Irradiating or Autoclaving Chitosan/Polyol Solutions: Effect on Thermogelling Chitosan-β-glycerophosphate Systems

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The effects of steam sterilization and γ-irradiation on chitosan and thermogelling chitosan-β-glycerophosphate (GP) solutions containing polyol additives were investigated. The selected polyols were triethylene glycol, glycerol, sorbitol, glucose and poly(ethylene glycol) (PEG). They were incorporated to chitosan solutions prior to sterilization in a proportion ranging from 1 to 5% (w/v). The solutions were characterized with respect to their viscosity, thermogelling properties, compressive stress relaxation behavior and chitosan degradation. All polyols reduced the autoclaving-induced viscosity loss and had a positive impact on the solution thermogelling properties and compressive performance of the gels. Steam sterilization in the presence of glucose resulted in a substantial increase in the solution viscosity and gel strength. This was associated with a strong discoloration suggesting chemical alteration of the system. PEG was the most effective agent in preventing hydrolytic degradation of chitosan chains. Gamma-irradiation strongly decreased the chitosan solution viscosity regardless of the presence of additives, even when sterilization was carried out at −80 °C. Moreover, the thermogelling properties were dramatically altered, and thus, γ-irradiation would not be an appropriate method to sterilize chitosan solutions. In conclusion, polyols are potentially useful additive to maximise the viscoelastic and mechanical properties of chitosan-GP after steam sterilization.

Key words chitosan; hydrogel; sterilization; autoclaving; irradiation

Partially N-deacetylated chitosans are derived from chitin, a natural *N*-acetyl-D-glucosamine polymer obtained from the exoskeleton of crustaceans. Chitosan is also produced and isolated from marine microorganisms and microbial strains.[1] Chitin and chitosan have been found to be attractive biopolymers for various applications in the textile,[2] food,[3] cosmetic[2,4] and pharmaceutical industries.[4,5] More particularly, chitosan has been evaluated as a haemostatic,[4,6] wound healing[4,5] and bone repairing material.[7,8] In drug delivery, chitosan is being investigated for the preparation of sustained release formulations[9,10] and drug/gene carriers.[11] Chitosan is relatively non-toxic, biocompatible and degradable,[6,10] and exhibits potential immuno-adjuvant properties.[5] Chitosan-based materials are generally transformed into films,[6] membranes,[12] sponges or foams,[5,13] particles or microspheres,[10] and covalently or ionically cross-linked hydrogels.[14] Recently, Chenite *et al.*[15,16] proposed the chitosan-β-glycerophosphate (chitosan-GP) system as a novel family of injectable thermogelling liquid solutions for biomedical applications. The chitosan-GP system is a neutral liquid solution at low temperatures, which spontaneously turns into a homogeneous gel when heated to body temperature. It is devoid of any organic solvents, chemical or ionic cross-linkers. Chitosan-GP formulations have been shown to be attractive for the encapsulation of living cells, the sustained release of therapeutic agents and the design of *in situ* self-forming implants.[15]

Sterilization is a major step in developing artificial implants and medical devices. It has been investigated on chitosan-based systems, but almost quite exclusively on solid materials, such as films.[17—20] We recently studied the effects of steam sterilization and γ-irradiation on chitosan solution and powder.[21,22] It was shown that both sterilizing methods generally induced important physicochemical changes, particularly in terms of average molecular weights, solution viscosity, and viscoelastic properties of chitosan-GP systems. Processing additives have been proposed for polymeric liquid solutions to control the level of heat-induced degradation during energetic sterilizing treatments. For instance, certain polyols have been shown to reduce the thermal denaturation of polypeptidic solutions (*e.g.* collagen).[23] Glycerol, for example, has been proposed as a processing agent to protect gelatin materials during γ-irradiation.[24] Such polyols were also claimed to stabilize chitin materials during steam sterilization.[25] Ethylene glycol oligomers or polymers, sorbitol, mannitol and glycerol have been commonly used as processing additives or stabilizers for biopolymers such as chitosan.[26—30]

In this study, different polyols were added to liquid chitosan solutions prior to steam sterilization or γ-irradiation, in order to evaluate their protecting action on the properties of the chitosan-GP system. The systems were characterized with respect to the molecular weight of chitosan, viscosity, thermogelling parameters, and gel mechanical strenght.

