Carbohydrate Polymers 103 (2014) 267–273

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Novel hydrogels of chitosan and poly(vinyl alcohol)-g-glycolic acid copolymer with enhanced rheological properties

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ARTICLE INFO

Article history:
Received 14 October 2013
Received in revised form
12 December 2013
Accepted 14 December 2013
Available online 21 December 2013

Keywords:
Chitosan
PVA grafting
Glycolic acid
Hydrogels
Rheology
Interactions

ABSTRACT

Poly(vinyl alcohol) (PVA) has been grafted with glycolic acid (GL), a biodegradable hydroxyl acid to yield modified poly(vinyl alcohol) (PVAGL). The formation of hydrogels at pH = 6.8 and physiological temperature through blending chitosan (CS) and PVAGL at different concentrations has been investigated. FTIR, DOSY NMR and oscillatory rheology measurements have been carried out on CS/PVAGL hydrogels and the results have been compared to those obtained for CS/PVA hydrogels prepared under the same conditions. The experimental results point to an increase in the number of interactions between chitosan and PVAGL in polymer hydrogels prepared with modified PVA. The resulting materials with enhanced elastic properties and thixotropic behavior are potential candidates to be employed as injectable materials for biomedical applications.

1. Introduction

Chitosan is an amino-polysaccharide obtained by alkaline deacetylation of chitin, a natural component of shrimp or crab shells. It is a biocompatible and biodegradable pH-dependent cationic polymer able to form hydrogels which are widely employed in biomedical applications (Boucard, Viton, & Domard, 2005; Chenite et al., 2000; Dash, Chiellini, Ottenbrite, & Chiellini, 2011; Hao et al., 2010; Islam, Yasin, Bano, & Riaz, 2012; Jayakumar, Menon, Manzoor, Nair, & Tamura, 2010; Khor & Lim, 2003). Nowadays, the development of responsive chitosan hydrogels to external stimuli such as pH, temperature, electric field, ionic strength, salt type, etc., is a very active area of research (Carreira, Gonçalves, Mendonça, Gil, & Coelho, 2010; Crompton et al., 2005; Giri et al., 2012; Klouda & Mikos, 2008). When chitosan aqueous solutions are combined with other components like polyol salts (Chenite et al., 2000) and polyhydroxy polymers (Tang, Zhao, Li, & Du, 2010), thermoresponsive chitosan hydrogels, which are responsive not only to pH but also to temperature, are obtained. These thermosensitive hydrogels are fluid at room temperature and become a transparent gel at temperatures above 35 °C and, therefore, they can be employed as injectable materials for applications such as drug delivery or tissue engineering.

PVA has a prime position in biomaterials science because of its inherent nontoxicity, noncarcinogenicity, good biocompatibility, and desirable physical properties such as rubbery or elastic nature and a high degree of swelling in aqueous solutions (Abdel-Mohsen, Aly, Hrdina, Montaser, & Hebeish, 2011; Hernandez, Lopez, Mijangos, & Guenet, 2002; Ma, Xiong, Miao, Zhang, & Peng, 2009; Pawde & Deshmukh, 2008). We have recently reported the results of the chemical modification of PVA with different hydroxyacids (Lejardi, Etxeberria, Meaurio, & Sarasua, 2012). The modification reaction moves away the hydroxyl groups bonded to the chain skeleton of PVA to the end of the lateral graft chains avoiding to some extent the tendency for intra-molecular association (auto-association) of PVA and favoring the intermolecular interactions via hydrogen bonds when blending with a specific polymer pair.

It is well known that chitosan and modified chitosan derivatives present antibacterial and antifungal activities (Kong, Chen, Xing, & Park, 2010; Xu et al., 2011), therefore CS/PVA blends

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0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.12.040

present applications as materials for wound dressing (Tahtat et al., 2011). Other applications of blends of PVA and chitosan include bioseparation (Yang, Su, Leu, & Yang, 2004), drug delivery (Zu et al., 2012) and tissue engineering (Koyano, Minoura, Nagura, & Kobayashi, 1998). Furthermore, aqueous solutions of blends of chitosan with poly(vinyl alcohol) give rise to thermosensitive chitosan/PVA hydrogels at physiological temperature and pH. In these materials, the PVA concentration and pH are directly related to the transition temperature sol–gel and the gel time. In the absence of PVA, chitosan precipitates at basic pH instead of forming a gel occupying all the volume offered by the solvent (Tang, Du, Hu, Shi, & Kennedy, 2007). Because of the simplicity of pharmaceutical formulation by solution mixing, biocompatibility with biological systems, and convenient administration, the pharmaceutical and biomedical uses of these hydrogels include solubilization of low-molecular-weight hydrophobic drugs, controlled release, labile biomacromolecule delivery, such as proteins and genes, cell immobilization, and tissue engineering (Bhattarai, Gunn, & Zhang, 2010; Jeong, Kim, & Bae, 2012).

