Influence of Starch and Glycerol on the Properties of Chitosan by Positron Annihilation Spectroscopy

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ABSTRACT: Two types of chitosan (CS), α and β, were blended with different concentrations of starch and cast to obtain films. The addition of 1% glycerol was used as a plasticizer to increase film flexibility. The properties of the obtained films were studied by positron annihilation lifetime spectroscopy, X-ray diffraction, and scanning electron microscopy. The results indicate that pure β-CS had smaller size free-volume holes with high fractions than pure α-CS; this was attributed to the difference in bonding of main chains in β-CS. The addition of starch (>20% up to 50%) reduced the size of the free-volume holes and increased their fraction because of the close packing of chain segments. The effect of 1% glycerol to the CS starch blends indicated that some modification took place. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2874–2883, 2010

Key words: films; biomaterials; biopolymers; blends; microstructure

INTRODUCTION

As most present-day polymers are synthetic materials, their biocompatibility and biodegradability are much more limited than those of natural polymers, such as cellulose, chitin, chitosan (CS), and their derivatives.

In this respect, chitin and CS are recommended as suitable functional materials because these natural polymers have excellent properties, such as biocompatibility, biodegradability, nontoxicity, and adsorption properties. Chitin and CS are of commercial interest because of their high percentage of nitrogen (6.89%) compared to synthetically substituted cellulose (1.25%).[1]

Chitin is usually isolated from the exoskeletons of crustaceans and, more particularly, from shrimp and crabs where α chitin is produced.[2,3] Squid is another important source of chitin, in which it exists in the β form, which has been found to be more amenable for deacetylation. It also shows a higher solubility, reactivity and affinity toward solvents and swelling than α chitin because of the much weaker intermolecular hydrogen bonding ascribable to the parallel arrangement of its main chains. The extraction of chitin from its natural sources followed by its deacetylation to obtain the much more useful CS material has been studied by many authors.[4–11]

The improvement of the properties of biopolymers is an important subject, in which the blending of polymers results in the preparation of new materials with improved physicochemical and mechanical properties. The final properties of the blends are determined by the miscibility of the polymers, which is greatly favored by the formation of intermolecular hydrogen bonds between the polymers components.[12,13]

To improve the physical properties of CS for practical utilization, the modification of CS by means of blending with other polymers is often used as a convenient and effective method. In the last few years, some publications have appeared on the blending of CS with various polymers, such as poly(vinyl alcohol),[14,15] starch,[15] poly(*N*-vinyl pyrrolidone),[16] poly(ethylene oxide),[17] and cellulose.[18,19]

Recently, much attention has been paid to CS as a potential polysaccharide resource with many important applications.[1,20–25] CS possesses immense potential as a packaging material because of its biodegradability and antimicrobial activity. Starch also is an attractive biopolymer as a packaging material, and the preponderance of amylase in starch gives rise to stronger films.[20,21]

Positron annihilation lifetime (PAL) spectroscopy has been used to study polymeric materials. PAL spectroscopy is considered a probe for subnanometer local free volumes in amorphous polymers that arise from their disorder structure.[26] Such free-volume holes play an important role in the

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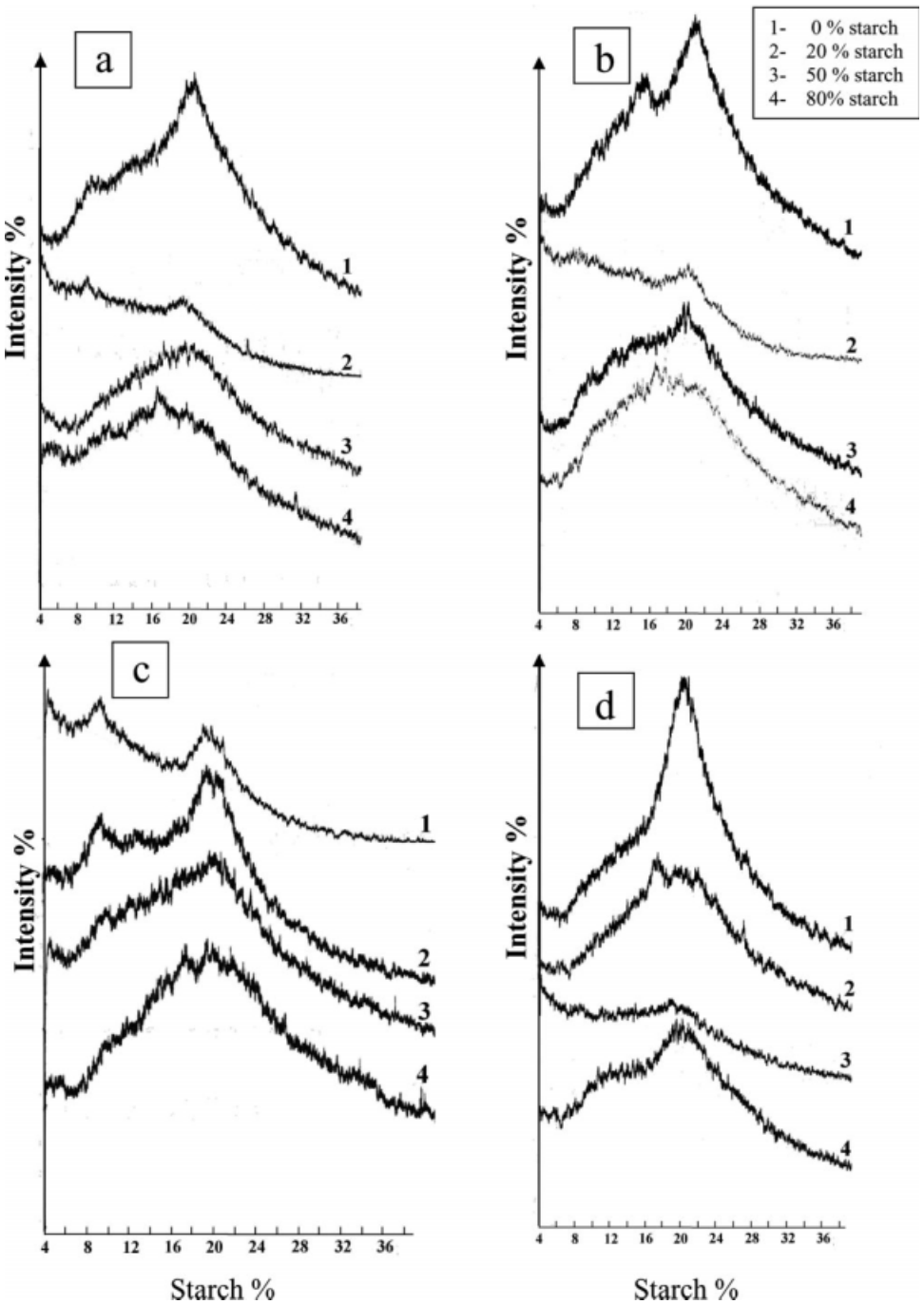

