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Quantitative spectrophotometric estimation of specific monosaccharides by DNSA method

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The determination of saccharide is widespread and has led to the development of numerous analytical techniques for measuring its concentrations. Analytical tool with various applications in different fields are tedious and have time-consuming protocols ranging from food analysis to biology. The overall purpose of this research is to develop easy methods for analyzing reducing sugars or saccarides. The most abundant monosaccharide in nature Dglucose sometimes referred to as dextrose in our research its shows a peak difference of 1nm as one water molecule is always loosely attached to dextrose ring structure. In this article it is seen that DNSA methods has advantage of its simplicity, low cost, sensitivity and adoptable during handling the analysis of a large number of samples at a time.

Keywords: - Monosaccharides, Reducing sugars, Spectrophotometric, DNSA method.

INTRODUCTION

Monosaccharides are the building blocks of all known biopolymer structures in which carbohydrates are the most abundant biomolecules on Earth. Insoluble carbohydrate polymers function as structural and protective essentials in the cell walls of plants and bacteria as well as in the connective tissues of animals. Besides, other carbohydrate polymers lubricate skeletal joints and play a part in recognition and adhesion between cells. More complex carbohydrate polymers covalently attached to proteins or lipids act as signals that determine the intracellular location or metabolic fate of these hybrid molecules, which are called glycoconjugates. Carbohydrates are polyhydroxy aldehydes or ketones or substances that yield such compounds on hydrolysis. There are three major size classes of carbohydrates: monosaccharides, oligosaccharides, and polysaccharides. 'Saccharide' means sugar or 'sweet in taste'. Monosaccharides of more than four carbons tend to have cyclic structures. Oligosaccharides consist of short chains of monosaccharide units, or residues, joined by

characteristic linkages called glycosidic bonds.

In cells, most oligosaccharides consisting of three or more units do not occur as free units but are joined to nonsugar molecules like lipids or proteins in glycoconjugates. The polysaccharides are sugar polymers containing more than 20 monosaccharide units, and some have hundreds or thousands of units. Polysaccharides like cellulose are in linear chains and

others, such as glycogen are branched. Both glycogen and cellulose consist of recurring units of D-glucose, but they differ in the type of glycosidic linkage and as a result have strikingly different properties and biological roles (Nelson et al, 2004).

MATERIALS AND METHOD

CHEMICALS USED

Dinitrosalicylic acid (DNSA), crystalline phenol and D-(−)-arabinose were obtained from HI-Media (India), and D-(+)-galacturonic acid monohydrate from Fluka Analytical-Sigma Aldrich (India). Dextrose (anhydrous), D-fructose, D-glucose (anhydrous), D-xylose, potassium sodium tartarate (Rochelle salt), sodium sulphite and sodium hydroxide were obtained from Loba Chemie (India).

PREPARATION OF DNSA REAGENT

Dinitrosalicylic Acid Reagent (DNSA Reagent) was prepared by dissolving 1 g DNSA, 200 mg crystalline phenol and 50 mg sodium sulphite in 100 mL 1% NaOH and was stored at 4°

C. The reagent deteriorates because of sodium sulphite so it is added at the time of use to enable prolonged storage which is followed by addition of 40% Rochelle salt solution

(Potassium sodium tartarate).

PREPARATION OF STANDARD GRAPH FOR SPECIFIC MONOSACCHARIDES

Stock solutions (mg/mL) of specific monosaccharides [D-(-)-arabinose, Dextrose (anhydrous), D-fructose, D-glucose (anhydrous), D-xylose and D-(+)-galacturonic acid monohydrate] were prepared in distilled water. Spectra were recorded with different concentrations of monosaccharides in the range 25 mcg – 1000 mcg with dilution. A peak was selected in visible wavelength 400–800 nm by mean data.

Reaction mixture of 3 mL was prepared from the extract stock solution of which 20mcg to 250mcg was used in test tubes and equalizes the volume to 2 mL with distilled water. DNSA reagent 0.5 mL was added and kept in water bath at 80°C to 85°C for 15 min. When the contents of the tubes were still warm, 0.5 mL of 40% Rochelle salt solution was added.

Reaction mixture was cooled and absorbance of the coloured complex was measured using a Jasco V-530 spectrophotometer. Standard graph was plotted with monosaccharide concentration (microgram) on X-axis against absorbance on Y- axis.

