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Carboxymethylation of cashew tree exudate polysaccharide

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Abstract

Cashew tree gum was carboxymethylated in aqueous alkaline medium using monochloroacetic acid (MCA) as etherifying agent. The effects of reaction parameters such as alkali concentration, MCA/cashew gum (CG) ratio and temperature on reaction yield and degree of substitution (DS) were investigated. Products were characterized by ^{13}C nuclear magnetic resonance, infrared Fourier transform spectroscopy, gel permeation chromatography and flow capillary viscosity. Carboxymethyl gum samples with DSs between 0.10 and 2.21 were prepared. The reaction yield was in the range of 31–70%. The best MCA efficiency towards production of carboxymethylated macromolecules was obtained in 5.5 M sodium hydroxide concentration, 55 °C, 3 h reaction time and 1:1 MCA/CG molar ratio. Degradation of the chain length of the starting material during carboxymethylation occurred with intensity dependent on reaction conditions.

Keywords: Anacardium occidentale; Cashew gum; Carboxymethylation; Polymer modification; Derivative

1. Introduction

Many polysaccharide derivatives have been prepared by carboxymethylation reactions using substances such as cellulose (Torul & Arslan, 2003), starch (Kooijman, Ganzeveld, Manurung, & Heeres, 2003), chitin (Kurita, 2001), gellan (Miyamoto, Tsuji, Nakamura, Tokita, & Komai, 1996), pullulan (Picton, Mocanu, Mihai, Carpov, & Muller, 1995), scleroglucan (de Nooy, Rori, Masci, Dentini, & Crescenzi, 2000) and inulin (Verraest, Peters, Kuzee, Batelaan, & Van Bekkum, 1995) as starting materials. The derivative obtained is usually a polyelectrolyte that can be applied in a wide variety of fields, e.g. in the chemical, food, pharmaceutical and cosmetic industries. Other advantages of carboxymethylation reactions are the ease of processing, the low cost of the chemicals and the non-toxicity of the products (Verraest et al.).

The reaction is based on the Williamson synthesis, whereby the polysaccharide alkoxide is reacted with monochloroacetic acid (MCA) (Ege, 1989) and the primary and secondary alcohol groups are substituted by carboxyl groups. The reaction occurs in two steps as shown in Fig. 1.

The dependence of the reaction yields and the degree of substitution (DS) (the amount of carboxymethyl groups inserted in the polymer per ring unit) on reagents concentration, reaction time, solvent and temperature has been studied for carboxymethylation reaction of several polysaccharides (Barai, Singhal, & Kulkarni, 1997; Bhattacharyya, Singhal, & Kulkarni, 1995; Kooijman et al., 2003; Tijsen, Kolk, Stamhuis, & Beenakers, 2001; Tijsen, Scherpenkate, Stamhuis, & Beenackers, 1999; Verraest et al., 1995; Ywata, Narui, Takahashi, & Shibata, 1985).

The most relevant carboxymethyl derivative is carboxymethylcellulose (CMC), with widespread uses as anti-redeposition agent in detergents, in the oil, paper, textile and mining industries, as thickener in foods and in pharmaceutical formulations (Verraest et al., 1995). Commercial CMC samples have DS in the range of 0.4–1.4 and degree of polymerization (DP) ranging from 20 to 100.

Cashew tree gum, denoted also as cashew gum (CG), is an exudate polysaccharide from *Anacardium occidentale* trees. The composition of Brazilian polysaccharide was

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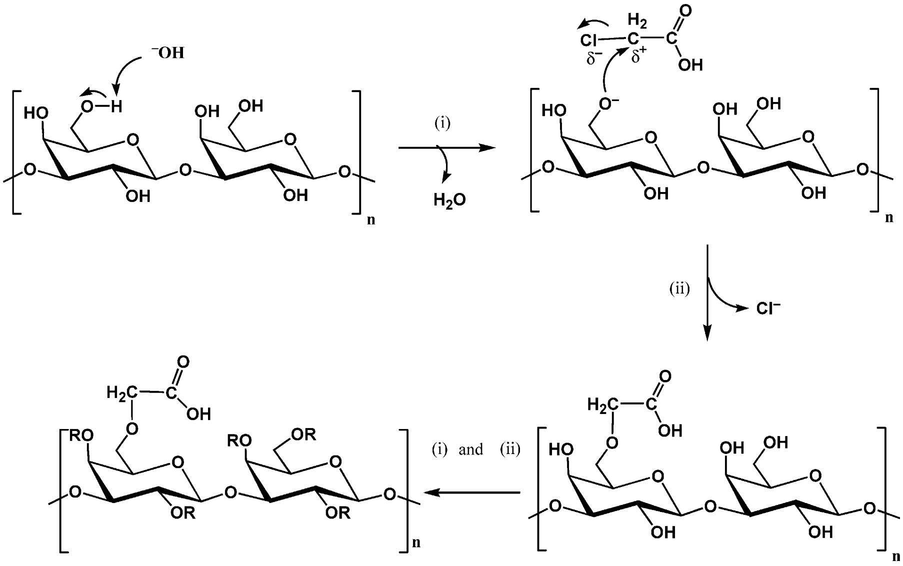

