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Synthesis and characterization of new unsaturated esters of Gellan Gum

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Abstract

A series of Gellan Gum unsaturated esters by addition of free radical polymerizable groups, which can be polymerized under mild conditions to design biodegradable three-dimensional networks having hydrogels properties, were synthesized by esterification under various conditions. Different degrees of functionalization were obtained either homogeneously in water with acrylic acid or heterogeneously with acryloyl chloride and maleic anhydride in organic solvents. Acrylate and maleate groups determination was carried out by ATR-FTIR and ^{1}H liquid NMR techniques. The degree of substitution as determined by ^{1}H NMR could be controlled by varying the chemical nature of functionalization agent, reaction time and temperature. Maleic anhydride presents a higher reactivity as compared to acrylic acid and acryloyl chloride.

Keywords: Polysaccharide esterification; Acrylate and maleate Gellan Gum; NMR spectroscopy

1. Introduction

Controlled drug delivery systems have been extensively investigated in recent years. The needs of biocompatible and biodegradable carriers for this purpose lead to the use of natural polymers as materials for drug delivery supports. Lately, microbial polysaccharides such as Gellan Gum have been used extensively in the food, cosmetics, pharmaceutical and biomedical industries (Chandrasekaran, Millane, & Arnott, 1988; Nishinari, Kremer, & Lagaly, 1999).

Gellan Gum (GG) is an anionic deacetylated exocellular polysaccharide secreted by *Pseudomonas elodea* with a tetrasaccharide repeating unit of one α-L-rhamnose (Rhap), one β-D-glucuronic acid (GlcpA) and two β-D-glucoses (Glcp) (Fig. 1) as proposed by Jansson et al. (Jansson, Lindberg, & Sandford, 1983).

Aqueous solutions of GG form gels in the presence of cations (Crescenzi, Dentini, & Coviello, 1990), the mechanism of gelation involving the formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water (Milas & Rinaudo, 1996; Ogawa, Matsuzawa, & Iwahashi, 2002; Takahashi et al., 2004). In pharmaceutical and biomedical industries, its gel forming properties in the presence of multivalent cations have been exploited for controlled release of drugs (Balasubramaniam, Thilek Kumar, Pandit, & Kant, 2004; Mukai-Correa, Prata, Alvim, & Grosso, 2004; Ottoboni, Ronald, & Stanley, 2000). The mechanical stability of the ionically crosslinked gel is provided by the combination of multivalent, generally divalent, cations, such as calcium (Crescenzi, Dentini,

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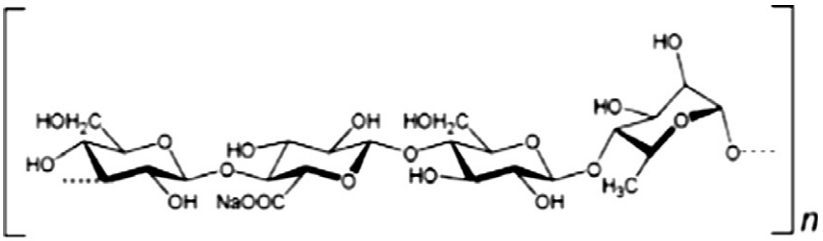


→3)-β-D-Glcp-(1→4)-β-D-GlcpA-(1→4)-β-D-Glcp-(1→4)-α-L-Rhap-(1→

Fig. 1. Repeating unit of deacetylated Gellan Gum sodium salt. (according to Ref. Bianchi et al., 2000).

& Dea, 1987b). For the applications as drug release systems in the physiological environment, extracellular concentrations of monovalent cations (such as sodium ions) exceed the concentration of divalent ones (such as calcium). Therefore, the ionic gels tend to lose their mechanic stability over the long term due to diffusion, leading to exchange of divalent cations for monovalent ones in the physiological fluid (LeRoux, Guilak, & Setton, 1999). To avoid these problems, chemical modifications of GG is proposed preferring covalent rather than ionic crosslinking. Thus, polysaccharide backbone carrying unsaturated substituents, which provides multiple centers for grafting/crosslinking, could result in stronger gels and more tightly controlled mechanical and degradation behavior than those ionically crosslinked gels (Bergera, Reista, Mayera, Feltb, & Peppas, 2004). If the connecting grafted chains are linked to the polysaccharide backbone, it can be assumed that those of acrylic type are hydrolysis resistant in physiological environment in contrast to the maleate semi-esters. Nevertheless maleate semi-esters could have the advantage for the design of hydrogels which crosslink density decreases with time and thus becoming more readily bioresorbable. In this context, the aim of this work was to design efficient methods for modification of GG in order to obtain derivatives carrying reactive double bonds for subsequent grafting and crosslinking to form hydrogels. Chemical modification of GG involves the reaction of hydroxyl groups of its backbone. Several methods will be employed for introducing double bonds into GG by esterification with acrylic acid or with reactive derivatives of unsaturated organic acids (acryloyl chloride, maleic anhydride) under homogeneous and heterogeneous conditions.

