Soy Protein Isolate Based Films: Influence of Sodium Dodecyl Sulfate and Polycaprolactone-triol on Their Properties

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Summary: We report on the preparation and properties of soy protein isolate (SPI)-sodium dodecyl sulfate (SDS)-polycaprolactone-triol (PCL-T) films obtained by solvent casting from solutions containing variable amounts of SDS or SDS/PCL-T. It is shown that the mechanical and thermal properties, and the morphology of SPI-based biofilms can be easily controlled by changing SDS, PCL-T, and moisture contents, enabling the fabrication of rigid and flexible materials as pure SPI films [Young's modulus $\sim$ 1 400 MPa, elongation at break (E) $\sim$ 2%, and glass transition temperature (T_g) $\sim$ 150 °C] and SPI/SDS/PCL-T films with [PCL-T] $\geq$ 18% (Young's modulus $\sim$ 50 MPa, $E \sim$ 90%, and $T_g \sim$ 135 °C), respectively. Micrographs taken at the cross-section of biofilms whose [PCL-T] $\geq$ 18% revealed the occurrence of a porous matrix, whereas a dense bulk phase was otherwise observed (pure SPI, SPI/SDS, and SPI/SDS/PCL-T films with [PCL-T] $<$ 18%).

Keywords: biofilms; mechanical properties; morphology; soy protein isolate; thermal properties

Introduction

Nowadays, one of the main goals in polymer research is to develop low-cost biodegradable plastics. In this framework, attempts have been focused on biodegradable polymer composites, new polymers, and chemical modification of already used synthetic polymers,[1] also with emphasis on the substitution of petroleum-based materials which present important concerns in terms of pollution and sustainability.[2,3] Polysaccharides and proteins have been shown to be suitable starting materials.[4] A number of investigations have shown the filmogenic properties of sunflower,[4] wheat gluten,[5,6] corn,[6] maize zein,[7] pea,[8] milk,[9] and soy proteins.[1–3,10–15] Special interest is herein placed on soy protein isolate (SPI), which is obtained from soybean seeds by a separation method based on chemical reactivity and solubility, and is a cream-colored powder with 90–95% protein content.[1,2,10,16] The ultracentrifugal pattern of SPI generally shows a wide range of molecular weights (8–600 kDa), with four major fractions identified as 2S (20–22%), 7S (37%), 11S (31–40%), and 15S (10–11%).[1,3,17]

However, SPI materials without other secondary components do not show, in most cases, satisfactory physical–chemical and mechanical properties adequate for industrial applications, namely because they tend to be very brittle due to the presence of strong intra- and intermolecular interactions.[1,11] To improve the above properties, fundamentally two approaches have been studied. The first method consists in modifying the protein structure in the film-forming solutions (i.e., before casting) through reactive blending with

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other biodegradable polymers,[2,4,18,19] denaturation,[12,13,16,18,20–24] and chemical[2,10,15,16,21,25] and enzymatic[2,26,27] modification. Another possibility, which is extensively employed in polymer research, is the addition of plasticizers to increase the flexibility and elasticity of plastics due to their ability to reduce internal hydrogen bonding between polymer chains while increasing molecular spacing. Protein films are better plasticized by compounds containing hydroxyl moieties as glycerol, glycols, and water.[2,3] Regarding SPI-based materials, the hydrophilic nature of the protein makes the influence of the moisture content (MC) (directly influenced by the relative humidity) on the film properties very important. From this point of view, the addition of a moderated hydrophobic polymer chains is a very interesting approach to obtain materials with better mechanical properties, presumably also enabling the control over the MC in the resulting film.

Polycaprolactone-triol (PCL-T) is a biodegradable, synthetic, low-molecular-weight aliphatic polyester. To the best of our knowledge, the use of PCL-T as plasticizer in biopolymer films was still not described in literature. In the present study, PCL-T is introduced as plasticizer in SPI/sodium dodecyl sulfate (SDS) films. The amphiphilic nature of SDS provided the solubility of PCL-T chains in the aqueous film-forming solutions. It is known, however, that the presence of SDS affects the properties of the resulting SPI films, since it is a surface-active agent known to dissociate and denature proteins by disrupting hydrophobic and electrostatic interactions that maintain the protein conformation, leading to partially unfolded structures.[12,20,22,24,28]

The aim of this work was to prepare SPI-based biofilms, and to characterize them in terms of thermal (glass transition temperature—T_g) and mechanical properties [Young's modulus, elongation at break (E), and tensile stress (TS)], MC and morphology [observed by scanning electron microscopy (SEM)]. SPI, SPI/SDS, and SPI/SDS/PCL-T films with different compositions were therefore investigated.

