**Qualitative testing for biological molecules – glucose (Benedict’s test) STUDENT**

**Introduction**

A qualitative test just gives us a positive or negative result to tell us whether a particular substance is present. It does not tell us how much of the substance is present (or at what concentration). A positive result for the Benedict’s test for reducing sugars is a colour change due to the reduction of Cu2+ to Cu1+.

**Aims**

* To test qualitatively for the presence of glucose (a reducing sugar) and sucrose (a non-reducing sugar).

**Intended class time**

* 60 minutes

**Chemicals**

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| --- | --- |
| Benedict’s reagent | Low hazard but avoid contact with eyes |
| 2.0 mol dm-3 hydrochloric acid | HSE warning symbol  Causes skin and eye irritation |
| Sodium hydrogen carbonate | Low hazard |
| B | No known hazard |
| C | No known hazard |
| D | No known hazard |

**Equipment**

* Benedict’s reagent
* 2 dropping pipettes
* 8 boiling tubes
* 8 x 5 cm3 syringes
* Samples in beakers labelled ‘**A**, **B**, **C** and **D**’  
  *These are 1% glucose, 1% sucrose, 1% protein and distilled water but not necessarily in that order. By performing the qualitative tests in this activity you will identify which is glucose and which is sucrose.*
* Water bath set to 100˚C
* 2 mol dm-3 hydrochloric acid
* Sodium hydrogen carbonate
* Small spatula
* pH paper
* Marker pen

**Health and Safety**

* Eye protection must be worn throughout the activity due to the presence of boiling water and acid.
* Benedict’s reagent is low hazard but contact with eyes should be avoided.
* The boiling water bath presents a scalding hazard.
* 2.0 mol dm-3 hydrochloric acid is an irritant.

**Method 1: Testing for reducing sugar**

1. Label four boiling tubes **A** – **D** and place 4 cm3 of the corresponding samples into each using a fresh syringe each time.
2. Use a dropping pipette to add ̴̴ 2 cm3 Benedict’s reagent to each of the four samples.
3. Record your observations of each sample immediately after adding Benedict’s reagent.
4. Place all four boiling tubes into the water bath.
5. After 2 minutes record your observations of each sample, replacing the samples in the water bath afterwards.
6. After a further 2 minutes (4 minutes total incubation time) remove the tubes from the water bath and record your final observations of each sample and your conclusions about the presence or absence of reducing sugar in the samples (and therefore which letter corresponds to the glucose solution).

**Method 2: Testing for non-reducing sugar**

*Note: to carry out a test for non-reducing sugars you must first test the sample for reducing sugars. Only by obtaining a negative result for reducing sugar followed by a positive result for non-reducing sugar can you be sure you have a non-reducing sugar in your sample.*

1. Put 4 cm3 of each of the samples, **A** – **D**, into its own labelled boiling tube using a fresh syringe each time.
2. Use a dropping pipette to add ̴̴ 2 cm3 of 2 mol dm-3 hydrochloric acid to each of the four samples.
3. Place all four boiling tubes into the water bath and incubate for 2 minutes.
4. Remove the tubes from the water bath and allow them to cool.
5. Next neutralise the acid by gradually adding sodium hydrogen carbonate to each sample until no more effervescence is observed.
6. Check the pH with indicator paper. If the pH is still lower than pH 7, repeat step 5. It does not matter if the pH has risen slightly above pH 7.
7. Use a dropping pipette to add ̴̴ 2 cm3 Benedict’s reagent to each of the four samples.
8. Record your observations of each sample immediately after adding Benedict’s reagent.
9. Place all four boiling tubes into the water bath.
10. After 2 minutes record your observations of each sample, replacing the samples in the water bath afterwards.
11. After a further 2 minutes (4 minutes total incubation time) remove the tubes from the water bath and record your final observations of each sample and your conclusions about the presence or absence of non-reducing sugar in the samples (and therefore which letter corresponds to the sucrose solution).

**Extension questions**

1. What improvements can you suggest to the method you were given for this qualitative test?
2. What changes would you make to obtain semi-quantitative results?
3. Outline a method for a fully quantitative test for reducing sugars.
4. Suggest how you could distinguish a sample of glucose from a sample of maltose using Benedict’s reagent. *[Hint: maltose is a disaccharide reducing sugar]*.

**To submit**

For this piece of work to count towards Practical Activity Group 9 of the GCE Biology Practical Endorsement, you need to have evidence of the positive and negative results of the Benedict’s tests as described above. You also need to have considered the above questions as the answers to these will aid you in preparation for your written examinations.