

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



Sudan University of Science and Technology
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**Preparation and characterization of carboxymethylated
gum from *pilliostigama reticulatum* seeds gum**

تحضير وتشخيص ودراسة خواص الكربوكسي ميثيل من صمغ بذور الخروب

**A Dissertation Submitted in Partial Fulfillment of the Requirements of the
Master Degree in Chemistry**

By

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Dedication

To who teach us the deeply meaning of love,

To who teach us how to be strong,

To whom I need them all my life,

To whom I find them when I need help,

To My continuous support in this life,

My parents, husband, brother and daughter

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Abstract

Carboxymethylated *Piliostigama reticulatum* seed gum (CM-PRSG) was prepared by treating the PRSG seed gum with monochloroacetic acid (MCA) in the presence of sodium hydroxide (NaOH). Various reaction conditions were optimized. The influence of the reaction temperatures and times, concentrations of sodium hydroxide and MCA on the degree of substitution (DS) were investigated. The result shows that Optimum preparation conditions for a CM-PRSG with DS of 0.0453 are: reaction temperature, 35°C; reaction time, 30 min; NaOH concentration was 4.15 mmol and MCA concentration was 4.15 mmol. The yield was 6.5 grams. Fourier-transform infrared analysis of the products indicates the successful carboxymethylation by the presence of the characteristics absorption bands at 1741 and 1000-1100 cm^{-1} .

المستخلص

تم تحضير كاربوكسي ميثايل صمغ البيلوستغما روتكولاتم من صمغ البيلوستغما روتكولاتم عن طريق المعالجة بحمض المونوكلورواسيتك اسيد في وجود هيدروكسيد صوديوم تحت ظروف تفاعل مختلفة . تمت دراسة تأثير درجة الحرارة و الزمن و تركيز حمض المونوكلورواسيتك اسيد و تركيز هيدروكسيد الصوديوم علي درجة الاستبدال. أظهرت النتائج ان الظروف المثلى لتحضير كاربوكسي ميثايل صمغ بذور البيلوستغما روتكولاتم بدرجة استبدال 0.0453 هي : درجة حرارة 35 و زمن تفاعل 30 دقيقة و هيدروكسيد صوديوم بتركيز 4.15 مل مول وحمض المونوكلورواسيتك اسيد بتركيز 4.15 مل مول وكان وزن الناتج 6.5 جرام .تحليل المركب النهائي بالأشعة تحت الحمراء أكد حصول تفاعل الإستبدال بمجموعة الكاربوكسي ميثايل وذلك بظهور الإمتصاصات المميزة لها عند 1741 والمنطقة⁽¹⁾(1000-1100 cm⁻¹).

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List of Abbreviations

PR	<i>Piliostigama reticulatum</i>
PRS	<i>Piliostigama reticulatum</i> seed
PRSG	<i>Piliostigama reticulatum</i> seed gum
CM-PRSG	Carboxymethyl <i>Piliostigama reticulatum</i> seed gum
AGU	Anhydrous glucose unit
DS	Degree of substitution
FT-IR	Fourier-transform infrared spectroscopy
MCA	Mono-chloro-acetic acid
SMCA	Sodium mono-chloro-acetic acid
CTG	<i>Cassia tora</i> gum
CM-CTG	Carboxymethyle <i>cassia tora</i> gum
GG	Guar gum
ER	reaction efficiency

Chapter one

Introduction and literature review

1.1 Polysaccharides

Polysaccharides are consist of many monosaccharide units joined together through glycosidic linkages, which can be broken by hydrolysis they are widely and abundantly distributed in nature, fulfilling roles as: Structure-forming skeletal substances (cellulose, hemicellulose and pectin in plants; chitin, mucopolysaccharides in animals).. There may be hundreds or even thousands of monosaccharide unites per molecule. Their acidic hydrolysis yields monosaccharaides. Partial chemical and enzymatic hydrolysis, in addition to total hydrolysis is important for structural elucidation. Enzymatic hydrolysis produces oligosaccharides, the analysis of which elucidates monosaccharide sequences and the positions and types of linkages. Polysaccharides (glycans) can consist of one type of sugar structural unit (homoglycans) or of several types of sugar units (heteroglycans). The monosaccharides may be joined in a linear pattern (as in cellulose and amylose) or in a branched fashion (amylopectin, glycogen, guaran). The frequency of branching sites and the length of side chains can vary greatly (glycogen, guaran) (Cui ,2000, Morrison, *et al.*, 2002, Huang, *et al.*, 2004 , Crini, 2005, Anklam, *et al.*, 2005, Coviello, *et al.*, 2007, McMurry, 2008, Harden, *et al.*, 2009, Mukherj, *et al.*, 2010).

The outlined functions of polysaccharides are based on their highly variable properties. These properties are related to their structures in a general way.

1.1.1 Perfectly Linear Polysaccharides

Compounds with a *single* neutral monosaccharide structural unit and with one type of linkage (as occurs in cellulose or amylose) are denoted as, perfectly, linear polysaccharides. They are usually insoluble in water and can be solubilized only under drastic conditions, e.g., at high temperature, or by

cleaving H-bonds with alkalies or other suitable reagents (Cui, 2000, Anklam, *et al.*, 2005).

1.1.2 Branched Polysaccharides

Branched polysaccharides (amylopectin, glycogen) are more soluble in water than their perfectly linear counterparts since the chain–chain interaction is less pronounced and there is a greater extent of solvation of the molecules. Solutions of branched polysaccharides, once dried, are readily rehydrated. Compared to their linear counterparts of equal molecular weights and equal concentrations, solutions of branched polysaccharides have a lower viscosity (Yang,*et al.*, 1998,Anklam, *et al.*, 2005).

1.1.3 Linearly Branched Polysaccharides

Linearly branched polysaccharides, i.e., polymers with a long “backbone” chain and with many short side chains, such as guaran or alkyl cellulose, have properties which are a combination of perfectly linear and of branched molecules. The long “backbone” chain is responsible for high solution viscosity. The presence of numerous short side chains greatly weakens interactions between the molecules, as shown by the good solubility and rehydration rates of the molecules and by the stability even of highly concentrated solutions (Anklam, *et al.*, 2005, Dakia, *et al.*, 2008).

1.1.4 Polysaccharides with Carboxyl Groups

Polysaccharides with carboxyl groups (pectin, alginate, carboxymethyl cellulose) are very soluble as alkali salts in the neutral or alkaline pH range. The molecules are negatively charged due to carboxylate anions and, due to their repulsive charge forces, the molecules are relatively stretched and resist intermolecular associations. The solution viscosity is high and is pH-dependent (Okamoto, *et al.*, 1994, Ralph, *et al.*, 1995, Anklam, *et al.*, 2005).

1.1.5 Polysaccharides with Strongly Acidic Groups

Polysaccharides with strongly acidic residues, present as esters along the polymer chains (sulfuric, phosphoric acids, as in furcellaran, carrageenan or

modified starch), are also very soluble in water and form highly viscous solutions. Unlike polysaccharides with carboxyl groups, in strongly acidic media these solutions are distinctly stable (Anklam, *et al.*, 2005).

1.1.6 Modified Polysaccharides

Modification of polysaccharides, even to a low substitution degree them brings about substantial changes in their properties.

