

Radiation Engineering of Xyloglucan Hydrogels

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Xyloglucans (XGs) are interesting substrates for the production of scaffolds for tissue engineering, drug delivery depots and hydrogel dressings, thanks to their ability to gel in appropriate conditions, such as in the presence of hydro-alcoholic solvents or by addition of sugar molecules. Due to their natural source, they are characterized by high average molecular weights and broad molecular weight distributions. High energy irradiation is a suitable tool to reduce polysaccharides molecular weight without a dramatic alteration of the polymer chemical structure and gelation ability. In this work, the effect of the radiation dose on the molecular weight of a XG derived from Tamarind seeds is investigated. The rheological properties of the gels obtained by adding ethanol to the polymer water solutions are also described. The effects of alcohol content, storage time and irradiation dose on gel strength are discussed.

1. Introduction

Xyloglucan is a highly branched, hydroxyl-rich polyglucan that can be found either as a structural polysaccharide in the primary cell walls of higher plants or as storage polysaccharide in plant seeds. Its backbone is formed by β -(1,4) D-glucan, partially substituted by α -(1 \rightarrow 6)-linked xylose units. Some of the xylose residues are β -D-galactosylated at the O-2 (Fry, 1988).

Research activities are mostly focused on xyloglucan extracted from tamarind seeds, due to its commercial availability. Besides, it is already FDA approved for use as food additive, due to its ability to act as thickener and stabilizing agent (Dea, 1989).

In its native form, xyloglucan is a film forming polymer (Occhiuzzi et al., 2015), it can undergo gelation when its aqueous solutions are mixed with appropriate substances, such as polyphenols (Nitta et al., 2004), iodine solutions (Yuguchi et al., 2005) and alcohols (Yamanaka et al., 2000), and upon a temperature increase when it is partially degalactosylated (Todaro et al., 2015). In particular, xyloglucan can undergo gelation in presence of mono and polyhydric alcohols, the gelation behavior being strongly dependent on the type of alcohol (Yuguchi et al., 2004). The gel structure obtained upon mixing with ethanol was observed by time-resolved small angle X-rays scattering measurements. In this case, the cross-linking domain is formed by random aggregation of xyloglucan chains (Yamanaka et al., 2000).

Being naturally-occurring polymers, xyloglucans are characterized by broad molecular weight distributions and high average molecular weights. (Nishinari et al., 2007). The reduction and control of polysaccharides molecular weight distribution is a method to tailor their water solubility and, when required, their gelling ability. Various depolymerization treatments have been tested: acid hydrolysis, enzymatic degradation, (Strickland et al., 1999) microwave irradiation, (Galema, 1997) ultrasonication (Basedow and Ebert, 1977) and high energy irradiation treatments (Phillips, 1961). There are only a few studies concerning the effect of irradiation on xyloglucans, in particular Vodenicarová et al. (2006) compare the effects of various radiation sources on molecular and chemical properties, Patel et al. (2008) investigate gamma-irradiation effects on polymer

conformation, while Todaro et al. (2014) study the influence of irradiation on temperature-induced aggregation at the mesoscale of partially degalactosylated xyloglucan.

In this work, molecular and physico-chemical properties of e-beam irradiated xyloglucan at various doses (namely 10, 20, 40 and 60 kGy) are investigated by gel filtration chromatography and Fourier-transform infrared spectroscopy measurements. Then, the gelation behavior of the polymer solutions in presence of ethanol is analyzed by visual inspection and rheological measurements. The effects of alcohol content, storage time and irradiation dose are investigated.

2. Materials and methods

Tamarind seeds xyloglucan (XG) was purchased from Megazyme International (Ireland). Ethanol with purity $\geq 99.8\%$ was purchased from Sigma Aldrich.

XG powder was irradiated in air at room temperature with the linear electron accelerator of "Lodz University of Technology" (Lodz, Poland), with doses of 10, 20, 40 and 60 kGy and a dose rate of 300 kGy/h. Samples are coded as EX, where E means e-beam irradiated and X is the integrated irradiation dose in kGy.

Polymer aqueous solutions were prepared by overnight magnetic stirring at room temperature. Xyloglucan gels were prepared by mixing the polymer aqueous solutions with ethanol at 10%, 20% and 50% in volume.