RESULT AND STANDARD GRAPH

Spectrum and Standard graph of monosaccharides

Figure 1.1: D-(+)-Galacturonic acid monohydrate spectrum

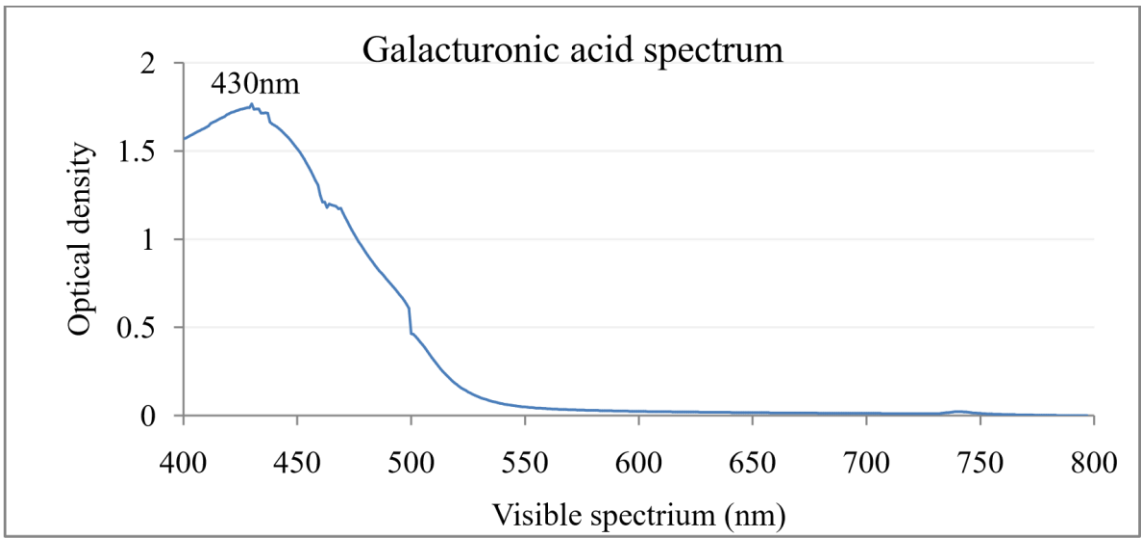


Figure 1.2: D-(+)-Galacturonic acid monohydrate standard graph at 430 nm

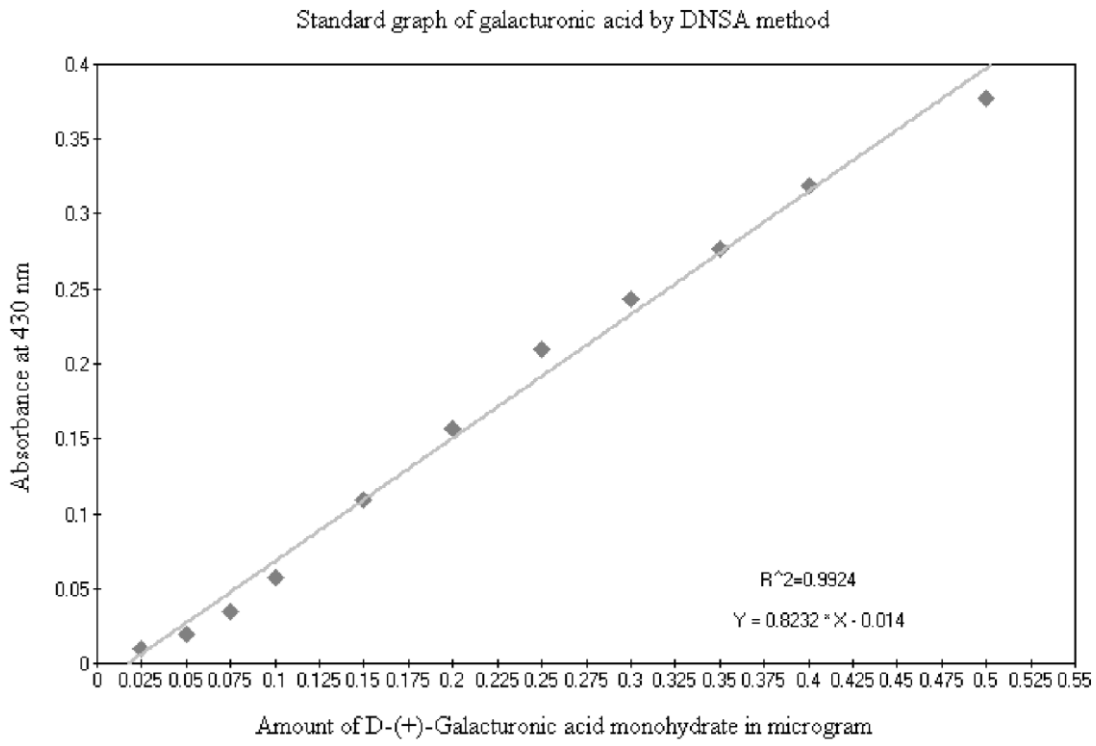


Figure 2.1: D - Fructose spectrum

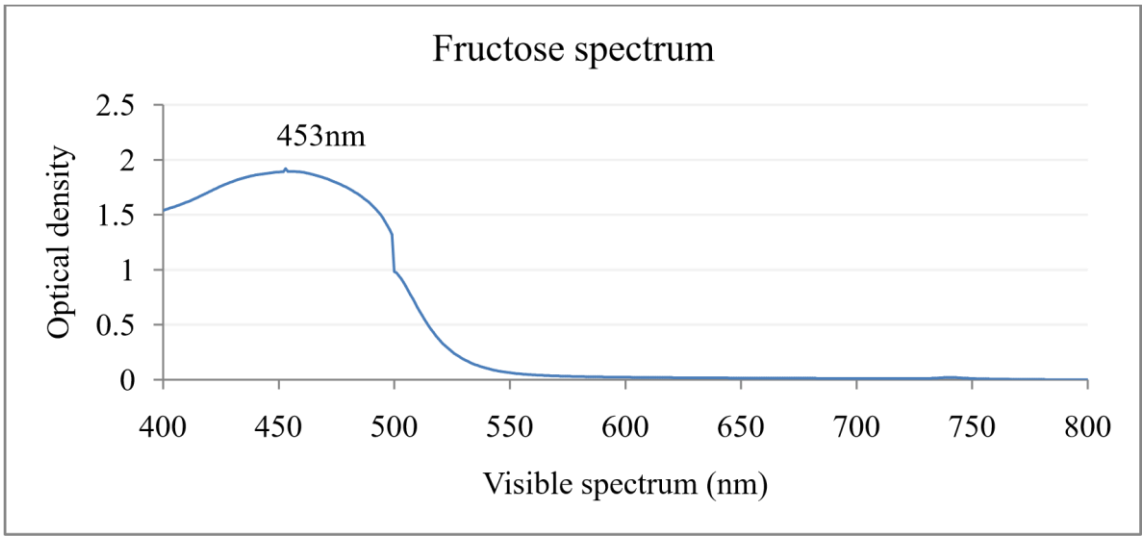