The solubility in water, viscosity and stability of solutions are all increased by binding neutral substituents to linear polysaccharide chains. Thus the properties shown by methyl, ethyl and hydroxypropyl cellulose correspond to those of guaran and locust bean gum. The effect is explained by interference of the alkyl substituents in chain interactions, which then facilitates hydration of the molecule. An increased degree of substitution increases the hydrophobicity of the molecules and, thereby, increases their solubility in organic solvents. Binding acid groups to a polysaccharide (carboxymethyl, sulfate or phosphate groups) also results in increased solubility and viscosity for reasons already outlined. Some derivatized polysaccharides, when moistened, have a pasty consistence (Tolstoguzov, 1991, Okamoto, *et al.*, 1994, Ralph, *et al.*, 1995, Anklam, *et al.*, 2005).

1.1.7 Uses of polysaccharides

polysaccharides are utilized to a great extent in food processing, pharmaceutical industry and cosmetics either in native or modified form, as thickening or gel-setting agents stabilizers for emulsions and dispersions; film-forming, coating substances, and it also used as a stabilizers in preparation of metallic nanocomposites (Anklam, *et al.*, 2005 Gupta, *et al.*, 2014, Gupta, *et al.*, 2015) .

1.2 Galactomannans

Galactomannans are heterogeneous polysaccharides composed of a β (1–4)-d-mannan backbone with a single d-galactose branch linked α (1–6). They differ from each other by the mannose/galactose (M/G) ratio, another definition was done by Aspinall that is, those mannans containing more than 5% of d-galactose.

These gums are mostly obtained from the endosperm of dicotyledonous seeds of numerous plants, particularly from Leguminosae. The endosperm has several functions: it serves as food reserve for germinating seeds and it retains water, preventing the complete drying of the seeds (McCleary, *et al.*, 1974, Dea, *et al.*, 1975, McCleary, *et al.*, 1975, McCleary, *et al.*, 1976, Robinson, *et al.*, 1982, Wielinga, 2009).

There are four major sources of seed galactomannans: locust bean gum (LBG) (*Ceratonia siliqua*), guar gum (GG) (*Cyamopsis tetragonoloba*), tara (*Caesalpinia spinosa Kuntze*), and fenugreek (*Trigonella foenum-graecum*). Among these, only locust bean and guar gums are of considerable industrial importance. The use of tara and fenugreek gums is limited due to availability and price. Other sources of galactomannans have also been explored (Dea, *et al.*, 1975, Wielinga, 2009).

The two main groups of galactomannan polysaccharides are those derived from

(a) The endosperm of plant seeds, the vast majority of which originate in the *Leguminosae*.

(b) Microbial sources, in particular, the yeasts and other fungi.

The majority of galactomannans originate from *Leguminosae* family. 70 species of the *Leguminosae* have been identified containing galactomannans. True galactomannans, have also been extracted from members of *Annonaceae*, the *Convolvulaceae* and the *Palmae*, *Ebenaceae* and *Loganiaceae*. The general procedure to obtain galactomannans from seeds combines extraction and purification processes. Briefly, the seed hull is removed from the seeds and the germ is separated from the endosperm. The most used separation procedures are: filtration, sifting and in some cases (e.g., lab scale) they are separated manually. The endosperm is dissolved in water (at temperatures that range between 20 and 120 °C), followed by a precipitation step using alcohol (in ratio water: alcohol ranges between 1:1 and 1:3). No effects of the alcohol on galactomannans' structure have been reported. Ground endosperms are the source of trade

galactomannan gums. The recovered endosperm halves, of the required purity, of carob, tara and guar, and fenugreek seeds, are ground to obtain a fine off-white powder (McCleary, *et al.*, 1974, Dea, *et al.*, 1975, McCleary, *et al.*, 1975, McCleary, *et al.*, 1976, Wielinga, 2009).

1.2.1 Physical properties of Galactomannans

Galactomannans forms viscous, colloidal dispersions when hydrated in water. The molar ratio of galactose to mannose varies with plant origin but is typically in the range of 1.0:1.0–1.1, 1.0:1.6–1.8, 1.0:3.0 and 1.0:3.9–4.0 for fenugreek, guar, tara, and locust bean gums, respectively. The conformation of the 1→4-linked β -d-mannan backbone is similar to that of cellulose, so that it does not dissolve in water. The galactose side groups are considered to sterically disturb the interchain association and crystallization, thereby imparting certain water solubility to galactomannans. As a result, the solubility of the galactomannans increases with the degree of galactosyl substitution: fenugreek and guar gums are readily dissolved in cold water, but heating is needed to reasonably solubilize locust bean gum in water (Dea, *et al.*, 1975, Wielinga, 2009, Prajapati, *et al.*, 2013).

1.2.2 Physico-chemical properties of galactomannans

For a better understanding of properties of galactomannans one should know the basic structure of their building units. It is obvious that mannose is occupied with cis-OH groups in the galactomannan polymer. Therefore, an enhanced deposition to form hydrogen bonds between the polymannan chains is expected as long as neighboring groups like galactose do not develop steric hindrance to prevent galactomannan chains from coming too close together and prevent mannose cis-OH groups in forming hydrogen bonds. Comparing different galactomannans, it is obvious that, since additional cross linking via hydrogen bonds goes hand in hand with less solubility, an increase in substitution leads to higher solubility. The best solubility is found with 1,1-galactomannan (related to the position of the galactose in the galactomannan backbone) in which the high

substitution by galactose obviously establishes so much steric hindrance that it dissolves easily in cold water and delay attack of degrading enzymes for some time .However, 1.,2-galactomannan where on average each second mannose is blocked by galactose, shows so much steric hindrance and so little hydrogen bonding between molecular chains that it hydrates instantly. 1,4-Galactomannan does not dissolve in cold water but its solubility in boiling water is already better than that of 1,5-galactomannan due to slightly higher degree of substitution by galactose side groups compared to 1,5-galactomannan. M/G ratio varies considerably depending on the galactomannan physico-chemical properties. Variations in galactomannans' structure, particularly in M/G ratio and fine structure, cause significant changes in solubility, viscosity and in the interactions between galactomannans and other polysaccharides. Usually, galactomannans with higher galactose content (such as GG) are ready soluble in water and exhibit low tendency to form gels as a result of synergistic interactions, when compared with galactomannans with a lower M/G ratio (such as LBG). The higher solubility of GG has been attributed to the presence of a higher number of side chains, which keep the main mannose chains far enough to prevent effective intermolecular interactions. On the other hand, galactomannans with few side chains (higher M/G ratio) can interact with other polysaccharides due to their long blocks of unsubstituted mannose units. From the foregone discussion, it can be concluded that galactomannans are rich in hydroxyl groups; this enables them to bind and take up water and rich in cis-OH groups, which allow chain-chain aggregation via hydrogen bonding so that hydration becomes more complicated if interchange cross linking takes place. By substitution with galactose, nature establishes steric hindrance between the molecules and thus enhances water solubility (Prajapati, *et al.*, 2013).

1.2.3 Uses of galactomannans

They are used as industrial hydrocolloids (thickening and gelling agents) beside their wide uses as additives in food products (mainly to make them appealing

and attractive to the consumer) and in pharmaceutical as well as cosmetic products. Furthermore, they should also improve shelf-life by binding water, control the texture, influence crystallization, prevent creaming or settling, improve the freeze-thaw behaviors, prevent syneresis and the retrogradation of starch products, maintain turbidity. This means that these food additives find applications mainly in convenience food, dairy products, including frozen products (ice cream), soft drinks and fruit juices, bread and pastry, fruit preserves, baby food, and as household gelling agents in puddings, flans and pudding powder, as dietary fibres, and in pet foods. Other non-food applications of galactomannans are found in the textile industry (carpet dyeing and textile printing), and in the paper, mining, explosive, drilling, construction, oil field and chemical industries (Kapoor, *et al.*, 1998,Chaubey, *et al.*, 2001, Srivastava, *et al.*, 2005, Prajapati, *et al.*, 2013).