Experimental

Materials The chitosans used (85% deacetylated) were from Natural Biopolymer Inc. (Raymond, WA, U.S.A.) (free base, SC342) or Pronova Biomedical (Oslo, Norway) (hydrochloride salt, UPCL 213). The polymer was stored under anhydrous conditions (relative humidity <10%) until use. Triethylene glycol (TEG) was purchased from ACP Chemicals Inc., (Montreal, Qc, Canada). β-glycerophosphate (GP), glycerol (GLY) 99.5%, D-sorbitol (SOR), poly(ethylene glycol) (PEG, M_w 1000, 3400), and D(+)-glucose (GLU) were supplied from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada).

Preparation of Polymer/Additive Solutions for Autoclaving A 2.35% (w/v) SC342 chitosan solution was prepared in HCl 0.1 N. The additives were then incorporated at different concentrations (0, 1, 2, 5% w/v). Two samples of each formulation were prepared for each test, and half of the samples were autoclaved, while the other half served as control. All the samples were stored at 4 °C until use. For rheological and mechanical testing, a 55% (w/v) aqueous solution of GP was progressively added to the chitosan solution under cool conditions (4 °C) and stirring. The final GP concentra-

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tion in all samples was 8.2% (w/v) and the final chitosan concentration was 2% (w/v). The pH was measured before and after sterilization, and after GP addition (Table 1). The solutions were steam sterilized in a Steromaster MK II autoclave (Consolidated Stills & Sterilizers, Boston, MA, U.S.A.). Autoclaving was performed under a liquid cycle for 15 min at 121 °C.

Preparation of Chitosan Solutions for γ-Irradiation The UPCL 213 hydrochloride salt chitosan was dissolved in distilled water to obtain a final chitosan concentration of 2.0% (w/v). The selected additives (TEG and GLY) were added to the chitosan solutions at a concentration of 1.0% (w/v). Chitosan samples were γ-irradiated, using a ^{60}Co source at a dose of 5 or 25 kGy (MDS Nordion, Laval, Qc, Canada). The irradiated samples were either liquid chitosan solutions, or frozen chitosan/additive solutions (−80 °C). During the irradiation process (10—45 min), frozen solutions were kept in dry ice. GP was added under stirring to the sterilized or non-sterilized chitosan and chitosan/additive solutions pre-cooled at 4 °C. Some samples were also directly irradiated in presence of GP. The final GP concentration in all samples was 8.2% (w/v).

Physicochemical Characterization Physicochemical characterization was carried out on chitosan or chitosan/additive solutions, as well as chitosan-GP or chitosan/additive-GP solutions. All chitosan solutions were examined visually to notice changes in coloration or aspect.

Gel Permeation Chromatography: The average molecular weights were determined by gel permeation chromatography (GPC) on a Waters Alliance GPCV2000 chromatographic station (Waters Corporation, Milford, MA, U.S.A.). Chitosan solutions were prepared at a concentration of 0.5 mg/ml. The eluent was a 0.2 M CH_3COONa and 0.3 M CH_3COOH buffer, the flow rate was 0.8 ml/min and the temperature of the columns (ultrahydrogel 120, 250, 1000, 7.8×300 mm) was 35 °C. Pullulans (Shodex, Showa Denko K.K., Japan) were used as standards.