Figure 1 X-ray diffractograms of α-CS and β-CS blend films: (a) α-CS, (b) β-CS, (c) α-CS with 1% glycerol, and (d) β-CS with 1% glycerol.

determination of the properties of polymers, such as changes in their behavior under the influence of plasticizers.[24] In polymeric materials, positrons preferentially form and annihilate from a bound state called *positronium* (Ps). Ps forms either in the so-called *para*-positronium (*p*-Ps) state (antiparallel electron and positron spins) or *ortho*-positronium (*o*-Ps) state (parallel electron and positron spins). The lifetime of a *p*-Ps is 125 ps *in vacuo*, whereas an *o*-Ps lives approximately 142 ns. The free volume in polymers for *o*-Ps has a finite probability of annihilating with an electron other than its bound partner (and of opposite spin) during the numerous collisions that it undergoes with the molecules of its surroundings, a process generally termed as the *pickoff*. The result is a drastically reduced *o*-Ps lifetime compared to its vacuum value. *o*-Ps reflects the sensitivity to the electron density in the nearest surroundings of the Ps, and in this way, the size of free-volume holes where *o*-Ps is localized can be

TABLE I
τ_3, I_3, R, and F Values

Sample	τ_3 (ns)	I_3 (%)	R (nm)	F (%)
α-CS	1.83 ± 0.012	8.86 ± 0.28	2.67 ± 0.15	1.295 ± 0.005
β-CS	1.56 ± 0.025	13.6 ± 0.26	2.40 ± 0.14	1.424 ± 0.005
α-CS with 1% glycerol	1.84 ± 0.025	9.44 ± 0.26	2.70 ± 0.12	1.394 ± 0.004

estimated. One can use PAL spectroscopy to measure the mean free volume and the size distribution of these holes.

In this study, two different kinds of CS, α-CS produced from shrimp shells and β-CS obtained from squid pens, were chosen as bulk polymers. The objective of this study was to determine the influence of starch concentration on the structure of two different types of CS and the effect of glycerol as plasticizer on these with PAL spectroscopy and to compare the results with those of traditional techniques, such as X-ray diffraction (XRD) and scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

Isolation of chitin

Chitin was extracted from two different sources; α-chitin was obtained from marine shrimp, and β-chitin was obtained from squid pens. The raw materials were obtained in solid form, washed with water, desiccated at room temperature (RT), and cut into small pieces. Demineralization was carried out at RT with 1*M* hydrochloric acid baths. The number of baths and their duration were dependent on the source. Deproteinization was performed with alkaline treatments with 1*M* sodium hydroxide solutions at 105–110°C. This treatment was repeated several times. The number of baths depended on the clarity of the solution; the absence of protein was indicated by the absence of color of the medium at the end of the last treatment. Washing with distilled water was then carried out up to neutrality, after which the samples were dried.

Deacetylation of chitin

We converted the obtained chitin into more useful soluble CS by steeping it in solutions of 40% NaOH for 24 h, and then, the alkali chitin was heated in an autoclave for 0.5 h. β-Chitin from squid pens did not require steeping in the sodium hydroxide solution.

Determination of the deacetylation percentage (potentiometric titration)

CS (0.5 g) was dissolved in 25 mL of a standard 0.1*M* hydrochloric acid aqueous solution. The solution was then topped off to 100 mL with distilled water, and a calculated amount of KCl was added (0.1*N*) to adjust the ionic strength. The titrant was a solution of 0.05*M* NaOH; a pH meter was used for pH measurements under continuous stirring. The titrant was added until the pH value reached 2.00, the standard NaOH was then added stepwise, the pH values of the solution were recorded, and a curve with two inflection points was obtained.

The difference in the NaOH solution volumes between these points corresponded to the acid consumed for salification of the amine groups of CS and allowed us to determine the degree of deacetylation [DDA (%)] of the CS.

DDA (%) was calculated as follows:[27]

$$\mathrm{DDA}(\%) = \frac{1 - 161Q}{1 + 42Q}$$

where Q is equal to $N \times \Delta V/m$ [where N is the concentration of NaOH (mol/L), ΔV is the volume of the NaOH solution between the two inflection points (L), and m is the dry weight of CS (0.5 g)]. The DDA values for α-CS and β-CS were 83.9 and 93.35%, respectively.