1.3 Botanical classification of *Piliostigma reticulatum* (DC) Hochst

Family: *Caesalpinaceae* (*Leguminosae* – *Caesalpinioideae*)

Synonyms: *Bauhinia reticulata* DC, *Bauhinia glabra* A. chev, *Bauhinia glauca* A. chev [El Amine, 1990, Brink and Achigan-Dako, 2012].

1.3.1 description of *Piliostigma reticulatum*

Piliostigma reticulatum is a *leguminous* plant belonging to the family *Caesalpinaceae*, a family that comprises of trees, shrubs or very rarely climbers. The tree is perennial in nature and its vernacular names include Abefe, Monkey bread, Camel's foot, Kalgoand Okpoatu and these vary according to locality. *P. reticulatum* has a close resemblance to *P. thonningii* to such an extent that they may easily be confused with each other, although when examined very closely, distinguishing morphological features become readily observable. *P. reticulatum* has smaller leaves and smoothness on the lower side whereas *P. thonningii* has larger leaves and hairiness on the lower side. *P. reticulatum* is widely distributed in Africa and Asia. Ethno medically, the bark, root, pod, young stem or leaves have been used for treating leprosy, smallpox,

coughs, ulcer, heart pain, gingivitis, snake bite, dysentery, fever, wounds and a variety of closely related disease conditions (Diack, *et al.*, 2000, Babajide, *et al.*, 2008, Bright, *et al.*, 2017, Hernandez, *et al.*, 2015, Mustifa, *et al.*, 2017).

1.3.2 *Piliostigma reticulatum* gum

P. reticulatum gum is a type of polysaccharide gum which consists mainly of mannose (42.58%) and galactose (20.27%). It contains a protein which has an average value of 17.19%. Furthermore, the gum contains more than eight elements with potassium, calcium, silicon, phosphorous and iron represent the major ones. The gum is almost insoluble in water and in a number of organic solvents such as acetone, ethanol, methanol and isopropanol. On the other hand, the gum shows noticeable solubility in alkali at 60 °C like sodium carbonate and bicarbonate while in sodium hydroxide it forms a gel. The Solubility of *Piliostigma reticulatum* gum is shown in Tables (1.1) and 1.2 [Abow, 2017].

Table 1.1: the solubility of *Piliostigma reticulatum* gum at 60 C°

Solvent	Gum	Gum pH
H ₂ O	8.2%	6.28
0.5 M Na ₂ B ₄ O ₇	Gel	8.28
0.1 M Na ₂ CO ₃	48.9%	10.59
0.5 M NaHCO ₃	80%	8.53
0.1 M NaOH	Gel	12.53
0.5 M NaOH	Gel	12.80
1 M NaOH	Gel	12.94

Table (1.2): Solubility of *Piliostigma reticulatum* gum in organic solvents

Solvent	Solubility
Ethanol	Insoluble
Acetone	Insoluble

1.4 Carboxymethylation

Carboxymethylation is the introduction of a carboxymethyl group into various side chains. It is one of the various strategies used for functionalization of natural polymers. It is, widely, employed modification approach because of its ease of application, lower cost of chemicals and versatility of the product. Carboxymethyl derivatives are, usually, polyelectrolyte with better aqueous solubility. Carboxymethylation of polysaccharides is based on the William synthesis in which the polysaccharide alkoxide is reacted with monochloroacetic acid and the primary and secondary alcohol groups are substituted by carboxymethyl group (Eyler, *et al.*, 1947, Gurd ,1967, Gurd, 1972, Hebeish, *et al.*, 1990, Novák, *et al.*, 1993, Sharma, *et al.*, 2003, Adinugraha, *at el.*, 2005,Pushpamalar, *et al.*, 2006,Varshney, *et al.*, 2006, Kamel, *et al.*, 2007, Chen, *et al.*, 2010,Badwaik, *at el.*, 2016, Sahin, 2018).

Carboxymethylation reaction of gums is a two-step process accompanied by an undesired side reaction (Tijssen, *et al.*, 1999, Sharma, *et al.*, 2003, Gupta, *et al.*, 2004 ,Wu, *et al.*, 2017,Rajpand , *et al.*, 2015).

I. Generation of alkoxide of gum by the action of NaOH.



II. SN₂ reaction of the alkoxide formed with SMCA to form CM-CAG.



The side reaction occurs in both liquid bulk and gum phase and results in the formation of sodium glycolate from NaOH and SMCA.



1.5 Previous studies on carboxymethylation of gums

Sharma, *et al.*, [2003], studied the factors influencing carboxymethylation of cassia tora gum. The reaction was optimized with respect to concentrations of sodium hydroxide , MCA , reaction times and temperatures. The highest degree of substitution (0.099) was obtained for 0.03 mol/l cassia tora gum, 0.084 mol/l monochloroacetic acid, 0.125 mol/l sodium hydroxide, 75°C temperature,

reaction time of 60 minutes and solvent medium of 80:20 (methanol:water) ratio. The rheological studies of carboxymethylated cassia tora gum solutions of 1-2% displayed a non-Newtonian pseudo-plastic behavior.

In another study [Gupta, et al., \[2004\]](#) investigated the optimization of carboxymethylation of *Cassia occidentalis* seed gum. Concentrations of monochloroacetic acid and sodium hydroxide, solvent ratio, reaction time, and reaction temperature were studied. Optimum conditions which give a DS of 0.464 were found to be 0.053 mol monochloroacetic acid, 0.100 mol sodium hydroxide, reaction temperature of 80 °C, reaction time of 60 min, solvent ratio of 80:20 (methanol: water) and gum liquor ratio is 1:10. The viscosity of carboxymethylated *Cassia occidentalis* seed gum of 2% solution was about 38 times greater compared to native gum. Rheological studies showed the non-Newtonian pseudo plastic nature of carboxymethylated *Cassia occidentalis* seed gum.

[Kumar, et al., \[2012\]](#) studied the modification of kondagogu gum (GK) by carboxymethylation and the potential application of the product for pharmaceutical purposes. Carboxymethylation of gum was carried out by reaction of the gum with monochloroacetic acid under alkaline conditions. The results revealed that carboxymethylation of GK provided means of preparing mucoadhesive, sustained-release bead formulation of metformin which releases the drug by zero-order release kinetics.

Carboxymethylated seeds of *Cassia angustifolia* gum were utilized in wide, industrial applications ([Rajput, et al., 2015](#)). The gum was etherified with sodium monochloroacetate (SMCA) in a methanol-water system in the presence of alkali (NaOH) at different reaction conditions. The optimum DS of 0.474 was obtained at reflux temperature using 80% methanol, 0.03 mol (as AGU) of *C. angustifolia* seed gum, 0.100 mol of NaOH, 0.05 mol of SMCA, 10:1 liquor/gum ratio and a reaction time of 60 min. The microbial resistance and paste quality of carboxymethylated *C. angustifolia* gum was found to be better

than the unmodified gum. The increase in the viscosity of 1% and 2% Carboxymethylated *C. angustifolia* gum solutions is about 32.19 and 35.65 times respectively in comparison to 1% and 2% unmodified *C. angustifolia* gum solutions. Furthermore, the rheological studies revealed the non-Newtonian pseudo plastic behaviour of Carboxymethylated *C. angustifolia* gum.