Viscosity: The dynamic viscosity was determined at 20 °C on chitosan solutions using a Brookfield model RVDV-II+ cone-plate viscometer with cone plate geometry and CPE-52/CPE-42 spindles (Brookfield Engineering Laboratories Inc., Middleboro, MA, U.S.A.). The viscosity (mPa·s) was recorded *versus* the shear rate (s^{-1}) applied onto the solution.

Viscoelasticity: The thermogelling properties were determined by measuring the elastic modulus (G') of chitosan-GP and chitosan/additive-GP solutions. These parameters were recorded on a Bohlin CVO rheometer (Bohlin Instruments Inc., Cranbury, NJ, U.S.A.) equipped with C-25 coaxial cylinders. The solution (*ca.* 12.5 ml) was kept at 37 °C, or first stabilized 10 min at 15 °C and then heated to 60 °C at a rate of 0.5 °C/min. The analyses were carried out in a small oscillation mode at a frequency of 1 Hz. The rheological curves were then analyzed to determine the maximal elastic modulus (G'_m). At least, two samples were analyzed for each solution.

Mechanical Testing: Compressive indentation tests were used to characterize the mechanical properties of the gels. The mechanical tests were applied to autoclaved chitosan-GP gels, with or without TEG (1%), GLY (5%), GLU (1%), PEG_{1000} (1%) or SOR (2%). The gels were molded in 35-mm petri dishes at 37 °C for 24 h. Indentation tests were carried out on a MACH-1™ (BioSyntech Canada Inc., Laval, Qc, Canada) micro-mechanical tester equipped with a 150 g load cell (precision: 7.5 mg), a high-precision actuator (precision: 25 nm) and a cylindrical flat-ended indentator about 9.55 mm in diameter. The applied indentation consisted in a compressive stress-relaxation with 25% deformation, a displacement rate of 1.0%/s, and a relaxation time determined by a change of load less than 0.05 g/s. For each gel sample, the load, position and time were recorded at 0.05 s intervals. The determined mechanical parameters were the transient and equilibrium compressive loads and the relaxation time.

Results

pH Values and Molecular Weight Determination Table 1 summarizes the chitosan/additive compositions and pHs of the autoclaved and non-autoclaved solutions. The pH values of chitosan/additive samples were around 5.0—5.5 prior to sterilization. After sterilization, the pH values did not show any significant changes. After GP addition (with or without steam sterilization) pH ranged between 6.7 and 7.2, which is physiologically acceptable for injectables. At high PEG concentrations, the addition of GP resulted in the precipitation of chitosan. This may indicate that the protective effect of GP in preventing precipitation of chitosan at neutral pH[16] is diminished in the presence of PEG. Indeed, PEG may decrease the chitosan solubility by promoting hydrogen bonding between the hydroxyl groups of chitosan and ethylene oxide units. The changes in molecular weights between non-autoclaved and autoclaved chitosan/additive solutions are presented in Table 2. After autoclaving control chitosan solutions, the weight average molecular weight (M_w) decreased by about 30%. The addition of polyols in chitosan solutions could, in some cases, protect partially chitosan against degradation. The protective effect of polyols on chitosan during autoclaving can be summarized as follows (for 1% additive): PEG_{1000}=PEG_{3400}>GLY>TEG>SOR>GLU.

