Mechanical and Drug Delivery Properties of a Chitosan–Tartaric Acid Hydrogel Suitable for Biomedical Applications

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Received 28 January 2011; accepted 16 March 2011
DOI 10.1002/app.34513
Published online 8 August 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The present investigation describes the synthesis and the characterization of a novel highly stable polysaccharidic hydrogel system, designed for modified drug delivery. Gels and films based on the biodegradable and biocompatible chitosan (CS) were prepared by a crosslinking reaction between the polysaccharide amino groups with tartaric acid, using the short-range crosslinkers 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as coupling agents. The aim of the study is to characterize the novel CS hydrogel by means of rheological and mechanical measurements; *in vitro* swelling, release, and degradation studies were also carried out. Obtained results show how the structure of the obtained networks can deeply affect dynamomechanical properties of the hydrogel as well as the delivery rate of loaded model drugs. The mechanical characterization of the hydrogel, in the form of films, indicates that the film elasticity increases as the water content in the hydrogel increases. Rheological studies evidenced that the different network structures can affect the elastic modulus of the system. Release studies of two model molecules, i.e., vitamin B12 and blue dextran, of different steric hindrance were carried out using both the bulk gel and the film. *In vitro* release of both drugs was evaluated in water and in Hepes to assess the suitability of this novel drug delivery system for pharmaceutical applications. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 842–849, 2012

Key words: chitosan; hydrogel; film; rheology; mechanical properties

INTRODUCTION

During the last decades, within the field of drug delivery, much effort has been devoted to the development of special systems capable to avoid or minimize the side effects of drugs and to improve the efficacy of therapies and patient compliance. In this sense, hydrogels appear to be very attractive for their peculiar physicochemical characteristics[1] and also because, under mild conditions, they can incorporate drugs that can then be released at a controlled rate. In particular, hydrogel-forming polysaccharides are attractive materials, because they can be obtained from natural sources and are, in most cases, biocompatible.[2] Chitosan (CS) is a well-known polysaccharide, and it is one of the most studied polymers in the field of pharmaceutics. CS is obtained by the alkaline partial deacetylation of chitin, which is an important constituent of the exoskeleton of animals, such as crustaceans, molluscs, and insects,[3] and it can be produced at low costs.[4] CS is a linear copolymer (Fig. 1) of β(1–4) linked 2-acetamide-2-deoxy-β-D glucopyranose (*N*-acetyl-glucosamine) and 2-amino-2-deoxy-β-D-glucopyranose (glucosamine).

CS exhibits several interesting biological activities: hypocholesterolemic, antimicrobial, and wound healing properties have been reported. It can also be useful for other applications such as waste water treatment, functional membranes, flocculation, metal chelation, as well as in food, cosmetics, and agricultural industries.[5–8] Because of low production costs, good biocompatibility, low immunogenicity, low or no toxicity, and biodegradability,[9,10] CS gained importance also in the pharmaceutical field for various

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Contract grant sponsor: UCM (Spain); contract grant number: PR27/05-13878.
Contract grant sponsor: FIRB, Fondo per gli Investimenti della Ricerca di Base, Research Program: Ricerca e Sviluppo del Farmaco (CHEM-PROFARMA-NET); contract grant number: RBPR05NWWC_003.

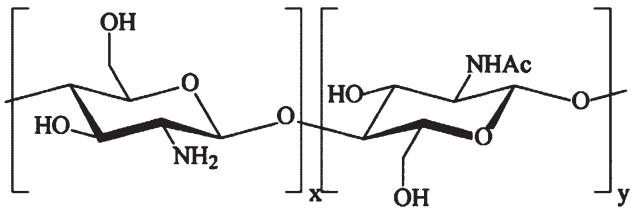

Figure 1 Repeating unit of chitosan.

technological and biomedical applications such as excipient for tablet formulations,[11] topical ocular applications,[12] implantation,[13] intra-articular injection,[14] tissue engineering, and drug or gene delivery systems.[15–17]

Numerous CS hydrogels were developed by means of crosslinking of CS chains. Actually, CS can form both physical and chemical hydrogels by ionic interactions[18,19] or by means of chemical crosslinking using low molecular weight moieties,[20–23] respectively. Hydrogel-based pharmaceutical formulations have been developed for both systemic and local applications, such as implants and oral, buccal, rectal, ocular, topical, and transdermal delivery.[1,24]

