A UV-VIS SPECTROPHOTOMETRIC METHOD FOR THE CALCULATION OF ALDEHYDE CONTENT IN POLYSACCHARIDES BY USING 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE.

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ABSTRACT: *In this study, a simple technique was developed to determine the aldehyde content on an oxidized-polysaccharide. 2,3,5-triphenyltetrazolium chloride (TTC) was used to measure aldehyde analytically by UV-Vis spectrophotometric assay. Comparable results were obtained when tested against the known aldehyde contents of formaldehyde, acetaldehyde, and glutaraldehyde. The TTC UV-Vis assay was further employed to check the aldehyde produced during oxidation of natural polysaccharide konjac glucomannan (KGM). It was found that TTC UV-Vis assay was accurate even at low concentrations of aldehyde and thus this assay could be used to optimize the reaction parameters of oxidation.*

1. INTRODUCTION

Polysaccharides are the biopolymers that contain an important group of compounds and plays a vital role in the fields of food, paper, textile and fibers [1]. In the last two decades, it has been shown that the study of polysaccharide has increased in terms of their application and usefulness of many biotechnological aspects. It obtained from different sources like plant-extracted, animal extracted and microbial polysaccharides. A number of functional groups are attached to the polysaccharide molecules as hydroxyl, amine, carboxymethyl, carboxyl and many more that are liable to make it active for different other groups reaction.

Amorphophallus konjac and salep glucomannan from the orchidaceous family are the major available source of the konjac glucomannan (KGM). This is a natural polysaccharide that made of β-1,4 and β-1,3 linked D-mannose and D-glucose with the ratio of about 1.6:1 respectively and there are some branching points at the C-3 position of the mannoses. The degree of branching is about 8% and 5-10% acetyl group substitutes are present [2]. The nutritional supplement is a potential use of this polysaccharide and as an impenetrable dietary fiber that is resistive against digestive enzymes in the human gut. In the pharmaceutical field, it is being used in the synthesis of DNA-controlled release hydrogel matrix. Among many polysaccharides, KGM is preferably used in a drug delivery system and in other biomedical fields [3].

Aldehyde groups can be produced on polysaccharides by different methods in which oxidation processes are very common nowadays. For this, 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion (TEMPO) produces a high content of aldehyde by oxidizing the primary hydroxyl groups on the polysaccharide. TEMPO can be used in different ways, and one of them is the combination with sodium hypochlorite/sodium bromide. This is a basic medium reaction as the pH is more than 10 in this reaction [4]. Oxidation of polysaccharides was also done by chemo-enzymatic modification, where TEMPO is used as mediator and laccase as a biocatalyst. The conditions in this modification are mildly acidic and the temperature is 30 $^{\circ}$C. The benefits of this process are the selective introduction of aldehyde groups on saccharides in mild aqueous conditions and in fact, the process is eco-friendly [5]. But in some cases, the high content of oxidation is not useful and for that, a process was described using oxoammonium ion producing reagent along with the oxidase [6]. When an aldehyde-containing reagent reacts with the polysaccharide, it also produced aldehyde polysaccharide. This is a non-oxidative method and involved two steps. In first step, polysaccharide reacts with derivatizing acetal reagent in a highly basic medium and then in the second step, need to hydrolyze that acetal in a highly acidic medium. The cons of this method are the involvement of an extra step and opposite mediums in both of the steps [7]. Another method to produce the aldehyde on a polysaccharide was when Kulkarni and Mehta induced redox polymerization with the ceric ions. It was assumed that the Ce^{+4} ions break the C_2-C_3 bond and then by further reduction it produced three primary hydroxyl groups on the oxidized glucose unit. This technique did not get so eminence as ceric ion Ce^{+4} was difficult to separate from the leftover [8].

