



## Graft-Copolymerization of Acrylate Monomers onto Chitosan Induced by Gamma Radiation: Amphiphilic Polymers and Their Behavior at The Air-Water Interface

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### ABSTRACT

Graft polymerization induced by ionizing radiation is a powerful tool in materials science to modifying the physical properties of polymers. Chitosan is a biocompatible, biodegradable, antibacterial, and highly hydrophilic polysaccharide. In this work, we report the obtaining of amphiphilic polymers through graft polymerization of acrylic monomers (methyl acrylate, t-butyl acrylate, and hexyl acrylate) onto chitosan. The polymerization reaction was carried out by simultaneous irradiation of monomers and chitosan using a gamma radiation source of <sup>60</sup>Co. The formation of Langmuir films of amphiphilic polymers was studied at the air-water interface through surface pressure versus main molecular area isotherms (Π-A) and hysteresis cycles of compression and decompression. Finally, it was analyzed the transferring of Langmuir films towards solid substrates to obtaining Langmuir-Blodgett films with potential application as an antibacterial coating. The microstructure of the Langmuir-Blodgett films was characterized by AFM microscopy observing a regular topography with roughness ranging between 0.53 and 0.6 μm.

## 1. Introduction

Ionizing radiation produces different effects over polymers. For example, degradation by cleavage of the polymer backbone, crosslinking and grafting [1]. Thus ionizing radiation is a powerful tool for modifying the physical and chemical properties of polymers. Graft polymerization induced by ionizing radiation is an easy way to obtain copolymers with a synergy of properties.

Chitosan is a polysaccharide obtained by deacetylation of chitin, which is a widely occurring polymer in nature. Chitin is the most abundant polymer after cellulose and it is a renewable resource. The most important source of chitin is the crustacean exoskeletons, which contain a large amount of this biopolymer. The physical and chemical properties of chitosan have been modified employing ionizing radiation.

Chitosan has several applications and uses. It is employed to enhancement the plant growth because it promotes the defense against diseases caused by fungi and other pathogens [2]. Chitosan is biocompatible, biodegradable and innocuous for humans due to its natural origin. These properties, are useful in the food industry. For example, in the casein coagulation and cheese production [3]. Chitosan

is used as a coagulant in the clarification of beer and wine [4]. Besides, chitosan decreases the turbidity, z potential, viscosity and the content of phenolic compounds in the beer [5]. Also, chitosan removes almost the totality of ochratoxin A in the wine [6]. Further, chitosan is employed for the clarification of other beverages such as fruit juice (apple, orange, pomegranate, and passion fruit) and enhancements its quality [7, 8, 9, 10].

Chitosan is added to the calcium phosphate cement and it is used in vivo for refilling bone defects due to its biocompatibility, and its capacity of osteoconduction, reabsorption and remodeling [11]. Further, the addition of chitosan to calcium phosphate cement contributes to creating macropores obtaining a better material for osteoconduction [12]. Currently, there is a growing interest in the modification of physical and chemical properties of chitosan to obtain new materials with high value-added. The graft-polymerization has been investigated with this purpose. Several polymers have been grafted on chitosan including polyacrylamide, poly (olygoethylene glycol mono methyl ether methacrylate), and some polypeptides. These grafted polymers have been obtained in the presence of

redox-systems, by ring-opening reactions or using plasma or UV radiation as energy sources. The grafted polymers have been applied in the metallic ions removal [13] and algae [14], as super-plasticizers for concrete [15], and for enhancement the tissue adhesion over metallic surfaces [16].

