

PRACTICAL BIOCHEMISTRY

for BDS

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JAYPEE

Foreword
Bharat M Modi

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Section 2

Qualitative Exercises



The qualitative exercises form an important section in practical biochemistry for the learners of biochemistry. Biochemistry is the study of biomolecules like carbohydrates, lipids, proteins, amino acids, vitamins, enzymes, etc. To study these biomolecules in laboratory we should be able to identify their presence or absence in a sample by chemical analysis. Qualitative analysis basically is concerned with this procedure of identification of the presence of a particular biomolecule in a given sample along with the analysis of the basic chemical nature of that biomolecule.

The dental students perform the biochemical analysis of biomolecules like carbohydrates, lipids and proteins.

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CHAPTER 5

Carbohydrate Analysis

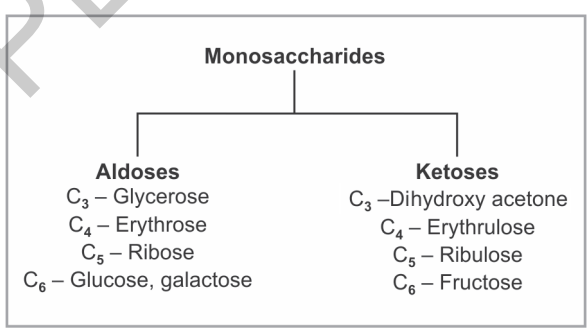
DEFINITION

Carbohydrates can be defined as aldehyde or ketone derivatives of polyhydroxyl alcohols and compounds which yield these on hydrolysis.

Classification

Carbohydrates can be classified as follows:

1. **Monosaccharides:** These are the simplest sugars which cannot be hydrolyzed further, e.g. Glucose, fructose. These can be further classified on the basis of number of functional group into aldoses and ketoses.
   a. **Aldoses:** Those monosaccharides which contain an aldehyde group, e.g. Glucose, galactose, ribose, etc.
   b. **Ketoses:** Those monosaccharides which contain a ketone group e. g. Fructose, ribulose, erythrulose, etc.
   Furthermore the aldoses and ketoses are classified on the basis of number of carbon atoms into:



2. **Disaccharides:** Those carbohydrates which can be hydrolyzed further to produce 2 monosaccharide units are called disaccharides, e.g. lactose, maltose.

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3. **Oligosaccharides:** Those carbohydrates which can be further hydrolyzed into 3–9 monosaccharide units are called oligosaccharides, e.g. raffinose.
4. **Polysaccharides:** Those carbohydrates which are made up of more than 10, i.e. 100s and 1000s of monosaccharide units are called as polysaccharides.

Polysaccharides can be further classified as:

a. **Homopolysaccharides:** Those polysaccharides which are made up of similar monosaccharide units, e.g. Starch, dextrin, glycogen, etc.
b. **Heteropolysaccharides:** Those polysaccharides which are made up of repeating units of [–amino sugar + uronic acid –] derivatives, e.g. Chondroitin, hyaluronic acid, etc.

The common carbohydrates used for analysis in the undergraduate laboratory are such that they include monosaccharides (glucose and fructose), disaccharides (***nonreducing***—sucrose, ***reducing***—lactose and maltose) and polysaccharides (homopolysaccharides—starch and dextrin).

TESTS FOR ANALYSIS OF CARBOHYDRATES

1. Molisch's Test

This test is given by all soluble carbohydrates and similar color is developed at the surface of insoluble carbohydrates under appropriate conditions. Thus, it is the group specific test for carbohydrates.

Reagents

Molisch reagent—1% α-naphthol in 95% ethyl alcohol
Concentrated sulfuric acid (conc. H_2SO_4).

Principle

Strong acids like sulfuric acid, hydrolyze polysaccharides and disaccharides into monosaccharides. The monosaccharides then are dehydrated to corresponding furfural derivatives. The reaction is due to the formation of furfural and hydroxyl methyl furfural by the action of concentrated acid on pentoses and hexoses. The furfural derivatives then condense with a naphthol to give the violet colored ring at the junction of the two liquids.

