Detection and Spectrophotometric Determination of Mono and Disaccharides

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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BY
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Syed Taufeeq Ahmad
## CONTENTS

<table>
<thead>
<tr>
<th>I. LIST OF TABLES</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. LIST OF FIGURES</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>III. CHAPTER I</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>1</td>
</tr>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>3</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IV. CHAPTER II</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DETECTION AND SPECTROPHOTOMETRIC DETERMINATION OF MONO AND DISACCHARIDES</td>
<td></td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>11</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td>14</td>
</tr>
<tr>
<td>RESULTS</td>
<td>16</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>30</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>34</td>
</tr>
</tbody>
</table>
LIST OF TABLES

TABLE I LIMITS OF IDENTIFICATION OF CARBOHYDRATES 17

TABLE II FORMATION OF CHARACTERISTIC COLOURS WITH ORGANIC COMPOUNDS 19

TABLE III CONFIRMATORY WITH BEER'S LAW OF CARBOHYDRATES 26

TABLE IV STUDY OF PRECISION OF CARBOHYDRATES 28

TABLE V LIMITS OF TOLERANCE OF FOREIGN SUBSTANCES IN THE DETERMINATION OF CARBOHYDRATES 29

TABLE VI MAX. VALUES OF DIFFERENT POSSIBLE SPECIES FORMED BY THE COMBINATION OF ANY THREE/TWO OR INDIVIDUAL SPECIES 33
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Absorption spectrum of the colour formed with glucose</td>
<td>20</td>
</tr>
<tr>
<td>II</td>
<td>Effect of time on absorbance</td>
<td>22</td>
</tr>
<tr>
<td>III</td>
<td>Effect of the amount of 1-chloro-2,4-dinitrobenzene</td>
<td>23</td>
</tr>
<tr>
<td>IV</td>
<td>Effect of the amount of sodium carbonate</td>
<td>24</td>
</tr>
<tr>
<td>V</td>
<td>Calibration curve of glucose at 350 nm</td>
<td>25</td>
</tr>
</tbody>
</table>
CHAPTER I

GENERAL INTRODUCTION
ABSTRACT

This dissertation comprises of two chapters. In the first chapter a detailed and up-to-date literature survey of the subject has been reviewed.

The second chapter includes the detection and spectrophotometric determination of carbohydrates. The term carbohydrate covers a vast array of chemical substances. A meaningful definition of the term is difficult to provide, but it may be stated that carbohydrates are polyhydroxy compounds containing aldehyde or ketone carbonyl group. Several methods have been described for their detection and determination. In this chapter the detection of carbohydrates is based on the reaction with 1-chloro-2,4-dinitrobenzene and sodium carbonate to give an orange yellow colour. Place a few drops of carbohydrate solution on a white spot plate followed by addition of 1-chloro-2,4-dinitrobenzene and sodium carbonate. Heat the mixture an orange yellow colour appears indicating a positive test of carbohydrates. The carbohydrates giving a positive test are glucose, fructose, rhamnose, lactose, galactose, maltose, xylose, mannose and arabinose. The limits of identification of these carbohydrates are determined. Sucrose gave a negative test. Negative test is also given by the following functional groups amines,
ethers, alcohols, acids, amino acids, aldehydes, nitriles, amides, phenoles, ketones, hydrocarbons and their derivatives. Certain organic compounds under given conditions gave their characteristic colours.

For the determination of carbohydrates the procedure is as follows. To the different carbohydrate solutions containing milligram amounts of carbohydrates (which follow the Beer's law) add 0,1 ml of 1-chloro-2,4,6-dinitrobenzene and 0,1 ml of sodium carbonate in a standard volumetric flask (10 ml capacity). Make up the contents of the flask with demineralized water and transfer them to the boiling tubes. The yellow colour developed after heating on a water bath is allowed to stand at room temperature for 30 - 40 minutes. The absorbance of the coloured solution is measured at 350 nm against a blank. The ranges within which Beer's law is obeyed is given in the table III. The interaction of foreign substances in the determination of carbohydrates has also been studied and their limits of tolerance has been presented. The detection and determination of carbohydrates can not be performed in presence of certain transition metal ions.
The detection and determination of organic compounds containing different functional groups has been one of the most demanding problems. The analysis of organic compounds can be made by using non-instrumental methods viz. spot tests via colour reactions or by instrumental methods such as IR spectrometry, UV-visible spectrophotometry and mass spectrometry etc. Both the methods of analysis have their advantages and disadvantages. Non-instrumental methods are fast, simple and inexpensive while instrumental methods are fairly sensitive, specific but suffer some disadvantages of being less simple and time consuming. Of the non-instrumental methods of analysis, spot test technique has been found to be the most versatile and widely used. This technique of spot test was extensively used by Feigl (1). Using this technique Qureshi et al. (2) described a new specific colour reaction for the detection of substituted aromatic aldehydes with diphenylamine.

