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Polymer 48 (2007) 1921–1929
polymer
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New unsaturated derivatives of Xanthan gum: Synthesis and characterization

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Received 22 August 2006; received in revised form 24 January 2007; accepted 24 January 2007
Available online 30 January 2007

Abstract

Unsaturated Xanthan derivatives, which can be used for the development of biodegradable three-dimensional networks having hydrogel properties, were prepared by esterification under various conditions. Xanthan gum derivatives with different degrees of substitution were obtained by reaction with an unsaturated organic acid (acrylic acid) or with acid reactive derivatives (acryloyl chloride, maleic anhydride). The presence of acrylate and maleate groups in the modified structure of Xanthan gum was detected by ATR–FTIR, ^{1}H liquid NMR and ^{1}H HRMAS NMR spectroscopies. The degree of substitution as determined by ^{1}H NMR could be controlled by varying the chemical nature of functionalisation agent, reaction time and temperature.

The results proved that this polysaccharide can be modified by esterification with acids or unsaturated acid derivatives for further synthesis of hydrogels. Maleic anhydride presents a higher reactivity as compared to acrylic acid and acryloyl chloride.

Keywords: Polysaccharide esterification; Acrylate or maleate Xanthan; NMR spectroscopy

1. Introduction

During the second half of the 20th century, new and useful polysaccharides of medical and commercial interest have been discovered which can be obtained by microbial fermentations. The usefulness of water-soluble carbohydrate polymers relies on their wide range of functional properties: the ability to modify the properties of aqueous environments that is their capacity to thicken, emulsify, stabilize, flocculate, swell and suspend or to form gels, films and membranes. Another very important aspect is that polysaccharides obtained from natural, renewable sources are both biocompatible and biodegradable.

Among these polysaccharides, Xanthan gum (Xan), a microbial biopolymer produced by the *Xanthomonas campestris* seems potentially useful for such purpose. When Xan was commercialized (around 1964), it was known that it consisted of D-glucose, D-mannose, D-glucuronic acid acetal linked pyruvic acid and *O*-acetyl [1]; later, the currently accepted structure was identified (Fig. 1) [2,3].

The acetate and pyruvate contents appear to vary due to the culture conditions and the post-fermentation processing as well. Some properties of the Xan gum depend on those contents. Therefore the knowledge of the acetate and pyruvate degrees of substitution is important [4–6].

Xan is known for being biocompatible allowing its use in various medical applications such as topical ocular application [7–9], implantation [10–14] or controlled release devices [11,15]. Moreover, Xan is considered as biodegradable [16–18] and as bioadhesive, which increases retention at the site of application [19–22]; it also promotes wound-healing effects [23]. In medical and pharmaceutical applications, Xan is also used as a component in hydrogels [24–27].

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doi:10.1016/j.polymer.2007.01.048

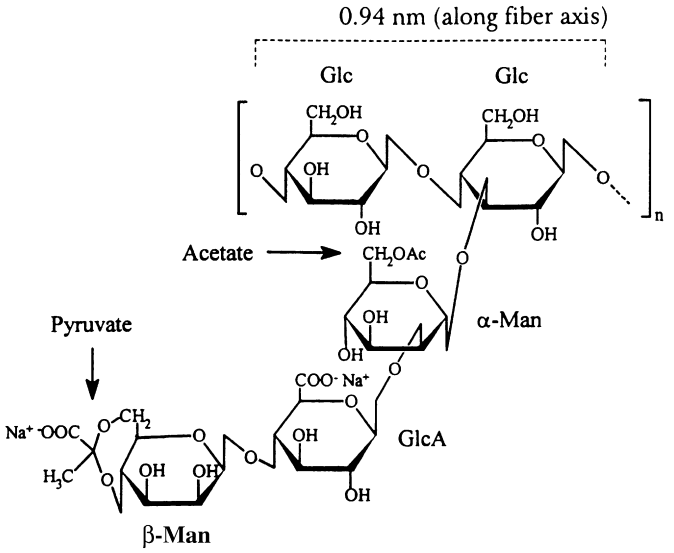


Fig. 1. Chemical structure of Xanthan (according to Refs. [2,3]).

Xan can form both physical and chemical gels. Typical physical gels formed simply from polysaccharide concentrated solutions are unstable; physical gelation process of Xan being usually reversible (sol–gel transition). By heating or dilution, Xan physical gels can easily be solubilized in aqueous solution [28], so they are not interesting as drug controlled release carrier. As a rule, chemical gels (crosslinked by covalent bonds) can be obtained either by employing crosslinking agents such as epichlorohydrin, or by grafting/crosslinking with acrylic monomers. Epichlorohydrin, as many other crosslinking agents, is known to be cytotoxic [29]. Moreover, short crosslinking bridges introduced by such a crosslinking agent between Xan backbones would lead to a final product having a high rigidity in dry state. The increase of crosslinking bridges' length can be accomplished by grafting/crosslinking of polysaccharide with monomers, among which, acrylics occupy a significant position [30,31]. For this purpose, the polysaccharide should be at first modified with unsaturated binding functionalities. There are several advantages of this method, including introduction of longer and/or exhibiting temperature or pH sensitive behavior bridges between polysaccharide chains. A less rigid structure, and an easier control of network density, as a function of number of double bonds introduced on the polysaccharide backbone, may thus be attained [32].

