

A Microstructural Study of Acrylic-Modified Chitosan by Means of PALS and SAXS

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Abstract. Chemical modification of chitosan, specially grafting with different polymers is an important strategy in the production of bio-based materials with enhanced properties. In the present study, chitosan was grafted with *n*-butyl acrylate, in a surfactant-free emulsion polymerization. Stable dispersions with high grafting efficiency were obtained and the microstructure of the casted films was analyzed by positron annihilation lifetime spectroscopy and small angle X-ray scattering. Results are discussed in terms of the number and distribution of the grafting sites.

Introduction

Chitosan is the partially deacetylated form of the chitin, a natural polysaccharide extracted from the crab, shrimps and other crustacean skeleton. This biopolymer has great potential as packaging material, due to its inherent antimicrobial activity, non-toxicity and biodegradability. Chitosan is obtained by alkaline N-deacetylation of chitin and it involves the removal of acetyl groups from the molecular chain of chitin leaving behind a complete amino group (NH₂). Chitosan versatility depends mainly on this high degree chemically reactive amino group. In general, chitosan occurs as N-acetylglucosamine and N-glucosamine copolymer that are randomly or block distributed throughout the biopolymer chain [1]. Chitosan is one of the most interesting biopolymers obtained from natural sources. However, as other biopolymers, chitosan have relatively poor mechanical properties, mainly involving low flexibility and brittleness at room temperature, and reduced performance against water. Chemical modification of chitosan, specially grafting with different polymers is an important strategy in the production of bio-based materials with enhanced properties. Chitosan presents two types of reactive sites which can be potentially modified by grafting: the C-2 free amino groups on deacetylated units and the C-3 and C-6 hydroxyl groups in either acetylated or deacetylated units [2].

In the present study, two chitosan polymers with different deacetylation degree (DD%) and molecular weight were subject to grafting reactions with *n*-butyl acrylate in different proportions. Microstructure of chitosan and grafted samples films were studied and compared by means of PALS and SAXS.

Materials and methods

Materials. The materials and chemicals used in this work were:

Chitosan: medium molecular weight chitosan (QS) with a 78.2 % deacetylation degree and M_v 450,000 (Sigma); chitosan (QP) with a 95.2 % deacetylation degree and M_v 310,000 (Parafarm); *n*-butylacrylate (BuA, Sigma); acetic acid solution 0.165 M (1 % v/v); acetone (Merck); sodium hydroxide (NaOH, Anedra) and potassium persulphate (KPS, Anedra). All reagents were of analytical grade and used without purification, except BuA which was purified to eliminate the inhibitor (hydroquinone 100 ppm).

Chitosan-graft-butyl acrylate synthesis. Chitosan solution (2 % w/v in 1 % v/v acetic acid) was placed in a flat bottomed three necked flask. A nitrogen purge was made throughout one hour at room temperature through the stirred solution. Then, the temperature was set at 60 °C and a freshly prepared KPS solution (0.741 mM in the minimum quantity of water, around 1 ml) was added. Five minutes later, drop-wise addition (78 - 156 mM) of *n*-butyl acrylate was made. The reaction was conducted at 60 °C for 24 h with stirring and N₂ purge. Finally, another 0.370 mM KPS was added to ensure the complete polymerization of the acrylic component. Samples nomenclature indicate the original chitosan (QS or QP), if it is grafted with the acrylic polymer (A), and the chitosan:BuA ratio (1:0.5 = 50, 1:1 = 100). In this way, QSA50 identify a Sigma chitosan (78.2 DD%) grafted with a 1:0.5 chitosan:BuA weight ratio.

Film preparation. 12 ml of the solution were cast on a 10 cm diameter glass Petri dishes, dried at room temperature for 48 h and then another 4 h at 60 °C. After cooling, the films were neutralized with 1 N NaOH to allow the peeling off from the Petri dishes, washed with distilled water and dried at room temperature in a PTFE plate. Film thicknesses obtained were around 100 μm.

