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Degradation of pullulan irradiated in hydro-methanolic blends: influence of cinnamyl alcohol at low absorbed dose of radiation

*Dhriti Khandal and Xavier Coqueret**

Institut de Chimie Moléculaire de Reims, CNRS UMR 7312
Université de Reims Champagne Ardenne - 51687 Reims, France
*Corresponding author: xavier.coqueret@univ-reims.fr

ABSTRACT. This study reports on the effects of electron beam radiation on pullulan in both the dry state and hydro-methanolic blends containing cinnamyl alcohol (CA). The radiation chemical yields of scission (*G(S)*) and crosslinking (*G(X)*) were determined using Saito's formalism applied to the evolution of molecular weight (MW) with increasing absorbed dose of radiation. To satisfy the requirements of the statistical treatment, commercially available pullulan was fractionated to obtain a monomodal MW distribution with a dispersity close to 2. The changes in MW profiles were monitored by SEC with differential refractive index and UV detection. The introduction of small quantities of CA in the pullulan blends led to a significant decrease in *G(S)* and *G(X)*, protecting pullulan against radiation-induced effects likely via energy and electron transfer. In presence of larger amounts of CA, irradiation at higher dose induced an increase in molecular mass with concomitant grafting of the aromatic additive.

Keywords: Scission, Crosslinking, Pullulan, Cinnamyl Alcohol, Radiolytic yields, Radiation protection.

1. Introduction

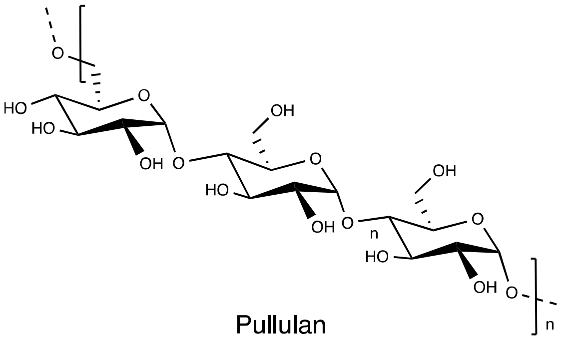
High-energy radiation treatments applied to polysaccharides continue to arouse considerable attention within the scientific community as a green method for modifying a broad class of natural and renewable resources (Al-Assaf et al., 2016). The resulting structural transformations are dependent not just on the induced chemical transformations at molecular level. Physical interactions and molecular arrangements at the nano- and mesoscale levels can have a strong influence on reactivity when complex blends are processed under high energy radiation. As a result, there is a great technological potential for tailoring the properties of polysaccharides (Phillips, 1962; Bhat & Karim, 2009; Brașoveanu & Nemțanu, 2018).

The behavior of polysaccharides submitted to ionizing radiation is roughly described by their general trend towards chain scission with a degree depending primarily on the structure of the polymer, on chemical treatments possibly performed prior to irradiation, and on the degree of hydration (Ivanov, 1992; Bhat & Karim, 2014). Some applications aim at achieving a controlled decrease of the polysaccharides molecular weight (MW) by radiation-induced chain scission for biomass deconstruction (Driscoll et al., 2009; Driscoll et al., 2014) or to produce chitosan oligomers (Choi et al., 2002, Hai et al., 2003). Yet, the degradative behavior is often a limitation in developing new applications based on radiation-induced modification. To overcome the radiation-induced degradation of polysaccharides while achieving the desired structural and chemical modifications, alternative processing methods with corrective actions have been proposed. These methods either consist of blending them with monomers that polymerize and form crosslinks under ionizing radiation or convert reactive side-groups with appropriate functionalities, for example, acetic or acrylic esters. Both approaches try to balance chain scission with crosslinking that results in gel formation upon irradiation (Fei et al. 1999; Wach et al. 2001; Ogawa et al. 2002; Wach et al. 2003; Della Giustina et al. 2019).

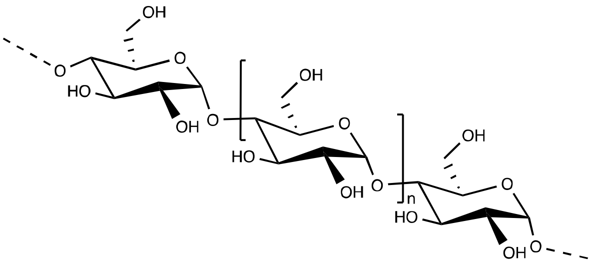
This type of treatment strongly modifies the architecture of the polysaccharide, hence its properties are likely to be changed, but it is an effective way to counteract the formation of low MW fragments, when this is undesirable. In the case of the radiation-induced modification of starch, a major contributor to bio-based plastics with a limited environmental footprint, our group has reported on original methods to mitigate the water sensitivity and to reduce retrogradation of amorphized starch by blending it with lignin or with allyl urea followed by electron beam (EB) processing (Olivier et al., 2000, Olivier et al. 2001; Lepifre et al., 2004a, Lepifre et al., 2004b). Further studies on starch and maltodextrin including low MW aromatic additives such as cinnamyl alcohol (CA) revealed a strong dependence of the radiation-induced degree of modification (i) on the chemical composition of the blend, and (ii) on inter-molecular associations between the constituents with formation of inclusion complexes in helical domains of the glucan (Khandal et al., 2012; Khandal et al., 2013). The occurrence of such supramolecular interactions does not allow for a simple interpretation of the radiation-induced evolution of MW distribution and gel fraction as a function of blend composition and absorbed dose of radiation (Khandal et al., 2015).

In this context, we wished to pursue a more basic investigation with a quantitative assessment of the role and the degree of influence of aromatic additives such as CA on the competing phenomena of scission and crosslinking along glucan chains, using the theoretical formalisms developed by several authors (Saito, 1972; Charlesby, 1980, Moad & Winzor, 1998; Rosiak, 1998) to quantify the radiation chemical yields $G(S)$ and $G(X)$ for scission and for interchain linking, respectively, defined as the number of occurrence for each type of event per unit absorbed dose of radiation.

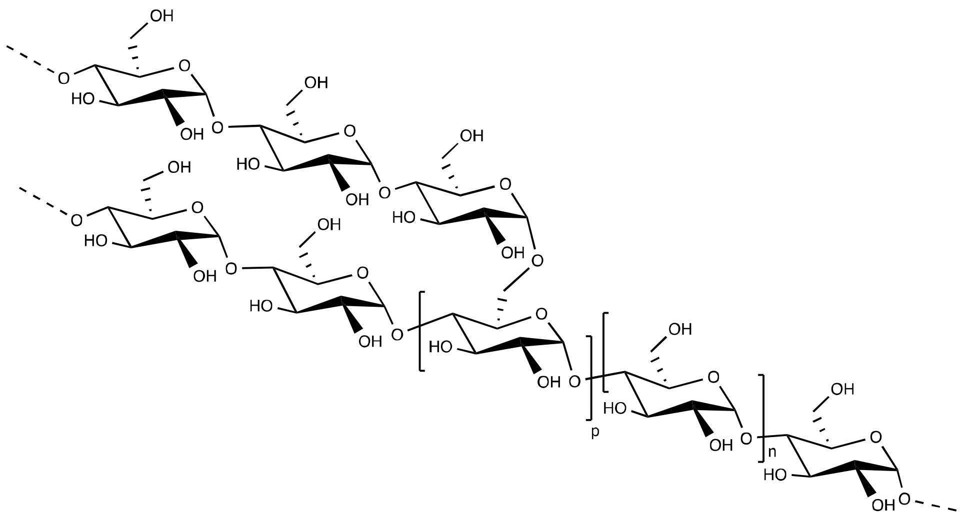
Since starch and starch-derived maltodextrins are multimodal mixtures of linear and branched polysaccharides, generally with a broad MW distribution, they cannot be used for the determination of *G(S)* and *G(X)*. Hence, it was necessary to select an appropriate model for glucans submitted to irradiation in such media, that would comply with the requirements of the theoretical formalism detailed in a forthcoming section of this article. Pullulan is an α-glucan constituted of 1,6-linked maltotriose units, hence including anhydroglucose units (AGU) linked by α-1,4 glycosidic linkages, the basic constituting unit and connecting bonds present in starch, as shown in Scheme 1.