Similar modification reactions were used for the functionalization of several polysaccharides such as starch (Stawski & Jantas, 2003), cellulose (Bianchi, Bonazza, Marsano, & Russo, 2000), dextran (Kim, Won, & Chu, 1999), xanthan gum (Hamcerencu, Khoukh, Desbrieres, Popa, & Riess, 2007) and disaccharides (Liu & Fan, 2003). Mention should also be made of Gellan benzyl esters studied by Crescenzi et al. (Crescenzi et al., 1995; Dentini et al., 1999) and of double bond functionalized Gellans obtained by gamma irradiation in the presence of glycidyl acrylate (Paparella & Park, 1996) for the preparation of hydrogels. Gellan based hydrogels, with covalent crosslinks, could further be obtained by a self-crosslinking reaction of the polysaccharide in the presence of carbodiimide derivatives (Dentini et al., 2001).

As an extension to our work carried out on xanthan (Hamcerencu et al., 2007) for the preparation of drug release hydrogels, it was of interest to examine the possibilities of preparing double bond functionalized Gellan by esterification with acrylic and maleic derivatives. Among exopolysaccharides, Xanthan and GG are remarkable due to their stiffness, which limits the accessibility of hydroxyl functional groups and hence their ability to chemical modification. Gellan Gum stiffness is due first to the conformation of the macromolecular chains and second to the involvement of many hydroxyl groups in intramolecular (leading to stability of this conformation) or intermolecular hydrogen bonds. As a consequence the access to these hydroxyl groups is limited and some of these functional groups cannot be involved in chemical reactions. Based on this consideration, the reactivity of these polymers was compared by introducing unsaturated substituents on the backbone using different processes. As GG is less rigid than xanthan it may be assumed that the degree of substitution will be larger with the former.

2. Materials and methods

2.1. Materials

Gellan Gum (GG) was supplied by CPKelko KELKOGEL® ($M_w = 2{,}351{,}000$ g/mol as determined at 25 °C by automatic continuous mixing (ACM) technique Grassl & Reed, 2002). Acryloyl chloride (AC) (⩾96.0% (HPLC)) and isopropyl alcohol (IPA) were obtained from Fluka Chemie AG, Buchs, Switzerland. Maleic anhydride (MA) (99%), acrylic acid (AA) (anhydrous, 99%), triethylamine (TEA) (99.5%), *N*,*N*-dimethyl formamide (DMF) (99%), acetone (99.5%), *N*′-[3-(dimethylaminopropyl)]-*N*-ethylcarbodiimide hydrochloride (DEC) (98+%), sodium acetate (99+%) (NaA) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

2.2. Synthesis of unsaturated esters of GG

Several working methods have been applied for GG modification. Two types of esters were prepared, in particular the acrylate and maleate of GG. The syntheses

were performed in homogeneous (aqueous) and heterogeneous (acetone; DMF) medium, in the presence of different esterification agents. The esterification of GG proceeds in accordance with Scheme 1.

2.2.1. Synthesis of acrylate GG (A-GG) under homogeneous condition (Scheme 1a)

Acrylate GG derivatives were synthesized according to the previously published procedure (Hamcerencu et al., 2007). Briefly, GG (1 g, 1.49×10^{-3} monomoles considered as repeat unit) was dissolved in double distilled water (100 mL) under magnetic stirring. To the obtained solution, the corresponding volume of AA (10 moles/monomole GG, defined as the average repeating unit) was added dropwise. Finally, the DEC (1.1 moles/mole AA) was added as an activator and stirred for determined times. Reactions were carried out for 24 h at 2–4 and 70 °C, respectively. After the precipitation with acetone, the reaction product was rinsed alternatively four times with IPA/water mixtures (75–90% IPA, v/v) and finally with IPA and then, dried at room temperature (RT) followed by drying in a vacuum oven (0.1 atm, RT, 24 h).

2.2.2. Synthesis of acrylate GG (A-GG) under heterogeneous conditions (Scheme 1b)

Esterification of GG with AC was performed in organic medium according to our previous paper (Hamcerencu et al., 2007). Gellan Gum (GG) (1 g, 1.49×10^{-3} mol) was suspended in DMF (15 mL) and stirred at RT, in order to swell the polysaccharide particles. Then, the corresponding volume of TEA (1.1 moles/mole AC) was added, TEA acting as a captor of hydrochloric acid. Finally, AC (10 moles/monomole GG) previously dissolved in DMF (10 mL) was added dropwise to the system, the reaction proceeding at 2–4 °C for 6 and 24 h, respectively. The obtained product was separated by filtration, dissolved in distilled water and precipitated again in IPA. The purification, precipitation and drying processes were the same as those described above.