Highly substituted carboxymethyl Cassia tora gum (CM-CTG) was prepared from Cassia tora gum (CTG) by the treatment with monochloroacetic acid (MCA) in ethanolic aqueous solutions after alkalization under different reaction conditions [Wu, *at el.*, 2017]. The influence of the etherification temperature, alkalization and etherification times, molar ratio of sodium hydroxide to MCA ($n_{\text{NaOH}}/n_{\text{MCA}}$), theoretical degree of substitution (DS_t), and weight percentage of water ($\text{W}_{\text{H}_2\text{O}}\%$) in the solution on the degree of substitution (DS) and reaction efficiency (RE) of the reaction were investigated. Optimum preparation conditions for a CM-CTG with DS of 1.05 are: etherification temperature, 60 C°; alkalization time, 60 min; etherification time, 180 min; $n_{\text{NaOH}}/n_{\text{MCA}}$, 2.1; DS_t , 1.4; and $\text{W}_{\text{H}_2\text{O}}\%$, 20%. Fourier-transform infrared analysis of the products indicated that carboxymethylation successful. The degree of crystallinity of CM-CTG decreases with increasing DS, as shown by X-ray diffraction measurements. Rheological studies showed that all the CM-CTG thickeners are pseudo plastic fluids, and, as the DS of the CM-CTG thickener increases, the pastes become less shear thinning and more like Newtonian fluids. Therefore, highly substituted CM-CTG has the potential to be applied as a dye printing paste for textile.

1.6 Objective of this research is

The main objective of the present study is to optimize the carboxymethylation reaction of *Piliostigma reticulatum* seeds gum by adopting the following specific objective

1. To prepare carboxymethylated PRSG
2. To study the effect of concentrations of MCA ,NaOH,temperature and reaction time on the Ds of the product

Chapter two

Materials and methods

2.1 Sample collection and pretreatments

Piliostigama reticulatum fruit pods were collected from Southern Darfur State (Nyala area) during seasons 2014-2016. The fruit pods were dehusked and *Piliostigama reticulatum* seeds were removed. The dried seeds were then packed in plastic containers and stored in a dry place for further treatments.

The dried seeds were kept in 60% sulphuric acid (H₂SO₄) solution for 30 minutes then it was soaked in distilled water for 24 h. The soft coat was then peeled off and the endosperm were separated manually from the germ and dried at room temperature [Dakia, *et al.*, 2008]. The separated endosperm were ground to obtain *Piliostigma retculatum* gum and kept in a labeled plastic container for experimental works.

2.2 Chemicals

Sulphuric acid (assay = 98.08%) was purchased from loba chemie. Monochloroacetic acid-MCA (assay = 95.5%), was purchased from SDFCL. Methanol (Density = 0.79 g/ml at 20°C, assay = 99.8%) was purchased from chem.-lab NV. Ethanol (Density = 0.808-0.812 g/ml at 20°C, b.p= 78.5 ° C, assay = 94.8-95.8%), was purchased from Duksan Reagents. Methyl orange indicator was purchased from SDFCL. Sodium hydroxide (Assay = 98%), was purchased from Nice Laboratory. Glacial acetic acid (Boiling point = 118 at 20°C, minimum assay = 99.7%), was purchased from Duksan Reagents.

2.3 Optimization of the carboxymethylation of *Piliostigma reticulatum* seed gum

Carboxymethylation of the *Piliostigma retculatum* seed gum (PRSG) was performed based on the previous studies [Rajput, *at el.*, 2015] with slight modifications in which the ratio of the reactants was kept as 1:1. In a typical experiment, 8.3 mmol per anhydrous glucose unit (AGU) of PRSG; was

dispersed in 10.38 mmol solution of NaOH at room temperature with continuous stirring for 25 min followed by addition of 16.6 mmol of MCA with continuous stirring for another 25 min. The reaction mixture was heated at 75 °C for 30 minutes. The reaction mixture was occasionally shaken during the course of the reaction. The reaction product was separated by centrifugation for 10 min, dissolved in water and neutralized by acetic acid (1:1 v/v). Finally, the reaction product was precipitated in ethanol and separated again by centrifuging for 5 min and washed twice with aqueous methanol followed by pure methanol. The product was dried at room temperature. Exactly similar steps were repeated by varying the concentration of NaOH, concentration of MCA, reaction time and temperature. Table 2.1 shows the detailed steps of optimization of the carboxymethylation process.

Table2.1: The replicates obtained using minitab program and the variable above to determine the optimum condition of the carboxymethylation.

No	Temp (°C)	Time (minute)	MCA (mmole)	NaOH (mmole)
1	75	60	16.6	10.38
2	35	60	16.6	10.38
3	75	60	4.15	10.38
4	35	30	10.38	10.38
5	35	60	10.38	4.15
6	55	60	10.38	10.38
7	75	60	10.38	16.6
8	55	60	10.38	10.38
9	55	60	4.15	16.6
10	55	60	10.38	10.38
11	55	30	10.38	4.15
12	55	30	4.15	10.38
13	75	60	10.38	4.15

Table 2.1 continued

14	55	60	16.6	4.15
15	35	60	4.15	10.38
16	55	90	16.6	10.38
17	35	60	10.38	16.6
18	55	90	10.38	4.15
19	75	30	10.38	10.38
20	55	60	4.15	4.15
21	75	90	10.38	10.38
22	55	60	10.38	10.38
23	55	90	10.38	16.6
24	35	90	10.38	10.38
25	55	60	16.6	16.6
26	55	30	10.38	16.6
27	55	90	4.15	10.38

Reaction conditions: [PRSG] = 8.3 mmol

Optimum conditions for 8.3 mmol gum are 4.15mmol NaOH,4.15 mmol MCA at 35°C for 30 mint.

2.4 Preparation of carboxymethyl *piliostigma reticulatum* seed gum at optimum conditions

Carboxymethylation of the *Piliostigma reticulatum* seed gum (PRSG) was performed as follows : 10g (55.5 m-mole) per AGU of PRSG were dispersed in distilled water containing 1.11 g (27.75 m-mole) NaOH at room temperature with continuous stirring for 25 min followed by the addition of 2.62 g (27.75 m-mole) MCA with continuous stirring for another 25 min. The reaction mixture was heated (35°C) for 30 min. The reaction mixture was occasionally shaken during the course of the reaction. The reaction product was separated by centrifugation for 10 min, dissolved in water and neutralized using acetic acid

(1:1 v/v). The reaction product was precipitated in ethanol and separated again by centrifuging for 5 min, washed twice with aqueous methanol followed by pure methanol. The product was dried at room temperature.

2.5. Degree of substitution

A 0.5 g sample of CM-PRSG, calculated as oven-dry product, was ashed at 700 °C for 15 to 20 min. The ash was dissolved in 40 mL boiling distilled water before being titrated with 0.01 N H₂SO₄, using methyl orange indicator. Boiling of the solution was done three times between repeated titrations to evaporate carbon dioxide. The DS value was then calculated from the amount of titrated acid (V/mL) and the amount of PRSG (G/g), using following equation:

$$A = \frac{C * V}{G}$$
$$Ds = \frac{0.162A}{1 - 0.08A}$$

Where

A is the milliequivalents of sulphuric acid required per gram of the sample.

C is the concentration of sulfuric acid (N).

V is the volume consumed of sulphuric acid (ml).

G is the weight of carboxymethylated *piliostigma reticulatum seed* gum (g).