As we mentioned before, ionizing radiation such as gamma radiation can be employed for the synthesis of grafted polymers. Ibrahim and co-workers reported the preparation of chitosan-g-poly(maleic anhydride) induced by gamma radiation and its application in the removal of Cu(II) and Ni(II) in water [17]. The graft-polymerization induced by gamma irradiation has been applied in the preparation of amphiphilic polymers. Zhou and co-workers grafted poly(sulfobetaine methacrylate) on chitosan to yield polymer micelles through self-assembly. The antibiotic alamethicin interacts with these micelles forming a complex useful for controlled drug-release [18]. Further, Pasaphanet al reported the preparation of nanoparticles of amphiphilic polymer grafting poly(polyethyleneglycol methacrylate) on chitosan-deoxycholate [19]. Rattanawongwiboon and co-workers realized the preparation of nanoparticles of chitosan-g-poly(stearyl methacrylate) with core-shell structure. The nanoparticles are suitable as nanofiller for the formulation of nanocomposites containing poly(lactic acid) matrix [20]. Gamma irradiation has been employed for preparing nanocomposites with polymer matrix formed by grafted copolymers of chitosan. Abdel Aziz and co-workers reported the one-step preparation of nanocomposites from chitosan-g-poly(*N*-vinylpyrrolidone) and montmorillonite. These nanocomposites exhibit better antibacterial activity than the grafted copolymer [21]. Finally, chitosan can be grafted on other polymers such as poly(3-hydroxybutyrate) using gamma radiation for modifying the crystallinity of the copolymers in a controlled way [22].

Based on this background, in this work, we synthesized copolymers grafting acrylic monomers on chitosan to obtain amphiphilic polymers. We studied the behavior of the copolymers in Langmuir monolayers (at the air-water interface) and Langmuir-Blodgett (LB) films (after transferring it onto solid substrate) to evaluate if the copolymers can be incorporated in thin coatings with potential antibacterial activity. LB film methods are perhaps the earliest example of what is now called 'supramolecular assembly', providing the opportunity to exercise molecular level control over the structure of organic thin films.

## 2. Materials and methods

### 2.1 Graft Polymerization

Chitosan, methyl acrylate, *t*-butyl acrylate, and hexyl acrylate were purchased from Sigma Aldrich Company and used as

received. Samples for bulk polymerization were prepared to mix 250 mg of chitosan and 1 cm<sup>3</sup> of monomer in a test tube. The mixture was bubbled with argon and the tube was closed and sealed. Graft-polymerization was performed with a Gamma Beam 651PT irradiator using gamma radiation from <sup>60</sup>Co as an energy source. The radiation doses were 2, 4, 6, 8, 10 and 12 kGy and the dose rate was 8 kGy/h.

After polymerization, the content of each tube was placed in a beaker and 20 cm<sup>3</sup> of dichloromethane was added. The mixture was stirred magnetically for 20 minutes and the solvent was separated by decantation to remove the unreacted monomer and the homopolymer. This procedure was repeated 3 times and finally, the grafted polymers were rinsed with acetone. The polymers were dried at 70 °C for 8 h and weighed. The percentage of grafted monomer was calculated using Equation 1:

$$\text{Grafted-monomer}(\%) = \left[ \frac{w_{\text{polymer}} - w_{\text{chitosan}}}{w_{\text{monomer}}} \right] * 100 \quad (1)$$

Where  $w_{\text{polymer}}$ ,  $w_{\text{chitosan}}$  and  $w_{\text{monomer}}$  represent the weight of the grafted polymer, chitosan, and monomer respectively.

For the experiments of solution polymerization, 250 mg of chitosan was dissolved in 10 mL of aqueous acetic acid solution (2% v/v). Then, 1 mL of monomer dissolved in 2 mL of *N,N*-dimethylformamide was added. The mixture was sonicated for 10 min obtaining a homogeneous mixture and bubbled with argon. The samples were irradiated with a dose of 2 kGy of gamma radiation with a dose rate of 8kGy/h. After that, acetic acid was neutralized with a NaHCO<sub>3</sub> saturated solution and the grafted polymers were precipitated in methanol. The polymers were filtered, rinsed and dried at 70°C. The grafted polymers were characterized by FTIR spectroscopy using a Perkin Elmer Spectrum 100 spectrometer equipped with an attenuated total reflectance (ATR) accessory.

