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Production and Physicochemical Characterization of Microspheres Made from Sericin and Alginate Blend

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Sericin is a protein extracted from silkworm´s cocoons (*Bombyx mori*) and sodium alginate is an anionic polysaccharide found in species of marine brown algae. Both are biomaterials usually studied because of their biodegradability and biocompatibility properties that enable the use of these materials for many applications like medical biomaterials and functional biomembranes production. Nowadays, researches with particles produced from sericin and alginate blend are focused mainly in developing new drug delivery systems. However, other applications can be purposed, for example, in the environmental area. The goal of this study is to evaluate the particles produced from the blend between sericin and alginate. The dripping technique, in aqueous and alcoholic solutions of $CaCl_2$, was applied to produce particles with 1 %, 2 % and 3 % w/v of alginate in sericin solution (2.5 % w/V). The formed particles from different blends were evaluated by humidity, percentage of solubilised matter (water solubility), SEM - Scanning Electron Microscopy, FTIR – Fourier transform infrared spectrometry and Zeta Potential. The results demonstrated that the cross-linking process by ionic gelation and by heating could reduce the water solubility of particles and promote changes in their chemical structure, as seen in SEM analyses and FTIR spectra.

1. Introduction

Recently, many studies are centering in development of material based in natural biopolymers, specially proteins and polysaccharides. These materials are able to produce a wide range of materials with properties concurring to molecular structural alterations. Furthermore, proteins and polysaccharides are easily available, cheap and biodegradable, which is the most important characteristic (Khandai et al., 2010).

Sericin is a group of glue proteins produced in the middle silk gland of the silkworm, *Bombyx mori*, that surrounds fibroin fibers and fixes them to each other in the cocoons (Takasu et al., 2002). During the silk manufacturing, sericin is removed from the cocoons by a degumming process and it is usually discarded in wastewater. This is a macromolecular hydrophilic protein that consists of 18 amino acids most of which have strong polar side chain made of hydroxyl, carboxyl and amino groups that enable easy cross-linking, copolymerization, and blending with other polymers to form improved biodegradable materials with silk (Dash et al., 2009) and sericin (Turbiani et al., 2011). Besides that, the amino acids serine and aspartic acid constitute approximately 33.4 % and 16.7 % of sericin, respectively (Zhang, 2002). The sericin structure occurs mainly in an amorphous random coil and to a lesser extent, in β-sheet organized structure. The randomly coiled structure easily changes to β-sheet structure, as a consequence of repeated moisture absorption and mechanical stretching (Pandawar and Pawar, 2004). Due its properties sericin is used in cosmetics and fabrics and shows promising application for protein adsorption, drug delivery and tissue engineering (Chen et al., 2011).

Alginate is a water-soluble linear polysaccharide extracted from brown seaweed and it is composed of regions of sequential β-D-mannuronic acid monomers (M-blocks), regions of α-L-guluronic acid (G-blocks),

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and regions of interspersed M and G units. Alginates undergo gelation in aqueous solution under mild conditions through interaction with divalent cations such as calcium that can cooperatively bind between the G-blocks of adjacent alginate chains creating ionic inter-chain bridges (Khandai et al., 2010). This polysaccharide is a biopolymer with many applications in drug delivery systems, cell encapsulation, food industry, cosmetics, etc. In wastewater treatment it could play an important role for heavy metal ions removal due its advantages, such as easy obtaining procedure, biodegradability and biocompatibility, making alginate an economical and environmental friendly material (Nita et al., 2007). Moreover, alginate as an anionic polymer with carboxyl end groups is a good mucoadhesive agent, that improve the drug delivery system because of the increasing of the residence time of drug dosage in small intestine, for example (Khandai et al., 2010).

In the present work, particles made from sericin and alginate, in different concentrations, were evaluated. It was investigated the humidity percentage, percentage of solubilised matter (water solubility), the structure by SEM - Scanning Electron Microscopy, FTIR – Fourier transform infrared spectrometry and Zeta Potential. The dripping gelation technique (in aqueous and alcoholic solutions of $CaCl_2$) was selected towards to prepare the microspheres due the simplicity and low costs involved. Alcoholic solutions are used due the fact that sericin and alginate are insoluble in it what can decrease the solubility in water after the alginate cross-linking process with $CaCl_2$.