Positron Annihilation Lifetime Spectroscopy. The positron lifetime spectrometer used consists of a "fast-fast" timing coincidence system, with a temporal resolution of 340 ps. Positron lifetime spectra were recorded at room temperature with a total number of 1.5×10^6 coincidence counts using a 0.2 MBq sealed source of ²²NaCl deposited on a thin Kapton foil (1.08 mg/cm²). The samples were prepared stacking several layers of the film in order to stop all the injected positrons, following the same procedure used by Consolati *et al.* (2001) [3]. PALS spectra were deconvoluted into three discrete lifetime components using the LT10 program. A correlation between τ_{o-Ps} and the size of the hole (in a spherical approximation of nanoholes of radii *R*), was obtained using the Tao-Eldrup model [4-6]:

Small angle X-ray scattering (SAXS). Small-angle X-ray scattering (SAXS) experiments were performed at the SAXS2 beamline at the National Laboratory of Synchrotron Radiation in Campinas (Brazil) using a monochromatic beam with a wavelength of 1.608 Å. Scattering intensity was registered using a two-dimensional detector with a sample detector distance of 2314.8 mm for values of the scattering vector (*q*) of $0.07 \text{ nm} < q < 1.38 \text{ nm}$ [$q = (4\pi/\lambda) \sin(\theta/2)$, where θ is the scattering angle]. One dimensional curves were obtained by integrating the two-dimensional data with the program FIT2D. The acquired data were corrected by subtracting the background contribution of the empty cell.

Results

Free volume plays an important role in different polymer films properties like permeability and mechanical properties. To further analyze the free nanohole volume and nanostructural variations in the chitosan and grafted samples PALS was carried out, in which the τ_{o-Ps} and I_{o-Ps} were obtained from the deconvolution of PALS spectra. The o-Ps lifetime observed for pure chitosan samples are in good agreement with those reported by Sharma *et al.* (2013) [7].

In grafted samples, τ_{o-Ps} is higher than that measured in pure chitosan. It was observed that this lifetime increases for higher grafting ratios.

Free nanohole volume (v_h) measurements are plotted in Fig 1. As can be seen, QP sample has smaller v_h values than that for the QS sample. This feature could be assigned to the lower molecular weight and also to the different deacetylation degree, because of the more voluminous groups present in the acetylated moiety. Furthermore, in the figure it can be seen that grafted samples show a noticeable increment in v_h with respect to the values of this parameter for pure chitosan samples.

The increment in the o-Ps lifetime, and therefore of v_h , obtained for the grafted samples could be attributed to the breaking of specific interactions (i.e. H-bonds) between polar groups in the biopolymer due to the graft of pBuA in those functional groups (NH₂, OH), with the consequent separation of the chitosan chains.

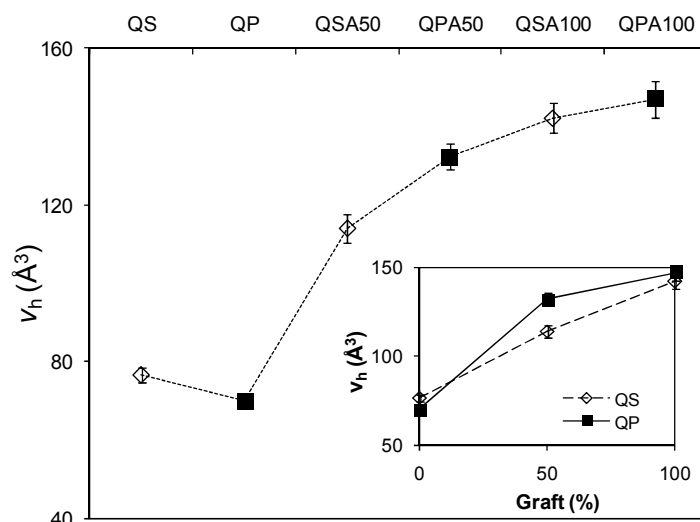


Figure 1. Free nanohole volumes (v_h) for studied samples and its evolution with grafting ratio.

Lizama et al. (2001) [8] analyzed poly(*n*-butyl acrylate) homopolymer and reported a v_h value similar to the obtained in QA100 samples. Under this scenario, we could assign the main contribution to the long-lived lifetime to o-Ps annihilation in rich-acrylate regions. Even our chitosan/poly(*n*-butylacrylate) samples are chemically bonded and are not completely immiscible, a possible contribution to o-Ps annihilation coming from the interphase zones cannot be discarded [9,10].