Based on this approach, and through the study of polymer-polymer interactions, the purpose of this work is to develop and characterized novel hydrogels based on blends of modified PVA/chitosan. Initially, PVA was modified with glycolic acid since it can be easily functionalized due to its various hydroxyl groups (Islam & Yasin, 2012). Then, hydrogels were prepared by blending modified PVA with chitosan. The formation of intermolecular hydrogen bonds should be favored due to reduced steric hindrances in PVA. Therefore intermolecular interactions between chitosan and chemically modified PVA should increase leading to a miscibility improvement in regard to those between chitosan and neat PVA.

The gel structure (or properties) arising from the interaction between two polymers as in the case of PVA and CS is not easy to determine by Fourier Transform Infrared Spectroscopy (FT-IR). Nuclear magnetic resonance (NMR) spectroscopy is a suitable technique for studying interactions because a single analysis can yield a vast amount of structural information without destroying the sample. Nearly all molecules have NMR-active nuclei, meaning the technique is universal and analyze derivatization is unnecessary. Furthermore, analyzing blends intact without effecting a separation simplifies and quickens the analysis (Lucas & Larive, 2004). This technique has already been used by other researchers to determine carbohydrate-carbohydrate (Santos et al., 2009) and π–π stacking interactions (de Arenaza et al., 2013) in different systems. In this paper, diffusion-ordered NMR spectroscopy (DOSY) will be employed as an alternative technique to determine the interactions present in the modified PVA/chitosan hydrogels. The results obtained will be corroborated through oscillatory dynamic rheology.

2. Materials and methods

2.1. Materials

Chitosan (CS) of low molecular weight (degree of deacetylation = 75%) and poly(vinyl alcohol) (PVA) powder with an average of molecular weight 30,000 g/mol and a saponification degree of 98% were employed. The hydroxy acid selected to modify the PVA chains was the glycolic acid. All of them were supplied by Sigma–Aldrich. The chemical structures corresponding to all reagents are shown in Scheme 1.

2.2. Chemical modification of PVA

Poly(vinyl alcohol) was modified with glycolic acid. 10 g of PVA were added to 50 mL of distilled water and dissolved for 1 h at 80 °C. 40 g of glycolic acid were slowly added to the reactor. The esterification process was carried out in two stages, firstly the solution was maintained at 100 °C for 2 h, and then water was removed till dryness by evaporation at about 120 °C. The product was redissolved in water at 25 °C and reprecipitated twice in acetone (10 times in volume) to remove unreacted acid and the poly(glycolic acid) chains formed as by-product. Finally, the modified PVA was dried under vacuum at 30 °C for 24 h and named PVAGL.

2.3. Preparation of chitosan/PVA hydrogels

Hydrogels were prepared using the method described by Tang et al. (2007). In a specific example, 200 mg of chitosan were dissolved in 0.1 M HCl (10 mL) to yield a 2% (w/w) aqueous solution and cooled in an ice bath for 15 min. PVA or modified PVA (PVAGL) were dissolved in distilled water at various concentrations ranging from 1 to 0.250% (w/w) PVA. 1.0 M $NaHCO_3$ was added to each of the PVA solutions in order to basify the solutions to pH = 6.8. Aliquots of the chitosan solution and PVA solution were slowly mixed under constant agitation in an ice bath. Afterwards samples were kept in an oven for 37 °C to form the gels. Samples were denoted as PVAGL/CS or PVA GL/CS and the weight ratio between both components is reported in Table 1.

2.4. FT-IR analysis

Infrared spectra of samples were recorded on a Nicolet AVATAR370 Fourier transform infrared spectrophotometer (FTIR). Chitosan, PVA and the dried gel were triturated with KBr in the ratio of 1:100 and pressed to form pellet samples.

