A New Sensitive, Eco-Friendly and Effortless Colorimetric Method for the Estimation of Reducing Sugars

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ABSTRACT

Quantitative organic analysis (Volumetric analysis) is an integral part of the university chemistry laboratory curriculum. This training type is essential as a student develops basics of reactions, experimental skills, calculative mind, analytical skill and systematic analysis. However, the conventional method of analysis such as gravimetric, volumetric methods generates considerable waste containing a mixture of hazardous chemicals. The scraps are difficult to reuse, which pollutes the atmosphere to a greater extent and commercial disposal of this invites a considerable expense. Further, student uses a large volume of reagents (60 mL) for one reading of titration and must take concordat readings by performing at least four sets (240 mL). Thousands of students produce about (300mL /student) waste while conducting these experiments. In conventional methods, complex and expensive hazardous reagents chemicals like Fehling's solution in volumetric analysis, potassium ferrocyanide and zinc in Hagedorn-Jenson Method, H_2SO_4, KI and KIO_3 in Somogyi's method were used which will now be replaced with green, less hazardous, inexpensive, easily available in contrast to mentioned in the literature. Keeping these factors in mind a new colorimetric analysis of reducing sugar is developed, which is sensitive and can detect in micrograms with UV-Visible spectrophotometer or colorimeter instruments. This experiment will be fruitful to study as it will modify the tedious, hazardous, waste generating methods to simple, eco-friendly, energy and time-efficient process**.** The method will train the students to deal with micro volumes of reagents and develop environmental sensitisation.

Key Words

Reducing sugar, metadinitrobenzene, colorimetric method, economic, green method, quantitative estimation,

INTRODUCTION

The determination and quantification of sugars are significant for university laboratory experiments and quality control, assurance of horticultural products[1,2]. Quantitative organic analysis (Volumetric analysis) is an integral part of the university chemistry laboratory curriculum. This training type is essential as students develop basics of reactions, experimental skills, calculative mind, analytical skill, and systematic analysis. However, the conventional method of analysis such as gravimetric, volumetric methods generates considerable waste containing a mixture of hazardous chemicals. The wastes are difficult to reuse, which pollutes the atmosphere to a greater extent and commercial disposal of this incurs a vast expense.

Further, student uses a large volume of reagents (60 mL) for one reading of titration and must take concordat readings by performing at least four sets (240 mL). Thousands of students produce about (300mL /student) of waste while conducting these experiments. Sweetness in many fruit and vegetables is a looked-for and is often overseen, in part, by sugar concentration. The level of reducing sugars in wine, juice, and sugarcane is symbolic of the quality of these food products. Monitoring the levels of reducing sugars during food production has improved the market quality of food. The sugar content and sweetness of fruit and vegetables are commonly quantified by sensory evaluation and instrumental assessment.[3] Colorimetry or refractometry are generally preferred for estimating monosaccharides and disaccharides sugar such as glucose, fructose. The conventional method for doing so is the Lane-Eynon method[4], which involves titrating the reducing sugar with copper(II) in Fehling's solution in the presence of redox indicator, methylene blue. However, it is inaccurate, expensive, and sensitive to impurities.[5,6]

The instrumental techniques, such as chromatography and Visible to near-infrared spectroscopy (vis/NIRS), are exact and beneficial. They require extensive sample preparation based on solvent extraction and are expensive, time-consuming and initial validation and calibration steps are needed[2]. The use of organic solvent in these techniques further contributes to the risk of health hazard due to exposure to volatile organic compounds. Current trends, therefore, favour analytical methods that are simple to use, quick and non-destructive. The increasing demand for internal quality assurance in the food industry has encouraged developing a wide range of advanced rapid, real-time, reliable and non-invasive technologies for quality assessment of sugars. Thus, this study attempts to compare the results obtained from new method of glucose estimation with existing laboratory methods.

This study aims to simplify the quantitative estimation of glucose as representative example of reducing sugar. The present titrimetric methods are tedious, time-consuming and requires many reagents. This present MDN method requires to minimise time, fewer reagents and a relaxed working procedure. The intensity of the wine-red colour developed in the reaction depends on the concentration of glucose, and the standard curve of glucose is plotted using MDN method. The method is accurate, detect upto 100 mmoles of glucose and reproducible. This MDN reagent has high sensitivity and has λ Max of 390nm and explore the reducing property of glucose for many applications in qualitative and quantitative chemical analysis.

Apparatus and Reagents

Reagent 1. Prepare the standard solution of anhydrous glucose 0.1M in double distilled water by dissolving 1.8 g of dextrose in 100 mL of double-distilled water. The different dilute concentrations were prepared from this stock solution (see Table 1).

Reagent 2. Prepared the MDN reagent by dissolving 1g of 1,3-dinitrobenzene in 2N NaOH and make up the volume to 100 mL with double distilled water. For each dilution of 0.1 M glucose solution, 2 mL of this reagent were used.

Experimental procedure: To 2 mL of glucose solution of known concentration added 2 mL of MDN reagent in each test tubes in accordance with Table 1.

