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CHEMICAL AND PHYSICAL CONSIDERATIONS OF CARBOHYDRATES

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR DEPARTMENT HONORS

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and

PHYSICAL CONSIDERATIONS OF CARBOHYDRATES
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GLUCOSE - C₆H₁₂O₆

The following tests were made on glucose as a typical carbohydrate, and are not specific for this sugar.

1. SOLUBILITY -

Glucose is soluble in all ordinary solvents (H₂O; 10% NaCl; 5% Na₂CO₃; 2% HCl; concentrated KOH; concentrated HCl) and in alcohol.

2. NAPHTHOL REACTION (MOLISCH) -

When 5 c.c. concentrated H₂SO₄ were placed in a test tube and 5 c.c. sugar solution to which 2 drops of Molisch's reagent (15% alcoholic solution of naphthol) was added slowly down the inclined tube, a reddish violet zone was produced at the point of contact of the two solutions.

3. PHENYLHYDRAZINE REACTION -

5 c.c. of the sugar solution were added to a small amount of phenyl hydrazine mixture (2 parts of phenyl hydrazine hydrochloride; 3 parts of sodium acetate by weight) and shaken well. After heating on a water bath for about one-half hour, and allowing the tube to cool, yellow crystals formed. These were prepared on a slide and examined microscopically.

\[
\text{CH}_2\text{OH} + 3 \text{CH}_3\text{NH}_2 \rightarrow \text{CH}_2\text{OH} + \text{C}_4\text{H}_6\text{N}_2\text{H}_5 + 2\text{H}_2\text{O}
\]
4. CIPOLLINA'S TEST -

4 c.c. of dextrose solution, 5 drops of phenylhydrazine and \( \frac{1}{2} \) c.c. of glacial acetic acid were thoroughly mixed in a test tube and heated for about one minute. A few drops of NaOH were added, the solution remaining acid, and it was again heated and then cooled. Yellow crystals formed very similar to the glucosazone were formed and revealed when the crystals were mounted on a slide and examined microscopically.

5. PRECIPITATION BY ALCOHOL -

Dextrose solution added to 95% alcohol showed no reaction. As compared with dextrin, this was a distinction since 95% alcohol throws dextrin out of solution as a gummy white precipitate.

6. IODINE TEST -

Iodine added to glucose showed no reaction. This was in contrast to starch which yields a blue color with iodine.

7. DIFFUSIBILITY OF GLUCOSE -

Glucose solution was diffusible through an animal membrane or parchment paper. A dialyzing bag of collodion was not prepared.

8. MOORE'S TEST -

An equal volume of NaOH was added to 2/3 c.c. of glucose solution and boiled. The solution darkened and finally assumed a brown color. The odor of caramel was apparent.

9. REDUCTION TESTS -

To their aldehyde or ketone structure many sugars owe the property of readily reducing alkaline solutions of oxides of metals. Upon this property of reduction the most widely used tests for sugars are based. As an example -

\[
2 \text{Cu} \overset{\text{OH}^-}{\longrightarrow} \text{sugar} \rightarrow \text{Cu}_2\text{O} + 2\text{H}_2\text{O} + [\text{Cu}]^+ 
\]
a. TROMMER'S TEST -

2 c.c. of NaOH were added to 5 c.c. of sugar solution and a dilute solution of copper sulphate added until there was a slight permanent precipitate of cupric hydroxide. When heated, the cupric hydroxide was reduced to yellowish red cuprous oxide. Trommer's test was not very satisfactory.

\[ 2 \text{Cu(OH)}_2 + \text{C}_6\text{H}_12\text{O}_6 \rightarrow \text{Cu}_2\text{O} + 10\text{I} + 2\text{H}_2\text{O} \]

b. FEHLING'S TEST -

(Fehling's solution is a mixture of copper sulphate, caustic soda, and Rochelle salt which holds the cupric hydroxide in solution.) Good Fehling's solution was added to sugar solution a few drops at a time and the mixture heated after each addition. The cupric hydroxide was reduced to cuprous oxide which varied in color from yellow through green to red, due to the different sizes of the particles.

