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New way to crosslink chitosan in aqueous solution

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ABSTRACT

In this paper, the reaction between *o*-phthaldialdehyde and free —NH_2 of chitosan is investigated; at a very low molar ratio between the two reactants ([dialdehyde]/[—NH_2] ~ 2.5×10^{-4}), an increase of the apparent molecular weight is obtained as evidenced from the rheological behaviour. Then, three non-ionic polysaccharides (galactomannan, maltodextrins, methylcellulose) are oxidised to 10% with sodium metaperiodate to obtain polyaldehydic derivatives able to react with free —NH_2 of chitosan after their direct dissolution into chitosan solution at a molar ratio [monosaccharide units]/[—NH_2] ~ 0.6. Stable swollen porous gels are obtained with an excellent yield in the presence of a reducing agent ($NaBH_3CN$) chosen to reduce the Schiff base; nearly no influence of the structure of the initial non-ionic polysaccharides is observed when the polysaccharides are oxidized in the same conditions. Different parameters for the reaction of oxidized methylcellulose (Me-ox) with chitosan are tested: influence of the degree of oxidation (up to 50%), and of the oxidised methylcellulose concentration. The larger is the degree of oxidation or the Me-ox concentration, the lower is the degree of swelling (i.e., the larger is the degree of chitosan cross-linkage). The swollen gels formed immediately after reaction are isolated and re-swell in aqueous acidic conditions, a good solvent of initial chitosan, to purify the gel and determine the yield of the reaction and the swelling degree. At the end, preliminary tests of biodegradability of these new gels are performed using specific enzymatic degradation with lysozyme and cellulase in the case of chitosan/Me-ox cogels chosen as example.

1. Introduction

Chitosan (Chit) is a pseudo natural polysaccharide extracted mainly from crustaceous shells. It becomes water soluble under acidic conditions as soon as pH is lower than 6 and if the average degree of acetylation (DA) is equal or lower than 0.5. A review on its characterization and properties was recently published [1]. Its biocompatibility and biodegradability are important advantages for this polymer which is now used in many biomedical and pharmaceutical applications. For that purpose, it is often used not as a single component but blended with other polymers with or without cross-linkage [2]. Controlled cross-linkage of chitosan may be of interest to prolong the life time over a desired period of time as needed in a large range of applications (especially for implantable drug-delivery systems) and also prepare chitosan under stable gel or membrane devices. Crosslink was often obtained using well-known glutaraldehyde, or *m*- and *p*-phthalaldehydes (but not with *o*-phthalaldehyde) [3]. An original cross-linkage was recently investigated using genepin as natural cross-linker. Genepin-cross-linked chitosan (by an amide linkage) is especially convenient for biomedical applications due to its lower toxicity and slower degradation rate compared to the glutaraldehyde-cross-linked chitosan [4–8]. The exact mechanism of reaction depends on pH which plays a role on the degree of cross-linkage and on the degree of condensation of genepin in the connection [9].

In the present work, it is suggested to crosslink chitosan by a reductive-amination mechanism with uncharged multifunctional polymers obtained after partial chemical oxidation of non-ionic polysaccharides [10]. Firstly, a low

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molar mass dialdehyde (*o*-phthaldialdehyde) is reacted with chitosan. Secondly, three polysaccharides are oxidized to aldehyde by periodic oxidation as developed previously on starch [11–14], alginates [15–17] and methylcelluloses [18]. This reaction gives dialdehyde carbohydrate units on C-2 and C-3 positions with opening of the sugar unit. Then, these partly oxidized derivatives (polyaldehydic derivatives) are used to react with the —NH_2 groups of chitosan in direct reductive amination process in the presence of sodium cyanoborohydride used as reducing agent [19]. The first results obtained for cross-linkage of chitosan are discussed in this paper and a preliminary enzymatic test allows to evidence (bio)degradability of such cross-linked materials.