The molecular weight of CS was calculated with the value of intrinsic viscosity measured by an Ubbelohde viscometer (Cairo, Egypt).[28] The value of the molecular weights of α-CS and β-CS were 3.43×10^3 and 3.98×10^3 g/mol, respectively.

Starch

Commercial starch was obtained from the local market.

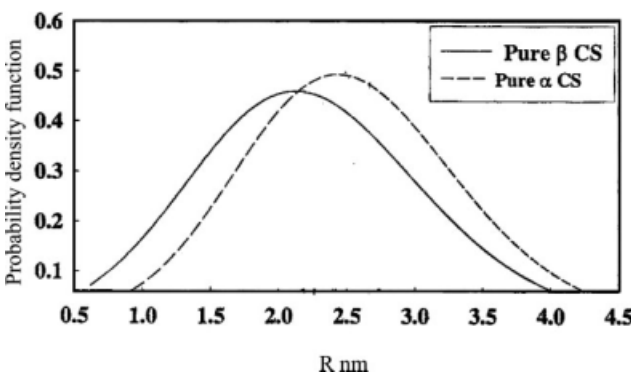

Figure 2 Distributions of R for pure α-CS and β-CS.

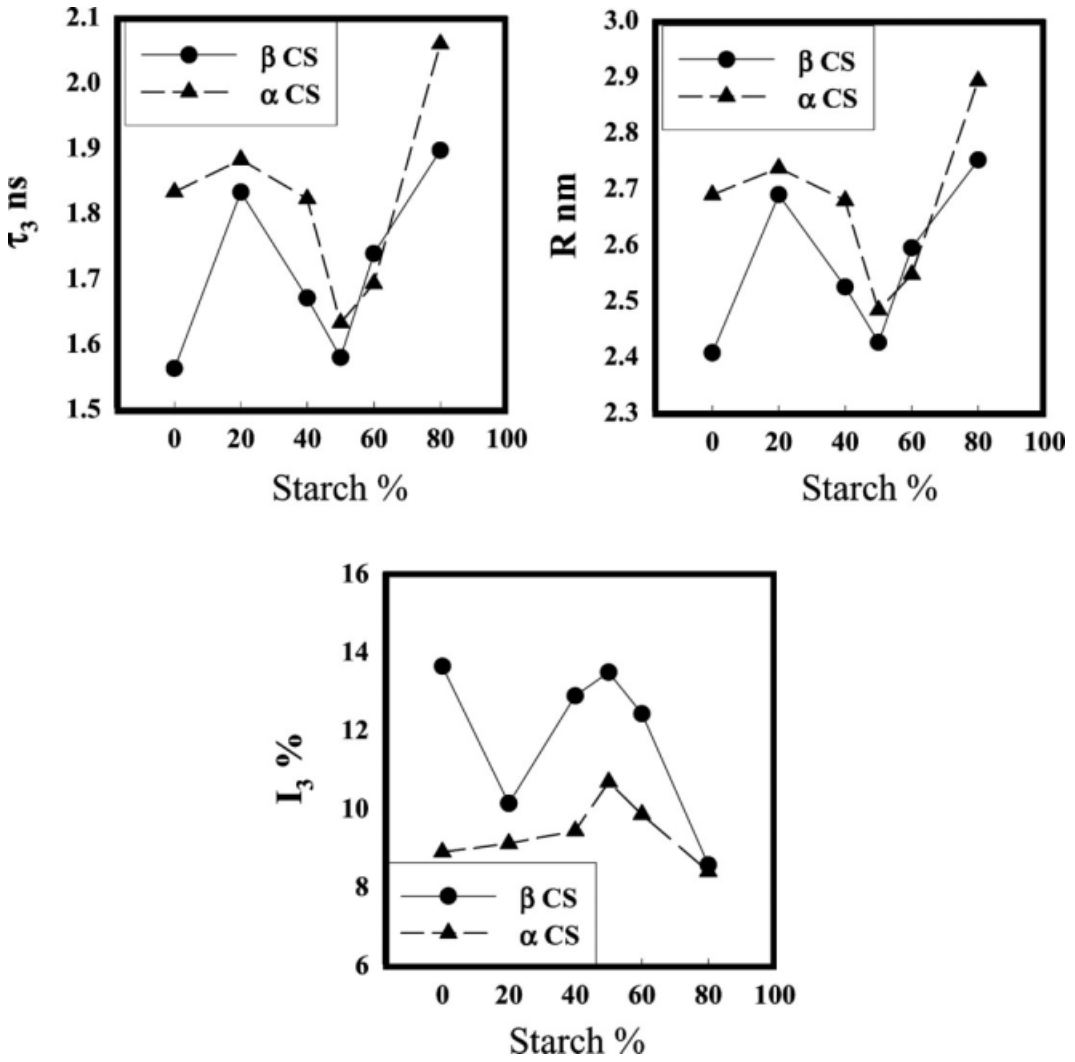

Figure 3 Free-volume parameters (τ_3, R, and I_3) of the α-CS/starch and β-CS/starch blends versus the starch percentage.

Film preparation

We dissolved the starch in distilled water at a concentration of 2 g/100 mL by heating the mixture on a hot plate and stirring it until it gelatinized (90°C for 15 min). The mixture was then left to cool to RT. The CS solutions from two different sources (α-CS and β-CS) were prepared by the dispersion of 10 g of CS in 500 mL (2 g/100 mL) of acetic acid (5% v/v). Two series of starch/CS blend films were prepared by the mixture of different ratios of starch and CS (v/v) from 20% starch to 80% starch. One of them contained 1 vol % glycerol as a plasticizer to make the formed films more flexible to study the effect of glycerol on the free-volume measurements. The mixtures were cast onto flat, leveled, nonstick Teflon plates; then, the plates were held undisturbed at 50°C for 10 h and cooled to the ambient temperature before the films were peeled off of the plates. The moisture contents of all of the samples were almost the same, about 8%.