Fujimoto (3) first demonstrated the possible use of resin beads as a reaction medium and to concentrate the colour on resin surface particularly for the detection of inorganic ionic species and a few organic compounds of known colour reactions. Several methods for the detection of amides, imides, anilides, esters and selective detection of diphenylamine have been described using p-dimethylaminobenzaldehyde and methyl red in the presence of ion exchange
resins (4,5,6). A new test for the detection of ketones on
the microgram scale has been described by Qureshi et al. (7)
The detection is based on the reaction with 1-chloro- 2,4-
-dinitrobenzene in the presence of an anion exchange resin in
OH form to give a violet colour on resin bead's surface.

Qureshi et al. (8) developed a capillary solid state
spot test for some aldehydes and sugars with diphenylammonium
chloride.2,2, Bicinchonate (9) reagent has been used for the
detection of reducing sugars. Using this reagent the detection
of sugars can be done in the presence of borate ion. Sodium,
potassium tartrate (10) added basic tetrazolium blue is used
as a reagent for the detection of reducing sugars. By this
method sensitivity is increased and reaction time is decreased.
This modified reagent can detect as little as 1 n mole of
neutral as well as 2-amino and N - acetylamino sugars. Ruppolt
(11) used 2,6-dichlorphenolidophenol as a reagent for the
detection of reducing sugars. It produces various colours with
sugar solutions according to the nature of the reducing sugar.
Glucose, fructose etc. give a blue colour but ketose solutions
turn colourless on warming.

Spectrophotometric determination of organic compounds
has received much attention especially in trace analysis. To
be able to work in the visible region, spectrophotometric
determination is a very important and suitable method for the
determination of many substances that give a particular colour on reaction. Very few reactions are specific but a large number of reactions are selective or can be made selective by the proper adjustment of suitable conditions and hence can be applied for the determination of organic compounds.

Qureshi et al. (12), used this technique for the determination of salicylaldehyde with concentrated sulphuric acid and absorbance measurements are carried out at 560 nm. Beer's law is obeyed within the range of 50-300 μg of salicylaldehyde per ml. Szymczak (13) used 1% 2,3,5-triphenylchloride solution for the determination of reducing sugars in an alkaline medium at an increased temperature, giving a colour. Measurements are carried out at 480 nm. The method is suitable for the detection of reducing sugars in presence of lactic acid. 1,5-dihydroxy-4,8-dinitroanthraquinone-2,6-disulphonic acid is reduced by sugars such as glucose and fructose in 0.15 N sodium hydroxide at 70 - 100°C. Absorbance is measured at 620 nm and the Beer's law is obeyed within 0.3 - 9 mg of reducing sugars (14). Bubica (15) determined reducing sugars in cereals after extracting ground sample with boiling water, mixing with copper sulphate, sodium hydroxide and the reagent and measuring copper concentration in the filtrate spectrophotometrically at 574 nm followed by calculation of amount of reducing sugars from an equation. An automated method using p-hydroxybenzoic acid hydrazide and 2-thiobarbituric acid for the determination of total reducing sugars is described by Tawfik et al. (16). Using an automated analyzer the determination range is changed from
50-500 mg/L to 1 - 30 mg/L without affecting the linearity of the calibration curves. A spectrophotometric method based on the reaction with alcoholic p-aminobenzoic acid solution for determining free reducing sugar in surface water, is used to determine certain sugars by condensation at 70 - 75° for 60 minutes with the addition of glacial acetic acid. The calibration graph for the determination of aldoses is sharply divided into mono and disaccharides (17). A method for estimating reducing sugars glucose, xylose, maltose involves addition of 0.5 ml ammonia to 1 - 2 mg sugar followed by addition of standard copper solution and 2 ml of oxalyldihydrazide solution and volume is made up to the mark. After heating for fifteen minutes, the colour intensity of the complex is measured at 410 nm and Beer's law is obeyed for 0.2 - 2 mg of sugar (18). Singh et al. described the kinetics and mechanism of oxidation of certain sugars by aqueous Ag solution in the presence of ammonium hydroxide. The oxidation of the sugars shows that the reaction rate is independent of silver ion concentration and directly proportional to the reducing sugar concentration and inversely proportional to the square of the ammonium hydroxide concentration (19).