Procedure

Add 2 drops of Molisch reagent (5% solution of α - naphthol in 95% ethyl alcohol) to 2 ml dilute sugar solution (1%) in a narrow bore test tube. Mix thoroughly. Incline the test tube and add about 3 ml of concentrated

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sulfuric acid down the side of the test tube, forming a layer beneath the sugar solution. Be sure that the acid does not mix to a large extent with the solution. A purple violet color at the interface of the two liquids is an indication of positive test.

Precautions

- Always use a narrow test tube for obtaining a good ring at the junction.
- Do not confuse brown ring as a positive test.
- Do not add the acid too rapidly else the sugar would get charred and give a false-negative result.

2. Reduction Tests

Many sugars have the property of readily reducing the alkaline solution of the ions of certain metals such as copper, bismuth, iron and silver. The alkali in all these reagents brings about a considerable decomposition of the sugar molecule into reactive fragments (the enediols) which may also reduce the metal ions. This, while the total reduction for a given concentration of sugar may be constant under carefully defined conditions and is, therefore, utilizable for quantitative purposes.

a. Fehling's Test

Reagents

Fehling's solution A: 34.65 gm copper sulfate dissolved in water and made up to 500 ml.

Fehling's solution B: 125 gm potassium hydroxide and 174 gm Rochelle salt dissolved in water and made up to 500 ml.

These solutions are prepared separately. The Rochelle salt helps to prevent the precipitation of cupric hydroxide.

Procedure

Take 1 ml each of Fehling's solution A and B in a test tube and boil for 2 minutes. Now add 1 ml of sugar solution and boil for about a minute. Reducing sugars will give a precipitate of Cu_2O with the oxidation of sugars to their respective aldonic acids.

Drawbacks

- The reagent has low stability, therefore has to be prepared and stored separately.

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- The procedure is cumbersome since it involves two steps.
- The alkali used, i.e. potassium hydroxide is strong and causes destruction of the sugar.

b. Benedict's Test

Reagents

Copper sulfate – 17.3 gm

Sodium citrate – 173 gm

Sodium carbonate – 100 gm

Distilled water (to make) – 1 liter.

Dissolve sodium citrate and sodium carbonate in about 800 ml distilled water by application of heat. Dissolve copper sulfate in about 100 ml distilled water. Now add copper sulfate slowly to the carbonate citrate mixture with constant stirring. Make the volume up to 1 liter.

Principle

Both Fehling's test and Benedict's test are based upon the principle of reduction of cupric ions in hot alkaline medium (KOH in Fehling's test and Na_2CO_3 in Benedict's test) by reducing carbohydrates having a potentially free aldehyde or ketone group to Cu_2O and the carbohydrate gets oxidised to corresponding aldonic acid.

Procedure

Heat 5 ml Benedict's reagent with 8 drops of sugar solution. Boil the solution to then allow it to cool. There is a formation of red/yellow/green precipitate indicating a positive test.

Advantages of Benedict's Test

- The reagent is stable, i.e. has a longer shelf life.
- The procedure is single step procedure.
- The alkali used is a weak alkali therefore the sugar does not get charred/destroyed.

Clinical Importance of the Benedict's Test

The test is a semiquantitative test when performed in a standard way and gives an idea of the percentage of sugar in urine sample.

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Standard Way to Perform the Benedict's Test with Urine Sample

Take 5 ml of the Benedict's reagent in a test tube. Add 8 drops of urine sample to it and boil for one and half minute. If sugar is present in urine sample yellow/green/orange/brick red colored precipitate is observed. The color of the precipitate formed gives idea of the percentage of sugar in the given urine sample. (0.5% - yellow, 1% - green, 2% - orange, >2% - brick red).