### 2.2 Langmuir films

The behavior of amphiphilic copolymers of chitosan at the air-water interface was analyzed through the construction of isotherms of surface pressure as a function of the molecular area (Π-A) and the hysteresis cycles of compression and decompression. The experiments were carried out in a KSV 5000 alternate multilayer LB system (KSV Finland). ASTM type 1 ultra-pure water was employed for the subphase (Millipore Direct-Q3 Smart system, 18.2 MΩ cm). Spreading solutions of grafted copolymers with a concentration of 1 mg cm<sup>-3</sup> were prepared in a ternary mixture of ethyl acetate, DMF and HOAc (17:2:1). Then, 0.2 cm<sup>3</sup> of solutions were slowly spread over the water surface. The system was maintained undisturbed for 15 minutes to achieve complete

evaporation of the solvent. Afterward, the monolayer was symmetrically compressed at a barrier speed of 10 mm/min and the  $\Pi$ -A isotherms were recorded at 22°C applying the Wilhelmy method. Finally, the stability of monolayers was analyzed through compression-decompression cycles (hysteresis loops) below the collapse pressure.

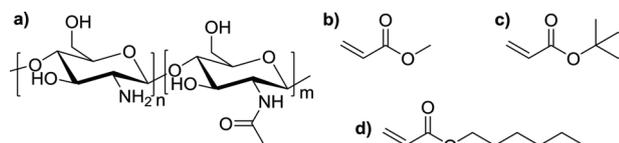
### 2.3 Langmuir-Blodgett films

Langmuir monolayers of amphiphilic polymers were transferred onto glass substrates (36 x 12 x 1 mm) using the vertical lifting method at a lifting speed of 10 mm min<sup>-1</sup> (transfer ratio of 1 ± 0.3) at 22°C. Glass substrates were previously treated with piranha solution, ultrapure water, ethanol and chloroform in order to remove impurities. Z-type multilayer structures ( $n = 1 - 3$  layers) were prepared by vertical deposition. The surface of polymeric coatings was characterized by atomic force microscopy (AFM). AFM images were recorded with a JEOL JSPM-5200 probe microscope.

## 3. Results and discussion

### 3.1 Amphiphilic Copolymers

The structures of chitosan and the monomers employed for the synthesis of amphiphilic copolymers are shown in Fig. 1. Chitosan contains in its structure OH and NH<sub>2</sub> groups which confer hydrophilic character, while the acrylate monomer does not have hydrophilic groups and they provide hydrophobic character. The combination of hydrophilic and hydrophobic parts in the grafted copolymers gives, as a result, amphiphilic copolymers.

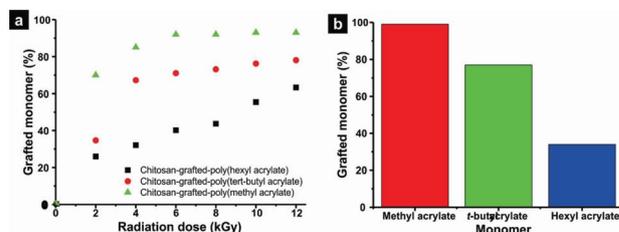


**Figure 1:** Chemical structures of a) chitosan, b) methyl acrylate, c) *t*-butyl acrylate and, d) hexyl acrylate.

As mentioned before, the graft polymerization of acrylic monomers onto chitosan was carried out applying the bulk and solution polymerization techniques. The grafted polymers obtained by bulk polymerization were heterogeneous materials showing two phases. The first one was a chitosan-rich phase and the second one was a polyacrylate-rich phase. Chitosan is poorly soluble in the acrylic monomers. Thus, the diffusion of monomer towards the chitosan matrix is limited and the graft occurs at the surface of chitosan. These grafted polymers are insoluble in organic solvents such as hexane, dichloromethane,

chloroform, acetone, ethyl acetate, methanol and *N,N*-dimethylformamide. Also, they are insoluble in aqueous solutions of acetic acid where chitosan is highly soluble.