Certain other techniques such as paper chromatography, thin layer chromatography, ion exchange chromatography and high performance liquid chromatography have also found their importance for the detection and determination of organic compounds.
Rf values and colours with naphthoresorcinol are tabulated in the literature for eighteen sugars with nine different solvents on cellulose MN - 300, boric acid impregnated silica gel chromatographic plates. Separation of the mixture of lactose and succrose is achieved (20). Cerbulis (21) used p- anisidine phosphoric acid as a reagent for the detection of sugar derivatives and halogen compounds. The developed thin layer chromatograms are sprayed with the reagent and heated for ten minutes at 110 -120°. Various compounds with their characteristic colour spots with p- anisidine -phosphoric acid reagent are listed. N - (1 - Naphthyl) ethylenediamine- 2 hydrochloric acid dissolved in sulphuric acid - methanol, O - aminobenzenesulphonic acid - phosphoric acid and sulphosalicylic acid (22,23,24) are used as reagents for the detection of sugars on thin layer chromatographic plate. In another method chromatograms on 0.25 mm layer of silica gel are developed for two hours. The developed chromatograms are stained with ammonical silver nitrate (25). The concentrations are estimated from the spot chains. The method is suitable for the detection of 0.1 μg of sugar. Separation of sugars has been done by ion exchange methods (26,27) and their detection is performed by different methods. Davies et al. (28) described a system for the ion exchange chromatography of reducing sugars in neutral borate buffers by using sulphate ion as counter ion. The eluted sugars are detected by using p- hydroxybenzoic acid hydrazide.
Rocca (29) has used high speed liquid chromatographic columns for the fast separation of sugars. Elution takes place by solvent and is detected by a refractometer. Gyon et al. (30) described the separation of sugars by column packed with copper silica gel by using water–methylcyanide containing ammonia. The change of the capacity factor as a function of the water and ammonia contents of mobile phase is also studied.
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CHAPTER II

DETECTION AND SPECTROPHOTOMETRIC DETERMINATION OF MONO AND DISACCHARIDES
Feigl (1) has described a number of tests for the detection of carbohydrates. Carbohydrates when heated with an alkaline solution of triphenyltetrazolium chloride give a red colour. This test is supposed to be an extremely sensitive test because a large number of other substances such as hydroxylamine, hydrazine, sulphites, tartaric and citric acid do not interfere much in this test. Filatova et al. (2) have described a method for the detection of carbohydrates. The method is based on the reaction of anthrone with carbohydrate in concentrated sulphuric acid to form coloured furfural compounds. The use of this method eliminates the hydrolysis of carbohydrates and detection of pentose. Qureshi and Anwar (3) have given a selective test for the detection of fructose and sucrose, based on the reaction with 1-chloro-2,4-dinitrobenzene in the presence of cation exchange resin in H⁺ form. Glucose, lactose, xylose, rhamnose and arabinose give negative tests. Qureshi et al. (4) have described a solid state method for the detection of glucose, lactose, galactose, xylose, rhamnose, sucrose trehalose, fructose and starch by applying several tests. Glucose can be distinguished from other carbohydrates using anthrone test in the solid state. A new spot test for the detection of carbohydrates and vitamin C when present individually or together has been described by Hashmi et al. (5). Chloranric acid was used for quantitative determination.
of vitamin C. The standardized spectrophotometric conditions for the thymole - ferric chloride - hydrochloric acid reagent has been developed by Patil et al. (6) for the determination of total carbohydrate on a microgram (1 - 14 ug) scale. The convenience of the procedure and the stability of the reagent offers advantage over the other procedures. A spectrophotometric method for estimating the carbohydrate content of acid hydrolysed glycoproteins is described by Krystal (7). The method is based on alkaline ferricyanide reaction with carbohydrate. It is accurate within 1 - 25 n mole range.