Fig. 1. Carboxymethylation reaction for polysaccharide. R could be H or CH_2COOH group, if the carboxymethylation progresses.

investigated, whereby, it is shown that CG is rich in β-D-galactose (72%) and α-D-glucose (14%), arabinose (4.6%), and also contains rhamnose (3.2%) and glucuronic acid (4.7%) (de Paula, Heatley, & Budd, 1998). It is a low viscosity gum, comparable in many aspects to arabic gum (de Paula & Rodrigues, 1995). CG structural characterization by gas–liquid chromatography and ^{13}C nuclear magnetic resonance (NMR) showed that the polysaccharide is mainly composed of three types of galactan units within the core, linked by C-1 and C-3; C-1 and C-6 and C-1, C-3 and C-6. The glucose is present as a side chain, with up to five units long (de Paula et al., 1998).

This paper reports on preparation and characterization of carboxymethylated cashew gum (CMCG) with different degrees of substitution.

2. Experimental

2.1. Origin and purification

Crude samples from CG were collected from native trees at Fortaleza, Ceará, Brazil. They were purified as a sodium salt using the method previously described (de Paula, santana & Rodrigues, 2001). Nodules free of bark were selected and dissolved in distilled water at room temperature to give a 5% (w/v) solution. The solution pH was adjusted to approximately 7.0 by addition of diluted aqueous NaOH. The clear solution was successively filtered through sintered glass and the polysaccharide precipitated with ethanol.

2.2. Carboxymethylation reaction

The purified gum (5 g, 0.0278 mol) was mixed with 5 ml of water until a homogeneous paste was formed. A 10 M NaOH solution (volume in the range of 2.7–16.0 ml) was added and the mixture was kneaded for 10 min. After that, MCA (2.62, 5.25 or 7.87 g) was mixed thoroughly with the paste. The mixture was maintained at room temperature (30 °C) or heated at 55 and 70 °C, during 3 h. The conditions applied to each sample are reported in Table 1. The system was neutralized with 1 M HCl and dialyzed against distilled water until all remaining reagents or added salt was eliminated (4–5 days). The solid carboxymethylated products were recovered by freeze-drying.

2.3. Degree of substitution (DS)

The absolute degree of substitution (DS_{abs}) was determined by potentiometric back-titration. In order to titrate all carboxymethylated samples, either in acid or salt form, 20 ml of the CMCG solutions (2.5% w/v) were passed through Amberlite IR-120H$^+$ column and freeze-dried subsequently. The acid CMCG solution (10 mg/ml) was then titrated with 0.0182 M HCl after addition of known amount of NaOH. DS_{abs} was calculated by the molar ratio of carboxymethyl acid groups to monosaccharide units,

Table 1
Carboxymethylation reaction conditions

Products (CMCG)	Volume of 10 M NaOH (ml)	MCA mass (g)	Molar ratio, CG/NaOH/MCA	Temperature (°C)
1	2.7	2.62	1:1:1	55
2	5.5	2.62	1:2:1	55
3	8.3	2.62	1:3:1	55
4	11.0	2.62	1:4:1	55
5	5.5	2.62	1:2:1	30
6	5.5	2.62	1:2:1	70
7	11.0	5.25	1:4:2	55
8	16.0	7.87	1:6:3	55

Mass of cashew gum was maintained in 5 g.

as follows:

$$DS_{abs} = \text{mol of –}CH_2COOH\text{ groups/mol of monosaccharide unit} \quad (1)$$

The molar mass of galactose (180 g/mol), as major constituent of CG (de Paula et al., 1998), was used to calculate the mols of monosaccharide units. A first value of DS was then determined. The new molar mass of average monosaccharide units was then recalculated taking into account the addition of 58 g/mol (molar mass of CH_2COOH) for each DS increase of 1.0 unity. The method of successive approximation was applied to obtain the final absolute DS.

2.4. Molar mass distribution

Molar mass distribution was determined by gel permeation chromatography (GPC) at room temperature using an Ultrahydrogel linear 7.8×300 mm column and 0.1 M $NaNO_3$ as solvent. A differential refractometer was used as detector and the elution volume was corrected to the internal marker of ethylene glycol at 11.25 ml.

2.5. Infrared spectral analysis

The Fourier transform IR spectra (FT-IR) were recorded with a Shimadzu IR spectrophotometer (model 8300) between 400 and 4000 cm^{-1}. The samples were analyzed as KBr pellet.

