Structural Evidence of Grafting in Electron Beam Irradiated Starch-Allylurea Blends

*A. Olivier, J. Luna-Carlos, F. Cazaux, X. Coqueret**

Laboratoire de chimie macromoléculaire – UPRESA CNRS 8009
Université des Sciences et Technologies de Lille
F-59655 Villeneuve d'Ascq Cedex, France

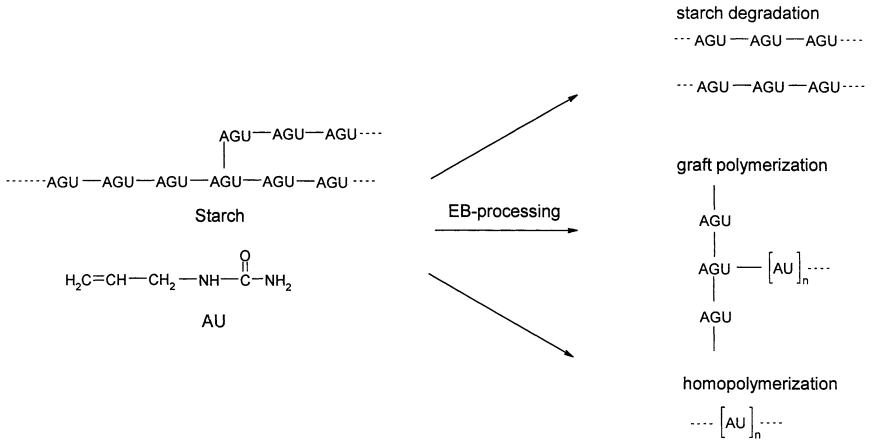
Summary: The effectiveness of covalent grafting in mixtures of N-allylurea (AU) and amorphized starch submitted to electron beam irradiation was studied for elucidating the chemical changes at the origin of the physical stabilization observed after radiation processing. FTIR and nmr analysis were used to quantify the progress of AU conversion upon irradiation of the mixtures and to gain information on the structure of the products. The grafting yield was determined by gravimetry after selective solubilization that extracts AU monomer and its homopolymer from the covalently modified polysaccharide. MALDI-TOF spectrometry applied to irradiated mixtures of AU and maltotriose, as a model compound, affords unambiguous evidence of covalent grafting. The obtained data allow to estimate the size of the AU grafts and to propose mechanistic pathways for radiation grafting.

Introduction

The development of biodegradable materials exhibiting most of the use properties of the conventional thermoplastics is still a major challenge. Starch-based materials are particularly attractive because they can be produced from a cheap and annually available resource.[1] For obtaining thermoplastic starch materials, native granular starch is amorphized at temperatures above 100°C and plasticized with the aid of additives of relatively low molecular weight.[2] We have recently reported that the electron beam (EB) irradiation of amorphous blends of potato starch and N-allylurea (AU) efficiently impedes the spontaneous blooming of mixtures including a large wt-fraction of the low molecular weight additive.[3] The physical instability of untreated blends was evidenced not only by the occurrence of blooming at film surface, but also by internal phase separation and by recrystallization of the starch and AU constituents monitored by X-ray diffraction.[4] The observed physical stabilization of the blends obtained after irradiation was confirmed by thermomechanical analysis of films submitted to various aging conditions.[3,4]

 CCC 1022-1360/00/$ 17,50+.50/0

AU was purposely selected as a reactive additive, claimed to undergo radiation induced polymerization.[5] Thus, the physical changes observed after irradiation of the blended materials possibly originate (i) from AU conversion into free polymer, (ii) from AU grafting polymerization onto the anhydroglucose units of starch (AGU), (iii) from polysaccharide chains degradation or (iv) from various combinations of these three basic chemical reactions (see Scheme 1).



Scheme 1 - Simplified representation of the reactions induced by the EB-treatment in starch - allyllurea (AU) blends [AGU for anhydroglucose unit].

A detailed understanding of the chemical effects induced by high energy radiation into the starch - AU blends conditions the optimization of formulations and of processing with the view of meeting a set of desired requirements. The present report is devoted to the effectiveness of AU grafting polymerization in the amorphous blends with starch, with the objective to determine the grafting yield to get structural and mechanistic information from MALDI-TOF mass spectrometry.

