CARBOXYMETHYLATION OF GUAR GUM: SYNTHESIS AND CHARACTERIZATION

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Recent studies focus on the chemical modification of polysaccharides for biomedical applications. Guar gum is a natural polymer composed of linear chains of (1-4)-β-D mannopyranosyl units with α-D-galactopyranosyl units attached by (1-6) linkages, in a ratio of about 1.5-2, possessing hydroxyl groups available for the attachment of biologically active compounds. The modifications here discussed include the introduction of carboxymethyl groups into the molecule, for extending the applications of partially carboxymethylated guar gum. The carboxymethylation conditions with respect to the volume and concentration of sodium hydroxide, weight of chloro-acetic acid and reaction temperature were optimized. The resulting products were characterized by NMR and FTIR spectroscopy and by thermal gravimetric analysis (TGA). The carboxymethylated guar gum may provide an efficient alternative approach for the oral delivery of hydrophilic macromolecules.

Keywords: guar gum, chemical modification, carboxymethyl group, drug delivery

INTRODUCTION

In recent years, the development and utilization of polysaccharides isolated from natural sources have attracted increasing attention in biochemistry and pharmacology, due to their sustainability, biodegradability and biosafety. Guar gum is a natural nonionic polysaccharide extracted from the refined endosperm of cluster bean seeds, chemically classified as galactomannan. It is basically composed of a straight chain of D-mannose units, united by β(1-4) glycoside linkages, and bearing a single D-galactose unit on approximately every alternate mannose, joined to it by an α(1-6) glycoside linkage.[1] (Fig. 1)

Even if guar gum and its derivatives are well-known for a wide range of applications, like other polysaccharides, they evidence some drawbacks, such as uncontrolled rates of hydration, pH-dependent solubility and high susceptibility to microbial attack.[2] Chemical modification provides an efficient route not only for removing such drawbacks but also for improving swelling and solubilization.

Possible processing methods for guar gum depend on the chemical modifications aimed at developing functional characteristics that make this material versatile and useful in a variety of applications. Native guar gum can be modified into various water-soluble derivates by using reactive groups, to substitute the free hydroxyl groups along the macromolecule backbone.[3] Upon dissolution in water, guar gum may give rise to as much as 10-14% insoluble residue, as depending on gum purity.[4] The formation of such residues, mainly composed of heavily intertwined polysaccharide chains, proteins and ashes, together with the tendency of guar gum to form aggregates in solution, are indesirable characteristics in some applications.

Some investigations have been undertaken to deal with the solubility properties of guar gum, as faster and better solubility in water, as well as multifunctional characteristics, can be obtained by the introduction of different compounds.[5]

The simplest change is achieved by a purification method for removing the insoluble fractions,[6] as well as by the synthesis of guar gum derivatives, such as hydroxypropyl-guar[7] and carboxymethyl-guar,[8,9] which may obviate the above-mentioned problems and, quite naturally,

lead to obtaining new products/materials with possible biomedical applications. The carboxymethylation of gums increases their hydrophilicity and solution clarity and makes them more soluble in aqueous systems. Site-specific targeted release can be also implemented by attaching pH-sensitive groups, such as carboxylic acid groups, to the guar polymeric structure, through a carboxymethylation process. Carboxymethylation was selected as a chemical means of attaching pendant carboxylic acid groups (COOH), due to its technical simplicity, low cost of chemical reagents, and wide applications, to produce carboxylmethylcellulose (CMC),[10] carboxylmethyl starch,[11] carboxylmethylinulin[12] and carboxylmethyl xylan.[13] The reaction conditions of carboxymethylation vary among different types of polysaccharides.

The objective of the present work was to optimize the reaction conditions and to characterize a water-soluble polymer based on unsubstituted and substituted guar gum, by using simple techniques and instruments.

EXPERIMENTAL

Materials and method

Guar gum (Mw ≈ 220 kDa) and sodium hydroxide were obtained from Sigma-Aldrich (Sigma-Aldrich, Germany); chloro-acetic acid was purchased from Merck (Merck, Germany); the solvents and the glacial acetic acid were of analytical grade.

Unless stated otherwise, reagents were used as purchased, without further purification. All solutions were prepared with distilled water.

Native guar gum was modified for analyzing the ability of the new products to dissolve in water.