The kinetics of the chloramine - T oxidation with xylose, arabinose, mannose, and galactose in highly alkaline medium has been described by Mushran and Agrawal (8). The oxidation rate follows the order xylose > arabinose > galactose > mannose. Carbohydrates have been separated as their borate complexes on an anion exchange column at 55° C by using concentration gradient elution with a mixture of borate, sodium chloride and sodium bicarbonate. The carbohydrates in the effluent are detected by sulphuric acid - orcinol method (9). Palmer (10) has described a simple rapid and versatile method for the separation and determination of carbohydrates via high performance liquid chromatography. As little as 20 ug of an individual carbohydrates can be detected by this method. Takemoto et al. (11) have described a method for quantitation of picomole amount of neutral and amino sugars in glycoconjugates. Glycoconjugates are hydrolyzed with a
mixture of equal amount of 4 M trifluoroacetic acid and 4 M hydrochloric acid and the free amino groups are coupled with 2-aminopyridine. After the excess reagents have been removed by high performance gel chromatography. The fluorescent pyridylamino derivatives of sugars are separated and quantified by reverse phase HPLC. Bonn (12) has described the high performance liquid chromatographic separation of oligosaccharides, monosaccharides, sugar degradation products and alcohols by using a series connected system of different ion exchange columns with water as the eluent.
EXPERIMENTAL

Apparatus:

Bausch and Lomb spectronic - 20 (U.S.A.) and Pye Unicam PU 8800 (England) spectrophotometers were used for spectrophotometric analysis.

Reagents

All chemicals used were of reagent grade.

Test Solutions

0.01M solutions of each carbohydrate like glucose, fructose, rhamnose, lactose, galactose, maltose, xylose, mannose, arabinose and sucrose were prepared in demineralized water.

Reagents

(a) 0.01M 1-chloro-2,4-dinitrobenzene solution was prepared in pure dimethylsulphoxide as a stock solution. From this stock solution a 0.001M solution of 1-chloro-2,4-dinitrobenzene was prepared in water-dimethylsulphoxide mixture in the ratio 80:20. This solution was used throughout the experimental procedure in the detection and determination of carbohydrates.

(b) A 1.0M solution of sodium carbonate was prepared in demineralized water.
Procedure for the detection of carbohydrates

Place a few drops of carbohydrate solution on a white spot plate. Add a few drops of 1-chloro-2,4-dinitrobenzene and sodium carbonate. Heat the mixture, an orange yellow colour is obtained in the presence of carbohydrates.

Procedure for the determination of carbohydrates

To the different carbohydrate solutions, containing milligram amounts of carbohydrates (which follow the Beer's law) add 0.1 ml of 1-chloro-2,4-dinitrobenzene and 0.1 ml sodium carbonate in a standard volumetric flask (10 ml capacity). Make up the contents of the flask with demineralized water and transfer them to the boiling tubes. Heat on a water bath for 20 - 25 minutes to develop a yellow colour. Now allow to stand the mixture contents at room temperature for 30 - 40 minutes. Transfer the yellow coloured solution into flask (10 ml capacity) and volume is made up, if there is any change in volume. Measure the absorbance of each of the carbohydrate solutions against a blank (prepared under the same conditions but without carbohydrates) at a maximum absorbance wave length 350 nm.
RESULTS

The recommended procedure for the detection of available carbohydrates gives a positive response for the carbohydrates such as glucose, fructose, rhamnose, lactose, galactose, maltose, xylose, mannose and arabinose. Sucrose gave a negative test. The limits of identification of different carbohydrates have been presented in table I.

The following organic compounds gave a negative test.

