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Carbohydrate Polymers 59 (2005) 501–506
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Chemical modification of *Cassia occidentalis* seed gum: carbamoylethylation

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Received 3 June 2004; revised 21 September 2004; accepted 9 November 2004
Available online 16 December 2004

Abstract

The seeds of *Cassia occidentalis*, an annual weed occurring throughout India, is a rich source of galactomannan gum. The gum derived from seed endosperm can be potentially utilized in a number of industries to replace the conventional gums. With a view to utilize the gum for broader applications, carbamoylethylation of *C. occidentalis* seed gum was carried out with acrylamide in presence of sodium hydroxide under different reaction conditions. Variables studied were concentration of sodium hydroxide, acrylamide, gum–solvent ratio, reaction time and temperature. The nitrogen content, carboxyl content and total ether content were determined. The optimum condition for preparing carbamoylethyl *C. occidentalis* seed gum (%N = 2.57) comprised concentration of acrylamide (0.070 mol), sodium hydroxide (0.125 mol), *C. occidentalis* seed gum (0.03 mol) at 30 °C for 3 h. Rheological properties of carbamoylethyl *C. occidentalis* seed gum solution showed non-Newtonian pseudo-plastic behavior, relatively high viscosity, cold water solubility and solution clarity vis-à-vis unmodified *C. occidentalis* seed gum.

Keywords: Cassia occidentalis; Seed gum; Carbamoylethylation

1. Introduction

Cassia occidentalis Linn. (Kasondi) is a common herbaceous annual weed occurring throughout India up to an altitude of 1500 m (Anonymous, 1992). Every part of the plant is used in different applications in Indian Ayurvedic system of medicine (Anonymous, 1992). The pods are 10–13 cm long and up to 0.8 cm in diameter containing dark olive green seeds. The *C. occidentalis* seeds are rich source of galactomannan (~30% endosperm) (Kapoor, Khan, & Farroqi, 1991). The structure of the backbone of the seed polysaccharide, β-(1→4) linked D-mannopyranose with random distribution of α-(1→6) linked D-galactopyranose units as side chain (M/G:3.1), was established by partial hydrolysis (Gupta & Mukherjee, 1975), permethylation (Gupta & Mukherjee, 1973) and NMR studies (Gupta, Chougule, & Pakadalkar, 1995).

Galactomannans are important industrial products used in various industries (Whistler, 1994). *C. occidentalis* seed gum has a potential on account of its wide availability, and can be utilized to replace the conventional gums like guar and locust bean. In our laboratory seed gums (Tamarind/*Cassia tora*/Guar) were modified via carboxymethylation (Sharma, Kumar, Soni, & Sharma, 2003; Soni & Sharma, 2000a,b), carbamoylethylation (Sharma, Kumar, & Soni, 2003a), cyanoethylation (Sharma, Kumar, & Soni, 2003b) and grafting (Sharma, Kumar, & Soni, 2002, 2003c,d). The present work aims to modify the COG chemically via carbamoylethylation under a variety of conditions to improve the solubility and rheology of solutions prepared therefrom and make it more useful/industrially exploitable.

The main reaction parameters, which influence the carbamoylethylation process, are the gum–liquor ratio, concentration of sodium hydroxide and acrylamide (AA), temperature and duration of reaction (El-Molla, Abdel Rahman, & El-Thalouth, 1998; Khalil, Bayazeed, Farag, & Hebeish, 1987; Khalil, Beliakova, & Aly, 2001). In the present work, carbamoylethylation of COG was performed

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0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.11.010

in aqueous system, and the reaction parameters were studied.

2. Materials and methods

2.1. Materials

C. occidentalis seed gum was obtained from M/S Shri Nath Gums and Chemicals, Jodhpur, India. Sodium hydroxide, acrylamide, and acetic acid were of laboratory grade (SD Fine-Chem. Ltd, Mumbai, India).