1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) represent a chemical crosslinking system (EDC/NHS) frequently used in CS hydrogel formation; in this case, the amidic group of CS is crosslinked by means of a low molecular weight species bearing two or more carboxylic moieties.[25–28] In this sense, tartaric acid (TA) was used as low molecular weight covalent crosslinker to obtain biodegradable CS nanoparticles, which can act as bioadhesive cationic polyelectrolytes systems being good candidates for carrying negatively charged bioactive molecules. The degradability of polysaccharides multilayer films of chitosan and hyaluronan crosslinked with EDC and sulfo-NHS in the rat oral environment was investigated[29]: these chemically crosslinked films were resistant, *in vitro*, to enzymatic degradation up to 18 h; while *in vivo*, this degradation was much slower leading to a 3-days resistant system. Phosphorylated chitosan, synthesized by graft copolymerization by using EDC as a crosslinker, was employed for the preparation of beads suitable as controlled drug release systems for oral administration and as scaffolds for tissue engineering.[30]

In the present investigation, an easy and simple procedure for the preparation of chemical hydrogels (CHs) based on CS chains crosslinked with TA using the EDC/NHS system is described. Chemically crosslinked chitosan films (CFs) were also obtained starting from CH. The two systems, CH and CF, were characterized in terms of water uptake/degradation, rheological, and mechanical properties. The network was also tested for its suitability as drug delivery system, using vitamin B12 (Vit.B12) and blue dextran (BD) as model drugs of remarkably different steric hindrance. Collected data suggest that the novel systems can be proposed for biomedical applications.

MATERIALS AND METHODS

Materials

Chitosan (CS) was supplied by Guinama (Valencia, Spain) with a deacetylation degree of 97.0% and a viscosity of 92.0 cSt. The molecular weight, 1.0×10^5, was estimated by viscometric measurements. 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), and tartaric acid (TA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Vitamin B12 (Vit.B12) (molecular weight: 1355) was purchased from Fluka (Germany). Blue dextran (BD) (molecular weight: 2×10^6) was provided by Sigma-Aldrich Co. (St. Louis, MO, USA). All other products and reagents were of analytical grade. Distilled water was always used.

Preparation of chitosan chemically crosslinked hydrogels

CHs were prepared following a three-step procedure:

Polymer solution preparation

An appropriate amount of CS (typically 0.15 g) was dissolved in 4.3 mL of 0.1*M* sodium acetate/acetic acid buffer solution (pH 4.0) under magnetic stirring at room temperature for 12 h.

Crosslinking agents and tartaric acid solutions preparation

EDC (0.492 g)/NHS (0.289 g) and TA (0.0126 g) were separately dissolved in two vials, under gentle magnetic stirring at ambient temperature in 0.3 and 0.1 mL of sodium acetate/acetic acid pH 4.0 buffer solution, respectively, to obtain the stoichiometric ratios r of 3/1 (r: moles of crosslinkers/mol of CS glucosamine units and r' of 0.1/1 (r': moles of TA/mol of CS glucosamine units). These r and r' values correspond, according to preliminary tests, to the minimum values to obtain self-sustaining hydrogels at the tested CS concentration.

Chemical hydrogel formation

After complete solubilization, EDC/NHS and TA solutions were added to the CS solution kept under vigorous magnetic stirring for 1 min. The resulting homogeneous solution was immediately poured into glass mold (diameter: 35 mm), and the formed CH was cured overnight, until the reaction completion

