Available online at http://www.ijims.com
ISSN: 2348 – 0343

Osazones of the Uncommonly Encountered Reducing Sugars

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Abstract

Carbohydrates are polymers of sugar molecules that serve as storage molecules or as structural molecules. Chemically, carbohydrates are either aldehydes or ketones derivatives of polyhydroxylic compound known as aldoses and ketoses. Osazones are characteristic crystals resulting from the reaction of reducing sugars with phenylhydrazine. When detected, these osazones can be correlated with their associated disorders such as arabinose in autism & alzheimer's disease and galactose in Galactosemia. This study is to demonstrate the Osazone crystals of glucose, fructose, mannose, galactose, arabinose xylose, maltose & lactose sugars.

One gram percent of the carbohydrate solution was used in the study. Osazone test was performed by treating carbohydrates with phenylhydrazine at 100°C and pH 4.3 for 10-20 mins. Each carbohydrate was identified by the shape of the crystals seen under Nikon eclipse 600 microscope. Osazone test has been one of the simplest means to differentiate between sugars, however it is exclusively being done only for certain sugars such as glucose, fructose, maltose & lactose. Studies show that in children with autism, arabinose concentrations may exceed 50 times the upper limit of normal. For many other disorders related to carbohydrate, there is a need to analyse the reducing sugars qualitatively also. For further evaluation, HPLC technique which gives high specificity can be used. These sugars can be identified qualitatively in biochemistry lab and can also be identified by paper chromatography or by thin layer chromatography.

Introduction

Carbohydrates are polymers of sugar molecules that serve as storage molecules or as structural molecules[1]. Carbohydrates classified as simple or complex. Simple carbohydrates, often called monosaccharides or simple sugars, cannot be broken down into smaller carbohydrate molecules. Complex carbohydrates are further broken down into smaller carbohydrate units through a process known as hydrolysis[2].

Starch and glycogen are examples of storage molecules. Hydrolysis of either starch or glycogen releases the monosaccharide glucose, which may then serve as fuel for cellular work or as a carbon source for synthesis of other organic molecules[3].

Chemically, carbohydrates are either aldehydes or ketones derivatives of polyhydroxylic
compound known as aldoses (Fig.1-A) and ketoses (Fig.1-B) Carbonyl group give aldoses and ketoses the reducing properties[4].

Osazones are characteristic crystals resulting from the reaction of reducing sugars with phenylhydrazine. All sugars having free carbonyl group (reducing sugars) can form osazone crystals[5]. Monosaccharides are highly reducing compounds compared to disaccharides. Sucrose and trehalose being non-reducing sugars do not form any crystals.

When detected, these osazones can be correlated with their associated disorders. (e.g) Xylose in Small Bowel Dysfunction[7] (xylose absorption test – diagnostic test), arabinose in autism & alzheimer’s disease, galactose in Galactosemia[6], glucose in conditions with impaired glucose tolerance and renal glycosuria, fructose in people on high fruit diet, in patients with hereditary fructose intolerance and essential fructosuria and may other disorders related to carbohydrates. The objective of the present study was to demonstrate the Osazone crystals of glucose,fructose, mannose, galactose, arabinose xylose, maltose & lactose

Materials and Methods

This study was conducted using commercially available monosaccharides and disaccharides. [Merck specialities (P) Ltd. and Sisco Research Laboratories (P) Ltd.]

1.PREPARATION OF TEST SOLUTION :

One gram of the substance, either monosaccharide (glucose, fructose, mannose, galactose, arabinose, xylose) or disaccharide (maltose and lactose) is taken in a sterile test tube. After labeling, one hundred milliliters of distilled water was added to each test tube and shaken well. This mixture is called the test solution with concentration 1 gm %.

2 .Phenyl hydrazine(SRL chem)

3. Sodium acetate (Merck Laboratories)

4. Glacial acetic acid (SRL chem)

Since the study was completely done in department PG lab and materials were used from the manufacturers mentioned above, ethical committee clearance was not obtained for the same and was done under the permission & guidance of department head.

