

### Practical 3 - The identification of biological chemicals present in solutions






This practical focuses on making decisions about measurements and observations, recording and presenting data and observations, interpretation, drawing conclusions and suggesting improvements. You will also develop other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Decide what tests to carry out and what observations to make
- Use an appropriate means to record your observations, constructing any tables before you make the observations
- Describe and summarise the key points of your observations
- Draw conclusions in terms of the presence or absence of different chemicals in the solutions
- Suggest alternative strategies for identifying some of the materials

#### Safety information

	You should wear eye protection throughout this practical.
	Amylase is <b>harmful</b> , avoid contact with eyes and skin.
 	Benedict's solution is <b>harmful</b> and <b>dangerous to the environment</b> .
	1 mol dm <sup>-3</sup> hydrochloric acid is <b>harmful</b> .

#### Background information

- Make sure that you know how to carry out Benedict's test, what it is used for and what the positive and negative results should be.
- Make sure that you know how to carry out biuret test, what it is used for and what the positive and negative results should be.
- Think about how Benedict's test and the enzyme amylase can be used to confirm the presence of a polysaccharide such as starch.
- Think about how acid hydrolysis, neutralisation and Benedict's test can be used to confirm the presence of the non-reducing disaccharide, sucrose.

You will use the materials provided to identify the unknown materials in the solutions A, B, C and D

- Read and think about the information above.
- The solutions A, B, C, D and E each contain only one of the following materials, but not necessarily in this order
  - A reducing sugar
  - A non-reducing sugar
  - A polysaccharide that can be hydrolysed by amylase
  - Proteins including amylase
  - No dissolved material
- You are also provided with materials for biuret test and for Benedict's test, as well as dilute hydrochloric acid, calcium hydrogen carbonate powder and a waterbath at 35°C

### Method

#### Preparations and making observations

1. You need to decide what tests to do and in what order so that it is possible to use the amylase to test some of the other solutions.
2. Decide how you are going to record your observations so that it will be absolutely clear what you did to which solutions, what you observed and your interpretation of the observations.
3. Prepare a piece or pieces of paper in accordance with your decisions.
4. Make a risk assessment of your proposed methods and decide what precautions to take to reduce the likelihood of an accident and to reduce the damage any accidents might cause – ask your teacher to confirm that you may go ahead with the tests.
5. Carry out the tests with full regard to safety, recording your observations and interpretations.
6. Record the identity of the unknown solutions.

#### Write-up

- hand in your original laboratory records, including your methods, observations and interpretations.
- suggest improvements to the method including some of the following:
  - a simpler way of testing for the presence of starch,
  - starch would also be hydrolysed by acid. Suggest a better order to do your tests if this caused you difficulties, or a way of using amylase to confirm that it is non-reducing sugar rather than starch that is present,
  - if a solution contained a small amount of reducing sugar and also non-reducing sugar, suggest how it might be possible to use repeated benedict's tests, filtering the precipitate out after each, to remove the reducing sugar before testing for non-reducing sugar.

### Practical 3 - Lesson Plan

#### The identification of biological chemicals present in solutions

##### Context

A practical investigation set in the context of 9700 syllabus – The identification of biological chemicals present in solutions

##### Key aims of the lesson

This practical is designed to develop the skills of decision-making, observation, interpretation and evaluation.

##### Intended learning outcomes

By the end of this practical the student should be able to:

- Decide what tests to carry out and what observations to make
- Use an appropriate means to record your observations, constructing any tables before you make the observations
- Describe and summarise the key points of your observations
- Draw conclusions in terms of the presence or absence of different chemicals in the solutions
- Suggest alternative strategies for identifying some of the materials

##### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

##### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – student worksheet given out for students to read in preparation for the practical lesson. To ensure understanding of methods and their use as well as to consider decisions about which observations to make and in what order, and the means of recording
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review of biochemicals and biochemical tests - key points written on board
5 - 8	<b>Introduction to method</b> – Teacher briefly reminds students of methods of tests, demonstrating as necessary. Teacher answers any student questions on procedure. Teacher emphasises safety concerns with acidic and harmful solutions and heating of solutions
8 - 45	<b>Carrying out the practical</b> – students carry out the practical work and record their observations and interpretations.
45 - 50	<b>Clear up</b> – Students clear away apparatus as soon as they have finished

