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RESEARCH ARTICLE

Synthesis, Characterization and Optimization of Reaction Parameters for Sodium Salt of Partially Carboxymethylated Okra Gum

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Abstract: Okra is an associate of the mallow family which is related to cotton, hibiscus and hollyhock. It is a tall annual tropical herb cultivated for its edible green seed pod. Okra gum was extracted from the pods of hibiscus esculents using acetone as a solvent. The solution of okra gum in water has the highest viscosity among all the natural polysaccharide exposed till date. Etherification of extracted okra gum was carried out to produce carboxymethylation okra gum. Reaction parameters such as reaction temperature, reaction time, volume of sodium hydroxide and amount of monochloroacetic acid were optimized. Physical and chemical characteristics such as solubility, pH, moisture content and viscosity were observed for carboxymethylation okra gum. Further extracted okra gum and carboxymethylation okra gum were characterized by Fourier transform infrared spectroscopy.

Keywords: Okra gum, Extraction, Carboxymethylation, Monochloroacetic acid, Degree of substitution

Introduction

Today okra is popular in India, Africa, Middle East, Greece, Turkey, Caribbean, South America and Southern United States. It is not a very common vegetable in most European countries, except in Greece and some parts of Turkey. The major okra producing states in India are Gujarat, Uttar Pradesh, Bihar, Orissa, West Bengal, Andhra Pradesh and Karnataka. In West Bengal, 0.6 mmt of okra is produced from 58,400 ha with an average productivity of 11.4mt/ha.[1,2]

Okra or ladies finger (Bhindi) is one of the important vegetables in India. It is grown throughout the tropical and sub-tropical regions and also in the warmer parts of the regions. The nutritional value of 100 g of edible okra is 1.9 g protein, 0.2 g fat, 6.4 g carbohydrate, 0.7 g minerals and 1.2 g fibers. Okra has a good potential as an external exchanger crop and accounts for 60% of the export of fresh vegetables. It is cultivated in 0.3 mha area with the production of 3.3 mmt and productivity of 9.6 mt/ha[3,4].

Okra gum is a glactomannan and is derived from seeds of *abelmoschus esculents*. It has biodegradability and biocompatibility. Okra gum derived from the pods of hibiscus esculents (Figure 1) is one of the advantageous polysaccharides that are currently being studied in the pharmaceutical industry as a hydrophilic polymer in pharmaceutical dosage forms[5]. Okra plant is grown in all soil types and is among the most heat and drought -tolerant vegetables[6].

It has been investigated as a binding agent for tablets and has also been shown to produce tablets with good hardness, friability and drug release profiles[7]. It has advantage over most commercial synthetic polymers as it is safe, chemically inert, non-irritant, biodegradable, biocompatible and eco-friendly. It is economical as it is widely harvested and does not require toxicology studies[8,9]. The okra gum is also used in transdermal drug delivery systems as synthetic cervical mucus and as a viscous supplementation agent in osteoarthritis treatment, tablet binding and disintegration agent-controlled drug delivery systems, slimming aids, nutritional foods *etc*.[10]. Biodegradability, biocompatibility and easy availability of Okra gum encourage most scientists to focus their work on it. Okra gum is an economical thickener and stabilizer[11,12].

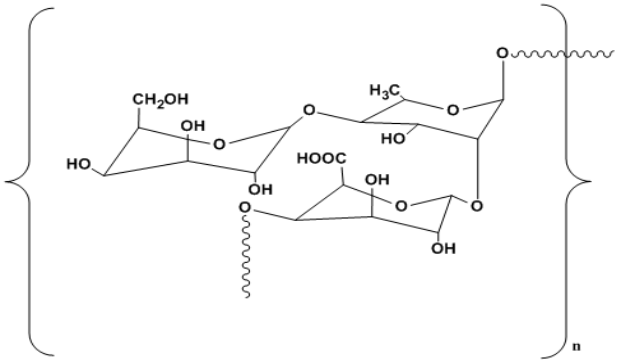

Figure 1. Chemical structure of polysaccharide of Okra mucilage

Okra gum is modified to improve its application in control release drug delivery system. The derivatives of okra gum are prepared by reaction like alkylation, esterification, *etc*. The derivatives of okra gum are prepared under different experimental conditions. Different properties such as moisture regain, rate of hydration, solubility, viscosity and rheology of the derivatives have been studied[13,14]. The properties depend upon polysaccharide chain length, the nature and degree of chemical modification. Chemical modification such as oxidation, acetylation, hydroxylpropylation and cross-linking provide efficient route not only to reduce the drawbacks but also to improve on the physicochemical properties and to introduce new properties for different applications[15-17].