Fourier transform infrared (FTIR) spectroscopy was carried out with a Perkin Elmer-Spectrum 400 apparatus. Samples were prepared by dispersing the dry polymer in potassium bromide and compressing into pellets. Spectra were recorded at 30 scans per spectrum and 1 cm^{-1} resolution in the $4000\text{--}450\text{ cm}^{-1}$ range. All spectra have been normalized with respect to the peak correspondent to the stretching of methylene groups (2956 cm^{-1}).

Low concentrated polymer solutions (0.1 wt%) were analyzed by gel filtration chromatography (GFC) after $0.45\text{ }\mu\text{m}$ filtration by using two Shodex SB HQ columns in series (806 and 804) thermostated at $15\text{ }^{\circ}\text{C}$ with a Knauer oven and connected to a HPLC device (LC-2010 AT Prominence, Shimadzu, Kyoto, Japan) equipped with a $50\text{ }\mu\text{l}$ sample loop. All samples were eluted with 0.02% sodium azide solution at 0.5 ml/min and the refractive index was recorded with a Smartline RI detector 2300 Knauer.

XG aqueous solutions at 2 wt% were mixed with 20% ethanol and a qualitative test was performed by putting them into transparent glass containers that were tilted at various time intervals.

Rheological measurements were performed with a stress-controlled Rheometer Ar G2 (TA Instruments) equipped with an acrylic parallel plate (diam. 40 mm) and a gap of $500\text{ }\mu\text{m}$ was set. Preliminary strain-sweep tests were performed at 1 Hz frequency in the shear strain range $0.001\text{--}10$ to identify the linear viscoelastic region of the gels, frequency-sweep tests in the frequency range $0.01\text{--}100\text{ Hz}$ were performed at 0.004 strain, i.e. within the linear viscoelastic region of the gels. Measurements were performed on 5 different samples for each investigated system. Standard deviations were always lower than 10% (bars not reported for ease of representation).

3. Results and Discussion

3.1 Effect of irradiation treatments on XG molecular structure

Gel filtration chromatography measurements were performed on 0.1 wt% XG aqueous solutions to gather information about the modifications induced by high energy irradiation on the molecular weight distribution of the biopolymer (see Figure 1a). The effect of radiation dose is a gradual shift of the maximum of the chromatograms towards the higher times, which correspond to the lower molecular weights. This result suggests that radiation dose-dependant molecular degradation phenomena occur, involving the polymer backbone and possibly also its lateral branches. Eventual chemical modifications induced by the irradiation treatment were investigated by FTIR measurements: the spectra of nonirradiated and irradiated XGs are reported in Figure 1b. While the nonirradiated and the 10 kGy systems have identical spectra, the 20 kGy and 40 kGy systems are at all similar to the 60 kGy one. For the higher doses, no new vibration bands or disappearance of XG characteristic peaks are observed, but only a reduction of the heights of the stretching vibrations of both hydroxyl groups ($3600\text{--}3200\text{ cm}^{-1}$) and the C-O groups of alcohols and/or ethers ($1200\text{--}800\text{ cm}^{-1}$). This effect can be possibly associated to the loss of lateral branches carrying the more hydroxyl-rich galactose rings.