2.2. Methods

2.2.1. Carbamoylethylation

The carbamoylethylation reaction was performed as follows: in a 500 ml stoppered conical flask (equipped with magnetic stirring bar) COG (0.03 mol, 100 mesh) was dispersed in alkaline (0.05–0.175 mol, NaOH) aqueous solution (250 ml) varying the gum–liquor ratio from 1:25 to 1:100. After 10 min, acrylamide (AA) (0.028–0.113 mol) was added with stirring. At this end, the reaction was allowed to proceed for the desired temperature (20–60 °C) and time (60–240 min). The reaction mixture was cooled and neutralized with dilute acetic acid (1:1, v/v) and precipitated by pouring the reaction contents in the ethanol with stirring. The precipitated product was centrifuged, filtered, and washed twice with aqueous methanol (methanol:H_2O::80:20) followed by pure methanol. The products were initially dried at room temperature followed by in electric oven at 60 ± 2 °C for 2 h.

2.2.2. Analysis and measurements

☐ The nitrogen content of the carbamoylethyl derivative of COG (CB-COG) was determined by Kjeldahl's method (Jeffery, Bassett, & Mendham, 1989).

☐ The carboxyl content of the CB-COG was determined according to a reported method (Daul, Reinhardt, & Reid, 1953).

☐ The degree of substitution (DS) and reaction efficiency (RE) were calculated as follows:

$$\text{DS (as carbamoylethyl)} = \frac{162 \times \%\text{N}}{1400 - (71 \times \%\text{N})}$$

$$\text{RE} = \frac{\text{Weight of reacted acrylamide} \times 100}{\text{Original weight of acrylamide}}$$

☐ The rheological properties were determined using Brookfield Digital Viscometer model 'RVTD', Stoughton, USA by adopting the following experimental conditions:

(a) Range of shear rate between 2.5 and 34.0 s^{-1}

(b) Temperature 25 ± 1 °C.

☐ FT-IR (Fourier transform infrared) analysis was carried out using JASCO FT/IR-5300.

3. Results and discussion

Carbamoylethylation was carried out by reacting COG with acrylamide in presence of sodium hydroxide under a variety of conditions. The variables studied were concentration of acrylamide and sodium hydroxide, gum–liquor ratio, temperature and duration of reaction. The change in chemical structure of COG as a result of carbamoylethylation was confirmed by FT-IR spectroscopy (Fig. 1). From the IR spectra it is evident that CB-COG shows a broad band at 3441 Cm^{-1} for OH stretching vibration, and symmetrical and asymmetrical N–H stretching vibrations, which are merged with OH group stretching vibrations. Another band appear at 1668 Cm^{-1} due to the amide I band of carbonyl stretching and at 1653 Cm^{-1} due to the amide II band of N–H bending. The peak at 1092 Cm^{-1} is also observed due to C–O–C stretching vibration of the ether group. On reacting COG with acrylamide in the presence of sodium hydroxide using the wet process the following reactions are expected to occur (El-Molla et al., 1998; Khalil et al., 1987).

$$\text{COG–OH} + \text{CH}_2 = \text{CH–CONH}_2 \xrightarrow{\text{NaOH}} \text{COG–O–CH}_2\text{–CH}_2\text{–CONH}_2 \quad (1)$$

$$\text{COG–O–CH}_2\text{–CH}_2\text{–CONH}_2 + \text{H}_2\text{O} \xrightarrow{\text{NaOH}} \text{COG–O–CH}_2\text{–CH}_2\text{–COONa} + \text{NH}_3 \quad (2)$$

$$\text{CH}_2 = \text{CH–CONH}_2 + \text{H}_2\text{O} \xrightarrow{\text{NaOH}} \text{CH}_2 = \text{CH–COONa} + \text{NH}_3 \quad (3)$$