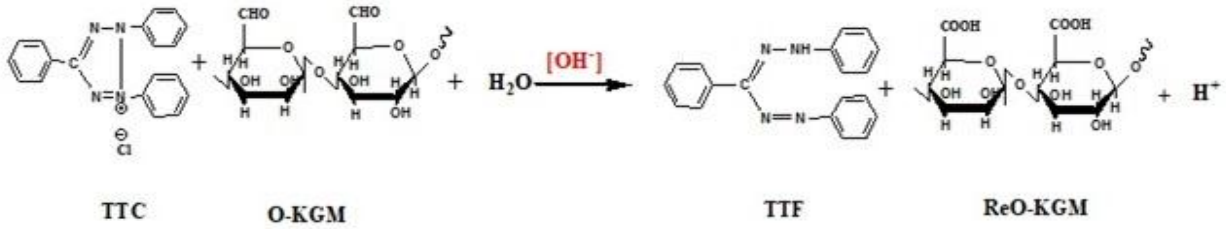

Figure 1. Reaction mechanism of 2,3,5-triphenyltetrazolium chloride with oxidized polysaccharide (KGM) and formation of red colored triphenyltetrazolium formazan

To determine the aldehyde content there were a number of methods reported. Maekawa used UV-Vis spectrophotometric analysis of residual periodate ions to calculate the degree of oxidation in partially oxidized 2,3-dicarboxy cellulose [9]. Another important procedure is finished by hydroxylamine hydrochloride. This method is known as Oxime conversion method and it involves the Schiff base reaction on the generated aldehyde groups and converts it to oxime. Another testing technique is known as the titrimetry, but it has a drawback of uncertainty to determine the endpoint by the inspector. On the other hands, all the above methods gave accurate aldehyde content values but they were costly and tiresome [9]. Infrared spectroscopy was also a commonly used method to determine the carboxyl group, but many of the aldehyde groups are bound as hydrates and cannot be identified in this technique. Aldehydes, carboxylic acid, and ketones absorb Infrared in a narrow range of 1730-1780 cm^{-1}, and due to this narrow range, it cannot be identified and distinguished among these three groups [10]. Another method to identify the aldehyde and ketone content is by using 2,4-dinitrophenylhydrazine (DNPH). This process involves the derivatizing of aldehyde into hydrazones and detecting them by the change in depth of color [11]. This method required low reaction pH that is not favorable to all saccharides. Recently a single step aldehyde content determination method was developed and announced its vast application on more than 95% compounds containing aldehydes. Oximes were produced by the combination of O-2,3,4,5,6-pentafluorobenzyl hydroxylamine hydrochloride (PFBHA.HCl) with methanol. Less labor-intensive and loss of samples are the benefits for this method in contrast to the previously described methods. As well as this method was tested on a wide range of chemical including mono, di and polycarboxylic acids and aromatic acid [12].

In the current research, a method was formulated for the determination of aldehyde content on polysaccharide by reaction with 2,3,5-triphenyltetrazolium chloride (TTC) and the formation of the corresponding formazan. The method was fully elaborated with the help of chemical reaction taking place and tested on some known aldehyde content chemicals. Then, depending upon the results, an oxidized polysaccharide KGM with unknown aldehyde content was tested.

2. MATERIALS AND METHODS

2.1. Materials

We used konjac glucomannan as a testing polysaccharide extracted from konjac gum obtained from Wuhan Qingjiang konjac Products Co. Ltd. It was purified and oxidized in Eco Textile Dyeing and Finishing laboratory, Wuxi. From Sigma-Aldrich Inc., 2,3,5-Triphenyl tetrazolium chloride (TTC) was obtained. Formaldehyde (35%), acetaldehyde (25%) and glutaraldehyde (25%) were purchased from Sinopharm chemicals ltd (Shanghai, China). Methanol absolute and potassium hydroxide (KOH) were obtained from Shanghai chemical reagent co. ltd. All the other chemicals used were of analytical grade and obtained from reliable sources.