Experimental Part

Native potato starch (NPS) was obtained from Roquette Frères (Lestrem, France). N-allylurea (reagent grade) was purchased from Sigma-Aldrich. Thermoplastic starch (TPS) materials were prepared in an internal Brabender mixer at 125°C with a screw rotation speed of 50 rpm for 15 min. The blends were chopped into small cubes and quenched at –17°C immediately

after processing in order to avoid physical evolution during storage. TPS films of thickness ranging between 20 and 40 μm were prepared by grinding the material into powder and by compression molding at 130°C (6 MPa for 5 min). The materials named SAU20 and SAU50 were prepared from NPS (100 parts) and AU, 20 or 50 parts (w/w), respectively. The desired water content in the pressed materials was achieved by conditioning the films in cabinets where a constant relative humidity was maintained (58 % RH at R.T. over saturated aqueous NaBr solution).

Irradiation of blend films or of crystalline AU was carried out under the EB of an Electrocurtain CB150 generator (Energy Sciences Inc.) operating at 175 kV. The radiation was performed in air at a dose rate of 200 $kGy.s^{-1}$. Current and conveyor speed were adjusted to obtain 25 or 50 kGy per pass. Higher doses were obtained by increasing the number of passes in the chamber of the EB processor.

Thermogravimetric analyses were performed with a Shimadzu TGA apparatus in an air flow, with heating rate of $10°C.min^{-1}$. Infrared and nmr spectroscopic measurements were performed as described elsewhere.[6] For grafting yield determination, the procedure was as follows. Irradiated films (100 mg) were stirred in a methanol / water mixture (v/v 75/25, 20 mL). After stirring for 24 h, the turbid suspension was filtered with a Büchner funnel with a paper filter (Whatman No 1005 070). The liquid fraction was concentrated in vacuo. The solid residues of both fractions were then allowed to dry for 1 week at room temperature (constant weight) before analysis by ^{1}H nmr spectroscopy. Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectra were obtained on a Vision 2000, Finnigan Mat Spectrometer. The matrix was prepared by dissolving 2,5-dihydroxybenzoic acid (10 mg) in a 50:50 v/v mixture of water and methanol. Aqueous NaCl was added so as to obtain a 5 mM salt concentration. Equal volumes of the matrix and of the sample solution (0.1 wt-% in water) were mixed before filling the target pit. SEC analyses were performed with a Waters apparatus equipped with Ultrahydrogel columns (1 linear, 1 DP) and a refractometric detector. Water was used as the eluent with a flow rate of $1mL.min^{-1}$.

Results and Discussion

Dose Dependence of AU Conversion

The polymerizability of allylurea under ionizing radiation is strongly dependent on the physical state of the monomer. It has been reported to undergo polymerization in the crystalline state with formation of long chain polymer.[5] Its reactivity in solution is believed

to be poor, on the basis of low polymer yield after precipitation.[7] Various effects can be expected from the differences in molecular organization (crystal *vs.* amorphous blend) and in chemical purity of irradiated medium (recrystallized monomer *vs.* intimate mixture with the polysaccharide) that exist between the various forms of AU we have to consider. We have monitored by infrared spectroscopy the progress of AU conversion, irradiated under various solid states at room temperature with increasing doses of accelerated electrons. The two allotropic crystalline forms of AU were recrystallized in the appropriate solvents, so as to obtain the monoclinic and tetragonal crystals with high purity, from methanol and water solutions, respectively.

The conversion profiles shown in Figure 1 were deduced from infrared absorbance measurements at 929 cm^{-1}. The lines corresponding to the two allotropic forms of crystalline AU indicate that the polymerization proceeds with similar rate. This only suggests that the molecular organization within the crystal has no overall effect on polymerization. Keeping in mind the possible differences of behavior in each step of the chain addition mechanism, a more detailed study would be necessary to further comment on the compared reactivity of the crystalline forms of AU. The point of interest for the present discussion seems to be the apparently higher reactivity observed when AU (20 wt-parts) is blended with destructurized starch (80 wt-parts), if reasoning on fractional conversion.