Figure 2a shows the graft percentage of acrylic monomers onto chitosan as a function of the radiation dose. Methyl acrylate showed higher reactivity than *t*-butyl acrylate and hexyl acrylate for the graft-polymerization. The results showed that a radiation dose of 4 kGy is enough to yield a high percentage of grafted poly(methyl acrylate) onto chitosan. Higher radiation doses do not increase significantly the grafted monomer percentage. For hexyl acrylate, the grafted polymer percentage was proportional to the radiation dose. Thus, the highest graft-percentage was obtained at 12 kGy. However, under this radiation dose, the graft percentage is lower than the values obtained for methyl acrylate. The reactivity of *t*-butyl acrylate was intermediate between hexyl acrylate and methyl acrylate. Radiation doses higher than 4 kGy do not increase significantly the percentage of grafted monomer. Finally, 78 % of grafted monomer was reached after the irradiation with a dose of 12 kGy.



**Figure 2:** a) Percentage of the grafted monomer as a function of the radiation dose, and b) percentage of grafted monomer obtained in homogeneous polymerization.

Figure 2b shows the percentage of grafted monomer on chitosan obtained by solution polymerization with a radiation dose of 2 kGy. As it was observed before, methyl acrylate is more reactive than *t*-butyl acrylate or hexyl acrylate. Thus, a higher percentage of grafted monomer was obtained with methyl acrylate which is close to 100%. It is well known that the reactivity of a monomer during the chain polymerization decreases as the molecular weight of the monomer increases. The results showed that gamma radiation does not change this low tendency for the polymerization in high molecular weight monomers such as hexyl acrylate. These copolymers are homogeneous materials and they are soluble in a mixture of ethyl acetate-DMF-AcOH (17:2:1) and they were employed for the building of Langmuir and Langmuir-Blodgett films.

Chitosan and the grafted-polymers were characterized by FTIR-ATR spectroscopy and their spectra are shown in Figure 3. The spectrum of chitosan showed the typical absorption bands at 1578 and 3370 cm<sup>-1</sup> attributed to  $\delta$ NH

and  $\nu\text{OH}$  vibrations of amino and hydroxyl groups present in the structure of chitosan. The absorption band of  $\nu\text{C}=\text{O}$  vibration of amide group was observed at  $1645\text{ cm}^{-1}$  and it is due to the presence of residual *N*-acetylamino groups. Finally, the absorption bands of  $\nu\text{C}-\text{O}-\text{C}$  were observed at  $1027\text{ cm}^{-1}$  and they are due assigned to acetal groups from glucoside bonds which are forming the polysaccharide backbone. These absorption bands are observed after graft polymerization which indicates that the polysaccharide backbone is conserved after being exposed to gamma radiation. The most important evidence of the obtaining of chitosan-g-polyacrylate copolymers were the absorption bands observed at  $1730\text{ cm}^{-1}$  attributed to the  $\nu\text{C}=\text{O}$  vibration of carbonyl groups from polyacrylates. These bands are absent in the spectrum of pristine chitosan.

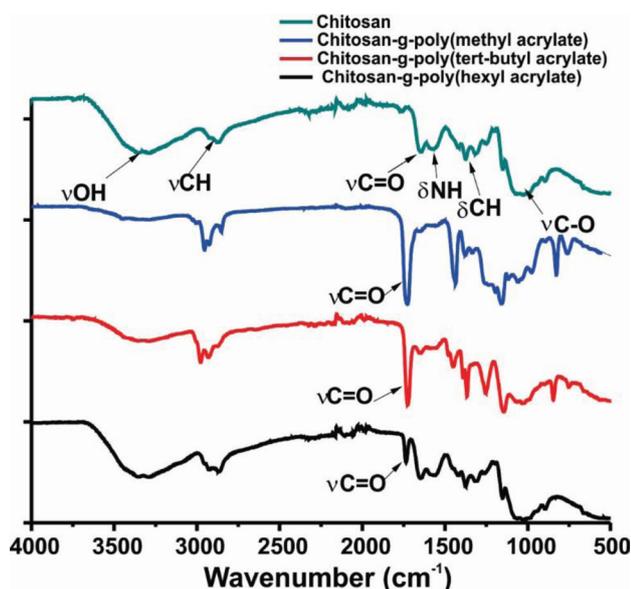


Figure 3: ATR-FTIR spectra of grafted copolymers.