\[ 2 \text{Cu(OH)}_2 + \text{C}_6\text{H}_12\text{O}_6 \rightarrow \text{Cu}_2\text{O} + 10\text{I} + 2\text{H}_2\text{O} \]

c. BENEDICT'S MODIFICATION OF FEHLING'S TEST -

Benedict's solution is modified in that sodium carbonate replaces soda and sodium citrate is used instead of Rochelle salt to hold the cupric hydroxide in solution.

When a few drops of dextrose solution was added to Benedict's solution, boiled vigorously, and cooled, a precipitate formed which was bluish green changing through green to yellow. The above modification will serve to detect sugar when present in as small quantity as .015 to .02 percent.

d. BISMUTH REDUCTION TEST (NYLANDER'S TEST)

Nylander's Reagent is prepared by digesting 2 grams of bismuth subnitrate and 4 grams of Rochelle salts in 100 c.c. of 10% KOH. When Nylander's Reagent was added to sugar solution and heated in a boiling
water bath, the solution darkened, and upon standing for a few moments, a black color due to the precipitation of bismuth formed.

$$\begin{align*}
\text{Bi} (\text{OH})_2 \text{NO}_3 + \text{KOH} & \rightarrow \text{Bi} (\text{OH})_3 + \text{KNO}_3 \\
\text{Bi} (\text{OH})_3 + \text{C}_6\text{H}_5\text{O}_2 & \rightarrow 2 \text{Bi} + 3 \text{H}_2\text{O}
\end{align*}$$

### e. Barfoed's Test -

Barfoed solution is prepared by dissolving 9 grams of neutral crystallized copper acetate in 100 c.c. of H$_2$O and adding 1.2 c.c. of 50% CH$_3$COOH.

When Barfoed's solution was added to sugar solution and heated, reduction was indicated by the formation of a red precipitate of cuprous oxide.

### f. Picric Acid Test -

When 2/3 c.c. of saturated picric acid solution and 1 c.c. of 10% KOH was added to about 5 c.c. of sugar solution and warmed, a mahogany red color formed due to reduction of picric acid with the formation of picramic acid.

$$\begin{align*}
\text{C}_6\text{H}_2\text{OH} (\text{NO}_2)_3 + \text{C}_6\text{H}_5\text{O}_2 & \rightarrow \text{C}_6\text{H}_2\text{OH} \text{H}_2 (\text{NO}_2)_2
\end{align*}$$

### 10. Alcoholic Fermentation -

A concentrated solution of glucose to which a fragment of yeast was added was placed in a test tube and inverted over H$_2$O. CO$_2$ collected at the top of the tube and was identified by addition of Ba(OH)$_2$ which formed a white precipitate of BaCO$_3$.

Alcohol was revealed by the iodoform test which consisted of rendering the solution alkaline with KOH and adding a few drops of iodine. Yellow crystals of iodoform were formed and recognized by their characteristic hospital-like odor.
11. FORMATION OF CARAMEL -

When a small amount of pulverized glucose was melted and continued to be heated, a dark brown color formed which was soluble in H₂O. This coloring matter produced is known as caramel.

12. The polariscope was examined as to mechanism and method of operation. Trial readings were also taken.

FRUCTOSE

1. FRUCTOSE was identical with glucose in its solubility and in its reaction to the Fehling's, Phenylhydrazine, Barfoed's, Nylander's, and Fermentation tests.

2. RESORCINOL - HYDROCHLORIC ACID REACTION (SELIWANOFF)

Selivanoff's reagent is prepared by dissolving .05 gram of resorcinol in 100 c.c. of dilute HCl.

When Selivanoff's reagent was added to fructose solution and heated, a red color was produced and a brown red precipitate separated. The precipitate, when dissolved in alcohol, imparted a striking red color.

