

Manual of Practical Biochemistry *for* **Dental Students**

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VAYU EDUCATION OF INDIA

2/25, Ansari Road, Darya Ganj, New Delhi-110 002

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ISBN: 978-93-82174-94-3

First Edition: 2013

Rs. 180.00

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Printed & bound in India

Published by:

An ISO:9001:2008 (Certified Company)

VAYU EDUCATION OF INDIA

2/25, Ansari Road, Darya Ganj, New Delhi-110 002

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Manual of Practical Biochemistry
for
Dental Students

Name:

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Preface

This manual is written with an objective to provide brief, but complete and easily understandable, various aspects of Practical Biochemistry to Dental students.

The manual covers the syllabi of almost all the Indian Universities and also covers the practical syllabus as prescribed by Dental Council of India (DCI). This manual would be equally beneficial for the students who are pursuing their career in various branches of Dental Sciences.

The author has also included one chapter on the preparation of various types of solutions, buffers and the terms which are commonly used in this field, have been explained with suitable examples.

The manual is written in a students friendly language. Separate chapter "important points to remember" emphasizes on maintenance of accuracy during Biochemical experimentation, which is the backbone for biochemical work, has also been included.

For qualitative analysis, scheme for the identification of carbohydrates, proteins, lipids and biologically important compounds is given so that students can themselves perform experiments by following that scheme.

For quantitative analysis, observation tables are given along with complete procedure in a systematic manner. Method for the preparation of standard curve, calculations are given in a very simple, easily understandable manner and space has also been provided to record the readings and other data.

In each chapter, important points are highlighted separately and at the end of the manual, a list of important points has been summarized. This would be a ready reference for the students appearing for various entrance tests.

Some experiments have been modified keeping in mind the normal availability of the equipment facility in the laboratory. Our aim is to give the students, solid foundation for understanding the practical aspects of Bio-chemistry and make them understand each and every step in a clear way.

The author also wishes to thank to her family members for their full support.

The author acknowledges with thanks to Dr. Rajiv K. Jain, Mr. Vijay Kumar Saini, Ms. Shahina, Mr. Kaushik and other members of **Vayu Education of India**, Daryaganj, New Delhi-110002, for their full support and dedication in bringing out this manual.

Suggestions for improvement of this manual from Dentists, research scholars, teachers, as well as students are most welcome.

Ritu Mahajan

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Biochemistry deals with the chemical changes which takes place in the living system (Here we are concerned with the human body) So whatever chemical reactions are taking place in the human body with the help of enzymes, all come under Biochemistry.

Clinical Biochemistry gives us status of various Components/ Metabolites/Enzymes present in body fluids (Blood, Urine, CSF etc.) and hence helps in the diagnosis of the disease.

Accuracy during biochemical tests is very very important aspect because wrong diagnosis can be made if the results are not correct. So accuracy is required at every step.

- (i) Weighing should be accurate.***
- (ii) Solutions of standards, reagents should be made properly.***
- (iii) Pipetting should also be accurate because all results depend upon the accuracy in weighing, pipetting and preparation of the solutions to the required volume.***
- (iv) Knowledge about the proper working of equipments is very essential.***
- (v) Protocol of various tests should be followed exactly in the same way as given for each test.***
- (vi) Calculations should be done very carefully.***
- (vii) Every test sample should be taken in duplicate. If more difference in the result in duplicate samples is observed, then repeat the test again in order to avoid wrong interpretation of the results.***

QUALITATIVE ANALYSIS

GENERAL REACTIONS OF CARBOHYDRATES

CARBOHYDRATES ARE ALDEHYDE/KETONE DERIVATIVES OF POLYHYDROXY ALCOHOLS OR COMPOUNDS WHICH YIELD THESE DERIVATIVES ON HYDROLYSIS

- Sugars such as glucose are amongst the major sources of energy whereas starch and glycogen function as storage polysaccharides in plants and animals respectively.
- They are also constituents of vital molecules like nucleic acids, coenzymes such as NAD(P), FAD etc.
- $(\text{CH}_2\text{O})_n$ carbo-hydrates are referred to as saccharides (**Greek : Sakcharon meaning sugar**).

Three major classes:

- 1. Monosaccharides:** They are simple sugars and consist of single polyhydroxy aldehyde or polyhydroxyketone unit. These can not be hydrolysed into simpler forms.

General formula – $(\text{CH}_2\text{O})_n$

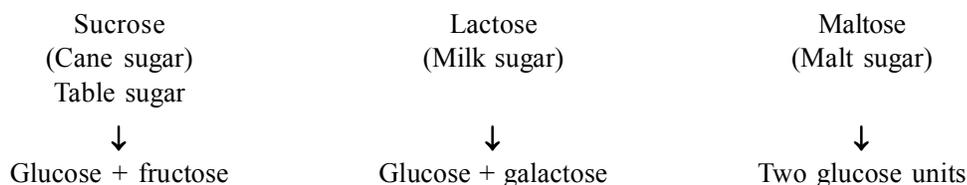
Reducing property is due to the presence of free aldehyde or keto groups

Most important monosaccharides are **hexoses** and **pentoses**. D-glucose, six carbon monosaccharide is the most common sugar found in nature. Examples of monosaccharides are glucose, fructose, ribose, galactose, arabinose etc.