The objective of this work is to prepare Xan derivatives carrying reactive double bonds for subsequent grafting and crosslinking to form hydrogels. Several methods will be employed for introducing double bonds into Xan by esterification with acrylic acid or with reactive derivatives of unsaturated organic acids (acryloyl chloride, maleic anhydride) under homogeneous and heterogeneous conditions.

To the best of our knowledge these esterification reactions have not yet been carried out on Xan. Similar modification reactions were used for the functionalisation of starch [33], cellulose and chondroitin sulphate [34,35], dextran [36] and disaccharides [37]. The preparation of Xan unsaturated derivatives with a high degree of substitution might be not as easy, mainly due to the insolubility of Xan in a suitable medium and the conformational structure at supermolecular level. That is why very few papers are regarding chemical modification of Xan, the present work is an attempt to bring a contribution in this direction.

2. Materials and methods

2.1. Materials

Xanthan gum (Xan, from *X. campestris*) was obtained from BioChemika (degree of substitution per side chain of 0.73 and 0.75 for acetate and pyruvate groups, respectively, as determined by proton NMR). Acryloyl chloride (AC) (≥96.0% (HPLC)) was 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 (ACT) (99.5%), *N*′-[3-(dimethylaminopropyl)]-*N*-ethylcarbodiimide hydrochloride (DEC) (98+%), sodium acetate (99+%) (NaA) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Ethanol (EA) was from Acros Chimica, Geel Belgium.

2.2. Synthesis of unsaturated esters of Xan

Several working methods have been applied for Xan modification. Two types of esters were prepared, in particular the acrylate and maleate Xan. For removing humidity, the Xan powder was previously dried, at 50 °C, for 12 h. The syntheses were performed in homogeneous (H_2O) and heterogeneous (ACT; DMF) medium, in the presence of different esterification agents.

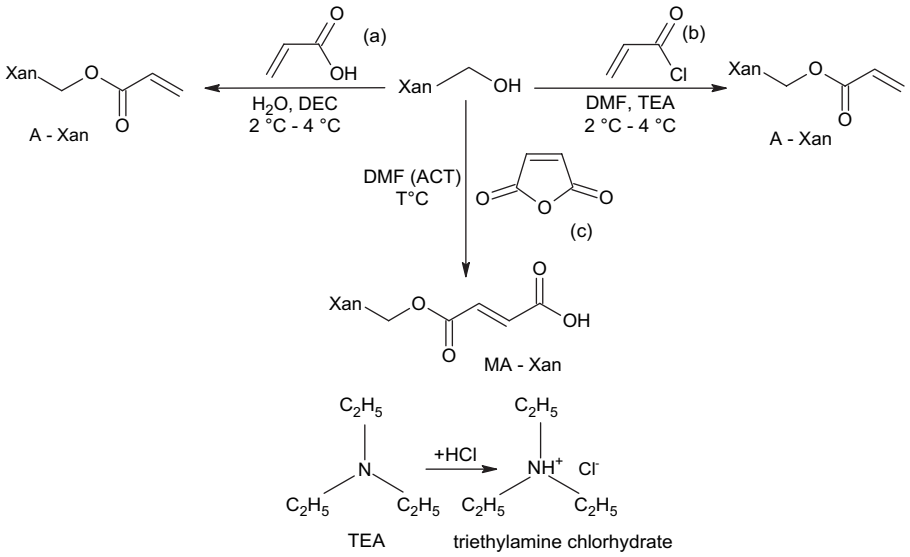
The esterification of Xan proceeds in accordance with the Scheme 1.

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

Xan (1 g) was dissolved in 100 mL water under magnetic stirring for 12 h. The system was either cooled down using an ice bath to a temperature of 2–4 °C or heated up to a temperature of 70 °C under stirring. When the temperature was reached, the corresponding volume of AA (11 mol/monomole Xan, defined as the average repeating unit) was added dropwise to the system. Finally, the DEC (1.1 mol/mole AA) was added as an activator and stirred for 6–24 h. Then, the obtained viscous solution was precipitated with ACT (150 mL), filtered, rinsed alternatively 4 times with EA/water mixtures (75–90% EA, v/v) (150 mL) and finally with EA (200 mL). The final product was dried at room temperature (RT) followed by drying in a vacuum oven, under a pressure of 0.1 atm, at ambient temperature for 24 h.