2. Materials and methods

A few polysaccharides with relatively low molecular weight (M_w < 50,000) are chosen as models to be oxidized to aldehydic derivatives: maltodextrins (delivered by Prolabo) with a $M_w \sim 25{,}000$ determined by steric exclusion chromatography (SEC), methylcellulose Methocel A15 Premium LV from Dow Chemical ($M_v \sim 31500$; DS ~ 1.8) and guar gum MLV (used as a model for galactomannans) with a mannose/galactose ratio equal to 1.6 from TIC Gums Inc., USA; guar molecular weight is calculated from intrinsic viscosity and we obtain $M_v \sim 43{,}000$ [20]. Sodium metaperiodate, sodium cyanoborohydride $NaBH_3CN$ and *o*-phthaldialdehyde in solution are obtained from Sigma–Aldrich. Enzymatic degradation was performed with lysozyme (EC 3.2.1.17, grade III from chicken egg white) and cellulase (EC 3.2.1.4 from Trichoderma viride) provided by Sigma.

Oxidation of neutral polysaccharides is performed following the conditions adopted previously for alginates [17]: 2 g of polysaccharide are dissolved in 150 mL water in a dark bottle; then, 50 mL of aqueous solution containing 264 mg sodium periodate are added under stirring (molar ratio [monosaccharide units]/[periodate] ~ 10, corresponding to samples at 10% oxidation). After 24 h at room temperature, the solutions are filtrated on porous glass filter No. 1 and the oxidised polysaccharides are recovered. Oxidized galactomannan (GM-ox) is precipitated with ethanol and isolated as usually for water soluble polysaccharides; modified maltodextrins (Malt-ox) and methylcelluloses (Me-ox) are dialyzed against pure distilled water, concentrated at 35 °C and freeze dried. The M_w of modified maltodextrins was found equal 20,000 indicating a relatively low degradation compared to what was obtained on alginates [17]. In the case of methylcellulose, the same reaction is performed with molar ratio equal to 10, 4 and 2, to examine the role of the degree of oxidation on the cross-linkage of chitosan. The degree of oxidation was controlled by infrared and NMR. The degree of oxidation (yield in reducing groups using maltose to calibrate the response at 420 nm) was determined quantitatively using the colorimetric technique with ferricyanide [21].

Chitosan Kitomer is provided by Marinard (Canada); it is characterized by a degree of acetylation DA ~ 0.2 and M_w = 500,000. It is dissolved in water in presence of the stoichiometric amount of HCl to obtain a 10 g/L solution at pH ~ 3.5. Two types of reaction were performed: firstly, chitosan was reacted with a low molar mass dialdehyde (*o*-phthaldialdehyde); secondly, oxidized polysaccharides (polyaldehydic polymers) in powder form are added into chitosan solution under stirring up to complete dissolution. An excess of reducing agent ($NaBH_3CN$ at a molar concentration five times the —NH_2 content) is added to reduce the intermediate imine as shown previously to obtain a stable linkage Chit—NH—CH_2—R (R—CH=O is used for the oxidized polysaccharide chain) (see Fig. 1) [19,22]. After addition of the reducing agent (here $NaBH_3CN$ but other less toxic reducers may be used), a very swollen gel forms in the same time as pH increases up to ~6.

IRTF spectroscopy was realized on a Perkin Elmer Model SpectrumRX1 spectrophotometer; 10 mg of each sample was ground with 100 mg KBr.

^{1}H NMR experiments were performed using a Bruker DRX400 spectrometer operating at 400 MHz. 1D NMR spectra were collected at 30 °C using 32 K data points and 32 scans. Deuterium oxide (99.90% D) was obtained from Euriso-top (France).

Molecular weight of maltodextrins was characterized by size exclusion chromatography (SEC) using a Waters Alliance GPCV2000 (USA) equipped with three detectors on line: a differential refractometer, a viscometric detector, and a multi angle laser light scattering (MALLS) detector from Wyatt (USA). The concentration of the sample injected was 5 g/L with an injection volume of 100 μL using two columns in series (Shodex OH-pack 802 and 803); before injection, the samples were filtrated on a 0.2 μm pore membrane ("Sartorius AG" cellulose acetate filter) in order to retain large aggregates. The eluent used was a 0.1 M $NaNO_3$ aqueous solution, at 30 °C as elution temperature and a flow rate of 0.5 mL/min; the molecular weight distribution and weight-average molecular weight (M_w) are determined using refractive index increment dn/dc = 0.142.

Degrees of swelling of gels are calculated in different conditions from the ratio of weight of solvent absorbed by the swollen material divided by the dry weight of the gel obtained after weight stabilisation at 70 °C.