Table 1. Description of the Chitosan/Additive-Based Solutions

Additive	Additive concentration (w/v)	Autoclave	pH before GP addition[a)]	pH after GP addition[a)]
—	—	Yes	5.84	7.15
		No	5.41	7.14
TEG	1.0%	Yes	5.21	7.10
		No	5.32	7.09
	2.0%	Yes	5.36	7.11
		No	5.45	7.12
	5.0%	Yes	5.47	7.15
		No	5.55	7.19
GLY	1.0%	Yes	5.25	7.14
		No	5.25	7.15
	2.0%	Yes	5.26	7.13
		No	5.26	7.13
	5.0%	Yes	5.25	7.16
		No	5.25	7.09
SOR	1.0%	Yes	5.11	7.10
		No	5.12	7.11
	2.0%	Yes	5.06	7.10
		No	5.13	7.12
	5.0%	Yes	5.09	7.00
		No	5.13	7.04
GLU	1.0%	Yes	5.21	7.17
		No	5.08	7.13
	2.0%	Yes	5.15	7.10
		No	5.10	7.10
	5.0%	Yes	5.02	N.A.
		No	5.08	7.11
PEG_{1000}	1.0%	Yes	5.52	7.08
		No	5.50	7.07
	2.0%	Yes	5.45	6.94
		No	5.43	6.93
	5.0%	Yes	5.42	6.72[b)]
		No	5.46	6.73[b)]
PEG_{3400}	1.0%	Yes	5.46	7.02
		No	5.55	7.02
	2.0%	Yes	5.64	N.A.
		No	5.52	7.0[b)]
	5.0%	Yes	5.70	N.A.
		No	5.45	N.A.

a) pH is measured at room temperature (21 °C) on a clear solution. *b*) The solution is turbid: presence of precipitates. N.A.: not available.

Viscosity Steam Sterilization: Except for TEG, the addition of polyols to chitosan solutions generally lowered the viscosity (Fig. 1). The addition of GLY, GLU and SOR decreased the viscosity by about 20% (*ca.* 100 mPa·s), regardless of the amount added. PEG initially decreased the solution viscosity, but it came back to its initial level with increasing PEG concentrations.

All autoclaved chitosan/additive samples experienced a major viscosity drop, with a remarkable exception for the samples containing GLU (Fig. 1). After autoclaving, the vis-

Table 2. M_w and Polydispersity Index (PD) of Autoclaved and Non-autoclaved Chitosan Samples

Additive (1%)	M_w			PD	
	Non-autoclaved	Autoclaved	%[a)]	Non-autoclaved	Autoclaved
0	388300	270300	−30.4	5.37	4.78
TEG	395000	306500	−22.4	5.59	3.36
GLY	414200	324400	−21.7	4.15	3.17
SOR	408600	313300	−23.3	3.73	4.17
GLU	404800	295600	−26.7	4.19	3.99
PEG_{1000}	336700	278900	−17.2	4.28	3.85
PEG_{3400}	375100	307700	−18.0	3.17	2.94

a) indicates a percent change.

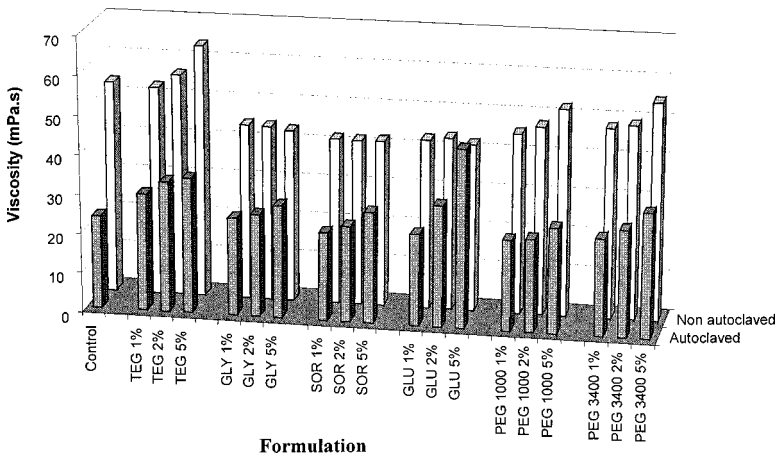

Fig. 1. Dynamic Viscosity of Autoclaved and Non-autoclaved Chitosan/Additive Formulations at a Shear Rate of 38.4 s^{-1}, (n=5)
All standard deviations <16 mPa·s (not represented). Chitosan/additive samples contain no GP.

cosities of chitosan solutions with TEG, GLY, SOR or PEG were found to increase progressively with the additive concentration. However, the viscosities of all autoclaved samples were generally 1.5 to 2.2-fold lower than that of the corresponding non-autoclaved samples. In nearly all cases, the autoclaved chitosan/additive (TEG, GLY, SOR or PEG) solutions had higher viscosities than the autoclaved control. GLU was found to apparently stabilize chitosan viscosity during sterilization since there was no difference in viscosity between autoclaved and non-autoclaved chitosan solutions containing 5% GLU. However, these samples experienced discoloration upon sterilization. The other additives had a marginal impact in minimizing the viscosity decrease during steam sterilization.