3. BORCHARDT'S REACTION

An equal volume of 25% HCl and a few crystals of resorcinol were added to a solution of fructose. When heated to boiling, a red color was produced. After cooling, the mixture was made slightly alkaline with solid KOH and 2/3 c.c. of acetic ether added. The acetic ether was colored yellow.
GALACTOSE - C₆H₁₂O₆

1. PHLORETIN - HYDROCHLORIC ACID REACTION (TOLLENS)

A little phloroglucinol was added to equal volumes of galactose solution and HCl and the mixture heated on a boiling water bath. A red color was produced.

2. MUCIC ACID TEST

When a solution of galactose was treated with concentrated HNO₃, and the mixture evaporated on a water-bath, a clear fluid containing a fine white precipitate of mucic acid resulted.

3. PHENYLHYDRAZINE REACTION

This test was carried out according to the same directions as for glucose. When the yellow crystals were mounted on a slide and examined microscopically, it was revealed that they have a striking resemblance to phenyl glucosazone.

DISACCHARIDES - C₁₂H₂₂O₁₁

MALTOSE

1. Maltose was very soluble in the "ordinary solvents". It reduced Fehling's, Nylander's, and Barfoed's Reagents. However, its reducing power was only about 2/3 that of glucose. Maltose gave a characteristic osazone with phenylhydrazine.

LACTOSE

1. Lactose was much less soluble in water than glucose. It was insoluble in alcohol and soluble in dilute acid and alkali. Lactose reduced Fehling's,
Barfoed's, and Nylander's Reagents. Its reducing power was about 7/10 that of glucose.

This sugar formed a characteristic osazone with phenylhydrazine.

2. MUCIC ACID TEST

Lactose solution, when treated with concentrated HNO₃ and evaporated, yielded a clear fluid and a fine white precipitate of mucic acid.

Sucrose - C₁₂H₂₂O₁₁

Sucrose was soluble in the "ordinary solvents" and did not reduce Fehling's, Nylander's, or Barfoed's Reagents. It did not yield a compound with phenylhydrazine.

2. INVERSION OF SUCROSE.

When a few drops of H₂SO₄ were added to sucrose solution, boiled, and neutralized, and the resulting solution tested, the following results were obtained. An osazone, phenylglucosazone, was formed with phenylhydrazine, and Fehling's, Nylander's, and Barfoed's Reagents were reduced. This was due to the inversion of the sucrose into glucose and fructose by the acid.

\[
\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O} \xrightarrow{\text{acid}} \text{C}_6\text{H}_{12}\text{O}_6 + \text{C}_6\text{H}_{12}\text{O}_6
\]

STARCH \((\text{C}_6\text{H}_{10}\text{O}_5)_x\)

1. A potato was pared, grated, mixed with water, and strained through cheese cloth. After filtering, it was dried in the air.

2. SOLUBILITY.

Starch was insoluble in cold water and in alcohol. It was soluble in dilute alkali and acid.
3. MICROSCOPICAL EXAMINATION -

Starch grains under the microscope appeared as round, concentric granules.

4. IODINE TEST -

A few granules of starch were treated with a drop of a dilute solution of iodine in potassium iodide. The granules were colored blue due to the formation of so-called iodide of starch. The cellulose of the granule was not stained as shown when examined microscopically.

5. IODINE TEST ON STARCH PASTE -

Starch paste was treated with dilute iodine solution producing a blue color. When heated, the color disappeared but it reappeared on cooling.

Alcohol and alkali also removed the blue color which did not return.

6. FEHLING'S TEST -

Starch paste reacts negatively to Fehling's solution. The blue color of the reagent was not altered.