2.2.3. Synthesis of maleate GG (MA–GG) under heterogeneous conditions (Scheme 1c)

Two working methods have been applied differing by the chemical nature of reaction medium either acetone or DMF. MA–GG esters were synthesized according to our previously published procedure (Hamcerencu et al., 2007) with some modifications. The syntheses were performed corresponding to MA/GG molar ratio of 1 and 10 moles/monomole, respectively. The purification, precipitation and drying processes were the same as those described above.

The modified GG samples were designated as *aGb-c*, *a* being the reaction time in hours, *b* the esterification agent and *c* the temperature of reaction.

2.3. FT-IR spectroscopy

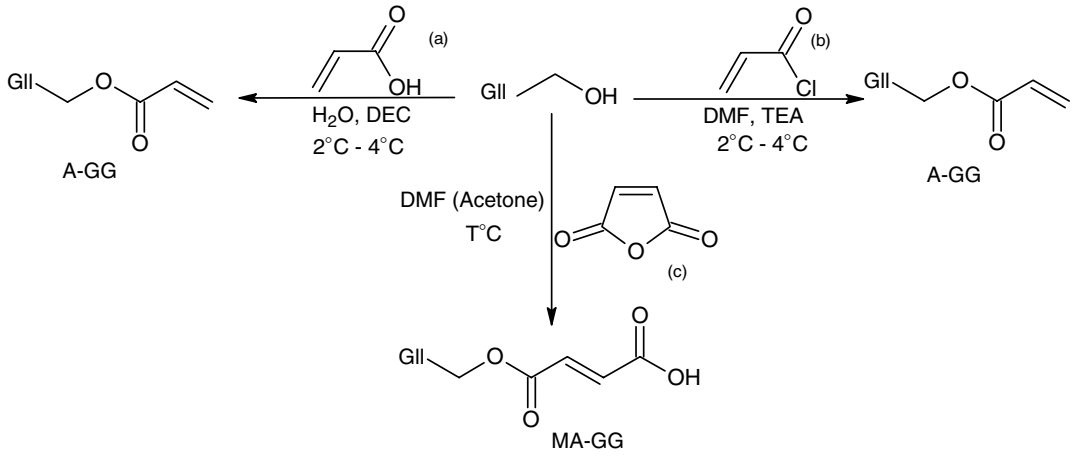
FTIR-ATR spectra were recorded with a Bruker IFS 66/S spectrometer with a "Golden Gate" unit (IIa type diamond crystal). For each sample, 100 scans were recorded between 4000 and 600 cm^{-1}, with a resolution of 4 cm^{-1}.

2.4. ^1H NMR spectroscopy

NMR spectra were recorded with a Bruker Avance 400 spectrometer operating at 400.13 MHz equipped with a 5 mm indirect detection gradient probe and a variable temperature system. ^1H NMR spectra were obtained in D_2O solution having a polymer concentration of 3 g/L at temperatures from 25 up to 85 °C. Chemical shifts were referred to NaA as internal standard, which is at 1.8 ppm. An interpulse time (D_1) of 2 up to 60 s was used.

The degree of substitution (DS, the fraction of modified hydroxyl groups per average repeating unit (RU)) of the unsaturated GG derivatives was calculated as

$$\left(\frac{I_{DB}}{\frac{I_{CH_3}}{3} \cdot n_{H_{DB}}}\right) \Big/ n_{OH}, \quad (1)$$



Scheme 1. Strategies for modification of Gellan Gum: (a) acrylic acid, (b) acryloyl chloride, (c) maleic anhydride.

where I_{DB} is the average integral of the protons at the double bound, I_{CH3} the integral of the methyl proton of the Rhap unit at δ 1.23 ppm, $n_{H_{DB}}$ the number of protons at the double bond and n_{OH} is the number of GG hydroxyl groups (GG comprises 10 hydroxyl groups/RU). DS of the unsaturated GG derivatives was assigned as 100% when all of the ten hydroxyl groups of GG RU are substituted. The NMR DS values of modified GG, as determined by ^{1}H NMR, are given within an error of ±5%.

3. Results and discussion

3.1. Determination of the optimal conditions for the quantitative evaluation of the double bonds content by ^{1}H NMR analysis

The properties of GG depend on their degree of acetylation, which is one of most important factors for specifying GG. It is therefore critical to control the structure and purity of used unmodified GG. Subsequently, optimal conditions for ^{1}H spectra recording for the quantitative evaluation of the double bonds content were determined. Samples were prepared as indicated in *Experimental* part. The influence of temperature and interpulse time (D_1) on the spectra resolution was examined.