2.5. Nuclear magnetic resonance (NMR) measurements

^{1}H and ^{13}C NMR spectra were recorded in a Bruker Avance DPX 300, which corresponds to 300.16 and 75.5 MHz frequencies for ^{1}H and ^{13}C, respectively, in 5 mm sample tubes (o.d.) using 0.7 ml of deuterated dimethyl sulfoxide (DMSO-d6) at room temperature (≈30 °C).

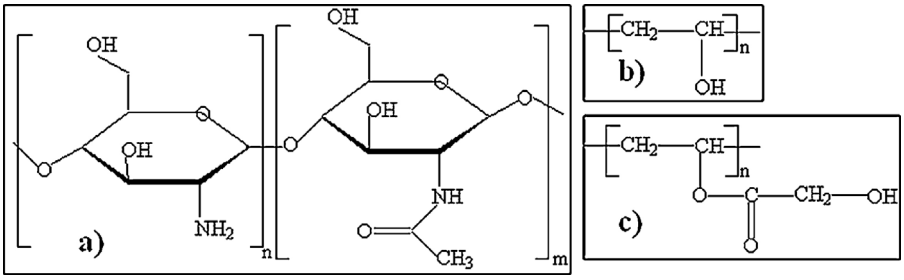
DOSY experiments were recorded on a Bruker 400 MHZ instrument at 4 °C. Concentrations of about 0.17 mM of PVA, 0.72 mM of CS and 0.17 mM of PVAGL were used. Chemical shifts were referenced to external 2,2-dimethyl-2-silapentane-5 sulfonic acid (DSS) in D_2O. 1D ^{1}H spectra were acquired by use of 32 K data points, which were zero-filled to 64 K data points prior to Fourier transformation. Spectra were processed with the aid of the Bruker TOP-SPIN program.

2.6. Rheological measurements

Oscillatory viscoelastic measurements were performed in a AR-G2 TA Instruments rheometer, using the 40 mm parallel-plate shear mode to measure the storage modulus, G', the loss modulus, G'' and the loss tangent, $\tan\delta$. To avoid evaporation of solvent in the rheometer geometry, a special solvent trap from TA Instruments was used. Given the fact that the formation of the gel takes place immediately after the solution is left upon to rest at 37 °C, the gel was directly formed on the lower rheometer plate by placing the CS/PVA or CS/PVAGL aqueous solutions at this temperature.

The linear viscoelastic region, defined as the region in which the storage and the loss modulus are independent of the strain amplitude, was located with the aid of strain sweeps (0.01–2000% strain) at constant frequency of 1 Hz. Once the % strain reached 2000% a second strain sweep from 2000 to 0.01% strain was applied to evaluate the thixotropic properties of the gels under study.

Isothermal frequency sweeps extending from 0.01 to 20 Hz were carried out at a constant temperature (37 °C) and a constant 1%



Scheme 1. Chemical structures of (a) chitosan (CS), (b) poly(vinyl alcohol) (PVA) and (c) modified poly(vinyl alcohol) (PVAGL).

Table 1
Concentration of poly(vinyl alcohol), chitosan in different blends and ratio between both components.

Sample name of the gel	Ratio between PVA (or PVAGL)/CS (v/v) in the gel	Concentration of PVA (or PVAGL) (% w/w) in the gel	Concentration of CS (% w/w) in the gel
CS/PVA (or PVAGL) 0.125	50/50	0.125	1
CS/PVA (or PVAGL) 0.250	50/50	0.250	1
CS/PVA (or PVAGL) 0.375	50/50	0.375	1
CS/PVA (or PVAGL) 0.500	50/50	0.500	1

strain located in the linear viscoelastic region. Each measurement was repeated three times to ensure reproducibility of the results.

3. Results and discussion

3.1. Degree of modification of PVAGL

Modified poly(vinyl alcohol) was thoroughly characterized by ^{1}H and ^{13}C based in the paper recently published by our group (Lejardi et al., 2012).

Table 2 lists relevant information regarding modified poly(vinyl alcohol), such as glycolic acid molar fraction, sequence distribution, degree of substitution of PVA (DS), random character (η) and degree of polymerization (DP). The analysis details of the ^{1}H and ^{13}C NMR spectra of modified PVA can be found in a previous reference from the group (Lejardi et al., 2012).