The rheological behaviour of chitosan and modified chitosan was determined using an AR 1000 rheometer from "TA Instruments" (USA) at 25 °C using a cone and plate geometry (the cone has 4 cm diameter and 3.59°). For chitosan solution and loosely cross-linked chitosan in solution, dynamic experiments were performed in the linear viscoelastic region where G' and G'' are independent on the frequency applied. Dynamic moduli (storage modulus G' and loss modulus G'' in Pa) as well as complex viscosity $|\eta^*|$ were determined as a function of the angular frequency (ω) expressed in Hz. Steady state viscosity η (in Pa s) was determined as a function of the shear rate γ (in s^{-1}). The solvent used is 0.05 M HCl.

3. Results and discussion

Our purpose is to crosslink linear chitosan to different degrees based on the well-known direct reaction of reductive amination in which —NH_2 groups react with aldehyde

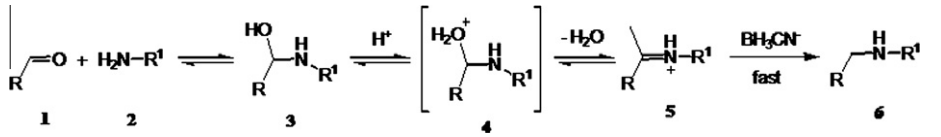

Fig. 1. Schematic representation of the reductive amination reaction involving an aldehydic group from partly oxidised non-ionic polysaccharides **1** and amine group of chitosan—NH_2 **2** in presence of the reducing agent $NaBH_3CN$. The accepted mechanism involves the formation of an intermediate aminoalcohol **3** which is then protonated and looses one molecule of water to give an iminium ion **5**. The latter adds a hydride (H^-) anion from the reducing agent, and is thus transformed into the final amine **6**. This last step is fast and irreversible, thus driving the equilibrium of the reaction towards the final product **6**.

in mild acidic conditions (pH ~ 6) in the presence of an excess of reducing agent $NaBH_3CN$. In these conditions, reduction of aldehydic groups is negligible. The mechanism of reaction proposed is recalled in Fig. 1 [19]. The advantage of such a reaction is that it is performed in mild conditions (slightly acid aqueous solution) and that via this reaction a stable linkage is formed avoiding possible hydrolysis in dependence with local pH.

3.1. Reaction of chitosan with a dialdehyde

o-Phthaldialdehyde (20 μg in 2 mL solution as received) was added under stirring into 9 mL of a 10 g/L solution of chitosan at initial pH 3.5; the molar ratio *R* between [dialdehyde] and [glucosamine repeat unit] is very low ($R \sim 2.5 \times 10^{-4}$) and the final concentration in —NH_2 is 5×10^{-2} monomol/L. Then, 200 mg of $NaBH_3CN$ was added and the solution was stirred during 24 h. The pH at the end of reaction was pH 7. Modified chitosan precipitates at such pH. The precipitate is washed with water and redispersed directly in 0.05 M HCl for purification; the viscous clear solution is neutralized and the modified chitosan is recovered and dried with ethanol and acetone. The yield of reaction is around 50 ± 5% estimated from the amount of cross-linked chitosan recovered. Results obtained for rheological behaviour are given in Figs. 2 and 3 for two separate experiments on 10 g/L solution of modified chitosan dissolved in 0.05 M HCl showing the good reproducibility.

From these results, it is clear that the behaviour of the modified chitosan in HCl is that of a viscoelastic solution but *G′*, *G′* and complex viscosity values are much increased after reaction compared with the behaviour of initial linear chitosan prepared in exactly the same conditions; these data indicate a loose degree of cross-linkage. Especially, it is observed that a characteristic non-Newtonian behaviour appears in Fig. 3. It is concluded that such a reaction at low yield of low molecular weight dialdehyde increases the apparent molecular weight of chitosan preserving its solubility in acid conditions. This reaction, in controlled conditions, may be of interest to get better thickening properties of chitosan in aqueous solution. In the following, higher degrees of cross-linkage allowing us to get hydrophilic gels are proposed by reaction of chitosan with multifunctional polymers prepared separately.

