4. Determination of the content of reducing sugars using Benedict' s test

Benedict' s solution is designed to detect the presence of reducing sugars. In hot alkaline solutions, reducing sugars reduce the blue copper(II) ions to brick red copper(I) oxide precipitate. As the reaction proceeds, the colour of the reaction mixture changes progressively from blue to green, yellow, orange and red. When the conditions are carefully controlled, the colouration developed and the amount of precipitate formed depends upon the amount of reducing sugars present. Hence, in most conditions, a sufficiently good estimation of the concentration of glucose-equivalent reducing sugars present in a sample can be obtained.

Procedure

1. Use 2% glucose solution and distilled water to prepare a series of standard glucose solutions of different concentrations : 0.4%, 0.6%, 0.8%, 1.0%, 1.2% and 1.4%.
2. Pipette 0.5 cm^3 of each of the standard glucose solutions into labelled test tubes, each containing 5 cm^3 of Benedict' s solution. Mix by shaking.
3. Make a serial dilution of a sample containing reducing sugar(s) (e.g. an unknown glucose solution or grape extract) of 1:2, 1:5, 1:10, 1:20, 1:50 and 1:100 with distilled water.
4. Pipette 0.5 cm^3 of each of the original sample and its dilutions prepared in step (3) into an appropriately labelled test tube containing 5 cm^3 of Benedict' s solution. Mix by shaking.
5. Heat all test tubes in a boiling water bath for 3 minutes.
6. Remove the test tubes from the water bath. Read and grade the colour of the reaction mixtures immediately without setting aside to cool.

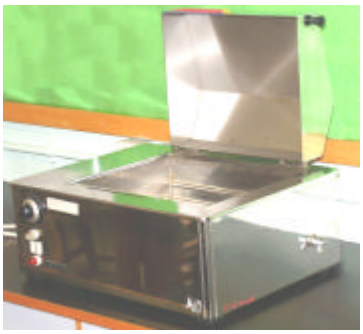

Fig. 1 Water bath

7. Match the colour of the reaction mixtures containing the sample and its dilutions with those containing the standard glucose solutions to determine the concentration of glucose-equivalent reducing sugars present in the sample or its dilutions.
8. Calculate the concentration of glucose-equivalent reducing sugars present in the original sample.

Note

1. This method measures all reducing substances present. Hence compounds, other than reducing chemicals (e.g. ascorbic acid), that can reduce the hot alkaline copper(II) ions may cause a positive error.
2. The extent of this reaction depends markedly upon the conditions of temperature, duration of heating and degree of alkalinity. Hence the specified conditions must be followed in all determinations, and standards should always be heated together with the sample and its dilutions in the same boiling water bath.

6-8 pieces of pumice stones could be added to reduce bumping of boiling water.

An Alternative Method

Procedure

1. Use 2% glucose solution and distilled water to prepare a series of standard glucose solutions of different concentrations : 0%, 0.4%, 0.8%, 1.2%, 1.6% and 2%.
2. Pipette 1 cm^3 of each of the standard glucose solutions into labelled test tubes, each containing 3 cm^3 of Benedict' s solution. Mix by shaking.
3. Make a serial dilution of a sample containing reducing sugar(s) (e.g. an unknown glucose solution or grape extract) of 1:5, 1:10, 1:50, 1:100, 1:500 and 1:1000 with distilled water.
4. Pipette 1 cm^3 of each of the original sample and its dilutions prepared in step 3 into labelled test tubes, each containing 3 cm^3 of Benedict' s solution. Mix by shaking.
5. Heat all test tubes in a boiling water bath for 5 minutes.
6. Remove the test tubes from the water bath. Place them in a test tube rack and let the precipitates settle.
7. Match the appearance and the relative amount of precipitates in the test tubes containing the sample and its dilutions with those in the test tubes containing the standard glucose solutions to determine the concentration of glucose-equivalent reducing sugars present in the sample or its dilutions.
8. Calculate the concentration of glucose-equivalent reducing sugars present in the original sample.