Gellan Gum (GG) presents, like Xanthan, a conformational transition depending upon the temperature, the ionic strength and polymer concentration (Majeti & Kumar, 2000; Milas, Shi, & Rinaudo, 1990). This transition was studied quite extensively by spectroscopic techniques such as specific optical rotation, circular dichroism, NMR, etc., or by measurement of the hydrodynamic and rheological properties (Brownsey et al., 1984; Crescenzi and Dentini, 1987; Crescenzi et al., 1987a, 1986, 1987b; Grasdalen and Smidsrod, 1987; Kwon et al., 1987; Veeder & Kang). The ordered and rigid conformation produces strong dipolar interactions between proton or carbon nuclei and an increase in viscosity (leading to a decrease in mobility), causing such severe broadening that the NMR spectra cannot be detected under the high-resolution conditions at room temperature. The increase of temperature leads to a greater mobility which is evidenced on the spectrum by an improved spectra resolution (Hamcerencu et al., 2007; Milas & Rinaudo, 1996). Moreover, at room temperature, the presence of the D_2O signal in the H-1 signals region does not allow a quantitative analysis of such spectra.

Quantitative yields were calculated from the integral of ^{1}H signals with respect to the methyl groups of the Rhap unit groups by reference to NaA (3×10^{-3} M) used as external standard. Spectra obtained at 85 °C, above the GG transition temperature, (Chandrasekaran, Millane, Arnot, & Atkins, 1988; Chandrasekaran & Thailambal, 1990) show the presence of four anomer protons corresponding to Rhap (δ 5.06 ppm), GlcpA (δ 4.64 ppm) and Glcp (δ 4.4–4.46 ppm). The integrals of these peaks are in agreement with the theoretical values corresponding to envisaged protons, i.e. 1:1:2 and 3, respectively, attributed to the methyl proton of the Rhap unit at δ 1.23 ppm (Fig. 2).

Therefore, by choosing 85 °C as temperature where the spectra had the best resolution, the influence of interpulse time, D_1, between two scans was studied. ^{1}H liquid NMR spectra for different values of D_1 (2, 10, 20, 30, 40, 50 and 60 s, respectively) were recorded. For a 40 s and higher pulse intervals the percentage of the methyl groups of the Rhap unit tends to stabilize to 100% (100% refers to the methyl groups of Rhap present in the solution). Consequently from these results the optimal conditions for

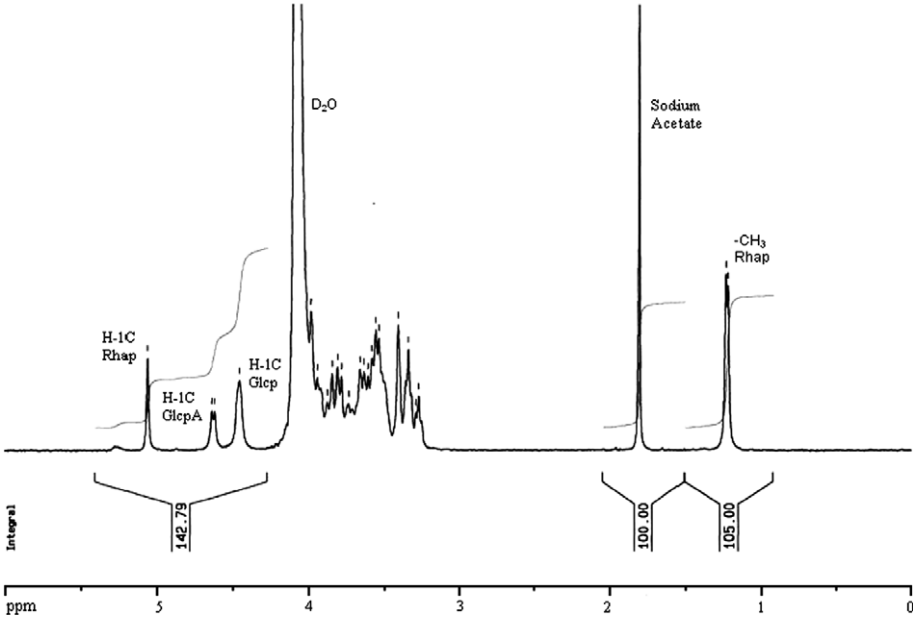


Fig. 2. ^{1}H liquid NMR spectrum of GG performed in D_2O (c_{pol} = 3 g/L, T = 85 °C; D_1 = 50 s).

recording ^{1}H spectra were chosen as following: 85 °C, interpulse time: 50 s, internal standard: NaA (3×10^{-3} M) and polysaccharide solution concentration around 3 g/L.

3.2. Synthesis of unsaturated GG precursors (A-GG, respectively, MA–GG)

Among microbial polysaccharides, GG is a high molecular weight polymer presenting an important rigidity of backbone. Because of this, it is much less accessible to potential reagents than cellulose. In order to prepare derivatives with well defined structures and thereby to develop advanced materials, it is crucial to perform the reactions in a well controlled manner.

