

Structural characterization of chitosan-grafted polyurethanes

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ABSTRACT

Biohybrid polyurethane-grafted-chitosan hydrogels were synthesized by a heterogeneous synthetic method. Chitosan-grafted polyurethane hydrogels were prepared. Their analogous, without chitosan, were studied as control.

Hydrogels were prepared using bis(cyclohexyl)methane-4,4'-diisocyanate, 1,4-butanediol, a crosslinking agent and, alternatively, polyethylene-oxide or polyethylene-adipate as macrodiol. Chitosan powder, in a preset molar ratio, was added to the reacting mixture. Structure and thermal properties were investigated by FT-IR, DSC and dynamic mechanical analyses (DMA).

The obtained biohybrid hydrogels show interesting structural characteristics: the insertion of chitosan in the polymeric structure induced an increase of swelling degree, modification of the structure (by FTIR), and thermal properties (by DSC and DMA). DMA analyses, performed on dry and swollen state, showed a significant increase of storage modulus with the insertion of chitosan; swelling induced a decrease of the storage modulus. Obtained results indicate the possibility to produce a range of materials with substantially different properties to be targeted to different applications.

1. Introduction

Chitosan, a polysaccharide composed by N-acetyl-D-glucosamine and N-deacetylated units connected by β (1 \rightarrow 4) linkage, is obtained from the partial deacetylation of chitin. Chitin is a characteristic compound found in fungi, in crustaceans and mollusks shells, in the backbone of squids and in the cuticle of insects.

The deacetylation of chitin produce primary amino-groups positively charged at physiological pH, thus leading to a polysaccharide structure which can interact with the majority of natural polymers, mainly negatively charged. This peculiar characteristic of chitosan, and the presence of groups able to form secondary interactions, allows to obtain also physical networks via complexation or aggregation. Physical hydrogels are reversible and their solubility can be tuned by environmental parameters, such as temperature or pH [1].

The partially deacetylated structure, and the specific distribution of the acetylated units within the polymer backbone, is also fundamental for the solubility of the biopolymer in aqueous acidic solutions, promoted by the protonation of the amino-groups. But solubility in acidic environments can be viewed as a limit for many applications of this biopolymer. This limitation is partially overcome by specific modifications of this polymer, most of them affecting the amino group.

Chemical crosslinking can be achieved similarly as for many natural polymers. Diisocyanates were used to crosslink chitosan, obtaining urea groups, in alternative to glutaraldehyde or other common crosslinkers to overcome a too fast degradation of chitosan resulting in loss of mechanical properties. For crosslinking, different diisocyanates are employed, mainly 1,6-hexamethylenediisocyanate (HDI) for nerve regeneration [2]: HDI-crosslinked film degradation rate was decreased and greatly enhanced the spread and proliferation of Schwann cells if compared to not crosslinked chitosan. Toluenediisocyanate (TDI) is also employed in preparation of membranes for different applications, e.g. dehydration of organic solvents [3] or nanofiltration [4]. However TDI can not be accepted in biomedical uses.

Even if crosslinking was achieved, reacting isocyanate with chitosan is not straightforward. Solubility problems are the major obstacle employing isocyanate: isocyanate readily degrades in acidic environments and in presence of water, leading to the formation of a no more reactive amine and CO₂ as byproduct. Moreover at acidic pHs the amino groups in chitosan are protonated, and they can not act as nucleophiles. Unfortunately, the preferred solvent for chitosan is dilute acetic acid and the partial protonation of the amino-groups is on the basis of solubilization. For these reasons, crosslinking reactions are usually carried out in heterogeneous conditions, for example immersing a preformed film of chitosan, or its derivatives, in diisocyanates solutions [2-4]. Swelling of the chitosan films in the appropriate solvent can improve crosslinking, as crosslinking efficiency is very low if mobility of the reacting diisocyanates in the solid films is poor.

Reaction with diisocyanates is also the basic reaction for polyurethane synthesis. Among polyurethanes, PEG-based polyurethane hydrogels [5] possess the

favorable characteristics of the family of polyurethanes as well as the properties typical of hydrogels. Polyurethane hydrogel formulations, introducing a low crosslinking degree, allowed to obtain high swelling, not water soluble, hydrogels (PUHy). These materials also present an elastomeric behavior in the swollen state [6].

In this work chitosan grafted polyethylene oxide and polyethylene adipate polyurethane hydrogels were prepared. Facing to the solubility problems of chitosan, polyurethane-grafted-chitosan were synthesized by a heterogeneous synthetic method, aimed to induce polyurethane grafting on the surface of chitosan particles of small dimensions. The chitosan powder, with a controlled granularity, was prepared to obtain a high surface/volume ratio, increasing the availability of reactive groups.

