axis. The point on the x-axis where this line touches will be the concentration of glucose in the urine sample.
Results

Record the absorbance of each known glucose solutions in the table.

<table>
<thead>
<tr>
<th>Concentration of glucose (M)</th>
<th>0.32</th>
<th>0.16</th>
<th>0.08</th>
<th>0.04</th>
<th>0.02</th>
<th>0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Plot your calibration curve on the graph paper.

Record the absorbance of the urine sample in this table. Use the calibration curve to work out the concentration of the glucose and record it in the table.

<table>
<thead>
<tr>
<th>Absorbance of urine sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of glucose in urine (M)</td>
</tr>
</tbody>
</table>

Questions

1. The normal concentration of glucose in urine is 0–0.0008 M.
   a. What was the concentration of glucose in the urine sample?
   b. Does this person have diabetes?
   c. Is this a good indication of the person’s blood glucose level?
   d. Why do you think that?

2. You carried out a serial dilution of a 0.32 M glucose solution.
   a. How do you make sure that you measured a volume accurately?
   b. Why was that important?
   c. Why did you invert the test tubes?
   d. If you took 1 cm$^3$ of a 5 M glucose solution and added it to 99 cm$^3$ of distilled water, what would the concentration be?

3. You carried out the Benedict’s test for reducing sugars using Benedict’s quantitative solution.
   a. What colours did the Benedict’s reagent change into after being in the water bath?
   b. What do these colours indicate?
   c. Can these colours tell you the exact concentration of the glucose?
   d. Explain why it was important that you did not leave the test tubes in the water bath for longer than 5 minutes.

4. You measured the absorbance of the glucose concentrations using the colorimeter.
   a. Describe how a colorimeter works.
   b. Explain why it was important to use a blank cuvette to set the absorbance to 0.0.
   c. Why did you set the filter to 680 nm?
   d. Explain how a colorimeter allows you to obtain a quantitative measure of the concentration of an unknown solution.