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A simple non-aqueous method for carboxymethylation of galactomannans☆

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Abstract

A simple non-aqueous method for the preparation of carboxymethyl (CM) derivatives of galactomannans and other biopolymers has been developed. Galactomannans such as guar, tara and locust bean gums were subjected to carboxymethylation using monochloroacetic acid under the catalytic influence of $NaHCO_3$ in dry state. The ratios of reagent and catalyst as well as the temperature and duration of the reaction were varied to prepare CM derivatives of different DS values ranging from 0.065 to 0.675. The progress of the reaction was followed by FT-IR and ^{13}C NMR spectral data. The reaction carried out in the presence of a small amount (<0.01%) of EtOH (just to surface wet the gum powder) gave CM derivatives having relatively a higher viscosity (3500 cps) than the unmodified gum. Being a mild alkali, removal of $NaHCO_3$ was easier, and the possibility of alkaline degradation caused by strong NaOH and also the effect of elevated temperature could be minimized. Overall the described method adds to the cost effectiveness as well as eco-friendly characteristic of the reaction.

Keywords: Guar gum; Tara gum; Galactomannans; Carboxymethylation

1. Introduction

Galactomannans, the polysaccharides found especially in the endosperm of several leguminous seeds, are composed of a mannan backbone linked together by β−(1→4) glycosidic linkages and having galactose side chain residues linked α−(1→6) (Dea and Morrison, 1975). Their solubility in water is mainly due to the presence of the latter. These semi rigid polymers with a persistent chain length in the range of 100 Å are found to be good thickening and stabilizing agents. Industrially viable and the most commonly used galactomannans are from guar (*Cyamopsis tetragonoloba*) (Davidson, 1980), tara (*Caesalpinia spinosa*) (Beak, 1977) and locust bean gum (*Ceratonia siliqua*), which differ in the ratio of galactose to mannose, viz. 1:2, 1:3 and 1:4, respectively. Chemical modifications of polysaccharides by etherification, esterification, oxidation and hydroxypropylation (Sierakowaski, Milas, Desbrieres, & Rinacedo, 2000) are generally done for preparing custom-made derivatives having desirable functionality attributes. Carboxymethylation (CM) generally increases the hydrophilicity and solution clarity of the polysaccharide and makes it better soluble in aqueous systems. In a majority of the reported methods, carboxymethylation is done using strong NaOH and monochloroacetic acid (MCA) in aqueous medium at elevated temperature (Green, 1963; Moe, 1951; Yuch, 1972). Inadvertent molecular as well as reagent degradations induced by strong alkaline pH at elevated temperature cannot be underestimated under such conditions. No information is available on such reactions being carried out in absolutely dry, anhydrous conditions (without the addition of external water) at ambient or slightly elevated temperatures. The present study was carried out to fillin this gap.

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2. Experimental

2.1. Materials

Guar gum and tara gum were commercial food grade samples procured from Aqualon (Wilmington, DE, USA). Locust bean gum (food grade), cornstarch, monochloroacetic acid (MCA) and $NaHCO_3$ were from Sigma Chemical Co., Mo, USA. The various other chemicals used were of analytical reagent quality. Total sugars were determined by phenol–sulphuric acid (Rao and Pattabhiraman, 1989) and reducing sugars by DNS method (Miller, 1959). The sugar composition was determined by acid depolymerization followed by alditol acetate derivatization and GLC (Soltzberg, 1970). A Schimadzu gas chromatograph equipped with FID and an OV-225 ss column (3% on Chromosorb W (100–120 mesh), 5 ft$\times$1/8″) operated at 200 °C, with N_2 as the carrier gas was used.

2.2. Carboxymethylation

In a typical reaction, to weighed samples (0.1–1 g) of galactomannan and finely powdered $NaHCO_3$ (1–5 g) mixed well manually using a pestle and mortar, were added ~0.1–0.2 ml of ethanol (100% for surface wetting), and solid MCA (0.5–2 g). The reaction was carried out at ambient or elevated temperature (60, 80, 98 °C) for 2 h, with intermittent manual mixing with a glass rod, followed by the addition of dilute acetic acid to phenolphthalein end point. The salts (Green, 1963) formed were removed by repeated washings with 70% aqueous ethanol (3$\times$10 ml, kept for 15 min) followed by 100% ethanol and solvent exchange drying. The derivatized gums tested for Na^+ using uranyl magnesium acetate (Vogel, 1989) spot test as well as AAS, were found to be completely free of Na^+ ions, etc. by these washings. A number of CM derivatives of differing degree of substitution (DS) values were prepared by varying the ratio of catalyst and the reagent. In a similar way carboxymethylation was carried out without prior surface wetting with alcohol, and the derivatized dry products were recovered as mentioned above.