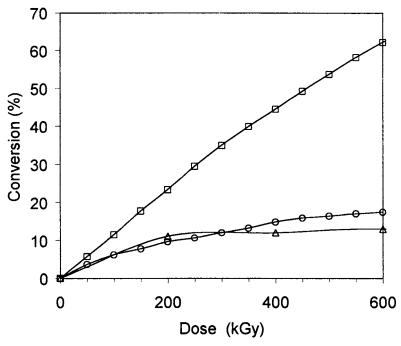


Figure 1. Progress of fractional conversion of AU as a function of EB-irradiation dose, in a SAU20 blend (□) and in the two crystalline forms of AU (○, monoclinic; Δ: tetragonal).

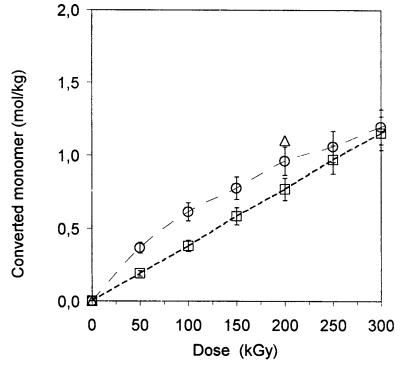


Figure 2. Progress of the amount of converted AU as a function of EB-irradiation dose, in a SAU20 blend (□) and in the two crystalline forms of AU (○, monoclinic; Δ: tetragonal).

However, since AU is diluted in the solid starch blend, the absolute amount of converted

monomer is the pertinent criterion for the comparison of monomer reactivity. The plots of Figure 2 indicate that the actual polymerization profiles are not so different. The topochemical control acting strongly over some polymerization conducted under radiation in the solid state does not seem to operate. One could have expected that the presence of starch in the amorphous and homogeneous blends could lead to the generation of additional initiating centers favoring graft polymerization. Thus, this study of AU reactivity does not afford any conclusive information nor any indication on the possibility of graft formation.

Thermogravimetric Analysis

One of the claimed characteristic effects of grafting polymerization onto starch is the modification of the material sensitivity to thermal degradation in air.[8] Several examples concern the γ-radiation induced grafting polymerization of acrylonitrile. The degradative behavior in the high temperature range (300°C-700°C) strongly modified, as an expected consequence of polyacrylonitrile grafts rearrangement. The changes in the low temperature range (50°C-300°C) are said to constitute a strong evidence for grafting.[9] We have recorded the thermograms of native starch for comparison with that of EB-irradiated starch-AU blends.

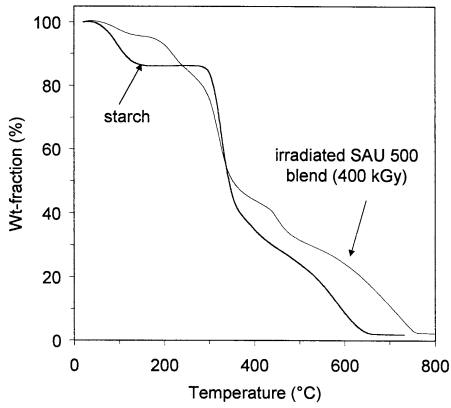


Figure 3. Plots representing the weight loss of native starch and irradiated SAU50 blend submitted to thermogravimetric analysis in air at a heating rate of 10°C.min^{-1}.

The profiles we observe with our samples are quite similar to that described for PAN modified starch, in which covalent grafting has been demonstrated by independent methods. Though we consider the argument to be indirect and questionable in a number of examples, our material exhibits the typical behavior of other grafted starches.