Materials and Methods

Film Preparation

Film-forming solutions were prepared by dissolving 1 g of SPI (92% protein content on dry basis, SUPRO 500 E, Solae) in a 20 mL aqueous solution with different PCL-T (Aldrich) and SDS (Aldrich) contents while stirring. The solution pH was adjusted to 10.0 with 1.0 $mol \cdot L^{-1}$ NaOH (Vetec). Alkaline conditions favor SPI film formation, presumably by aiding the protein dispersion in the film-forming solution.[12] The solutions were then cast onto leveled polystyrene plates ($A = 177$ cm^2). Film thickness was controlled by casting the same amount (18 mL) of film-forming solution per plate. Castings were left to stand at room temperature (25 °C) for 24 h, and then dried films were peeled from the plates. Prior to the experiments, the SPI-based films were stored in desiccators for at least 24 h. The chemical composition of the resulting films studied in this work is thereafter given in wt.-%.

Fluorescence Measurements

Steady-state fluorescent spectra were measured using a Hitachi F-4500 spectrometer in the right-angle geometry (90° collecting optics). For the fluorescence measurements, 2 mL of the film-forming solution was placed in a 10-mm square quartz cell. All spectra were run on air-equilibrated solutions. For fluorescence emission spectra, λ_{ex} was 280 nm. Spectra were accumulated with an integration time of 1 s per 0.5 nm.

Thermal Analysis

The experiments were carried out using a DSC-50 Shimadzu system at a heating rate of 10 °C · min^{-1} under nitrogen atmosphere (50 $cm^3 \cdot min^{-1}$). The samples with average mass of 7 mg were initially heated from 25 up to 200 °C and cooled down to 25 °C prior to registering the second run in the same temperature range.

Moisture Content

After conditioning in desiccators under vacuum during 4 d, the samples were weighed (±0.0001 g) into dishes and dried in an air-circulating oven at 100 °C for 4 h. MC was determined as the percentage of initial film weight lost during drying. Triplicate measurements of MC were obtained for each film with individually prepared films.

Mechanical Tests

An EMIC Universal Machine model DL 500 equipped with a 1 kN static load cell was used to evaluate the tensile strenght (TS), percentage of elongation at break (PE), and Young's modulus of films according to ASTM standard D-882-95a.[29] Sample films were cut into 20 mm wide strips, and the grip separation was 50 mm. At least ten samples of each film were tested.

Results and Discussion

Fluorescence Measurements

The tryptophan amino acid residue is present in several protein structures found in SPI, namely in the glycinin fraction. Its fluorescence emission peak is dependent on the polarity of the local molecular environment. When it is in a hydrophobic microenvironment, the wavelength of maximum fluorescence emission (λ_{max}) is approximately 330 nm while in a polar environment such as water, λ_{max} is around 350 nm. The observed emission peak will thus reveal the polarity of the medium around the tryptophan amino acid residue.[30]

Figure 1 shows the variation of λ_{max} (A) and intensity (B) of the tryptophan fluorescence as a function of the solution composition measured using $\lambda_{ex} =$ 280 nm. In these experiments, dilute solutions as compared to those used to obtain the biofilms (indicated in the figure) were also examined in order to better understand the SDS and PCL-T effects on the tryptophan microenvironment, and ultimately on the protein structure. In Figure 1(A), a 3 nm blue shift in the λ_{max} (from 338 to 335 nm) is observed for [SDS] (here and throughout the text, the square brackets refer to concentration) higher than the critical micellar concentration (CMC), which is about 8 mmol · L^{-1} for SDS in pure water. In parallel, the tryptophan fluorescence intensity [Figure 1(B)] also undergoes an evident decrease. In both cases [Figure 1(A) and (B)], the results demonstrate that the local