2.3. FT-IR spectroscopy

Both native and modified gum samples (5 mg) were blended with solid KBr, (100 mg, Sigma-Aldrich, USA) and about 40 mg of the blend was used to prepare a pellet (Delta press, Tetra Scientific, USA). The spectra were scanned from 4000 to 400 cm^{-1} in a Perkin Elmer 2000 FT-IR spectrometer under dry air at room temperature.

2.4. DS by FT-IR spectroscopy

The degree of carboxymethylation was quantitatively determined by calculating the ratio between the intensity of hydrogen bonded –OH groups at about 3400 cm^{-1} and the intensity of carboxyl –C=O stretching of ether at 1740–1745 cm^{-1}. $DS = A_{1740–1745\ \bar{c}m1}/A_{3400\ \bar{c}m1}$ (A is intensity of absorption) was calculated after suitable baseline drawing for these absorbances (Nahalka, Nahalkova, Germeiner, & Blanarik, 1993)

2.5. CP MAS ^{13}C NMR spectroscopy

For solid state CP MAS ^{13}C NMR, approximately 300 mg of samples were inserted into the ceramic rotor on a Bruker DSX 300 spectrometer. The spectrum, at 75.5 MHz was recorded using the cross polarization pulse sequence, spun at 7.5 kHz magic angle. A contact time of 1 ms and a pulse repetition time of 5 s were used with more than 2000 scans accumulated for each sample.

3. Results and discussion

Carboxymethylation(CM) is the most commonly used chemical derivatization method to transform water insoluble polymeric materials into a water-soluble form. The Williamson's etherification reaction carried out invariably in aqueous medium using strong NaOH and MCA at elevated temperature for different periods of time may lead to non-specific degradation by β-elimination and/or peeling reaction initiated at the reducing sugar unit due to high alkaline pH (Whistler and BeMiller, 1958), which in turn reduces the molecular weight of derivatized material. Reduction in solution viscosity, due to subtle disturbances in the water–macromolecule interaction is also reported for polysaccharides in the presence of high alkaline pH. Guar gum, even in oxygen-free alkaline solution is reported to generate saccharinic acids (Whistler and BeMiller, 1958).

CM reaction is highly dependent on several factors including the ratio of base catalyst, MCA, reaction medium, time and temperature. Hence, attempts were made to carryout the reaction (Fig. 1) in anhydrous conditions (without using external water) using solid $NaHCO_3$, a permitted food grade chemical, at both ambient as well as slightly elevated temperatures (Table 1). It was observed that as the concentration of base was increased beyond a ratio of 1.0, both solution viscosity as well as DS of the CM derivative were seriously affected, both showed a decreasing trend. Guar gum showed a relatively better DS, which increased slowly as the temperature was increased, but latter it decreased (Fig. 2). The reaction took place to some extent even at room temperature for extended period of time. The decrease in viscosity of CM guar was more as the temperature and catalyst concentration was increased.

In the case of tara gum a different trend in DS was noticed (Fig. 2), as the temperature was increased, the DS was increased, which then slightly decreased but later further increased. However, an increase in MCA concentration though did not change the DS that significantly, but gave products with considerable retention of viscosity.

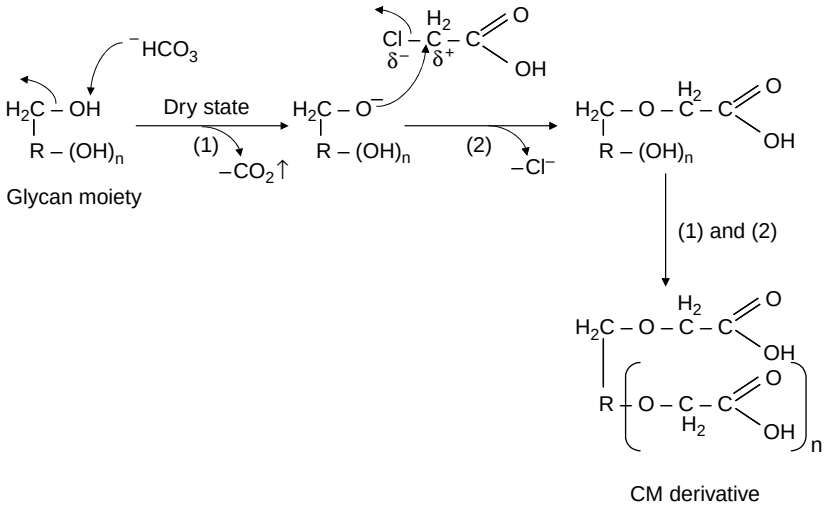

Fig. 1. Reaction mechanism, where R is the glycan moiety of galactomannan.