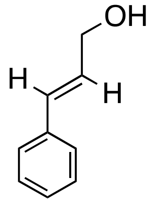
a) Pullulan



b) Amylose



c) Amylopectine d)



Cinnamyl alcohol

Scheme 1. Molecular structure of pullulan (a), of the main components of starch, amylose (b) amylopectine (c), and of cinnamyl alcohol (CA).

Being a well-defined linear polysaccharide (Singh et al., 2008; Trinetta & Cutter, 2016, Singh et al. 2019), pullulan can be easily fractionated by chromatography or precipitation techniques to obtain a desirable MW distribution. Another crucial aspect is its tendency to adopt a random coil conformation in aqueous blends (Nishinari et al., 1991), rather than forming helices with hydrophobic internal cavities exhibiting strong affinity for aromatic solutes, such as CA. Hence, it can be regarded as a good model for monitoring changes in chain length and for the statistical formalisms that allow for the quantification of radiation chemical yields associated with scissioning and interchain coupling.

2. Experimental

2.1 Materials

Pullulan was obtained from ABCR GmbH & Co. and was fractionated for the higher molecular mass portion and freeze-dried before use. Water was de-ionized using the Waters Milli-Q purification system. Reagent grade methanol and cinnamyl alcohol (CA) were purchased from Sigma-Aldrich and used as received.

2.2 Fractionation of pullulan

The commercial sample of pullulan (14 g) was dissolved in distilled water (300 mL) to obtain a homogeneous solution. Methanol (200 mL) was added slowly to this solution under constant magnetic stirring until it became turbid and milky. The mixture was stirred for a few minutes to allow complete precipitation of the large molecular chains of the pullulan. The precipitate was then allowed to settle before filtration. The fractionated pullulan was rinsed two times with water and freeze-dried to yield a white cotton-like material. The pullulan samples were sealed in poly(ethylene) bags under the exclusion of air and stored at 4°C.

2.3 Preparation of pullulan-based formulations

The lyophilized fractionated pullulan was found to have a moisture content of 2.5 wt-% after storage for 4 weeks over P_2O_5 in a desiccator, as determined by thermogravimetric measurements at a constant temperature of 120°C for 72 hours. The dried samples after such a treatment have a moisture content lower than 1 wt-%. Samples were stored and irradiated as such in sealed air-tight vials.

Hydro-methanolic blends were prepared by placing the desired amount of lyophilized pullulan in a porcelain dish and by adding water over the entire volume, keeping the ratio pullulan : water = 70 : 60 parts by weight. The hydrated pullulan formed a viscous paste after conditioning at 50°C for around 30 min. The paste was allowed to cool to room temperature. Methanol was then added dropwise and gently mixed manually, giving the final blend composition of pullulan : water : methanol in 70 : 60 : 30 parts by weight, respectively. The blends containing CA were prepared by adding the aromatic alcohol in small fractions (0.002 to 0.20 equivalents per mole of AGU) to prevent any macrophase separation. Each formulation was prepared, treated and analyzed in duplicate.

2.4 *Irradiation conditions*

The electron beam accelerator used for irradiation was a 10 MeV Circe II industrial scale accelerator (Linac Technology, France) operated at IONISOS, Chaumesnil, France. The samples were sealed in glass vials in ambient air and irradiated with an average dose rate of 3 kGy s^{-1} at 10, 25, 50, 75, 100 and 200 kGy doses, by applying 25 kGy per pass for doses of 25 kGy and above. Dose values were controlled by using calibrated calorimeters with an accuracy of ± 2 %.

2.5 SEC analysis

The size exclusion chromatography (SEC) system used for this study was based on a DMSO and water mixture (20 : 80 v/v) as mobile phase containing 0.02 percent in weight (wt-%) NaN_3, as an anti-bacterial agent and background electrolyte (308 mM). The sample solutions were prepared by weighing 80 mg of formulation and adding 4 mL of the mobile phase followed by heating at 60°C for 30 min. Complete solubility of the formulation in the mobile phase resulted in a clear solution that was filtered using 0.45µm PTFE syringe filters before injection into the SEC system. The separation was carried out on two Polargel columns M and L kept in series (obtained from Varian- Agilent Technologies) behind a Polargel-specific guard column (Varian-Agilent Technologies). The mobile phase flowing at a constant rate of 1 mL min^{-1} was analyzed by two detectors in series, first a UV Absorbance detector (Jasco UV-1575) set to 282 nm and then a differential refractive index (RI) detector (Precision Instruments IOTA 2). Samples were injected with a 50 µL-volume using an auto-sampler (Jasco AS-350) .

The calibration curve was established from the retention times based on RI detection of 9 pullulan standards of low dispersity (Đ ~ 1.05) covering a broad MW range between 700 and 800000 g mol^{-1} (Varian-Agilent Technologies). Every sample was analyzed by at least 3 injections for calculating the average MWs of the polymers.

3. Results and discussion

3.1. Theoretical considerations

To apply the formalisms developed independently by Charlesby and Pinner, and by Saito, to the changes in molecular mass for a given type of polymer as a function of absorbed dose of radiation, the treated polymer must comply with certain critical requirements that can be summarized as follows: (i) the initial polymer structure should be topologically linear, (ii) the events of scission and crosslinking should occur randomly along the chains and their extent must directly be proportional to the dose, (iii) the number of monomer units affected by chemical events should be considerably lower than the number-average degree of polymerization (Saito, 1972; Charlesby, 1980, Moad & Winzor, 1998; Rosiak, 1998).