**Hydrocarbons and their derivatives:** Benzene, toluene, chloroform, nitrobenzene.

**Amines:** Triethanolamine, triethylamine, trimethyamine, ethylamine, diethylamine, benzylamine, diphenylamine.

**Ethers:** Anisol, 1-4 dioxane, ether.

**Alcohols:** Methylalcohol, ethylalcohol, amylalcohol, butylalcohol.

**Acids:** Acetic acid, benzoic acid, phthalic acid, ascorbic acid, barbituric acid, cinnamic acid.

**Amino acids:** Aspartic acid, leucine, lysine, glycine, histadine.
Table I

Limits of identification carbohydrates

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Molar concentration</th>
<th>Volume taken (ml)</th>
<th>Amount detected (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>$1 \times 10^{-3}$</td>
<td>0.05</td>
<td>9.0</td>
</tr>
<tr>
<td>Fructose</td>
<td>$1 \times 10^{-3}$</td>
<td>0.04</td>
<td>7.2</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>$1 \times 10^{-3}$</td>
<td>0.04</td>
<td>7.28</td>
</tr>
<tr>
<td>Lactose</td>
<td>$1 \times 10^{-3}$</td>
<td>0.05</td>
<td>17.12</td>
</tr>
<tr>
<td>Galactose</td>
<td>$1 \times 10^{-3}$</td>
<td>0.04</td>
<td>7.2</td>
</tr>
<tr>
<td>Maltose</td>
<td>$1 \times 10^{-3}$</td>
<td>0.03</td>
<td>10.27</td>
</tr>
<tr>
<td>Xylose</td>
<td>$1 \times 10^{-3}$</td>
<td>0.04</td>
<td>6.0</td>
</tr>
<tr>
<td>Mannose</td>
<td>$1 \times 10^{-3}$</td>
<td>0.05</td>
<td>6.0</td>
</tr>
<tr>
<td>Arabinose</td>
<td>$1 \times 10^{-3}$</td>
<td>0.04</td>
<td>9.0</td>
</tr>
</tbody>
</table>
Aldehydes: 4 -dimethylaminobenzaldehyde, anisic aldehyde, 4 -diethylaminobenzaldehyde, formaldehyde, acetaldehyde.

Nitriles: Benzonitrile, acetonitrile.

Amides: Acetamide, formamide, benzamide.

Phenols: Phenol, O - cresol, m - cresol, orcinol.

Ketones: Benzophenone.

Certain organic compounds under given conditions give their characteristic colours which are shown in the table II.

Determination of carbohydrates:

Absorption spectrum

The absorption spectrum of solutions containing some milligram of carbohydrates were studied against a blank. The maximum absorbance was obtained at 350 nm for each carbohydrate. The absorption spectrum of glucose is shown in fig. I.

Optimum conditions

The optimum conditions for the formation of yellow colour was studied and maintained throughout the studies.
### Formation of Characteristic Colours with Organic Compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Colour</th>
<th>Compounds</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resorcinol</td>
<td>yellow colour</td>
<td>Butane -2- one</td>
<td>pinkish</td>
</tr>
<tr>
<td>Diphenyl-carbazide</td>
<td>red</td>
<td>N,N-diethylformanide</td>
<td>light yellow</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>pinkish</td>
<td>Acetone</td>
<td>pinkish</td>
</tr>
<tr>
<td>α-Naphthol</td>
<td>light green</td>
<td>β-Naphthol</td>
<td>light green</td>
</tr>
<tr>
<td>Thiourea</td>
<td>light yellow</td>
<td>Methyl n-propyl ketone</td>
<td>pinkish</td>
</tr>
<tr>
<td>Pyrocatechol</td>
<td>dirty yellow</td>
<td>Piperidine</td>
<td>greenish yellow</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>chocolate</td>
<td>2-merceptopropionic acid</td>
<td>red</td>
</tr>
<tr>
<td>Pyridine</td>
<td>pinkish violet</td>
<td>m-chloroaniline</td>
<td>greenish blue</td>
</tr>
</tbody>
</table>
FIGURE I

ABSORPTION SPECTRUM OF GLUCOSE
The time versus absorbance studies showed (fig.II) that the yellow colour is stable for the period of half an hour. Therefore it is recommended that absorbance of the solution should be measured within this period.

The effect of reagent concentration was studied by adding different volumes of 1-chloro-2,4-dinitrobenzene and it was found that 0.1 ml of reagent was most suitable volume for determination studies as shown in fig. III. Similar effect of volume variation of sodium carbonate suggested that the optimum volume for the spectrophotometric studies should be 0.1 ml as shown in fig. IV.

Confirmatory with Beer's law

The absorbance measurement of yellow colour was made at 350 nm for each carbohydrate. The calibration curve of glucose has been shown in fig. V and the range of each carbohydrate within which Beer's law is obeyed is given in table III.