2. Materials and Methods

The *Bombyx mori* silkworm´s cocoons were kindly provided by Bratac Silk Mills Company, located in the State of Paraná – Brazil.

2.1 Preparation of particles

The cocoons were manually cleaned and cut into small pieces (about 1 cm^2). Subsequently, they were carefully washed with tap water and three times rinsed with deionized water. The cocoons were dried overnight at 50 °C, weighed and immersed in ultrapure water, in the ratio 4.5:100 w/V, in order to be used in the degumming process. The aqueous sericin solution (SS) was extracted using an autoclave at 120 ^oC (1 kgf/cm^2) for 40 min. The processing time started to be measured after the system had reached the desired temperature and pressure. The SS was vacuum filtered to remove the fibres from the solution, stored in a sealed container, and posteriorly maintained at room temperature for a minimum of 12 h to stabilize the hydrogel. After this period, the SS was frozen in a conventional freezer for a minimum of 24 h and then it was thawed at room temperature. The precipitated sericin was vacuum filtered, heated in autoclave (120 ºC, 10 min) to solubilise the protein again, and then it was adjusted to 2.5 % w/V. The sodium alginate was added in concentration of 1 %, 2 % and 3 % w/V in the adjusted sericin solution. The particles were prepared by ionic gelation process, where each blend was dripped, with an infusion bomb, in aqueous and alcoholic (ethanol) solution of $CaCl_2$ (3% w/V) and then it was magnetic stirred for 24 h. The percentage of solubilised matter (water solubility) was evaluated for wet and dry particles. The other analyses were performed only in dry particles.

2.1 Percentage of humidity (ω) and Solubility in water (S_W and S_D)

After stirring period in $CaCl_2$ solutions, the particles were collected and washed with deionised water for 10 minutes in magnetic stirring. For calculation of ω e S_W the particles were placed on absorbent filter paper to remove the surface moisture and the initial mass was determined: m_{0_ω} and m_{0Sw}, to ω and S_W, respectively. The percentages of humidity were obtained by Eq(1), where $m_{F\omega}$ is the weight of dry particles after 24 h (105 ºC) in continuous flow oven.

$$\omega = (m_{0_\omega} - m_{F\omega}) / m_{0_\omega} \quad (1)$$

The percentage of solubilised matter of wet particles (S_W) were determined by Eq(2), adapting the procedure adopted by Turbiani et al. (2011). Samples of wet particles (m_{0Sw}) were immersed in 100 mL of ultrapure water using Erlenmeyer of 125 mL and then these recipes were maintained under agitation (200 rpm) at 25 ºC for 24 h. After this period, the particles were dried for 24 h at 105 ºC and the final weight was measured (m_{FSw}). The percentages of solubilized matter of dry particles (S_D) are similarly measured by Eq(3). The particles after washing process were dried for 24 h at 105 ºC, and an initial sample (m_{S0D}) was immersed in 100 mL of ultrapure water. After agitation period (200 rpm, 24 h, 25 ºC) the particles were dried again (24 h, 105 ºC) and the final weight was measured (m_{SFD}). All analyses were performed in triplicate.

$$S_W = [m_{0Sw}.(1 - \omega) - m_{FSw}] / [m_{0Sw}.(1 - \omega)] \quad (2)$$

$$S_D = (m_{S0D} - m_{SFD}) / m_{S0D} \quad (3)$$

2.2 FTIR – Fourier Transformed Infrared Spectroscopy

A Transformed Infrared Spectrophotometer (Nicolet 6700 FTIR, Thermo Scientific) was used in order to obtain the FTIR spectra and to characterize the chemical properties of the particles. The pellets of test samples and KBr were prepared by compression at 7 t for 7 min. All analyses were performed with an average of 32 repeated scans and 4 cm^{-1} scan resolution taken between 400 and 4,000 cm^{-1}.

2.3 SEM – Scanning Electron Microscopy

The surface and morphology of sericin-alginate particles were observed by a Scanning Electron Microscope with Energy Detector (LEO Electron Microscopy, LEO 440i, MOD. 6070, Cambridge - England). For particle´s metal coating it was used a Polaron Sputter Coater (VG Microtech, MOD. SC7620, Uckfield - England) which provides an estimated thickness of 92 Aº of gold. The particles were coated two times.