Figure 2.2: D - Fructose standard graph at 453nm

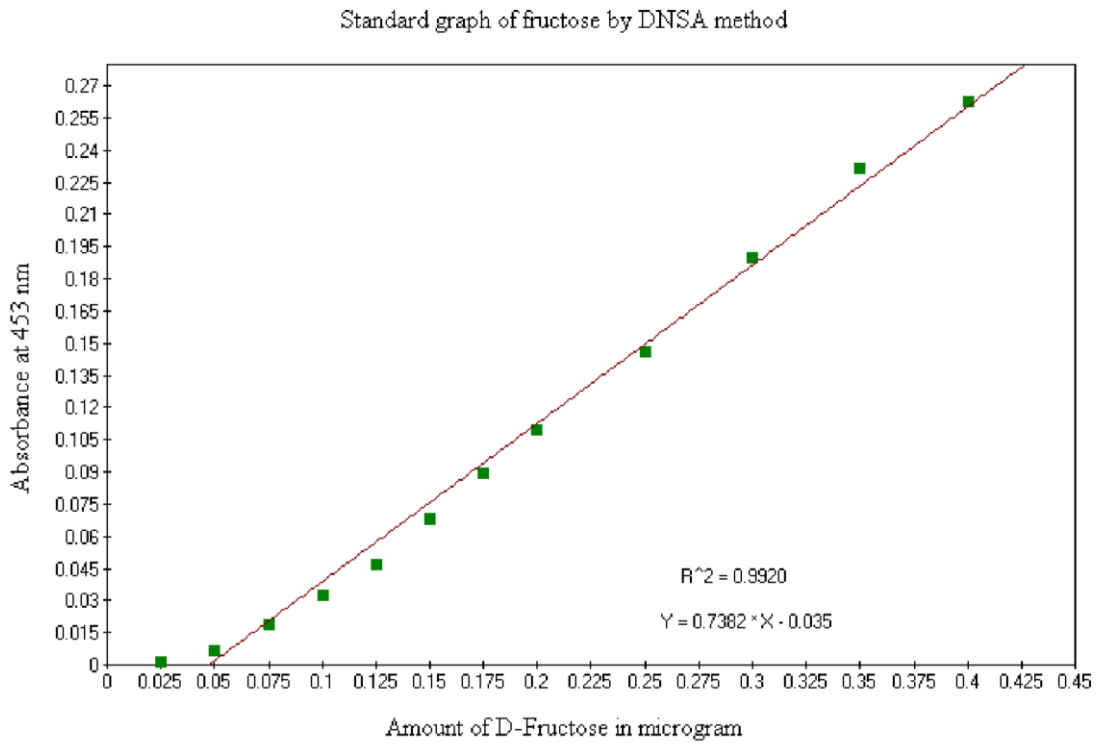


Figure 3.1: Dextrose (anhydrous) spectrum

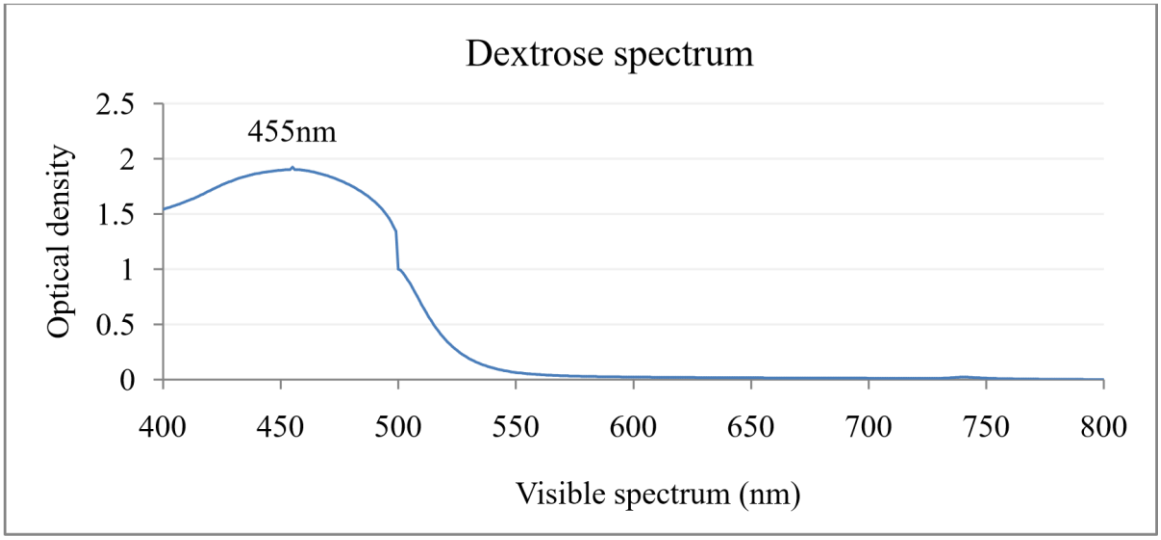


Figure 3.2: Dextrose (anhydrous) standard graph at 455nm

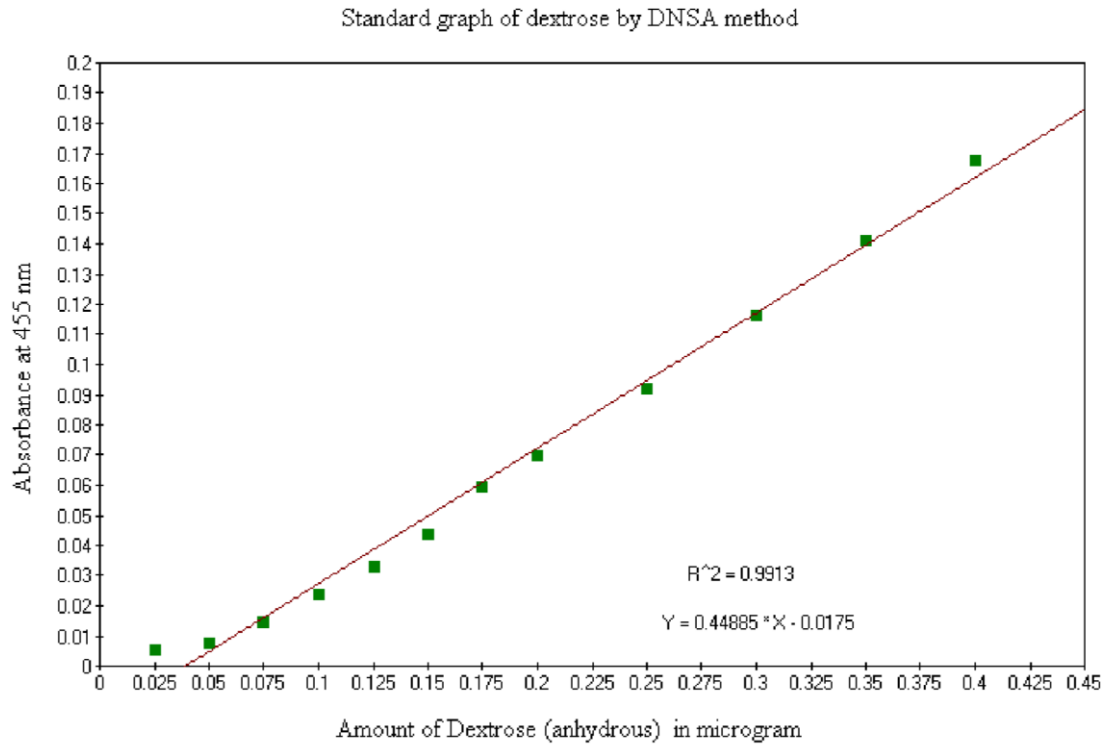


Figure 4.1: D - Glucose (anhydrous) spectrum