7. HYDROLYSIS OF STARCH -

10 drops of concentrated HCl were added to about 25 c.c. of starch paste and gently boiled. At regular intervals a drop of the solution was added to a test tablet containing iodine solution. As the testing proceeded, the blue color gradually faded and finally disappeared. After cooling and neutralizing with KOH, Fehling's test was positive yielding a red precipitate of cuprous oxide. Also, a portion of the solution yielded an osazone with phenylhydrazine. The osazone was phenyl glucosazone and this proved that the final hydrolysis product of starch was glucose.

8. An excess of tannic acid solution added to a small amount of starch paste yielded a yellowish white precipitate. This is a test which distinguishes
starch from dextrin.

9. Starch paste was non-diffusible through parchment paper and an animal membrane.

**DEXTRIN** - \((C_6H_{10}O_5)_x\)

1. Dextrin was insoluble in cold water. It formed a clear solution in hot water. This test distinguishes dextrin from glycogen which gives an opalescent solution.

2. **IODINE TEST**

   Iodine added to dextrin solution produced a brownish red color due to the formation of red iodide of dextrin. The color disappeared on heating, and reappeared very slowly upon cooling. Alkali and alcohol removed the color, which did not reappear.

3. **FEHLING'S SOLUTION**

   Dextrin solution responded negatively to Fehling's solution.

4. **HYDROLYSIS OF DEXTRIN**

   5 drops of dilute HCl were added to about 25 c.c. of dextrin solution in a small beaker and the solution boiled. At regular intervals a drop of the solution was added to a test tablet containing iodine. As the testing proceeded the red color gradually faded and finally disappeared. When neutralized and tested with Fehling's solution, there was a positive reaction, proving the dextrin had been hydrolyzed. By the phenylhydrazine test, it was proven that the dextrin was hydrolyzed to glucose.

5. **PRECIPITATION OF ALCOHOL**

   When 95% alcohol was added to dextrin, dextrin was thrown out of solution as a gummy white precipitate. This test distinguished dextrin from glucose.
6. INFLUENCE OF TANNIC ACID -

An excess of tannic acid had no effect on dextrin. This result differs from the result of a similar experiment upon starch.

7. DIFFUSIBILITY OF DEXTRIN -

Dextrin was non-diffusible through parchment paper and animal membrane.

CELLULOSE

1. Cellulose was insoluble in water and dilute acid and alkali. It was soluble in concentrated acid and alkali.

2. IODINE TEST -

Dilute iodine added to a few shreds of cotton in a test tube produced no color. Cellulose differs from starch and dextrin in this respect.

3. FORMATION OF AMYLOID -

When 10 c.c. of dilute and 5 c.c. of concentrated H₂SO₄ was added to some absorbent cotton, the cotton dissolved without heating. When some of this solution was cooled and diluted with water, a gummy precipitate of amyloid was formed.

When a portion of this same solution was neutralized with KOH, and tested with Fehling's solution, a positive reaction was obtained. Glucose was formed from the cellulose by the action of the acid.

4. AMMONIACAL CUPRIC HYDROXIDE SOLUBILITY TEST - (SCHWEITZER) -

When Schweitzer's reagent was added to cellulose and stirred, the solid dissolved. Upon acidification with acetic acid, an amorphous precipitate of cellulose was produced.
5. HYDROCHLORIC ACID - ZINC CHLORIDE SOLUBILITY TEST (CROSS AND BEVAN) -

When a solution of two parts of hydrochloric acid and one part of zinc chloride by weight (Cross and Bevan's reagent) was added to cellulose, the solid dissolved. 95% alcohol reprecipitated the cellulose.

GALACTAN

1. SOLUBILITY -

The galactan, agar-agar, was insoluble in cold water, soluble in hot water and had a marked property of imbibing water.

2. IODINE TEST -

Iodine added to agar-agar yielded no color reaction.

3. HYDROLYSIS OF AGAR - AGAR -

Agar-agar boiled with strong HCl and neutralized yielded a positive reaction with Fehling's solution, proving that the product of hydrolysis was a reducing agent. This reducing substance was galactose.
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