2.4 Zeta Potencial (pH_{ZPC})

The zeta potentials of sericin-alginate particles were determined using a Sur-PASS Electrokinetic analyzer (Anton Paar GmbH, Austria) equipped with a cylindrical cell. For each measurement, approximately 0.3 g of particles was transferred into the glass cylinder of the measuring cell. Measurements were carried out with a 0.001 mol/L KCl aqueous solution at 25 ± 1.0 °C and pH=6.3.

3. Results and Discussion

Table 1 shows the results for water solubility, percentage of humidity and zeta potential of the particles made from the blend between sericin (2.5% w/V) and alginate (1%, 2% and 3% w/V) dripped in aqueous and alcoholic $CaCl_2$ solutions (3% w/V).

Table 1 shows that water solubility of wet particles decreases when the blend was dripped in alcoholic solutions. Since sericin and alginate are less insoluble in alcohol (ethanol) than in water, it seems to contribute to the cross-linking process of sodium alginate in $CaCl_2$ solution. Similar results were obtained for the water solubility of dried particles, where the S_D decreases in same composition particles made by dripping process in alcohol solution. Only in particles E and F it was not observed a decrease of solubility (S_D) when the dripping process was proceed in alcoholic solution. According to Aranwit et al. (2012) alcohol changes the structure of proteins and for sericin induces β-sheet formation. Gimenes et al. (2007) explain that ethanol increases the aggregated strands (β-sheet), whereas both the random coil and α-helix components decrease in the secondary structure of the sericin. The increase of β-sheet structure is related to the decreasing of water solubility because the structure becomes more organized, and that justifies why these results were obtained when particles were dripped in alcoholic solutions.

Comparing the results of S_W and S_D for each particle it was observed that when the particle was dried at 105 ºC the solubility in water decrease. The thermal drying has improved the cross-linking process of sericin and alginate blend in particles. Excepting for F particles, for all others it was found a smaller S_D value in comparison with S_W. As related by Aranwit et al. (2012), heating favours crosslinking by disrupting the protein structure what exposes the sulphydryl and hydrophobic groups, resulting in formation of disulphide linkages. Gimenes et al. (2007), in a study about sericin/(Poly vinyl alcohol) blend membranes, found that higher temperature and longer period of time for thermal crosslinking generally improve the crosslinking degree, although the membranes become too brittle. In the membrane study, one of the targets was to produce flexible membrane.

It is important to notice that when the particles were produced with 3 % w/V of alginate and 2.5 % of sericin, particles E and F, the formed blend was very viscous which hindered the dripping process. A low drip flow (1.5 ~ 2 mL/min) was necessary in order to permit the droplets formation. In addition, it was observed that a large part of the particles cross-linked in alcoholic $CaCl_2$ solution (F particles) has broken during the stirring time.

The negative zeta potentials, shown in Table 1, indicate that the sericin/alginate particles had a net negative charge at pH=6,3. The varying zeta potential particles with the concentration of alginate indicated a varying degree of net negatively charged groups on the surface of sericin/alginate particles (Table 1). It was found that the alginate may cause an increase or a decrease in zeta potential of the blend when combined with proteins or other biopolymers (Xie et al., 2010). This net negative charge in particles' surface enables the use of these particles in the adsorption processes of positively charged compounds, such as adsorption of metals and some dyes.

Table 1: Humidity of wet particles (ω), Solubility in water of wet particles (S_W), Solubility in water of dried particles (S_D), and zeta potential (ζ - mV) of the particles of sericin (2.5% w/V) and alginate, in different proportions, prepared by ionic gelation process in $CaCl_2$ solutions (3% w/V)

Particle	Alginate (w/V)	$CaCl_2$ solution (3% w/V)	ω (%)	S_W (%)	S_D (%)	ζ-potential at pH=6.3 (mV)
A	1 %	Aqueous	94.44 ± 0.01	22.20 ± 6.36	18.13 ± 2.26	-0.963 ± 0.127
B	1 %	Alcoholic	93.83 ± 0.02	13.59 ± 2.05	10.59 ± 0.06	-0.494 ± 0.027
C	2 %	Aqueous	94.21 ± 0.03	21.85 ± 0.91	18.47 ± 1.08	-1.529 ± 0.171
D	2 %	Alcoholic	92.98 ± 0.03	11.51 ± 2.23	10.02 ± 0.56	-1.445 ± 0.126
E	3 %	Aqueous	93.10 ± 0.03	19.29 ± 0.54	16.50 ± 0.26	-1.059 ± 0.207
F	3 %	Alcoholic	92.44 ± 0.12	10.58 ± 2.21	16.45 ± 0.61	-1.550 ± 0.223

Figure 1 shows SEM photographs of the surface of sericin-alginate particles dried at 105 ºC.

