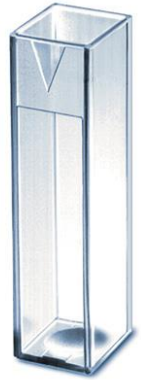
Carbohydrates Revision

Colorimetry – quantitative method to determine glucose concentration

- Colorimeter used to quantitatively measure the absorbance or transmission of light by a coloured solution.
- The more concentrated a solution is, the more light it will absorb and the less light it will transmit.
- This can be used to calculate concentration of reducing sugar present.

1) Filter placed in colorimeter
2) Colorimeter calibrated using distilled water
3) Benedict's test performed on range of known concentrations of glucose.
4) Resulting solutions filtered to remove precipitate.
5) % transmission of each of the solutions of glucose was measured using the colorimeter.
6) Calibration curve plotted.



cuvette

1. Explain why the transmission of red light increases as the glucose concentration in the sample rises.

As the concentration of glucose in the sample increases, more blue cu2+ ions (present in the added Benedict's solution) will be reduced to cu+ ions. These cu+ ions form a precipitate in the bottom of the centrifuge tubes. Therefore, as the quantity of precipitate increases, the remaining solution will be lighter – and a lighter solution will transmit more light. The more concentrated the glucose solution, the less unreacted cu2+ ions remain – therefore the solution is a paler blue colour. This means that the transmission of red light increases as the concentration of glucose increases.

Centrifuge tube

1. Why is it necessary to centrifuge the sample before taking colorimeter readings?

We centrifuge the samples in order to separate the coloured solution of cu2+ ions from the cu+ ions which form a precipitate. The solution is then added to a cuvette, leaving the precipitate behind in the bottom of the centrifuge tubes. The solution is filtered in this way, removing the solid precipitate that could disrupt readings if left in.

1. 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?

You could use extrapolation, referring to the calibration curve. Or, the test could be repeated with a glucose solution that is more concentrated than 1%. This larger sample would allow you to identify the unknown solution.