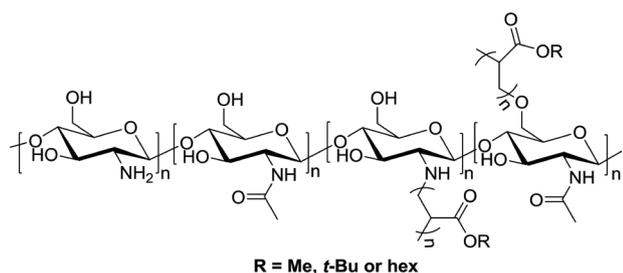


Figure 4: Structure of chitosan-g-polyacrylate.

The ATR-FTIR spectra of chitosan-g-poly(methyl acrylate) and chitosan-g-poly(*t*-butyl acrylate) show a decrease in the intensity of the absorption bands attributed to  $\nu\text{OH}$  and  $\delta\text{NH}$  vibrations. This suggests that the grafting of acrylic

monomers occurs through the rupture of O-H and N-H bonds, which are the most labile. The proposed structure of the grafted copolymers is shown in Figure 4.

### 3.2 $\Pi$ -A Isotherms and Stability of Monolayers

The  $\Pi$ -A isotherms of the grafted copolymers are shown in Figure 5a. The  $\Pi$ -A isotherms of chitosan-g-poly(*t*-butyl acrylate) and chitosan-g-poly(hexyl acrylate) are very similar. They showed the presence of solid, liquid and solid phases as a function of the surface pressure. The solid phase was observed at values of surface pressure higher than  $6.1$  and  $4.3\text{ mNm}^{-1}$  for chitosan-g-poly(*t*-butyl acrylate) and chitosan-g-poly(hexyl acrylate), respectively. The collapse of the monolayer was observed when the surface pressure increase above  $20.4$  and  $15.9\text{ mNm}^{-1}$  for chitosan-g-poly(*t*-butyl acrylate) and chitosan-g-poly(hexyl acrylate), respectively. According to these isotherms, both amphiphilic copolymers can form compact monolayers at the air-water interphase. The  $\Pi$ -A isotherm of chitosan-g-poly(methyl acrylate) does not show a clear transition from liquid to the solid phase. Further, the collapse of the monolayer is not observed clearly. This isotherm showed that chitosan-g-poly(methyl acrylate) form a less dense and compact monolayer at the air-water interphase. The capability of grafted copolymer derivatives from chitosan to form stable monolayers at the air-water interphase was attributed to their amphiphilic character. At the air-water interphase, the OH and  $\text{NH}_2$  groups of chitosan are oriented towards the water-subphase while the polyacrylate chains are oriented towards the air-subphase.

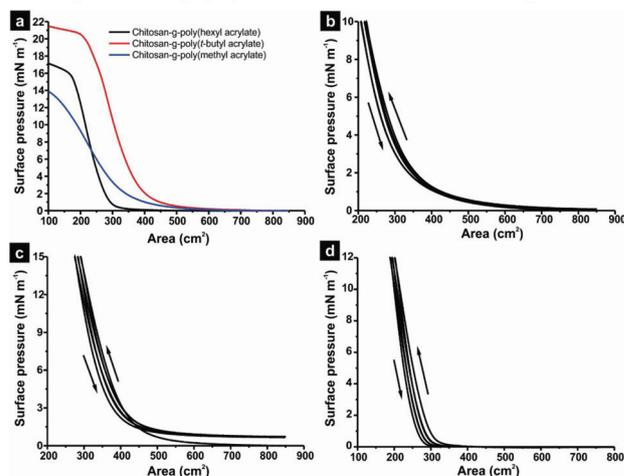
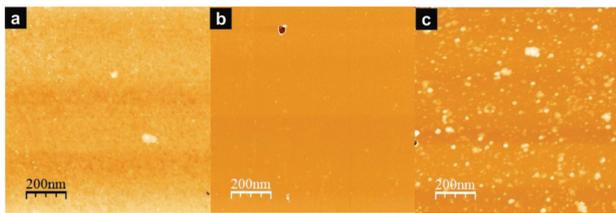