NMR Analysis of Grafting

At the molecular level, the changes in spectroscopic properties related to the sites modified by the covalent attachment of grafts may afford unambiguous information. We have tried to obtain such arguments by using ^{13}C and ^{1}H nmr spectroscopies. The anomeric carbon atom (C_1) of the AGU unit is a most plausible site for a free radical generation on starch.[10] Owing to the presence of the overlapping broad signal due to the NH_2 group of monomeric and polymeric AU in the ^{1}H nmr spectra, there is no possibility to determine, even qualitatively, the effectiveness of grafting from changes in the intensity for the AGU anomeric protons at δ = 5.5 ppm. The signals assigned to poly(AU) units, irrespective to their free state in the blend or of their grafting onto starch can be used to determine accurately AU conversion. It was not possible to obtain conclusive information from ^{13}C nmr spectra, in spite of careful examination for the presence of quaternary C atoms, as the probable grafting sites.[6]

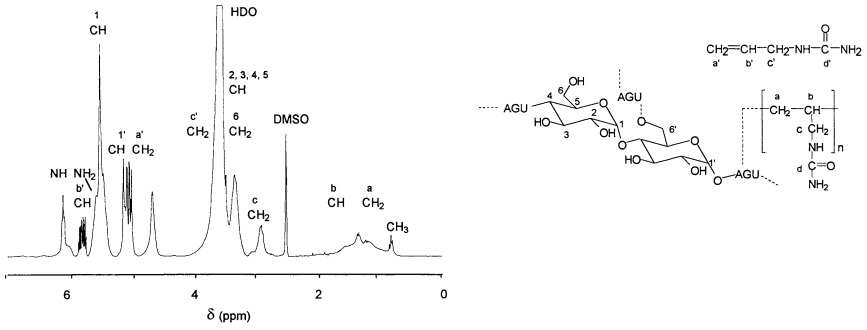


Figure 4. ^{1}H nmr spectrum (300 MHz, d-DMSO) of an SAU50 blend after EB processing with a 750 kGy dose.

Selective Solubilization of Free Poly(AU)

In order to quantify the relative extent of grafting and of AU homopolymerization, we have developed a procedure for achieving the selective solubilization of the different constituents. The solubility of amorphized starch and of AU - poly(AU) mixtures were tested separately at 1 wt-% concentration in water – methanol solutions. It was possible to solubilize efficiently AU together with free poly(AU) in water-methanol mixtures of composition ranging from 25:75 to 34:66 v/v, with only 2 wt-% of dry residue, whereas starch was not dissolved in similar conditions, as confirmed by gravimetry of the dried filtrate, indicating 100 % recovery.

From the ^{1}H nmr spectra of the crude irradiated samples and of the separated constituents it was possible to determine the overall conversion of AU as well as the fraction of polymerized AU present in grafts. The spectrum in Figure 5 shows the presence of poly(AU) grafts that can be quantified by integration of appropriate signals.

The results collected in Table 1 indicate that grafting is in competition with the formation of free homopolymer. The relative grafting yield is higher for the SAU20 films, in which AU is present in amounts lower than the limiting solubility.

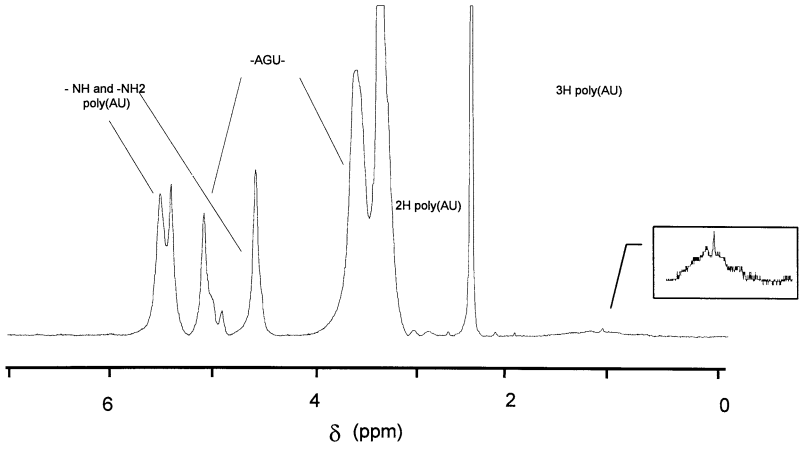


Figure 5. ^{1}H nmr spectrum (300 MHz, d-DMSO) of the water - methanol insoluble fraction of an irradiated SAU50 treated with a 400 kGy EB dose.

Table 1. Graft yield in SAU20 and SAU50 samples treated by a 400 kGy EB dose.