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

Esterification of Xan with AC has to be carried out in organic medium. Xan (1 g) suspended in 15 mL DMF was



Scheme 1. Strategies for modification of Xanthan: (a) acrylic acid; (b) acryloyl chloride; (c) maleic anhydride.

stirred for 12 h at ambient temperature in order to swell the polysaccharide particles. One hour before the beginning of the reaction, the corresponding volume of TEA (1.1 mol/mole AC) was added. TEA acts as a captor of hydrochloric acid evolved during reaction. The system was cooled down using an ice bath to a temperature of 2–4 °C and maintained at this temperature for 30 min. In parallel, AC (11 mol/monomole Xan) had been dissolved in other 10 mL DMF and then added dropwise to the system, the reaction proceeding under magnetic stirring for 2, 6 and 24 h, respectively, at 2–4 °C. The reaction was stopped by adding 150 mL ACT. The formed precipitate was separated by filtration, dissolved in distilled water (150 mL) under stirring for 6 h (to hydrolyze the traces of AC and triethylamine chlorhydrate) and precipitated again in EA (200 mL). After 2 h, the obtained precipitate is filtered. This purification cycle is repeated 4 times. After the last purification procedure the formed precipitate is washed with EA/water mixtures (75–90% EA, v/v) (150 mL) and finally with EA (200 mL). The product was dried at RT, normal pressure and then under vacuum for 24 h at ambient temperature. It was finally ground by hand in a mortar and subjected to analysis.

2.2.3. Synthesis of maleate Xan (MA-Xan) under heterogeneous conditions (Scheme 1(c))

Two working methods have been applied for such Xan modification. The difference is the chemical nature of reaction medium either acetone or DMF.

Two syntheses were performed corresponding to MA/Xan molar ratio of 1 and 11 mol/monomole. MA was dissolved in 25 mL ACT and 1 g Xan was added. The system was heated up to a temperature of 50 °C under magnetic stirring. The reaction was conducted at 50 °C for 24 h and the product was filtered. The obtained modified powder was suspended in 150 mL ACT (to remove the unreacted MA) then, the suspension was filtered and washed alternatively several times with EA/water mixtures. After the last washing in EA, the product was dried initially at RT, normal pressure, and then in vacuum at RT, for 24 h.

Xan (1 g) was introduced into 15 mL DMF. The resulting suspension was stirred for 12 h at ambient temperature, so that the polysaccharide particles swelled well in solvent. In parallel, MA (11 mol/monomole Xan) was dissolved in other 10 mL DMF and then added dropwise to the system, the reaction proceeding under magnetic stirring. A range of temperatures from 25 to 70 °C were examined to determine the optimal reaction conditions. The reaction was generally conducted for 6–24 h. After adding 150 mL ACT, the formed precipitate was separated by filtration, dissolved in water (150 mL) under stirring for 6 h and then precipitated in EA (400 mL). After 2 h, this precipitate was filtered. The purification procedure was repeated 4 times. After the last purification procedure the precipitate was washed with EA/water mixtures (75–90% EA, v/v) (150 mL) and finally with EA (200 mL). The product was dried at RT, first at normal pressure and then under a pressure of 0.1 atm in a vacuum oven, for 24 h.

The modified Xan samples were designated as *a*Xb*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 spectrophotometer 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. ^{1}H NMR spectra were obtained in D_2O solution having a polymer concentration around 3 g L^{-1} at temperatures between 35 and 85 °C. Chemical shifts were referred to sodium acetate (NaA) as external standard, which is at 1.8 ppm. Acetate (n_A) and pyruvate (n_P) content was determined by reference to NaA (3×10^{-3} M) [38].

The degree of substitution (DS, the fraction of modified hydroxyl groups per average repeating unit (RU)) was calculated as detailed in Supplementary data part. The acrylate, respectively maleate content values were assigned as 100% when all of the 11 hydroxyl groups of Xan RU are substituted. The DS values of native and modified Xan, as determined by ^{1}H NMR, are given within an error of ±5%.

2.5. ^{1}H HRMAS NMR spectroscopy

^{1}H HRMAS NMR spectra were recorded in D_2O at room temperature. Samples, each of approximately 3 mg, were soaked in D_2O and were packed into separate 4 mm diameter ZrO_2 rotors with spherical inserts and Kel-F caps. D_2O was added to the polymer directly inside the rotor. All ^{1}H HRMAS NMR spectra were recorded on a Bruker Avance instrument operating at 400.13 MHz for ^{1}H and using a 4 mm HRMAS ^{1}H/^{13}C probe head. The samples were spun at 6 KHz. A typical ^{1}H HRMAS NMR spectrum consisted of 1024 transients using 16K data points over an 18 ppm spectral width. The 90° pulse duration was 13.5 μs. Sodium 2,2-dimethylsilapentane sulfonate (DSS) was used as internal reference to calibrate the spectra in aqueous solution. An interpulse time of 2 s up to 30 s was used. Each spectrum was phased and linearly baseline corrected.

3. Results and discussion

3.1. Characterization by ^{1}H NMR analysis

NMR spectra of Xan have been studied by several groups [39–41]. It is important to characterize the acetate and pyruvate content because some properties of Xan depend on this; moreover the culture conditions and the fermentation processing appear to influence it.

In this study, proton NMR spectroscopy was used both for acetate and pyruvate contents' determination and literature data confirmation. Subsequently, optimal conditions for ^{1}H spectra recording for the quantitative evaluation of the double bonds' content were determined. Samples were prepared as detailed in Section 2.4 part and ^{1}H NMR spectra were recorded at temperatures from 35 up to 85 °C. The influence of temperature on spectra resolution and the effect of interpulse time (D_1) on the acetate and pyruvate degrees of substitution calculation were examined. The use of an external standard seems the best way to determine the substituents' yields by taking in consideration only the peaks of acetate and pyruvate groups apart from the rest of the spectrum (see Supplementary data).