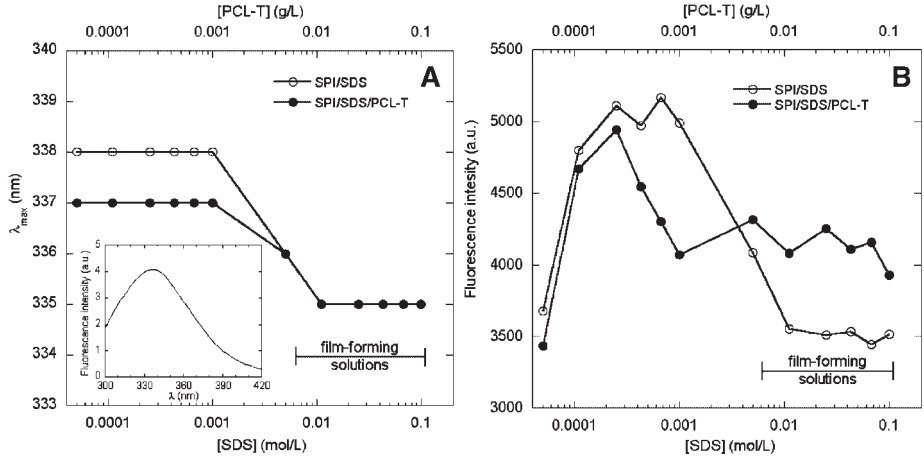

Figure 1.
Tryptophan fluorescence behavior in the film-forming solutions: (A) λ_{max} and (B) intensity variation as a function of the solution composition. The inset in (A) shows a typical fluorescence spectrum of tryptophan aminoacid residue; $\lambda_{ex} = 280$ nm.

environment of tryptophan is changed into a more polar one upon increasing the [SDS] above the CMC.[31]

Therefore, the most important structural changes in the protein are related to the existence of SDS micelles in solution, which interact electrostatically (through $-SO_3^-$ groups) and hydrophobically (through the dodecyl chain) with the protein. Such interaction promotes the rupture of low-energy intra- and intermolecular bonds that maintain the protein conformation and disrupt the continuity of the protein matrix,[3,12,13,22] leading to partially unfolded structures. As pointed out in Figure 1, all the film-forming solutions contained denaturated (unfolded) protein structures. Consequently, changes in the film properties (see below) are most likely associated to intermolecular interactions rather than to the protein structure itself.

Thermal Properties

The glass transition temperature (T_g) can be interpreted as the range of temperatures at which segment motion of macromolecules becomes thermally activated. The T_g increases with the chain rigidity and the energy of intra- and intermolecular interactions, including hindrance to internal rotation along the macromolecular chains. An effective plasticizer has to shield these interactions, facilitating the molecular mobility and decreasing the internal friction in the polymer.

Figure 2A shows a typical DSC thermogram for a pure SPI biofilm, which undergoes a glass transition at 143 °C. It is agreed that in the case of pure SPI not submitted to external modifications this process usually takes place at 150 °C.[1,32] The 7 °C-lowering observed for pure SPI films in the present work is attributable to changes in the native protein structure that occurred during the film preparation. The absence of endothermic transitions in the temperature range of 70–90 °C also indicates the protein denaturation.

The T_g-dependence on the film composition is given in Figure 2(B), where it can be seen that films formed by SPI/SDS showed an approximately constant T_g-value around 151 °C, while SPI/SDS/PCL-T films displayed a considerable decrease in the T_g-value for [PCL-T] > 9%, reflecting the plasticizer effect of PCL-T. In the case of SPI/SDS and SPI/SDS/PCL-T films containing up to 9% PCL-T, the results suggest that the chain rigidity and extent of intra- and intermolecular interactions remained practically unchanged.

However, we have observed from the mechanical tests (hereinafter discussed) that SPI/SDS films are in fact less rigid than pure

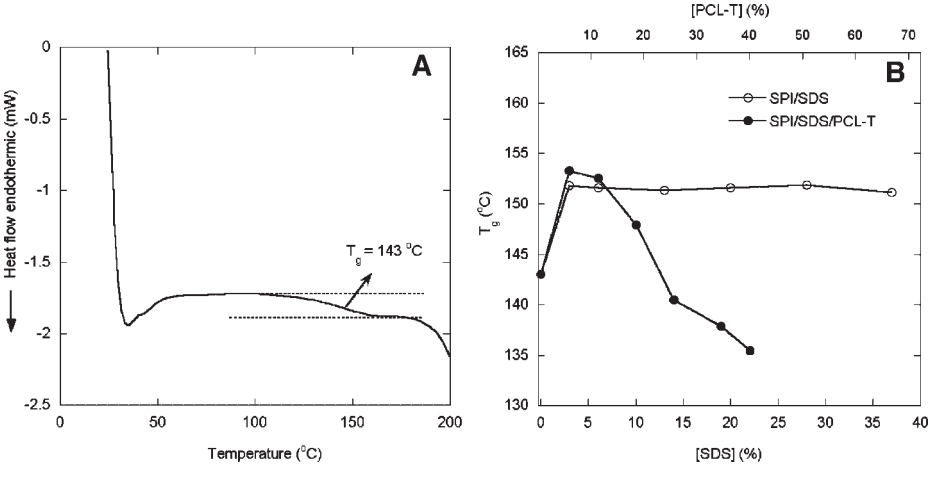

Figure 2.
DSC thermogram of a pure SPI biofilm as prepared in this work (A) and glass transition temperature (T_g) dependence on the composition of SPI/SDS and SPI/SDS/PCL-T films, as determined by DSC measurements (B).