2. Materials and Methods

2.1 Synthesis

Molecular weight (1260 Da) and degree of deacetylation (78%) of chitosan (Fluka, medium viscosity) were obtained by intrinsic viscosity and FT-IR. Chitosan powder (granulometry $\leq 500 \mu\text{m}$) was previously prepared by phase inversion, extensively washed with ethanol, and dried to constant weight. Residual water content of all reagents, including chitosan powder, was carefully controlled by potentiometric titration and stoichiometrically balanced accounting its reaction with diisocyanate.

Polyurethane hydrogels, not containing chitosan, were prepared in a one-step bulk polymerization process, at 80°C , using bis(cyclohexyl)methane-4,4'-diisocyanate (HMDI, Desmodur W, kindly provided by Bayer), 1,4-butanediol, and a trifunctional crosslinking agent and alternatively poly(ethylene oxide) (PEO, MW 1500) or poly(ethylene adipate) (PEA, MW 1000) respectively to obtain hydrogels named Hy G100 for PEO based polyurethane and Hy A100 for PEA based polyurethanes.

A similar procedure was followed for the synthesis of chitosan containing polyurethanes, either PEO or PEA based (Hy G50 and Hy A50), but dispersing chitosan powder into the reacting mixture chitosan powder in the preset molar ratio ($\mu\text{OH}_{\text{macrodiol}} : \mu\text{NH}_2_{\text{chitosan}} 1:1$). The reaction kinetic was followed by FT-IR.

2.2 Characterization

Differential scanning calorimetry (DSC)

Analyses were performed on 5-10 mg samples in the temperature range -150° to $+250^\circ\text{C}$ at a heating rate of $20^\circ\text{C}/\text{min}$ under N_2 , with a DSC 6200 SII Seiko Instruments calorimeter, calibrated with indium. A first run was performed from $+25$ to $+160^\circ\text{C}$ at $20^\circ\text{C}/\text{min}$ to eliminate residual moisture.

For the evaluation of free and bound water, equilibrium swollen samples were cooled at -50°C and equilibrated at this temperature for 10 minutes, then heated up to 40°C at 10°C/min. Free water was calculated by the formula [7]

$$\%W_{frozen} = \left(\frac{\Delta H}{\Delta H_0} \right) \cdot 100$$

where ΔH is the melting enthalpy of the sample and ΔH_0 is water melting enthalpy (334 J/g).

FT IR spectroscopy

ATR FT-IR spectra were performed with a FT-IR Magna 560 Nicolet spectrometer equipped with Omnic 4.1 Software. ATR Spectra-Tech attachment, mod. 300, was used.

Dynamic mechanical analysis

The mechanical properties were analyzed in compression mode by using a dynamic mechanical analyzer (DMTA 2980, TA instruments) on cylindrical samples (diameter 8 mm, h 5 mm).

Frequency sweep DMTA: a cyclic compressive force was applied with increased frequencies from 0.25 to 5 Hz with constant amplitude displacement of 15 μm deformation and the response (storage modulus, E' , and loss modulus E'') was determined. The experiment was performed at 37°C on previously swollen samples.

Temperature sweep DMTA: samples were tested at a frequency of 1 Hz with constant amplitude displacement of 15 μm at temperatures from 0°C to 250°C at a heating rate of 1°C/min.

Swelling degree and weight loss

The swelling degree and weight loss were measured by immersion in water at 37°C from 30 min up to 10 days and in acidic environment (1M acetic acid and 0.5M nitric acid) up to 48h. The volume of adsorbed solvent, W.U., versus time, was calculated as $\%W.U. = (W_s - W_d)/W_d \times 100$, where W_s and W_d are respectively the weight of the swollen and dry samples. Weight loss was calculated as $\%W.L. = (W_t - W_d)/W_d \times 100$ where W_t is the dried sample after immersion in water for each time point.

3. Results and discussion

Biohybrid polyurethane/chitosan hydrogels containing polyether (PEO) and polyester (PEA) segments were synthesized. Polyester-polyurethane hydrogels, characterized by hydrolytic degradation, were synthesized in order to obtain a second level of degradability in addition to chitosan degradation. PEO and PEA PU-hydrogels were synthesized as control.