Ten different dilutions of 0.1M solution of glucose (**N_1**) in final solution volume of 2 mL (**V_2**) were prepared in test tubes whose concentrations can be calculated using the equation 1. To each diluted solutions in separate test tubes, added 2mL of MDN reagent in accordance with Table 1. The test tubes were plugged with marble or cotton and kept in a boiling water bath for 5 minutes. Let the test-tubes to cool at room temperature since the optical density is sensitive to temperature. Recorded the optical density of the different room temperature cooled solutions at 390 nm in UV-Visible spectrophotometer. Standard curve is plotted between concentration of glucose in micromoles and absorbance.

$$M_1V_1 = M_2 V_2 \qquad \textbf{Eq. 1}$$

Where, **M_1** = 0.1 N (Normality of stock solution of glucose);

V_1 = Volume of glucose stock solution used for dilution

M_2 = unknown ; **V_2** = 2 mL (final volume after dilution)

Table 1 Preparation of different dilute solutions of glucose

S.No	Double distilled water (mL)	Volume of 0.1M glucose stock solution **V_1**(mL)	Concentration of glucose solution **M_2** **(moles/L)**	MDN reagent (mL)	Concentration of glucose in (µmoles/mL)	Optical density (a.u)
1	1.9	0.1	0.095	2	95	0.13
2	1.8	0.2	0.19	2	190	0.24
3	1.7	0.3	0.29	2	290	0.35
4	1.6	0.4	0.38	2	380	0.45
5	1.5	0.5	0.48	2	480	0.56
6	1.4	0.6	0.57	2	570	0.73
7	1.3	0.7	0.67	2	670	0.79
8	1.2	0.8	0.76	2	760	0.86
9	1.1	0.9	0.85	2	850	0.87
10	1	1	0.95	2	950	0.95

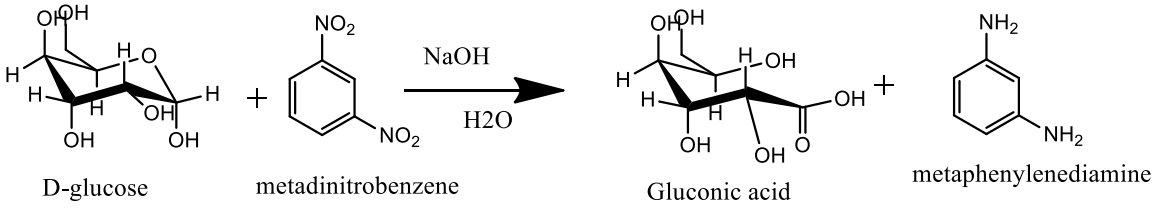
RESULTS AND DISCUSSION

Standard curve of glucose: The standard graph is plotted and is shown in Fig. 1. Plotted the standard curve by taking a concentration of glucose (µmoles/mL) along X-axis and absorbance at 390 nm along Y-axis. From the standard curve calculated the concentration of glucose in the various samples. The experiment was repeated by multiple students in triplicates on different days to check the method's validity. In all the experiments, results, including the readings in triplicates of the same experiment set, varied between 0.01 to 0.02 units in absorbance values. Absorbance (A) is a dimensionless quantity and is given by the following equations 2 and 3, where, b is the path length of light in centimetres, T is the per cent transmittance and c is the concentration in µmoles/mL of glucose.

$$A = \text{Ɛ} \times b \times c \qquad \text{Eq. 2}$$

$$A = \log_{10} (T_{solvent} / T_{solution}) \qquad \text{Eq. 3}$$

Mechanism of the reaction: Glucose an aldohexose, is a reducing agent and is well known to reduce many compounds. Reduction of dinitrosalicyclic acid to 3-amino-5-nitro salicylic acid is shown (Scheme 1). We propose the reduction of MDN reagent takes place in alkaline medium via ene-diol form of the glucose. The MDN reagent is reduced from its pale-yellow coloured solution to its wine red reduced form in alkaline medium and the intensity of the colour produced is found to be dependent on the concentration of glucose and other reducing sugars. Ketoses like fructose first comes in enediol form to get converted to aldose form and finally acting as reducing sugar. The chromogenic product produced in the reaction has maximum wavelength of 390 nm. The lambert beer's law is followed to give linear relationship between concentration of glucose and absorbance recorded.



Scheme 1

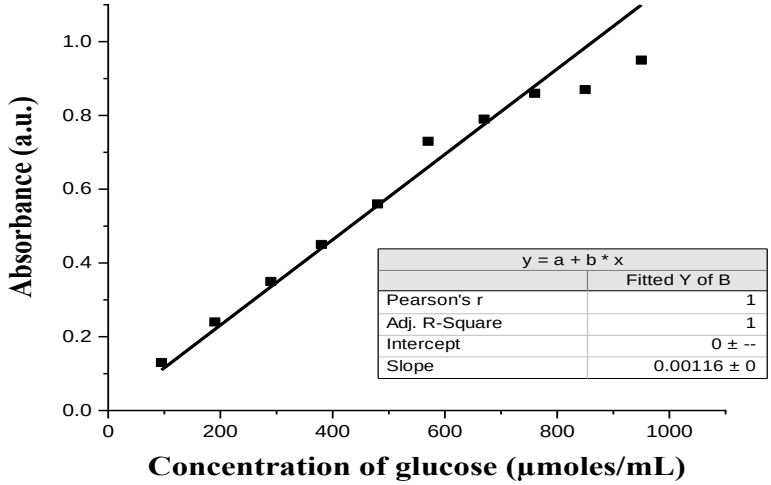


Fig. 1 Standard curve of glucose with MDN reagent at 390 nm.