3.2. Reaction of cross-linkage of chitosan with oxidized polysaccharides

To obtain higher degree of cross-linkage, chitosan was reacted with multifunctional polysaccharides (oxidized

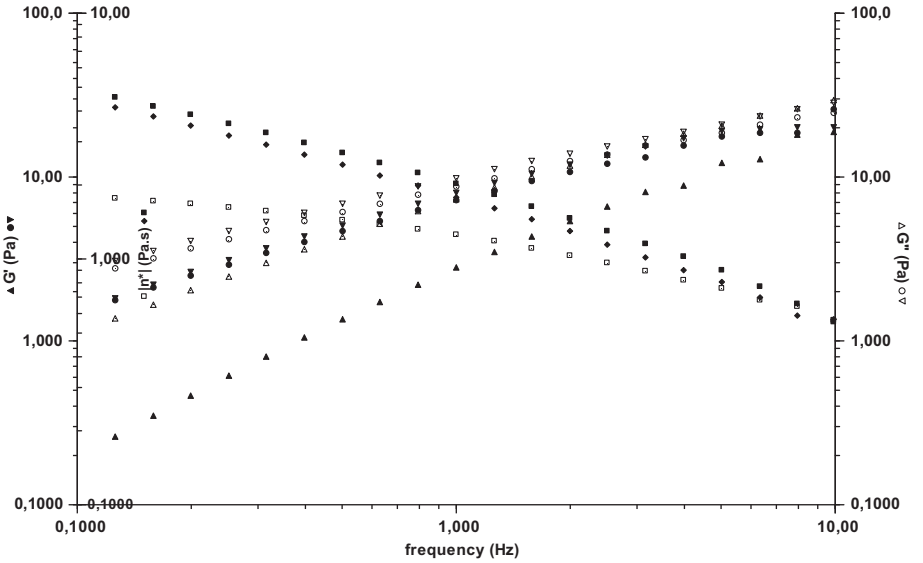

Fig. 2. Dynamic rheology of initial and modified chitosan dissolved at 10 g/L in 0.05 M HCl, at 25 °C. Initial chitosan *G′* ▲, *G″* △, $|\eta^*|$ □; chitosan derivative (with *o*-phthaldialdehyde) *G′* ● and▼, *G″* ○ and ▽, $|\eta^*|$ ♦ and ■ (respectively, for the two sets of experiments).

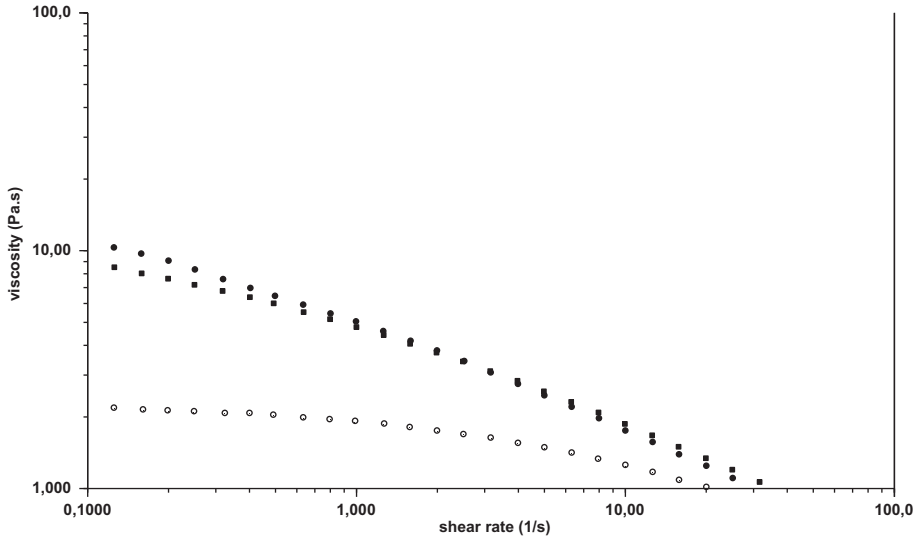

Fig. 3. Steady state viscosity as a function of the shear rate for chitosan (○) and chitosan derivative (with *o*-phthaldialdehyde) (■ and ● for the two series of separate experiments) at 25 °C. Polymer concentration 10 g/L in 0.05 M HCl.