SPI films, but this behavior is strictly dependent on the MC. Water molecules are known to reduce hydrogen bonding, electrostatic, and dipole-dipole interactions within the protein structure with ultimate improvement in the mobility of chains. Therefore, we attribute the constant T_g-value of 151 °C to the low water content in all SPI/SDS films due to evaporation during heating in a DSC experiment.

Mechanical Properties

Figure 3 shows typical stress–strain curves of SPI/SDS films at 75% RH and SPI/SDS/PCL-T films at 75 and 40% RH. A remarkable effect of PCL-T on the mechanical properties of the film is evident in this figure, as characterized by the difference between the solid and dotted lines at 75% RH in terms of TS, E, and Young's modulus. Likewise, this figure also reveals that the relative humidity of the surroundings to which the SPI-based biofilms are exposed plays a decisive role on material features. Table 1 summarizes the dependence of TS, E, and Young's modulus on the film composition. For biofilms whose brittleness allowed their handling, the measurements were done at three different relative humidities. It is noteworthy to mention that the mechanical properties measured for pure SPI films are in very good agreement with those listed by Mo and Sun.[22]

In the case of SPI/SDS films, the results obtained at 75% RH revealed a rather stable plateau for the Young's modulus around 800 MPa for [SDS] $\geq$ 13% after an initial decrease from 1 424 MPa (pure SPI films) (Table 1). For RH $\leq$ 54%, on the other hand, the SDS influence on the resulting mechanical properties was less obvious. At 54% RH the Young's modulus first decreased from ca. 1 720 MPa (pure SPI films) down to 985 MPa for SPI/SDS 80/20 films, and the opposite behavior was observed for [SDS] > 20%, while at 40% RH it was practically unaffected by the SDS presence.

As previously mentioned, the interaction between SDS and SPI takes place simultaneously in the hydrophilic and hydrophobic domains, disrupting the continuity of the protein matrix and reducing interaction between peptide chains. In principle, this scenario would lead to materials of lowered stiffness.[22] We have observed, alternatively, that such an effect is dependent on the MC in the film, provided that it is indeed found for the

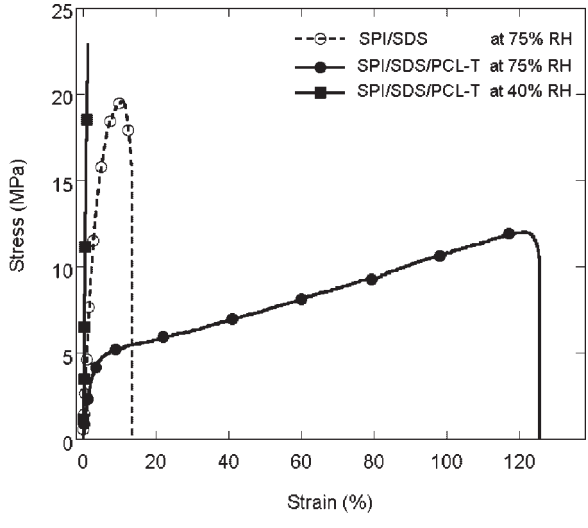

Figure 3.
Typical stress–strain curves of SPI/SDS films at 75% RH and SPI/SDS/PCL-T films at 75 and 40% RH.

Table 1.
Young's modulus, TS, and *E* measured for pure SPI films and as a function of the composition for SPI/SDS and SPI/SDS/PCL-T films conditioned at different relative humidities.