To the best of our knowledge studies concerning the variation of n_A and n_P with D_1 have not yet been published.

Xan presents a conformational transition depending upon the temperature, ionic strength, pH and polymer concentration [42–44]. 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 a severe broadening that the NMR spectra cannot be detected under the high-resolution conditions at room temperature. As reported by Morris et al. [44], Xan spectra present a better resolution at high temperature (85 °C) when Xan is entirely in a random conformation (as confirmed by our study, too, and shown in Fig. 2) leading to a precise determination of the pyruvate and acetate contents.

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 temperature of 85 °C and for 30 s and higher pulse intervals, n_A and n_P values tend to stabilize in the range which is consistent with the reported values [5,6]. Leaving from these results, the optimal conditions for recording ^{1}H spectra were chosen as following: $T = 85$ °C, interpulse time: 30 s, external standard: NaA (3×10^{-3} M) and polysaccharide solution concentration around 3 g L^{-1} (the temperature for conformational transition being above 50 °C for such polymer and salt concentration [45]).

3.2. ^{1}H HRMAS NMR spectra of native and unsaturated Xan

High-resolution magic angle spinning (HRMAS) has become an extremely versatile tool to study heterogeneous systems. This method is rapid, requires only small amounts of samples and avoids heating of the sample. Due to the higher viscosities of Xan derivative solutions the characterization by ^{1}H HRMAS technique was also proposed. This technique makes it possible to obtain sufficiently resolved spectra

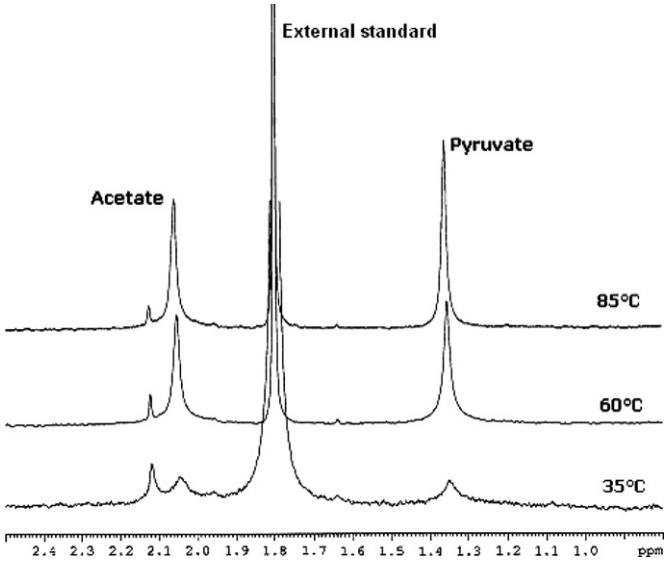


Fig. 2. Evolution of ^{1}H NMR Xan spectra resolution with temperature ($c_P = 2.92$ g L^{-1} in D_2O–Na acetate 3×10^{-3} M).

starting from solvent swollen samples, even if the movements of spin are limited in comparison with the liquid state, as shown in Fig. 3.

A slight shift of D_2O peak between spectra recorded by liquid state and by ^1H HRMAS NMR can be noticed. This phenomenon is explained by the high recording temperature in ^1H liquid NMR (85 °C). Two peaks close to those of acetate and pyruvate groups can be observed, which might be due to the impurities grown from Xan culture conditions and fermentation processing, as mentioned before. It is further of interest to mention that the interpulse time has no influence on ^1H HRMAS NMR spectra resolution in contrast to solution NMR, this technique being quite interesting as a qualitative method.

3.3. Synthesis of unsaturated Xan precursors (A-Xan, MA-Xan)

The Xan modification by esterification with unsaturated organic acids (such as acrylic acid) or their reactive derivatives (acryloyl chloride, maleic anhydride) was performed using different procedures in view of establishing the optimal reaction conditions. The esterification proceeds predominantly to the primary hydroxyl groups (C6), although participation of some secondary ones is not wholly excluded. Preliminary trials of synthesizing Xan 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/Xan molar ratio of 11/1 was generally used (Xan presents 11 hydroxyl group/RU) corresponding to an equimolar ratio of reactive sites. The syntheses were performed under various conditions such as homogeneous system for the esterification with AA (Xan aqueous solution, 1% wt/vol) and heterogeneous system for the modification with AC and MA (dispersion medium: DMF and ACT).