Okra gum or its derivatives are used in pharmaceutical industries as gelling, viscosifying, suspension, stabilization, emulsification, preservation, water retention/water phase control, binding, clouding/bodying, process aid, pour control for suspensions and anti-acid formulations. However, okra gum derivatives are used for controlling the release of drugs in the gastrointestinal tract such as, carrier for colon targeted drugs for anticancer drugs and for oral rehydration solutions in the treatment of cholera in adults. Also, it has many applications in various industries such as paper, food, textile, cosmetic, nutrition and animal feed[18-20].

In the present work, okra gum was extracted from okra pod. The extracted okra gum was characterized by moisture content, pH, solubility test, viscosity, DS. Carboxymethylation of extracted okra gum was carried out. Effect of different parameters *viz*. time, temperature, effect of NaOH and effect of monochloroacetic acid on carboxymethylation was studied.

Experimental

Okra fruit was purchased from local vegetable market. Sodium hydroxide, monochloroacetic acid, methanol and isopropyl alcohol were purchased from Atul Chemical, Anand. Solvents and other laboratory chemicals were used after routine purification and they were of analytical reagent grade. Double distilled water was used.

Methods

Extraction of okra gum

500 g of unripe and tender okra fruit was washed and sliced with a knife. The sliced mass was soaked in distilled water overnight. After soaking, white muslin cloth was used to filter out the viscous gum extract. Acetone was added to precipitate the gum at a ratio of 1/3 parts of acetone to 1 part of the gum extract. The precipitated gum was dried in a desiccator containing anhydrous calcium chloride for approximately 15 days. Size reduction and screening of the dried gum were carried out using a stainless steel grinder and no. 30 stainless steel mesh sieve. Airtight powder bottles were used to store the undersize fractions.

Purification of okra gum

2 g of the okra gum was boiled with 8 mL of 70% (v/v) ethanol for 1 h with reflux. The sample was filtered, washing with 95% (v/v) ethanol and dried at 60 ^{0}C for 3 h in vacuum oven.

Carboxymethylation of okra gum

Carboxymethylation of okra gum was carried out by mixing extracted okra gum with 4 mL of distilled water which was heated to 80 ^{o}C for 15 min. After that, 56% w/v of ice-cold sodium hydroxide solution was added drop by drop over a period of 45 min. Monochloro-acetic acid solution was added slowly for a period of 60 min. to the above mixture and maintained the temperature at 15 ^{o}C. The temperature of the mixture was raised slowly to 65 ^{o}C and stirred for another 6 h. The wetted mass was washed with methanol for 30 min. The pH of the suspension was adjusted to neutrality with glacial acetic acid, and then it was dried at 50-60 ^{o}C.

Purification of carboxymethyl okra gum

Carboxymethyl okra gum was purified with water. Product remained dialyzed as compared to distilled water for 48 h and then carboxymethyl okra gum was precipitated with methanol. Finally product was dried in oven at temperature of 40-45 ^{o}C.

Characterization of extracted okra gum

Moisture content

The moisture content of the sample is calculated using the following equation:

$$\%W = \frac{A-B}{A} x100 \quad (1)$$

Where, %W = Percentage of moisture in the sample, A = Weight of wet sample (g), B = Weight of dry sample (g)

pH Determination

1% w/v dispersion of the sample in water was stirred consistently for 5 min. and pH was determined using a pH meter with built in magnetic stirrer model Equiptronics-614.

Solubility test

Solubility of the extracted okra gum was evaluated qualitatively by stirring 10 mg of okra powder in 10 mL water, acetone, chloroform, dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) and methanol. Solubility was determined by visual observation of the solute.

Viscosity

Viscosity of okra gum at 1% and 0.5% concentrations was measured using the Gardner – bubble viscometer.

Characterization of extracted carboxymethylation okra gum

The moisture content of the sample was calculated using Eq. 1. The pH determination, solubility test and viscosity measurments were carryout as per the procedure explained in the previous section.

Degree of substitution in carboxymethylation okra gum

1 g of Na-carboxymethyl okra gum was dissolved in required amount of water. Then this solution was passed through regenerated Amberlite anion exchange resin several times till it became acidic. As a result, solution was divided into two equal parts labeled as solution 1 and solution 2. The exhausted resin was regenerated by passing 1N HCl solution (3-4 times) followed by washing with distilled water to remove any excess acid.

Solution 1 was taken into previously weighed beaker. The solution was heated until all solvents on hot plate evaporated. Then the solution was cooled and Na- Carboxymethyl okra gum was weighed.