2.6. Nuclear magnetic resonance spectroscopy

^{13}C NMR spectra of 5% w/v solutions in D_2O at 80 °C were recorded in a Varian 500 spectrometer. Chemical shifts were given in values relative to internal acetone at 31.07 ppm. A distortionless enhancement through polarization transfer (Dept) spectrum was recorded in order to determine the multiplicity of carbon peaks; the acquisition and delay times were 1.0 s. DEPT spectrum was obtained with final ^{1}H pulse flip angle of 135° (DEPT 135). The signals in the ^{13}C NMR spectrum were assigned based on literature data for correlated compounds (de Paula et al., 1998; Glinel, Sauvage, Oulyadi, & Huguet, 2000).

2.7. Viscosity measurement

Viscosity measurements were performed in a Canon–Ubbelohde viscometer with capillary diameter of 0.5 mm and 0.1 M NaCl as solvent at 25 °C. All flow times were averages of at least five replicates.

3. Results and discussion

3.1. Effect of reaction condition on DS_{abs} and product yield

3.1.1. Effect of NaOH concentration

The effect of NaOH concentration on DS_{abs} and yield, for a given MCA/polysaccharide molar ratio and temperature is shown in Table 2 (CMCG 1–4). The DS increases with alkali concentration up to 6.2 M NaOH and then decreases sharply. Similar results have been found for starch (Bhattacharyya et al., 1995; Tijsen et al., 2001). This is explained by the occurrence of competitive reaction of sodium hydroxide with MCA, leading to less polymer degradation and higher reaction yield.

3.1.2. Simultaneous effect of monochloroacetic acid and NaOH concentration

The simultaneous effect of MCA and NaOH concentration was investigated keeping temperature and solvent constant. The results can be seen in Table 2 (CNCG 1, 4, 7 and 8 samples). MCA efficiency was calculated as follows:

$$\text{MCA efficiency} = \text{DS/MCA–CG molar ratio} \quad (2)$$

This parameter measures the extent to which the carboxymethylation is favored compared with competitive reaction, such as hydrolysis of MCA into glycolate (Verraest et al., 1995). DS and MCA efficiency increase with MCA and NaOH concentration (CMCG 1, 7 and 8 samples). Increasing MCA concentration, keeping NaOH/CG constant (CMCG 4 and 7 samples), also led to

Table 2
Efficiency of reaction conditions in the production of carboxymethylated CG materials

Products (CMCG)	Molar ratio, MCA/CG	Temperature (°C)	[NaOH] (M)	DS_{abs}	Yield (%)	MCA efficiency[a]	MCA total efficiency[b]
1	1	55	3.5	0.10	70	0.10	0.07
2	1	55	5.2	0.48	45	0.48	0.22
3	1	55	6.2	0.90	40	0.90	0.36
4	1	55	6.9	0.22	67	0.22	0.15
5	1	30	5.2	0.75	32	0.75	0.24
6	1	70	5.2	0.16	57	0.16	0.09
7	2	55	6.9	0.86	32	0.44	0.14
8	3	55	7.6	2.21	31	0.74	0.23

[a] MCA total is calculated by Eq. (2).
[b] MCA total efficiency is calculated by Eq. (3).

Table 3
Effect of monochloroacetic acid/monosaccharide unit molar ratio on DS_{abs}

Carboxymethylated polysaccharide	MCA/gum	DS_{abs}	Reference
Scleroglucan	1	0.16	de Nooy et al. (2000)
	2	0.26	
Inulin	1	0.40	Verraest et al. (1995)
	2	0.66	
Cashew gum	1	0.48	This work
	2	0.86	

an increase in DS and in MCA efficiency, but to a less extent when compared to simultaneous increase in NaOH and MCA concentration. Table 3 shows the effect of MCA/CG ratio on DS for inulin, scleroglucan and CG. By increasing twice the MCA/polysaccharide molar ratio, the DS increases about 1.7 ± 0.1 times, for all three carboxymethylated polysaccharides.

3.1.3. Effect of temperature

The effect of temperature on product yield and DS_{abs} was investigated keeping CG/NaOH/MCA ratio at 1:2:1 (CMCG 2, 5 and 7 samples). The increase of reaction temperature results in a DS_{abs} decrease (Table 2). Considering that MCA/CG ratio is equal to 1, MCA efficiency has the same numerical value of DS_{abs}. The maximal DS_{abs} and MCA efficiency is obtained at 30 °C, although with a smaller product yield. Carboxymethylation of inulin shows a DS_{abs} decrease (0.73–0.65) with increase in temperature from 55 to 95 °C (Verraest et al., 1995). The authors also found that the MCA efficiency for inulin decreases from 0.43 to 0.38, when temperature was increased in the same range. For starch samples the optimal temperature depends on the starch source. For Amaranth starch, the better temperature is 30 °C. For corn and potato polysaccharides, higher temperatures are required (65 and 40 °C, respectively) (Bhattacharyya et al., 1995; Tijsen et al., 2001).