For the heterogeneous phase polymerization reaction, the polyurethanes were synthesized in presence of chitosan powder dispersed into the reagent mixture, then the following addition of diisocyanate starts polymerization process and the grafting onto chitosan. A progressive decrease (Fig.1) of the anti-symmetrical

stretching of isocyanate group ($2285\text{-}2250\text{ cm}^{-1}$) is associated with the increase of stretching of urethane carbonyl band ($1720\text{-}1705\text{ cm}^{-1}$) and -NH stretching ($3340\text{-}3250\text{ cm}^{-1}$). Infrared spectroscopy of the products showed an increase of the hydroxyl stretching, accounting the hydroxyl groups of chitosan part, while the increase of the amide I area may be attributed to the reaction of some chitosan amino groups and subsequent urea formation.

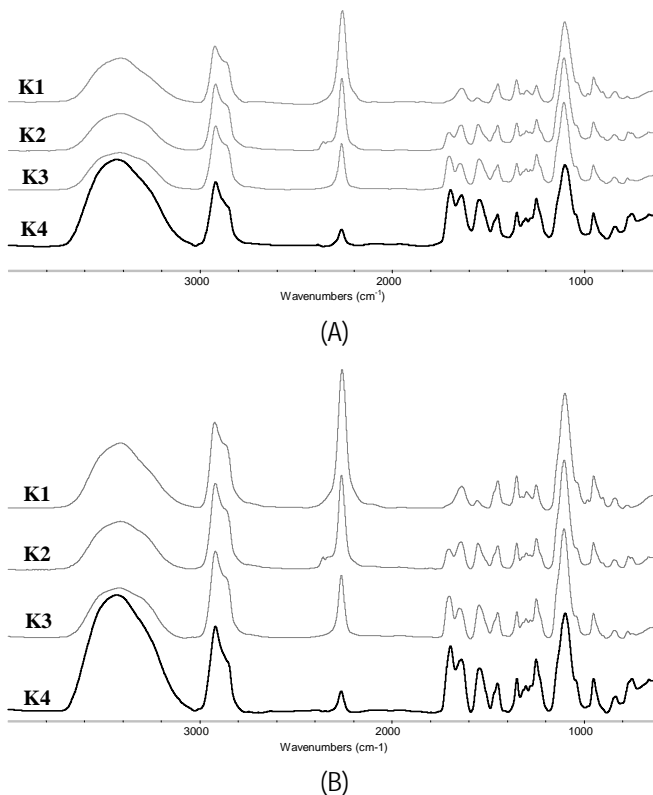


Fig.1 – Infrared spectra of the reacting mixtures at different time points (K1= immediately after adding diisocyanate, K2= after 30 minutes, K3= after 3 hours, K4= after 20 hours) for chitosan containing polyester PUHy (A) and polyether PUHy (B).

Urea formation can be also attributed to the reaction of water and isocyanate. Residual water is present mainly in the PEO and PEA and, at minor extent, in chitosan, so urea formation can be expected in all the polymers. Infrared spectra of the reaction products (Fig. 2) did not show an evident urea band for A50 and A100 (Fig. 2B), indicating that either amino-groups of chitosan reacted with isocyanate at a very low amount and, eventually, water present in chitosan introduce a negligible contribution to the urea band. On the opposite, spectra of G50 (Fig. 2A) indicate a higher urea band if compared to G100, and this finding may support the hypothesis of an extensive reaction of the amino-groups of chitosan with isocyanate.

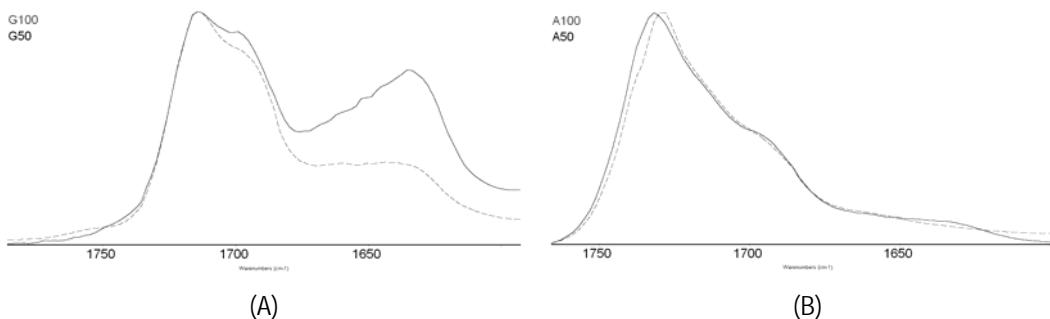


Fig.2 – Infrared spectra ($1800\text{-}1600\text{ cm}^{-1}$) of polyether PUHy G100 and G50 (A) and polyester PUHy A100 and A50 (B).