Gellan Gum (GG) modification by esterification with unsaturated organic acids (acrylic acid) or their reactive derivatives (acryloyl chloride, maleic anhydride) was performed using different procedures. The esterification proceeds predominantly to the primary hydroxyl groups (C-6), although participation of some secondary ones is not wholly excluded. Preliminary trials of synthesizing GG acrylates and maleates led to the conclusion that a considerable excess of esterification agent is necessary for attaining acceptable transformation degrees. For experiments a functionalization agent/GG molar ratio of 10/1 was generally used (GG presents 10 hydroxyl groups/RU) corresponding to an equimolecular ratio of reactive sites. The syntheses were performed either under homogeneous

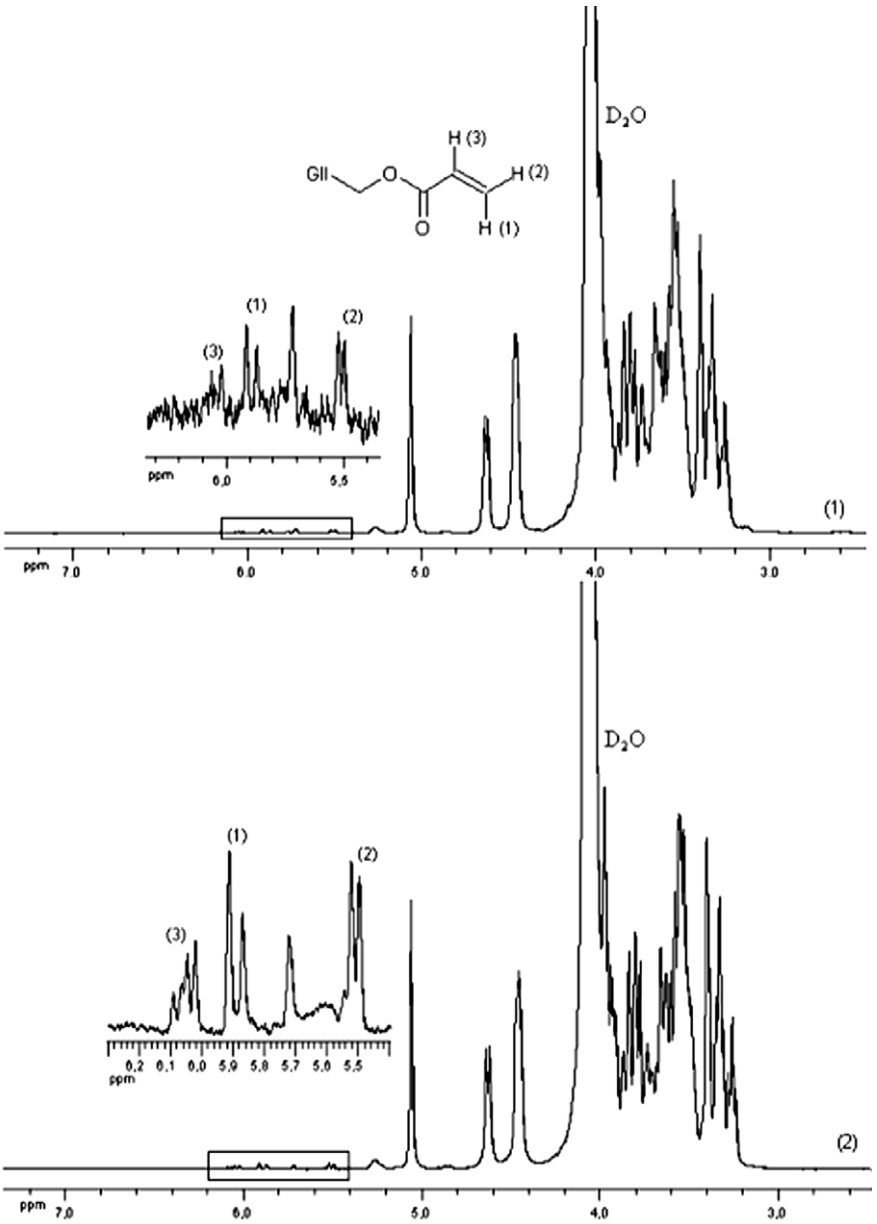


Fig. 3. 400 MHz partial ^{1}H NMR spectra of 24GAA-2 (DS = 0.5%) (1) and 24GAC (DS = 0.7%) (2) esterification products of GG (reaction conditions: 2–4 °C; 24 h), recorded in D_2O at 85 °C, $D_1 = 50$ s, c_{pol} = around 3 g/L.

conditions for the esterification with AA (GG aqueous solution, 1% wt/v) or under heterogeneous conditions for the modification with AC and MA the dispersion medium being DMF and acetone.

3.2.1. Synthesis of GG acrylates (A-GG)

Gellan Gum (GG) acrylates were synthesized by esterification reaction with AA and AC, respectively.

3.2.1.1. Esterification of GG with AA. Because GG is a water soluble polysaccharide, the chemical modification was at first performed under homogeneous conditions by using AA as esterification agent in the presence of DEC as activator, according to the reaction *a* presented in Scheme 1. The influence of time (6 and 24 h, respectively) and reaction temperature (2–4 and 70 °C, respectively) on DS of polysaccharide was studied. The molar ratios of AA/GG and DEC/AA were fixed at 10/1 and 1.1/1, respectively.