XRD

XRD analysis was applied to detect the crystallinity of the prepared films. A Scintag powder diffractometer was used for this purpose between 2θ angles of 5 and 40°. Nickel-filtered Cu Kα radiation was used as the X-ray source.

PAL measurements

The PAL measurements were carried out in air with a fast–fast coincidence system with a lifetime resolution of 266 ps full width at half-maximum. A positron source, ^{22}Na, was prepared by the deposition of about 20 μCi of NaCl solution on a 7 μm thick Kapton foil of 1 cm^2, dried, and covered by another foil of the same size. Then, the positron source was sandwiched between two similar pieces of the sample. PAL spectra containing 10^6 counts were collected for each spectrum. All of the lifetime spectra

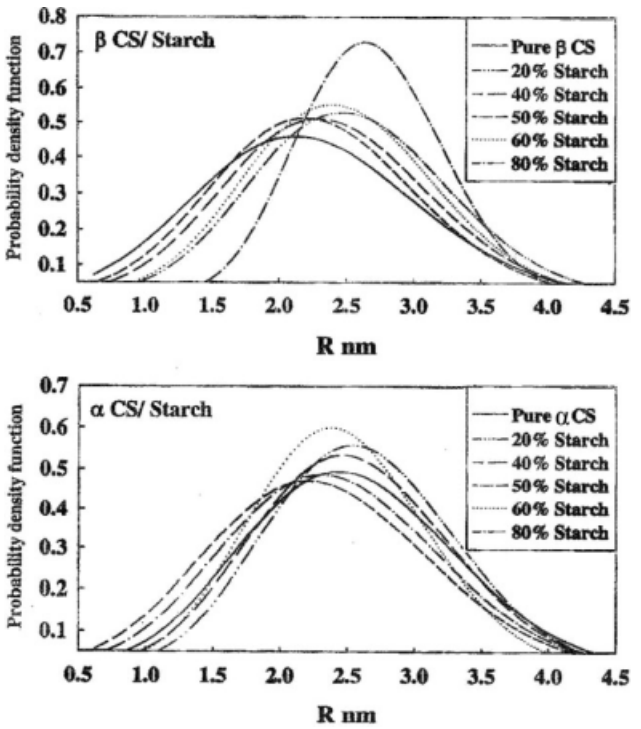

Figure 4 Distributions of R for the β-CS/starch and α-CS/starch blends.

were resolved into three components with the LT 9.0 analyzing program (Portsmouth, NH),[29] which allows both discrete and long normal distributions of the annihilation rate.[30,31] The shortest component, with mean lifetime τ_1 and intensity I_1, is related to the annihilation of *p*-Ps. The second lifetime component, with mean lifetime τ_2 and intensity I_2, is attributed to the annihilation of free positrons in the polymer matrix. The longest lifetime, with mean lifetime τ_3 and intensity I_3, is attributed to the pickoff annihilation of *o*-Ps in the nanoscale free-volume holes. Only this component was considered in our study.

The mean nanoscale free-volume hole radius (R) was derived from the measured *o*-Ps lifetime (τ_3) with the following semi-empirical equation:[32]

$$\tau_3 = 0.5\left[1 - \frac{R}{R+\Delta R} + \frac{1}{2\pi}\sin\left(\frac{2\pi R}{R+\Delta R}\right)\right]^{-1} \quad (1)$$

where $\Delta R = 1.656$ Å is an empirical parameter obtained through the fitting of the measured lifetimes of cavities of a known size.[33,34]

The relative intensity of the *o*-Ps lifetime component (I_3) is assumed to be proportional to the number of the nanoscale free-volume holes because it gives information on the *o*-Ps formation probability. Wang et al.[35] proposed a semi-empirical relation that can be used to evaluate the fractional free volume [F (%)]:

$$F(\%) = A \times I_3 \times V \quad (2)$$

where $V = 4\pi R^3/3$ is the size of the nanoscale free-volume hole. The R value is taken from eq. (1), and A is the normalization constant.

The free-volume radius distribution was calculated with the LT 9 program.[29]

Microstructure studies by SEM

The CS blend film morphology was studied by SEM (SM5400, JEOL). The samples were mounted on metal discs and coated with gold.

RESULTS AND DISCUSSION

XRD

The X-ray diffractograms of the α-CS and β-CS blend films with the addition of starch (without glycerol) are shown in Figure 1(a,b), respectively. The results demonstrate that both pure α-CS and β-CS films had a crystalline state. Pure α-CS had a main diffraction peak ($2\theta = 21°$), whereas β-CS showed two diffraction peaks ($2\theta = 15$ and $21°$); this agreed with the findings of Nunthanid et al.[36] and Xu et al.[37] After the addition of starch, the crystallinty decreased with increasing starch percentage, where the intensity of the peaks decreased and became flatter. The decrease in crystalline peaks with increasing starch percentage might have been due to the incidence of a molecular miscibility between these two components.

The addition of glycerol as a plasticizer did not alter the crystallinity of both α-CS and β-CS, as shown in Figure 1(c,d), respectively. The results show that α-CS had two main diffraction peaks ($2\theta = 9$ and $20°$), whereas there was one sharp diffraction peak at $2\theta = 20°$ for β-CS. Also the degree of crystallinity decreased with increasing starch percentage; this indicated the same behavior of α-CS and β-CS without glycerol.