3.2. Gel formation

A simple test tube inverting method was employed to determine the occurrence of sol–gel transition. The sol phase was defined as a flowing liquid, and the gel phase, as a non-flowing gel when the hydrogel solution in the test tube was inverted.

Fig. 1 shows the images obtained after keeping CS/PVA and CS/PVAGL hydrogels in an oven for 24 h at 37 °C. It can be verified the formation of gel for all the samples under study for the different concentrations of PVA while the concentration of chitosan was kept constant.

3.3. Analysis of the interactions between chitosan and PVA or PVAGL

The inter-molecular interactions through hydrogen bonding can be characterized by FTIR, because the specific interactions affect the local electron density and the corresponding frequency shift can be observed (Marsano, Vicini, Skopinska, Wisniewski, & Sionkowska, 2004). FTIR spectra of CS, PVA, PVAGL and their blends are shown in Fig. 2. A FTIR spectrum of pure PVA (Fig. 3 black curve) shows —OH stretching at about 3400 cm^{-1}, —CH stretching as in alkanes at 2900 cm^{-1} and —C—O stretching at about 1096 cm^{-1} (Pawde & Deshmukh, 2008). In the case of pure chitosan, the main absorption peaks of chitosan are attributed to C=O stretching (amide I) at 1635 cm^{-1}, to N—H bending (amide II) at 1530 cm^{-1}, and to C—N stretching (amide III) at 1320 cm^{-1}. The presence of these bands confirms that chitosan is a partially deacetylated product. The absorption peak at 1050 cm^{-1} is assigned to C—O stretching and broad and strong overlapped bands around 3430 and 3360 cm^{-1} were caused by stretching vibrations of hydroxyl and NH functional groups (Kadir, Aspanut, Majid, & Arof, 2011).

Respect to the modified PVA (PVA-GL), both the intense band located at about 1750 cm^{-1}, characteristic of the carbonyl stretching mode of esters, and the band located at about 1200 cm^{-1}, attributed to the C—O stretching mode of the ester moiety, suggest successful esterification. In the FTIR spectrum of CS/PVA and CS/PVA-GL blends the characteristic absorption band of chitosan at about 1530 (amide II) is shifted indicating the transition of $-NH_3^+$ to $-NH_2$ when $NaHCO_3$ is added to the chitosan-HCl solutions. In our case, the peak of the hydroxyl groups could not be used to evaluate the interactions because the OH band of the chitosan and the PVA were overlapped. Therefore, it was very difficult the measurement of the displacement of this band.

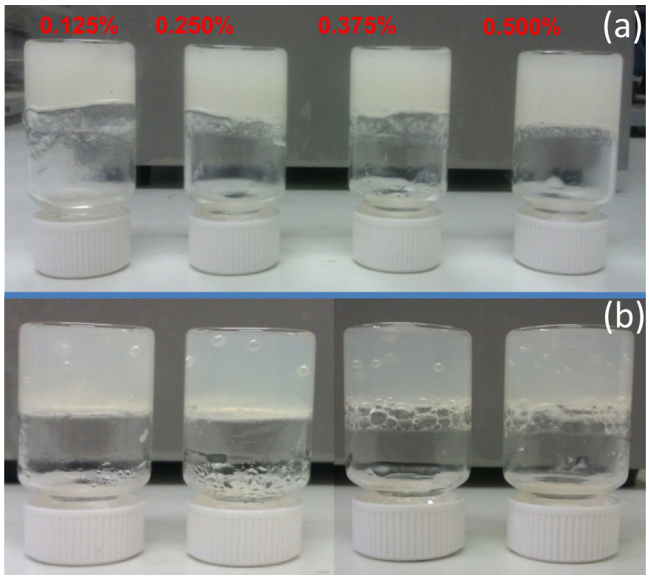


Fig. 1. Photographs of the hydrogels after keeping for a day in the oven at 37 °C. (a) PVA/chitosan and (b) PVAGL/chitosan.

Table 2
Hydroxyl acid molar fraction, sequence distribution, degree of substitution of PVA(DS), random character (η) and degree of polymerization (DP) in the grafted poly(vinyl alcohol).