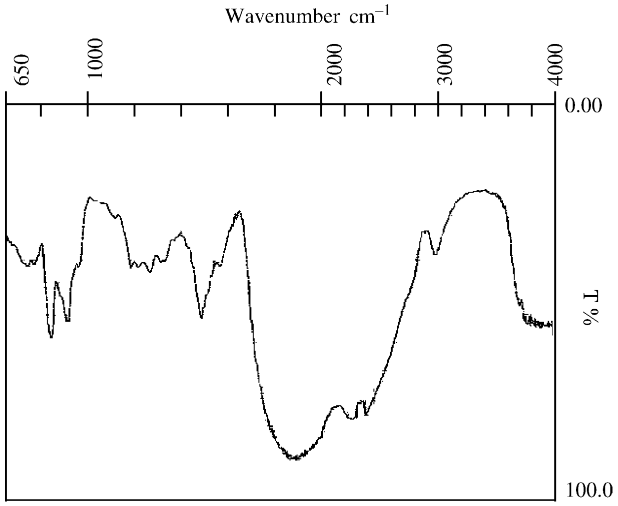

Fig. 1. FT-IR spectrum of CB-COG.

Furthermore, it has also been found that water adds to acrylonitrile in the presence of alkali to give ethylene cyanohydrin, which further reacts with acrylonitrile yielding bis-2-cyanoethyl ether (Bruson & Riener, 1943). In a similar way acrylamide in presence of alkali gives β-hydroxypropionamide, which further reacts with acrylamide to give bis-2-carbamoylethyl ether Eqs. (4) and (5).

$$CH_2=CH\text{–}CONH_2 + H_2O \xrightarrow{NaOH} HO\text{–}CH_2\text{–}CH_2\text{–}CONH_2 \qquad (4)$$

$$CH_2=CH\text{–}CONH_2 + HO\text{–}CH_2\text{–}CH_2\text{–}CONH_2 \rightarrow CONH_2\text{–}CH_2\text{–}CH_2\text{–}O\text{–}CH_2\text{–}CH_2\text{–}CONH_2 \qquad (5)$$

During the carbamoylethyl reaction, the fission of ether linkage (Moe, Miller, & Buckley, 1952) also takes place in alkaline medium as shown in Eq. (6).

$$COG\text{–}O\text{–}CH_2\text{–}CH_2CONH_2 + H_2O \xrightarrow{NaOH} COG\text{–}OH + CH_2=CH\text{–}CONH_2 \qquad (6)$$

The magnitude of the above reactions would be expected to depend upon temperature and duration of the reaction as well as sodium hydroxide concentration. Therefore, carbamoylethylation reaction was studied with respect to nitrogen and carboxyl contents of the modified COG.

3.1. Effect of sodium hydroxide concentration

The effect of variation of sodium hydroxide concentration from 0.050 to 0.175 mol at 30 °C on the percent nitrogen and carboxyl content of CB-COG was studied and the results are given in Table 1. It is seen that the extent of carbamoylethylation reaction (expressed as %N) of COG increases significantly by increasing sodium hydroxide concentration up to 0.125 mol. Further increase in sodium hydroxide concentration is accompanied by a decrease in the nitrogen content. Sodium hydroxide has three functions in carbamoylethylation reaction of COG: (a) catalyzing the reaction, (b) swelling the COG, and (c) hydrolyzing the amide groups in acrylamide and CB-COG Eqs. (2) and (3) (Feit & Zilkha, 1963; Khalil et al., 1987). Current data suggest that factors (a) and (b) are favored upon using sodium hydroxide concentration within the range 0.05–0.125 mol thereby giving rise to higher extents of reaction. Above 0.125 mol sodium hydroxide, factor (c) prevails over (a) and (b) and, as a result extent of reaction decreases and carboxyl content increases.