The reaction performed beyond 2 h did not show any significant improvement either in DS or solution viscosity (data not shown). In fact, a further increase in their ratio caused a competition between the main and the side reactions, the latter resulting in the formation of sodium glycolate and consequently lowering DS. Being a very mild base quantitative removal of $NaHCO_3$ after the reaction by repeated washings with aqueous ethanol was much easier and did not pose any problems. Qualitative test as well as atomic absorption spectroscopy showed the absence of Na^+ ions in the derivatives. FT-IR spectra (Fig. 3) indicated the introduction of carboxymethyl moiety (absorption band at 1743–1748 cm^{-1}), whose intensity increased with increase in DS. The absorption band at 1022 cm^{-1} (of native gum) was shifted to 1027 cm^{-1} in the case of modified gum due to C–O stretching in C–O–C linkage, which also indicated a possible increase in molecular crystallinity (Atalla et al, 1979). ^{13}C NMR spectra (Fig. 4) showed resonances for methylene ($–CH_2$) around 44.84 and 44.54 ppm and –C=O signal around 174.95 and 174.536 ppm, respectively, in CM tara and CM guar derivative. The C_1 (Man) appeared around 101.76 and C_1 (Gal) at 94.98 ppm. Strong signals between 60 and 85 ppm were assigned to ring carbons of mannose backbone and galactose side chains. The characteristic splitting of 70 ppm signal in the derivatives is yet another evidence for carboxymethylation (Atalla et al., 1979). Increase in the sharpness of the peaks together with splitting of the peaks over those of native unmodified gums indicated increased crystallinity of the modified gum samples

Moreover, unlike starch or cellulose, galactomannan gums are water-soluble with very high solution viscosity. Therefore, use of water in such reactions with gums may lead to a semi solid gel-like mass, which may resist effective penetration of the catalyst and reagent, and thus resulting in incomplete reaction with very low DS. Additionally, use of water demands its complete removal before drying, which may be laborious, energy intensive and therefore not cost effective. Feasibility of this method of carboxymethylation was verified by derivatizing other biopolymers such as starch and other galactomannans (locust bean gum).

Table 1
Carboxymethylation of guar gum and tara gum

G:C:R (g)	Temperature (°C)	Duration (h)	DS	
			Guar gum	Tara gum
0.1:1:1	Ambient	4.0	0.062	0.040
0.1:1:1	60	2.0	0.110	0.100
0.1:1:1	80	2.0	0.675	0.098
0.1:1:2	98	1.0	0.360	0.098
0.1:1:1	98	1.0	0.053	0.12
0.1: 1:1	98	1.5	0.390	0.231

G:C:R, Gum:Catalyst:Reagent. DS = $A_{–OH}$, $cm^{-1}/A_{–C=O}$, cm^{-1}, where A is the absorbance at these wavelengths, calculated from baseline drawing.

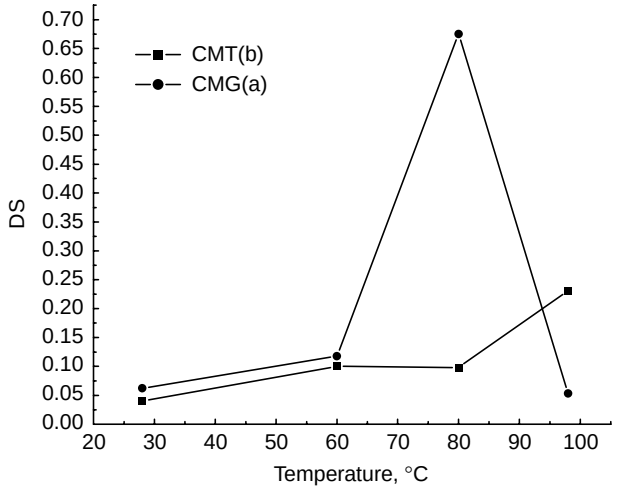

Fig. 2. Variation of DS with temperature of CM guar (a) and tara (b) gums.