Depending on the initial MW distribution and on the relative importance of scission and crosslink probabilities in the treated polymer, several relations have been derived from the statistical models to correlate the radiation chemical yields *G(S)* and *G(X)* to the change of MW distribution. If the starting polymer has a most probable MW distribution ($\overline{M}_w$ / $\overline{M}_n = 2$), the two models provide relatively simple equations relating to the dose-dependence of quantities deduced from experimental data. If gelation occurs, the Charlesby-Pinner relation (Equ. 1) predicts a linear variation of the quantity ($s + s^{0.5}$), where s is the sol fraction, with the reciprocal value of the dose D (kGy), giving access to *G(S)* and *G(X)* values in mol J^{-1} for the polymer of initial weight-average MW, $\overline{M}_w^0$ expressed in g mol^{-1}.

$$s + \sqrt{s} = \frac{G(S)}{2G(X)} + \frac{1}{\bar{M}_w^0 G(X) D} \qquad (1)$$

The second method is based on the evolution of polymer molecular masses, the reciprocal values of $\bar{M}_n$ and $\bar{M}_w$ being predicted to vary linearly with the applied dose D (kGy) and this relation, the $G(S)$ and $G(X)$ values in mol J^{-1} as well as the initial molecular masses ($\bar{M}_n^0$and $\bar{M}_w^0$) appearing as constants in equations 2 and 3, respectively.

$$G(S) \quad - G(X) \quad = \frac{1}{D}\left(\frac{1}{\bar{M}_n} - \frac{1}{\bar{M}_n^0}\right) \qquad (2)$$

$$\frac{G(S)}{2} - 2G(X) \quad = \frac{1}{D}\left(\frac{1}{\bar{M}_w} - \frac{1}{\bar{M}_w^0}\right) \qquad (3)$$

In practice, if the corresponding plots of $(\bar{M}_n)^{-1}$ and $(\bar{M}_w)^{-1}$as a function of dose are acceptably linear over a statistically relevant number of experiments, the values of the two slopes allow for the calculation of the $G(S)$ and $G(X)$ values for the system.

Despite certain limitations (Güven, 1990), size exclusion chromatography (SEC) is a particularly suitable method used for determining the changes in the average MWs of a polymer sample before the gel point, in the eventuality of predominant crosslinking. This analytical technique has been reported for the study of pullulan degradation by γ-irradiation (Shingel et al., 2000).

3.2 Fractionation of Pullulan

The commercially obtained pullulan is a multimodal and highly polydisperse sample with two well separated domains in the SEC-RI profile, one representing the larger molecular mass fraction ($\bar{M}_w$ ~ 250,000 g mol^{-1}) and the other a smaller molecular mass fraction ($\bar{M}_w$ ~ 4,400 g mol^{-1}) (Fig. 1 a). Since the commercial sample did not satisfy the requirements of the statistical model, the separation of the larger molecular mass fraction of pullulan was performed to obtain a pullulan with random molecular weight distribution.

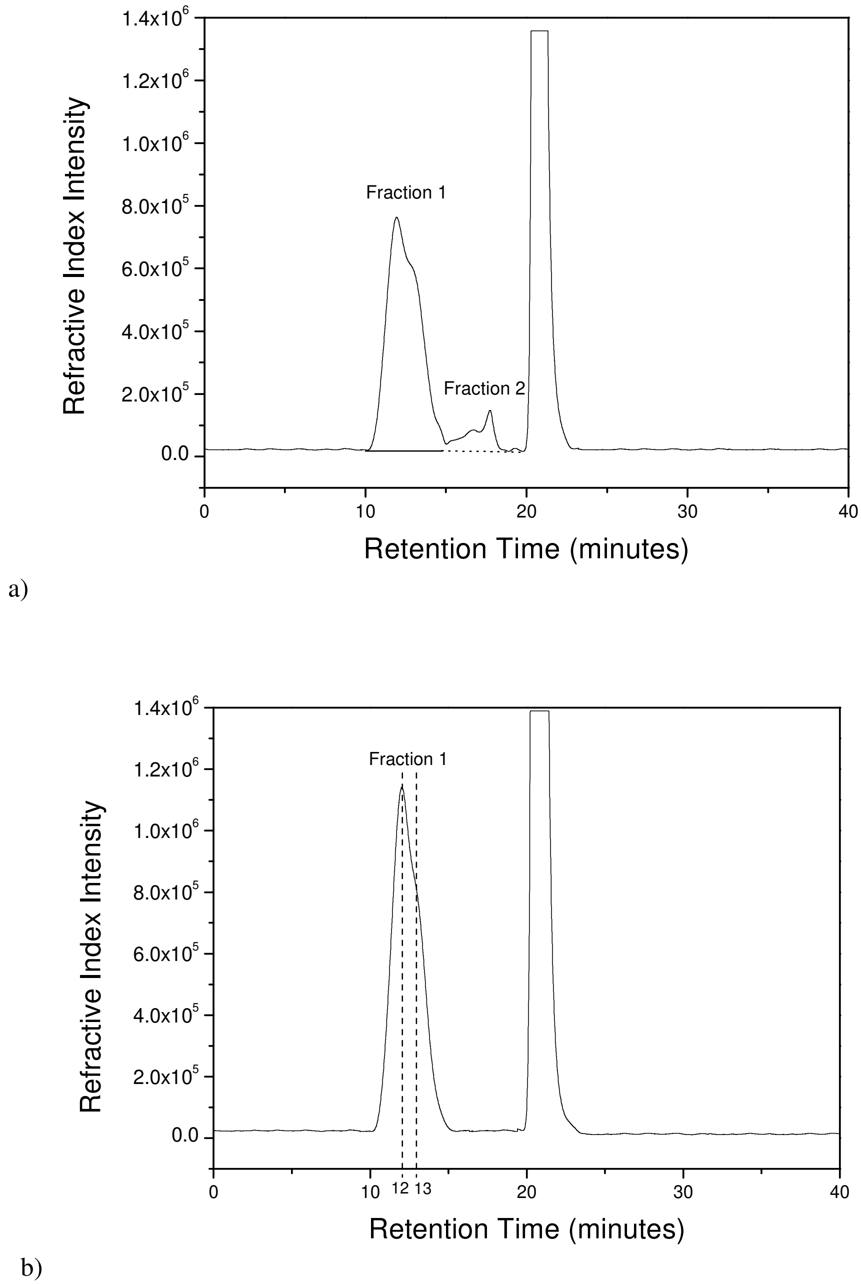


Fig. 1. SEC traces of the original (a) and of the fractionated pullulan (b) recorded with refractive index detection.

Fractionation of the commercial pullulan sample was attempted using different methods of separation such as preparative chromatography and precipitation by the addition of poor solvents to the aqueous solution (methanol, ethanol, acetone, isopropanol, THF and acetonitrile). It was found that the solvent-selective precipitation method was faster and more efficient in terms of final dispersity Đ ~ 2. The chromatographic separation method on a

Sephadex column was shown to result in much narrower MW distribution, the dispersity Đ being typically between 1.2 and 1.5. Precipitation with methanol was found most convenient and standardized over several trials with varying sample sizes. Good reproducibility was achieved when the concentration of aqueous commercial pullulan solution was 5 % (w/v) and the final methanol : water ratio remained close to but did not exceed the ratio of 6 : 4 (v/v). After decantation and washing the precipitate with water, the fractionated pullulan samples were freeze-dried. SEC analysis with the RI detection of this material (Fig. 1-b) showed a single peak with a slight shoulder. The peak profile did not change for various pullulan obtained from different batches, with retention times for the peak onset and the maximum differing only by a few seconds. The average MWs for the entire peak of the fractionated pullulan were $\overline{M}_w$ ~ 280,000 g mol^{-1} and $\overline{M}_n$ ~ 130,000 g mol^{-1}, leading to a dispersity value $\dot{D}$ ~ 2.15 ± 0.03. The small shoulder in the peak is considered of limited consequence in this study because we are concerned with the evolution of the molecular mass of the entire peak, independent of local variations of the distribution.