It can be seen in Fig. 1 that the particles present homogeneous composition. When the sericin solution was thawed, it occurred a sericin precipitation that made difficult the solubilisation of sericin. The SEM photographs show that the experimental procedure adopted in this study was able to produce a homogeneous blend composition. Also, it is possible to verify that the drying process of the particles cross-linked in aqueous $CaCl_2$ solutions (A, C and E) formed particles with a greater roughness surface than the ones cross-linked in alcoholic solutions (B, D and F).

The FTIR spectra of the particles are shown at Figure 2.

At Figure 2, it was possible to observe the containing of amide absorption bands of protein in the FTIR spectra. Characteristic amide absorption bands of protein are 1,710-1,590 cm^{-1} for amide I; 1,570-1,480 cm^{-1} for amide II and 1,270-1,200 cm^{-1} for amide III (Teramoto et al., 2008). The differences in spectra observed specially in interval of amide II and amide III shows that the blend, with different compositions of alginate, promoted changes in the chemical structure of particles. According to Teramoto and Miyazawa (2005) amide I absorption primarily represents the C=O stretching vibration of the amide group. Also, amide II absorption contains contributions from N-H bending and C-N stretching vibrations and amide III arises mainly from the C-N stretching vibration coupled to the N-H in-plane bending vibration. The spectra show the presence of β-sheet in the produced particles. The β-sheet structure presents characteristic peaks at 1,698-1,623 cm^{-1} (Gil et al., 2011) and this kind of organized structure is related to the decreasing of water solubility, as previously discussed.

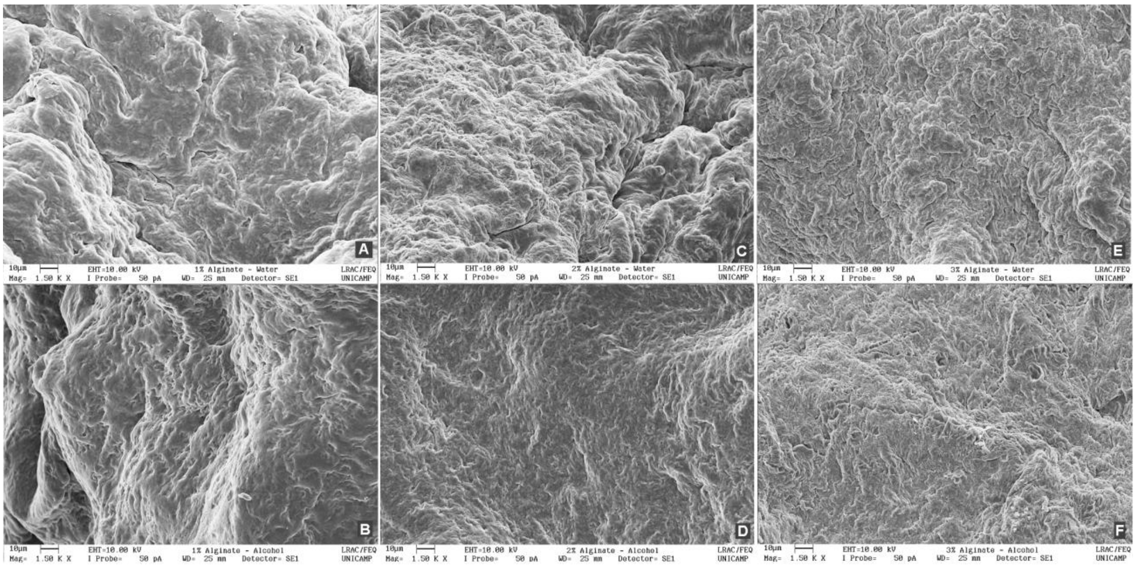

Figure 1: SEM photographs of sericin (2.5% w/V) and alginate (1% - A,B; 2% - C,D; 3% w/V- E,F) particles dripped in aqueous (A, C, E) and alcoholic (B, D, F) $CaCl_2$ solutions. (Mag. 1500X, 10 kV)