Film	Relative humidity %								
	40			54			75		
	TS	*E*	Young's modulus	TS	*E*	Young's modulus	TS	*E*	Young's modulus
	MPa	%	MPa	MPa	%	MPa	MPa	%	MPa
SPI	-	-	-	17 ± 3	2.10 ± 0.8	1 726 ± 152	14 ± 5	1.5 ± 0.8	1 424 ± 131
SPI[a)]	-	-	-	-	-	-	15 ± 3	1.8 ± 0.5	1 545 ± 124
SPI/SDS									
94/06	-	-	-	-	-	-	19 ± 3	2.6 ± 0.9	1 188 ± 159
87/13	-	-	-	9 ± 5	1.0 ± 1.4	1 409 ± 396	11 ± 2	11.3 ± 6.6	717 ± 167
80/20	21 ± 5	2.2 ± 0.8	1 950 ± 231	2 ± 2	6.2 ± 3.1	985 ± 145	10 ± 4	6.3 ± 2.3	899 ± 157
72/28	19 ± 4	2.2 ± 0.9	1 914 ± 233	7 ± 2	3.8 ± 2.1	1 173 ± 111	10 ± 3	10.0 ± 4.1	800 ± 196
63/37	16 ± 6	1.8 ± 0.7	1 819 ± 282	23 ± 4	2.5 ± 1.2	1 718 ± 201	12 ± 2	6.3 ± 3.6	858 ± 199
63/37[a)]	-	-	-	-	-	-	12 ± 3	8.8 ± 5.5	900 ± 117
SPI/SDS/PCL-T									
93/03/04	-	-	-	13 ± 4	3.8 ± 1.5	1 433 ± 200	15 ± 3	4.5 ± 1.6	1 138 ± 200
85/06/09	-	-	-	12 ± 2	3.7 ± 1.2	1 333 ± 129	11 ± 3	10.4 ± 8.7	982 ± 235
72/10/18	13 ± 2	1.4 ± 0.6	1 566 ± 143	12 ± 4	3.8 ± 1.0	1 038 ± 107	6 ± 2	121.4 ± 32.1	171 ± 41
60/14/26	10 ± 2	1.2 ± 0.3	1 228 ± 112	9 ± 3	5.6 ± 2.1	713 ± 148	2 ± 1	84.0 ± 18.7	66 ± 17
49/19/32	7 ± 1	1.5 ± 0.3	798 ± 063	6 ± 2	4.7 ± 2.2	661 ± 123	3 ± 1	85.0 ± 17.9	58 ± 29
39/22/39	5 ± 2	4.0 ± 1.4	526 ± 105	3 ± 1	6.9 ± 1.9	436 ± 91	1 ± 1	91.5 ± 18.0	44 ± 20
39/22/39[a)]	-	-	-	-	-	-	4 ± 2	135.4 ± 32.1	48 ± 15

[a)] Films submitted to heating at 70 °C within 1 h soon after the casting.

experiments done at 75% RH. The high hygroscopic nature of SPI enables water molecules to enter the amorphous interior of the biopolymer structure and disrupt hydrogen bonds and other forces still holding the chains together. Thus, the absorbed moisture acts as a plasticizer facilitating the movement of macromolecules to past each other, with consequent decrease in the Young's modulus.

On the other hand, SPI/SDS/PCL-T films exhibited a huge difference in the mechanical properties in comparison to those discussed above. As seen in Table 1, for SPI/SDS/PCL-T films conditioned at 75% RH, the Young's modulus underwent an abrupt decrease from 1 424 MPa (pure SPI films) to only 171 MPa for 72/10/18 SPI/SDS/PCL-T films, and this latter value further decreased slightly down to 44 MPa for higher [PLC-T]. In general, the same comments also apply to the Young's modulus recorded at 54 and 40% RH, except that in those cases its diminution occurred gradually as the [PCL-T] increased over the studied range. With respect to E and the TS associated to the SPI/SDS/PCL-T films, one observes in Table 1 that the former also increased abruptly for [PCL-T] $\geq$ 18% (up to an average value of 90%) for films conditioned at 75% RH, the opposite behavior being found in terms of TS. However, for RH $\leq$ 54%, the E-values are comparable to those obtained for SPI/SDS films, whereas a slight increase in the TS seemed to take place in presence of PCL-T.

The results showed hitherto for SPI/SDS and SPI/SDS/PCL-T films suggest that the mechanical properties of SPI-based materials can be tailored by adjusting the SDS, PCL-T, and MC, as clearly illustrated by textbook-like stress–strain curves characteristic of very distinct (rigid and flexible) materials (Figure 3).