3. Test for Detecting Monosaccharides

Barfoed's Test

Reagents

Dissolve 12.3 gm of neutral crystalline copper acetate in 200 ml water, filter if necessary and add 1.8 ml of glacial acetic acid.

Principle

Strongly reducing carbohydrates like the monosaccharides will cause reduction even in a mildly acidic medium (provided by glacial acetic acid) and form the brick red precipitate of Cu_2O.

Disaccharides do not give this test positive on heating for 30 seconds. However, on boiling for 15 – 20 minutes, the disaccharides are hydrolyzed to their constituent monosaccharides and will give a false positive test.

Procedure

Take 3 ml of Barfoed's reagent in a test tube and add 1 ml of reducing sugar to it. On boiling this solution for 30 seconds exactly, a brick red colored precipitate is obtained at the bottom of the test tube, indicating the presence of reducing monosaccharides.

Precautions

Do not boil the solution for a longer duration else the disaccharides would give a false positive test.

4. Test for Keto—Sugars

Seliwanoff's Test

Reagents

Dissolve 0.05 gm of resorcinol in 100 ml of dilute HCl to form Seliwanoff's reagent.

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Principle

The keto sugars react with dilute HCl form hydroxylmethylfurfural derivatives which, then condense with resorcinol to give a pink-red colored complex.

Procedure

Take 3 ml of Seliwanoff's reagent in a test tube and add 1 ml of keto sugar solution to it. Boil for 2 minutes. A pink red colored complex is formed indicating the presence of keto sugar.

5. Tests for Disaccharides

Inversion Test for Sucrose

Reagents

- Concentrated HCl
- Solid sodium carbonate
- Benedict's reagent
- Seliwanoff's reagent.

Procedure

Boil 3 ml of the sucrose solution with few drops of conc HCl. Now cool this solution and add solid sodium carbonate to it for neutralizing the solution. Keep adding Na_2CO_3 till effervescence ceases. This is now termed as the hydrolysis mixture/hydrolysate. Divide the hydrolysate into 2 parts and perform the Benedict's test with one part and Seliwanoff's test with the second portion. A positive test indicates the that sucrose is now hydrolysed into reducing sugars glucose and fructose which give the positive test respectively Benedict's test and Seliwanoff's test.

6. Osazone Test

Reagents

Osazone Mixture

It is prepared by mixing 1 part phenyl hydrazine and 2 parts sodium acetate by weight.

Glacial Acetic Acid

Principle

When reducing carbohydrates are heated with osazone mixture, they undergo specific chemical reactions giving rise to crystals of characteristic shape on cooling.

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Procedure

Take 5 ml of reducing carbohydrate solution, i.e. glucose, fructose, maltose, lactose in separate test tubes and label them. Now Osazone mixture is added. Add 5 drops of glacial acetic acid into each test tube and warm gently to dissolve the solids. Keep the test tubes in boiling water bath for the following time period:

Glucose and fructose—2 to 5 min

Lactose and maltose—45 min

Then cool it gradually, pore precipitate on slide and observe under microscope for different types of crystals.

Glucose and fructose—Needle shaped/Hay stack like crystals.

Lactose—Cotton puff shaped crystals.

Maltose—Sunflower shaped crystals (See Figure 5.1).

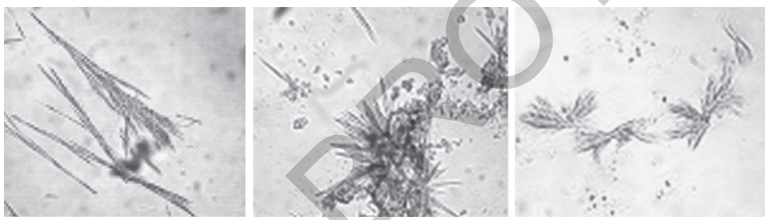

Fig 5.1: Osazone crystals

TESTS FOR POLYSACCHARIDES

7. Iodine Test

Reagents—I_2 Solution

Principle

Polysaccharide being macromolecules have affinity to adsorb I_2 giving characteristically colored complexes.