	HA mole fraction	Main chain sequence mole fraction			DS	η	DS
	^{13}C	(PVA-PVA)	(PVA-PVAGL)	(PVAGL-PVAGL)			
PVAGL	0.32	0.556	0.385	0.059	0.25	1.02	1.35

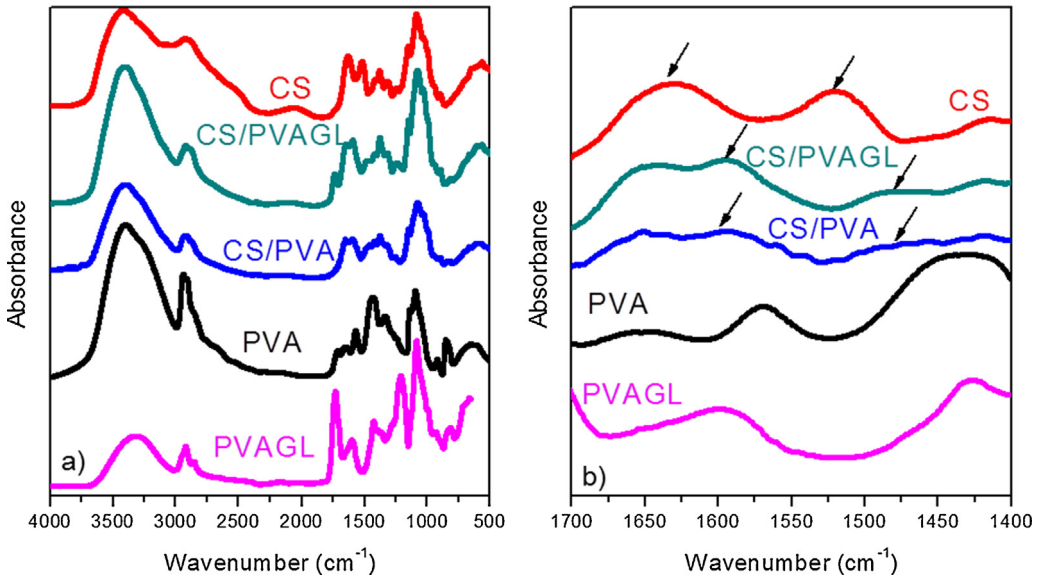


Fig. 2. (a) FTIR spectrum of PVA, CS, PVAGL, CS/PVA0.5, and CS/PVAGL0.5. (b) A magnification of the wavenumber region between 1700 and 1400 cm^{-1}.

As can be seen in Fig. 2b, the carbonyl stretching (amide I) of chitosan is located at about 1635 cm^{-1}, in good agreement with reported values (Hashemi Doulabi, Mirzadeh, & Imani, 2013). However, the blends exhibit two well resolved bands located at about 1639 cm^{-1} and 1593 cm^{-1}, that can be attributed to free and hydrogen bonded C=O groups. These results indicated that interactions were present between the carbonyl groups of chitosan and the hydroxyl groups of poly(vinyl alcohol) or modified poly(vinyl alcohol). In both blends the displacement of the band is similar but in the case of CS/PVAGL, this band is additionally shifted by about 8 cm^{-1} suggesting that hydrogen bonds are stronger in this system.

In order to demonstrate more accurately the interactions between the polymers DOSY experiments were carried out. Experiments were performed at 4 °C, since at this temperature the mixture was maintained in solution. DOSY (diffusion-ordered spectroscopy) is a pseudo two-dimensional (2D) spectrum that displays chemical shift along the horizontal axis and the calculated diffusion coefficients along the vertical dimension. The experimental