Positron lifetime results

Pure α-CS and β-CS

The values of the *o*-Ps lifetime components (τ_3 and I_3), which were related to R and F of the free-volume holes in the pure α-CS and β-CS, are listed in Table I. Also, the free-volume radius distributions of pure α-CS and β-CS are shown in Figure 2. The results show that β-CS had smaller size free-volume holes with high fractions than α-CS. This was because of the much weaker intermolecular hydrogen bonding ascribable to the parallel arrangement of the main chains in β-CS.[4]

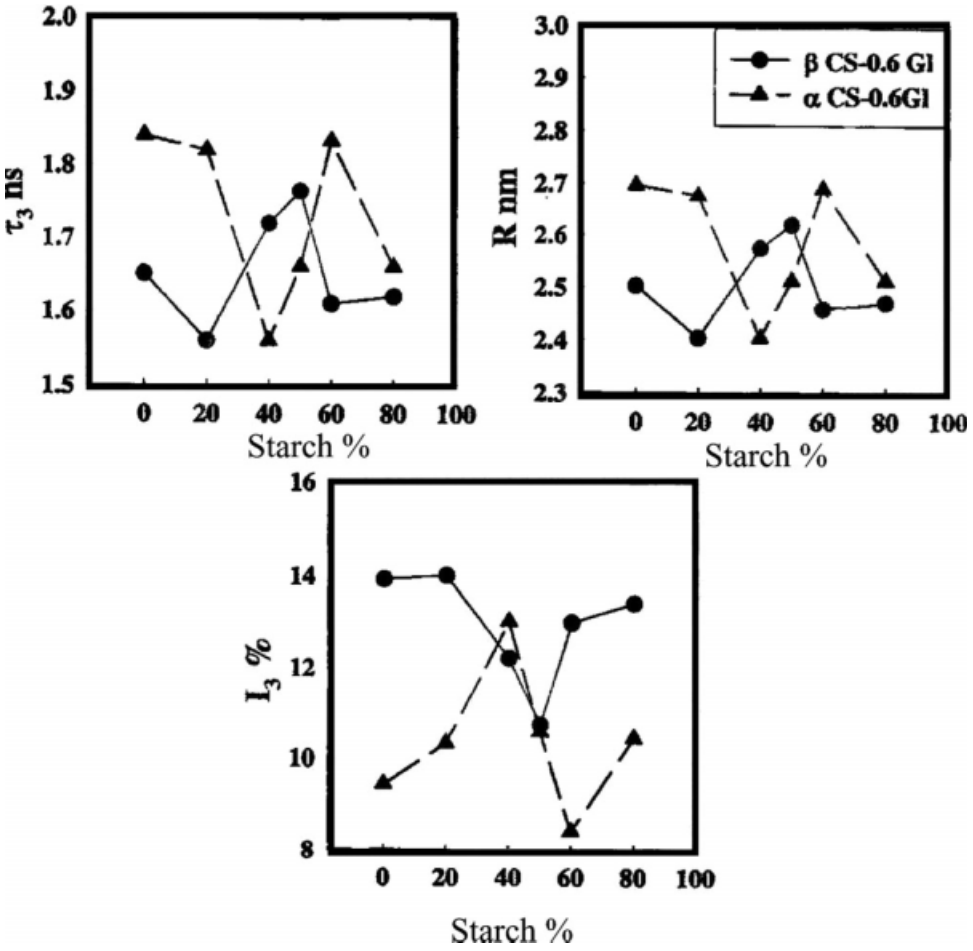

Figure 5 Free-volume parameters (τ_3, R, and I_3) of the α-CS–1% glycerol (Gl)/starch and β-CS–1% Gl/starch blends versus the starch percentage.

The free-volume parameters from the PAL measurements in both α-CS and β-CS with 1% glycerol indicated higher values compared to those without glycerol. The addition of plasticizers such as glycerol are intended to decrease the intermolecular forces along polymer chains and import an increase in the polymer flexibility that leads to an increase in the free-volume parameters, as shown in Table I.

Effect of starch on the α-CS and β-CS blend films

The results for τ_3, I_3, and R are summarized in Figure 3 for the α-CS/starch and β-CS/starch blends with different concentrations of starch. For β-CS/starch, we noticed that at 20% starch, the size of the free-volume holes (τ_3 or R) increased, and its fraction (I_3) decreased, whereas no significant changes in the free-volume parameters (τ_3 and I_3) were observed for α-CS/starch. The size of the free-volume holes showed a decrease, whereas its fractions increased, as the starch concentration was increased up to 50% in both α-CS and β-CS; this indicated comparatively close packing of the chain segments. Thereby, the starch chain segments were apt to migrate into the free-volume holes of the CS matrix because of the hydrogen bonding between the OH and amide groups (NH_2) of CS, and the OH groups of starch were stronger than the interactions among CS chains. These interactions inhibited the CS chain mobility and enhanced the stresses at the interface, which caused a decrease in the free-volume holes and an increase in its fractions.[23]

The further addition of starch (50–60%) led to an increase in the free-volume size for the blends. Such a result was reasonably attributed to the weaker hydrogen-bonding interactions that were formed between CS and starch compared with the interactions among the CS chains.[23] These interactions would have destroyed partial hydrogen bonds between the CS chains and then loosened the CS chains near the starch surface. Consequently, the free-volume hole size for these blends increased, and its fractions decreased.