As can be seen in Fig. 1 inset, the free nanohole volume in QS series films linearly increase with the grafting ratio increment, whereas in QP series v_h values increase for about 90%, with respect to the pure chitosan, with a 1:0.5 chitosan:acrylic ratio and 110% with the 1:1 ratio. This behavior can be attributed to the major number of available grafting sites (related with DD%) along the chitosan molecule and the presence of N-acetylated units forming blocks throughout the chitosan chain.

SAXS analysis reveals that, in contrast to the pure chitosan, grafted samples contains heterogeneity micro regions in its bulk and these micro regions differ in electron densities. As can be seen in Fig. 2a it is evident a higher intensity of small-angle X-ray scattering from the copolymer samples, compared to that of chitosan. In Fig 2b, Kratky representation, $I(q)q^2$ versus q , of pure chitosan samples is shown. Using the general equation $q^* = 2\pi/L$ we can find a characteristic distance (L) which we have related with the persistence length (L_p) of chitosan molecules. The obtained L_p value ($L_p \approx 20$ nm) is in agreement with the values reported by Terbojevich et al (1991) [11]. In grafted samples, this characteristic distance is not observed, confirming a rupture of the rigid rod structure of the chitosan, a stretching of the folded structure of chitosan chains and, therefore, an increment in L_p , making q^* not observable in the used q -range.

The referred heterogeneity of electron densities is related to the mentioned partially immiscible phases of the grafted samples. Core-shell morphology with pBuA-rich cores and chitosan shells was observed by TEM [12]. As can be seen in Fig 2.a, when the dispersion is casted to obtain the chitosan-based film, the core-shell morphology of the particles is almost lost.

Conclusions

In this work micro and nanostructure of chitosan and acrylate-grafted chitosan films were studied by means of PALS and SAXS. The free nanohole volume of the samples increased with the grafting percentage.

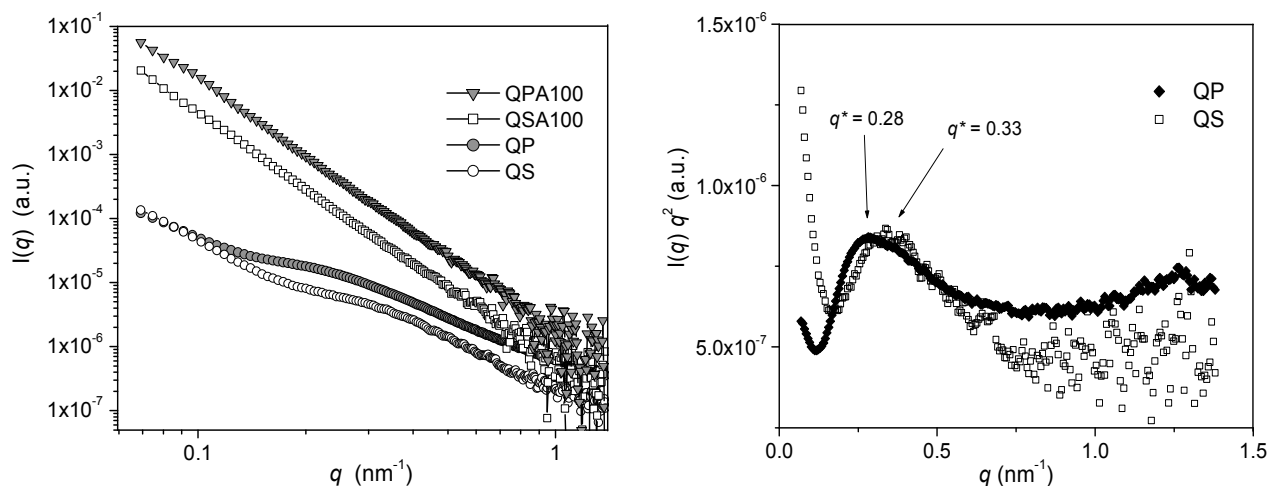


Figure 2. SAXS plots of chitosan and grafting samples (a) and Kratky plots for chitosan samples (b).

Also, different deacetylation degree and molecular weight of the pristine chitosan plays an important role in v_h values of grafted samples. From SAXS data, a persistence length of about 20 nm was obtained in pure chitosans, whereas this characteristic distance is not present in grafted samples. Micro regions with different electron densities were also found in acrylate-chitosan copolymers. The rupture of the typical rigid rod-structure of the polysaccharides because of the grafting is evidenced by SAXS and PALS.

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