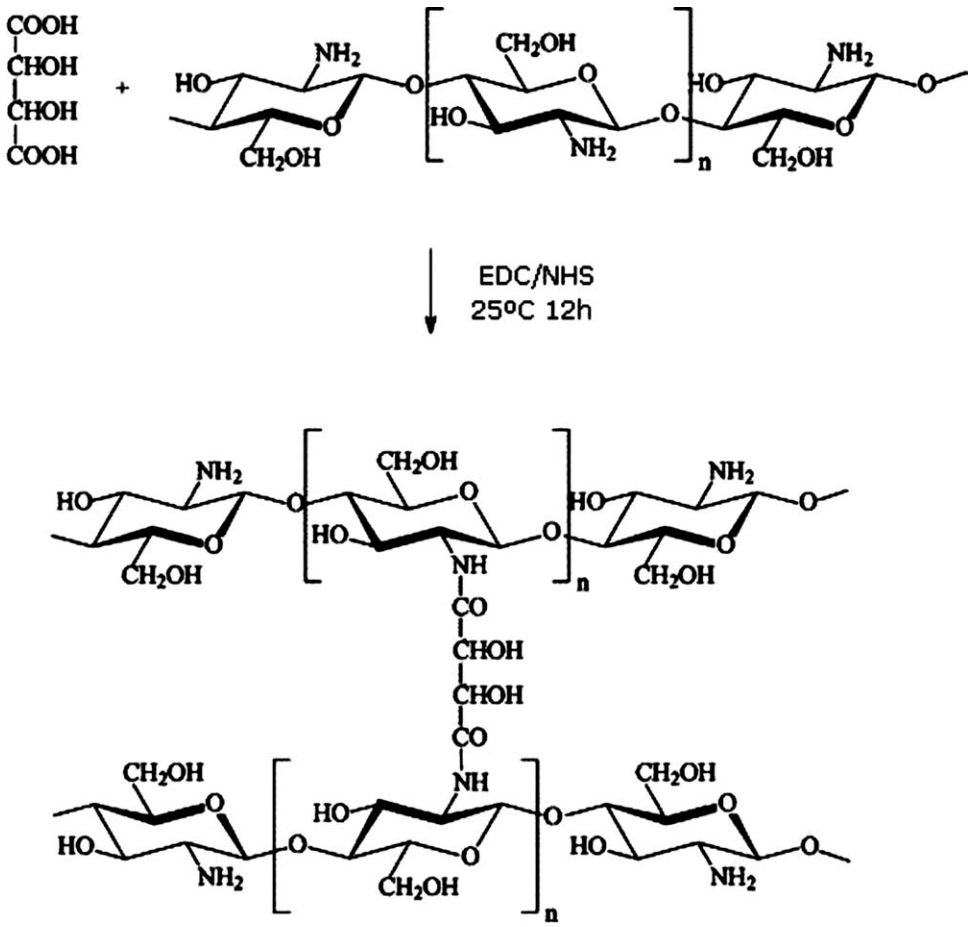

Figure 2 Scheme of the crosslinking reaction for the preparation of CHs.

was achieved and the mechanical properties reached equilibrium values. The obtained self-sustaining hydrogels showed the geometry of a disk with a height of 3.0 mm. The final polymer concentration, *cp*, was 3.0% (w/v). The scheme of the overall reaction is reported in Figure 2.[26]

CH loading was obtained by dissolving an appropriate amount of model drug (10 mg of Vit.B12 or 30 mg of BD) in the CS solution before the addition of the EDC/NHS and TA solutions to reach the final concentration of 1 or 3 mg/mL, respectively.

Preparation of chemically crosslinked chitosan films

The prepared CHs were put on a sieve covered with a paper filter, and 2-kg weight (in a glass cylinder) was allocated on the hydrogel sample to drain imbibed water. The system was left in an aired oven at 30°C overnight to obtain the dried film, CF. Drug-loaded CFs were obtained from the CHs loaded with the model drug following the same procedure. For an appropriate comparison, CS films without the presence of the crosslinker (no-CF), thus actually not hydrogels, were also obtained according to the same procedure.

Capillary viscometry

Viscosity measurements were performed at 30°C using a Haake automatic viscometer equipped with an Ubbelhode capillary (diameter: 0.53 mm) and a water thermostated bath. The solvent, 0.83 mol/L CH_3COOH and 0.3 mol/L NaCl,[31] was filtered through 0.22 μm Millipore filters, while the polymeric solutions were filtered through 0.45 μm Millipore filters. Intrinsic viscosity measurements were carried out, and the Mark Houwink relation was used to determine the chitosan molecular weight (1.0×10^5):

$$[\eta] = K \times M_r^a,$$

where $[\eta]$ is the intrinsic viscosity, K and a are constants, the values of which depend on the nature of the polymer and solvent as well as on temperature ($a = 0.885$; $K = 1.464 \times 10^{-4}$), and M_r is usually one of the relative molecular mass averages.[32]

Thickness measurements

The thickness of the CF systems (dried crosslinked and not crosslinked films) was measured using the

micrometer Positector 6000 Defelsko (Ogdensburg, NY, USA). The thickness of the CHs was measured by means of a caliper.