3.3 Effect of ionizing radiation on G(S) and G(X) of pullulan treated in the solid state

Besides the macromolecular effects of ionizing radiation to polysaccharide in general, and polyglucans more specifically, the basic direct and indirect reactions that affect the monomer units produce new functional groups on the polysaccharide that absorb in the UV region, such as aldehydes, ketones, carboxylic acids, etc. (Phillips et al. 1966; Scherz, 1970; Ershov, 1998; Shingel, 2002). Therefore, SEC analysis with double detection, UV absorption and differential refractive index, brings additional information on the degree of modification in constituting units as a function of pullulan chain length and as a function of dose.

The chromatograms of Fig. 2 were recorded with differential refractive index detection of fractionated pullulan irradiated in the dehydrated solid state for absorbed doses of radiation ranging from 25 to 200 kGy. The evolution of the traces illustrates the modifications occurring at the macromolecular and molecular levels.

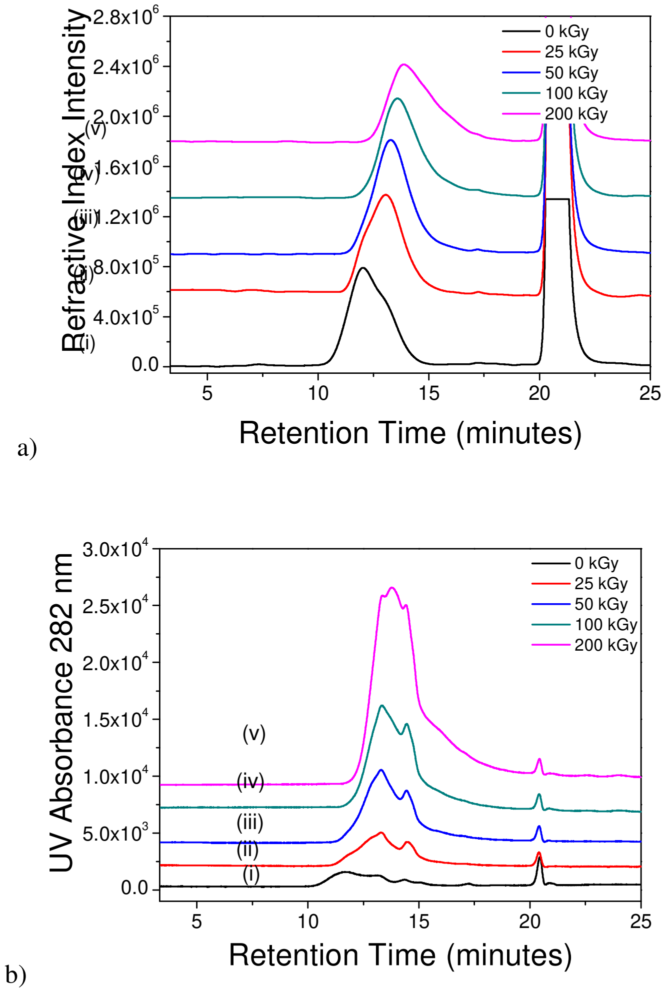


Fig. 2. Evolution of SEC traces for the fractionated pullulan after EB irradiation as a dehydrated powder with doses up to 200 kGy (0 kGy (i), 25 kGy (ii), 50 kGy (iii), 100 kGy (iv), 200 kGy (v)) recorded with differential refractive index detection (a) and with UV detection at 282 nm (b).

The SEC traces obtained by differential refractive index detection confirmed the strong propensity of pullulan to degrade, with a gradual shift toward longer retention times of the whole distribution. The dose-dependence of the reciprocal values of $\bar{M}_n$ and $\bar{M}_w$ calculated from the SEC traces is represented in the plots of Fig. 3.

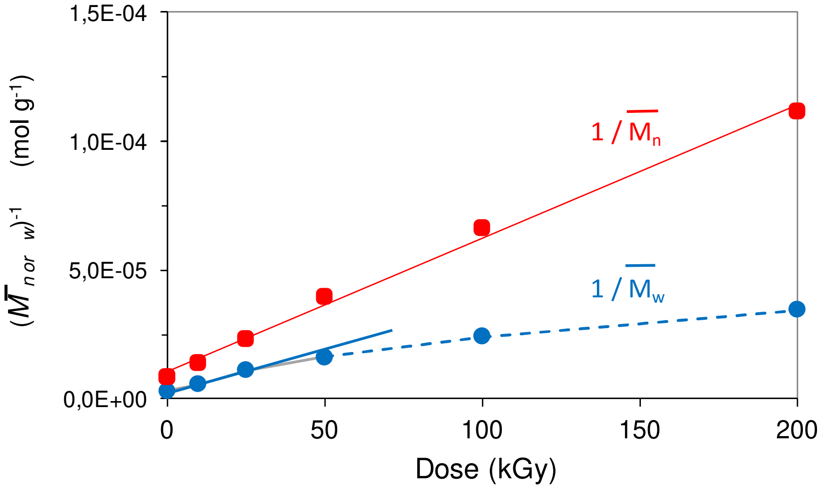


Fig. 3. Evolution of reciprocal values of $\bar{M}_n$ (■) and $\bar{M}_w$ (●) calculated from SEC traces recorded with refractive index detection for samples of fractionated pullulan irradiated in the dry state with doses up to 200 kGy.

The reciprocal number-average mass $\bar{M}_n^{-1}$ varies linearly across the whole range of doses explored for these experiments, following the predicted behavior expressed in equation 2, whereas the linearity of the reciprocal weight-average mass $\bar{M}_w^{-1}$ is lost for absorbed radiation doses higher than 40-50 kGy. The explored dose range, up to 200 kGy, is quite high for a polysaccharide. The SEC traces obtained by UV detection at 282 nm (Fig. 2-b) indeed reveal the concomitant formation of chromophores, likely carbonyl groups, that are known to result from the rearrangement of the initial free radicals generated by direct radiolysis of the polysaccharide, and also by their subsequent oxidation in the presence of air (Ershov, 1998; Bhat et al., 2014).

This confirms that the initial structure of pullulan has been profoundly modified. Its reactivity as well as its macromolecular properties should then be affected accordingly. Such chemical changes may explain the deviation from linearity, since the basic hypotheses of the statistical model are no longer fulfilled.

The linear domains of the plots covering the low dose range (from 0 to about 50 kGy) were then used to calculate the *G(S)* and *G(X)* values, by combining equations 2 and 3. The results in entry a of Table 1 show that the slopes are calculated in the domain from 0 to 25 kGy with acceptable correlation coefficients, yielding a *G(X)* value close to zero and *G(S)* of about 0.6 µmol J^{-1}.

Table 1. Determination of the radiation chemical yields *G(S)* and *G(X)* using SEC data measured for fractionated pullulan samples irradiated in the solid state.