Purification procedure

The method applied is based on a procedure described by Cunha *et al.*,[6] with some modifications. Crude guar gum (5 g) was treated by Soxhlet extraction with ethanol, for three days. The obtained product was hydrated in distilled water (500 mL) and mixed through magnetic stirring for 3 h, followed by centrifugation at 1500 rpm for 15 min. The supernatant was precipitated in acetone, filtered and washed successively with ethanol, then dialyzed against distilled water for 3 days and freeze-dried (GDp).

Carboxymethylation of guar gum

The method is based on a previous procedure, involving substituted gum synthesis.[8] For carboxymethylation, 2 g of purified guar gum (GDp) were dispersed in 100 mL distilled water, in a 250 mL jacketed glass reactor connected to a thermostated bath, and equipped with magnetic stirrer and gas-purging system. After the gum was well dispersed, an appropriate volume of sodium hydroxide solution of a certain concentration was added, as indicated in Table 1, at a rate of 1 mL within 15 min, with continuous stirring at room temperature. An aliquot of 15 mL chloro-acetic acid of specified weight (Table 1) was then added to the reaction mixture, over a period of 10 min. The reaction mixture was heated to a specified temperature (Table 1) with continuous stirring for 4 h, to drive the reaction process to completion.

The reaction product was repeatedly extracted with ethanol and separated by centrifugation. After the third extraction, the pH was adjusted to 7 with several drops of glacial acetic acid. Finally, the precipitate was washed with water and dialyzed against distilled water for a couple of days, and finally freeze-dried.

Characterization of modified guar gum

The resulting products were characterized by ^{1}H-NMR (Bruker Avance DRX, 400MHz), FTIR (Bomem MB 104 spectrometer) spectroscopy and thermal gravimetric analysis (Metler Toledo TGA-SDTA851) in nitrogen atmosphere, at a heating rate of 10 K/min.

RESULTS AND DISCUSSION

Purification of crude guar gum

A purification method was applied to commercial guar gum, to obtain well-defined products. Purification was carried out by Soxhlet extraction, followed by repeated dissolution, precipitation and centrifugation steps. The purification yield was high (86%). The purified product was further used in future synthesis.

Carboxymethylation of guar gum

Carboxymethylation of guar gum employed the Williamson ether synthesis procedure, which is a consecutive two-step reaction,[14] proceeding with a strong base – such as sodium hydroxide – that deprotonates the free hydroxyl groups (particularly, the hydroxyl group of (-CH_2OH) in guar gum) to form alkoxides, thereby increasing their nucleophilicity. Carboxymethyl groups are then formed in a reaction between guar alkoxides and chloroacetic acid. The overall reaction is briefly shown in Scheme 1.

A side reaction simultaneously takes place, resulting in the formation of sodium glycolate from sodium chloroacetate and sodium hydroxide.

The four selected process factors that influence the efficiency of the carboxymethylation process were the following: volume and concentration of sodium hydroxide (V_{NaOH}, C_{NaOH}), weight of the chloroacetic acid powder (W_{CA}), and reaction temperature (T). Table 1 summarizes the process factors selected in the sample sets.

In this paper, the dependent variables include the degree of substitution (DS), the swelling ratio and the percentage of gel fraction in water.

Degree of substitution in modified guar gum

Figures 2A and 2B compare the ^{1}H NMR spectra for the purified guar and the representative carboxymethylated guar sample, CMG5. The chemical shifts of the proton peaks in the NMR spectra were consistent with previously published values.[15]

^{1}H-NMR analysis of the purified guar gum showed a peak at δ = 4.7 ppm for anomeric protons, overlaid by the solvent peak (D_2O-d), while the peaks located at δ = 3.5-4.2 ppm are due to sugar protons (Fig. 2A). The spectrum for CMG5 revealed the occurrence of new proton peaks at δ = 3.7, 3.9 and 4.21 ppm, attributed to the methylene protons in the carboxymethoxy substituents in position C-6 of the α-D-galactose unit and position C-3 of the β-D-mannose unit. The partial degree of substitution (carboxymethylation) was calculated[16] from the ^{1}H NMR spectrum, using the integral of the proton peaks between the chemical shifts of 3.5-5.2 ppm, according to Eq. 1 (Table 1):

DS = A (protons of carboxymethylated guar in position O-i)/A (protons of carboxymethylated guar in position O-i) + A (proton(s) of purified guar in position O-i) (Eq. 1)

where A represents the peak area, O is the oxygen atom in position *i* (*i* = positions 6 and 3 of the α-D-galactose unit and of the β-D-mannose unit, respectively), and DS is the partial degree of substitution.