3.3.1. Synthesis of Xan acrylates (A-Xan)

3.3.1.1. Esterification of Xan with AA. Due to the water solubility of Xan 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

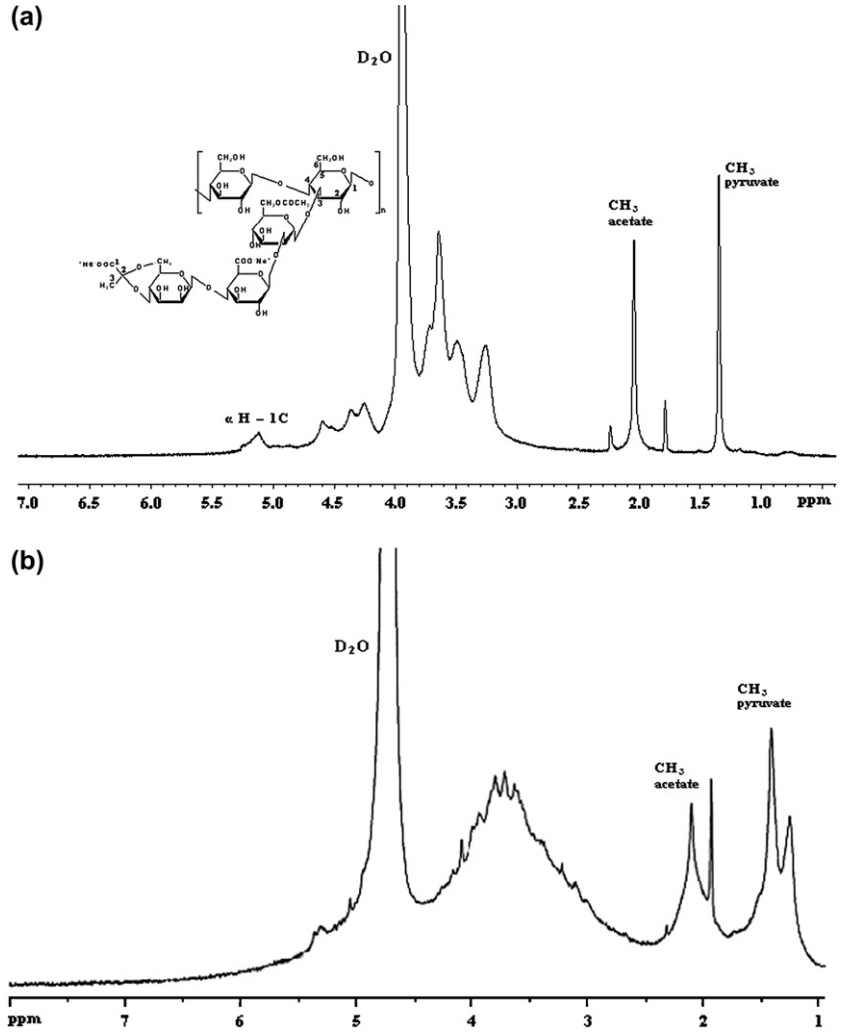


Fig. 3. Comparative NMR spectra of Xan performed in D_2O: (a) ^1H liquid NMR ($c_{pol} = 2.92$ g L^{-1}, $T = 85$ °C; $D_1 = 30$ s); (b) ^1H HRMAS ($T = 25$ °C; $D_1 = 30$ s).

reaction (a) presented in Scheme 1. The influence of time (6 and 24 h, respectively) and temperature reaction (2–4 °C and 70 °C, respectively) on DS of polysaccharide was studied. The molar ratio of AA/Xan and DEC/AA was established at 11/1 and 1.1/1, respectively.

The synthesis of AA modified Xan was evidenced by FTIR and ^{1}H NMR spectroscopies. The carbonyl peaks at 1733 cm^{-1} (24XAA-2) and 1736 cm^{-1} (24XAA-70, data not shown) which are characteristic of unsaturated ester absorption band are present. Additional double bond stretches were also observed at 1654 cm^{-1} (24XAA-2) and 1648 cm^{-1} (24XAA-70, data not shown).

The evidence of AA substitution on Xan was also observed by ^{1}H liquid NMR as shown in Fig. 4.

In comparison with the spectrum of Xan (Fig. 3(a)) there are weakly developed peaks within the range of δ 5.5, 5.8 and 6.0 ppm, which can be ascribed to the protons of vinyl groups (C*H*=C*H*$_2$). It should be mentioned that the integration and the normalization of the double bond peaks in the acrylate or maleate segments and the pyruvate group proton peaks of the Xan (1.35 ppm) gave a sufficient value to calculate the DS of acrylate or maleate Xan, as shown in Tables 1 and 2.

The DS was low at reaction time below 24 h, and after 24 h of reaction the obtained DS is around 1 double bond/30 RU of Xan.

Table 1 shows the effect of reaction temperature on substitution of Xan hydroxyl groups by AA. At temperatures above 50 °C, Xan in aqueous solution presents a transition from an ordered conformation to a disordered one [42,45]. 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 favorable factor considering the esterification reaction efficiency.

This behavior might be attributed, on the one hand to the fact that the reactivity of DEC is higher at low temperature [46], and on the other hand that the reaction rate increases with the increasing temperature [47]; therefore, a competition between these two 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.