Entry	Pullulan sample	$(\bar{M}_w)^{-1}$versus dose		$(\bar{M}_n)^{-1}$versus dose		*G(S)*	*G(X)*
		m_w a)	R_w b)	m_n a)	R_n b)	(µmol J^{-1})	(µmol J^{-1})
a	Powder dehydrated over P_2O_5	5.8 10^{-7}	0.996	2.7 10^{-7}	0.997	0.59	0.015
b	Powder with a moisture content of 2.5 wt-%	4.0 10^{-7}	0.999	1.8 10^{-7}	0.994	0.41	0.015

a) m_w and m_n represent the slopes of the linear regression for the plots of Fig. 3 and 4.

b) R_w and R_n are the coefficients of correlation of the corresponding linear regressions.

Similar experiments were conducted with fractionated pullulan irradiated in the solid state with a moisture content of 2.5 wt-%. Not surprisingly, the *G(X)* value is again close to zero for the neat polysaccharide (entry b of Table 1), but the *G(S)* value of 0.41 µmol J^{-1}, is somewhat lower than for the dehydrated sample. Similar protective effects by moisture has been reported in the early studies of the effects of radiation on polysaccharides (Chapiro, 1962), amylose (Ehrenberg et al., 1957) and cellulose derivatives (Leavitt, 1961; Leavitt, 1962).

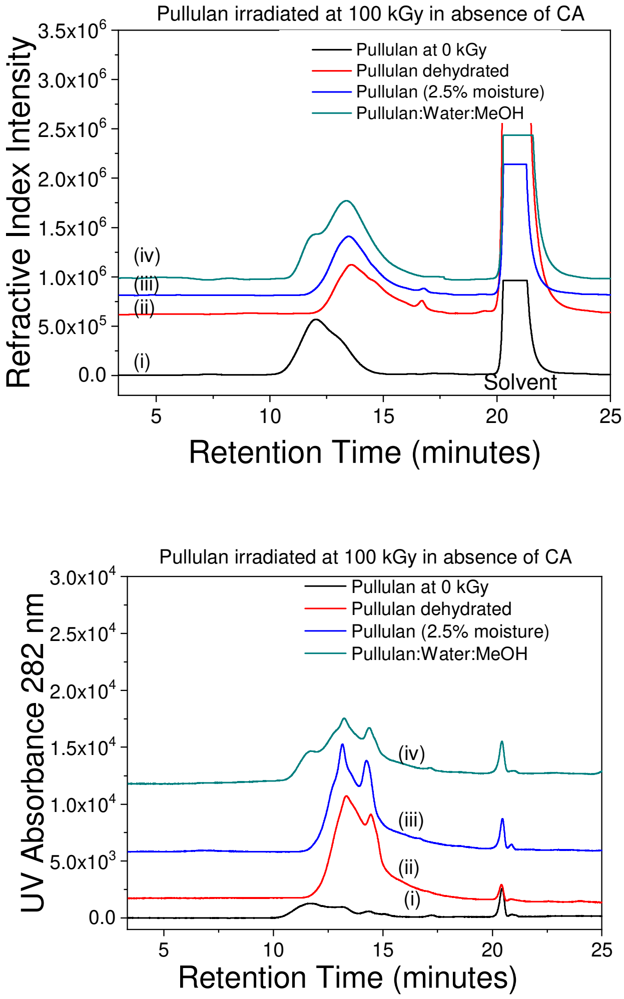
Few articles report reliable data using the theoretical models to quantify the behavior of polysaccharides under radiation. Among the glucose-based polysaccharides, amylose was shown to degrade with *G(S)* values between 0.5 and 1.35 µmol J^{-1} (Ehrenberg, 1957) and cellulose with a *G(S)* value of 0.56 µmol J^{-1} (Ershov, 1998). The *G(S)* values reported for other polysaccharides, based on modified glucose units or other types of sugar units carrying ionic groups, were found to be between 0.09 and 2.1 µmol J^{-1} for chitosan samples irradiated in the solid state, depending on their degree of deacetylation (Ulanski & Rosiak, 1992;

Taskin et al., 2014), between 0.15 and 0.25 for carboxymethyl cellulose (Fei et al., 2000), and from 0.1 to 0.4 µmol J^{-1} for various sodium alginates (Sen et al., 2014). Despite the differences in the conditions for irradiation treatments and in the analytical methods that were used, the scissioning yield that we report with a $G(S)$ value of 0.59 for dry pullulan compares well with values determined for amylose and cellulose, the linear polysaccharides showing the closest structural similarities with pullulan.

Strictly speaking, the next coming part of our work is somehow on the borderline of the basic frame of the theoretical models describing the radiation behavior of irradiated polymers. We wanted to apply this approach to study the influence of minor amounts of additives in protic formulations including water and methanol. This was motivated by our previous studies on the influence of an aromatic alcohol, CA, and on glycerol in the maltodextrine-based blends, these additives having shown a strong impact on the gelation of maltodextrine blends (Lepifre et al., 2004; Khandal et al. 2012, Khandal et al., 2013; Khandal et al., 2015). Various formulations containing the same pullulan at the same concentration were thus considered as a whole, to examine the influence of the composition in the additives on the apparent radiation chemical yields $G_{app}(S)$ and $G_{app}(X)$ values for pullulan irradiated in complex blends. The influence of the hydro-methanolic dispersing medium was examined at first by comparing pullulan-based formulations having a paste-like consistency due to the use a limited amount of solvents. The relatively high concentration of pullulan in these blends not only favors crosslinking by enhancing the occurrence of intermolecular coupling between chains, but also allows to achieve an acceptable degree of dispersion of the components within the slurry, even in the presence of a relatively hydrophobic additive such as CA (see section 3.5).

3.4 G(S) and G(X) of pullulan in hydro-methanolic blends irradiated in absence of CA

The SEC traces recorded with differential refractive index and UV (282 nm) detection of pullulan and pullulan-based slurries after irradiation at 100 kGy are shown in Fig. 4-a and Fig. 4-b, respectively.



a)

b)

Fig. 4. SEC traces with differential refractive index (a) and UV detection at 282 nm (b) for pullulan unirradiated (i), and irradiated at a 100 kGy dose in the dry state (ii), with 2.5 wt-% of moisture (iii), in paste-like blend of composition pullulan : water : methanol (70/60/30 parts by weight) (iv).

In comparison with the unirradiated pullulan, the hydro-methanolic blend reveals an overall increase in the retention times for the polysaccharide component, indicating a marked decrease in the chain length resulting from the radiation treatment. However, the profiles show a lower level of chain scission than for pullulan irradiated in the dry state or with a low moisture content. Also, the presence of shoulder on the high-MW side of the main peak suggests the occurrence of some intermolecular coupling reactions, whereas the formation of chromophores absorbing at 282 nm, appreciated from the area of chromatograms recorded for samples prepared with the same concentration in pullulan, is somewhat lower for the paste-like hydro-methanolic blend.