3.1.4. Correlation between DS_{abs} and product yield

The plot of product yield versus DS_{abs} is shown in Fig. 2. For low DS (up to ~0.7), the product yield seems to be inversely proportional to DS. The yield is certainly a function of the amount of material lost during dialysis step. As more drastic reaction conditions (higher temperature and NaOH and MCA concentration) were applied, more degradation occurred and larger amount of low molecular weight material were released. As a consequence, smaller amount of carboxymethylated polymeric product remained to be recovered by freeze-drying. The curve profile also suggests that the yield becomes independent of DS for values higher than 0.7. In the present reaction conditions, the limit value of yield tends to 33%.

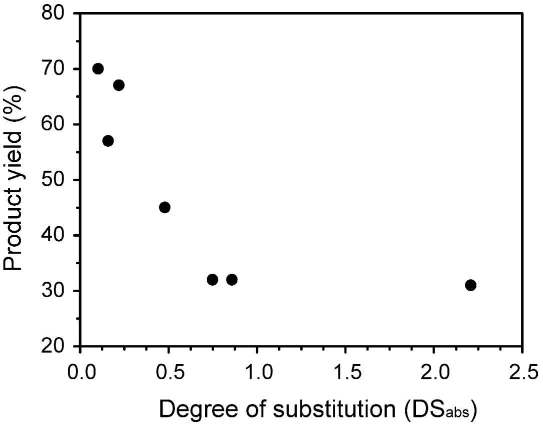

Fig. 2. Correlation between product yield and DS_{abs}.

To measure the extent to which carboxymethylated macromolecular products are favored, the yield have to be considered. A parameter defined here as MCA total efficiency is calculated using the following formula:

$$\text{MCA total efficiency} = (\text{MCA efficiency}) \times \text{yield}/100. \quad (3)$$

Table 2 summarizes the data for all samples. The CMCG-3 reaction, carried out under the following condition: MCA/CG = 1, [NaOH] = 6.2 M, at 55 °C, resulted in the highest efficiency of MCA in the promotion of polymeric carboxymethylated CG. This indicates that a small amount of by-product (glycolate and degraded low molecular weight) was formed.

3.2. Derivative characterization

3.2.1. FT-IR spectroscopy analysis

FT-IR spectra of CG and carboxymethylated samples are shown in Fig. 3. The presence of band around 1730 cm^{-1} in spectrum A is characteristic of C=O stretching vibration of carboxylic acid of glucuronic acid of the starting polysaccharide (de Paula et al., 1998). A substantial increase in the intensity of this band was observed after carboxymethylation (spectra B–D), caused by the introduction of new carboxylic groups per macromolecule (Fig. 1). As more groups are introduced (higher DS) more intense the absorbance at 1730 cm^{-1} becomes. The intensity increase in the 1730 cm^{-1} band and not in the ~1580 cm^{-1} band (assigned to symmetric stretching vibration of COO^-) indicates that the CMCG samples are predominantly in acid form.

Miyamoto et al. (1996) suggested an alternative method to quantify the carboxymethyl group for CM-gellan based on IR spectra. This DS, denominated as relative DS (DS_{rel}), could be calculated, in the present case, by the following formula

$$DS_{rel} = A_{1730}/A_{2920} - B \quad (4)$$

where A_{1730} and A_{2920} are, respectively, the absorbances of C=O (1730 cm^{-1}) and C–H (2920 cm^{-1}) stretching vibrations of the carboxymethylated products. B is a numerical constant correspondent to A_{1730}/A_{2920} ratio of