50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Teacher to emphasise importance of handing in original laboratory records – (these can be collected in at the end of the lesson, photocopied and given back to students if they are thought likely to 'copy them up'). Practical write up to be completed in following lesson or as homework activity.
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### Useful information

The most effective methodology is likely to be

- Test a small sample of each of the solutions with biuret test to identify the protein including amylase – set aside solution B for later use (identified with this test)
- Carry out Benedict's test, heating the tubes gently to 90°C until one gives a positive result, on small samples of each of solutions A, C, D and E to identify which contains reducing sugar – set aside solution A (identified with this test)
- Mix small samples from each of solutions C, D and E with equal volumes of solution B and incubate at 35°C for a few minutes. Test sample from each of the incubated tubes with benedict's test to identify in which the polysaccharide has been hydrolysed to give a reducing sugar – set aside tube E (identified with this test)
- Mix small samples from tubes C and D with an equal volume of hydrochloric acid and boil carefully for two minutes. Add a small excess of calcium hydrogen carbonate (until it just stops effervescing) to neutralise the acid and test with Benedict's test to identify which contains non-reducing sugar (C) and therefore which contains only water (D).

### Summary of contents of the five solutions

solution	material present in solution
A	reducing sugar
B	proteins including amylase
C	non-reducing sugar
D	no dissolved material
E	polysaccharide that can be hydrolysed by amylase

- To improve the methodology, iodine solution would be a more elegant way of confirming the presence of the polysaccharide.
- To detect non-reducing sugar in the presence of reducing sugar – react the latter with excess Benedict's and filter out the resulting precipitate. Re-test to confirm the absence of reducing sugar. Now hydrolyse with acid, neutralise and re-test.

### Practical 3 - Technical information

#### The identification of biological chemicals present in solutions

The apparatus and materials required for this practical are listed below.


The amount of apparatus listed is for one student or one group of students if they are to work in groups.



- 1 5 small beakers or other containers as follows


label on beaker	20 cm <sup>3</sup> of solution in beaker
A	1 mol dm <sup>-3</sup> glucose solution made by dissolving 18 g of glucose in 80 cm <sup>3</sup> of distilled water and making up to 100 cm <sup>3</sup>
B	10% egg white and 1% amylase solution made up by dissolving 10 cm <sup>3</sup> of fresh egg white (or 1 g of dried egg white) and 1 g of amylase in 80 cm <sup>3</sup> of cold distilled water, mixing until dissolved and making up to 100 cm <sup>3</sup>
C	0.5 mol dm <sup>-3</sup> sucrose solution made by dissolving 17 g of sucrose in 80 cm <sup>3</sup> of distilled water and making up to 100 cm <sup>3</sup>
D	no dissolved material
E	polysaccharide that can be hydrolysed by amylase

- 2 5 test tubes in a rack and a means of washing the tubes such as a sink and running water
- 3 a glass marker, such as a wax pencil or a permanent OHP pen or small labels and pencil
- 4 the usual materials that the students are used to using for biuret test, labelled appropriately
- 5 the usual materials and heating arrangements that the students are used to using for Benedict's test, labelled appropriately
- 6 1 mol dm<sup>-3</sup> hydrochloric acid in a small dropper bottle, labelled hydrochloric acid
- 7 Sodium hydrogen carbonate powder in a small specimen tube with a stopper and a spatula that fits in the tube to dispense it
- 8 A thermostatic waterbath or plastic trough containing water at about 35°C for use as a waterbath

#### Safety Precautions/Risks

Amylase = H 

Benedict's solution = H, N  

Hydrochloric acid (1 mol dm<sup>-3</sup>) = H 

A risk assessment should be carried out as a matter of course.