Gamma-Irradiation: Gamma-irradiation of unfrozen chitosan solutions systematically resulted in a major degradation of the polymer (brownish discoloration, ultra-low viscosity, no gelling) (data not shown). At 5 and 25 kGy, the presence of TEG or GLY in the frozen solutions significantly reduced the radiation-induced viscosity drop (Table 3). The decrease in viscosity of all samples was more pronounced at 25 than 5 kGy.

Rheology Figure 2 illustrates the value of the G'_m of autoclaved and non-autoclaved chitosan/additive samples. Sterile or non-sterile chitosan/PEG-GP samples with 5% PEG_{1000} or 2–5% PEG_{3400} were not rheologically examined as precipitated polymer was present.

Non-autoclaved Samples: All non-autoclaved chitosan/additive-GP solutions presented the characteristic thermogelling property of the chitosan-GP system (Fig. 2). In general, the presence of polyols did not increase the G'_m, except for GLU samples. The chitosan-GP solutions containing 1 and 2% GLU clearly showed an enhanced G'_m (7479 and 10248 Pa, respectively) in comparison to the chitosan-GP solutions (5318 Pa).

Autoclaved Samples: The presence of polyol additives during sterilization had a minimal effect on G'_m. In some instances, a slight increase of G'_m was observed. In comparison to sterilized and non-sterilized control chitosan-GP solutions, apparent increases of G'_m were observed for sterilized chitosan-GP solutions containing 1% TEG (6429 Pa), 5% GLY (6405 Pa), 2% SOR (6173 Pa), 1% PEG_{1000} (5739 Pa) and 1% PEG_{3400} (5511 Pa). A particular behavior was again obtained with GLU. After sterilization, the 1% GLU solution demonstrated a very high G'_m (11038 Pa). Higher GLU contents systematically resulted in instantaneous gel-formation of the sterilized chitosan-GP/GLU samples, thus making rheological characterization impossible. In most cases, polyols did not modify the apparent gelling temperature of autoclaved chitosan-GP/additive solution, except for 5% GLY and 2% GLU which gelled at room temperature (fast gelling systems, data not shown).

Gamma-Irradiated Samples: Gamma-irradiation had strong negative effects on the gelling properties of chitosan-GP systems, whether polyols were present or not. Irradiation significantly reduced G' (Table 3). Generally, the irradiation

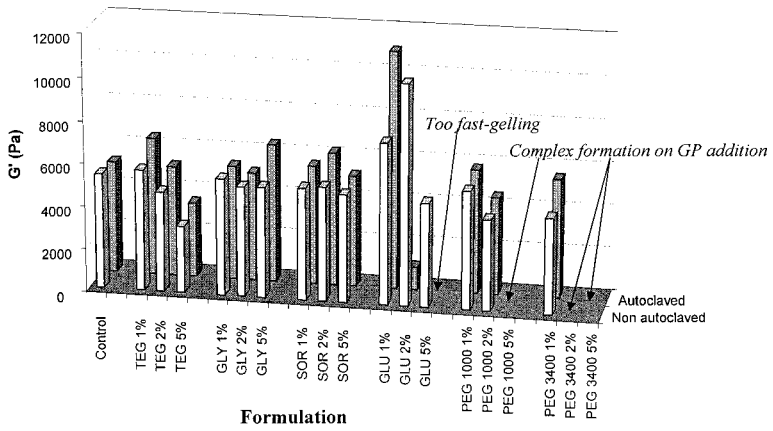

Fig. 2. Maximal Elastic Modulus (G'_m) of the Autoclaved and Non-autoclaved Chitosan/Additive Formulations

When precipitation upon GP addition was observed, the sample was not rheologically analyzed. The sample that gelled too rapidly were also not analyzed.