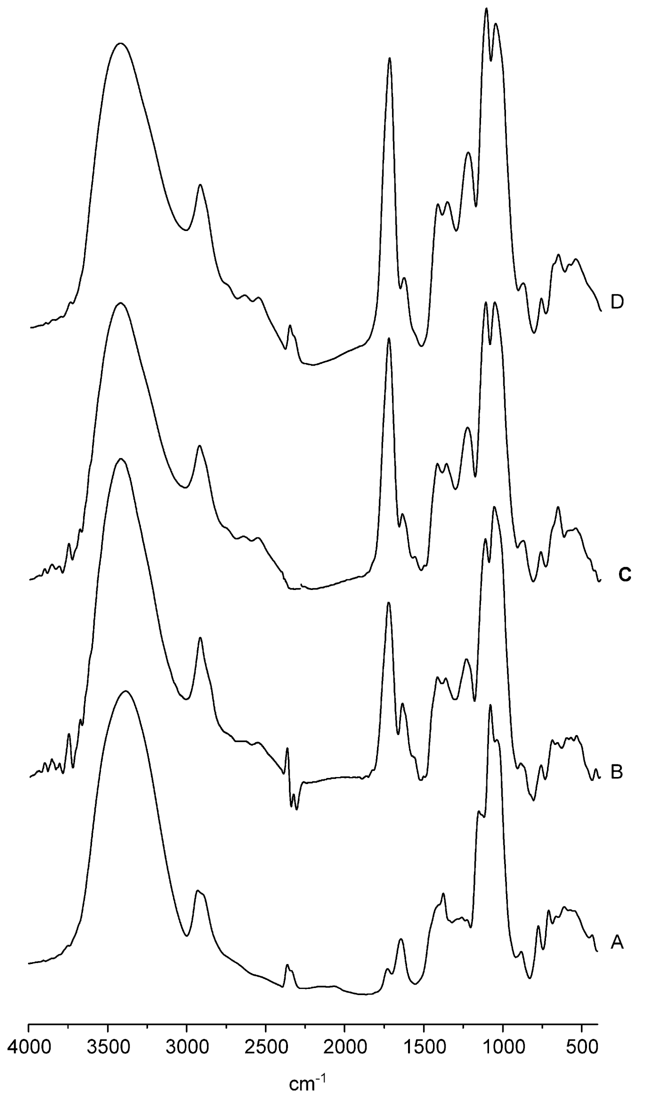

Fig. 3. FT-IR spectra of cashew nut gum (A) and carboxymethylated samples: CMCG-2, DS = 0.48; (B) CMCG-7, DS = 0.86 (C); and CMCG-8, DS = 2.21 (D).

the starting CG material. B value of 0.23 was determined from Fig. 3A.

DS_{rel} for CM-CG samples obtained in different reaction conditions are calculated from Eq. (4). The relationship between calculated DS_{rel}, determined by FT-IR, and DS_{abs}, determined by potentiometric titration, is depicted in Fig. 4. At low DS, a linear correlation was observed ($DS_{abs} = 0.12DS_{rel}$). Deviations from linearity with high carboxylate content may be attributed to high absorbance and/or ion-pair formation. The ion-pair, formed in function of higher charge density of the polyelectrolyte (i.e. the –COOH content), increases with carboxymethylation (Miyamoto et al., 1996). Similar explanation was given to justify non-linear behavior of DS versus 1250/2920 cm^{-1} ratio in high sulfated carrageenan (Rochas, Lahaye, & Yaphe, 1986). The linearity was observed up to DS 0.2 for CM-gellan (Miyamoto et al.) and 0.5 for carrageenan (Rochas et al.). CMCG exhibit the same behavior of CM-gellan, showing linearity up to 0.2.

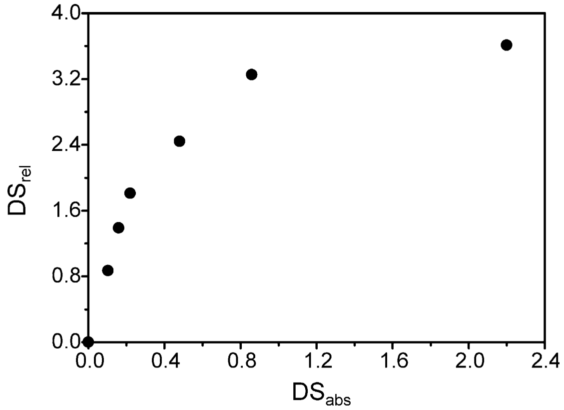

Fig. 4. Correlation between relative and absolute degree of substitution for CMCG samples.

3.2.2. ^{13}C nuclear magnetic resonance (NMR)

^{13}C Nuclear magnetic resonance (NMR) spectra of CG and two CMCG samples are shown in Fig. 5. CG spectrum is similar to that obtained by de Paula et al. (1998). In both spectra, the peak due to C=O of glucuronic acid was not detected by NMR. However, its presence is undoubtedly detected by FT-IR spectroscopy and determined by potentiometric titration as 4.7%. ^{13}C NMR spectrum of CMCG-2 (DS_{abs} 0.48) and CMCG-8 (DS_{abs} 2.21) shows peak due to carbonyl groups at 178 ppm, as evidence of carboxymethylation reaction. It can be observed that an elimination of monosaccharide units as the number of signals of anomeric carbons (98–110 ppm) has decreased after reaction. An increase in peak intensities between 85 and 70 ppm, attributed to carbons from C-2 to C-5 of monosaccharide residue, may be due to CH_2 groups of –CH_2COOH of MCA and also due to the shift of primary carbons (C-6) from region around 60 to 66–69 ppm, after substitution of –CH_2COOH group on primary carbon.

In DEPT 135 experiment, the methylene carbons show opposite amplitude to methyl and methyne carbons. DEPT spectra of CG and CMCG-2 and CMCG-8 (Fig. 6) shows a increase of CH_2 peak intensities, in the regions of 69–70 ppm, attributed to galactose primary carbon substituted in C-6 position by the carboxymethyl groups with DS increases.