Rheological characterization

Rheological properties of CH and swollen CF samples were analyzed in shear oscillatory experiments. CH samples were tested after their preparation, while swollen CF was analyzed after 24-h soaking in NaCl 0.9% (w/v). Experiments were carried out by means of a controlled stress rheometer Haake RheoStress 300 Rotational Rheometer (Germany) provided with a Haake DC10 thermostat. CH samples, with a thickness of 2.0–3.0 mm, were removed with the aid of a small spatula from the molds where they were prepared, and they were laid with care on the lower plate of the rheometer. The swollen CF samples, with a thickness of 1.5–3.0 mm, were removed from the Petri dish where they were swelled, and they were laid with care on the lower plate of the rheometer. The upper plate was then lowered until it reached the sample surface. Gap setting optimizations were undertaken according to the procedure described elsewhere.[33] Mechanical spectra were recorded in the linear viscoelasticity regime, previously obtained from stress sweep experiments; a $\gamma = 0.01$ deformation was used. Frequency sweep experiments were performed at 25.0 ± 0.5°C in the frequency range 0.01–1 Hz. A grained plate-plate device (Haake PP35 TI: diameter: 35 mm) was used to reduce the extent of the wall slippage phenomena.[34] Shear flow measurements of the TA–CS solution were performed at 25.0 ± 0.5°C using a cone-plate geometry (Haake CP60Ti: diameter: 60 mm; cone: 1°; gap between plates: 0.053 mm) in the range 0.001–1000 s^{-1}. A stepwise increase of the stress was applied, with an equilibration time of 40 s.

Texture analysis

A software-controlled dynamometer, TA-XT2*i* Texture Analyzer (Stable Micro Systems, United Kingdom), with a 5-kg load cell, a force measurement accuracy of 0.0025%, and a distance resolution of 0.0025 mm (according to instrument specifications) was used for the evaluation of the mechanical properties of the samples.[30,35] The Young's modulus, *E*, of CH and swollen CF, was evaluated in compression experiments, according to the procedure described by Meyvis et al.[36] at 10% deformation (in the linear viscoelastic regime) at 25°C. An aluminum cylinder probe with a diameter of 35 mm (P/35) was used. The pretest speed, the test speed, and the post-test speed were set up at 1 mm/s. All analyses were performed on four replicate samples, which were cut into cylindrical shaped disks (diameter: 2.3 cm; height: 1.5–3.0 cm). Penetration tests were performed on CH samples, dried and swollen CF samples [24-h soaking in NaCl 0.9% (w/v)], and on dried no-CF films, using a cylindrical stainless steel probe with a diameter of 5.0 mm (P/5). The probe, after reaching the sample surface, was forced to penetrate into the samples until rupture was achieved. The pre-test speed was set up at 0.50 mm/s, the test speed at 0.10 mm/s, and the post-test speed at 0.10 mm/s. Sample heights were always in the range 0.5–3.0 cm. Five replicates of the experiments were carried out for each sample at 25°C.

Solvent uptake

Solvent uptake was determined evaluating the relative increase of the sample weight in different experimental conditions. CF samples, each of ~ 500 mg, were incubated at 37°C in Petri dishes containing 200 mL of a NaCl 0.9% (w/v) solution.

Solvent uptake was also evaluated for CH, CF, and no-CF samples by incubating two disks (diameter and height 1.0 cm) in 10 mL of phosphate buffer solution (pH 7.4), containing 0.2 mg/mL of sodium azide, in a vial kept in a thermostatic bath at 37°C. At predetermined time intervals, the samples were taken out from the medium, rapidly dried on filter paper to remove the excess of solvent, immediately put into a closed vial, and weighed. The medium was replaced with the same volume of fresh solution. Water uptake degree (*D*), followed by slow dissolution in the case of no-CF, was calculated by measuring the weight variations according to the equation:

$$D = ((W_t - W_0)/W_0) \times 100,$$

where W_t is the weight at time t, while W_0 denotes the initial weight. Each experiment was repeated three times.

In vitro release studies

The drug-loaded CH and CF samples were suspended in a vessel with 200 mL of 1 m*M* Hepes (pH 7.4) at 37.0 ± 0.1°C, kept at a fixed distance (5.0 cm) from the bottom of the vessel by means of a thin web, while the release medium was gently magnetically stirred. This thin web was composed by an inert synthetic material that acted as a sample holder. By using such web, no filtration or centrifugation of the samples withdrawn from the dissolution medium was needed. For this purpose, 5-mL aliquots were directly taken at scheduled time intervals and replaced by an equal volume of fresh buffer. The released amount of Vit.B12 and BD was spectrophotometrically detected at 361 and 618 nm,