2.2. Apparatus

All the testing was done at UV-Vis spectrophotometer-1800 (Shimadzu, Japan), TDZ6B-WS centrifugal machine (Hangzhou Summer Instruments, China) and Eppendorf Thermomixer C (Eppendorf ThermoMixer® Instruments AG, Germany) operating at 15 °C to 100 °C with a max speed of 3000 rpm.

2.3. Methods

2.3.1. Oxidation of Konjac glucomannan (KGM)

Periodic acid (1.2M, 3mL) was added in 1.5% (w/v) solution of KGM at pH 2.5 with hydrochloric acid. The flask was covered with aluminum foil and kept under constant shaking for 4 h at 60 °C. The reaction was seized by pouring 10mL absolute ethanol then, centrifuge at 4000rpm for 10min to obtained oxidized konjac glucomannan (O-KGM) [13].

Table 1. D-Glucose concentration and corresponding absorbance values for standard line formation

Concentration (mmol/L)	2.657	5.314	7.972	10.629	13.286	15.944	18.601	21.259	23.916	26.574	29.231
Absorbance value (a.u)	0.092	0.381	0.805	1.19	1.71	1.98	2.35	2.8	3.2	3.6	3.99

2.3.3. Preparation of TTC reagent

The reagent was prepared by adding 10mL (0.3M) potassium Hydroxide (KOH) into a small test tube and then added 10mL (0.01M) TTC into it. Mixed the solution well for 10 min at 25 °C.

2.3.4. Aldehyde content calculation

Take freshly prepared 1mL TTC/KOH solution in four bottles and add 10ml of 37% formaldehyde, 40% acetaldehyde, and 40% glutaraldehyde into each test tubes. Keep the forth tube without any addition and used as a blank. Put these tubes into an Eppendorf thermomixer for 10min at 80 °C shaking at the speed of 700rpm. TTC reacts with the aldehyde and makes red-colored corresponding water-insoluble formazan as shown in Figure 1. Then, the 1mL reaction mixture was added into 9 mL methanol and centrifuged at 4000rpm for 10min to remove insoluble formazan. The quantity of red colored formazan produced is directly proportional to the concentration of the available reaction reagent. This formazan is dissolved in acid pyridine and measured spectrophotometrically.

2.3.5. Preparation of standard line

In order to calculate the aldehyde content present on the given samples, an equation was formulated depending upon the calibration curve obtained from D-glucose presented in Figure 2. D-glucose was dissolved in water with ten different concentrations at room temperature and stayed there for 30 min for conditioning. All the solutions were observed spectrometrically and found the absorbance values at 480nm as shown in Table 1.

2.3.6. Measurement of absorbance value of KGM

Oxidized KGM with three different concentration was with 1 mL freshly prepared TTC solution. Kept the reactions tubes at 80 °C for 10 min constantly shaking at 700 rpm. Dilute the reaction mixtures with 9 mL absolute methanol and then centrifuge at 4000 RPM for 10 min to remove insoluble formazan. Absorbance was measured at UV spectrometer for all the solutions.

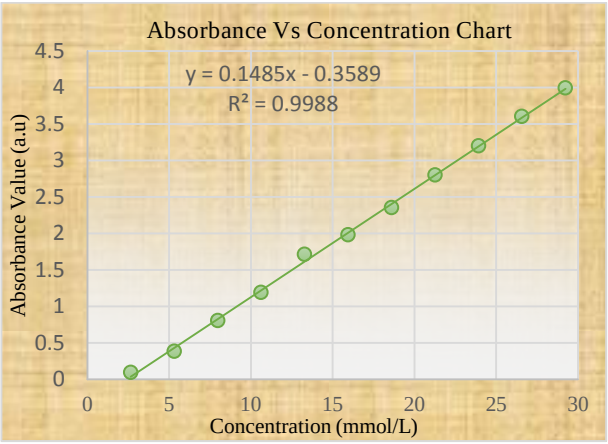

Figure 2. Calibration curve of D-Glucose with TTC at different concentrations

3. RESULTS AND DISCUSSION

In these experiments, UV-Vis-based analytical was used to calculate the aldehyde content on an oxidized polysaccharide and explained by a simple reaction mechanism. The TTC UV-Vis assay was verified against the reduction of known chemicals at different concentrations [14]. An equation for the calculation of aldehyde content percentage was formulated at 482nm with the help of standard curve drawn by D-glucose in Figure 2.