Sample	f_{AU} [a]	$f_{poly(AU)}$ [a]	f_{graft} [a]	$[AU]_0$ [b] mol.kg^{-1}	poly(AU) content [c] mol.kg^{-1}	graft content [c] mol.kg^{-1}
SAU20	0.25	0.15	0.60	1.6	0.24 (20%)	0.96 (80 %)
SAU50	0.70	0.175	0.125	3.3	0.58 (59%)	0.41 (41%)

[a] Fractional amount of unreacted AU, of free poly(AU) and of grafted AU in the blend after 400 kGy irradiation.
[b] Initial AU content in the blends.
[c] Amount of AU in the form of free or of grafted polymer after 400 kGy irradiation.

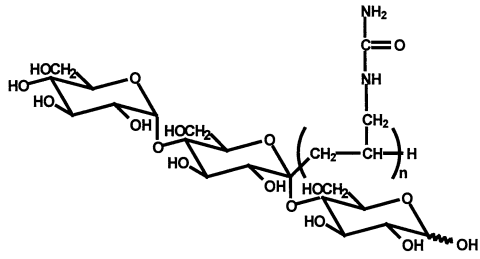
We see from these data that reacted AU is essentially under the form of graft polymer in the SAU20 sample, whereas less than one half of the reacted AU has been coupled to starch in the SAU50 film. However, it has been shown that, from the viewpoint of physical properties, a

400 kGy treatment applied to SAU50 samples induces sufficient stabilization.[3,4]

Maldi-TOF Mass Spectrometry

In order to gain a deeper insight into the grafting mechanism, amorphous mixtures of various maltodextrines and AU, as well as pure crystalline AU, were irradiated with accelerated electrons and subsequently analyzed by MALDI-TOF mass spectrometry.

The obtained mass spectrograms reveal the presence of short chain AU oligomers with DP < 15. A detailed analysis of the mass of the various series of homologous oligomers is underway that will allow us to propose different mechanisms for initiation and termination, and to discuss the particular issue of transfer reactions.[12] We will focus here on some selected results obtained from an irradiated blend of maltotriose and AU. A mixture including equal weight amounts of the two components was melted to obtain an homogeneous blend that was quenched over an aluminum plate cooled at a temperature of –15°C. The resulting solid film was then exposed to EB-radiation so as to obtain a dose of 435 kGy and was prepared for MALDI-TOF analysis. The obtained spectra reveal, in addition to the presence of unreacted starting materials, the formation of six oligomer families, recognized by peaks separated by a mass of 100 that corresponds to AU molecular weight. Interestingly, one series that was not found in the spectra recorded from irradiated pure AU, corresponds to maltotriose - AU adducts, with the possible structure depicted in Scheme 2.



Scheme 2. Possible structure for the maltotriose – AU adducts detected by MALDI-TOF MS.

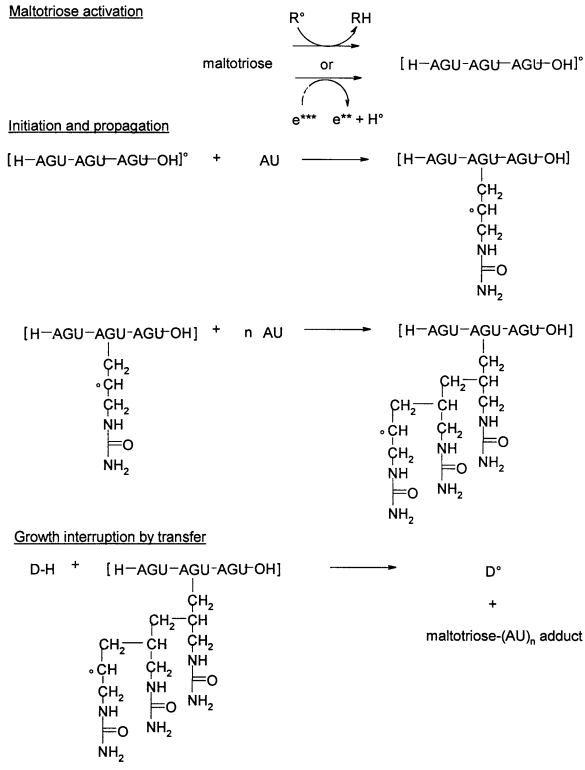
This series was identified with values for the number of grafted AU units ranging from 1 to 7, with maximum of the distribution for 3 repeat units (Table 2).