Principle

Osazone test was performed by treating carbohydrates with phenylhydrazine at 100°C and pH 4.3. A series of reactions takes place which results in the formation of hydrazone which on further reactions form the osazone[13]. This reaction is complete in 3 steps and consumes 3 moles of phenylhydrazine. During reaction with monosaccharides, additional phenyl hydrazine is consumed in oxidizing the adjacent OH-group to carbonyl group which then forms a second phenyl hydrazone. Such bisphenyl hydrazones are called osazones(Fig.3).

Osazone formation involves hydrazone formation at C-1 of an aldose (or C-2 of a ketose) and oxidation of C-2 (or C-1) of an alcohol group to a ketone (or an aldehyde). The new carbonyl group is also converted to a hydrazone.

A crystalline compound with a sharp melting point will be obtained. Since only C1 & C2 of a saccharide are involved in osazones, sugars with the same configuration at the remaining carbon atom give the same osazone. D-fructose and D-mannose give the same osazone as D-glucose.

Procedure

To 5 ml of the test solution in a test tube, 1 spatula (100-200 mgs) of phenyl hydrazine hydrochloride, 2 spatulas (300-400 mgs) of sodium acetate and 1 ml of glacial acetic acid were added and shaken well. The mixture with a pH of 4.3 was filtered and kept in boiling water bath at 100°C till the yellow crystals of osazones were formed. Disaccharides formed crystals on cooling at the end of 20 mins.

Fig.3 - NIKON eclipse 600 microscope

Microscopic Examination

Two drops of the formed crystals were placed were placed on a glass slide and a cover slip was placed above it and the slide was mounted on themicroscope.

The crystals were examined under high resolution NIKON eclipse E 600 microscope (Fig.3) & snapshots of the structures were taken.

Results

The study was performed on 6 monosaccharides and 2 disaccharides.

The osazone crystals formed at the end, either by boiling and cooling or only by boiling, after a particular time interval (Table 1) are obtained as follows

GLUCOSE, FRUCTOSE and MANNOSE

Glucose, fructose and mannose showed needle shaped crystals as shown in Fig.4-A, Fig.4-B, Fg.4-C respectively. For glucose and fructose, the crystals were formed in 3-4 minutes of boiling whereas for mannose it took 10 mins. It was difficult to distinguish between each other as all had the same shape.

GALACTOSE

Balls with thorny edge shaped crystals which were adjacent to each other, were obtained, as shown in Fig.4-D.

ARABINOSE

Less dense ball of needle shaped crystals present as clusters were obtained as shown in Fig.4-E.

XYLOSE

Fine needle shaped crystals which were long and intersecting were obtained as shown in Fig.4-F.

MALTOSE

Sun-flower shaped crystals were formed, when boiled for 20 minutes and cooled, as shown in Fig.5-A.

LACTOSE

Cotton-ball shaped crystals were formed, when boiled for 20 minutes and cooled, as shown in Fig.5-B.

Discussion

Osazone test has been one of the simplest means to differentiate between sugars, however it is exclusively being done only for certain sugars such as glucose, fructose, maltose & lactose. The possibility of obtaining different crystal shapes in the same test can lead to various queries as there are not many studies that describe them, nor is there any awareness about their significance Monosaccharides, when in hot solution, are highly reducing and formed the crystals earlier when compared to disaccharides, having only 40% reducing property than that of monosaccharides. It was found that fructose & mannose formed the same needle shaped crystals (Fig.4-B & Fig.4-C) as that of glucose (Fig.4-A). This is because the 1st and 2nd carbon atom take part in reaction with phenylhydrazine and at the end of the reaction, when osazones were formed, the osazone structure of glucose, fructose and mannose resemble each other. Fructose being a ketose sugar is more reactive when compared to glucose which is an aldose sugar.

Few of these uncommonly encountered reducing sugars, which also have the capacity to form osazones are xylose, galactose, mannose & arabinose

Confusions can be avoided when we come across the uncommon osazones during analysis. When detected, these osazones can be correlated with their associated disorders. Such as autism, galactosemia, hereditary fructose iintolerance, etc,.

Arabinose is a five carbon aldose, which is frequently elevated in autism. In few children with autism, arabinose concentrations may exceed 50 times the upper limit of normal[8]. Neurofibrillary tangles similar to those found in the brains of Alzheimer's victims have also been reported in the brain of an autistic person at autopsy[8].

Xylose absorption test as diagnostic use in Small Bowel Dysfunction. Similar results have been reported in the study conducted by Dennis L.Christie[6] and Buts JP et al[7].