Water uptake in deionized water showed for both chitosan-containing hydrogels an increase of the swelling equilibrium value compared with the hydrogels not containing chitosan. The introduction of chitosan is responsible for the increase of bound water/free water ratio for G50 and A50 evaluated by DSC analysis of melting enthalpy (Table 1).

| | % W.U. ($\pm s.d.$) | % W_{frozen} | % W_{bound} |
|---------|-----------------------|----------------|---------------|
| Hy G100 | 143 \pm 4 | 18,9 | 124,6 |
| Hy G50 | 158 \pm 3 | 9,4 | 148,9 |
| Hy A100 | 6 \pm 3 | 1,7 | 3,8 |
| Hy A50 | 48 \pm 1 | 11,2 | 36,7 |

Table 1 – Equilibrium water uptake (%W.U.) and fraction (%W) of frozen or bound water evaluated by thermal analyses of the swollen samples

Weight loss (%W.L.), followed up to 10 days, was low ($\approx 2\%$) for G100 and A100: the introduction of chitosan affected the %W.L. with an increase of 240% and 400% for G50 and A50 respect to the analogous hydrogels not containing chitosan. This can be also accounted to the increase of water uptake which can affect the degradation kinetic and mechanism. A different molecular weight of the products can also be responsible of the increase of weight loss in chitosan containing polymers.

Swelling and weight loss at acidic pHs were also evaluated at acidic pHs in nitric acid and acetic acid.

Swelling may be modified in acidic environment, as chitosan is protonated in acidic medium and is more hydrophilic than at basic or neutral pHs.

In our case, both polyether polyurethanes, with or without chitosan, became more hydrophilic at a similar extent at acidic environments, with an increase % W.U. up to about 200% for both materials in acetic acid, and 200% and 225% in nitric acid for G100 and G50 respectively. Weight loss of both polyether based

hydrogels was similar ($\approx 20\%$) with no dependence on the environment and higher than in water.

Acid environment are known to degrade ester bond, and acetic acid, but not nitric acid, is able to dissolve chitosan. For A100 almost no weight loss can be observed in acetic acid. Weight loss was higher ($\approx 16\%$) in nitric acid, indicating that ester bonds are probably degrading. This effect was also observable for A50 (W.L. $\approx 55\%$), where two possible processes resulting in weight loss are involved, i.e. degradation of the ester bond and solubilization of chitosan.

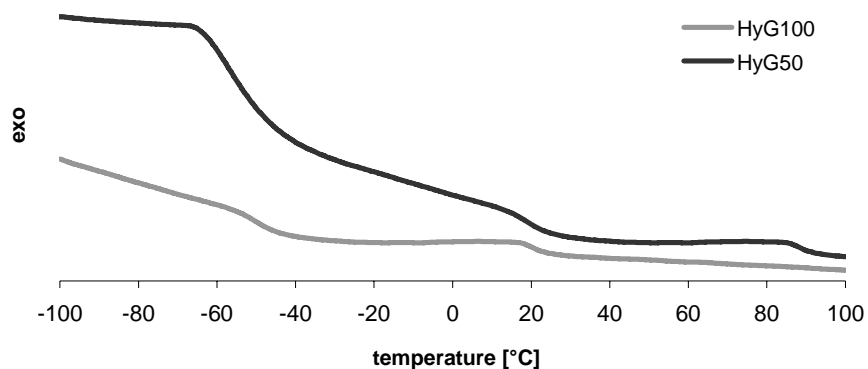


Fig. 3 - DSC thermograms of the PEO based polyurethane hydrogels, Hy G100 and Hy G50

Chitosan containing hydrogels showed slightly different thermal characteristics. In polyether hydrogels, the typical PEG glass transition can be observed around -50°C and a crystallization followed by the fusion of the soft segment can be noticed between 0°C and $+50^{\circ}\text{C}$, although it is more evident for G50. Differences can be observed in polyester hydrogels, with a T_g at 18°C in 100 no more observable for G50 (Fig.3).

Mechanical characterization of the hydrogels where conducted either varying temperature at a constant frequency or at a fixed temperature with a variable frequency by dynamic mechanical analysis.