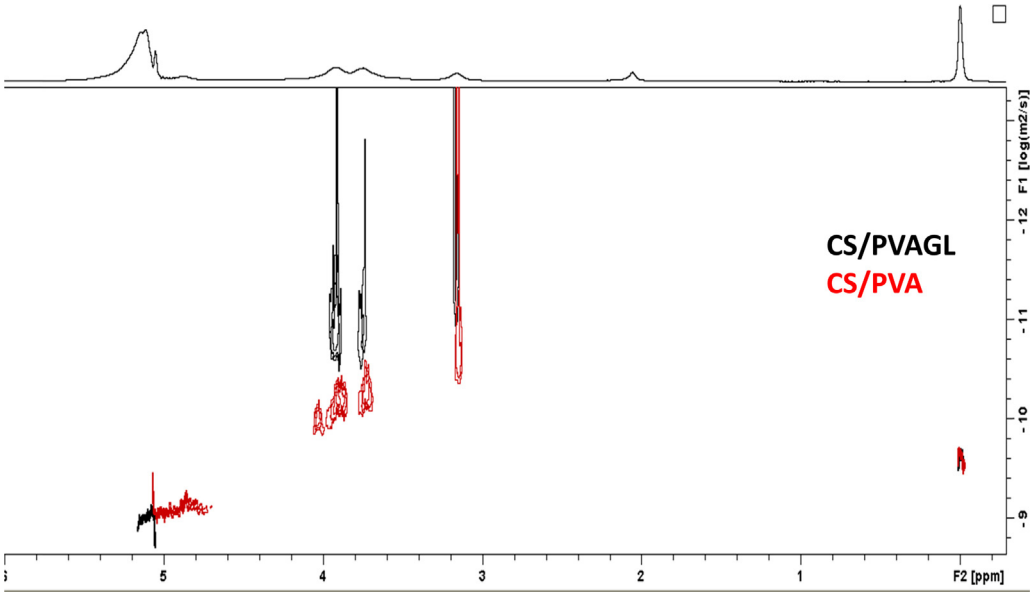


Fig. 3. The DOSY spectra of the solution CS/PVA0.5 (red) and the solution CS/PVAGL0.5 (black). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

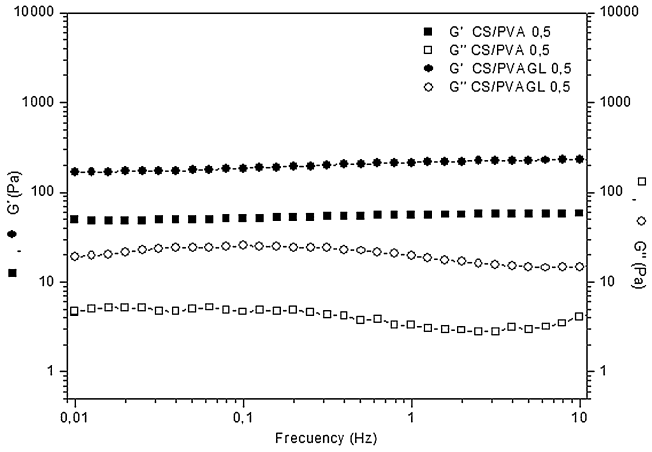


Fig. 4. Elastic modulus G' (closed symbols) and loss modulus G'' (open symbols) as a function of frequency for CS/PVA0.5 (■) and CS/PVAGL0.5 (●), at 37 °C.

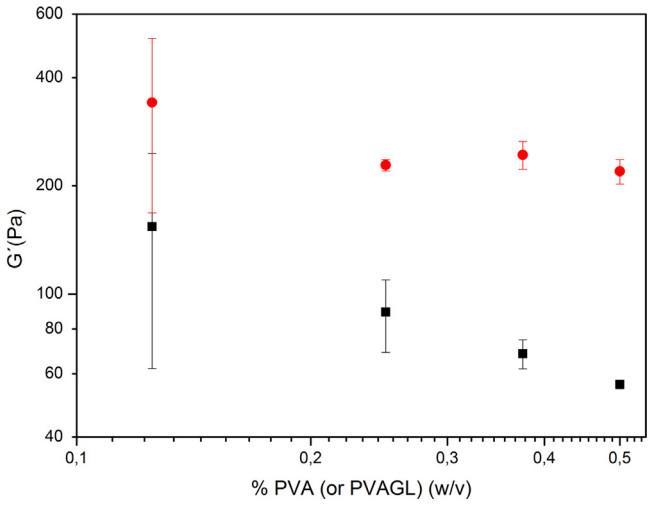


Fig. 5. Double logarithmic plot of the elastic modulus G' as a function of PVA concentration in the blend: (■) CS/PVA and (●) CS/PVAGL.

parameters δ and Δ (the delay time at which diffusion occurs) are optimized by the user and usually remain fixed throughout the experiment (Lucas & Larive, 2004). The purpose of this measurements was to determine the average value of diffusion coefficient and correlate these results with the interactions present in the different systems. If the interactions in the two systems are similar, the value of diffusion coefficient does not change. Fig. 3 shows the DOSY spectra of CS/PVA and CS/PVAGL solutions.