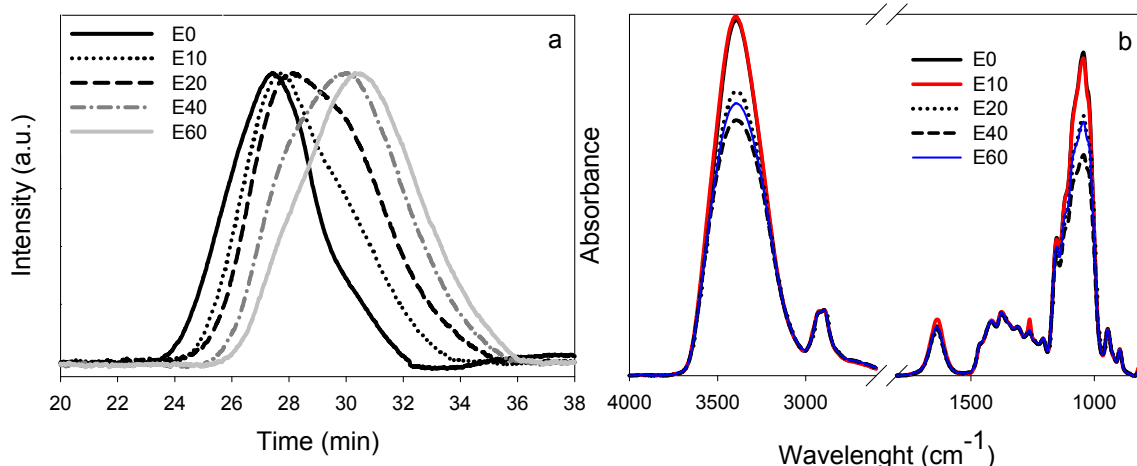


Figure 1: a) Chromatograms of 0.1 wt% XG aqueous solutions. b) FTIR spectra of the nonirradiated and irradiated polymers.

3.2 In-situ gelation properties

The possibility of obtaining in-situ gelling systems by mixing the aqueous solutions of a natural polymer with an alcohol is very appealing for a possible use of these gels as injectable scaffolds and drug delivery depots. The propensity of XG concentrated aqueous solutions to form gels upon mixing with ethanol was evaluated by rheological measurements. This preliminary study was carried out on the nonirradiated polymer. The effect of ethanol content was investigated by mixing a 2 wt% XG aqueous solution with ethanol in the 10 v/v%, 20 v/v% and 50 v/v% ratios. For the 50 v/v%, gelation is almost instantaneous and the system is characterized by high heterogeneity. A significant amount of the solution is not incorporated by the gelling material which is, in turn, too stiff to perform a meaningful rheological analysis. For the other two ethanol concentrations, dynamic mechanical spectra were recorded immediately after mixing and after 8 days storage at 25°C. Storage modulus, G' , and loss modulus, G'' , curves as function of the shear frequency are reported in Figure 2.

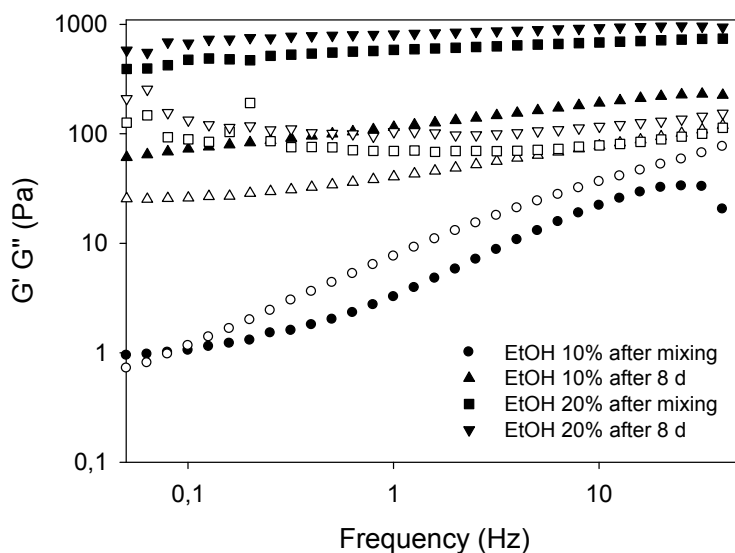


Figure 2: Dynamic mechanical spectra of E0 systems mixed with 10% and 20% ethanol, measurements recorded immediately after mixing and after 8 days. Full symbols: G' , empty symbols: G'' .