$$Aldehyde\ conc.\left(\frac{mmol}{g}\right) = \frac{Reacted\ TTC\ (mmol/g)}{Conc.(\%) \times 10^{-4}} \Big/ 334.8$$

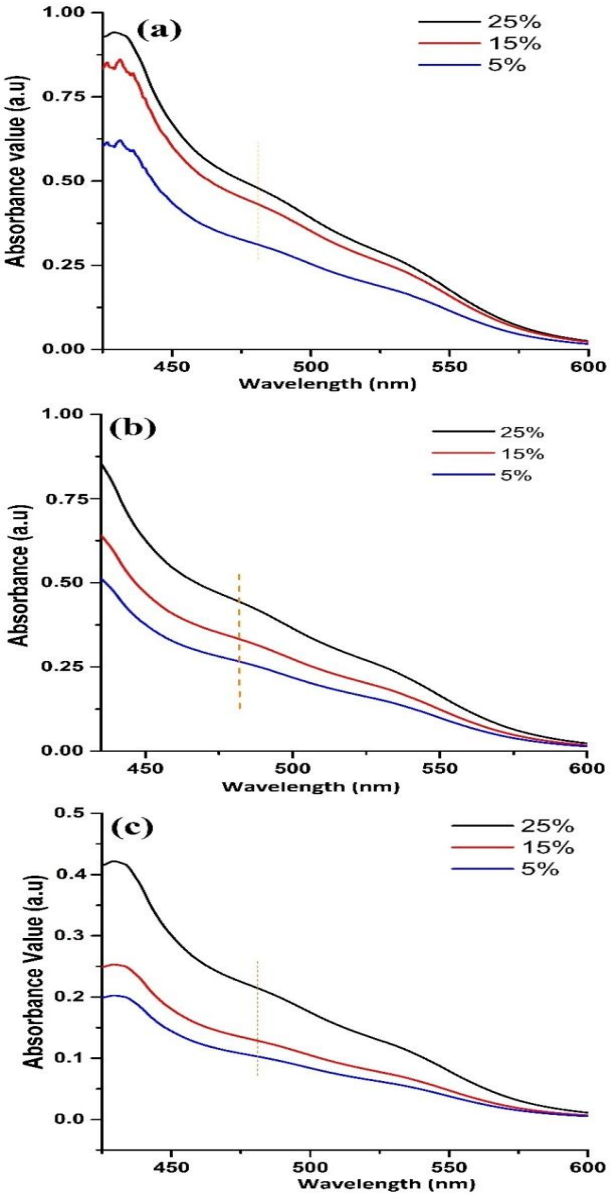

Figure 3. Absorbance values (a.u) of formaldehyde (a), acetaldehyde (b), and glutaraldehyde (c) at concentrations of 5%, 15%, and 25% each.

Where 334.8 is the molar weight of TTC. The TTC solution was colorless and readily soluble in water. Immediately after the reduction reaction with the saccharide molecule, it is been colored like red-purple. The depth of solution color depends upon the concentration of a chemical in the solution or the concentration of reduction taken place in the solution. This red color was produced due to the formation of formazan in the solution and lately, this color was measured against the standard colorless solution and comparing the values in the standard curve line produced some quantitative relationship.

The formaldehyde, acetaldehyde, and glutaraldehyde were reacted with the TTC with 5%, 15%, and 25% each and their absorbance values were checked by a spectrophotometer as shown in Figure 3. The comparison of these three chemicals showed that formaldehyde has greater absorbance value then acetaldehyde and glutaraldehyde has the least value among these three agents.