3.3. Molar mass distribution

Using GPC, no absolute value for the average molar mass of CMCG products could be determined (Verraest et al., 1995). The introduction of carboxymethyl groups affects the polymer charge and its hydrodynamic volume. And so the direct comparison of polysaccharide molecules with different charge density is not precise. The effect of NaOH concentration on molar mass distribution was shown in Fig. 7. The GPC curve of all

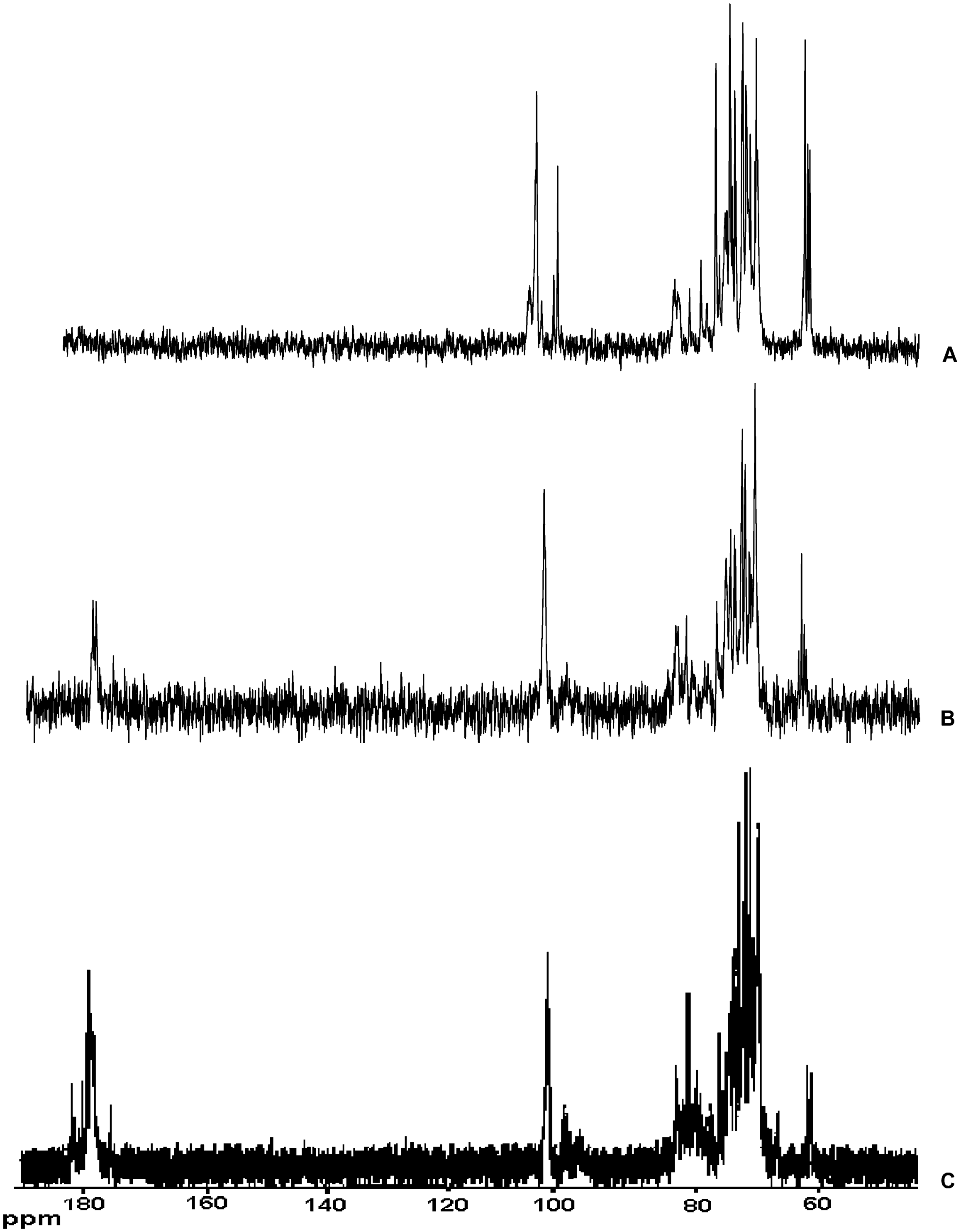

Fig. 5. ^{13}C NMR spectra in D_2O of cashew gum (A) and carboxymethylated samples: CMCG-2, DS = 0.48 (B) and CMCG-8, DS = 2.21 (C).

carboxymethylated samples (curves b–d) was shifted to higher elution volume than CG (curve a). If no chain length degradation occurs, the CM-polysaccharide is expected to elute at a lower volume than starting polymer. This is due to chain stiffening and extent due to the electrostatic repulsion of carboxymethyl groups (Verraest et al., 1995). Another contribution is the higher molar mass of CM product, due to introduction of $-CH_2COOH$/monosaccharide unit at each 1.0 unit increase in DS. It can be concluded that chain scission occurred during the carboxymethylation of CG under the reaction conditions 3, 4 and 7.