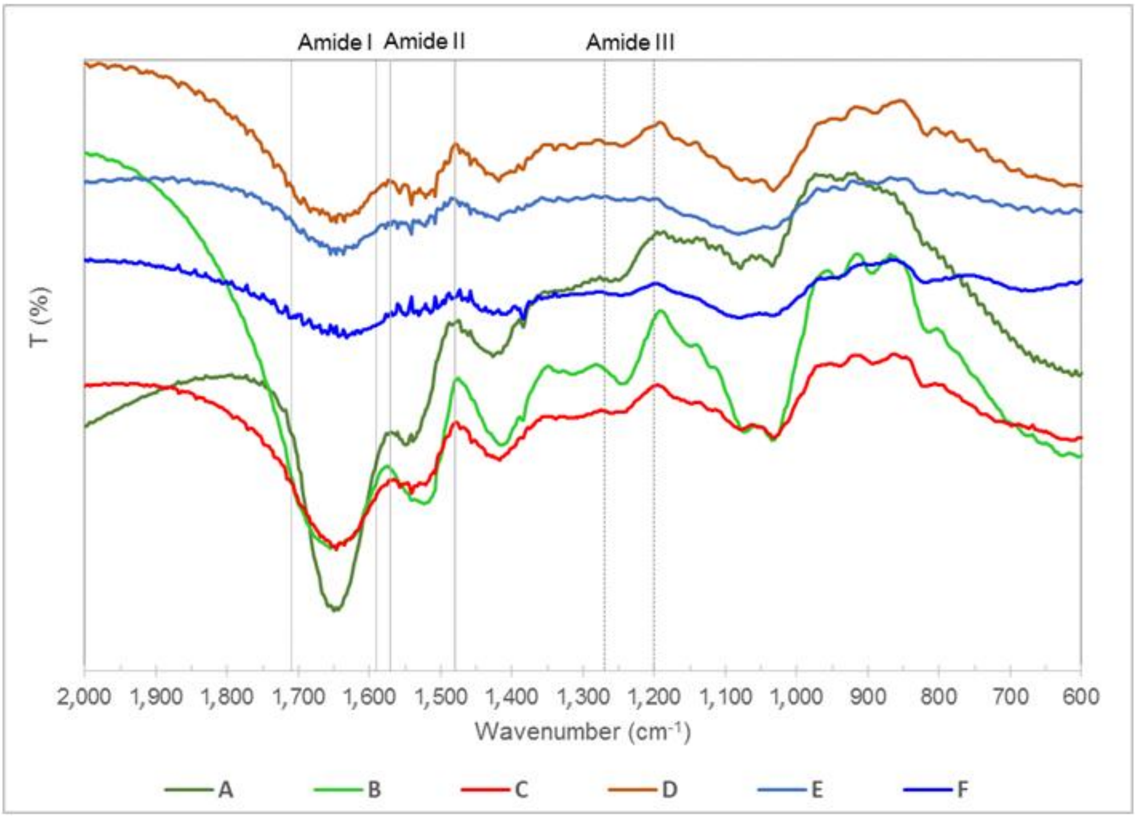

Figure 2: FTIR spectra of sericin (2.5 % w/V) and alginate (1% - A,B; 2 % - C,D; 3 % w/V- E,F) particles dripped in aqueous (A, C, E) and alcoholic (B, D, F) CaCl2 solutions

In the particles C, D, E and F the amount of alginate becomes larger than in the A and B ones, which makes the cross-linking process by calcium solution more representative. This may be related to the different FTIR spectra observed for these particles, besides possible reactions that occur during the formation of the blend and crosslinking process heat.

4. Conclusions

The particles produced in this study show lower water solubility when the blend was dripped in alcoholic (ethanol) $CaCl_2$ solution (ionic gelation process). The cross-linking process by heat also decreased the water solubility of particles. For environmental uses, like biosorption processes, this is a fundamental factor to be accomplished. The zeta potential had higher values in particles produced with 3% of alginate, but, the higher viscosity hindered the dripping process. The SEM photographs showed a greater roughness surface in particles cross-linked in water solutions, and that the blend had a homogeneous composition. The increase of alginate in composition and the cross-linking process (by ionic gelation and heating processes) promoted changes in the secondary structure of the blend, what could seem in FTIR spectra of the particles.

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