In Figure 4, the free-volume hole radius distributions in the β-CS/starch and α-CS/starch blends as a function of the starch percentage are shown. In this

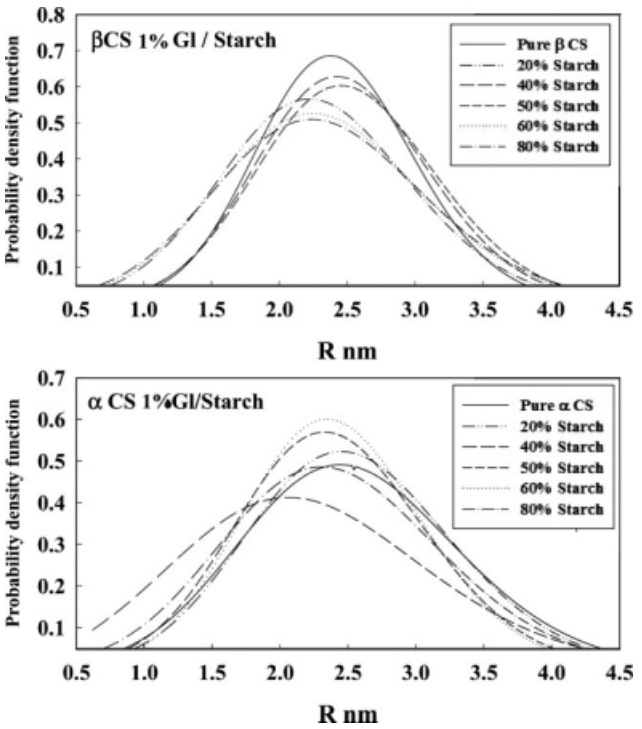

Figure 6 Distributions of R for the β-CS–1% glycerol (Gl)/starch and α-CS–1% Gl/starch blends.

figure, we observed that R increased up to 2.68 and 2.734 nm at 20% from the corresponding value of pure β-CS and α-CS, respectively. On the other hand, an increase up to 2.748 nm and a decrease up to 2.5 nm in the radius of the free-volume holes for β-CS and α-CS were observed at 80% of starch; this was confirmed by the discrete lifetime analysis.

Effect of 1% glycerol on the α-CS and β-CS blend films

The variations of τ_3, I_3, and R for both α-CS–1% glycerol/starch and β-CS–1% glycerol/starch are shown in Figure 5. From this figure, we can see that, the average size of the free-volume holes (τ_3 and R) decreased and its fraction (I_3) increased when the α-CS–glycerol matrix contained 20–40% starch. This means that α-CS–glycerol/starch blend was miscible at the molecular level.[25] This phenomenon was consistent with the partition effect to the free-volume holes of the α-CS–glycerol/starch blend, which resulted in a decrease in the free-volume size and an increase in the free-volume fraction of the blend. Also, in the α-CS–glycerol/starch blend containing greater than 40% starch, the average size of free-volume holes increased, and the fraction decreased almost to the pure α-CS–glycerol level. However, with in blend containing greater than 60% starch, τ_3 and R decreased, and I_3 increased. This means that with the addition of starch greater than 40%, the dispersion of starch in the α-CS–glycerol matrix was limited. Therefore, the free-volume fraction of the blends decreased. On the other hand, the addition of 80% of starch showed the reverse trend.

For β-CS–glycerol/starch blends, at 20–50% starch, the increase of τ_3 and R and the decrease of I_3 compared with pure β-CS–glycerol was mainly

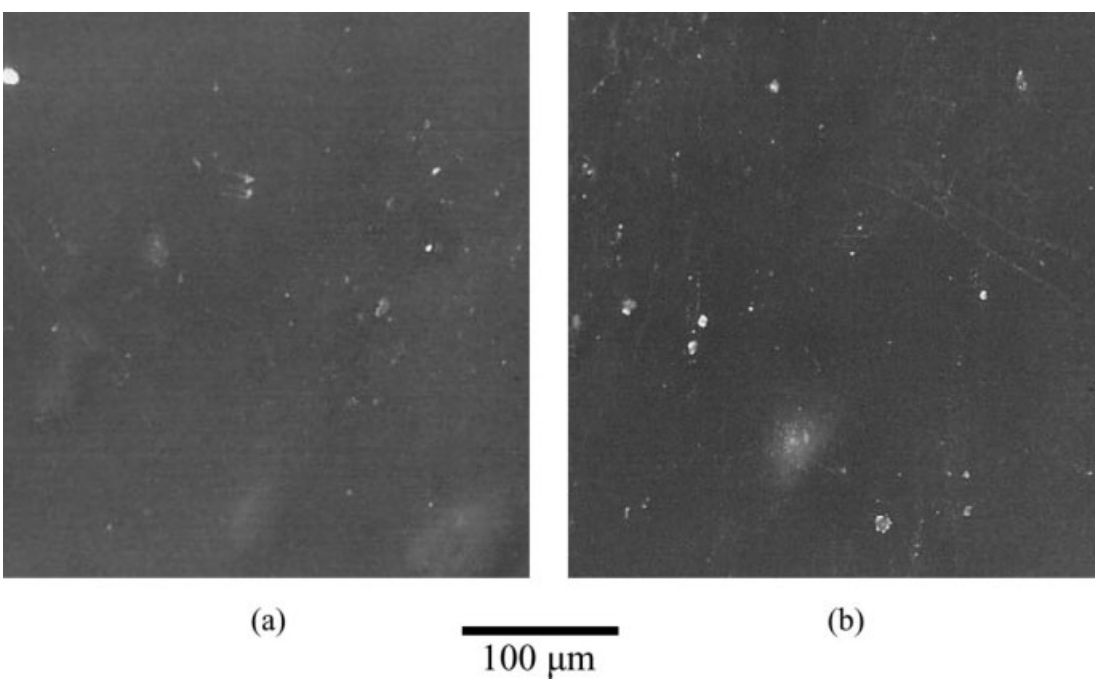

Figure 7 SEM images of the microstructure of (a) pure α-CS and (b) pure β-CS.