DS is the degree of substitution (Hong, *et al.*, 1978).

2.6. FT-IR analyses of crude and carboxy methylated samples

The samples of PRSG and CM-PRSG were subjected to FT-IR spectroscopy in a Fourier-transform infrared spectrophotometer (no of scans 8, resolution 4 [1/cm]) in range of 4000 cm⁻¹ to 400 cm⁻¹ and as KBr pellet.

Chapter three

Results and discussion

3.1 Determination of optimum reaction conditions

The optimization of the process of carboxymethylation with reference to DS was performed by varying the reaction parameters such as concentrations of NaOH and MCA, reaction temperature, reaction time using Minitab program. Each of the above parameter was varied (as shown in Table 3.1).

Table 3.1: The DS values obtained in each experiment

Sample No	DS	Sample No	DS
1	0.0354	15	0.0442
2	0.0356	16	0.0375
3	0.0342	17	0.0341
4	0.0373	18	0.0381
5	0.0359	19	0.0351
6	0.032	20	0.0376
7	0.041	21	0.0386
8	0.0395	22	0.0344
9	0.037	23	0.0336
10	0.032	24	0.0304
11	0.0382	25	0.0304
12	0.0377	26	0.0357
13	0.035	27	0.0344
14	0.039	28	0.0376

3.1.1 Effect of concentration of NaOH and MCA on the DS value

Figure 3.1 shows the dependence of DS on the two factors. The decrease in DS with the increase in the NaOH concentration up to 16.6 m-mol indicated that the

competitive side reaction which leads to glycolate formation increases. The increase in the DS on increasing MCA concentration up to 16.6 m-mol is

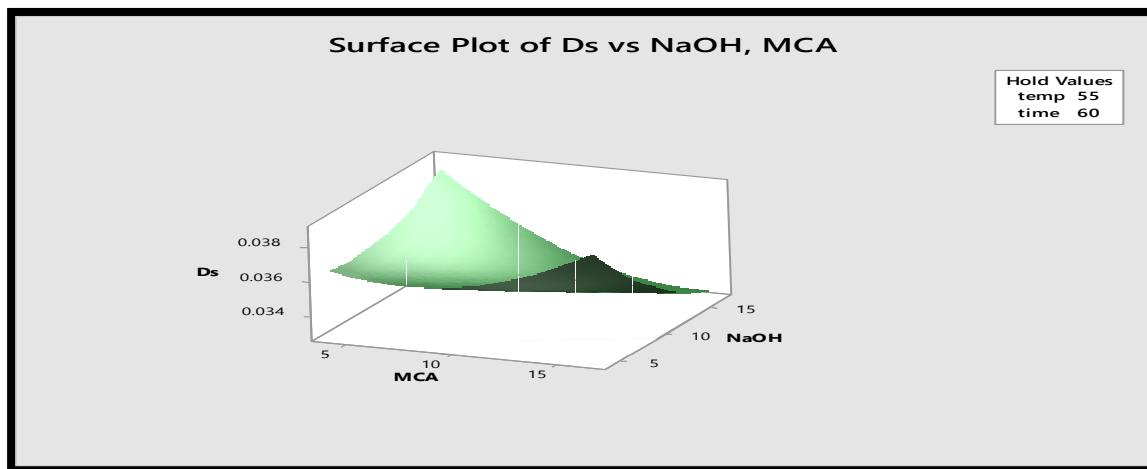


Figure 3.1: Surface plot of DS versus the concentrations of NaOH ; MCA because the increase in MCA concentration in the vicinity of the PRSG hydroxyls thereby facilitating the carboxymethylation reaction (Sharma, *et al.*, 2003, Gupta, *et al.*, 2004, Kumar, *et al.*, 2012, Rajput, *et al.*, 2015, Wu, *et al.*, 2017). At low MCA concentration and high NaOH concentration the DS value is high then the value increases again at low NaOH concentration and high MCA concentration. This means that at high NaOH the adsorption and swelling of the gum is at maximum (Abow, 2017) which leads to penetration of the reacting materials and hence better reactivity with MCA and the carboxymethylation reaction is preferred at this conditions. Abow (2017) has found that PRSG at all tested NaOH concentrations (0.1, 0.5 and 1M) forms gel. Also at high MCA and low NaOH the carboxymethylation is preferred because of the availability of MCA in the vicinity of -OH groups of the gum although the swelling is not at maximum.

3.1.2 Effect of time and temperature on the DS value

Figure 3.2 shows the dependence of DS on the two factors. The decrease in DS with the increase in temperature up to (75°C) is due to the fact that PRSG forms gel with NaOH (Abow, 2017) and the reaction system became sticky which reduced the shaking efficiency and the uniformity of the etherification reaction

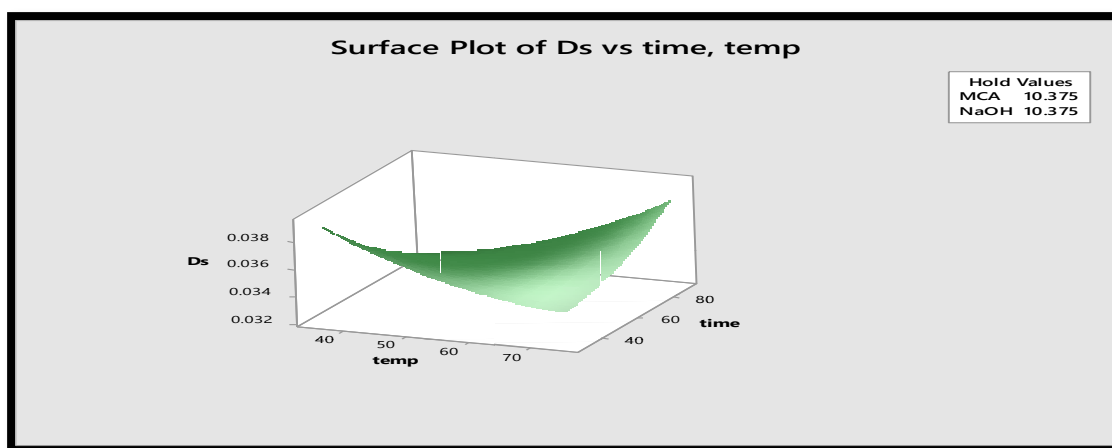


Figure 3.2: surface plot of Ds vs. time; temperature

. In addition, heating could increase the glycolate formation and thereby lowering the DS value. The enhancement of DS by prolonging the duration of reaction from 30 to 90 min is a direct consequence of the favorable effect of time on swelling of PRSG as well as the diffusion and adsorption of the reactants with the ultimate effect of better contacts between the etherifying agents and PRSG (Sharma, *et al.*, 2003, Gupta, *et al.*, 2004, Kumar, *et al.*, 2012, Rajput, *et al.*, 2015, Wu, *et al.*, 2017). At low temperature and low duration time as well as at high temperature and long duration time the DS value is high. High DS value at high temperature and long duration time could be interpreted by better swelling of PRSG resulting from diffusion and adsorption of the reactants at long duration time although the viscosity of the system is high. At low temperature and duration time, the increase in DS could be due to lower viscosity of the system which permits the accessibility of the reactants to $-OH$ groups of the gum despite the insufficient swelling at these conditions.