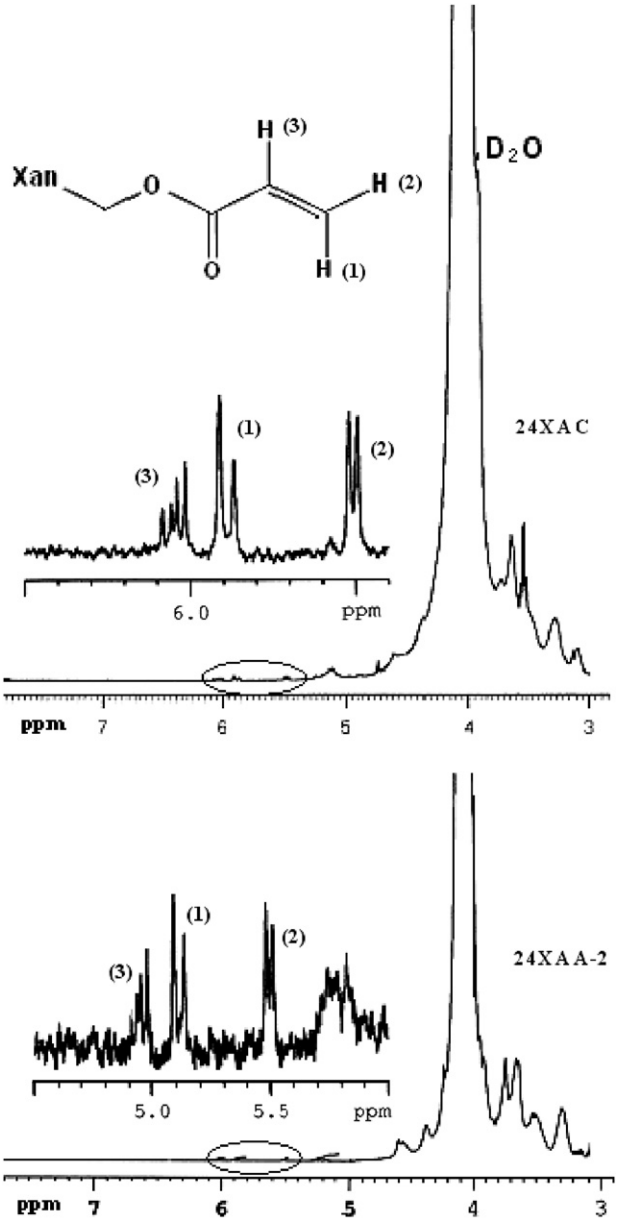


Fig. 4. 400 MHz ^{1}H NMR spectra of 24XAA-2 (DS = 0.3%) and 24XAC (DS = 0.4%) esterification products of Xan (reaction conditions: $T = 2$–4 °C; $t_r = 24$ h), recorded in D_2O at 85 °C ($D_1 = 30$ s, c_{pol} = around 3 g L^{-1}).

3.3.1.2. Modification of Xan 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 functionalisation agents hydrolyze easily, the reactions have to be carried out under heterogeneous conditions, in organic solvents.

The modification of Xan with AC is carried out in DMF as dispersion medium in the presence of an HCl acceptor (TEA), according to the reaction (b) presented in Scheme 1. The reaction products, obtained after 6 and 24 h reaction times, were characterized by spectroscopic methods (ATR–FTIR, ^{1}H NMR).

The esterification of Xan with AC was performed at constant temperature ($T = 2$–4 °C) and different reaction times (t_r) = 2 h (2XAC), 6 h (6XAC), 24 h (24XAC). The molar ratio of AC/Xan and TEA/AC was controlled at 11/1 and 1.1/1, respectively. The successful incorporation of AC on Xan was confirmed by ^{1}H NMR (Fig. 4) and FTIR–ATR. FTIR spectra of the region at around 1800–1200 cm^{-1} (where the modifications were more significant) of Xan and Xan esterification derivative are presented in Fig. 5.

The characteristic unsaturated ester absorption band ($\nu_{C=O} = 1715$–1730 cm^{-1}) appeared. Xan presents two carbonyl peaks at $\nu_{C=O} = 1723$ cm^{-1} corresponding to acetate or pyruvate groups, respectively, and at $\nu_{C=O} = 1600$ cm^{-1} characteristic of a carboxylate group. The shift of the carbonyl peak at 1736 cm^{-1} (24XAC) (Fig. 5(2)), 1730 cm^{-1} and,

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

Esterification agent	Reaction time (h)	Reaction temperature (°C)	DS[a] (%)	Double bond per repeating unit
AA	24	2–4	0.3	0.03
		70	0.3	0.03
AC	6	2–4	0.1	0.01
	24		0.4	0.04

[a] As determined by ^{1}H NMR spectroscopy (in D_2O, $T = 85$ °C, $D_1 = 30$ s, c_{pol} = around 3 g L^{-1}).

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

MA/Xan (mol/mol)	Reaction time (h)	Reaction temperature (°C)	DS[a] (%)	Double bond per repeating unit
DMF as dispersion medium				
11/1	6	20	1.7	0.2
	24		3.6	0.4
	24	50	4.5	0.5
	6	60	2.9	0.33
	24		9.7	1
	6	70	8	0.9
	24		10.8	1.18
ACT as dispersion medium				
1/1	24	50	0.4	0.045
11/1	24		1.7	0.19

[a] As determined by ^{1}H NMR spectroscopy (in D_2O, $T = 85$ °C, $D_1 = 30$ s, c_{pol} = around 3 g L^{-1}).

respectively, 1735 cm^{-1} (for the products obtained at 2 and 6 h of reaction, data not shown), in unsaturated Xan derivatives is obviously due to the ester linkage. The modification of spectra becomes more evident with an increase in the DS. FTIR peaks at 1650 cm^{-1} (24XAC) (Fig. 5(2)) and 1647 cm^{-1} (for 6 h reaction product, data not shown) were found in the modified Xan. These IR shoulders emerged from the stretch of *C*=*C* double bond of the attached acrylate segment.