Solution 2 was titrated against a standard solution of NaOH. The burette reading was noted down and the degree of substitution was found out by following equation (2).

$$\text{Degree of substitution} = 0.162\ B / (1 - 0.58\ B) \quad (2)$$

Where, B= Volume of NaOH / Weight of sample

Results and Discussion

Optimization study of parameters

Carboxymethylation reaction was carried out at variable amount of NaOH from 2-12 mL (Table 1). But degree of substitution (DS) increased as a result of increasing the amount of NaOH up to 10 mL.There was no change in the degree of substitution when NaOH was used in the amounts of 10 mL and 12 mL. So we found 10 mL NaOH is the best proportion. Increase in amount of NaOH leads to the alkali degradation of polymer. The lower amount of NaOH leads to lower number of free hydroxyl group deprotonated to form alkoxide which was resulted into lower value of degree of substitution.

Table 1. Effect of NaOH

S. No.	NaOH, mL, 30%	Temperature, °C	Monochloro-acetic acid, g	Time, h	Degree of substitution
1	2	60	10	6	0.35
2	4	60	10	6	0.36
3	6	60	10	6	0.39
4	8	60	10	6	0.55
5	10	60	10	6	1.11
6	12	60	10	6	0.99

Table 2. Effect of monochloroacetic acid

S. No.	Monochloro-acetic acid, g	NaOH, mL, 30%	Temperature, °C	Time, h	Degree of substitution
1	2	10	60	6	0.34
2	4	10	60	6	0.36
3	6	10	60	6	0.40
4	8	10	60	6	0.60
5	10	10	60	6	1.11
6	12	10	60	6	1.11

Carboxymethylation reaction was carried out by varying amount of monochloroacetic acid from 2-12 g (Table 2). But degree of substitution increase as a result of increasing the amount of monochloroacetic acid up to 10 g. The reaction remains constant by further increase in the amount of monochloroacetic acid there was no change in the degree of substitution with increase in monochloroacetic acid, So we found 10 g monochloroacetic acid is the best proportion. If the less amount of monochloroacetic acid used then it leads to unneutralization of alkali so the optimum monochloroacetic aid (10 g) was used to leads the favorable neutralization of alkali and to better formation of carboxymethylation okra gum having higher degree of substitution value.

Carboxymethylation reaction was carried out in the duration of 3-7 h, keeping other limits constant (Table 3). It can be settled from the table that degree of substitution improved with increase in time from 3-6 h. But degree of substitution was almost same at the end of 6 h and 7 h. So we selected 6 h is the best reaction time. Besides, carrying out the reaction for 7 h (*i.e*. one hour more) was likely to increase the production cost.

Table 3. Effect of time

S. No.	Time, h	NaOH, mL, 30%	Monochloro-acetic acid, g	Temperature, °C	Degree of substitution
1	3	10	10	60	0.36
2	4	10	10	60	0.40
3	5	10	10	60	0.56
4	6	10	10	60	1.11
5	7	10	10	60	1.12

Carboxymethylation reaction was carried out in the range of 30-70 ^{0}C, keeping other limits constant (Table 4). It can be perceived from the table that degree of substitution improved with increase in temperature from 30-60 ^{0}C. But the degree of substitution obtained at 70 ^{0}C was almost same as obtained at 60 ^{0}C. Beside maintaining temperature higher than 60 ^{0}C was likely to increase the production cost. So we selected 60 ^{0}C as the best reaction temperature.

Table 4. Effect of temperature

S. No	Temperature, °C	NaOH, mL, 30%	Monochloro-acetic acid, g	Time, h	Degree of substitution
1	30	10	10	6	0.37
2	40	10	10	6	0.42
3	50	10	10	6	0.60
4	60	10	10	6	1.11
5	70	10	10	6	1.13

Table 5. Characterization study of okra gum

Properties	Extracted okra gum	Carboxymethylated okra gum
Moisture content	10.96%	9.4%
pH*	6.59	9.10
Solubility	Soluble in water Insoluble in acetone, chloroform, methanol, DMSO and DMF.	soluble in water Insoluble in acetone, chloroform, methanol, DMSO and DMF.
Viscosity**	53.6 cSt (A tube) and 68.8 cSt (B tube)	31 cSt (A tube) 21.3 cSt (B tube)

**For1% w/v solution, ** 1% aqueous solution used in tube A & 0.5% in tube B (Gardner-bubble viscometer method)*

Characterization study

Characterization study for the extracted okra gum and extracted carboxymethylated okra gum was carried out and the values are listed in the Table 5.

FTIR spectrums of okra gum were shown in Figure 2. The absorption band at 3471.23 cm^{-1} in IR spectrum of okra gum showed intensity for the hydroxyl group present in okra gum. The sharp absorption band located at 2927.47 cm^{-1} may be attributed to CH group stretching.