Galactose can be measured in patients with Galactosemia as shown in study done by Gerard T Berry et al[10].

Fructose in people on high fruit diet and in hereditary fructose intolerance and essential fructosuria[11]

Glucose in conditions with impaired glucose tolerance and renal glycosuria[9] as in Type-2 Diabetes Mellitus, cushing's syndrome.

For further evaluation, HPLC (High Performance Liquid Chromatography) which gives high specificity can be done[12]. The main disadvantages being its high cost, time consuming and requirement of skilled personnel to operate instrument. In this case these sugars can be identified qualitatively in biochemistry lab and can also be identified by paper chromatography or by thin layer chromatography.

Acknowledgement

Special thanks to Mrs.Prabha in helping me to arrange materials in our Biochemistry lab.

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Figures & tables :

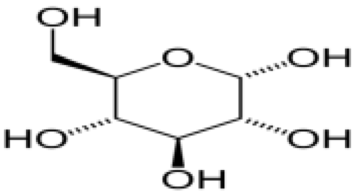


Fig.1-A. Structure of D-Glucose

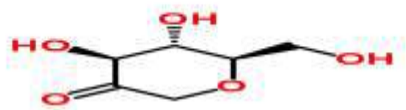
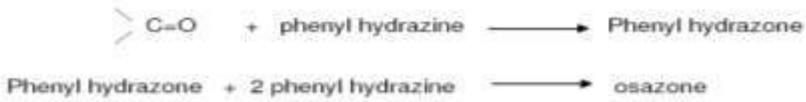


Fig.1-B. Structure of D-Fructose



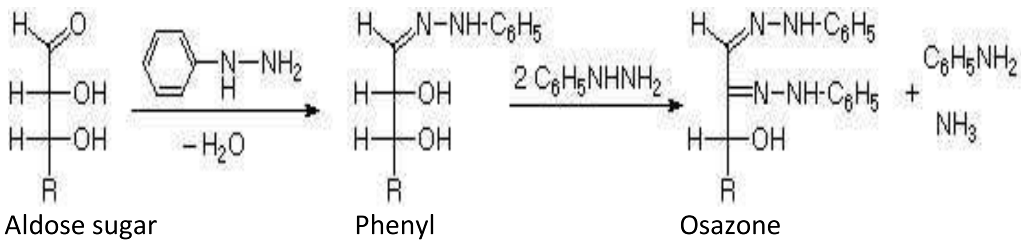


Fig.2 – Reactions showing formation of Osazone

Fig.4-A. GLUCOSE-Needle-shaped crystals.

Fig.4-B. FRUCTOSE- Needle-shaped crystals.

Fig.4-C. MANNOSE-Needle shaped crystals.

Fig.4-D. GALACTOSE - Balls with thorny edges.

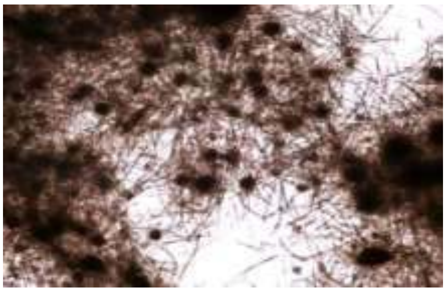


Fig.4-E. ARABINOSE-Less dense ball of needles.

Fig.4-F. XYLOSE-Fine needle shaped crystals

Fig.5-A. MALTOSE-Sunflower-shaped crystals.

Fig.5-B. LACTOSE- Cotton-ball shaped crystals.

Table 1: CHARACTERISTIC FEATURE & PHYSICAL CONDITIONS OF THE OSAZONES STUDIED.

NAME OF THE SUGAR	SHAPE OF CRYSTALS	TIME FOR FORMATION (IN MINS)	CONDITION		
			Boiling	Boiling & Cooling	Standing
Glucose	needle shaped	4	+	++	-
Fructose	needle shaped	3	+	++	-
Mannose	needle shaped	10	+	++	-
Galactose	small balls with cluster thorny edges	10	+	++	-
Arabinose	less dense ball of needles	11	+	++	-
Xylose	fine needle-shaped	6	+	++	
Maltose	sunflower-shaped	20	-	+	-
Lactose	cotton-ball shaped	20	-	+	-