Standardisation of the method:

The blank test of MDN reagent was performed with different bases to check whether this develops any colour see Fig. 2. It can be inferred from the figure that only the pale-yellow colour of the MDN reagent is observed, and there is no darkening of the colour either in the presence of NaOH or Na_2CO_3.

The reaction of various nitro compounds was investigated with glucose (see Fig.3). It was found that metadinitrobenzene took less time 1 minute for colour development than other nitro compounds is, therefore, the compound of choice (see Table2).

Fig. 2 MDN reagent reaction with NaOH and Na_2CO_3

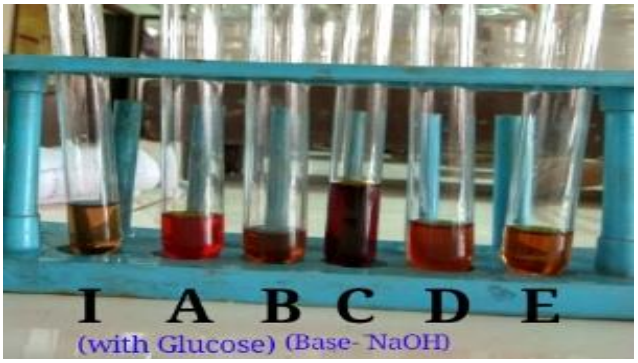


Fig. 3 Reaction of glucose with various nitro compounds**;** with NaOH (I); with 3,5- Dinitrosalicylic acid (A); with 2,4 -Dinitrophenylhydrazine (B.); with Nitrobenzene (C); with Picric acid(D); with m-Dinitrobenzene (E)

Table 2 Reaction of various nitro derivatives with glucose

S.No	Reagent	Time (minutes)	Colour
A	3,5- Dinitrosalicylic acid	3:00	Wine red
B	2,4 -Dinitrophenylhydrazine (in alco.)	2:30	Wine red
C	Nitrobenzene	3:00	Wine red
D	Picric acid	1:25	Wine red
E	m-Dinitrobenzene	1:00	Wine red
I	Blank test (Glucose + base)	5	Light brown

The linear dynamic range for glucose by our method is found to be 90 µ moles to 1000 µmoles at 22° C. The linearity of the graph of absorbance vs concentration are reproducible at the concentrations tested. The optimum wavelength of MDN reagent with glucose is determined to be 390 nm by plotting the graph between absorbance at different wavelength The molar absorptivity as seen from graph is 0.00116 micromoles $mL^{-1}cm^{-1}$. The intensity of the wine-red colour developed in the reaction is dependent upon the concentration of the glucose and other reducing monosaccharides and disaccharides such as fructose maltose, and lactose respectively. But non- reducing sugar, sucrose does not give any colour with MDN reagent as shown in Fig.4.

Fig. 4 MDN reagent reaction with fructose (F), lactose (L), sucrose (S) and maltose (M).

Concentration of metadinitrobenzene.
When the amounts of metadinitrobenzene were varied, the color intensity approached a maximum at a concentration of 1%. The metadinitrobenzene, had no effect on the loss of glucose over the range tested.

Accuracy of Experiments: This newly developed method is accurate to within ± 2%. This accuracy figure is obtained by plotting the results of concentration of glucose obtained with MDN reagent method and comparing the actual amount of glucose in the given solution. The percentage error is displayed in Table 3.

Concentration of reducing sugar =
(O.D. of Test-O.D. of Blank. / O.D. of Std.- O.D. of Blank) × Concentration of Std.

Table 3 Accuracy of MDN reagent method for glucose determination.

Concentration of glucose taken	Concentration of glucose found	% Error	Absorbance
100	101	1	0.98
50	49	2	0.53
20	20	0	0.24

Conclusions

The newly developed MDN method for the estimation of glucose as the reference sugar for reducing sugars gives reliable results for the estimation of the sugar content of pure sugar solutions. The colour developed in the method is quite stable and possess a definite absorption peak. The intensity of the colour produced at constant MDN reagent concentration is propotional to the amount of glucose present in the pure solution. The standard curve obtained by plotting the glucose concentration versus values of absorbance are readily reproducible. This experiment will be fruitful to study as it will modify the tedious, hazardous, waste generating methods to simple, eco-friendly, energy and time efficient process**.** The method will train the students to deal with micro volumes of reagents and develop environmental sensitivity. The MDN method is extended further to study the sugar level in blood and urine.

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