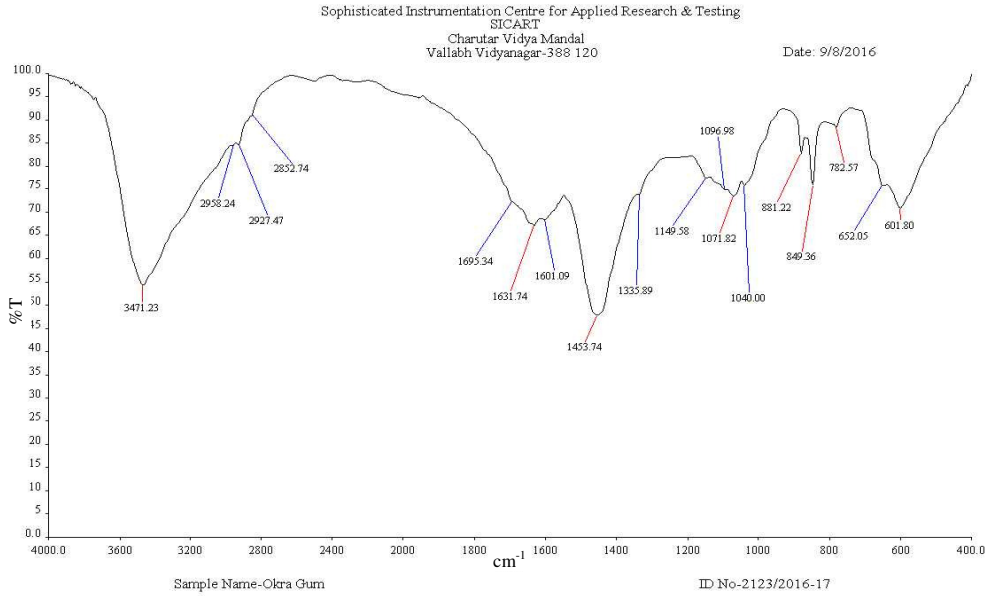

Figure 2. FTIR Spectroscopy of okra gum

FTIR spectrum of carboxymethylated okra gum is shown in Figure 3. The IR spectrum of carboxymethylated okra gum showed a reduced intensity of the absorption band located at 3436.51 cm^{-1}, as compared to okra gum IR spectrum. This happened due to -OH stretching which indicated that some -OH group were carboxymethylated. The sharp absorption band located at 2926.26 cm^{-1} may be attributed to CH group stretching. The C-O symmetrical and asymmetrical vibrations at a frequency of 1072.27 cm^{-1} confirms the incorporation of the carboxymethyl group to the okra gum molecule, which is absent in the okra gum spectra.

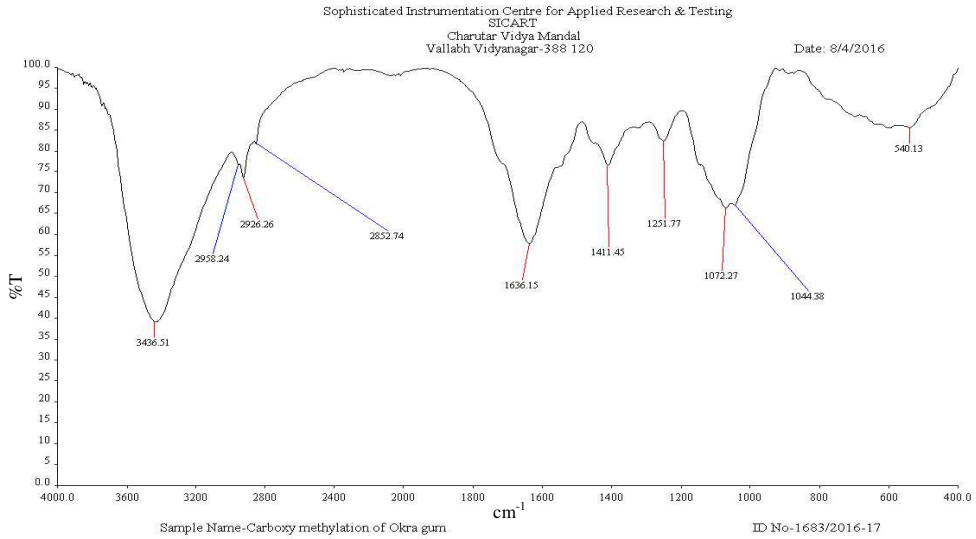

Figure 3. FTIR Spectroscopy of carboxymethylated of okra gum

Conclusion

The carboxymethylation of okra gum was carried out successfully and it is confirmed by FTIR. Carboxymethylation increases the properties of okra gum, moisture content and viscosity. The optimization study for carboxymethylation of okra gum was carried out. The optimum degree of substitution of 1.11 was obtained by carrying out reaction at 60 ^{o}C for 6 h by addition 10 mL (30%) of NaOH and 10 g of monochloroacetic acid.

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