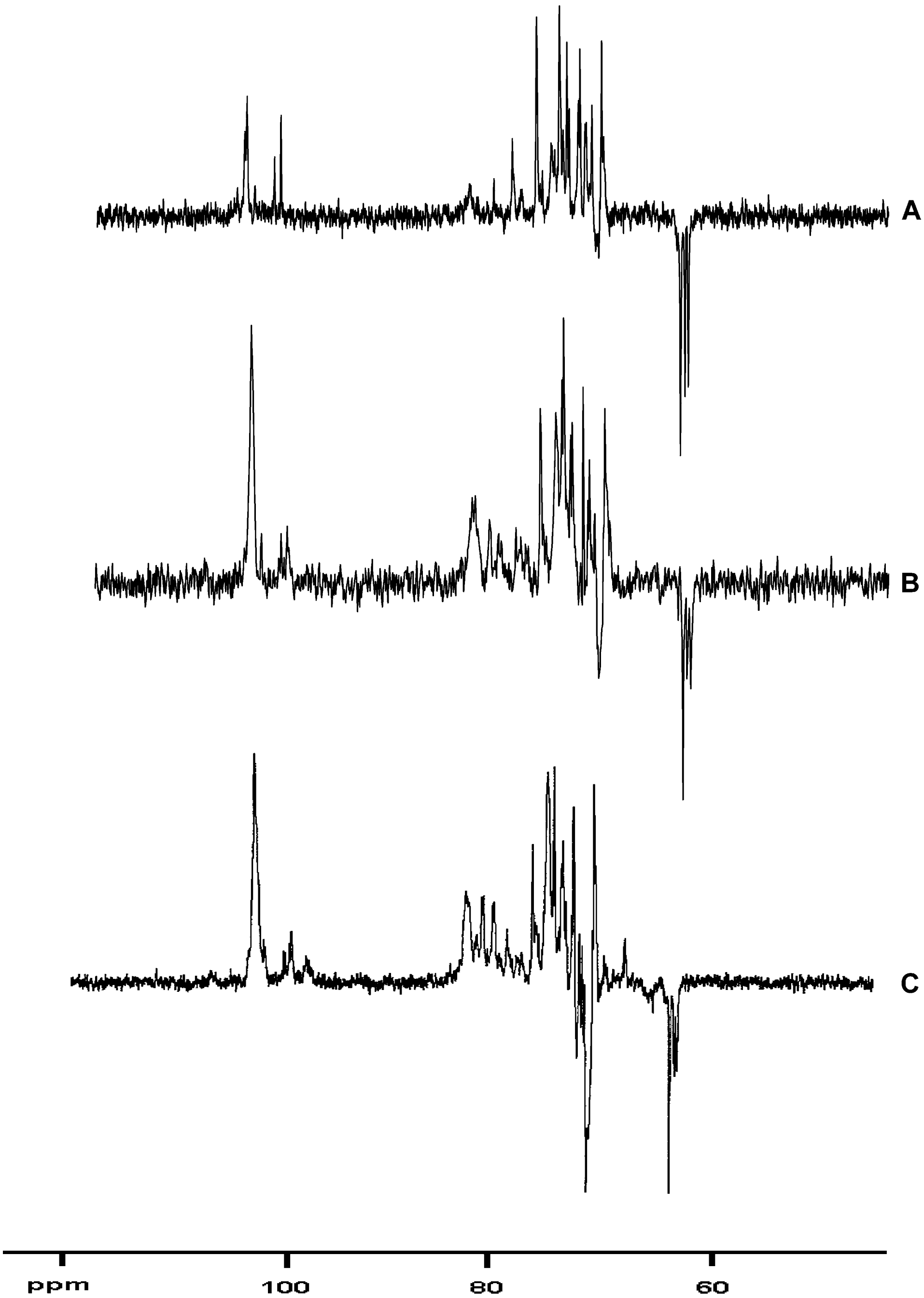

Fig. 6. DEPT spectra of cashew nut gum (A) and carboxymethylated samples: CMCG-2, DS = 0.48 (B) and CMCG-8, DS = 2.21 (C) in D_2O.

The new peak at elution volume 9.9 ml appears when NaOH concentration is increased from 6.2 (curve b) to 7.0 M (curve c). The bimodal distribution in curve c indicates that two different carboxymethylated products are being formed. The difference could be in the DS or in the average molar mass. The effect of MCA/CG ratio, at same NaOH concentration could also be verified in Fig. 7. The curve d (MCA/CG = 2) is slightly shifted to lower elution volume than curve c (MCA/CG = 1), probably due to difference in DS, amounting to 0.86 and 0.22, respectively. The higher intensity of peak at 9.8 ml elution volume (curve d), compared to peak at 9.9 ml (curve c), indicates that the eluted macromolecules are probably products of chain scission processes, promoted by high NaOH concentration (7.0 M) and intensified by high MCA/CG ratio.