The evolutions of the $(\bar{M}_w)^{-1}$ and $(\bar{M}_n)^{-1}$ values for pullulan calculated from the differential refractive index chromatograms for all the blends were plotted against the absorbed dose of radiation. For doses in the lower range, up to 50 kGy for $(\bar{M}_w)^{-1}$, and up to 100 kGy for $(\bar{M}_n)^{-1}$, the plots of Fig. 5 show an acceptable degree of linearity with correlation coefficients R equal to 0.996 and 0.999, respectively, allowing for the determination of apparent radiation chemical yields. To take into account, both from energetic and chemical viewpoints, the indirect chemical effects caused by radiation within the fluid medium onto the pullulan component, corrected chemical yields $G(S)_{corr}$ and $G(X)_{corr}$ are calculated by introducing the fraction in weight (f_w) of pullulan within the hydro-methanolic blend samples, using equations 4 and 5.

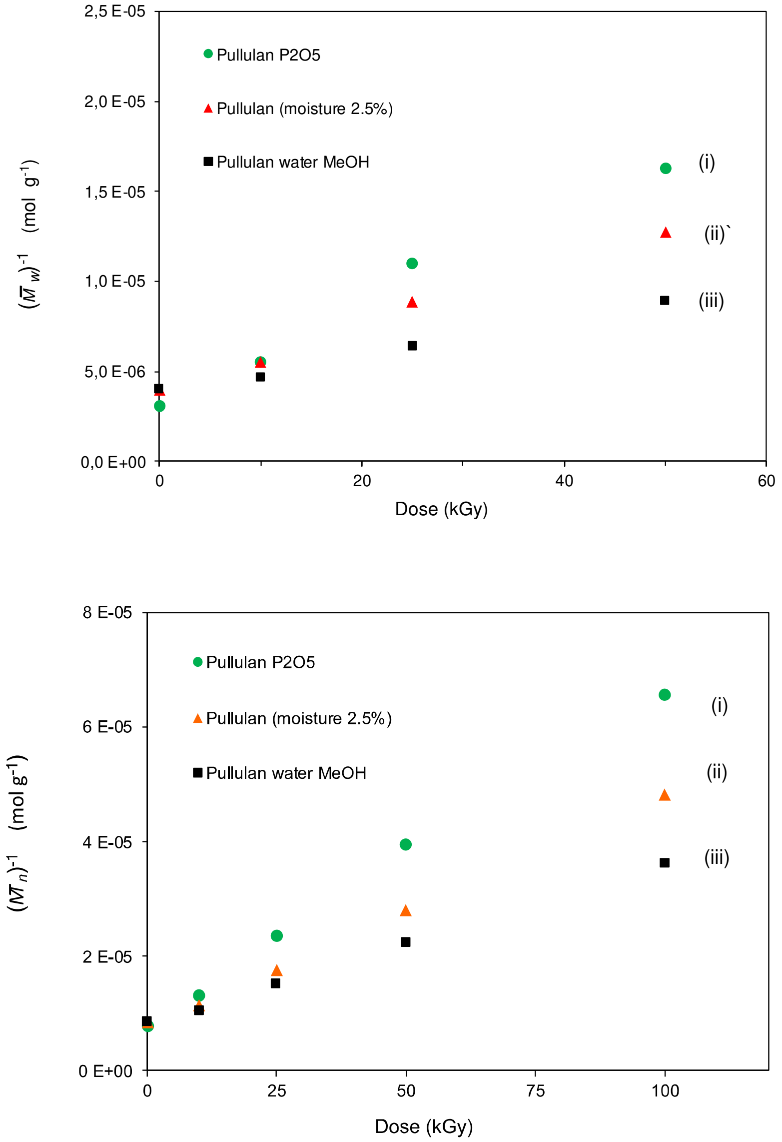
$$G(S)_{corr} - G(X)_{corr} = \frac{f_w}{D}\left(\frac{1}{\bar{M}_n} - \frac{1}{\bar{M}_n^0}\right) \qquad (4)$$

$$\frac{G(S)_{corr}}{2} - 2G(X)_{corr} = \frac{f_w}{D}\left(\frac{1}{\bar{M}_w} - \frac{1}{\bar{M}_w^0}\right) \qquad (5)$$

By this way, the number of events considered for each corrected G value are ratioed to the total energy deposited in the whole sample. In the present case, all blend samples include pullulan with the same weight-fraction, f_w = 0.4375, yielding the corrected values $G(S)_{corr}$ 0.134 µmol J^{-1} and $G(X)_{corr}$ 0.0117 µmol J^{-1}.

The deviation from linearity observed for higher doses can either originate from unsatisfied assumptions on the basic hypotheses required by Saito's model, or by a lack of accuracy of the MW calculated from the SEC traces for pullulan chains strongly modified by the radiation treatment, particularly if interchain branching takes place.

The *G(S)/G(X)* ratio determined for each blend sample is independent of the concentration in polymer. This ratio is interesting from a practical viewpoint, since the statistical models mentioned in the introduction predict that the irradiated polymer can undergo gelation if *G(S)/G(X)* < 4, or will not form a tridimensional network at any dose if *G(S)/G(X)* > 4.



a) b)

Fig. 5. Plots showing the linear part of the variations of $(\bar{M}_w)^{-1}$ (a) and $(\bar{M}_n)^{-1}$ (b) as a function of absorbed dose of radiation across the lower range, for pullulan dehydrated (i), pullulan with a moisture content of 2.5 wt-% (ii), hydro-methanolic paste of pullulan (iii).

Compared to pullulan irradiated in the dry solid state, with *G(S)* = 0.59, the *G(S)* values determined for the paste-like hydro-methanolic blend is lower by a factor approximately 4, with $G(S)_{corr}$ close to 0.15 for pullulan in water : methanol, whereas the $G(X)_{corr}$ value appears to be much less affected.

The direct and indirect effects of radiation-induced processes on pullulan are complex in such concentrated and multicomponent blends. Water radiolysis occurring in these blends generates various active species (Hart, 1965; Le Caër, 2011), among which $HO^{\bullet}$ radicals are known to efficiently abstract hydrogen atoms from the polysaccharide and induce the formation of C-centered free radicals at different positions of the AGU moieties (Von Sonntag & Schuchmann, 2001). Alcohols act as scavengers of $HO^{\bullet}$ radicals (Baugh et al., 1982; Buxton et al., 1988), therefore being expected to decrease the indirect radiolytic effects on both scission and crosslinking compared to simpler pullulan-water blends. Accordingly, the $G(X)$ and $G(S)$ values that were determined from the linear segments of the plots of Fig. 5 are significantly lower when an alcohol is present in the blend.