maltodextrins, oxidized galactomannan and oxidized methylcellulose at 10% oxidation); the selected polymers have no ionic charge and good water solubility to avoid phase separation or uncontrolled reaction (such as polyelectrolyte complex formation). The reaction was performed directly by adding 60 mg of each polysaccharide-ox under powder form into 10 mL of chitosan solution at 10 g/L and pH 3.5. In this case, the molar ratio [monosaccharide units]/[—NH_2] = 0.6 and the [—NH_2] equals 6×10^{-2} monomol/L. The oxidized polysaccharides solubilise progressively in the chitosan solution under stirring at ambient temperature. After 16 h at room temperature, a perfect solution is obtained with the different polysaccharides tested and the reducing agent (in saturated aqueous solution to allow homogeneous reaction) is added drop wise under stirring. After a few minutes, a porous gel is formed over the whole medium (around 10 mL of gel formed corresponding to 160 mg of polymers engaged) at an equilibrium pH around 6. It is left during one night and then swollen in a large excess of water. Gels are filtered on glass filter or on a 8 μm porous membrane. Swollen gels are weighted and dried to determine the initial degree of swelling in water at neutral pH (see Table 1). The apparent amount of material recovered (Ps mg) is around 100% of the total amount of polymers introduced but it contains eventually still some uncrosslinked products (see the case of Malt-ox in Table 1). Then, the initial dried gels are equilibrated in 0.05 M HCl, a good solvent of linear chitosan, during 4 days to extract the unreacted fraction of products and to determine the degree of swelling in a good solvent of chitosan (at this pH the —NH_2 groups remaining on chitosan are protonated and the highly charged gel is swollen). In a second step, the swollen gel is dispersed in a large amount of water to determine the swelling degree at low ionic concentration and the dried weight of the remaining gel in absence of unreacted polymers and impurities. Data indicate that a fraction of the firstly isolated material was solubilised which allows to determine the fraction of gel stable in acidic conditions (for two separate experiments, we found a minimum yield of 75 ± 5% on the basis of the total amount of initial polymers engaged) (see Table 1).

The amount of gel recovered looks nearly independent on the nature of polysaccharide-ox engaged when their degree of oxidation is the same (10%). In addition, the influence of electrostatic repulsion is demonstrated when the swelling degree in water is compared with that obtained in 0.05 M HCl solution which is always lower than in water (see Table 1). In addition, the swelling degree of Chit–Malt-ox in water is larger than for Chit–Me-ox as found in different conditions (not shown); this may be related to the

Table 1
Addition of polysaccharides oxidized at 10% (60 mg in powder form) into a solution of chitosan (10 mL at 10 g/L). Weight fractions of purified gels and degrees of swelling in 0.05 M HCl and in water are given with a precision of 5% between separate experiments.

	Initial swelling g/g in water	% purified gel in absence of drying	Swelling in 0.05 M HCl after drying (g/g)	Swelling in water after swelling in HCl (g/g)
Malt-ox	63	73	20	40
Me-ox	76	76	20	29
GM-ox	138	85	39	63

degree of branching of maltodextrins causing a lower degree of cross-linking (by steric hindrance) compared with methylcellulose which is a perfectly linear polysaccharide. In order to examine the influence of experimental conditions on the oxidised polysaccharide–chitosan cogels obtained, we focus our study on oxidized methylcelluloses.

3.3. Influence of the reaction conditions

3.3.1. Characterization of oxidized methylcelluloses

Oxidized methylcelluloses are prepared with different amount of periodate to vary the degree of oxidation as discussed previously for alginate [17]. The molar ratio [monosaccharide units]/[periodate] is chosen around 10, 4 and 2 which correspond to predicted degrees of oxidation of 10%, 25% and 50% (at maximum one sugar unit is oxidized over two units). As discussed before, it is difficult to determine the exact number of reactive aldehydic groups due to secondary reactions in which aldehydes are engaged [17]; nevertheless, using IRTF, it is shown that content in —C=O from aldehydic groups determined by IRTF at 1735 cm^{-1} increases linearly up to 25% oxidation and then levels slightly off (see Fig. 4) due to the presence of methylated and unreactive —OH in C-2 and C-3 positions [23]; it was previously determined that 40% of the glucose units are dimethylated in C-2 and C-3 positions in the methylcellulose used [24]. The direct titration of aldehyde groups follows the IRTF results and gives, respectively, 4.5%, 13.4% and 19.4% of monosaccharide units oxidised (assuming the presence of two C=O per oxidised sugar) [21].