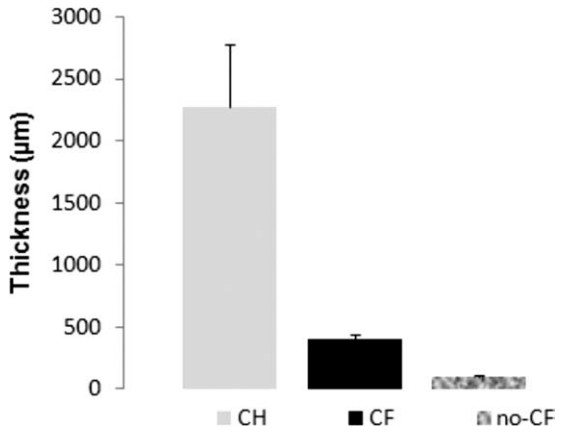

Figure 3 Thickness of CHs and of crosslinked (CF) and not crosslinked (no-CF) films. The values represent the mean of 10 different tests on four replicates of each system; standard deviations are also shown.

respectively, by means of a spectrophotometer (Perkin–Elmer, Lambda 3A UV/Vis Spectrophotometer) using quartz cells with pathlength of 1.0 cm. All experiments were performed in triplicate, and the obtained values in the present article always laid within 10% of the mean. The results are reported as the relative percentage release:

$$(M_t/M_\infty) \times 100,$$

where M_t indicates the amount of drug released at time t, and M_∞ indicates the total amount of the drug loaded in the formulation.

RESULTS AND DISCUSSION

The preparation of CS chemical hydrogels was carried out keeping constant CS concentration at 3% (w/v) and using the EDC/NHS and TA ratios (r, r') reported in the experimental section, being these values the lowest polymer and crosslinker concentrations capable to give a self-sustaining hydrogel. The EDC/NHS solution was used as a zero-length crosslinking agent that catalyzed the formation of short-range amide bonds between the carboxylic groups of TA and the amine groups of CS. The chemical crosslinking of CH is based on the reaction between the activated carboxylic sites with primary amine groups in the presence of EDC and of NHS, as reported in scheme of CH preparation summarized in Figure 2. The reaction proceeds in few minutes, leading to a homogeneous transparent hydrogel.

In Figure 3, the thickness of the various samples that were prepared is reported. Starting from CH, which showed a height of 2.2 mm, the dried crosslinked films, obtained after drainage, had an average thickness lower than 0.5 mm. Not crosslinked CS films (no-CF), prepared adopting the same experimental conditions used for CF but without the use EDC/NHS that consequently are not hydrogels, showed significantly lower values of their thickness. The difference observed between the crosslinked and not crosslinked CFs can be ascribed to the different water behavior because of the chemical network that avoids collapsing of the polymer chains, thus allowing to maintain a more open structure.

Rheological characterization

The various prepared systems were studied by means of rheological measurements to test their physicochemical properties.

The flow curve of the TA–CS solution, before addition of EDC/NHS, reported in Figure 4(a), indicates a pseudoplastic behavior. Mechanical spectra registered for the same solution, reported in Figure 4(b), showed that G' and G'' profiles are parallel, frequency dependent, and very close to each other, indicating a system near the gel point.[37]

The chemical reaction between CS and TA, using EDC/NHS as crosslinking agent, leads to the CS hydrogel (CH). The mechanical spectrum of this sample in the range 0.01–10 Hz is reported in Figure 4(c). Obtained data show that G' and G'', which differ for almost one order of magnitude, are parallel and that their profiles are almost independent on the applied frequency.

The data obtained for CFs are also reported in the same Figure 4(c): the mechanical behavior is typical of a hydrogel, but the absolute values of G' and G'' are much higher than those obtained with CH. Actually, in the case of the film, the G' value is higher than 10^4, whereas CH shows a remarkably lower G' value, roughly of one order of magnitude. A similar situation can be observed comparing G'' values of CFs and CH. The lower values obtained for the elastic modulus in the CH samples with respect to those of the film can be explained in terms of two concomitant contributions. In both cases, the gelation process occurs because EDC/NHS crosslinks CS and TA; nevertheless, during the film formation, the hydrogel looses water, thus forming a network with an increased amount of polymer chains per unit volume. This different packing of the polymer chains in the film leads to a more compact network, which was able to retain a lower amount of solvent, thus showing higher G' values than the CH systems.