A possible sequence of reactions yielding the observed adduct is depicted in Scheme 3. It includes maltotriose free radical generation, likely on one of the two anomeric C atoms at the

glucosidic junctions, by direct interaction with the energetic beam or by hydrogen abstraction caused by some free radical. Chain growth would result from repeated addition of the propagating free radical onto AU propenyl function. Finally abstraction of some hydrogen atom from monomer or saccharide would stop this growth by a transfer process.

Table 2. Main characteristics of the MS peaks assigned to maltotriose (MT) AU adducts.

m/z (observed)	Cation structure	Relative intensity	m/z (theoretical)
527.4	MT + Na^+	30	527.4
627.7	MT + 1AU+ Na^+	3.3	627.6
727.6	MT + 2AU+ Na^+	3.9	727.7
827.7	MT + 3AU+ Na^+	4.2	827.8
927.7	MT + 4AU+ Na^+	3.4	927.9
1027.7	MT + 5AU+ Na^+	3	1028.0



Scheme 3. Proposed mechanism for the graft polymerization of AU onto maltotriose under EB irradiation.

The evidence of grafting supported on a molecular basis by MALDI-TOF mass spectrometry now provides a reasonable explanation for the increase of SAU50 material solubility in water with higher processing doses (Figure 6).[3,5] We have suspected that polysaccharide degradation upon treatment with high energy radiation could induce chain scission at the origin of the higher soluble fraction. However, control measurements by SEC analysis performed with model maltodextrins of high solubility in water, blended with AU and EB treated in conditions similar to that used for starch blends, did not show any sign of degradation. The SEC traces of Figure 7 indeed indicate no significant modification of the distributions at large retention volumes that correspond to low molecular weights.

The change in water solubility observed with the starch AU blends therefore seems to be due to some effect impeding aggregate formation from the polysaccharide chains, as observed with gelatinized starch. The presence of short poly(AU) grafts along the polysaccharide chains reasonably explains this effect. It contributes also to the long term stability of the material, by avoiding retrogradation.

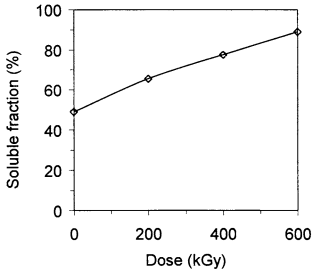


Figure 6. Variations of the soluble fraction of material in water, after irradiation of SAU50 blends.

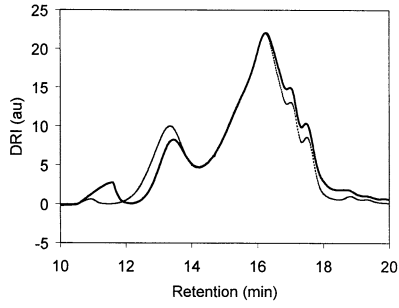


Figure 7. SEC traces of a mixture of maltodextrine (100 wt-parts) and AU (50 wt-parts) mixture before (---) and after (—) 400 kGy irradiation in the solid state.

Conclusion

The chemical modifications induced in starch-AU blends submitted to electron beam irradiation have been examined by various methods for addressing the important issue of the contribution of grafting in the observed physical effects. The selective solubilization in water - methanol mixtures of AU and free poly(AU) from irradiated blends allowed us to assess the extent of grafting onto starch that appeared dependent on the AU content. MALDI-TOF mass spectrometry affords unambiguous evidence of grafting that was suggested by other indirect

observations or measurements. The mechanism of AU polymerization under high energy radiation is currently investigated in more details by the informative MS analytical method.

Acknowledgements

The authors wish to thank Dr. U. Maschke for assistance during EB irradiations and Dr. G. Ricart for MALDI-TOF MS analyses. The Electron Beam Project conducted in Lille is supported by the Centre National de la Recherche Scientifique, the Conseil Régional Nord Pas de Calais and the EU program FEDER.

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