With ^{13}C NMR (data not shown), no C=O groups appears due to equilibrium in D_2O between the aldehydic form and the hydrated form as suggested in the literature [25]. From ^{1}H NMR spectroscopy on partly methylated cellulose and on the oxidised forms, the partial analysis of the spectra is performed using tables published for cellulose [26] and for trimethylated cellulose [27]. The signal located around 8.5 ppm attributed to —H of oxidized position (aldehydic group on C-2 or C-3 position) also increases as a function of the degree of oxidation; at 50% theoretical oxidation, a second signal appears around 9.32 ppm which may be attributed to modification on the C-2 and C-3 positions. An example of spectra obtained at different degree of oxidation is given in Fig. 5. A first estimation of the degree of oxidation is given from the decrease of the integral of H-2 signal compared to that of initial methylcellulose assuming that H-2 of methylated and free —OH units are both around 3.2 ppm (see Fig. 5) [26]. The agreement is good if compared with the colorimetric titration. A thin signal around 3.95 ppm develops also progressively when the degree of oxidation increases in the same time as the integral related to H-2 at 3.13–3.19 ppm decreases with opening of the glucosidic ring. From these characteristics, it is concluded that the predicted degree of oxidation follows perfectly the experimental conditions adopted (on the basis of metaperiodate amount involved in the reaction) even if the exact aldehyde content is not quantitatively determined by the metaperiodate used and needs quantitative titration; in fact, the NMR attribution and complete analysis was not the objective of this work. It must be recalled that it was demonstrated previously that some of the aldehyde groups are not available for reaction with amine groups: C=O groups react easily with —OH groups in the vicinity to give aggregates or gels [28,29] and/or do not react quantitatively (on the basis of oxidation degree imposed) with a low molecular weight amine

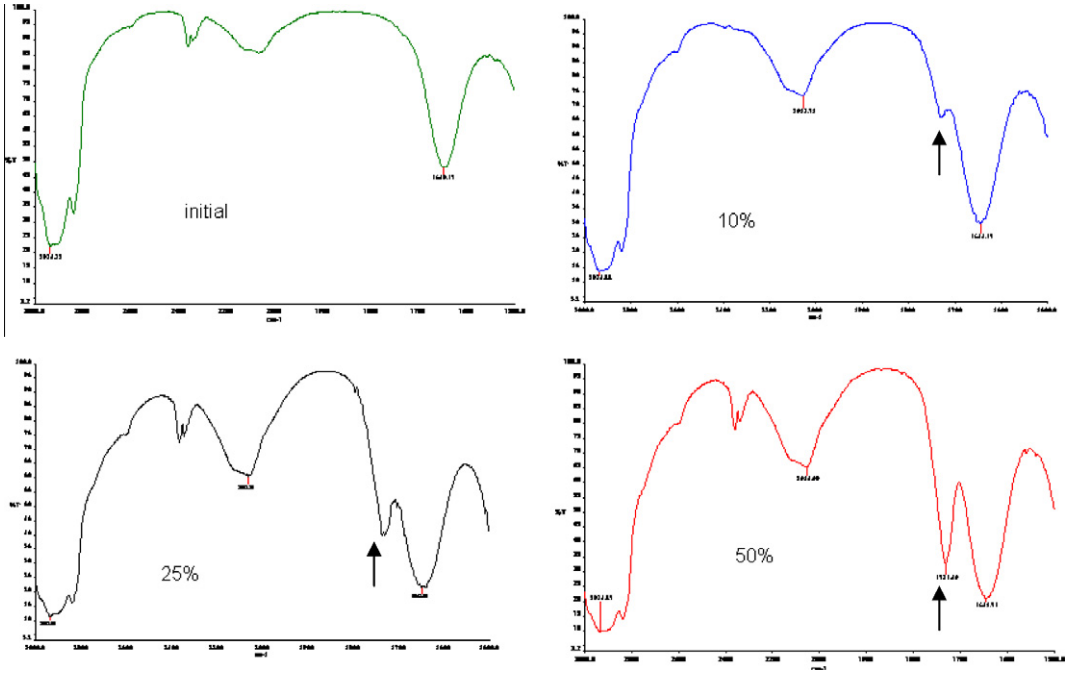

Fig. 4. Solid state IRTF spectra for initial methylcellulose and oxidised forms (10%, 25%, 50% oxidation) indicating the increase of amplitude of the —C=O stretching band around 1730 cm^{-1} (indicated by the arrow) directly related to the increase of oxidation degree.