Texture analysis

Texture analysis is a technique that has been extensively employed in the mechanical characterization of food materials, and in the last few years, it has emerged also as a useful technique in the field of

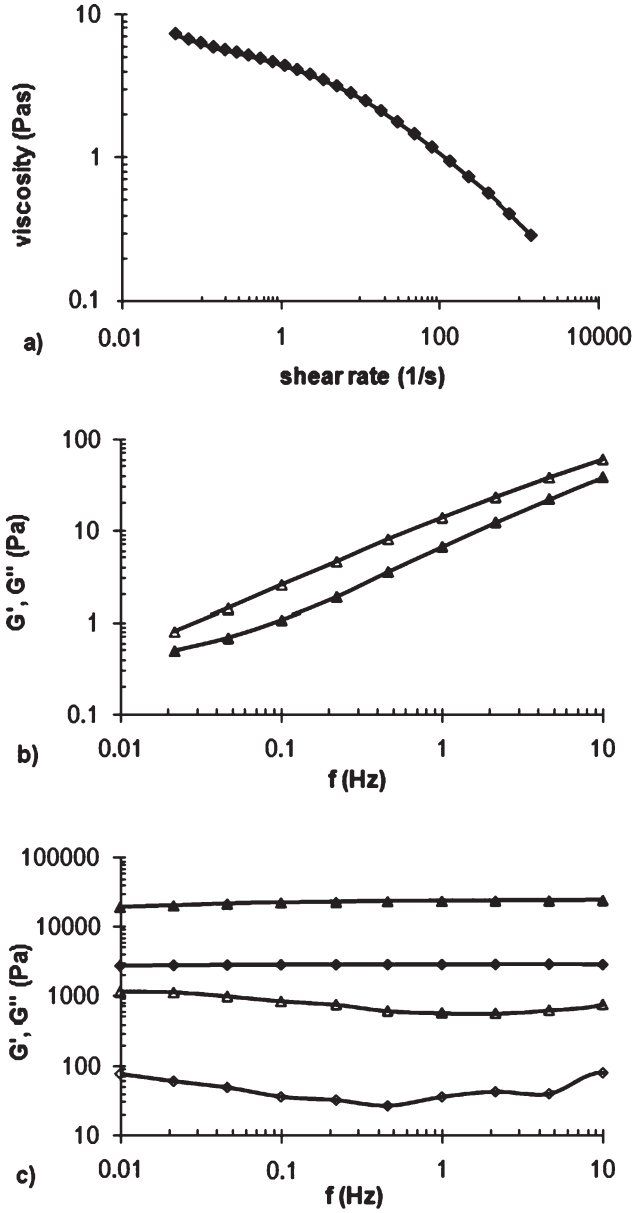

Figure 4 Flow curve of the TA–CS solution recorded at 25 ± 0.5°C (a); mechanical spectra of the TA–CS solution: G' (filled triangle) and G'' (open triangle) (b); and mechanical spectra of CHs (filled diamond: G' and open diamond: G'') and swollen CFs (filled triangle: G' and open triangle: G'') (25°C) (c).

pharmaceutical gel studies. Penetration tests are useful to characterize the mechanical properties of hydrogels.[38,39] In our case, experiments were carried out to assess the influence of crosslinking and water uptake on the mechanical properties of CH and CF.

The compression stresses and the strains measured at the fracture point of all tested systems are reported in Figures 5 and 6, respectively.

The obtained data show that the mechanical strength of dry films was higher than that of swollen films. This behavior can be related to the higher chain density in the dry state in comparison to the

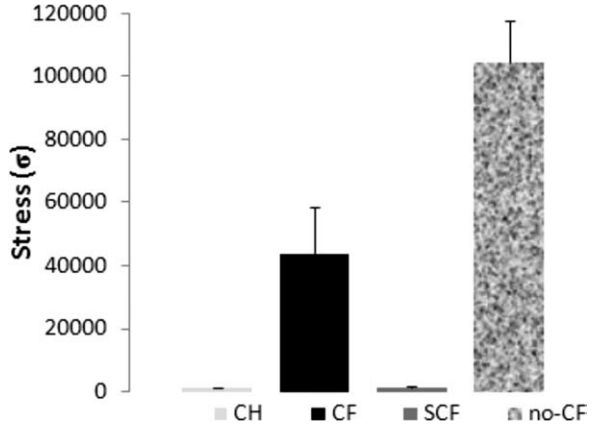

Figure 5 Compression stress at fracture point of the various samples [crosslinked hydrogels (CHs), crosslinked films (CFs), swollen crosslinked films (SCFs), and films obtained without EDC/NHS (no-CF)]. Reported values are the mean of five different tests carried out in four replicates for each sample; standard deviations are also reported.

swollen systems. Despite the chemical crosslinking of the polymer chains that usually enhances the mechanical performances of the polymer networks, in the present case, a reduction of the stress and strain at fracture point is observed, as no-CF shows stress at fracture point higher than CF. Similarly, the strain at fracture point is higher for no-CF. A reason for this somehow unexpected behavior can be ascribed to the formation of covalent bonds, provided by the short-range intramolecular/intermolecular crosslinker. The chemical crosslinking reduces the chain mobility, and consequently, a more brittle structure is obtained.