It has been described in the literature that crosslinking reactions may occur under certain conditions for protein-based materials, basically due to the presence of reactive side groups of amino acids in the protein such as hydroxyl groups, carbonyl groups, sulfyhydryl groups, and amino groups.[33] In our experiments, we noticed that the outcomes from mechanical tests depended on the elapsed time after casting for experiments done using specimens aged less than 1 week. The results shown above are from measurements carried out on SPI films after at least 1 week since their preparation. During this period, however, the mechanical properties were found to change considerably. Figure 4 shows the variation of TS and Young's modulus for SPI/SDS and SPI/SDS/PCL-T films as a function of the elapsed time after casting. For the sake of clarity, the parameters were normalized in relation to their initial values.

It is clearly observed in this figure that SPI/SDS specimens were stable during the period examined (TS $\sim$ 11 MPa; Young's modulus $\sim$ 840 MPa). In contrast, SPI/SDS/PCL-T films underwent an obvious evolution in terms of TS (from 0.3 to 3.0 MPa) and Young's modulus (from 7.5 to 53 MPa) during the first 4–5 days, remaining constant afterwards. These changes are attributable to the formation of crosslinkings within the film, provided that this process could be accelerated by heat curing fresh films at 70 °C during 1 h (see results in Table 1). Moreover, SPI and SPI/SDS/PCL-T films were insoluble in water after aging, further indicating the formation of internal bonds. Conversely, films formed by SPI/SDS (without addition of PCL-T) did not exhibit additional rearrangements, remaining stable and water-soluble even after 1 week.

Moisture Content

Figure 5 shows the MC present in SPI, SPI/SDS, and SPI/SDS/PCL-T films. These experiments revealed that the pure SPI films present 5.5% of MC. When SDS was added, this value first increased up to 8.8% at [SDS] = 3% apparently because protein structure had more exposed hydrophilic polar groups, which can interact with water molecules. Above this [SDS], the MC shifts down to its starting value. This was likely due to the hydrophobic portions of SDS

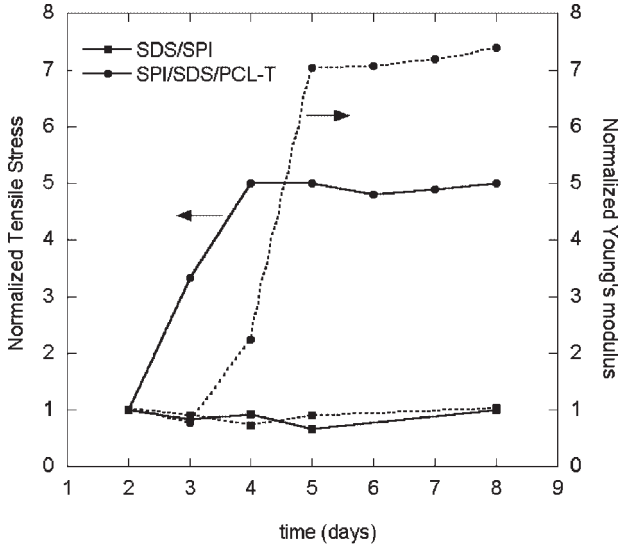

Figure 4.
Dependence of Young's modulus (dotted lines) and tensile strenght (solid lines) on the elapsed time after casting of SPI-based films (63/37 SPI/SDS and 39/22/39 SPI/SDS/PCL-T).

molecules that prevent water to be accommodated within the film structure.

Conversely, SPI/SDS/PCL-T films with [PCL-T] $\geq$ 18% ([SDS] $\geq$ 10%) were found to have a greater capacity to retain water, whereas for lower plasticizer concentrations the MC was similar to that determined for pure SPI films. Such behavior can be explained considering that PCL-T is a hydrophilic hydroxilated molecule, and

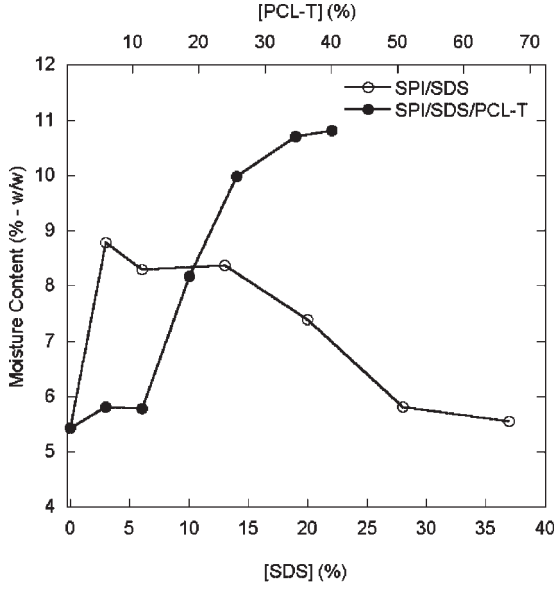

Figure 5.
Variation of the remaining MC as a function of the composition for SPI/SDS (A) and SPI/SDS/PCL-T (B) films.

therefore at low [PCL-T] water is not held owing to the fact that the PCL-T replaces it by forming intermolecular hydrogen bonding. Above a certain PCL-T concentration (>9%), the water absorption is then favored due to the augmented availability of hydrophilic sites (hydroxyl groups).