3.1.3 Effect of temperature and the concentration of NaOH on the DS value

Figure 3.3 shows the dependence of DS on the two factors. The increase in DS with the increase in the NaOH concentration up to 16.6 mmol indicated that the formation of carboxymethylated product prevails over its competitive side reaction (glycolate formation) (Sharma, *et al.*, 2003, Gupta, *et al.*, 2004, Kumar, *et al.*, 2012, Rajput, *et al.*, 2015, Wu, *et al.*, 2017). The value of DS decreases on

increasing the temperature due to the increase in the viscosity of the system which leads to the entanglements of the polymer chains and inhibition of movement and penetration of the reactants to the interior of the polymer.

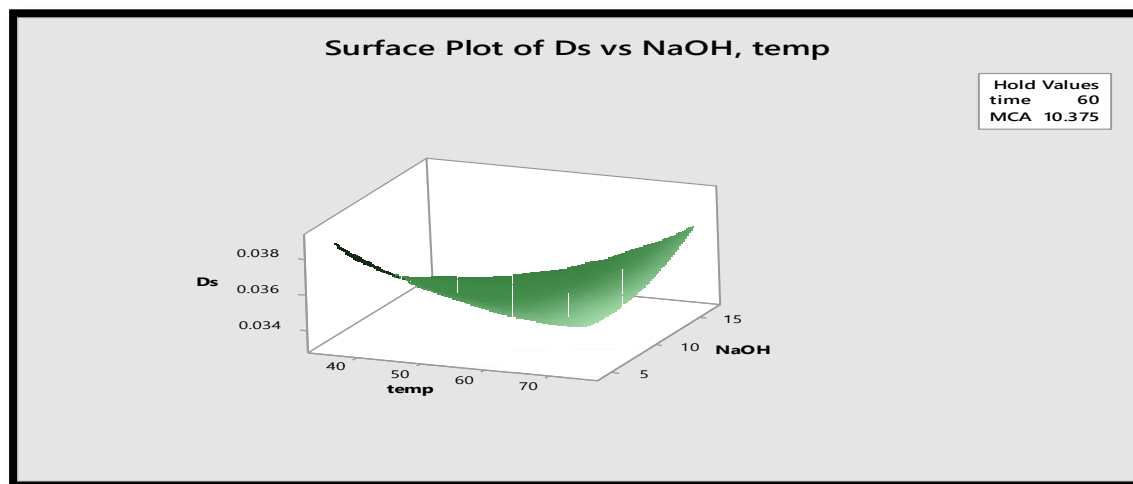


Figure 3.3: Surface plot of DS vs. the concentration of NaOH; temperature

All these lead to lower reactivity and possibility of domination of glycolate formation instead of carboxymethylation. The increase in DS value at low temperature and low concentration of NaOH is attributed to lower viscosity despite the low diffusion and adsorption of the reactants. This means that at these conditions the viscosity governs the reaction and become the dominant factor. Again, at high temperature and high concentration of NaOH the DS value increases which can be explained by the domination of high swelling and diffusion of the reactants to the interior of the polymer. This facilitates the carboxymethylation reaction.

3.1.4 Effect of temperature and the concentration of MCA on the DS value

Figure 3.4 Shows The dependence of DS on the two factors .The increase in DS with increasing MCA concentration up to 16.6 mmol is due to the availability of MCA molecules in the neighborhood of –OH groups of the gum. This enhances the carboxymethylation reaction thereby increasing the DS value (Sharma, *et al.*, 2003, Gupta, *et al.*, 2004, Kumar, *et al.*, 2012, Rajput, *et al.*, 2015, Wu, *et al.*, 2017). On the other hand, the value of DS decreases on increasing the temperature due to viscosity factor.

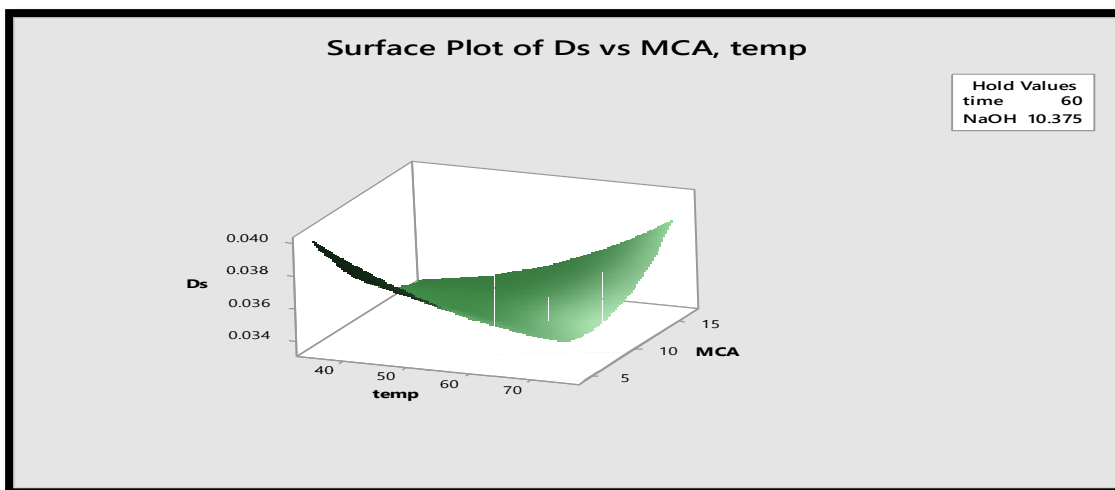


Figure 3.4: Surface plot of DS vs. the concentration of MCA; temperature

At low temperature and low MCA concentration and at high MCA concentration and high temperature the value of DS is high. This could be attributed to the effect of low viscosity in the first case and the better diffusion and swelling in the second case.

3.1.5 Effect of time and the concentration of NaOH on the DS value

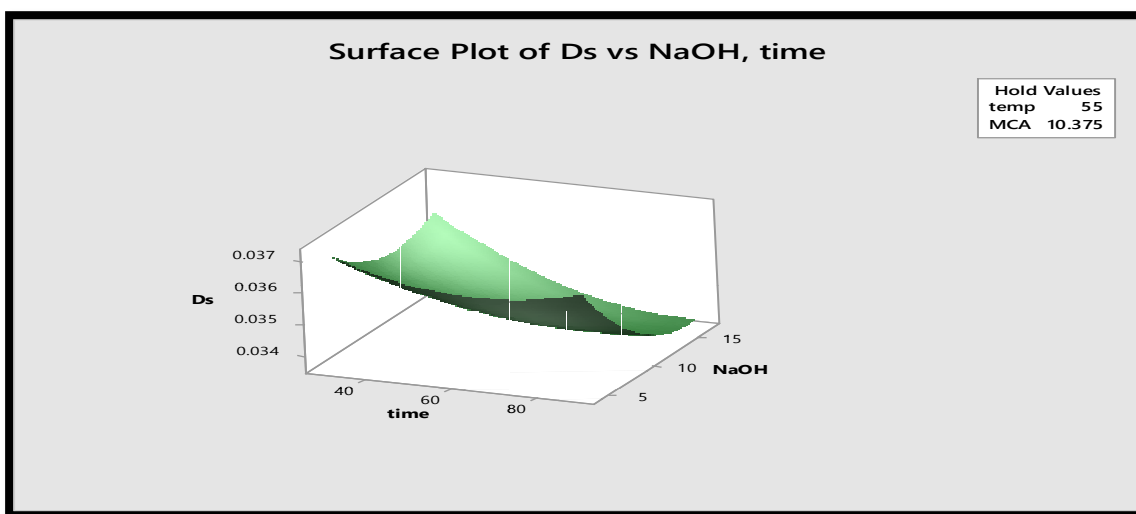


Figure 3.5: surface plot of DS vs time; the concentration of NaOH

Figure 3.5 shows the dependence of DS on the two factors. The decrease in DS with the increase in the NaOH concentration up to 16.6 mmol indicated that the competitive side reaction which leads to glycolate formation is dominated at these conditions thereby lowering the DS value (Sharma, *et al.*, 2003, Gupta, *et al.*, 2004, Kumar, *et al.*, 2012, Rajput, *et al.*, 2015, Wu, *et al.*, 2017) The

influence of time on DS alternatively has shown marginal increase with increasing reaction time.