The synthesis of AA modified GG was evidenced by FTIR (not shown) and ^{1}H NMR spectroscopy. The new carbonyl peaks at 1725 cm^{-1} (24GAA-2) and 1721 cm^{-1} (24GAA-70) which are characteristic of unsaturated ester absorption bands are present. Additional bands corresponding to double bonds were also observed at 1640, 925 cm^{-1} (24GAA-2) and 1643, 921 cm^{-1} (24GAA-70), respectively.

The evidence of AA substitution on GG was also observed by ^{1}H liquid NMR as shown in Fig. 3.

In comparison to the spectrum of GG (Fig. 2) there are weakly developed peaks within the range of δ 5.5, 5.9 and 6.1 ppm, which can be ascribed to the protons of vinyl groups (C*H*=CH_2). It should be mentioned that the integration and the normalization of the double bond peaks in the acrylate or maleate segments and the methyl group protons peaks of the GG (1.23 ppm) are sufficiently precise to calculate the DS of acrylate or maleate GG, as shown in Tables 1 and 2.

The DS was low at reactions time below 24 h, and after 24 h of reaction the obtained DS is around one double bond/20 RU of GG.

Table 1
Influence of reaction conditions (time, temperature and chemical nature of esterification agent, respectively) on DS for the synthesis of acrylate substituted Gellan Gum

Esterification agent	Reaction time (h)	Reaction temperature (°C)	DS[a] (%)	Double bond per repeating unit
AA[b]	24	2–4	0.4	0.04
		70	0.5	0.05
AC[c]	6	2–4	0.2	0.02
	24		0.7	0.07

[a] As determined by ^{1}H NMR spectroscopy (in D_2O, 85 °C, $D_1 = 50$ s, c_{pol} = around 3 g/L).
[b] Reaction performed in homogeneous conditions.
[c] Reaction performed in heterogeneous conditions (DMF).

Table 2
Effect of reaction parameters (time, temperature, nature of dispersion phase and MA/GG molar ratio) on DS for the synthesis of maleate substituted Gellan Gum

MA/GG (mole/monomole)	Reaction time (h)	Reaction temperature (°C)	DS[a] (%)	Double bond per repeating unit
DMF as dispersion medium				
10/1	6	25	0.6	0.06
	24		3.7	0.37
	6	50	2.6	0.26
	24		11.5	1.15
	6	60	3.8	0.38
	24		12.6	1.26
	6	70	5.6	0.56
	24		15.4	1.54
Acetone as dispersion medium				
1/1	24	50	0.48	0.05
10/1			1.6	0.16

[a] As determined by ^{1}H NMR spectroscopy (in D_2O, 85 °C, $D_1 = 50$ s, c_{pol} = around 3 g/L).

Table 1 shows the effect of reaction temperature on substitution of GG hydroxyl groups by AA. At temperatures above 40 °C, GG in aqueous solution presents a transition from an ordered conformation to a disordered one (Bosco, Miertus, Dentini, & Segre, 2000; Milas & Rinaudo, 1996). In this disordered form, the access of esterification agent to hydroxyl groups of polysaccharide is expected to be facilitated, thus a high DS should be attained. However, as shown in Table 1, the temperature was not a favourable factor considering the esterification reaction efficiency.

This behaviour might be attributed, on the one hand to the fact that the reactivity of DEC is higher at low temperature (Nenitescu, 1966), and on the other hand that the reaction rate increases with the increasing of temperature (Laidler, 1997); therefore, a competition between these opposite effects may be considered. The consequence is, under our conditions, the absence of a temperature effect on the efficiency of the reaction within the studied temperature range.

3.2.1.2. Modification of GG by AC. Due to low DS obtained by using AA under homogeneous condition, other esterification agents were investigated such as AC and MA. However, as these functionalization agents hydrolyse easily, the reactions have to be carried out in organic solvents under heterogeneous conditions.

The modification of GG with AC is carried out in DMF as dispersion medium in the presence of TEA as an HCl acceptor, according to the reaction *b* presented in Scheme 1. After AC was added, the reaction was performed different reaction times (2, 6 and 24 h, respectively) at constant temperature (2–4 °C). The molar ratio of AC/GG and TEA/AC were controlled at 10/1 and 1.1/1, respectively.