Table 1
Effect of sodium hydroxide concentration on carbamoylethylation of COG

Concentration of NaOH (mol)	Nitrogen (%)	DS	Carboxyl content (%)	RE (%)
0.050	1.55	0.194	1.035	6.92
0.075	1.72	0.218	1.230	7.11
0.100	1.81	0.230	1.548	7.38
0.125	2.57	0.342	1.624	13.42
0.150	1.80	0.229	2.052	7.38
0.175	1.80	0.229	2.452	7.37

Reaction conditions: [COG] 0.03 mol AGU; [AA] 0.07 mol; reaction time, 180 min; temperature, 30 °C; gum–liquor ratio, 1:50.

3.2. Effect of acrylamide concentration

The effect of acrylamide concentration on the extent of carbamoylethylation reaction of COG was studied using a concentration of 0.028–0.113 mol taking optimum concentration of sodium hydroxide 0.125 mol. Table 2 shows the effect of acrylamide concentration on percent nitrogen and DS of CB-COG and RE of carbamoylethylation. It is clear that the percent nitrogen and DS increase significantly as the concentration of acrylamide increases up to 0.070 mol. However, further increase in concentration of acrylamide beyond 0.070 mol, there is decrement in percent nitrogen and DS, and favor the side reactions Eqs. (3)–(5). The RE decreases continuously as the concentration of acrylamide increases.

This suggests that the enhancement in the extent of the reaction by increasing acrylamide concentration up to certain limit could be associated with greater availability of acrylamide molecule in the vicinity of COG. Since the COG hydroxyls are immobile, their reaction with acrylamide and sodium hydroxide would essentially depend upon availability of acrylamide and sodium hydroxide molecules in the proximity of the hydroxyl groups in gum molecule. However, on the other hand, after a certain acrylamide concentration, there is less accessibility of hydroxyl groups and further reaction becomes difficult under the conditions used, which favors the side reactions. As a result, the RE of carbamoylethylation reaction decreases as the concentration of acrylamide increases. Similar type of observation has also been reported in literature (Hebeish & Khalil, 1988; Khalil et al., 1987).

Table 2
Effect of acrylamide concentration on carbamoylethylation of COG

Concentration of acrylamide (mol)	Nitrogen (%)	DS	RE (%)
0.028	1.33	0.165	12.92
0.042	1.77	0.225	11.72
0.056	1.93	0.247	10.71
0.070	2.57	0.342	13.42
0.084	2.13	0.276	8.74
0.098	1.90	0.243	5.73
0.113	1.85	0.236	4.47

Reaction conditions: [COG] 0.03 mol AGU; [NaOH] 0.125 mol; reaction time, 180 min; temperature, 30 °C; gum–liquor ratio, 1:50.

Table 3
Effect of temperature on carbamoylethylation of COG

Reaction temperature (°C)	Nitrogen (%)	DS	Carboxyl content (%)	RE (%)
20	2.19	0.285	1.123	9.66
30	2.57	0.342	1.624	13.42
40	1.71	0.216	3.929	7.29
50	0.98	0.119	4.675	4.66
60	0.92	0.111	4.803	4.12

Reaction conditions: [COG] 0.03 mol AGU; [NaOH] 0.125 mol; [AA] 0.070 mol; reaction time, 180 min; gum–liquor ratio, 1:50.

Table 4
Dependence of percent nitrogen of CB-COG and carboxyl groups derived thereof on time and temperature of carbamoylethylation

Reaction time (min)	Reaction temperature					
	30 °C			60 °C		
	Nitrogen (%)	Carboxyl content (%)	DS	Nitrogen (%)	Carboxyl content (%)	DS
60	2.34	1.05	0.307	1.46	3.51	0.182
120	2.35	1.36	0.308	1.17	3.91	0.143
180	2.57	1.62	0.342	0.92	4.80	0.111
240	2.34	1.92	0.307	0.87	4.98	0.105

Reaction conditions: [COG] 0.03 mol AGU; [NaOH] 0.125 mol; [AA] 0.070 mol; gum–liquor ratio, 1:50.