The 10 v/v% ethanol system observed immediately after mixing shows the typical behavior of a viscous polymer solution, with G'' higher than G' and both moduli increasing at the increase of the frequency in the explored range. After 8 days storage, this system behaves like a “weak gel”, with G' higher than G'' and both moduli only slightly increasing with the frequency. When the polymer solution is mixed with a higher amount of ethanol (20 v/v%), the system becomes immediately a gel, yet weak, and only a slight increase of both G' and G'' is observed upon storage. It can be concluded that 10 v/v% ethanol is not enough to rapidly attain physical cross-linking of the system. Molecular diffusion of ethanol and macromolecular rearrangements are required for the system to gel, yielding a loose network. Conversely, when the alcohol content is too high (50 v/v%) the possibility of forming extended networks is impaired and small rubbery aggregates are formed. For this reason, the intermediate value of 20 v/v% of ethanol content was chosen for a more systematic investigation of storage time on the rheological properties of the gels with both the nonirradiated and irradiated xyloglucans. The effect of storage time after mixing with ethanol was systematically investigated for the nonirradiated system by dynamic mechanical frequency-sweep tests carried out after 4, 24 and 48 hours, and also after 6 and 8 days (see Figure 3). In all cases, the rheological behavior of a “weak gel” is observed. The effect of storage time is an initial increase of both G' and G'' , in particular going from 4 hours to 24 hours, while after 48 hours and 6 days no significant differences can be appreciated. After 8 days a slight decrease of both moduli is observed, instead. This effect can be ascribed to the onset of degradation phenomena. These results show that 24 h are definitely sufficient for this system to achieve its “equilibrium” state in the given conditions. The influence of radiation dose on XG aqueous solutions when mixed with 20 v/v% ethanol was visually evaluated by a “tilting” test. Results are shown in Figure 4a-b. After 5 minutes, the nonirradiated XG and the 10 kGy irradiated XG are the only systems that do not flow, although they show both transparent and opaque regions. After further 24 hours storage at room temperature all systems look like homogenous, white opaque, “wall-to-wall” gels (no flow upon tilting). Frequency-sweep tests were performed on all systems after 8 days storage at 25°C and the dynamic-mechanical spectra are reported in Figure 5. All systems can be considered weak gels. The effect of irradiation is only evident at the higher doses (40 and 60 kGy), as a reduction of both storage and loss moduli of the gels. Finally, the effect of storage time was investigated for the 20 and 60 kGy irradiated systems. Spectra were recorded after 4 and 24 h and compared with those shown for the 8 days storage time (see Figure 6). For the 20 kGy irradiated XG, the gel significantly increases both G' and G'' values during the first 24 h of storage, while no significant changes are occurring upon 8 days storage. A similar trend is observed for the 60 kGy irradiated XG, which confirms to be weaker than the 20 kGy also after 24 h storage.

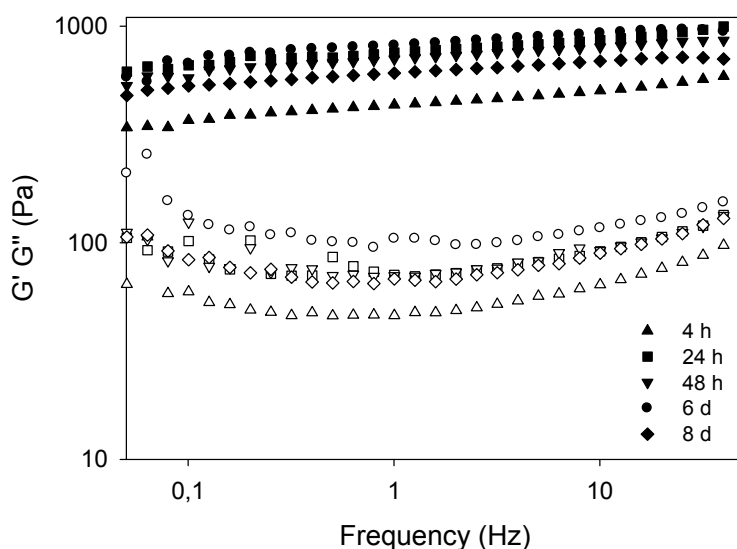


Figure 3: Dynamic mechanical spectra of E0 systems mixed with 20% ethanol, measurements recorded after various interval times from mixing: 4 hours, 24 hours, 48 hours, 6 days, 8 days. Full symbols: G' , empty symbols: G''

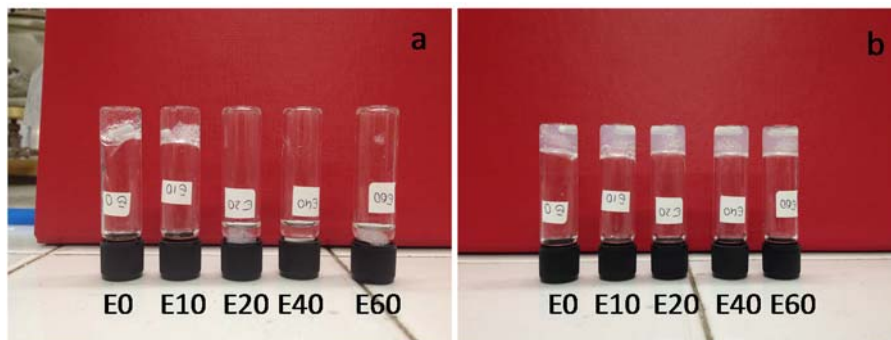


Figure 4: "Tilting test" results of XG aqueous solutions mixed with ethanol a) 5 minutes after mixing, b) 24 hours after mixing.