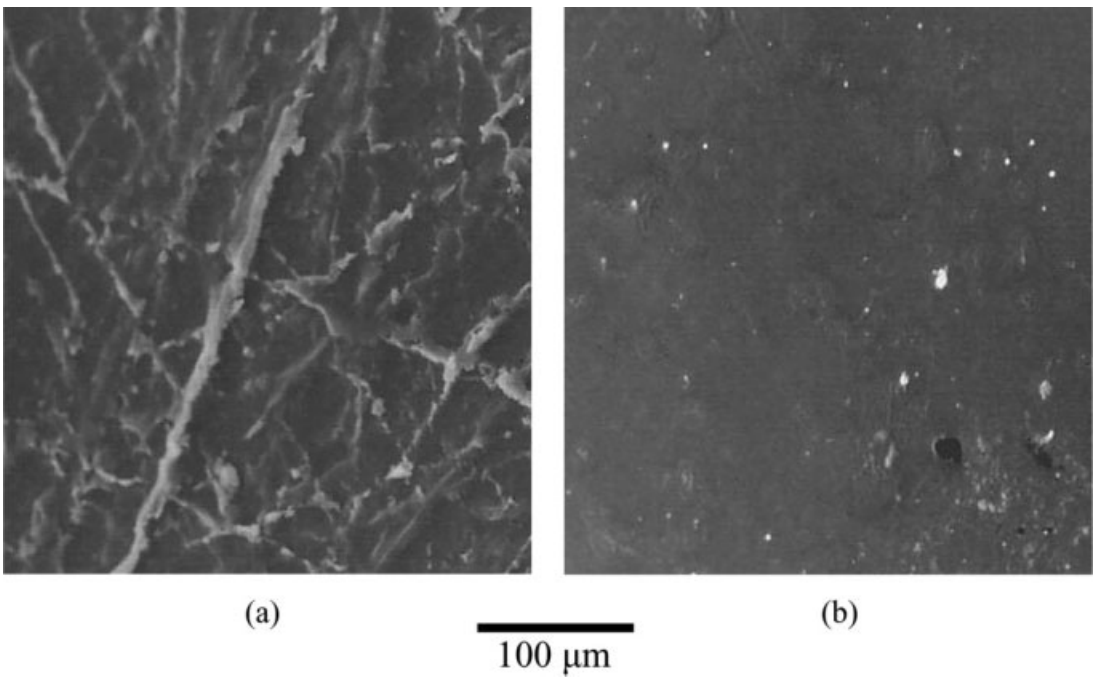

Figure 8 SEM images of the microstructure of (a) pure α-CS with 1% glycerol and (b) pure β-CS with 1% glycerol.

attributed to the formation of much larger free-volume holes in the interfacial zone between the β-CS–glycerol matrix and starch domain; this led to a connection effect of the free-volume holes and then a decrease in the free-volume fractions. With blends containing greater than 50% starch, τ_3 and R decreased and I_3 increased, and then, all parameters reached a saturation level. Like that in the α-CS–glycerol/starch blend, some starch molecules might have been retained in the β-CS–glycerol matrix, and they could have also entered the free-volume holes of the β-CS–glycerol matrix, which would have led to a decrease in the free-volume hole size in the blend. However, the interaction between β-CS–glycerol and starch was much poorer than that between α-CS–glycerol and starch. This was due to the main-

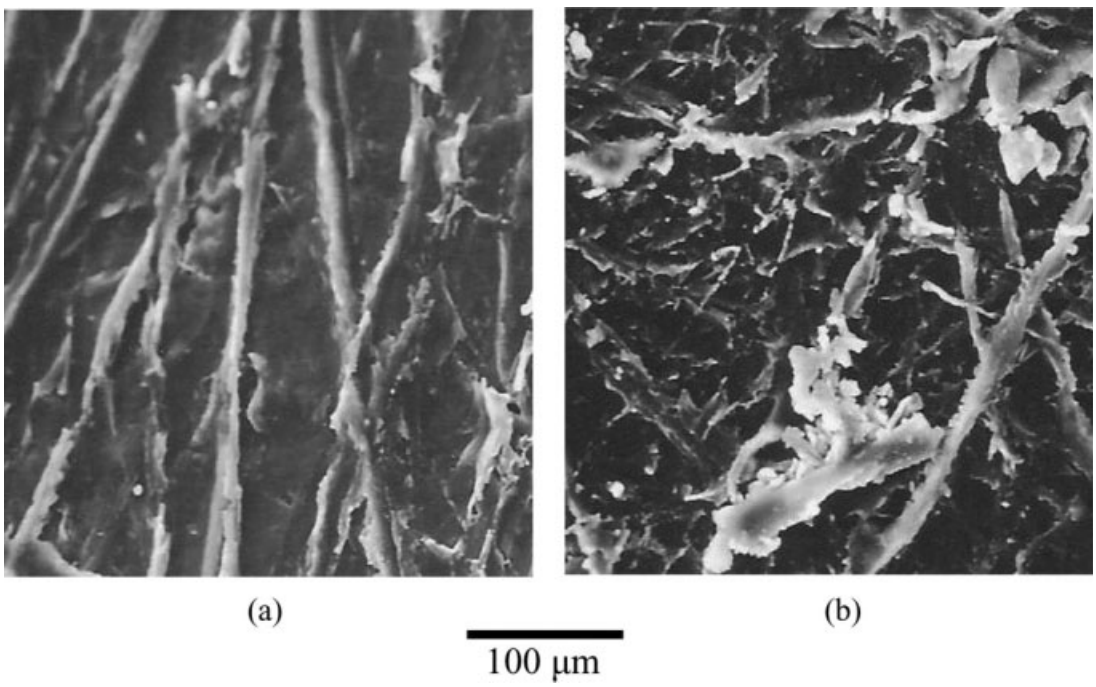

Figure 9 SEM images of the microstructure of CS with the addition of 50% starch for both (a) α-CS and β (b) pure β-CS starch.