The influence of reaction temperature on molar mass distribution is given in Fig. 8. No effect of temperature was detected in the range of 30–55 °C, curves a and b, respectively. The appearance of a shoulder at volume elution of 10.2 ml in curve c means that the degradation of chain length is more effective at 70 °C.

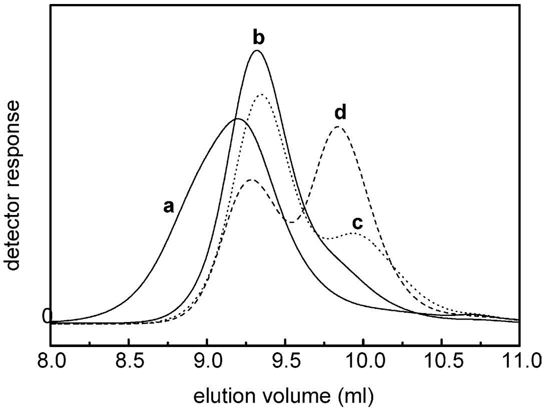

Fig. 7. Elution profiles from GPC (RI detection) of cashew gum (a) and carboxymethylated samples: CMCG-3, DS = 0.90, [NaOH] = 6.2 M and MCA/CG = 1 (b); CMCG-4, DS = 0.86, [NaOH] = 6.9 and MCA/CG = 1 (c); and CMCG-7, DS = 0.86, [NaOH] = 6.9 and MCA/CG = 2 (d). Temperature = 55 °C.

3.4. Intrinsic viscosity

CMCG samples of DS 0.10, 0.86 and 2.21 (CMCG 1, 7 and 8 samples) have intrinsic viscosity of 6.7, 5.5 and 5.2 ml/g, respectively. Intrinsic viscosities of three CMCG samples are smaller than the value for starting gum (8.4 ml/g). The decrease in intrinsic viscosity with increasing DS was observed also for carboxymethyl scleroglucan (de Nooy et al., 2000). The authors attributed this effect to polysaccharide degradation. The same explanation could be given in the case of CMCG. To calculate the viscosity-average molar mass, M_v, a Mark–Houwink equation proposed by de Paula et al. (1998) for CG could be used. The formula is

$$[\eta] = 5.2 \times 10^{-2} M_v^{0.42} \tag{5}$$

where $[\eta]$ is the intrinsic viscosity in ml/g in 0.1 M NaCl at 25 °C.

Considering the low degree of structural modification, the comparison between carboxymethylated sample and CG was made for CMCG-1 sample, which has the lowest DS (0.10). The calculated M_v value for CG is 1.8×10^5 g/mol, in good agreement with 1.9×10^5 g/mol, determined for Brazilian gum (de Paula et al., 1998). After carboxymethylation, the molar mass of the gum (CMCG-1) was reduced to 1.1×10^5 g/mol. The observed chain degradation, which occurs even under less drastic reaction condition (lower NaOH and MCA concentrations and low temperature), clearly indicates that degradation occurs in all CMCG samples (CMCG-1 to CMCG-8). This result corroborates the assumption of chain scission from GPC experiments.

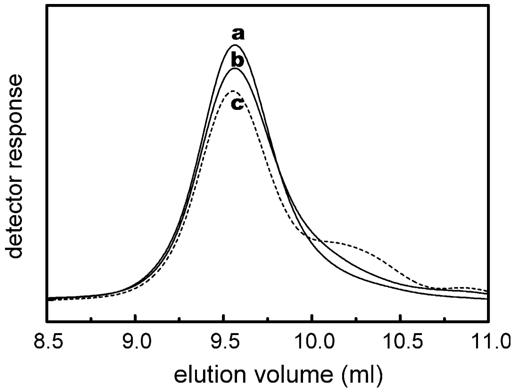

Fig. 8. Elution profiles from GPC (RI detection) of carboxymethylated samples: CMCG-5, DS = 0.75, temperature = 30 °C (a); CMCG-2, DS = 0.48, temperature = 55 °C (b); and CMCG-6, DS = 0.16, temperature = 70 °C (c). MCA/CG = 1. [NaOH] = 5.2 M.

4. Conclusions

CG was carboxymethylated with MCA in alkaline aqueous medium to produce CMCG in the acid form. The DS varied in the range from 0.10 to 2.21 depending on NaOH concentration, MCA/CG ratio and temperature. The DS increases with alkali concentration up to 6.2 M NaOH and then decreases sharply. At higher temperature and MCA concentration the DS was increased. The product yield seems to be inversely proportional to DS, for low DS (up to ~0.7). Degradation of chain length was verified in all reaction conditions studied. Taking into account the MCA efficiency towards production of carboxymethylated macromolecules from CG, the best resulting sample material is CMCG-3 (DS = 0.90), obtained with MCA/CG molar ratio equal to 1, temperature at 55 °C and 6.2 M NaOH. This reaction condition promotes, also, a less chain degradation.

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