Explanation

The test depends on the property of adsorption of I_2 by large polysaccharide molecules.

The small I_2 molecules adsorbed on the surface, form complex of lose physical combination. On heating the I_2-polysaccharide complex dissociates and therefore disappears on cooling the complex is reformed and the color

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reappears, except in the case of dextrin. Heating produces change on the surface of dextrin as the result of which they lose the property of adsorption.

Precaution

Excess of I_2 should not be added. It will give black color.

Procedure

Take 3 ml of carbohydrate solution and add few drops of I_2 solution, mix and observe the color. Starch gives a blue color which disappears on heating and reappears on cooling.

Dextrin gives a reddish purple color on addition of I_2 Which disappears or heating and does not reappears on cooling**.**

8. Half and Full Saturation Test

Reagents

Solid Ammonium Sulfate

Saturated solution ammonium sulfate in water.

Principle

Polysaccharides form colloidal solution in water. Colloids are kept in solution by two factors:

i. **Electric charges:** Similarly charged particles repel each other and therefore their coalescence is prevented**.**
ii. **Shell of hydration:** Each molecule is surrounded by shell of water known as shell of hydration; this prevents the coalescence of particle. Starch is a bigger molecule and easily precipitated by half saturation in ammonium sulfate while dextrin is smaller molecule so it is precipitated by full saturation.

Procedure

Half Saturation

Take 2 ml of carbohydrate solution and add equal volume of saturated solution of ammonium sulfate shake vigorously and allow standing. A white precipitate indicates that the carbohydrate under test is precipitated by half saturation. The test is given positive by starch.

Full Saturation

Take 2 ml of carbohydrate solution and shake and allow standing. A white precipitate indicates that the carbohydrate under test is precipitated on full saturation with solid $(NH_4)_2SO_4$. This test is given by both starch and dextrin.

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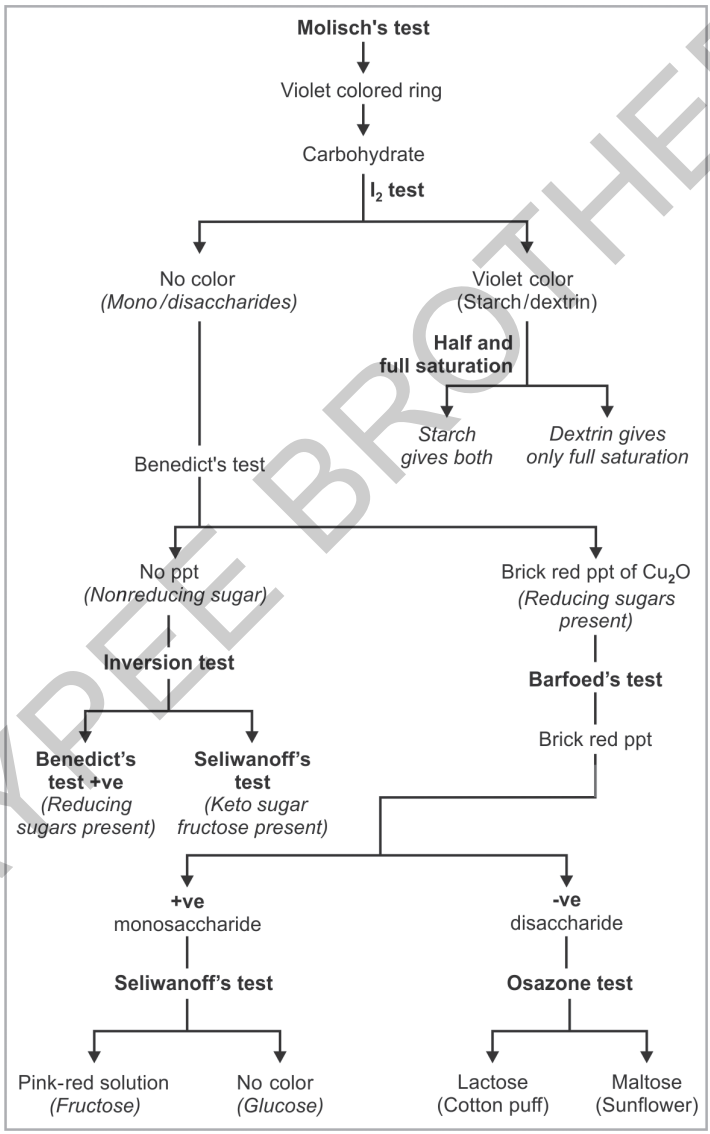
IDENTIFICATION OF UNKNOWN CARBOHYDRATE