The Young's moduli (E) of the CH samples and swollen CFs were also determined, and the corresponding data are shown in Figure 7.

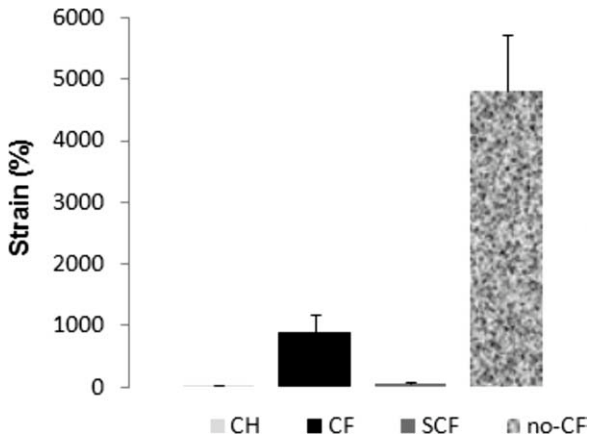

Figure 6 Strain at fracture point of the various samples (CH, CF, swollen crosslinked films, SCF, and no-CF). The values reported are the mean of five different tests carried out in four replicates for each sample; standard deviations are reported.

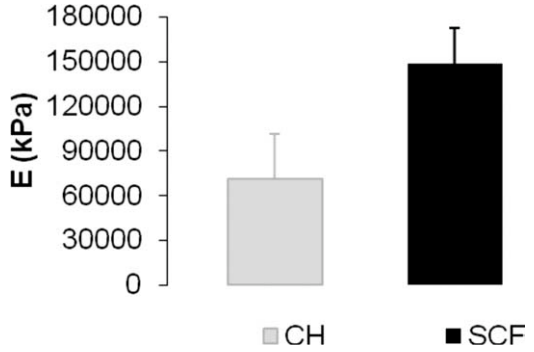

Figure 7 Young's modulus (E) of CH samples and swollen CFs. The values represent the mean of four different tests for each sample; standard deviations are also reported.

As expected, the CH sample shows E values lower than those of the swollen CF films. The strain necessary to achieve a compression of 10% was higher in the case of swollen CFs, because a higher chain density increases the capability to resist to a deformation on an applied stress. As reported for the G' values, also E values are higher for the swollen CFs, in agreement with the mechanical results obtained in shear experiments.

Water uptake

Water uptake experiments were performed in NaCl 0.9% at pH 5.5 and in phosphate buffer solution at pH 7.4 to test the behavior of the samples in different media.

In NaCl 0.9% at pH 5.5 and 37°C, no-CF, as expected due to the lack of crosslinking, showed a fast dissolution that was complete within 24 h, whereas the films prepared from the crosslinked samples (CF) showed a remarkable water uptake ($D = 222 \pm 15$). Equilibrium was reached within 24 h, and an increase of more than three times compared with the initial weight of the CFs was detected.

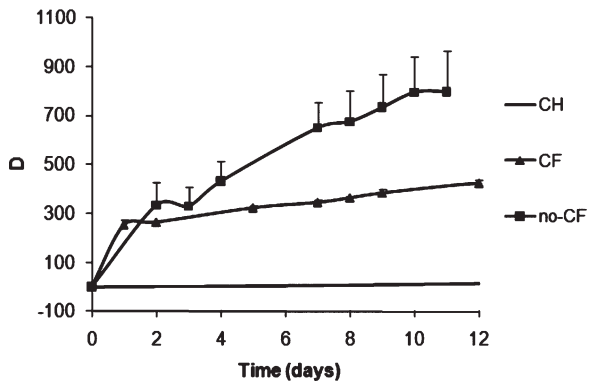

Figure 8 Solvent uptake degree (D) of CH, CF, and no-CF in phosphate buffer solution (pH 7.4, 37°C). Each value is the mean of three replicates; standard deviations are also reported.