The presence of the characteristic peak assigned to the carboxyl groups in the ^{1}H-NMR spectra confirmed the occurrence of the polysaccharide carboxymethylation reaction (Fig. 2B).

Swelling and gel fraction studies

Swelling and gel fraction studies were carried on the basis of a previously reported protocol.[17] Briefly, samples weighing 0.01 g purified or modified guar gum were placed in small dishes that were carefully inserted into glass flasks. A total volume of 60 mL distilled water was slowly poured into each glass flask. The samples were allowed to soak for 2 h at room temperature, after which the excess solution was carefully removed, and the gelled samples remaining in the glass bottle were weighed. The gelled samples were lyophilized for three days and then weighed again. The swelling ratio and percentage of gel fraction were calculated[18] using Eqs. (2) and (3):

Swelling ratio = Wwater/Wgel (Eq. 2)

Percentage gel fraction = (Wgel/Wsolid) x 100 (Eq. 3)

where *Wwater* is the weight of the sample after 2 h soaking, *Wgel* is the weight of the sample after lyophilization, and *Wsolid* is the initial weight of the sample.

The modified guar gum (CMG5) displayed a higher swelling ratio relatively to unmodified guar gum (Table 2). The carboxymethylation of carbohydrates relies on maximizing the degree of substitution.[19-22] However, the water solubility of carboxymethylated carbohydrates increases with increasing substitution, which, in turn, reduces the gelling properties,[14,23] which is the main reason for studying the gel fraction percentage.

Table 1
Synthesis parameters for carboxymethylation process

Batch	V_{NaOH} (ml)	C_{NaOH} (%)	W_{CA} (g)	T (ºC)
CMG1	15	3	3	50
CMG2	15	6	6	50
CMG3	15	9	6	50
CMG4	10	10	5	60
CMG5	10	30	10	60
CMG6	10	5	1	60

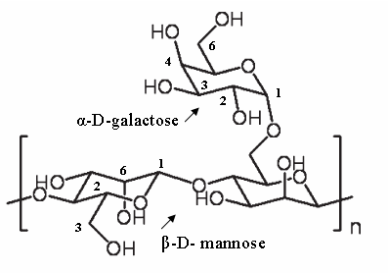
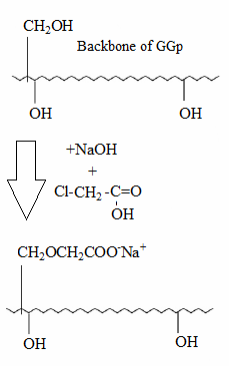


Figure 1: Structure of guar gum



Scheme 1: Reaction scheme for the carboxymethylation process of guar gum

Table 2
Substitution degree of samples and response (swelling ratio and gel fraction) of purified and carboxymethylated guar gum

Batch	DS^t (^{1}H NMR)	Swelling ratio	Gel fraction (%)
GGp	-	2.62	80
CMG1	0.600	2.96	73.87
CMG2	0.618	3.12	64.54
CMG3	0.649	3.22	62.31
CMG4	0.750	3.67	59.72
CMG5	1.230	5.32	54.60
CMG6	0.578	2.90	67.00

DS^t is the degree of carboxymethylation of the hydroxyl groups at C-6 and C-3 of the α-D-galactose unit and of the β-D-mannose unit in the guar gum samples as determined by ^{1}H NMR spectrum according to Eq. 1

FTIR analysis

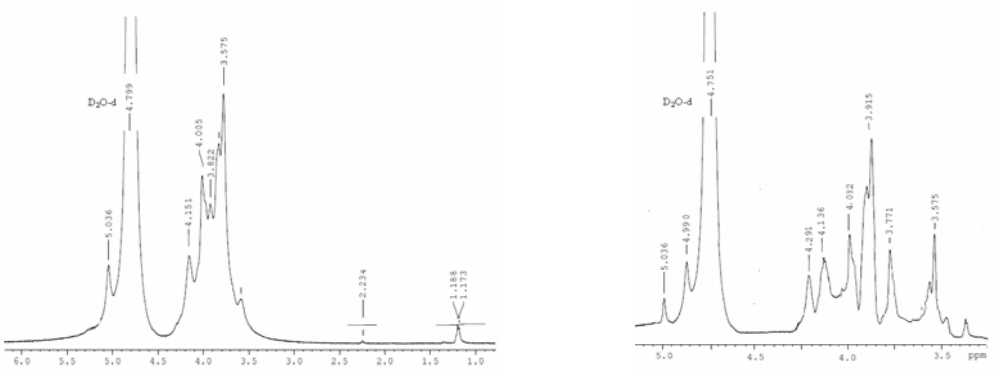
The overlaid IR spectra of raw guar gum (GG), purified guar gum (GGp) and carboxymethyl guar (CMG5) are shown in Figure 3.