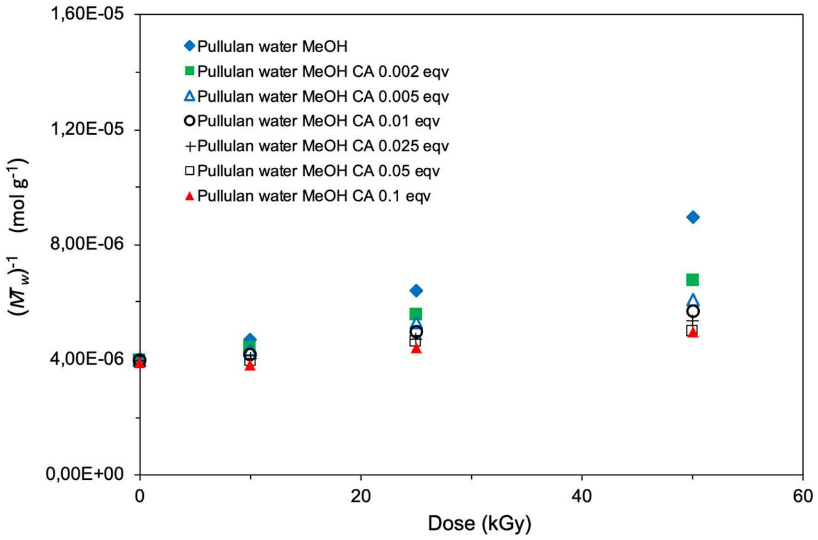
In addition to the chemical effects on the radiolytic and free radical processes, the hydro-methanolic medium has physical effects by enhancing chain mobility that would likely increase the possibility of interchain coupling between the macroradicals. Compared to the solid state and to dilute solution, paste-like blend samples offer much favorable conditions for ensuring gel formation (Wach et al, 2003; Yoshii et al., 2003).

At higher dose levels, the loss of linearity in the plots of the reciprocal MWs for pullulan in hydro-methanolic blends reveals a change in behavior, with stronger competition by intermolecular coupling. There is an inversion in the curves, indicating an overall increase of $\bar{M}_n$ and $\bar{M}_w$ values (Fig. 6 a-b, curves (iv)).

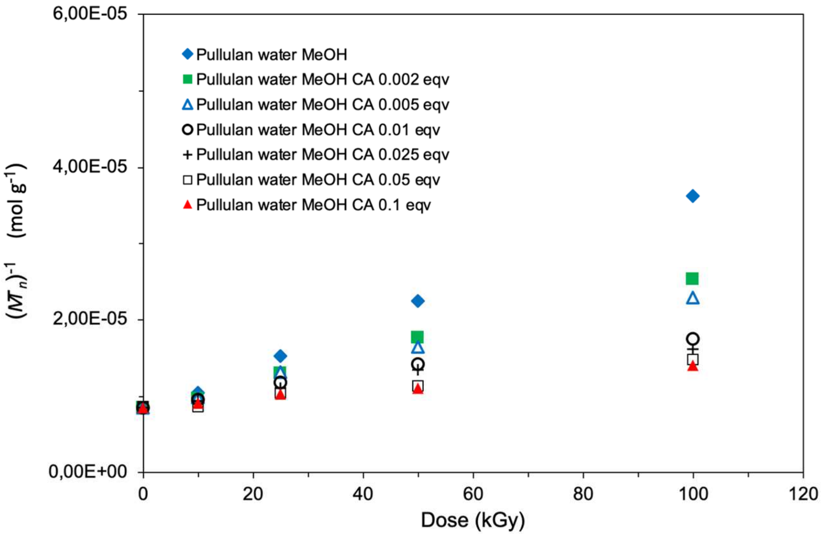
3.5 G(S) and G(X) of pullulan in hydro-methanolic blends irradiated in presence of CA

A remarkable feature of the pullulan blends including CA additive in the water-methanol solvent is the drastic increase in viscosity or even the occurrence of gelation when they are treated at a dose of 100 kGy or higher. The most interesting formulations in that respect were the water-methanol blends containing 0.1 and 0.2 equivalent of CA per mole of AGU that formed considerable amounts of gel when irradiated above 100 kGy, whereas the samples with lower CA content either showed an increase in viscosity or some signs of incipient gel formation.

To gain a better understanding of the effect of CA on the competition between radiation-induced scission and interchain coupling for pullulan in hydro-methanolic media, we have monitored the changes of MW distribution in blends with CA contents varying between 0.002 to 0.2 equivalents per mole of AGU from pullulan using the same analytical approach as was the one described in the previous section. The blends were prepared accordingly with CA concentration between 5.4 and 270.0 mmol kg^{-1} and a constant AGU concentration of 2.7 mol kg^{-1}.



a)



b)

Fig. 6. Evolution of (a) $(\bar{M}_w)^{-1}$, and (b) $(\bar{M}_n)^{-1}$ as a function of dose for pullulan blends in hydro-methanolic (70 : 60 : 30 parts by weight) including various amounts of CA.

Table 2. *G(S)* and *G(X)* values for pullulan in hydro-methanolic blends as a function of dose for various CA contents, based on the slopes of $(\bar{M}_w)^{-1}$ and $(\bar{M}_n)^{-1}$ in Fig. 7.

Pullulan in in water : methanol 70 : 60 : 30 (parts by weight)	Slope $(\bar{M}_w)^{-1}$ *vs D*		Slope $(\bar{M}_n)^{-1}$ *vs D*		*G(S)*$_{corr}$ (µmol J^{-1})	*G(X)*$_{corr}$ (µmol J^{-1})
	m_w	R_w	m_n	R_n		
no CA	1.01 10^{-7}	0.996	2.81 10^{-7}	0.999	0.1340	0.0115
CA 0.002 equiv. / 5.4 mmol kg^{-1}	0.57$10^{-7}$	0.990	1.70 10^{-7}	0.996	0.0826	0.0083
CA 0.005 equiv. / 13.5 mmol kg^{-1}	0.43 10^{-7}	0.996	1.44 10^{-7}	0.973	0.0715	0.0085
CA 0.01 equiv. / 27.0 mmol kg^{-1}	0.36 10^{-7}	0.978	0.89 10^{-7}	0.977	0.0416	0.0026
CA 0.025 equiv. / 67.5 mmol kg^{-1}	0.29 10^{-7}	0.977	0.76 10^{-7}	0.975	0.0360	0.0026
CA 0.05 equiv. / 135.0 mmol kg^{-1}	0.23 10^{-7}	0.924	0.54 10^{-7}	0.990	0.0295	0.0022
CA 0.1 equiv. / 270.0 mmol kg^{-1}	0.23 10^{-7}	0.921	0.62 10^{-7}	0.983	0.0249	0.0012

[a] m_w and m_n represent the slopes mol g^{-1} kGy^{-1} of the linear regression for the plots of Fig. 9.

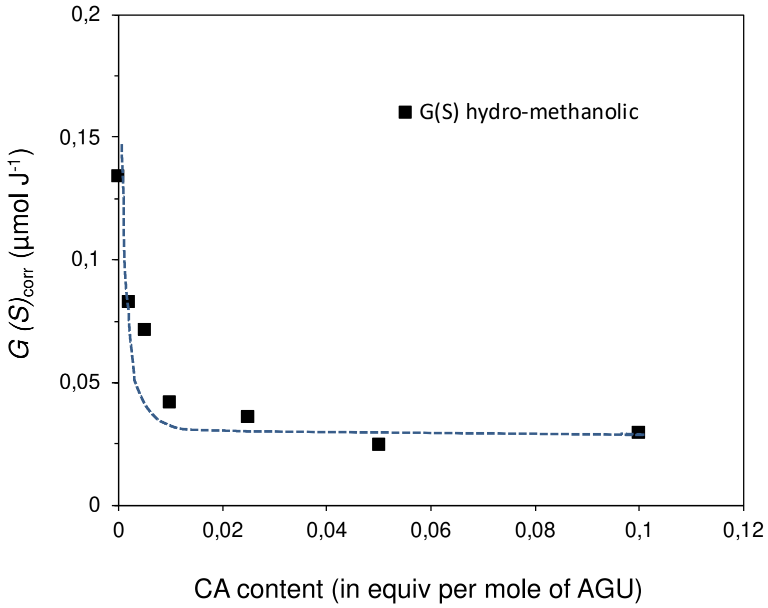
[b] R_w and R_n are the coefficients of correlation of the corresponding linear regressions.