3.1.6 Effect of time and the concentration of MCA on the DS value

Figure 3.6 shows the dependence of DS on the two factors. There is a gradual decrease in DS value with increasing MCA concentration up to certain range (10 to 14 mmol) then there is a gradual increase in DS up to 16.6 mmol. In contrary,

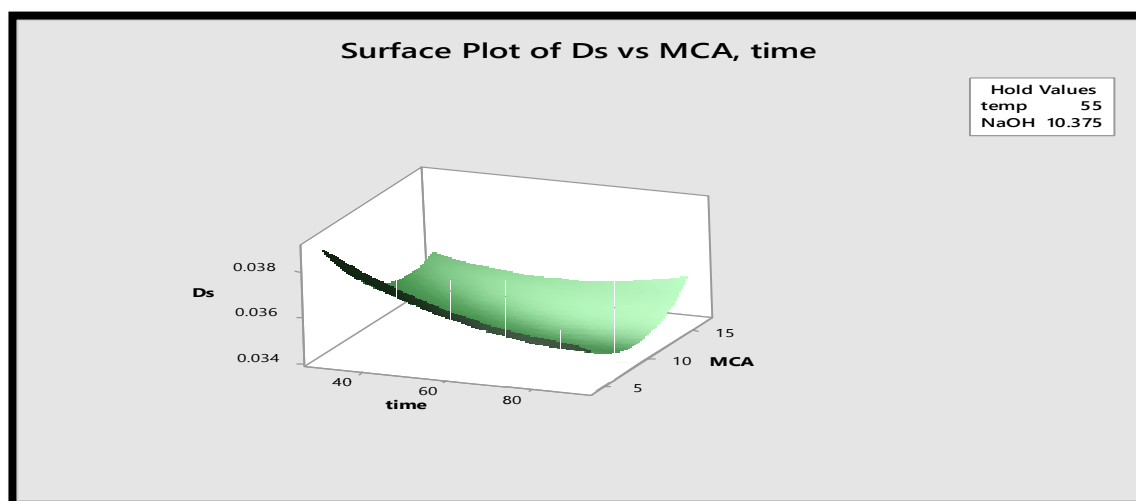


Figure 3.6: Surface plot of DS versus time and the concentration of MCA the value of DS decreases on increasing the time (Sharma, *et al.*, 2003, Gupta, *et al.*, 2004, Kumar, *et al.*, 2012, Rajput, *et al.*, 2015, Wu, *et al.*, 2017)

3.1.7. The optimum conditions

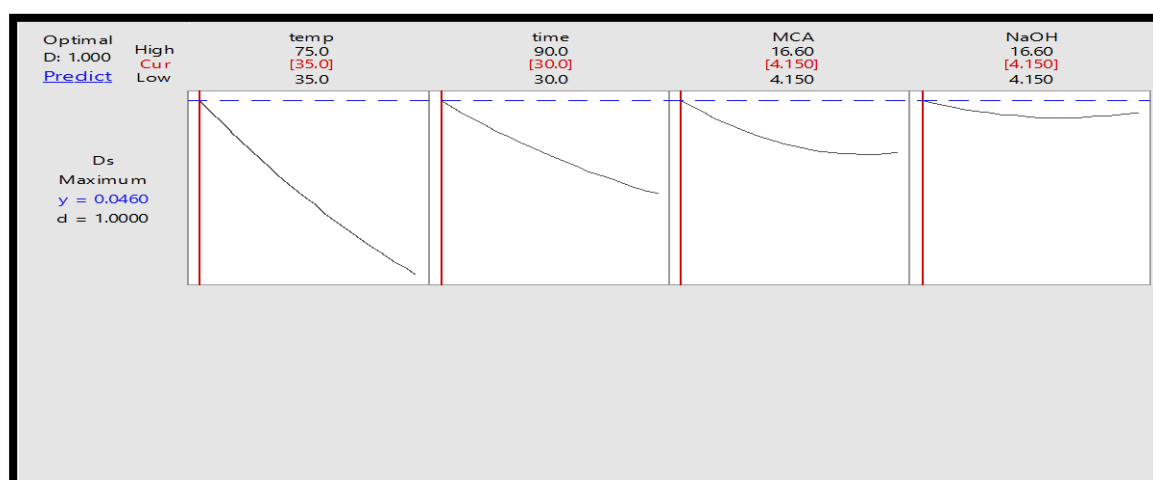


Figure 3.7: The optimum conditions

From figure (3.7) which is obtained by using minitab program the optimum condition of preparing CM-PRSG using distilled water as a solvent for PRSG 8.3 mmol per AGU are 4.15 mmol NaOH and 4.15 mmol MCA at 35°C for 30 minutes.

3.2 Degree of substitution at optimum conditions

The degree of substitution for the optimized CM-PRSG using ash method is (0.0453)