Morphology

Figure 6 shows SEM micrographs taken at the surface [Figure 6(A)] and cross-section [Figure 6(B)] of pure SPI biofilms. These specimens were characterized by minor surface heterogeneities originated from the solvent casting process, and by a dense bulk phase, which is initially well-compacted and becomes gradually less organized as the solvent evaporation occurs, as indicated by the arrow in Figure 6B.

Upon addition of SDS, the resulting films were found to become progressively more porous with a tendency to phase separation at high [SDS]. Figure 7 shows surface and cross-section SEM micrographs of SPI/SDS films containing variable amounts of SDS. At low SDS content (94/06 SPI/SDS), the morphological characteristics (not shown) remained practically unchanged as compared to pure SPI films. In contrast, a smoother surface [Figure 7(A)] and a continuous bulk phase [Figure 7(B)] were observed for films formed by 87/13 SPI/SDS. Above a certain [SDS] (20%), the bulk porosity of the films increased [Figure 7(C)], and micrographs taken at their surface revealed the presence of white regions as those seen in Figure 7(C). The melting point of the collected superficial white solid was characteristic of SDS.

SEM micrographs of SPI/SDS/PCL-T films are shown in Figure 8. At low PCL-T and SDS contents (85/06/09 SPI/SDS/PCL-T), the morphologies of the surface and cross-section were very similar to those observed in Figure 6(A) and (B) (pure SPI films). At [PCL-T] = 18%, the films had a rougher surface [Figure 8(A)] and a moderately porous bulk phase [Figure 8(B)]. The latter turned into a completely porous matrix for [PCL-T] > 18% as observed in Figure 8C and D. The increased bulk porosity of SPI/SDS/PCL-T films can be ascribed to the migration of the plasticizer from bulk phase to the film surface, as confirmed by the fact that one of the superficial phases corresponded to the plasticizer [Figure 8(A)]. The driving forces to this process are the relatively hydrophobic character of PCL-T and its large size, which apparently prevent it from becoming fully integrated into the network when very large quantities were used.[4]

Conclusion

According to the results, the thermal and mechanical properties and the morphology of SPI-SDS-PCL-T films prepared by solvent casting were greatly dependent on their composition. Stiff and flexible materials could be obtained from SPI, and the

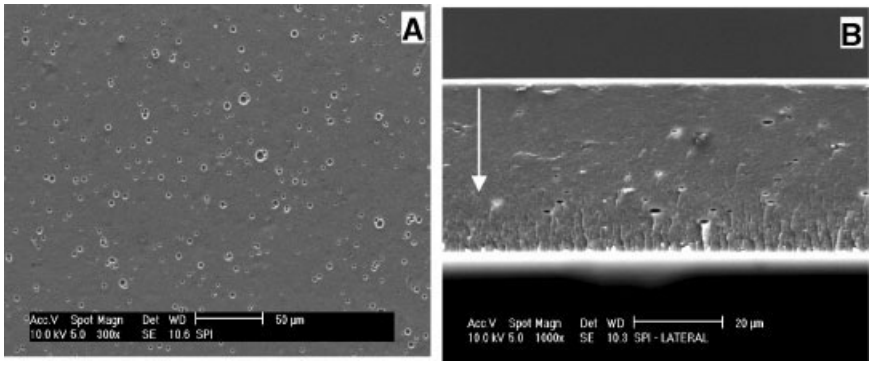

Figure 6.
SEM micrographs taken at the surface (A) and cross-section (B) of pure SPI biofilms.

