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Sugars
(identification of unknown saccharide)

Task 1

The aim of this task is to make selected tests to detect the presence of known sugars. The results of these tests will be useful in performing the second task. Therefore, note the results of performed tests.

Procedures

1. Molisch's test

Principles of determination

This is the general test for carbohydrate detection. Negative result of this test is more informative than a positive result. Negative result (lack of staining) informs that in a tested solution, there is no sugars, both in free form or attached to other compounds, e.g. glycoproteins and glycolipids. Positive result of this test can occur in presence of sugars and also other non-sugar substances, like acetone, formic acid, lactic acid, oxalic acid, citric acid. All these compounds can condense with alpha-naphthol and form colourful products (false positive results).

Procedure

Pipette 1 cm^3 of solution of chosen sugar to a tube and add 2 – 3 drops of Molisch reagent (solution of alpha-naphthol in an alcohol) and stir. Then, add **carefully** 1 cm^3 of concentrated sulphuric acid pouring it on the wall of tube set diagonally. As a result, acid layer on the bottom of the tube, under the water solution occurs. This kind of adding a reagent is called under layering. Wait a few minutes. On the border between two layers, red-purple ring is formed gradually.

Remember! Concentrate solutions of acids and/or bases cannot be piped by mouth!
They have to be dozen from a special injector.

2. Tollens's test for pentose detection

Principles of determination

Tollens reagent includes phloroglucinol {$C_6H_3(OH)_3$} dissolved in a concentrated chloric acid solution. Concentrated chloric acid solution works as dehydration factor. Pentose loses three molecules of water and is transformed to furfural. Phloroglucinol concentrates with furfural producing coloured product of reaction which has cherry colour.

Procedure

Measure 1 cm^3 of Tollens reagent to a tube and add 1 – 2 drops of arabinose solution. Heat it over the burner carefully and boil it over a dozen of seconds stirring briskly until cherry colour appears.

3. Seliwanoff's test

Principles of determination

This test detects the presence of ketoses. Seliwanoff's reagent includes resorcinol {$C_6H_4(OH)_2$} dissolved in 12% HCl. Under the influence of 12% HCl, only ketoses are transformed to furfurals (ketohexoses transform to 5-hydroxymethylfurfural), whereas aldoses do not transform in these conditions. Resorcinol concentrates with furfural producing cherry colour.

Procedure

Mix in a tube 1 cm^3 of Seliwanoff's reagent with 2 drops of fructose solution. Heat it carefully over the burner for 30 second or a little longer under cheery colour appears.

Attention: sucrose gives also the positive result of this test because sucrose includes fructose in its molecule, and sucrose disintegrates in 12% HCl solution.

4. Benedict's test

Principles of determination

The Benedict's reagent consists of $CuSO_4$, sodium citrate and Na_2CO_3, therefore, it is base. During reaction, cooper ions having +2 oxidation number are reduced to Cu^{+1} and precipitate in form of Cu_2O which has orange tint. Unfortunately, ketoses i.e. fructose gives also positive result of this probe. This phenomenon occurs because ketoses transforms in base solution to aldoses. This reaction is called epimerisation.

Procedure

Mix 1 cm^3 of Benedict's reagent with a few drops of examined sugar solution (glucose) in a tube and boil it over the burner. Observe the appearing of orange or red precipitate of cooper oxide.

5. Barfoed's test

Principles of determination

This test allows for the distinction of reducing disaccharides and monosaccharides.

Reducing reactions in organic chemistry occur easiest in base environment. As acidity of environment increases, the reducing properties of sugars decreases. This phenomenon is used to distinguish monosaccharides from reducing disaccharides. Barfoed's reagent includes Cu ions, similarly to Benedict's and Fehling's reagents but has higher pH than these two reagents.

Procedure

Measure 1 cm^3 of Barfoed's reagent to three tubes marked as G, L and S. Add 1 cm^3 of glucose solution to tube G, 1 cm^3 of lactose solution to tube L, and 1 cm^3 of sucrose to tube S. Put them into a boiling water bath for 3 minutes, get them out of the bath and observe the results. Then, put all three tubs into the boiling bath again for next 12 minutes. After this period of time, observe the results again.

Try to explain the results.

Task 2

The aim of task 2 is the identification of unknown, tested sugar. To do this, you need to make the tests described in task 1. Characteristic results of these tests are given in the table below.

Tests:	Arabinose	Glucose	Fructose	Lactose	Sacharose
Benedict's	+	+	+	+	-
Barfoed's	+	+	+	*	-
Tollens's	+	-	-	-	+/-
Seliwanoff's	-	-	+	-	+

+ positive result; - negative result; * the result is negative after 3 minutes of heating in boiling water bath, and positive after 15 minutes, however, you need to pour out the content of the tube to observe light precipitate on the bottom of the tube

Procedure

1. Take randomly selected tube with solution of unknown sugar. Note the number of your sample.
2. Make the Molisch's test to be sure that your sample includes sugar.
3. Make the test with iodine to confirm or exclude the presence of polysaccharides, like starch. Procedure of the test with iodine: mix 1 cm^3 of sugar solution with a few drops of iodine solution (aqueous solution of potassium iodide). In the presence of starch, navy blue colour appears. Iodine molecules (I_2) penetrate inside the starch molecule which has the form of spiral. Inside this spiral, molecules of iodine forms complex bounds, producing an intense blue/black colour.
4. Make all other tests described in task 1.

Note the results of your tests in the table below. Which sugar is in your sample? Explain your answer.

Tests:	your results:
Molisch's	
with iodine	
Benedict's	
Barfoed's	
Tollens's	
Seliwanoff's	