Study of precision

To test the reproducibility of the method, ten replicate determinations of each carbohydrate were carried out containing some milligrams of carbohydrates. The standard
FIGURE II

EFFECT OF TIME ON ABSORBANCE
FIGURE III

VOLUME OF 1-CHLORO-2,4-DINITROBENZENE (ml)

EFFECT OF THE AMOUNT OF 1-CHLORO-2,4-DINITROBENZENE

ABSORBANCE
FIGURE IV

EFFECT OF AMOUNT OF SODIUM CARBONATE
FIGURE Y

AMOUNT OF GLUCOSE (mg) ——>

CALIBRATION CURVE OF GLUCOSE AT 350 nm
Table III

Confirmatory with Beer's law of carbohydrates

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Concentration range (mg)</th>
<th>λ max. (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>2.70 - 9.0</td>
<td>350</td>
</tr>
<tr>
<td>Fructose</td>
<td>2.70 - 9.0</td>
<td>350</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>2.73 - 9.1</td>
<td>350</td>
</tr>
<tr>
<td>Lactose</td>
<td>1.71 - 10.26</td>
<td>350</td>
</tr>
<tr>
<td>Galactose</td>
<td>2.70 - 9.0</td>
<td>350</td>
</tr>
<tr>
<td>Maltose</td>
<td>3.42 - 10.26</td>
<td>350</td>
</tr>
<tr>
<td>Xylose</td>
<td>2.25 - 7.5</td>
<td>350</td>
</tr>
<tr>
<td>Mannose</td>
<td>2.70 - 9.0</td>
<td>350</td>
</tr>
<tr>
<td>Arabinose</td>
<td>2.25 - 6.0</td>
<td>350</td>
</tr>
</tbody>
</table>
deviation and the confidence intervals of 95% from the mean value lie within the ranges are tabulated in table IV for each carbohydrate.

**Study of charge on complex**

The charge on the complex was determined by adding two types of resins:

(a) Anion exchange resin

(b) Cation exchange resin

The anion exchange resin showed the adsorption of colour on its beads. Thus the complex formed was anionic in nature.

**Interaction of carbohydrates with transition metal ions**

The transition metal ions such as Co\(^{+2}\), Ni\(^{+2}\), Fe\(^{+3}\), Cu\(^{+2}\), and Cr\(^{+2}\) etc. were found to interfere with detection and determination of carbohydrates.

**Determination of carbohydrates in presence of foreign substances**

Carbohydrates were tested in presence of other functional groups and their limits of tolerance was calculated and presented in the table V.
Table IV

Study of Precision of Carbohydrates

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Amount taken for each carbohydrate solution (mg)</th>
<th>Standard deviation (mg)</th>
<th>95% Confidence interval (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>3.60</td>
<td>0.081</td>
<td>3.565 - 3.688</td>
</tr>
<tr>
<td>Fructose</td>
<td>3.60</td>
<td>0.075</td>
<td>3.494 - 3.606</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>3.64</td>
<td>0.091</td>
<td>3.587 - 3.725</td>
</tr>
<tr>
<td>Lactose</td>
<td>3.42</td>
<td>0.083</td>
<td>3.318 - 3.444</td>
</tr>
<tr>
<td>Galactose</td>
<td>3.60</td>
<td>0.078</td>
<td>3.559 - 3.677</td>
</tr>
<tr>
<td>Maltose</td>
<td>3.42</td>
<td>9.090</td>
<td>3.342 - 3.470</td>
</tr>
<tr>
<td>Xylose</td>
<td>3.00</td>
<td>0.056</td>
<td>2.975 - 3.059</td>
</tr>
<tr>
<td>Mannose</td>
<td>3.60</td>
<td>0.066</td>
<td>3.540 - 3.640</td>
</tr>
<tr>
<td>Arabinose</td>
<td>3.00</td>
<td>0.057</td>
<td>2.982 - 3.069</td>
</tr>
</tbody>
</table>
### Table V