Figure 5: (a)  $\Pi$ -A isotherms of amphiphilic copolymers. Hysteresis curves of compression-decompression of (b) chitosan-g-poly(methyl acrylate), (c) chitosan-g-poly(*t*-butyl acrylate), and chitosan-g-poly(hexyl acrylate).

The stability of these Langmuir monolayers was studied through the hysteresis behavior through compression and decompression cycles. The plots obtained after 3 cycles are shown in Figure 5b-d. For chitosan-g-poly(*t*-butyl acrylate) and chitosan-g-poly(hexyl acrylate), the hysteresis loop does not show important changes after each cycle of compression and decompression. This indicates that the floating monolayers of these copolymers are very stable at the air-water interphase and they can be transferred onto solid substrates by vertical deposition.

### 3.3 LB films

The Langmuir monolayers of chitosan-g-poly(*t*-butyl acrylate) and chitosan-g-poly(hexyl acrylate) were successfully transferred onto glass substrates by vertical deposition (*Z* type deposition) with the transferring ratio ranging between 0.898 to 1.174. The fabrication of multilayer coatings was realized making 3 depositions onto the same substrate. We observed that the transferring ratio does not vary significantly. From the above, the thickness of the polymeric coating may be controlled by the deposition of multiple Langmuir-Blodgett films. Otherwise, the monolayers of chitosan-g-poly(methyl acrylate) were transferred towards solid substrates with low transferring ratio (<0.7). This was attributed to the low affinity between the copolymer (highly hydrophobic due to the large graft percentage) and the glass substrate (highly hydrophilic). This implies that the glass substrate is not fully covered by chitosan-g-poly(methyl acrylate) and it could contribute to the formation of microstructural defects in the polymeric coating.



**Figure 6:** AFM images of thin films containing a) chitosan-g-poly(methyl acrylate), b) chitosan-g-poly(*t*-butyl acrylate) and c) chitosan-g-poly(hexyl acrylate).

The morphology and the topography of the LB films were characterized by AFM microscopy. In general, the microstructure of chitosan-g-poly(*t*-butyl acrylate) thin film is smooth in comparison with thin films of the other copolymers. The AFM image of chitosan-g-poly(hexyl acrylate) thin film shows a large number of agglomerates at the surface, while the AFM image of chitosan-g-poly(methyl acrylate) thin film shows agglomerates and a band-like microstructure formed by regions with different thickness. The roughness of thin films was 0.6, 0.55, and 0.53  $\mu\text{m}$  for

thin films of chitosan-g-poly(methyl acrylate), chitosan-g(*t*-butyl acrylate), and chitosan-g-(hexyl acrylate) respectively. Further, the height of the three thin films was close to 1  $\mu\text{m}$ . The irregular microstructure and the high values of height could be due to the effect of inter and intramolecular forces established in the polymer chains. These interactions promote the formation and transferring of large agglomerates in the LB films giving the before mentioned characteristics to the polymer coatings.

### Conclusions

Acrylic monomers were successfully grafted on chitosan using gamma radiation. A high percentage of grafted monomer was reached by copolymerization in homogenous and heterogeneous systems. Copolymers obtained by grafting-copolymerization in the homogenous system (with a radiation dose of 2 kGy) are amphiphilic and they are suitable for the fabrication of polymeric coatings through the Langmuir-Blodgett technique. The most suitable material for fabrication of polymeric coatings was chitosan-g-poly(*t*-butyl acrylate), which can be transferred from air-water interphase with transferring ratio close to 1. Further, the LB films of this material exhibit a regular microstructure, with a lower presence of agglomerates at the surface.

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