A comparison of aldehyde content was made between theoretical and measured content values of glutaraldehyde, formaldehyde, and acetaldehyde and a good agreement between these two was found. This comparison was graphically shown in Figure 4. Furthermore, the effect on aldehyde content by oxidation time of KGM was observed. For that purpose, aldehyde content was measured after every hour and presented in Figure 5.

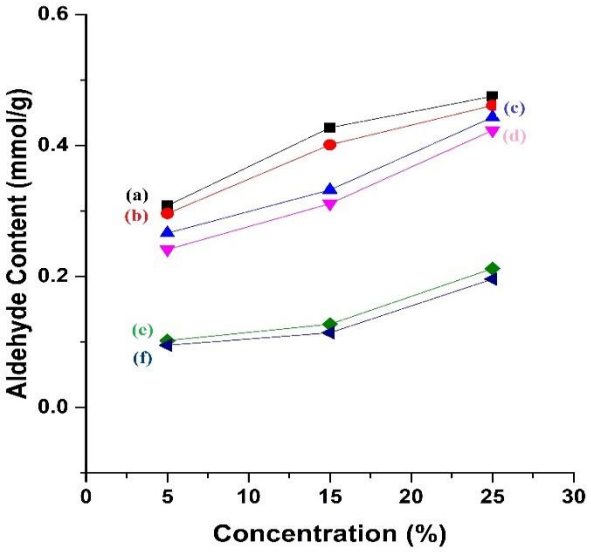

Figure 4. Concentration effect on the aldehyde content (mmol/g) measured for aldehyde (a), acetaldehyde (c) and glutaraldehyde (e) and calculated theoretically for aldehyde (b), acetaldehyde (d) and glutaraldehyde (f).

The trend shows that as time increases the aldehyde content was also increasing. At the start, it was increasing very fast and then the speed gradually decreases. It is obvious from the graph that at the start the reaction rate is higher and then it was decreasing. This could be attributed that at the start there were many reaction sights available but as the reaction goes on the sights were decreasing. The reaction rate is highest in the 2nd and 3rd hour that is 23% each and lowest in the 8th hour only 2.5%.

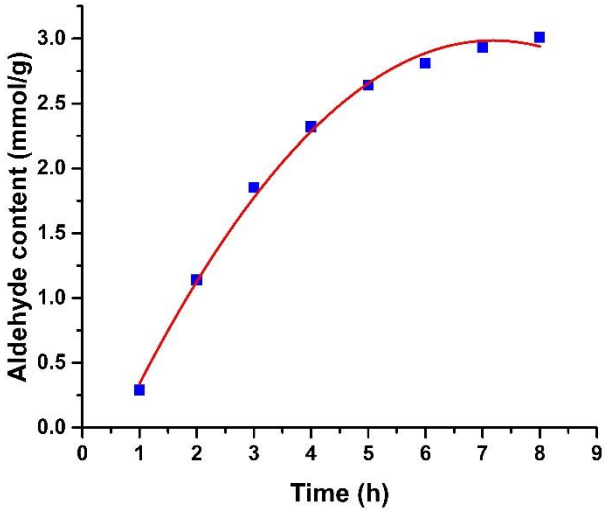

Figure 5. Effect on aldehyde content (mmol/g) values depending upon the oxidation time of KGM.

4. CONCLUSION

An economic and simple technique was developed by using 2,3,5-triphenyltetrazolium chloride and UV-Vis spectrophotometry to measure the aldehyde content on the pre-oxidized polysaccharide. The approach was further testified on the simplest aldehyde-containing compounds like formaldehyde, acetaldehyde, and glutaraldehyde and closeness in results was observed between the theoretical and measured values. The TTC reacts with the carbonyl groups to procedure insoluble red colored resultant formazan. The oxidation time study showed that the aldehyde formation increased by time up to some extent then start decreasing. Thus, during the polysaccharide oxidation process, the reaction parameters can also be optimized by TTC-UV-Vis assay.

5. REFERENCES

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