The presence of a very strong and broad absorption band at 3391cm^{-1} is assigned to OH bond stretching, while the sharp absorption band located at 2907 cm^{-1} may be attributed to CH group stretching. The absorption band appearing at 1649 cm^{-1} is due the OH bond belonging to water molecules. CH_2 group bending is assigned to an absorption band located at 1457 cm^{-1}, and the bending of CH_2–O–CH_2 appears in the 1025 cm^{-1} frequency region. A slight modification can be observed in the well-defined spectrum of purified guar gum.

The IR spectrum of carboxymethyl guar (CMG5-DS = 1.2) shows a reduced intensity of the absorption band located at 3418 cm^{-1}, due to OH stretching, indicating that some OH groups were carboxymethylated. The band due to water (bending of water), which appeared at approximately 1650 cm^{-1} in the GGp sample was absent in the CMG5 sample. The asymmetrical and symmetrical vibrations due to moiety were assigned to 1615 and 1429 cm^{-1}, respectively, which may be attributed to the incorporation of carboxymethyl groups into the guar molecule.

TGA

Differential thermogravimetric curves of the samples are presented in Figure 4. Thermogravimetric analysis of guar gum essentially reveals two distinct zones of weight loss. The initial weight loss occurred in the 25-115 °C range, due to the moisture traces present in the sample. The second step represents the degradation of the polymer backbone, having started at 230 °C and lasting until 350 °C. In addition to these zones of weight loss, the thermal degradation of carboxymethyl guar gum shows a third zone in the 408-478 °C range, due to the degradation of the carboxymethyl groups incorporated in the polymer moiety. This third step of weight loss, which was present only in CMG5, provided more proof on the insertion of carboxymethyl groups.



A B

Figure 2: (A)[1]H- NMR spectra of purified guar gum (GGp). (B) [1]H-NMR spectra of carboxymethylguar gum (CMG5)

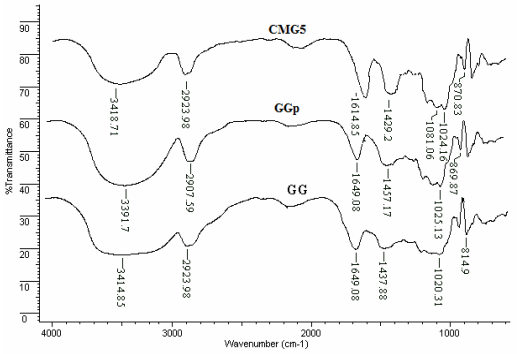


Figure 3: IR spectra of guar gum (GG), purified guar gum (GGp) and carboxymethyl guar gum (CMG5)

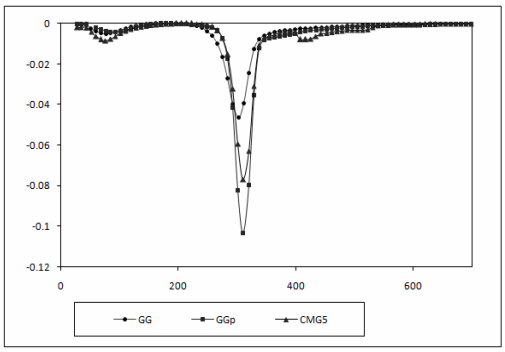


Figure 4: DTG curves of guar gum (GG), purified guar gum (GGp) and carboxymethyl guar gum (CMG5)

CONCLUSIONS

The study confirms that purification by extraction and carboxymethylation improves the properties of crude guar gum. The feasibility of the procedures was demonstrated by FTIR spectroscopy, [1]H-NMR spectroscopy and thermal analysis. Furthermore, the obtained products can have wider biological application as drug delivery carriers by grafting/crosslinking compounds of interest.

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