3.3. Effect of temperature and duration of the reaction

The magnitude of the etherification reaction Eq. (1) and side reactions Eqs. (2)–(6) would be expected to depend upon temperature and duration of the reaction. The dependence of carbamoylethylation of COG on temperature and duration can be monitored in terms of nitrogen content, carboxyl content and total extent of etherification of CB-COG samples.

3.3.1. Nitrogen content

Tables 3 and 4 shows the variation of nitrogen content of the carbamoylethyl COG at different reaction temperatures and time. As is evident, the nitrogen content is temperature and time dependent. For instance, the nitrogen content increases from 2.19 to 2.57 as the reaction temperature increases from 20 to 30 °C (Table 3) indicating that reaction suggested by Eq. (1) prevails over the other side reactions. Further increase in reaction temperature resulted in decrease in nitrogen content and increase in carboxyl content owing to conversion of some of the amide groups of the CB-COG samples to carboxyl groups Eqs. (2) and (3) via hydrolysis (Hebeish, El-Thalouth, & El-Kashouti, 1981; Hebeish & Khalil, 1988; Khalil et al., 2001) and ultimately affects the efficiency of the etherification reaction. However, at 30 °C, the nitrogen content increases from 2.34 to 2.57% (Table 4) by increasing the reaction time from 60 to 180 min. At 60 °C, the nitrogen content is maximum at 60 min and decreases thereafter. On comparing the data of the extent of the reaction at 30 and 60 °C, it was found that at higher temperature reaction is adversely affected. This shows that carbamoylethylation is favored at 30 °C.

3.3.2. Carboxyl content

The carboxyl content of the carbamoylethyl COG samples prepared at 30 and 60 °C for different periods of time (60–240 min) is shown in Table 4. It reveals that the carboxyl content increases significantly by increasing the duration of the reaction time as well as reaction temperature. It proves that hydrolysis of the amide groups to carboxyl groups Eq. (2) occurs early from the beginning of the reaction and concurrently with the carbamoylethylation reaction Eq. (1). Increasing the reaction temperature acts in favor of higher rate of hydrolysis Eq. (2) since the carboxyl content is higher at higher temperature with the concomitant decrease in percent nitrogen at higher temperature. Furthermore, the carbamoylethyl reaction catalyzed by higher concentration of NaOH acquires carboxyl content more than that by lower concentration (Table 1) (Hebeish et al., 1981; Hebeish & Khalil, 1988; Khalil et al., 2001).

3.3.3. Total extent of reaction

Table 5 shows the total extent of the reaction occurring between COG and AA, expressed as the sum of the amide and carboxyl groups (in mmol/100 g sample). It is seen that at 30 °C, the total extent of reaction increases by increasing reaction time up to 180 min and thereafter decreases. This indicates that etherification reaction prevails over deetherification up to 180 min. At 60 °C, reaction time 60 min,

Table 5
Total extent of reaction of the CB-COG samples prepared at different reaction temperature and time

Reaction time (min)	Reaction temperature					
	30 °C			60 °C		
	Amide content (mmol/100 g)	Carboxyl content (mmol/100 g)	Total ether content (mmol/100 g)	Amide content (mmol/100 g)	Carboxyl content (mmol/100 g)	Total ether content (mmol/100 g)
60	167.14	23.33	190.47	104.63	78.00	182.63
120	167.85	30.22	198.07	84.07	86.88	170.95
180	183.57	36.00	219.57	65.71	106.66	172.37
240	167.14	42.66	209.80	62.14	110.66	172.80

Reaction conditions: [COG] 0.03 mol AGU; [NaOH] 0.125 mol; [AA] 0.070 mol; gum–liquor ratio, 1:50.