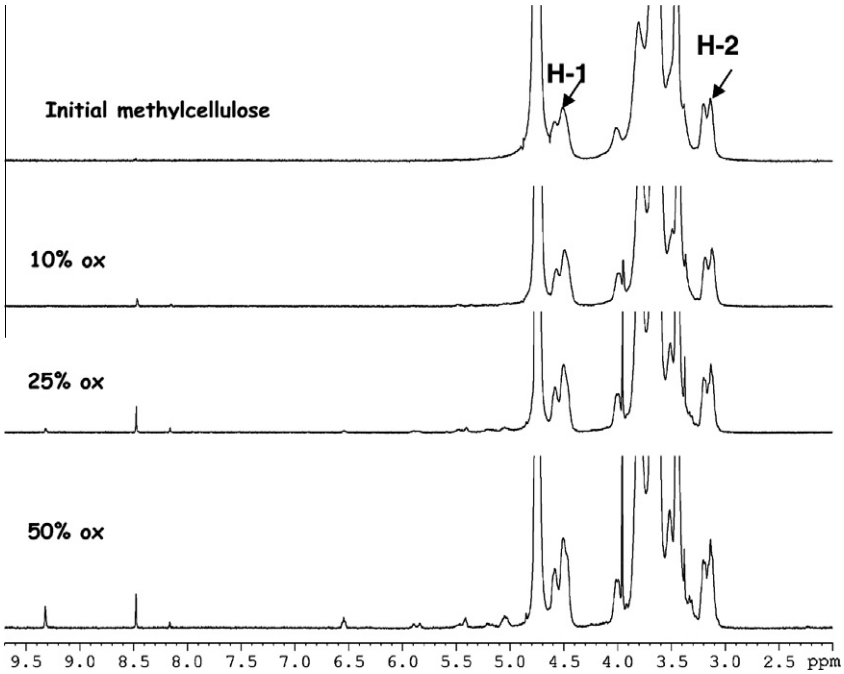

Fig. 5. ^{1}H NMR spectra for initial methylcellulose and the different oxidised forms in D_2O. Temperature 30 °C; concentration 6 mg/mL.

(propylamine) used as model to test the reactivity of oxidised alginates [17].

3.3.2. Influence of Me-ox amount added

Considering the previous results, the influence of the amount of polyaldehyde was tested to point out the role of the ratio polysaccharide-ox/chitosan. Me-ox (with 10% oxidation) is added to 10 mL of chitosan solution and stirred until perfect solubilisation. In this case, the molar ratio [monosaccharide units]/[—NH_2] varies from 0.25 to 1 and the —NH_2 concentration equals 6×10^{-2} monomol/L. After 16 h, the reducing agent is added (200 mg) and the gel forms. It is isolated at neutrality as previously and dried; 100% is initially recovered, dried and redispersed in acidic conditions to determine the exact amount of gel formed. Recovery after washing corresponds to 82–100% stable gels on basis of total initial polymer engaged. The main results are given in Table 2. These data demonstrate that the yield of reaction decreases when the amount of Me-ox added increases due to a limit of reaction due to steric hindrance when too many aldehyde groups compete for —NH_2 groups; in the same time, the initial degree of swelling decreases when the amount of Me-ox increases in relation with a higher average degree of cross-linkage. When the recovered material is dried and swollen again in acid, the swelling degree is lower than firstly obtained (as usually observed when polysaccharides are dried due to H-bonds formation) and in addition, the role of electrostatic is also demonstrated: the swelling in water being larger than in 0.05 M HCl (Table 2).

3.3.3. Influence of the Me-ox degree of oxidation

Samples of oxidised methylcellulose (around 23 mg) with the different nominative degrees of oxidation (namely 10%, 25%, 50% as imposed from experimental conditions) are solubilised in 10 mL of chitosan solution at 10 g/L in the usual conditions; the 200 mg of the reducing agent is added under stirring. The gels are swollen in large amount of water to eliminate the majority of impurities. The results are given in Table 3. From these data, it comes that the larger is the degree of oxidation (i.e., the density of aldehydic groups) the larger is the density of cross-linking points reflected by the lower initial degree of swelling in water. The gels are isolated and dried before re-swelling in 0.05 M HCl to deter-

Table 2
Gel properties in dependence of the amount of methylcellulose oxidized at 10% added in 10 mL of 10 g/L chitosan. Weight fractions of purified gels and degrees of swelling in 0.05 M HCl and in water are given with a precision of 5% between separate experiments.