When the DOSY experiment was carried out for the CS/PVA solution, the average value of log D was 8.90. However in presence of modified PVA in the solution, the value was changed substantially to an average value of $\log D = 9.68$. The existence of more hydrogen bonds in the CS/PVAGL solution makes increase the $\log D$ value, since having more interactions, the molecules are further linked together and their diffusion is lower. This indicates that the modification of PVA with glycolic acid increases the interactions between chitosan and poly(vinyl alcohol) improving the formation of hydrogen bonds. These results corroborate that the formation of hydrogen bonds is favored in the case of CS/PVAGL hydrogel blends.

3.4. Rheological study

To be considered as a gel, a material must meet some requirements according to its rheological behavior (Hernández, Sarafian, López, & Mijangos, 2004; Kavanagh & Ross-Murphy, 1998): (i) its dynamic elastic modulus (G') must be relatively independent of the frequency of deformation and (ii) G' must be greater than the loss modulus (G'') at all frequencies.

These two characteristics are observed in Fig. 4 where G' and G'' are depicted as a function of frequency for CS/PVA0.5 and CS/PVAGL0.5. As can be observed, both samples present a gel-like behavior at $T = 37$ °C which is characterized by the frequency-independence of G' and G'', being G' higher than G'' at all frequencies. The elastic modulus corresponding to the sample CS/PVA0.5 is ~50 Pa and increases to ~200 Pa in the case of sample CS/PVAGL0.5.

In Fig. 5, the elastic moduli of both type of gels, CS/PVA and CS/PVAGL are represented as a function of PVA concentration in the blend.

As observed, the elastic modulus of the samples prepared with PVAGL is higher than the corresponding value of samples prepared with PVA, at all PVA concentrations. The increase of pH produces the precipitation of chitosan above pH = 6.2, these precipitates are prevented by the addition of PVA since its presence promotes the association of hydrophobic chitosan chains to form the gel at 37 °C as previously reported (Tang et al., 2007). Taking into account that the modification of chitosan via grafting of glycolic acid is reported as hydrophobizing modification of chitosan (Qu, Wirsén, & Albertsson, 1999), an increase in the number of hydrophobic interactions may be expected when PVAGL is mixed with chitosan in CS/PVAGL hydrogels with respect to CS/PVA hydrogels.

On the other hand, the introduction of bulky lateral groups in PVAGL favors the intermolecular hydrogen bonds between PVAGL and chitosan as previously demonstrated through FTIR and DOSY experiments. In contrast, for CS/PVA hydrogels, the increase in the concentration of PVA leads to a higher auto-association of PVA chains through intramolecular hydrogen bonds. As a conclusion, the increase in the elastic modulus of CS/PVAGL hydrogels with respect to CS/PVA hydrogels can be explained because of the combined effect of the interactions of the hydrophobic side chains together with improved hydrogen bonding between the carbonyl groups of PVAGL and the amino groups of chitosan.

Shear-thinning of a colloidal suspension could enable a more homogeneous and easy delivery of the material to the body in the case of injectable materials (Guvendiren, Lu, & Burdick, 2012). Moreover, the recovery of the elastic properties immediately after injection may prevent the flow of the colloidal solution and facilitate that the material remains on the target site (Yan et al., 2012).

A possible recovery of the elastic properties after shear-thinning was explored by comparing up and down strain sweeps carried out on the same sample. Fig. 6 shows the up and down strain sweeps carried out on sample CS/PVA0.5 and CS/PVAGL0.5.

As a general behavior, both samples present, $G' > G''$ up to a critical deformation, γ_0, where G' starts to decrease, then the crossover of G' and G'' takes place indicating a change from a solid-like to a liquid-like behavior. The value of the elastic modulus plateau is higher for sample CS/PVAGL than for sample CS/PVA, in agreement with results obtained from frequency sweeps. The value of critical deformation, γ_0 for both samples is located at 90 Pa.

Interestingly, the sample CS/PVAGL0.5 presents a constant value of G'' up to a 90% of strain where G'' starts to increase giving rise to a maximum, G''_{max} of 98 Pa at a 180% of strain for 182% of strain followed again by a decrease (Fig. 6b). This behavior is known as a weak-strain hardening and it is directly related to the energy dissipation due to the breakage of a complex structure. The sample CS/PVA0.5 (Fig. 6a), does not present this maximum, therefore, the energy dissipated to break the rigidity of the network is lower for this sample which is related to its lower network elasticity.