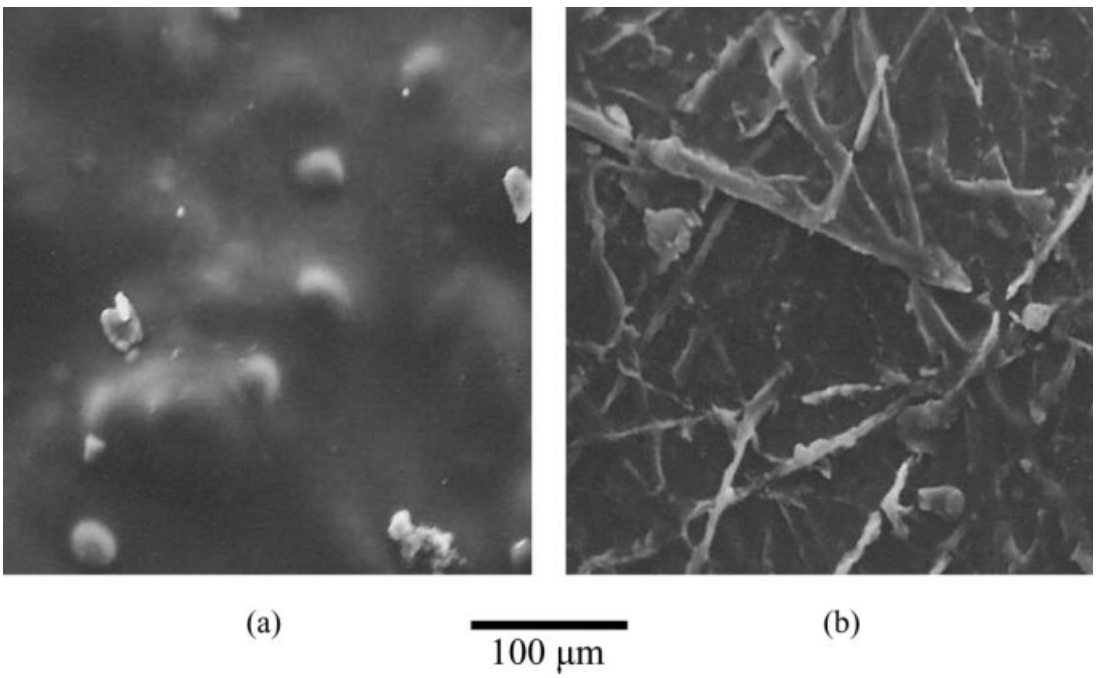

Figure 10 SEM images of the microstructure of CS with the addition of 40% starch and 1% glycerol for both (a) α-CS and (b) β-CS.

chain packing and parallel arrangement of the main chains of β-CS–glycerol, as mentioned previously. With regard to their much poorer interaction and phase separation, the molecules in the interfacial zone of the β-CS–glycerol/starch blend were less compact than those of the α-CS–glycerol/starch blend. This increase in the free-volume size of the β-CS–glycerol/starch blend was mainly attributed to poor interfacial adhesion and poor miscibility.

Figure 6 shows the distributions of the free-volume hole radius of the β-CS/glycerol/starch blends with different starch contents. The distributions of the free-volume hole radius shifted from a larger to smaller radius at 20% starch, and then, it became larger with increasing starch content up to 50%. These results confirmed the results shown in Figure 5.

SEM results

Figure 7 shows the SEM observations of pure α-CS and β-CS; these indicate that, generally, the surface of the microspheres in both α-CS and β-CS were uniform and smooth without appreciable defects. On the other hand, the shape of the microspheres became rounder, smoother, and smaller in β-CS compared with α-CS.

The observations of SEM (Fig. 8) indicated a distribution of microspheres through a crosslink shape for α-CS with 1% glycerol and a dispersed distribution of β-CS with 1% glycerol.

The SEM observations of α-CS and β-CS with 50% starch showed an interaction between the starch and CS microspheres with a crosslink, but it was more flexible for β-CS, as shown in Figure 9, which shows a good correlation with the PAL results.

The SEM observations at 40% starch for α-CS and β-CS with 1% glycerol are shown in Figure 10. This figure shows a reverse microstructure for α-CS and β-CS, that is, a distribution of microspheres through a crosslink shape for β-CS and a dispersed distribution of microspheres of α-CS. Thus, there was a positive correlation between the SEM observations and PAL results.

CONCLUSIONS

1. The addition of 20% starch to β-CS increased the size of the free-volume holes (τ_3) and decreased the fraction (I_3), whereas no significant change was observed for α-CS.
2. The behavior of both α-CS and β-CS with increasing starch up to 50% showed a decrease in the size of free-volume holes and an increase in the fraction; this indicated comparatively close packing of the chain segments.
3. Further addition of starch up to 80% led to an increase in the free-volume hole size and a decreases in the fractions for both α-CS and β-CS.
4. The addition of 1% glycerol to both α-CS and β-CS increased the flexibility, which led to an increase in the free-volume parameters.

5. Because of the packing and parallel arrangement of the main chains in β-CS glycerol/starch, the interaction between β-CS–glycerol and starch was much poorer than that between α-CS–glycerol and starch.
6. The addition of glycerol to the CS starch blends indicated that some modifications took place, but it did not reveal where the modification actually took place.
7. The XRD and SEM results show a decrease in crystallinity of the blend with the increase in starch percentage, and these were in agreement with the PAL results.

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