From the analysis of DMA charts of E' , E'' and $\tan\delta$, melting of polyether and polyester segments are clearly observable in chitosan containing polymers respectively at 37°C and 46°C . This transitions were not detectable in the hydrogels without chitosan. A sharp decrease of E' , E'' and $\tan\delta$ is also observable for G100 at 175°C , while on DSC thermograms only a slight endotherm is observed. A temperature shift of all the melting processes is detectable if compared to DSC results. Differences between results of these two techniques can also result from a different heating rate.

| <i>material</i> | $E'_{37^{\circ}\text{C}}$ (dry samples) [MPa] | $E'_{1\text{Hz}}$ (swollen samples) [MPa] |
|-----------------|---|---|
| Hy G100 | 2,87 | 1,29 ± 0,35 |
| Hy G50 | 1,73 | 0,20 ± 0,08 |
| Hy A100 | 7,26 | 1,80 ± 0,48 |
| Hy A50 | 73,22 | 0,20 ± 0,11 |

Table 2 – Storage modulus at 37°C, ($E'_{37^{\circ}\text{C}}$) obtained from temperature sweep analyses at the fixed frequency of 1Hz and storage modulus at 1Hz ($E'_{1\text{Hz}}$) obtained from frequency sweep analyses at the constant temperature of 37°C, first cycle

The conservative moduli of the four hydrogels ($E'_{37^{\circ}\text{C}}$) obtained from temperature sweep analyses at the fixed frequency of 1Hz (Table 2) indicate that polyether based hydrogels are on the overall more flexible than polyester hydrogels. The presence of chitosan in polyester hydrogels increase of an order of magnitude the conservative modulus of the resulting polymer.

DMA allowed an evaluation of the mechanical properties of the hydrogels in the swollen state under dynamic compression to mimic an in vivo physiological condition better than a typical static compression test. The range of employed frequencies (0,25-5Hz) were chosen to represent physiological frequencies in load-bearing applications.

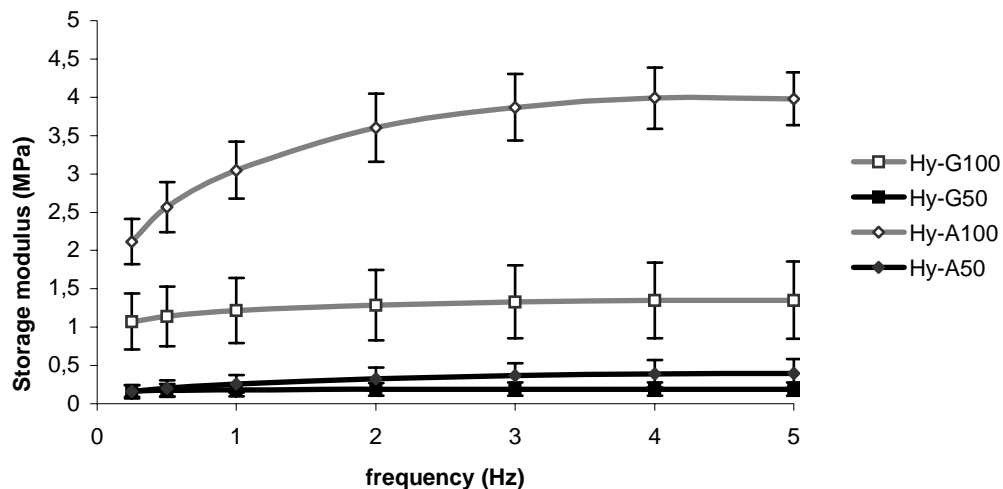


Fig. 4 - Dependence of storage moduli on frequency

Results indicate a dependence of the properties of E' and E'' on frequency with a progressive increase of the storage moduli (Fig. 4) for A100 and A50.

Storage moduli at 1Hz ($E'_{1\text{Hz}}$) obtained from frequency sweep analyses at the constant temperature of 37°C (first cycle) indicate that all the hydrogels possess interesting properties (Tab.2) if compared to typical values of swollen hydrogels. The decrease of moduli in the chitosan containing hydrogels in respect of their analogous without chitosan could be attributed to the higher swelling capabilities of these polymers.

Conclusions

The synthesized structures can be hypothesized as a result of a partial grafting on the surface of chitosan particles, an intermediate structure between a polyurethane grafted onto chitosan and a structure which where the natural polymer acts as “filler” and it is intimately bonded to a “matrix”, the polyurethane. The covalent anchorage could not be verified, within the analytical techniques considered in this paper, for the specific case of the polyester based polyurethane.

The biohybrid polyurethane/chitosan hydrogels show interesting structural characteristics and, potentially, degradation characteristics: the introduction of the polysaccharidic segment in the polymeric chain allows to obtain polymeric structures where chitosan degradation can be combined with hydrolytic degradation of ester moieties in poly-ester-urethane hydrogels, resulting in a double level of degradability.

Obtained results indicate the possibility to produce a range of materials with substantially different properties which can be targeted to different biomedical applications.

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