^{1}H NMR spectra of AC modified Xan derivatives showed several distinctive peaks in the double bond region (5.5–6.5 ppm) which were not present in the precursor Xan (Fig. 3(a)). 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. 4 (24XAC).

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 0.1% of the total hydroxyl groups of Xan. A DS of 0.4% was achieved after 24 h.

These results confirm that Xan can be esterified leading to unsaturated products with 1 double bond per 30, respectively, around 25 RU of Xan with AA and preferably with AC. As it will be discussed later, even if the degree of substitution is not very high, this does not prevent its use as precursor for hydrogels.

3.3.2. Synthesis of maleate Xan derivatives

Xan maleates were synthesized by esterification with MA according to Scheme 1. The reaction occurs in heterogeneous system. The hydroxyl groups of Xan 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 Xan modification. They differ in the chemical nature of reaction medium, in DMF and ACT, respectively. The reaction in DMF was performed at different reaction times (6 h, 24 h) and temperatures (20, 50, 60, 70 °C) with an MA/Xan molar ratio of 11/1. For the reaction in ACT, the influence of MA/Xan molar ratio (1/1; 11/1) at constant temperature (50 °C) and reaction time (24 h) was studied.

3.3.2.1. Xan esterification in N,N-dimethyl formamide. The successful reaction of Xan with MA in DMF was demonstrated using FTIR (Fig. 6), ^{1}H and ^{1}H HRMAS NMR spectroscopies (Fig. 7).

The presence of carbonyl typical bands at around 1736 cm^{-1} (24XMA-20), 1738 cm^{-1} (24XMA-50) and

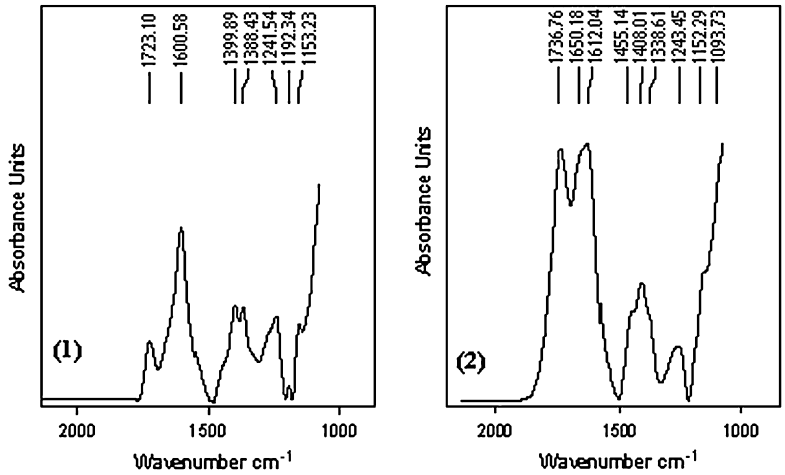


Fig. 5. Absorbance FTIR spectra of: (1) Xan; (2) Xan esterified with AC (reaction conditions: $T = 2$–4 °C; $t_r = 24$ h).

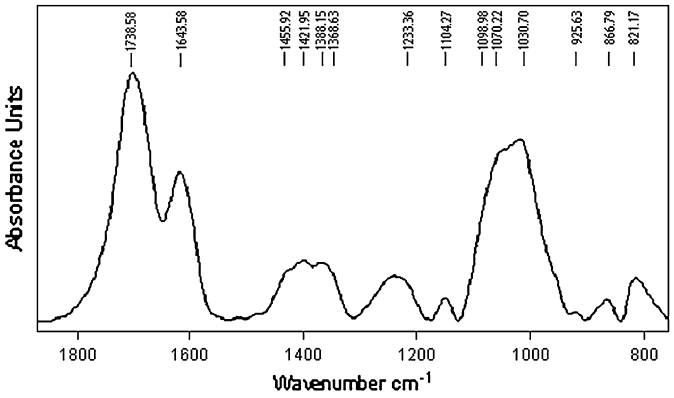


Fig. 6. MA/Xan absorbance FTIR spectra: 24XMA-70.

1739 cm^{-1} (24XMA-70) in modified Xan demonstrated the presence of ester and of the carboxylic acid groups (Fig. 6). The magnitude of this carbonyl group band increased significantly with the increasing DS. Each attached MA segment contains an ester and a carboxylic group. 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 1649–1655 cm^{-1} and 867–873 cm^{-1}.

The evidence of MA substitution on Xan was visualized by ^{1}H liquid NMR (data not shown) and ^{1}H HRMAS NMR (Fig. 7(1): 24XMA-70). New distinctive peaks in the double bond region (δ 6–7 ppm) are present.