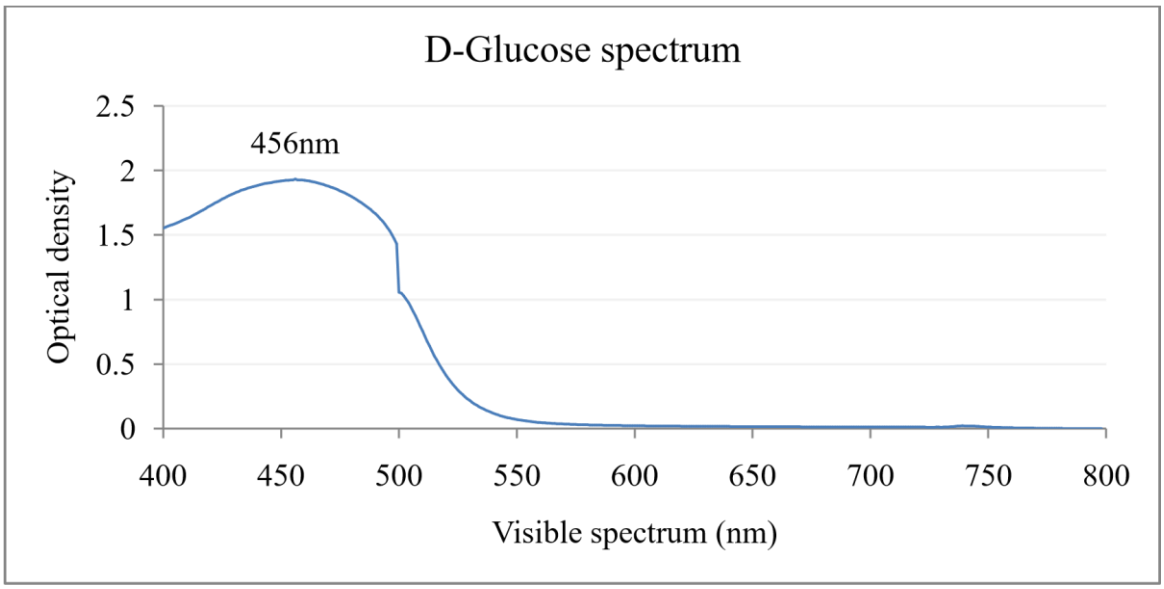


Figure 4.2: D – Glucose (anhydrous) standard graph at 456nm

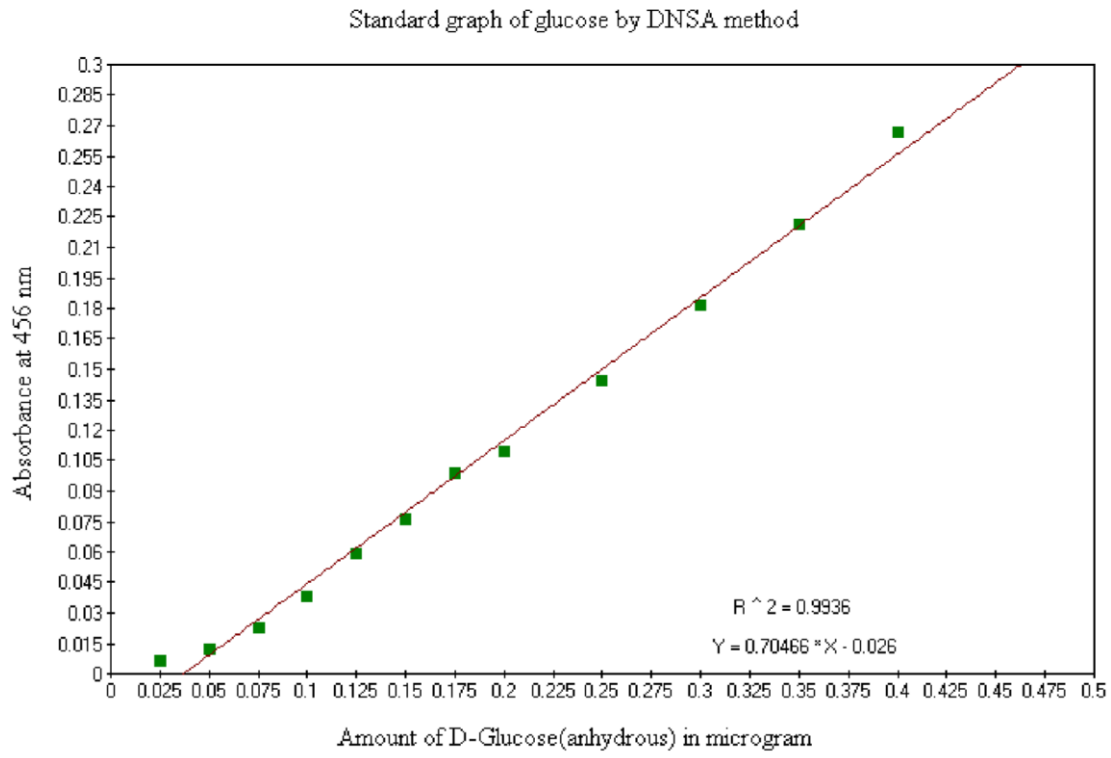


Figure 5.1: D-xylose spectrum

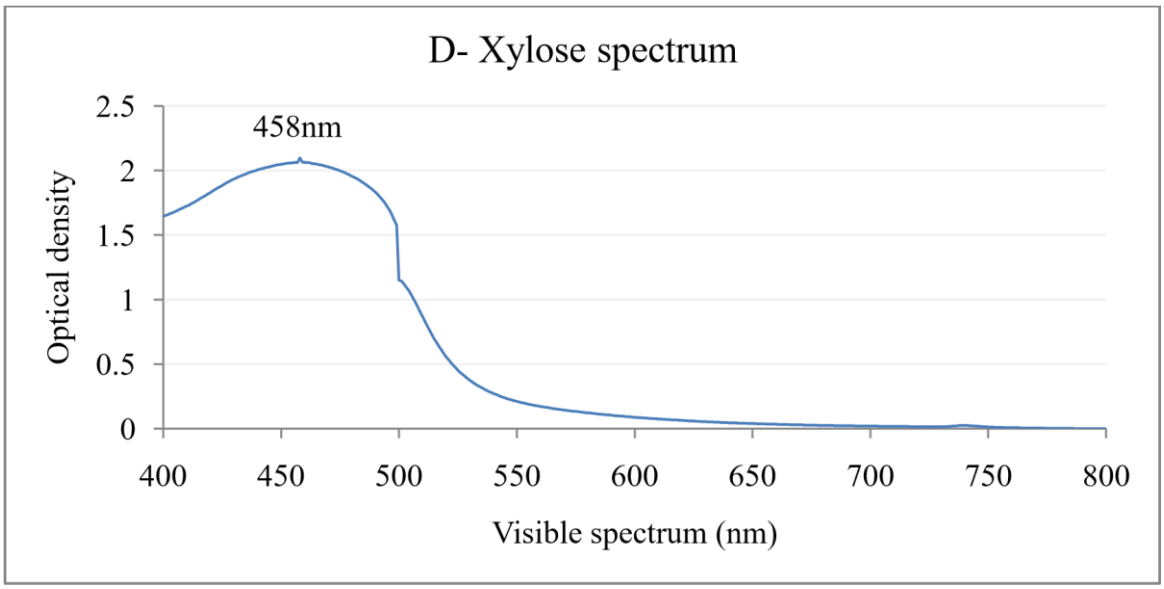


Figure 5.2: D- Xylose standard graph at 458nm

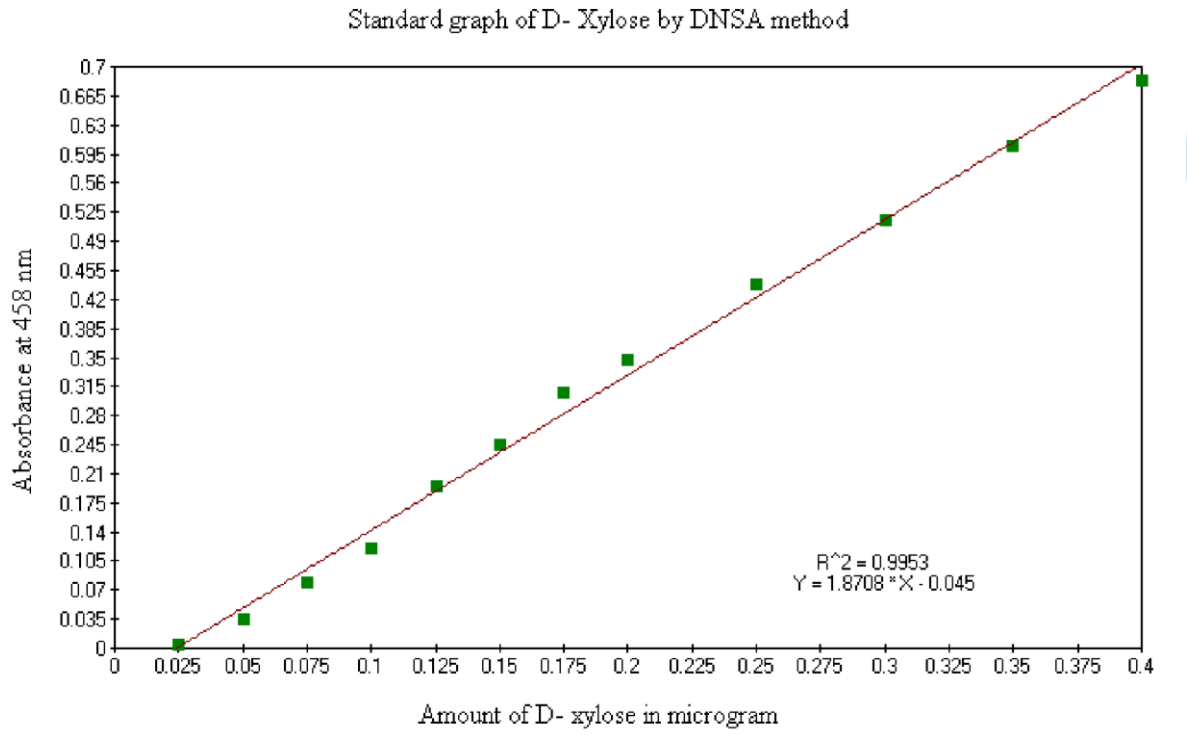


Figure 6.1: Arabinose spectrum