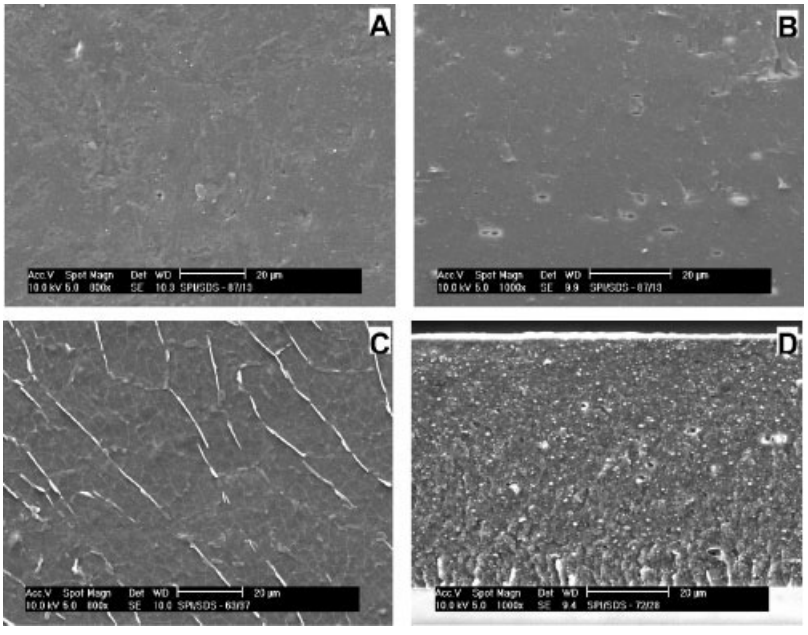

Figure 7.
SEM micrographs taken at the surface (left) and cross-section (right) of SPI/SDS films with different compositions: 87/13 (A,B); 72/28 (C,D).

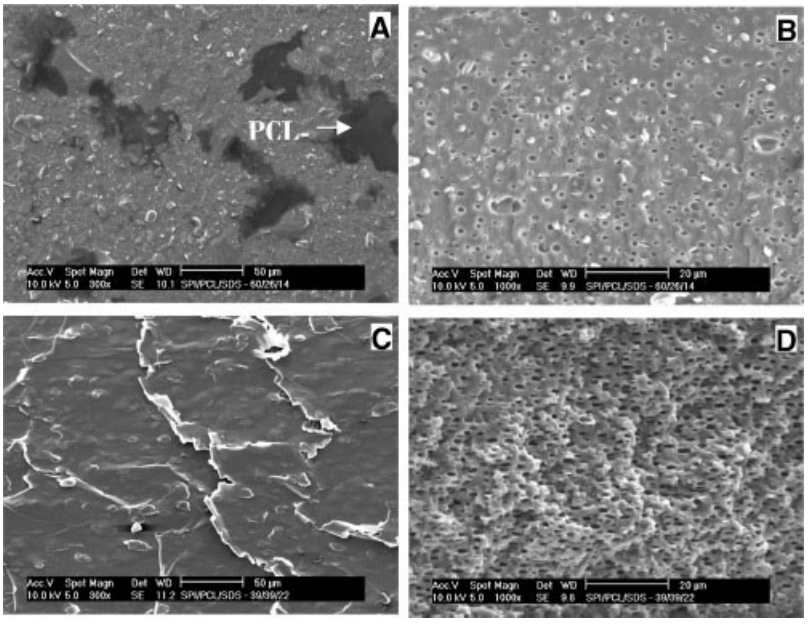

Figure 8.
SEM micrographs taken at the surface (left) and cross-section (right) of SPI/SDS/PCL-T films with different compositions: 60/14/26 (A,B); 39/22/39 (C,D).

differences between them were interpreted on basis of intra- and intermolecular interactions (electrostatic, dipole–dipole, and hydrophobic) between the film components, also taking into consideration the protein structure as accessed by fluorescence experiments.

The most important changes were observed for SPI/SDS/PCL-T films conditioned at 75% RH whose PCL-T content was at least 18%. In this case, a significant decrease in the Young's modulus from 1 424 MPa (pure SPI films) down to ca. 50 MPa was found, followed by an increase in E (from approx. 2 to 90%) and a parallel downwards shift in the T_g (from 150 down to 135 °C). Specimens exposed to 54% (or lower) relative humidity also put forward the plasticizer effect of PCL-T, but to a lesser extent (Young' modulus $\sim$ 650 MPa, $E \sim 5\%$, $T_g \sim 150$ °C). Micrographs taken at the cross-section of these films revealed the occurrence of a complete porous matrix. On the other side, SPI/SDS and SPI/SDS/PCL-T films with less than 18% PCL-T were in general more brittle, although some improvement was noticed. The extent of SDS and PCL-T effects on the selected properties depended on the MC in the film.

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