Limits of tolerance of foreign substances in the determination of carbohydrates

<table>
<thead>
<tr>
<th>Foreign substance</th>
<th>Maximum amount of the foreign substance added (mg)</th>
<th>Amount of Glucose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>0.22</td>
<td>3.6</td>
</tr>
<tr>
<td>Acetone</td>
<td>50.00</td>
<td>3.6</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>2.52</td>
<td>3.6</td>
</tr>
<tr>
<td>N,N\textsuperscript{-}diethylformamide</td>
<td>7.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Lysine</td>
<td>3.65</td>
<td>3.6</td>
</tr>
<tr>
<td>Thiourea</td>
<td>3.8</td>
<td>3.6</td>
</tr>
<tr>
<td>B- naphthal</td>
<td>1.44</td>
<td>3.6</td>
</tr>
</tbody>
</table>
DISCUSSION

The tentative reaction mechanism of the recommended test for the detection and determination of carbohydrates can be described in two different ways:

1. Nucleophilic reagents can displace chloride ion (although it is inert to these reagents) from 1-chloro-2,4-dinitrobenzene because the nitro group (strong electron attracting) attached to the benzene ring fulfills the required conditions of being at para or ortho position or both to the halide group. Such activation influence of two nitro groups attains a very high value that is to a factor of at least $10^8$ (13). In sodium carbonate, carbonate ion interacts with water to form a conjugate acid-base pair. The OH ion so produced has a nucleophilic attack on the carbon of halide group and thus causes its elimination (14).

\[
\begin{align*}
\text{Cl} & \quad \text{NO}_2^- \quad \text{Na}_2\text{CO}_3 \\
\text{Cl} \quad \text{NO}_2^- + \text{OH} & \quad \text{on boiling water bath} \\
\text{Cl} & \quad \text{OH} \quad \text{NO}_2^- \quad \text{Cl} \\
\text{Cl} & \quad \text{NO}_2^- \quad \text{Na}_2\text{CO}_3 \\
\text{Cl} & \quad \text{OH} \quad \text{NO}_2^- \quad \text{Cl}
\end{align*}
\]

The phenolic group of 2,4-dinitrophenol so produced undergoes an intramolecular dipole-dipole interaction with nitro group at ortho position. This produces polarized H-O and N-O bonds.
The carbon chain of carbohydrate is associated with polar OH group which forms hydrogen bonding with the above compound (I)

(2) As an alternate method the mechanism can also be proposed in the light of Meisenheimer complexes. In 80:20 v/v water - dimethylsulphoxide mixture, base (carbonate ion) addition at the different positions of ring - carbon atoms of 1 -chloro- 2,4 -dinitrobenzene may be expected as follows:

The above reaction is expected to be a parallel case of the reaction between sulphite ion (base) and 1 -chloro- 2,4 -dinitrobenzene in 80:20 v/v water - dimethylsulphoxide mixture (15).
The carbonate group of 0-adduct species (II) interacts with OH groups of carbohydrate chain forming weak hydrogen bonding.

\[ \text{O} - \text{H} - \text{O} \quad \text{NO}_2 \quad \text{C} \quad \text{NO}_2 \]

The sequence of the above two reactions is confirmed by spectroscopic studies. The \( \lambda \text{ max. for 1-chloro-2,4-dinitrobenzene which is found to be 250.9 nm is slightly shifted towards higher wavelength (} \lambda \text{ max. = 266 nm} \) when carbonate ions are added to it under the same conditions of the recommended procedure. Further when carbohydrate is added to this mixture, shifting of \( \lambda \text{ max. from 266 to 271.2 nm is observed (see table VI). This small shifting in } \lambda \text{ max may be attributed due to the formation of weak hydrogen bonding. This results in electron localization with a decrease in energy level to stabilize the reaction with small degree of magnitude. The quantitative studies have been carried out with Bausch and Lomb spectronic - 20 (U.S.A.) spectrophotometer. The complex is found to give a } \lambda \text{ max. at 350 nm and Beer's law holds good at monochromatic radiation.} \]
### Table VI

\(\lambda_{\text{max}}\) values of different possible species formed by the combination of any three/two or individual species

<table>
<thead>
<tr>
<th>Species</th>
<th>(\lambda_{\text{max}}) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-chloro-2,4-dinitrobenzene</td>
<td>250.9</td>
</tr>
<tr>
<td>1-chloro-2,4-dinitrobenzene and sodium carbonate</td>
<td>266.0</td>
</tr>
<tr>
<td>1-chloro-2,4-dinitrobenzene, sodium carbonate and carbohydrate</td>
<td>271.2</td>
</tr>
</tbody>
</table>
REFERENCES