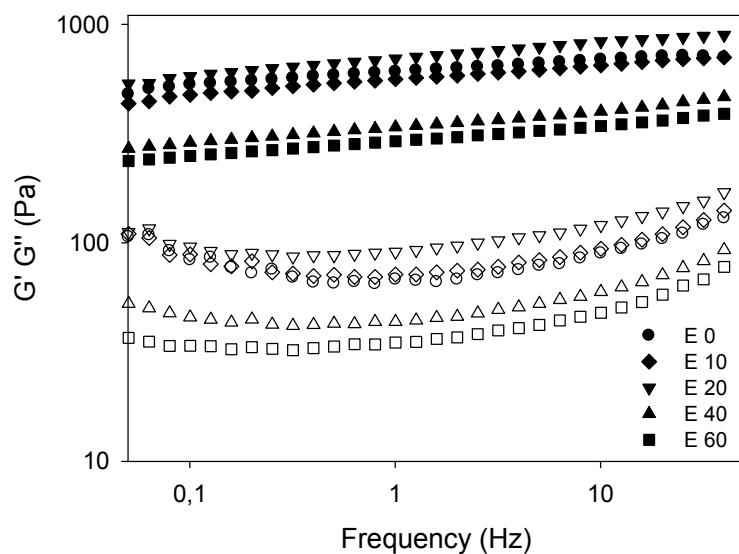


Figure 5: Dynamic mechanical spectra of the nonirradiated system and the irradiated systems obtained by mixing the polymer solutions (2 wt%) with 20% ethanol, measurements recorded 8 days after mixing. Full symbols: G' , empty symbols: G'' .

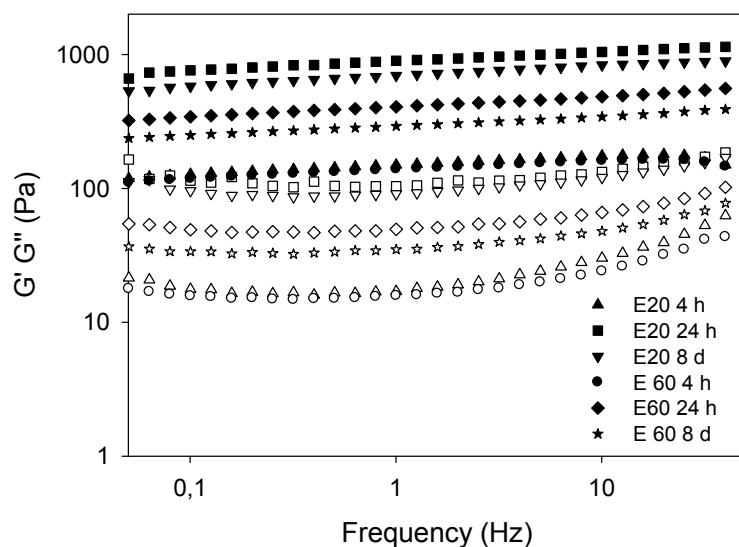


Figure 6: Dynamic mechanical spectra of the 20 kGy and the 60 KGy irradiated systems mixed with 20% ethanol at various storage times: 4 hours, 24 hours and 8 days. Full symbols: G' , empty symbols: G'' .

4. Conclusions

Electron beam irradiation of solid XG in air at room temperature induces a progressive reduction of molecular weight, without significant modification of polymer functionality especially at doses lower than 40 kGy. Irradiation of XG does not impair the ability of the polymer to rapidly undergo gelation when its aqueous solutions are mixed with alcohols. Gelation can be almost instantaneous, macroscopic and fairly homogeneous when polymer concentration is 2 wt% and the alcohol content is around 20 v/v%. Macromolecular rearrangements occur upon storage at room temperature towards the obtainment of stronger gels within one or two days. After that, degradation can occur if samples are stored in non sterile conditions. The stiffening effect is more pronounced for the 20 kGy irradiated XG, which shows an increase of G' of almost one order of magnitude. This result suggests that resizing the polymer molecular weight by irradiation may facilitate the rearrangements required for the local "condensation" that is at the basis of physical gelation.

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