Table 3. Influence of the γ-Irradiation on the Characteristics of Chitosan/Additive Systems as Compared to a Non-irradiated Chitosan System (Irradiation of Frozen Solutions)

Additive (% w/v)	5 kGy			25 kGy		
	Viscosity loss[a)]	G'_m variation[b)]	M_w loss	Viscosity loss[a)]	G'_m variation[b)]	M_w loss
No additive	−50.6%	−98.7%	41.7%	−88.6%	−21.3%	70.6%
1% GLY	−30.2%	−64.1%	15.6%	N.A.	−44.8%	34.8%
1% TEG	−32.9%	−62.9%	24.8%	−52.0%	−93.3%	36.9%

a) at 38.4 s^{-1}. *b*) Maximal elastic modulus after 170 min at 37 °C. N.A.: not available.

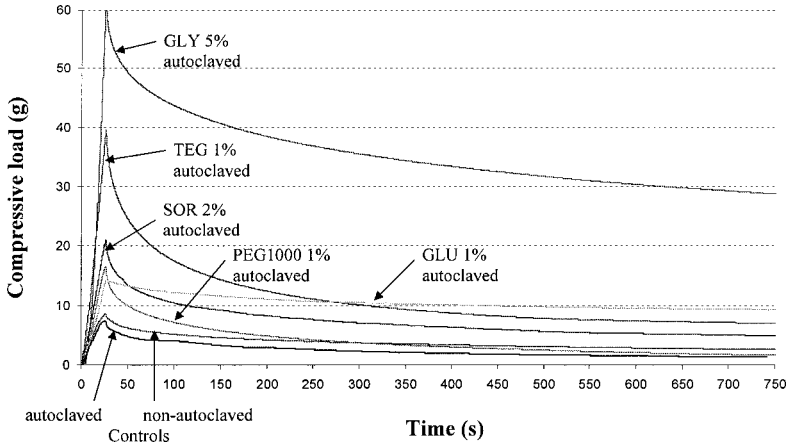

Fig. 3. Representative Examples of Autoclaved Chitosan/Additive-GP gels

The applied compressive deformation was 25%, and the compression rate was 1%/s.

process reduced the thermogelling properties of the chitosan-GP system (data not shown). Moreover, results were hardly reproducible, suggesting that the system is very sensitive to variations in the dose received during irradiation and storage time after sterilization.

Mechanical Testing With the exception of GLU samples, all specimen compression curves showed a similar profile (Fig. 3). However, variable stress-relaxation values in maximal and equilibrium load, and relaxation time were observed. The relaxation rate was slightly more important for autoclaved chitosan-GP, chitosan-GP/TEG and chitosan-GP/SOR samples, whereas the chitosan-GP/GLU system had the weakest relaxation rate (*i.e.* half the relaxation rate of autoclaved chitosan-GP gels). The relaxation factor R (where R equals equilibrium load/maximal load) was 0.18 and 0.30 for autoclaved and non-autoclaved control chitosan-GP samples, respectively (Table 4). R values of 0.18—0.47 were obtained with autoclaved chitosan-GP samples containing TEG, SOR or GLY. Chitosan/GLU-GP samples had a R factor of 0.65 and clearly showed a reduced relaxation capacity. Thus, the addition of polyols to chitosan solutions significantly improved the mechanical properties of the resulting gels, either before or after steam sterilization.