For both samples, immediately after shear-thinning, as the strain diminishes a recovery of the elastic properties is observed, moreover the recovery is almost complete in the case of the sample

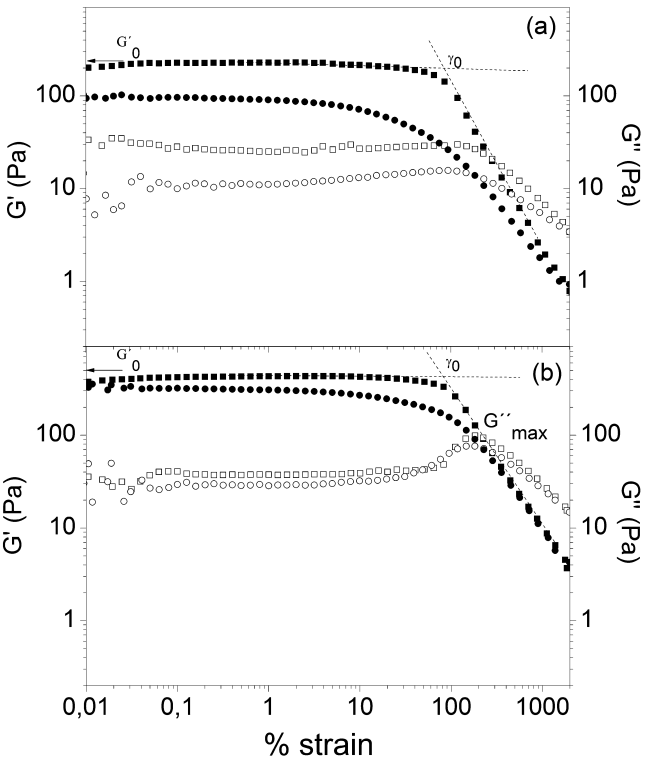


Fig. 6. Evolution of the elastic modulus (full symbols) and loss modulus (empty symbols) as a function of strain for (a) CS/PVA0.5 and (b) CS/PVAGL0.5. First strain sweep (squares) and second strain sweep (circles).

CS/PVAGL0.5 (Fig. 6b) whereas in the case of the sample CS/PVA0.5 (Fig. 6a) the elastic modulus reached in the second strain sweep diminishes with respect to the elastic modulus obtained during the first strain sweep.

4. Conclusions

Chitosan was successfully blended with modified PVA with glycolic acid (GL) yielding composite chitosan/PVA hydrogels at different PVA compositions ranging from 0.125 to 0.5 wt%. The hydrogel formation took place at pH = 6.8 and physiological temperature for all the compositions tested. Initially, a simple test tube inverting method verified the formation of gel for all samples at different PVA concentrations at 37 °C. That was corroborated by rheological measurements where both samples showed the frequency-independence of G' and G'', being G' higher than G'' at all frequencies.

FTIR was used to characterize the inter-molecular interactions through hydrogen bonding between CS and PVA or PVAGL and results showed that there were interactions between the carbonyl groups of CS and the hydroxyl groups of PVA or PVAGL. The displacement of the band is higher (by about 8 cm^{-1}) in the CS/PVAGL sample indicating that hydrogen bonds are stronger than in the CS/PVA sample. DOSY-NMR results corroborated that the formation of hydrogen bonds is favored in CS/PVAGL hydrogels. This resulted in a higher elastic modulus obtained for samples CS/PVAGL at all PVA concentrations as determined through dynamic oscillatory measurements. Thixotropic behavior was also analyzed for the samples under study and a recovery of the elastic properties was observed for CS/PVA and CS/PVAGL after shear-thinning, however only the sample CS/PVAGL presented an almost complete recovery of the initial elastic modulus.

Acknowledgements

Financial support from MEC (MAT 2011-24797), MICINN (BIO2010-21542-C02-01) and Basque Government (GIC13/161-IT-632-13 and IT618-13) are gratefully acknowledged. Rebeca Hernández thanks MEC for a Ramón y Cajal contract and Miryam Criado for a FPU fellowship.

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