3.3 FT-IR analysis

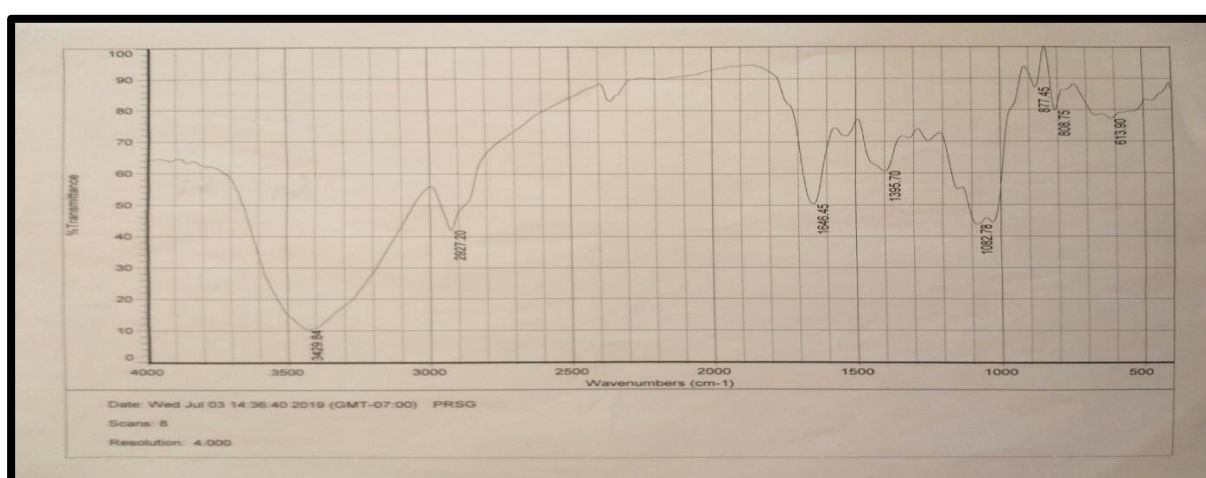


Figure 3.8: FT-IR spectrum for PRSG

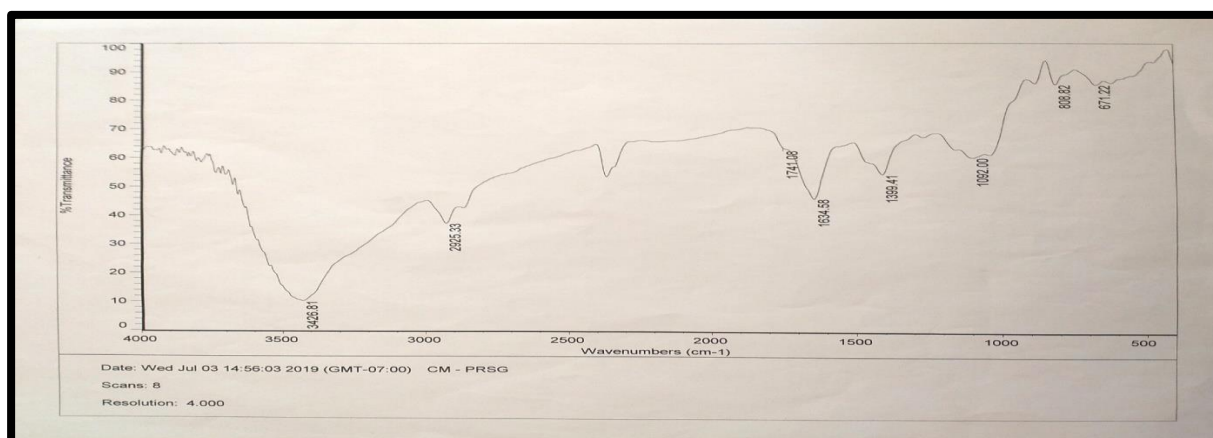


Figure 3.9: FT-IR spectrum for CM-PRSG

Figure 3.8 and 3.9 shows The FT-IR spectra of PRSG and CM-PRSG respectively. As can be seen from, a number of characteristics peaks were noticed. These peaks could be explained as follows: at 3430 cm^{-1} is attributed to O—H stretching vibration, at 2927 cm^{-1} is due to C—H saturated hydrocarbon

stretching vibration, at 1646 cm^{-1} is ascribed to O—H bending or adsorbed water. Furthermore, at 1396 cm^{-1} is due to C—H bending vibration, at 1083 cm^{-1} is attributed to C—O stretching vibration. On the other hand, the FT-IR spectrum of CM-PRSG is displayed in Figure 3.9 which has the characteristic absorption peaks almost typical to the ones noticed for PRSG and the observable difference is the presence of a weak absorption band which appeared as a shoulder peak at 1741 cm^{-1} . This peak could ascribed to carbonyl stretching vibration of carboxymethyl substituent.

Conclusion and recommendations

Conclusion

- In the present work, a CM-PRSG with DS of 0.0453 was prepared. The optimum conditions are as follows: reaction temperature, 35 °C; reaction time, 30 min; concentration of NaOH 4.15 mmol and concentration of MCA 4.15 mmol.
- Fourier-transform infrared analysis of the products indicated that carboxymethylation was successful at the above conditions .

Recommendations

for further studies it is recommended to change the solvent to a mixer of water and alcohols to reduce the viscosity of the reaction system

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Appendix



Figure: *Piliostigma reticulatum* tree



Figure: *Piliostigma reticulatum* branch with fruit pods



Figure: *Piliostigma reticulatum* dried seeds

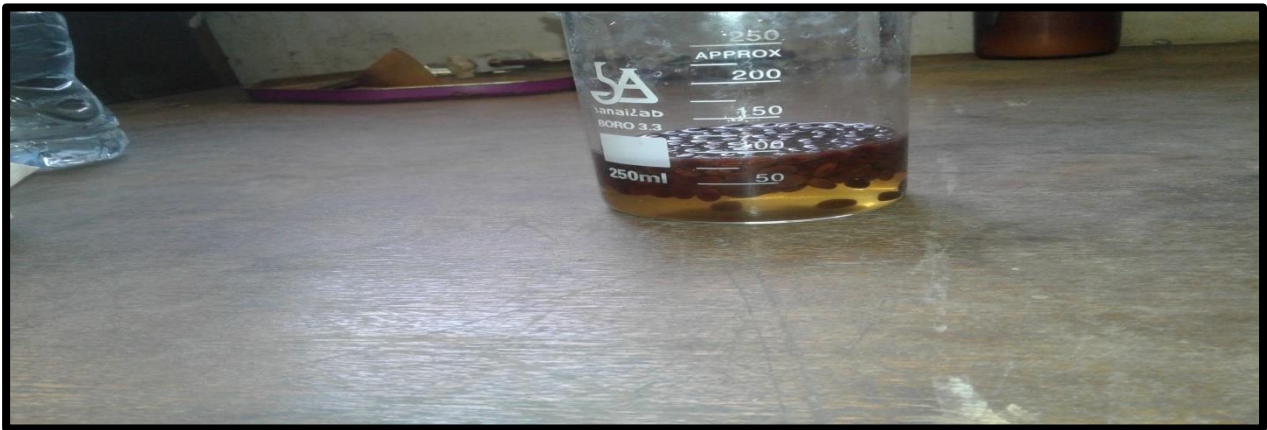


Figure: *Piliostigma reticulatum* seeds in 60% H_2SO_4 on minute 1

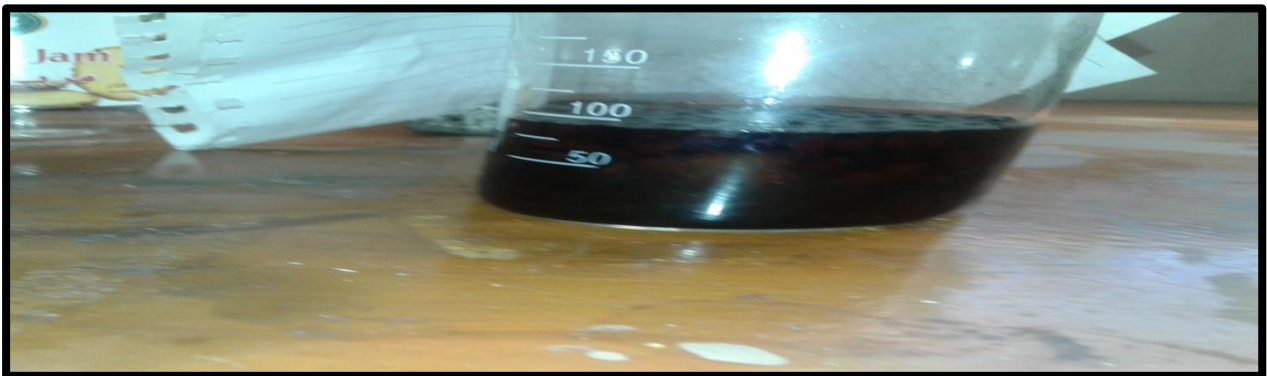


Figure: *Piliostigma reticulatum* seeds in 60% H_2SO_4 after 30 minutes



Figure: *Piliostigma reticulatum* seeds in water after 24h



Figure: *Piliostigma reticulatum* seeds before peeling the coat



Figure: *Piliostigma reticulatum* seeds after peeling the coat



Figure: *Piliostigma reticulatum* gum



Figure: *Piliostigma reticulatum* grem



Figure: *Piliostigma reticulatum* gum powder



Figure: carboxymethyl *Piliostigma reticulatum* gum