Discussion

Chenite *et al.*[16)] have shown that the mechanisms of gelation of chitosan-GP solutions is governed by delicate interplay between the pH and temperature. Usually chitosan solu-

Table 4. Maximal Compression and Equilibrium Load of Autoclaved Chitosan/Additives Samples

Additives	Maximal compression load	Equilibrium load	*R*
Autoclaved control	7.49	1.36	0.18
Non-autoclaved control	8.69	2.63	0.30
1% GLU	14.23	9.30	0.65
5% GLY	61.44	28.90	0.47
2% SOR	20.99	4.90	0.23
1% TEG	39.56	6.98	0.18
1% PEG_{1000}	16.55	1.65	0.10

R=equilibrium load/maximal compression load.

tions precipitate upon increasing the pH above 6.5. The precipitation of chitosan can be explained by a reduction of charge density along the polymer backbone reducing interchain electrostatic repulsion and allowing attractive and hydrogen-bonding force to predominate, and precipitate the polymer. However, at low temperatures when GP is used to increase the pH, chitosan remains in solution even at pH values of about 7.2. The glycerol moiety of GP is thought to separate the chitosan chains in solution and maintain the polymer solubility. Upon heating the chitosan-GP system undergoes sol–gel transition. Several hypothesis have been proposed to explain this rather unusual behavior. Indeed, this thermally induced shift may result from (1) reduced chitosan chain polarity and increased hydrophobicity upon heating; (2) reduced polarity and increased structuring of free water by the glycerol moiety of GP, thus dehydrating chitosan and also causing increased interchain hydrophobic attraction; and (3) thermally induced transfer of protons from chitosan amine groups to the phosphate moiety. Because chitosan-GP solutions are intended to be used as injectable delivery systems, it is prime importance to study the effect of sterilization on the gelling properties. However, owing to their thermosensitive behavior chitosan-GP solutions cannot generally be simply sterilized by autoclave. Thus, it is preferable to sterilize separately the chitosan and the GP solution.

In a previous study on autoclaving chitosan solutions, we reported a decrease of viscosity and a reduction by 30% of M_w, but the thermogelling character still remained clearly observable even after a 60-min autoclaving.[21] Yen and Sou[25] showed that mannitol, sorbitol and glycerol limited the temperature-driven viscosity reduction over time of a chitosan solution maintained at 55 °C. Thus, it was suggested that such polyols might act as stabilizing agents for autoclaved chitosan compositions. However, they did not give any measurements of molecular weights or mechanical properties. Glycerol was also found to protect gelatin during irradiation.[24] In this work, we wanted to evaluate whether the addition of such polyols to the chitosan solution prior to steam sterilization had a protective effect on molecular weight and viscosity, and had an influence on the thermogelling properties of our particular system. The driving hypothesis was that polyols, by modifying the structuring effect of water molecules around chitosan chains, might protect the polysaccharide from heat-induced degradation and/or improve the gel performances after sterilization.

In general, the addition of polyols had a modest impact on inhibiting chain scission upon heating, as demonstrated by molecular weight analysis (Table 2) and viscosity data (Fig. 1). Despite a reduction of viscosity following autoclaving, some polyols (1% TEG, 5% GLY, 2% SOR, 1% PEG_{1000} or 1% PEG_{3400}) did improve, at specific concentrations, the thermogelling properties of the chitosan-GP system. High GLU contents was also efficient in inhibiting the autoclaving-induced viscosity reduction, and provided the solution with a fast-gelling character. However, reducing sugars such as GLU are known to undergo Maillard reaction with amino groups under heat and acidic conditions.[31] Indeed, during steam sterilization, the initially transparent chitosan/GLU solutions turned yellowish/brownish. Thus, GLU should not be added to chitosan solutions during autoclaving as it fully changes their characteristics.