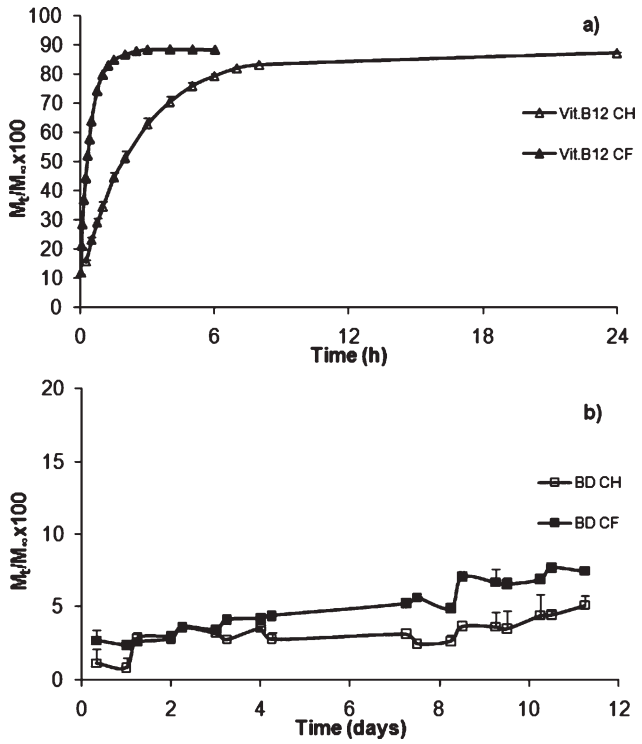

Figure 9 Release profiles of Vit.B12 and BD from CH (a) and CF samples (b) (Hepes, pH 7.4, 37°C).

The solvent uptakes of the different systems were investigated also in phosphate buffer at pH 7.4 and 37°C. The water uptake degree D as a function of time is reported in Figure 8.

Usually, the systems initially absorb the solvent and then reach an equilibrium. In these experimental conditions, although the CH samples kept their initial weights roughly for at least 25 days, the CF samples reached the maximum D value after 12 days, while the no-CF reached their maximum water uptake in 10 days. After equilibrium is reached, the samples start slowly to degrade.

The different ability (solvent uptake rate and D) to absorb solvent for CF in slightly acidic conditions (NaCl 0.9%, pH 5.5) and in phosphate buffer (0.1M, pH 7.4) can be easily attributed to the different ionization degree, in these environmental conditions, showed by the amine moieties not involved in the chemical crosslinking reaction.

In vitro release studies

Vit.B12 and BD, two water-soluble model drugs with remarkably different molecular weight and steric hindrance, were chosen as model molecules to test the release properties of the CH and CF samples. The delivery profiles of the drugs are reported in Figure 9.

Drug release from CH and CF samples is strongly affected by at least two factors such as swelling and

interactions between CS macromolecules and model drugs. In Figure 9, the different release behavior of Vit.B12-loaded CH and CF is clearly evidenced; at the same time, also the effect of both hydrogel networks on the almost negligible delivery of BD can be detected. This behavior can be explained on one side in terms of the different networks of the bulk hydrogel and of the film (as supported also by the rheological characterization) and on the other side in terms of the different steric hindrance of the two guest molecules. Although an almost complete delivery was detected within a few hours for Vit.B12, only a slight initial delivery of BD can be detected for both network systems. BD behaves as if it is permanently entrapped within the hydrogel, at least within the interval of time of the experiment (12 days). In this sense, it should be pointed out that the model drug was loaded before the crosslink reaction.

CONCLUSIONS

In this work, we showed that CS has been successfully crosslinked with TA by a condensation reaction using EDC/NHS. The obtained new system was studied both in the form of bulk hydrogel and in the form of film. Reported data evidenced that the network structure can deeply affect the dynamomechanical properties as well as the delivery rate of the model drugs. Films formed by CS, TA, and EDC/NHS were able to retain a higher amount of water in comparison with the not crosslinked ones. As far as the mechanical properties are concerned, the elasticity of the films is lowered by the chemical network formation, and a more brittle system is obtained in comparison with not crosslinked films. Despite reduced mechanical performances, degradation rate of the CF is lowered by the presence of chemical crosslinks in comparison with the no-CF.

The delivery of the model drugs was remarkably affected by the dimensions of the model molecules. Vit.B12 was delivered in a few hours from CH and CF; BD was almost unable to diffuse out of the hydrogels and films: only a small amount of the model drug was delivered within 11 days. From the collected data, we can conclude that the overall procedure used for the hydrogel and film formation is very simple and that the new material, both in the form of bulk hydrogel or as a film, can be proposed for pharmaceutical or biomedical applications.

Roberto Ruiz Caro thanks the UCM (Spain) for a scholarship.

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