The successful incorporation of AC on GG was checked with FTIR-ATR and ^{1}H NMR. FTIR spectra of the region around 2000–600 cm^{-1}, where the modifications were more

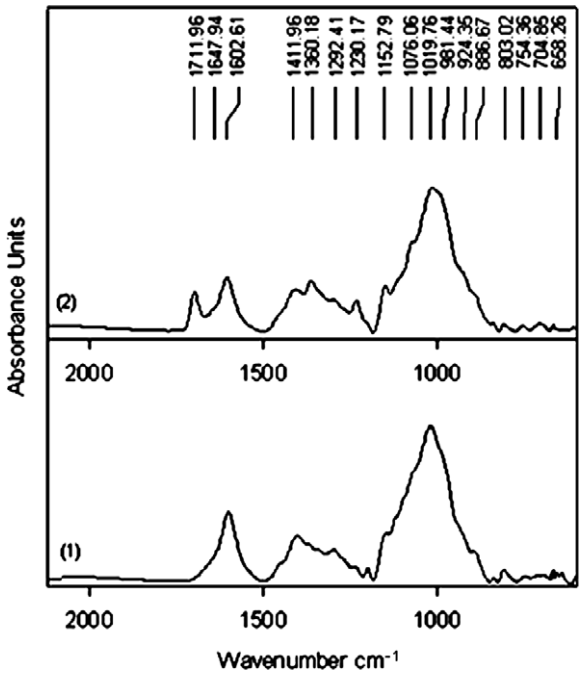


Fig. 4. Absorbance FTIR spectra of GG (1) and of GG esterified with AC (2) (reaction conditions: $T = 2$–4 °C; $t_r = 24$ h).

significant are presented in Fig. 4, for the GG precursor and its AC esterification derivative.

The characteristic unsaturated ester absorption band ($\nu_{C=O}$ 1710–1724 cm^{-1}) appeared. FTIR peaks at 1639–1652 cm^{-1} ($\nu_{C=C}$) and 921–925 cm^{-1}($\nu_{C=C-H}$) were found in the modified GG. These IR shoulders emerged from the stretch of C*H*═CH_2 double bond of the acrylate substituant.

^{1}H NMR spectra of AC modified GG derivatives showed several distinctive peaks in the double bond region (5.5–6.2 ppm) which were not present in the precursor GG (Fig. 2). Three distinctive peaks at δ 5.5, 5.9 and 6.1 ppm were attributed to the three protons linked to the double bond (C*H*═CH_2), as indicated on structural formula in Fig. 3b.

From Table 1 it appears that DS increases with reaction time. During the first 2 h an insignificant substitution was evidenced. After 6 h, acrylate groups substituted 0.2% of the total hydroxyl groups of GG. A DS of 0.7% was achieved after 24 h.

These results confirm that GG can be esterified with leading to unsaturated products. Apparently, the DS obtained after esterification by AA and AC are not very high but it should be reminded that these unsaturated esters of GG will be used for the synthesis of grafted/crosslinked structures having hydrogel properties. Theoretically, each double bond introduced by esterification represents a potential grafting/crosslinking centre. As it is known, the crosslink density affects the swelling degree, mechanical properties, drug inclusion and delivery characteristics of these crosslinked materials, as well. Thus, the mentioned properties can be controlled by obtaining GG with different degree of modification which will facilitate the preparation of both loose and tight networks.

3.2.2. Synthesis of maleate GG derivatives

GG maleates were synthesised by esterification with MA according to the Scheme 1 (reaction *c*). The reaction occurs in heterogeneous conditions. The hydroxyl groups of GG could perform nucleophilic attack on the carbonyl group of MA to form an ester linkage. This process also leads to a ring opening of the anhydride group of MA with generation of a carboxylic group.

Two methods have been applied for GG modification. They differ in the chemical nature of reaction medium, in DMF and acetone, respectively. The reaction in DMF was performed at different reaction times (6 and 24 h) and temperatures (25, 50, 60, 70 °C) with an MA/GG molar ratio of 10/1. For the reaction in acetone, the influence of MA/GG molar ratio (1/1; 10/1) was studied at constant temperature (50 °C) and reaction time (24 h).

3.2.2.1. GG esterification in N,N-dimethyl formamide. The successful reaction of GG with MA was demonstrated using FTIR (Fig. 5) and ^{1}H NMR spectroscopies.

The presence of carbonyl typical bands around ($\nu_{C=O}$ 1713–1721 cm^{-1}) in modified GG demonstrated the presence of ester and of the carboxylic acid groups. The magnitude of this carbonyl group band increased significantly with the increasing of DS. Each attached MA segment contains an ester and a carboxylic acid. Therefore, FTIR spectra should exhibit two adjacent split carbonyl peaks, but only one peak is observed and this could be explained by one carbonyl peak that was strong enough to merge with the other carbonyl peak. Double bond stretches were also observed at 1628–1643 cm^{-1} ($\nu_{C=C}$) and 921–925 cm^{-1} ($\nu_{C=C-H}$).

The evidence of MA substitution on GG was visualized by ^{1}H liquid NMR as well (Fig. 6).

Distinctive peaks in the double bond region (δ 6–7 ppm) are present.