- 2. Oligosaccharides:** oligo (Greek word means few).

Oligosaccharides consists of 2-10 units of monosaccharides linked to each other via glycosidic linkages.

Disaccharides: Lactose, maltose, sucrose



In Lactose – C-1 of galactose and C-4 of glucose is involved in glycosidic linkage.

In Maltose – C-1 of glucose and C-4 of glucose is involved in glycosidic linkage.

Lactose and maltose are reducing sugars because of free aldehyde group of one of the monosaccharide residues. In sucrose, glycosidic linkage is between C-1 of glucose and C-2 of fructose so free aldehyde or keto group is not available that's why sucrose is a **non-reducing sugar**.

3. Polysaccharides: Polysaccharides are made up of more than ten monosaccharide units, e.g., Dextrin, inulin, cellulose, starch, glycogen etc.

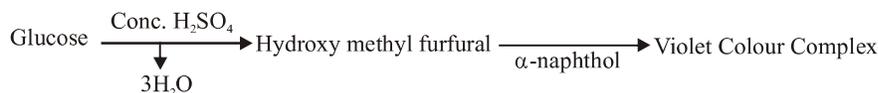
QUALITATIVE TESTS

Rapid tests are available to establish the presence or absence of a carbohydrate in a sample. These tests are based on specific colour reactions typical for their group.

| Name of Test | Application |
|---------------------|---|
| 1. Molisch Test | General Test for carbohydrates |
| 2. Anthrone Test | General Test for carbohydrates |
| 3. Iodine Test | For starch, glycogen |
| 4. Barfoed Test | Distinguish between monosaccharide from reducing disaccharides. |
| 5. Seliwanoff Test | For Ketohexoses |
| 6. Fehling Test | For reducing sugars |
| 7. Benedict Test | |
| 8. Picric Acid Test | |
| 9. Tommer Test | |
| 10. Tollen Test | For galactose |
| 11. Bial's Test | For pentoses |

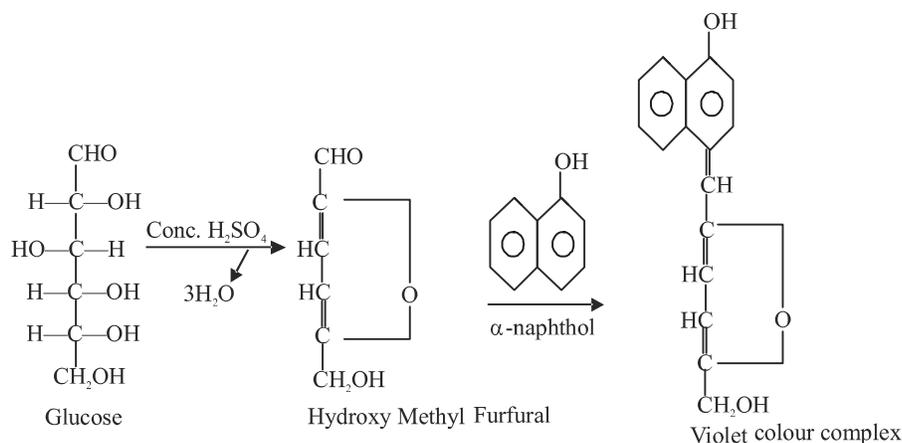
1. Molisch Test

It is a general test for all Carbohydrates. A positive test indicates the presence of carbohydrate in a test solution.



Carbohydrates when treated with concentrated H_2SO_4 (hydrolyses glycosidic bonds) and yield monosaccharides, which in the presence of an acid get dehydrated to form furfural or its derivatives which on condensation with α -naphthol form a violet colour complex. **All Carbohydrates except amino sugars give this test.**

Disaccharides and Polysaccharides are also hydrolysed by concentrated H_2SO_4 into monosaccharides which give a positive test.

**Reagents:**

1. Concentrated H_2SO_4
2. α -naphthol (5% w/v) in ethanol (prepare fresh)

Procedure: To 2 ml of sugar solution, add 2 drops of α -naphthol solution (Molisch's reagent). Mix thoroughly. Add 2ml of concentrated H_2SO_4 along the side of the test tube very gently by keeping the tube slightly in an inclined position so that two distinct layers are formed. Appearance of purple colour at the junction of two layers indicates the presence of carbohydrate in the sample.

Precautions

1. α -naphthol solution is unstable and should be prepared fresh.
 2. Concentrated H_2SO_4 should be added along the sides of the test tube causing minimal disturbance to the contents in the tube.
- 2. Anthrone Test:** This is also another general test for carbohydrates.