It is still unclear how polyols can protect chitosan chains from hydrolytic degradation during autoclaving and improve the mechanical strength of the gel. Back *et al.*[23] evaluated the protective action of polyols on proteins during heat treatment. They proposed that the stabilization of proteins to heat denaturation is due to the effect of polyols on hydrophobic interactions. Indeed, the presence of polyols in protein solutions induced a change in the structure of water that increased the strength of hydrophobic interactions. Na[32] explained that such a stabilizing effect of glycerol of native state of proteins was due to the repulsion between glycerol and the hydrophobic groups of the protein. In the native state, hydrophobic groups tend to form micelle core-like structures that are sequestered from the solvent, whereas in the denatured state, hydrophobic groups are more exposed to it. The presence of glycerol within the solvent helps maintaining the protein in its native form. Similarly, polyols may favor a more compact random coil conformation by structuring water molecules around chitosan chains. Such a compact conformation may be less prone to hydrolysis upon heating although, as demonstrated by our data, the protective effect of polyol on chain scission was minimal.

Since all chitosan solutions experienced significant hydrolysis upon autoclaving, the improved gelling properties of some systems cannot be attributed to a change in molecular weight. Indeed, tougher chitosan gels are generally obtained through the use of high molecular weight polymers (internal data). Hydroxymethyl chains of polyols have been shown to be an important factor for stabilizing and strengthening gels, and the stabilizing effect seems to be related to the number of hydroxyl groups.[33] It is however difficult to predict the effect of a polyol based on its chemical structure since polyols of distinct nature have been found to present similar stabilizing effect.[33] In the present thermogelling system, sorbitol, a linear polyol bearing more hydroxyl groups than glycerol, did not demonstrate better stabilizing and gel strengthening properties. In addition at low concentrations (1%), PEG stabilized and strengthened chitosan-GP gels, suggesting possible cross-linking of chitosan chains *via* hydrogen bonding with ethylene oxide units.

Polyols were found to improve the thermal stability of thermoresponsive carrageenan gels (increase in sol–gel transition),[33] as previously observed for proteins (*e.g.* collagen) in aqueous solutions.[34] The polymer–solvent interactions seemed to play an important role in the stabilization of polysaccharide gels in the presence of polyols.[33] These com-

pounds have been shown to be water-structuring and antagonist to hydrophobic groups, thus possibly enhancing the chemical potential of hydrophobic moieties of chitosan and favoring the random association of chitosan chains in solution. However, other interactions such as hydrogen bonding may be affected by the addition of polyols and thus, at this stage, it is difficult to propose a definite mechanism of action.

Gamma-irradiation of chitosan solutions at 4 or 20 °C degraded the polymer. In order to limit the hydrolysis occurring in aqueous solution during irradiation, all the samples were irradiated in the frozen state at −80 °C. At this temperature, degradation was slightly less important when TEG or GLY was added to the solution, but the results were variable, suggesting that there were some post-irradiation effects, even if the samples were stored at −80 °C. Rosiak *et al.*[35] also reported that, upon storage, irradiated chitosan samples had further reduction of their average molecular weights. Lim *et al.*[17] found that irradiation of chitosan films or fibers generated radicals within the polymer and led to an oxidative degradation. However they did not investigate the time-related evolution of this degradation. The presence of radicals in chitosan solution may induce a degradation, which persists after irradiation.

Conclusion

The degradation of chitosan following steam sterilization was not completely eliminated by the presence of polyol additives. The incorporation of some polyols to chitosan solutions prior to autoclaving and GP addition, allowed a slight reduction in viscosity loss, and improved mechanical properties of the resulting chitosan-GP based gels. Overall, the autoclaving process reduced the viscosity of chitosan solutions by 20—50%. Mechanical tests showed that polyol additives could enhance the mechanical properties of chitosan-GP materials. The ability of such polyols to influence the viscosity, gelling characteristics and mechanical properties after autoclaving may be very useful for the future development of biomedical chitosan-GP formulations. Gamma-irradiation would not seem to be a good alternative for the sterilization of chitosan solutions (even in presence of additives), since it massively degraded the polymer and failed to provide a chemically stable sterile product.

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