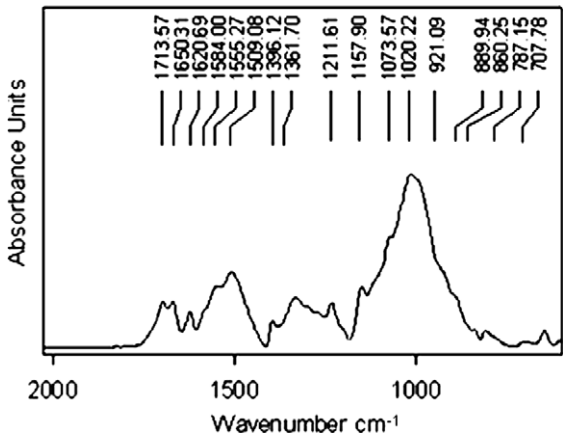


Fig. 5. Absorbance FTIR spectra of GG modified with MA: 24GMA-70.

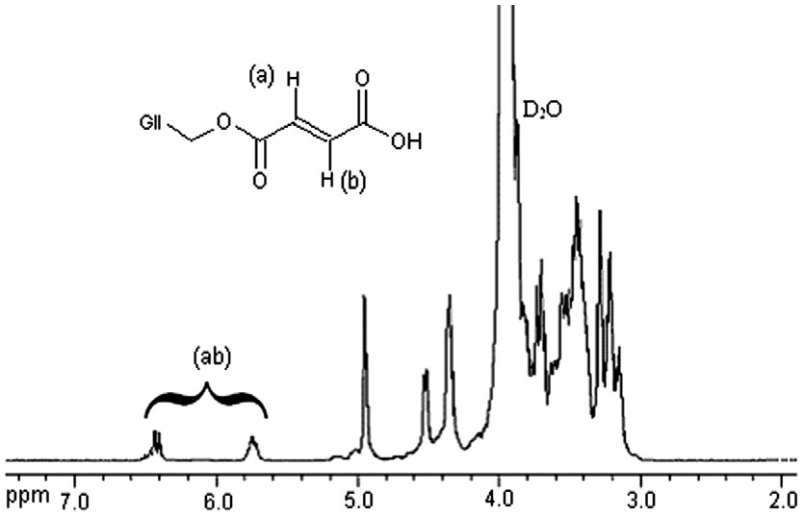


Fig. 6. Partial ^{1}H NMR liquid spectrum of 24GMA-70 synthesized in DMF (DS = 15.4%) recorded in D_2O, (c_{pol} = 3 g/L, 85 °C; D_1 = 50 s).

The effect of reaction time and temperature on the DS is presented in Table 2 which shows that the DS gradually increases with these two parameters. At 70 °C a DS of 15.4% was achieved. The DS values for 24 h reaction products obtained at higher temperatures (60–70 °C) are quite close.

Under identical reaction conditions, it could be confirmed that there is definitely an increase in the DS values for Gellan as compared to Xanthan [17]. This behavior can be attributed to the fact that Gellan is less rigid than Xanthan.

3.2.2.2. GG esterification in acetone. The GG precursor presents the characteristic peaks attributed to the two protons attached to the double bond (δ 5.5–6.5 ppm) in ^{1}H NMR spectrum. Carbonyl peaks at $\nu_{C=O}$ 1724 cm^{-1} and at $\nu_{C=O}$ 1726 cm^{-1} in unsaturated GG derivatives (data not shown) were evident due to the new introduced ester linkages characteristic of a carboxylate group. Double bond stretches are also present at 1635 and 1643 cm^{-1} ($\nu_{C=C}$).]

For the reaction products obtained in acetone the spectroscopic IR and NMR characteristics were identical to those obtained for the reactions carried out in DMF. From the results given in Table 2 it appears that the DS value increases as expected with the MA/GG ratio. Furthermore one can notice that DMF is more efficient than acetone when the reactions are carried out under the same conditions (24 h at 50 °C). This can be attributed to the fact that DMF is a better swelling agent of GG than acetone.

4. Conclusions

This work presents the preparation and the characterization of new reactive GG based macromonomers through esterification with an unsaturated organic acid (acrylic acid) or with reactive acid derivatives (acryloyl chloride, maleic anhydride) under homogeneous and heterogeneous conditions. The influence of some of the reaction parameters (temperature, reaction duration, chemical nature of esterification agent, molar ratio), on the degree of substitution were studied. Increase of the temperature and reaction duration leads to an increase of the degree of substitution. Maleic anhydride evidences a higher reactivity than the acryloyl chloride and acrylic acid, especially when the reactions of GG esterification are carried out in DMF. Preliminary copolymerization experiments performed with acrylic monomers such as *N*-isopropylacrylamide and unsaturated GG esters lead to thermo- and pH stimulable hydrogels with adjustable crosslink density. Early results revealed that modified GG/NIPAm based hydrogels are potential carriers in the design of controlled drug delivery systems, fully systematic study of this type of products being reported in a further article.

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