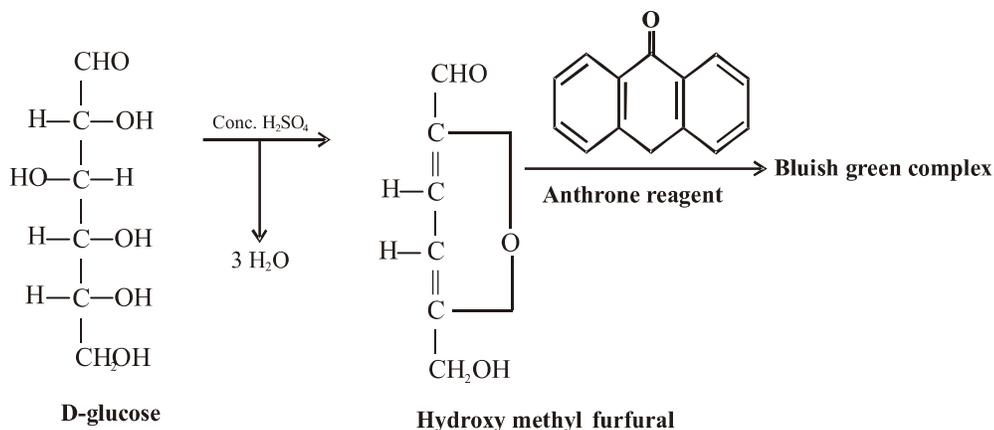


In this, the furfural produced reacts with Anthrone reagent to give bluish green coloured complex.

Reagents

1. Concentrated H_2SO_4
2. 0.2% w/v anthrone solution in concentrated H_2SO_4 .

Procedure: Add 1ml of test solution to 2 ml of anthrone reagent and mix thoroughly. Keep the tube in boiling water bath for 10 minutes. Bluish green colour would appear if test is positive.



3. Iodine Test: It is a test for polysaccharide which adsorb I_2 and form coloured complex. Starch gives blue colour, dextrin gives reddish purple colour, while glycogen gives reddish brown colour.

Reagents:

1. Iodine solution (0.1N)

Dissolve 1.27 g I_2 and 3 gm KI crystals in 100 ml distilled water. Dilute 1:10 in distilled water before use.

Procedure: Take 1ml of sugar solution in a test tube, add a drop of dilute HCl to acidify the solution or 5 drops of glacial acetic acid. Add 4-5 drops of I_2 solution to it and mix the contents gently. Note the colour of the product. Gently warm the solution and then cool it. Note the change in colour.

4. Benedict Test: All reducing sugars give this test positive. Reducing sugars have a free aldehyde or keto group which undergoes tautomerisation into enediol forms under hot alkaline condition. The enediols are strong reducing agents and they convert cupric ions of the benedict's solution into cuprous ions which ultimately form cuprous oxide as a red precipitate.

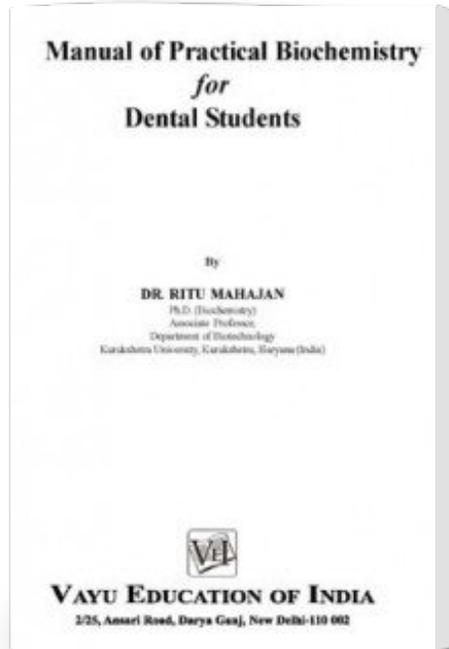
Common sugars like glucose, fructose, galactose, maltose, lactose are reducing sugars. Sucrose is non reducing sugar and does not give a positive Benedict test.

- Benedict reagent give different coloured precipitate ranging from green, yellow, orange, red depending upon the amount of sugar present. This is due to the larger particle size of the Cu_2O with gradual increase in concentration of the sugar. A very fine particle size produces yellow precipitate while large coarse particles give red precipitate.

Reagents

1. **Benedict Reagent:** Dissolve 173 g of sodium citrate and 100 g of anhydrous Na_2CO_3 in about 600ml of hot water. Dilute to 800 ml with water.
2. Dissolve 17.3 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 100 ml hot water. Cool and dilute to 150 ml.
3. Add reagent No. 2 to reagent No. 1 slowly with constant stirring. Make the final volume to 1.0 litre.

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Publisher : **Vayu Education**

ISBN : 9789382174943

Author : **Dr. Ritu Mahajan**

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