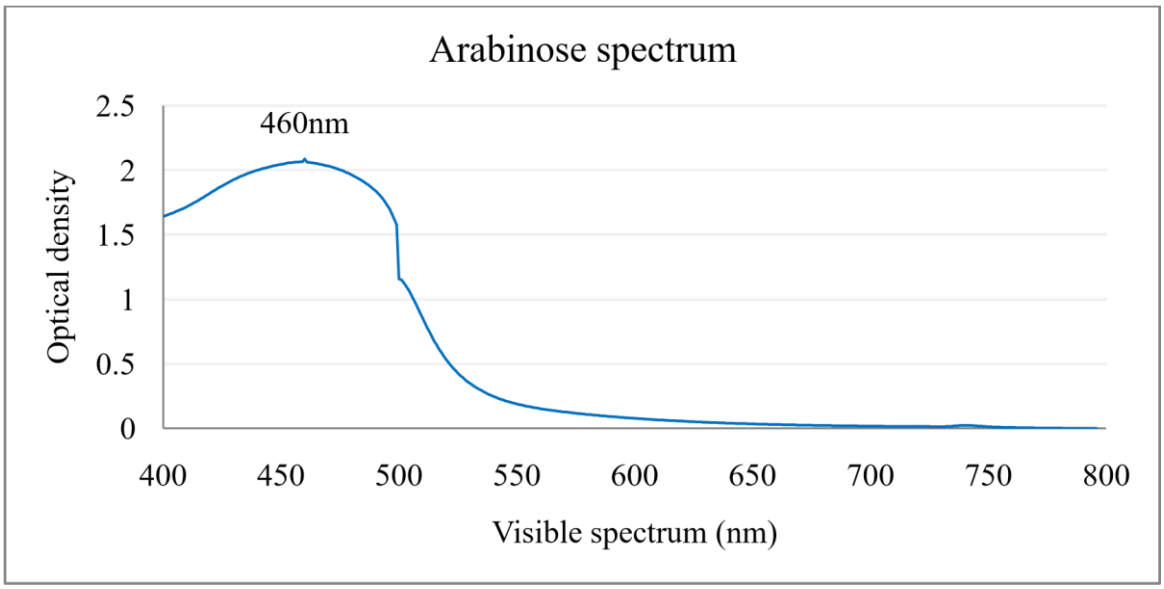
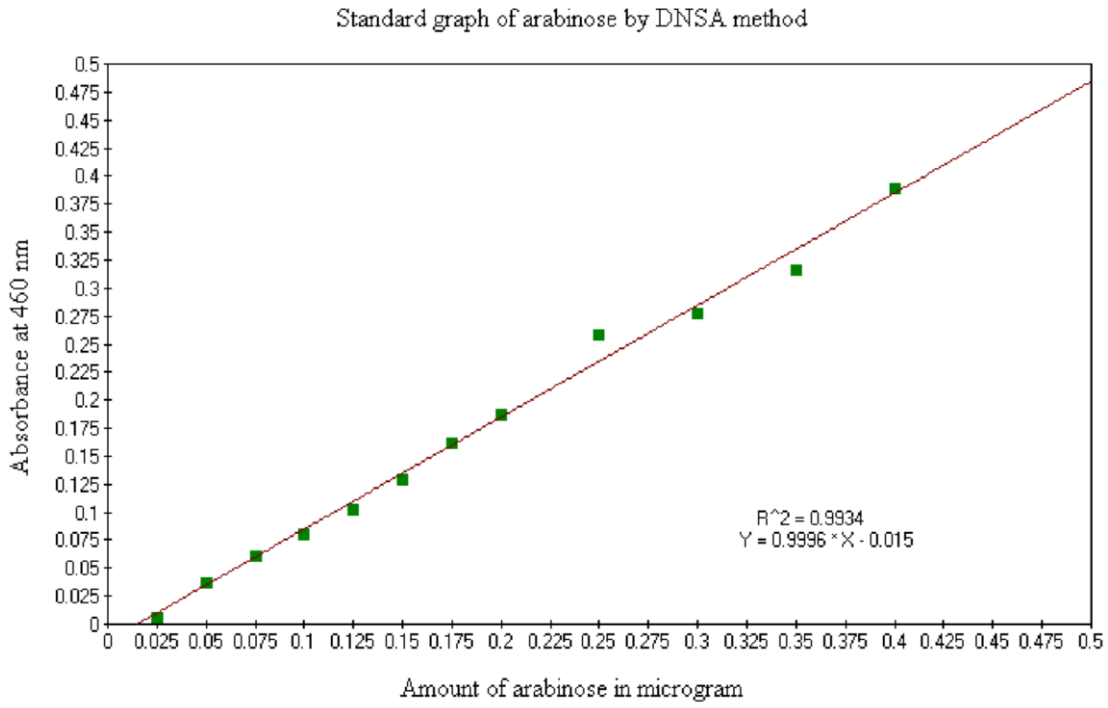


Figure 6.2: Arabinose standard graph at 430nm



DISCUSSION

The DNSA method for estimating the concentration of reducing sugars in a sample was originally described by G. Miller in 1959. Reducing sugars have the property to reduce many reagents. A reducing sugar is one that in a basic solution forms an aldehyde or ketone and which contains free carbonyl group. The oxidation of aldehyde group of all monosaccaride

(glucose, dextrose, fructose, xylose, arabinose and etc.) converts 3, 5-dinitrosalicylic acid (DNSA - yellow) to its only reduced product 3-amino-5-nitrosalicylic acid (ANSA - orange), which is the reduced in an alkaline solution. Water is used up as a reactant and oxygen gas is released during the reaction. The formation of 3-amino-5-nitrosalicylic acid results in a change in the amount of light absorbed, at wavelength recorded as maxima. The absorbance measured using a spectrophotometer is directly proportional to the amount of reducing sugar

(Miller, 1972).