Reference of gels	mg of Me-ox added	% purified gel recovered	Initial swelling degree (g/g) in water	Swelling degree in 0.05 M HCl after drying (g/g)	Swelling degree in water after swelling in HCl (g/g)
1	24.5	100	165	34	46
2	48.7	90	134	32.3	40
3	67.5	83.6	102	20	27
4	98.6	82	73	16.6	26

Table 3
Characterisation of gels obtained by cross-linking of chitosan with Me-ox at different degrees of oxidation. Degrees of swelling in 0.05 M HCl and in water are given with a precision of 5% between separate experiments.

Oxidation degree of Me-ox (in %)	Initial swelling degree in water (g/g)	Swelling degree in 0.05 M HCl after drying (g/g)	Swelling degree in water after swelling in HCl (g/g)
10	203	50.6	60.7
25	146	36.1	49.6
50	109	17.6	28.9

Table 4
Preliminary test of enzymatic degradability on cross-linked chitosan–Me-ox cogels.

References	% solubilized gel
Gel 1 + lysozyme	40
Gel 1 + cellulase	60
Gel 4 + lysozyme	31
Gel 4 + cellulase	40

mine their degree of swelling at equilibrium in this solvent; then, the gels are stabilized in water. At the end, the exact amount of insoluble material (namely, the gel fraction after extraction of impurities and linear polymers) is determined to calculate the swelling on the basis of the pure gel content. It is clear that the degree of swelling decreases when the degree of oxidation increases in acidic medium as well as in water.

3.4. Test of biodegradability

For different biomedical applications, it is interesting to demonstrate that the material produced remains biodegradable. The three polysaccharides chosen to react with chitosan are biocompatible allowing us to assume that the new gels remain biocompatible. The biodegradability of these gels is tested in preliminary experiments on chitosan–Me-ox systems using two separate enzymes: lysozyme specific for chitosan backbone [30] and cellulase specific for the cellulose derivative. Small pieces of gels characterized in Table 2 (gel 1 and gel 4 with different initial degrees of swelling) are swollen in water and added with enzymatic solution at neutral pH. After 24 h, gels are isolated, dried and weighted. The preliminary results are given in Table 4. From these results, it is concluded that accessibility of polysaccharides to enzyme degradation remains large even if it decreases slightly when the degree of cross-linking increases. These data allow us to conclude that the new chitosan based cogels produced remains biodegradable.

4. Conclusion

In this work, non-ionic polysaccharides (galactomannan, maltodextrins, methylcellulose) are oxidised with sodium metaperiodate to produce polyaldehydic reactants able to be coupled by reductive amination with the free $-NH_2$ position in chitosan. Advantage of this reaction is that the covalent bond between chitosan and aldehydic substrate ($-NH_2 + R-HC=O \rightarrow -NH-CH_2-R$) is stable whatever the pH. Firstly, a simple model (*o*-phthaldialdehyde) was chosen to react with chitosan at a low molar ratio ([dialdehyde]/[$-NH_2$] $\sim 2.5 \times 10^{-4}$). It is shown that an apparent increase of the molecular weight is obtained as evidenced from rheology; this may be of interest to get soluble chitosan with higher viscosity in acidic aqueous medium. Secondly, the reaction is studied with oxidised polysaccharides at higher molar ratio between partly oxidised sugar units and amino groups compared with the first experiment ([monosaccharide units]/[$-NH_2$] = 0.6). For that purpose, partly oxidised polysaccharides are dissolved directly into chitosan solution and the reaction allows us to get gels with nearly 100% yield. It is pointed out that the nature of the oxidised polysaccharides (when prepared in the same conditions) has relatively no influence on the gels obtained. Additionally, in presence of oxidised methylcellulose, it is shown that the degree of cross-linking of the cogels (based on the swelling degree) increases when its concentration and/or its degree of oxidation increase.

These new gels, prepared in mild conditions with an excellent yield, remain accessible to specific enzymes chosen against both polysaccharides engaged in the network indicating that they are still biodegradable in addition of being biocompatible. This may be of great advantage for biomedical applications or controlled drug release.

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