Table 6
Effect of gum–liquor ratio on carbamoylethylation of COG

Gum–liquor ratio	Nitrogen (%)	DS	RE
1:25	2.26	0.295	9.41
1:50	2.57	0.342	13.42
1:75	1.26	0.154	5.02
1:100	1.29	0.159	5.05

Reaction conditions: [COG] 0.03 mol AGU; [NaOH] 0.125 mol; [AA] 0.070 mol; reaction time, 180 min; temperature, 30 °C.

the maximum total extent of reaction is 182.63 which thereafter decreases by increasing the reaction time. Further, at 60 °C, the total extent of reaction is markedly low in comparison to 30 °C. Such decrease may be attributed to fission of the ether linkage Eq. (6) (Moe et al., 1952). The maximum total extent of etherification reaction occurs at 30 °C, reaction time 180 min, using the conditions indicated in Table 5. The sum of amide and carboxyl contents of the CB-COG corresponding to this total extent of reaction amounts to 219.57 mmol/100 g sample.

Thus, on the basis of foregoing discussion, it is evident that carbamoylethylation of COG is optimized at 30 °C, keeping reaction time 3 h.

3.4. *Effect of gum and liquor ratio*

The results obtained by investigating the effect of gum and liquor ratio on percent nitrogen are shown in Table 6. The amount of gum and reactants during these experiments were kept constant, however, the concentration of gum and reactants were proportionately decreased by increasing the amount of solvent. The maximum percent nitrogen (2.57) and DS (0.342) at gum–liquor ratio 1:50 signifies the role of volume of liquor in carbamoylethyl reaction. A critical amount of liquor helps the COG to swell and aids the diffusion and adsorption of etherifying agents on COG molecules. A decrease in the percent nitrogen at higher liquor volume was observed which can be attributed to the reduced probability of collision of the reactants or a decrease in collision due to dilution. Similar results were observed during the carbamoylethylation of *C. tora* gum (Sharma et al., 2003a).

4. Rheological properties

In order to evaluate the importance and feasibility of any gum, hydrocolloid or their derivatives in industries, the viscosity profile is generally considered as one of the parameters. The solutions of COG and CB-COG (N, 2.57%, DS, 0.342) were prepared in 2% concentration and their rheological properties were studied. It is seen (Table 7) that the viscosity of the modified sample (36,400 cps) was much higher than the native gum (250 cps). It is also observed that the viscosity increases with increase in % nitrogen and decrease in % carboxyl. However, the stability of the product decreases with increase in % nitrogen and increases with increase in percent carboxyl (Table 8).

Table 7
Rheological studies of *Cassia occidentalis* seed gum

Apparent viscosity (cps) 25±1 °C; 2% solution							
Unmodified gum			Modified gum (N=2.57%)				
Shear rate (s^{-1})	3 h	24 h	Shear rate (s^{-1})	3 h	24 h	48 h	72 h
3.5	250.0	Degradation	2.5	36,400	38,200	32,700	4200
6.8	212.0		5.0	26,600	28,300	24,600	3750
17.0	175.0		12.5	15,960	16,680	15,220	3080
34.0	162.5		25.0	9,950	9990	9600	2500

Table 8
Effect of nitrogen and carboxyl groups on viscosity and stability

Apparent viscosity (cps) 25±1 °C; 2% solution; shear rate 2.5 s^{-1}			
Time (h)	N=0.98%, –COOH=4.675%	N=1.8%, –COOH=2.052%	N=2.57%, –COOH=1.624%
3	13,400	31,200	36,400
24	15,700	33,600	38,200
48	17,700	34,600	32,700
96	20,000	33,000	4200
120	20,000	31,500	Degradation
168	20,100	30,900	Degradation

5. Conclusion

Carbamoylethylation of *C. occidentalis* seed gum was carried out with acrylamide in the presence of alkali as a catalyst. The optimum reaction conditions for carbamoylethylation of COG were obtained by using [COG], 0.03 mol as AGU; [NaOH], 0.125 mol; [AA], 0.070 mol; gum–liquor ratio, 1:50; temperature 30 °C; and duration 180 min. The paste viscosity of carbamoylethylated *C. occidentalis* seed gum shows it as pseudo-plastic and hence non-Newtonian, and increases manifold (∼145 times) vis-à-vis native *C. occidentalis* seed gum.

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