3, 5-dinitrosalicylic acid (Aldehyde group) → 3amino, 5nitro salicylic acid (carbonyl group)

Thus a set of standards with known concentrations can be made and a linear graph for specific monosaccharide can be obtained by which unknown concentrations of specific monosaccharides can be extrapolated by plotting a graph of concentration vs. absorbance.

Different reducing sugars generally yield different colour intensities; thus it is necessary to calibrate for each sugar. In addition to the oxidation of the carbonyl groups in the sugar, other side reaction such as the decomposition of sugar also competes for the availability of 3, 5dinitrosalicylic acid. Although this is a convenient and relatively inexpensive method, due to the relatively low specificity a blank is used to be interpret the spectrophotometric results

correctly and accurately (Sadasivam & Manickam, 1996).

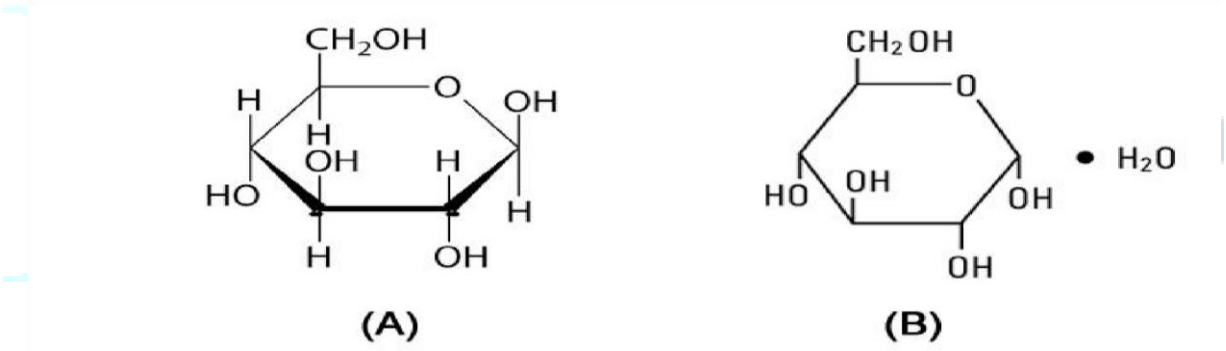
Table 1: Representative wavelength (nm) for each monosaccharide

Monosaccharide	**Wavelength (nm)**
D-(+)-Galacturonic acid monohydrate	430
D-Fructose	453
Dextrose (anhydrous)	455
D-Glucose(anhydrous)	456
D - Xylose	458
D-(-)-Arabinose	460

Monosaccharides are colourless, crystalline solids that are freely soluble in water but insoluble in non-polar solvents. Most have a sweet taste. The backbones of common monosaccharide molecules are unbranched carbon chains in which all the carbon atoms are linked by single

bonds and in open-chain form, one of the carbon atoms is double-bonded to an oxygen atom to form a carbonyl group; each of the other carbon atoms have a hydroxyl group. If the carbonyl group is at an end of the carbon chain than it is in aldehyde group and the monosaccharide is an aldose; if the carbonyl group is at any other position the monosaccharide is a ketose. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose. Hence, during analysis peak difference of 1nm is seen between glucose and dextrose. Compound A is glucose and compound B is dextrose as seen in below figure one water molecule in B is always loosely attached to its ring structure (Nelson et al, 2004) thus we see the peak difference of 1nm. It also shows

sensitivity of the DNSA method.



Several chemical reactions of the carbonyl groups of monosaccharides are seen of which the addition of a hydroxyl group from within the same molecule, generates the cyclic forms of five- and six-carbon sugars which are predominate in aqueous solution and creates a new chiral center, adding further stereochemical complexity to this class of compounds. Many of the carbon atoms to which hydroxyl groups are attached are chiral centers, which give rise to the many sugar stereoisomers found in nature. Monosaccharides with backbone of three to seven carbon form structure and stereoisomeric of sugars (Nelson et al, 2004).

Methods most commonly used for estimation of aldose-type sugars are based on their reducing action toward certain metallic salts. Compositional analyses or determination of individual sugars monosaccharides in samples requires additional advance techniques, among which principally used the non-chromatographic techniques are enzymatic assays, while most common chromatographic methods rely upon GC and HPLC. Hence, our proposed methods have some advantages of the simplicity, low cost, sensitive and adoptable during handling of a large number of samples at a time. For sugar estimation an alternative to simple dinitrosalicylic acid method is the Nelson-Somogyi's method which is unsuitable for large

number of samples on commercial basis (Gusakov et.al, 2011).

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