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 10.8% was achieved. The DS values for 24 h reaction products obtained at higher temperatures (60–70 °C) are quite close.

3.3.2.2. Xan esterification in acetone. A typical ^{1}H NMR spectrum of MA modified Xan, with the characteristic peaks attributed to the two protons attached to the double bond (δ 5.5–6.5 ppm), is shown in Fig. 7(2).

From the results given in Table 2 it appears that the DS value increases as expected with the MA/Xan ratio. Furthermore one can notice that DMF is more efficient than ACT 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 Xan than acetone.

Apparently, the DS obtained after esterification by AA and AC is not very high but it should be reminded that these unsaturated esters of Xan 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. The crosslink density substantially affects the characteristics (such as swelling degree, drug inclusion and mechanical properties) of these crosslinked materials. Thus, the network density, and hence the mentioned properties, can be adjusted through changes in the esterification degree of Xan ensuring variations from a loose network to a tight one.

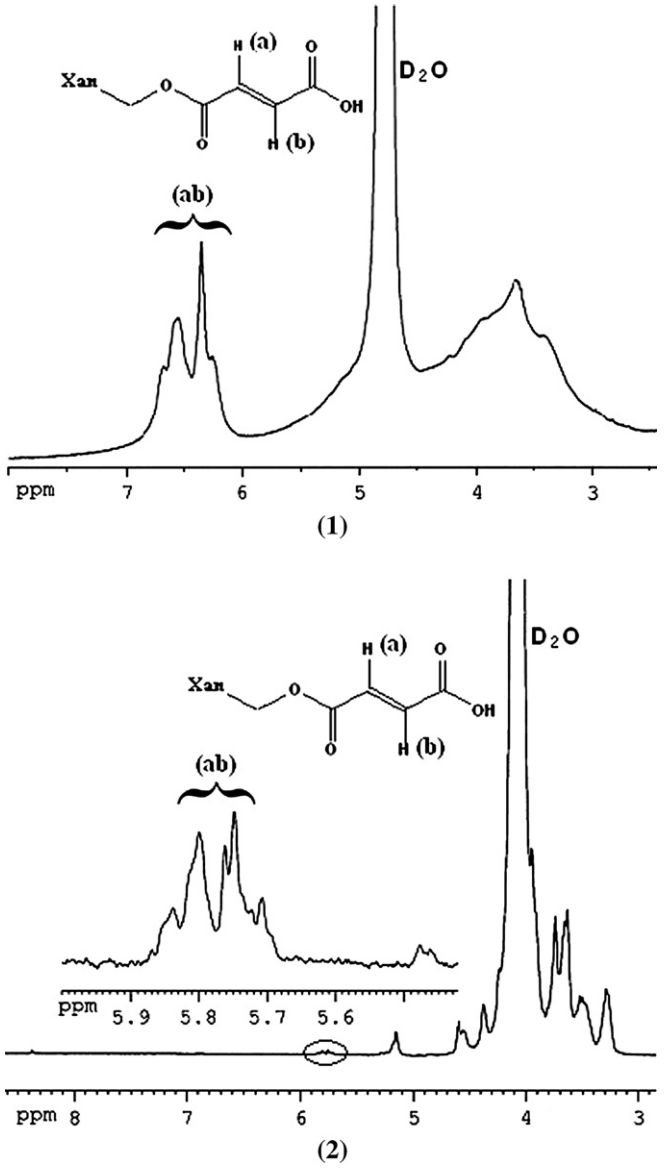


Fig. 7. (1) ^{1}H HRMAS NMR spectrum in D_2O ($T = 25$ °C; $D_1 = 30$ s) of 24XMA-70 synthesized in DMF (DS = 10.8%); (2) ^{1}H NMR liquid spectrum in D_2O ($c_{pol} = 3$ g L^{-1}, $T = 85$ °C; $D_1 = 30$ s) of MA esterification product of Xan ($T = 50$ °C; $t_r = 24$ h; MA/Xan ratio = 11/1 (mol/mol); synthesis in ACT) (DS = 1.7%).

4. Conclusions

This paper presents the preparation and the characterization of new reactive Xan based macromonomers through esterification with an unsaturated organic acid (acrylic acid) or with unsaturated acid derivatives (acryloyl chloride, maleic anhydride) under homogeneous and heterogeneous conditions. The influence of some reaction parameters (temperature, duration, chemical nature of esterification agent, molar ratio), on the degree of substitution was 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 Xan esterification are carried out in DMF.

With these unsaturated Xan esters, having variable grafting/crosslinking moieties, it was shown in preliminary copolymerization experiments with *N*-isopropylacrylamide, that thermo- and pH-stimulable hydrogels with adjustable crosslink density were obtained. The results clearly indicate that modified Xan/NIPAM based hydrogels are potential carrier in the design of controlled water-soluble drug delivery systems [48].

The fully systematic study of this type of products, with interest for potential applications in controlled-drug release applications, will be reported in a future article.

Acknowledgements

The authors would like to thank the Agence Universitaire de la Francophonie, for financial support. CLARIANT Company (Switzerland) is acknowledged for some reagents' supply.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.polymer.2007.01.048.

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