Identification of unknown carbohydrate can be done as shown in the Flow chart 5.1.

Flow chart 5.1: Identification of unknown carbohydrate



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Q1. What does the green fluorescence in Molisch's test indicate?

Q2. Why is Benedict's test preferred over Fehling's test?

Q3. Why is the Barfoed's test given by only monosaccharides and not by reducing disaccharides?

Q4. Can sucrose give a direct positive Seliwanoff's test?

Q5. Why do glucose and fructose have identical osazone crystals?

Q6. Why does starch give both half and full saturation tests whereas dextrin gives only full saturation test?

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PRACTICAL BIOCHEMISTRY
for BDS

Salient Features

- This book presents the extensive subject of Practical Biochemistry for the students of dentistry in a highly simplified format
- The complete syllabi of the Practical Biochemistry as prescribed by the Dental Council of India (DCI) have been covered in 5 different sections
- Each section begins with an introduction to it
- Various sections include–Introduction to Practical Biochemistry, Qualitative Exercises, Clinical Biochemistry involving Quantitative Exercises and Urine Analysis, Organ Function Tests
- A complete section has been included on demonstrations of some important biochemical techniques, food stuffs and very importantly on saliva. These practical exercises have been recommended by DCI for demonstration to the BDS students so as to give a foundation for basics of Clinical Biochemistry, Techniques and Nutritional Biochemistry
- Finally, a chapter on recent advances in the field of clinical biochemistry has been incorporated
- Each chapter is followed up by viva voce questions which would serve to brush up that chapter.



Purvi Purohit did BSc in Life Sciences with honours in Chemistry and Biology, paving way for her merit- based selection as the 1st batch for postgraduation MSc (Medicine) Biochemistry at Dr SN Medical College, Jodhpur, Rajasthan, India. After postgraduation in 2003, she joined as a lecturer in Biochemistry at Jodhpur Dental College, Rajasthan, India. Teaching has always been a passion for her and has evolved her as a human with great ethics and values.

She pursued her PhD (Medicine) Biochemistry at Dr SN Medical College, Jodhpur, Rajasthan, India under the able and enlightening guidance of Dr Ranjana Mathur (Professor and Head, Department of Biochemistry). She was conferred the PhD (Medicine) Biochemistry on a topic, which has got her research recognized at the 38th National Conference of Association of Clinical Biochemists of India (ACBI) with 2 papers included in two different categories—Endocrinology and Cardiovascular Disorders and Risk Markers. Internationally, her work has been recognized at the 10th Annual World Congress of Insulin Resistance, Diabetes and Cardiovascular Disease (CVD), Los Angeles, with 2 of her papers accepted for presentation.

Her keen interest in research in Clinical Biochemistry and Endocrinology has got to her credit research papers on Thyroid Disorders and its Correlation to Various Lifestyle Disorders, 5 of which have been accepted for publication in peer reviewed journals of both National and International acclaim (IJCB, IJEM, IJBMR).

Currently, she is holding the position of Associate Professor, Faculty of Medicine and Health, Jodhpur National University, Jodhpur, Rajasthan, India.

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Shelving Recommendation
BIOCHEMISTRY

ISBN 978-93-5025-907-8
9 789350 259078

Available at all medical book stores
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