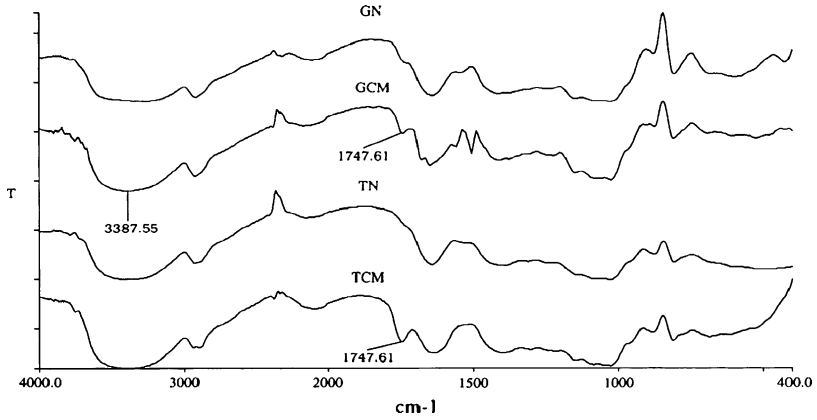

Fig. 3. FT-IR spectra of native (TN and GN) and derivatized (TCM and GCM) guar (G) and tara (T) gums.

Prior surface wetting of the powdered gum sample with a little (<0.01%) ethanol seemed to favor better carboxymethylation, as a similar reaction under the absence of alcohol gave a CM derivative with very low DS and considerably very low viscosity (1000 cps).

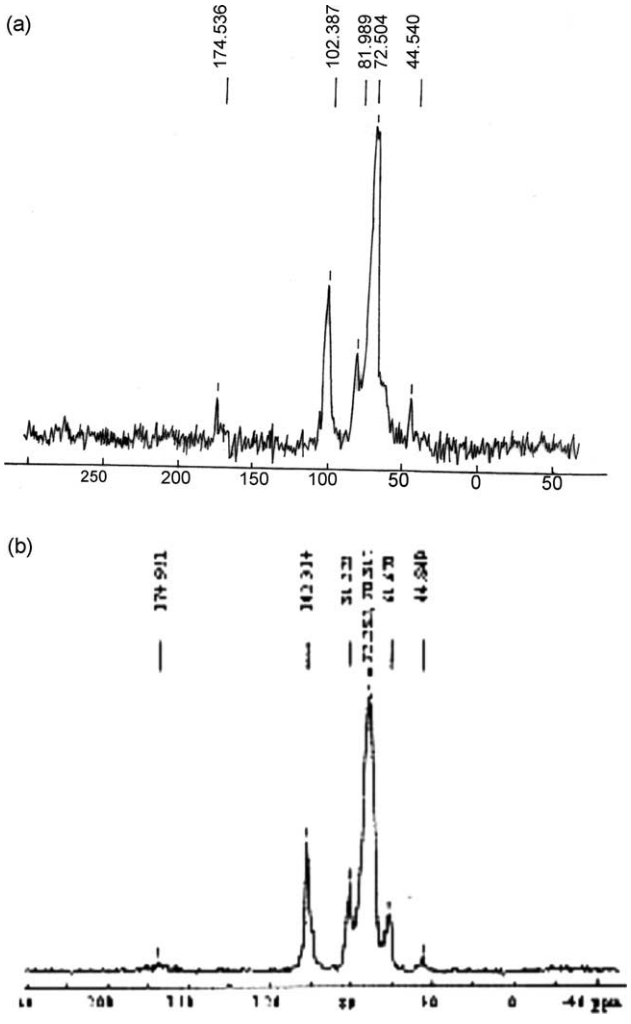

Fig. 4. CP MAS ^{13}C NMR spectra of CM guar (a) and (b) tara gums.

A variety of reaction media has been used for carboxymethylation of starch and it is stated that the amount of water required depends on the type of starch and solvent used (Tijsen, Kolk, Stamhuis, & Beenackers, 2001). For the various alcohols tested, isopropyl alcohol (containing 10% water) gave the highest DS. In most of the reported methods (Khalil, Beliakova, & Aly, 2001) the reaction was performed at >40 °C and for extended periods of time (~450 min) and the DS obtained was around 0.90. For ethyl and methyl alcohols, slightly higher water content (20 and 24%, respectively) was found to be optimal for CM derivatization of corn starch. To produce granular carboxymethyl starch with a higher DS (2.20), a stepwise modification procedure has been employed (Khalil, Hasehem, & Habiesh, 1990). It was also reported that prior oxidation (for example with potassium persulphate) enhanced the amenability of starch for better carboxymethylation (Habiesh, Khalil, & Hasehem, 1990). The latter proved to be a best sizing agent for cotton textiles.

In conclusion, the described method of carboxymethylation is simple, cost effective, ecofriendly and with slight manipulation of reaction conditions a range of custom-made CM derivatives for specific end uses can be prepared. At low DS values the CM derivatives show considerable solution viscosity and clarity.

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