Compared to the profiles recorded with irradiated samples that did not contain the aromatic additive, the first qualitative observation is the mitigated trend for chain degradation and the gradual emergence of bimodality in the MW distribution, with the persistence of a fraction with longer polymer chain. The average molecular masses were calculated for each of these formulations (see Supporting information) to plot the dose-dependence of $(\bar{M}_w)^{-1}$ and $(\bar{M}_n)^{-1}$ values for a large series of blends with various amounts of CA additive. The linearity of the plots was satisfactory for doses up to 50 kGy (Fig. 6).

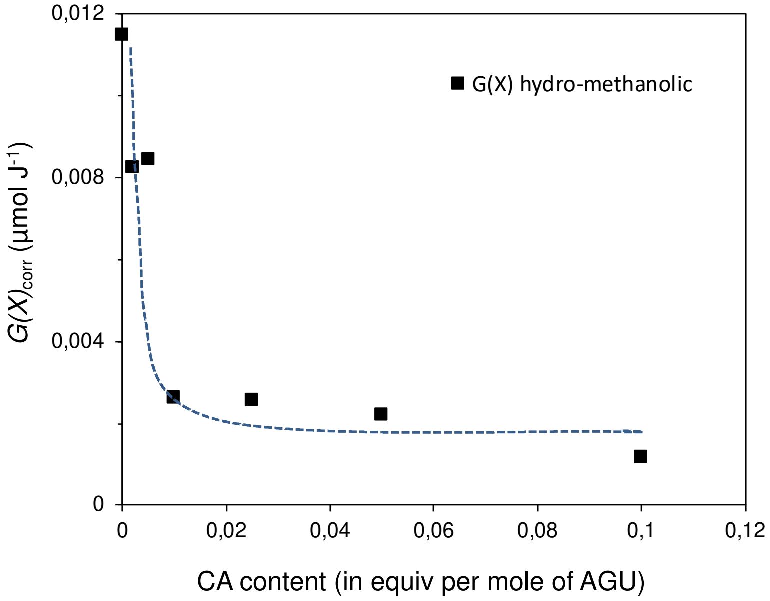
In both cases, the points corresponding to the 10 kGy dose appear slightly above the theoretical straight line. We assume that the presence of trace amounts of oxygen affects the radiolytic reactions at low doses, until the complete consumption of the dissolved O_2. The dependence over the dose range up to 50 kGy was considered more representative of the response of the system to the radiolytic treatment. The participation of CA to radiation-induced reactions could also affect the composition of the blends upon irradiation. However, we have determined by ^{1}H NMR spectroscopy that less than 40 mol-% of CA was modified by treatment at 200 kGy for the hydro-methanolic pullulan blend containing 0.2 equivalent of the aromatic alcohol (see Supporting information). We can therefore assume that the concentration of CA was approximately constant within all the samples during the treatment by much lower doses (up to 50 kGy).

The $G(S)_{corr}$ and $G(X)_{corr}$ values calculated for pullulan in hydro-methanolic blends are reported in Table 1 together with the correlation coefficient of associated linear variations.

The corresponding plots of Fig. 7-a and 7-b clearly show the drastic decline in $G(S)$ and $G(X)$ for amounts as small as 0.002 equivalents of CA per mole of AGU in the blends, corresponding to a concentration of 5.4 mmol per kg of the blend. This decrease in G-values suggests a strong reduction of the number of free radicals generated by direct or indirect radiolysis on pullulan chains.



a)



b)

Fig. 7. Comparison of the (a) *G(S)* and (b) *G(X)* values for pullulan : water : methanol (70 : 60 : 30 parts by weight) formulations containing varying amounts of CA and irradiated in the dose range of 0 to 60 kGy.

The presence of aromatic compounds in irradiated blends, as well as the presence of aromatic moieties in irradiated organic compounds, are known to mitigate radiolytic processes, particularly the formation of molecular hydrogen (Soebianto et al., 1992; Soebianto et al., 1993; Tabuse et al., 2001; La Verne & Dowling-Medley, 2015). Similar protective effects due to energy and charge transfer to aromatic additives seem to operate here, affecting with approximately the same attenuation factor both radiation chemical yields, therefore with limited impact on the *G(S)* / *G(X)* ratio in the low dose range (see Table 2).

4. Conclusion

The radiation chemical yields of scission and crosslinking for pullulan submitted to electron beam radiation in the dry state ($G(S) = 0.59$, $G(X) < 0.02$) complement the available sets of data on the radiolysis of polysaccharides. The method based on the monitoring of changes in molecular weight by SEC confirmed the influence of small amounts of moisture on both *G* values.

Our study emphasizes the effect of hydro-methanolic media on paste-like pullulan blends, showing that indirect effects of water radiolysis are overcome by alcohol-mediated scavenging of $HO^{\bullet}$ radicals, resulting in a decrease of *G(S)*. Introducing cinnamyl alcohol in the hydro-methanolic pullulan blends further induces a strong protective effect with a noticeable impact likely due to energy and / or electron transfer to the multifunctional aromatic additive. A significant influence of CA was observed at concentrations as low as 5 mmol kg^{-1} in the blend.

In another component of this study, we have analyzed by MALDI-ToF mass spectrometry and ^{1}H NMR spectroscopy, the modification occurring with pullulan and maltotriose. The Charlesby-Pinner model based on the gel fraction in irradiated pullulan provides access to the *G(S)* and *G(X)* values determined for the various blend compositions discussed here, but in another dose range beyond the gel point. Structural analysis of modified pullulan and other low molar mass model compounds bring additional information on the participation of CA to the radiation-induced gelation that we observed in the higher dose range for blends containing the aromatic additive at concentrations above 250 mmol kg^{-1}.

From a practical standpoint, the protective effect against main chain scissions demonstrated in this report opens a new perspective for the radiation treatment of polysaccharides which is generally considered to be strongly limited by the inherent sensitivity of this class of natural polymers to radiolytic degradation. The influence of other natural aromatic compounds, such as coumaric and ferulic acids, or of glycerol, as an alternative to methanol in the hydro-alcoholic blends, are under investigation.

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Highlights (3 to 5 bullet points - maximum 85 characters each)

- Pullulan was studied as a model for starch irradiated in dry or paste-like states
- Radiolytic yields for scission and cross-linking were determined using SEC data
- Cinnamyl alcohol exerts a radiation-protective effect even at low concentration
- Gelation is observed at high dose for cinnamyl alcohol exceeding 130 mmol kg^{-1}