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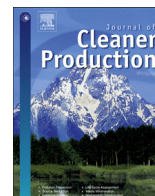
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Biosorption study of copper and zinc by particles produced from silk sericin – alginate blend: evaluation of blend proportion and thermal cross-linking process in particles production

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ABSTRACT

Sericin is a protein present in the cocoon of silkworm silk (*Bombyx mori*), usually discarded in the effluent from the spinning process, and alginate is a linear natural polysaccharide, extracted mainly from brown algae. Both biomaterials have been attracting attention due to their properties. Sericin isolated present weak structural properties, but its strongly polar side groups enable easy cross-linking, polymerization and blending. The use of blends provides an improvement in the physical characteristics of the materials produced with the protein, and the use of alginate has an advantage because it has affinity for a variety of cations. The aim of this paper is to evaluate the particles produced from the blend between sericin and alginate and its properties. The gelation technique, dripping blends 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). Posteriorly, the thermal cross-linking process at 40, 100, 125 and 150 °C was performed in sericin-alginate particles and the biosorption processes of these particles for zinc and copper metals were investigated. The evaluation of the data was carried out through the measurement of water solubility, Scanning Electron Microscopy (SEM) images, Fourier Transform Infrared Spectrometry (FTIR) spectra and adsorption capacity. Water solubility of particles decreased when they were produced in alcoholic solution, which is probably related to the formation of β -sheet structure confirmed by FTIR analyses. Also, it was observed that adsorption capacity increased when the particles were produced in alcohol solution. The thermal cross-linking process increased the adsorption capacity until 100 °C, with values around 75% and 65% of reduction of Cu^{2+} and Zn^{2+} , respectively. From temperature 100 °C all particles got higher degree of browning with an increase of solubility and decrease of adsorption capacity by particles because of the degradation of protein/polysaccharide.

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1. Introduction

The toxicity and persistence of heavy metals to environmental and humans are already well known. This kind of contamination is toxic to aquatic flora and fauna even in relatively low concentration.

Furthermore, some metals can be assimilated, stored and concentrated by organisms (Mohan et al., 2006) leading severe impacts in the environment and human health (Kwak et al., 2013). Some health problems related to toxic metals include accumulative poisoning, cancer, damages in brain (Nitā et al., 2007), kidneys and bones, among others, depending on the level of contamination (Mohan et al., 2006). Copper is an essential element but acute doses cause metabolic disorders. Inhalation of copper produces symptoms similar to those of silicosis and allergic contact dermatitis, besides chronic copper poisoning cause hemolytic anemia, neurological abnormalities and corneal opacity (Mohan et al., 2006).

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Industries, including mining, electroplating, fertilizer manufacturing and petroleum refining, have in their effluents high levels of heavy metals that can contaminate the environment (Mohan et al., 2006). For diluted metal concentrations, ion exchange, reverse osmosis and adsorption can be applied to decontamination (Nit̃a et al., 2007). The adsorption has the advantage of being most inexpensive among the other two methods, plus the ability to use the biosorbents from agriculture (Vieira et al., 2014), biomass (Lima et al., 2014) and industrial waste (Almeida Neto et al., 2014). The use of biosorbents in adsorption processes have been considered attractive because of the low-cost materials for treatment of wide range of pollutants, including dyes, pesticides and metal ions (Chen et al., 2011). The sericin and alginate are focus of many researches due to their properties and interaction with metals and dyes (Gimenes et al., 2014).

Bombyx mori silk is a natural protein fiber composed of parallel monofilaments of sericin and fibroin (Wu et al., 2014). Sericin is a globular water soluble protein that constitutes 25–30% of silk protein of silkworm cocoon (*B. mori*) (Zhang, 2002) and it acts as a gum binder to maintain the structural integrity of the cocoon (Mahmoodi et al., 2010). The sericin structure occurs mainly in an amorphous random coil and to a lesser extent, in β -sheet organized structure and the randomly coiled structure easily changes to β -sheet structure, as a consequence of repeated moisture absorption and mechanical stretching. The solubility of sericin in water decreases when the sericin molecules are transformed from random coil into β -Sheet structure (Padamwar and Pawar, 2004). Molecular weight of sericin ranges from 24 to values up to 400 kDa (Silva et al., 2014a, 2014b), with predominant amino acid group's serine and glycine, among others (Dash et al., 2009). These proteins consist of 18 kinds of amino acids, most of which have strong polar side chains such as carboxyl, hydroxyl and amino groups (Zhang, 2002) that allow easy cross-linking, copolymerization and blending with other polymers to form improved biodegradable environmental-friendly materials (Dash et al., 2009). Besides, many researches have now attributed to sericin several important properties such as excellent moisture absorption and release properties, UV resistance, anticoagulant properties, antioxidant activities among other properties (Gupta et al., 2013).

Most of the sericin is removed during the silk processing and it is usually discarded in the wastewater. Annually, it is estimated that about 50,000 tons of sericin is produced and not recovered from the effluents of degumming process (Zhang, 2002). The sericin, that surround fibroin fiber with sticky layers (Takasu et al., 2002), can be removed by boiling, use of alkali, soap, detergents, organic solvents, enzymes – which is an expensive method for degumming cocoons, and use high pressure and high temperature to solubilize sericin (Gupta et al., 2013; Zhang, 2002).

Alginic acid and its derivatives as biopolymers have two main sources: bacteria and mainly brown algae. Alginates with algae's sources show different structural and chemical properties with respect to their seasonal and growth conditions. These polymers can be described as linear binary copolymers of 1–4-linked M and G residues arranged with homopolymeric regions of α -L-guluronic acid residues (G-blocks) and a homopolymeric region of β -D-mannuronic acid sequences (M-blocks) interspersed by regions in which the two groups coexist in a strictly alternating sequence (MG-blocks) (Daemi and Barikani, 2012). Alginate polysaccharide has advantages, such as facile obtaining procedure, biodegradability, biocompatibility, viscosifying (Daemi and Barikani, 2012), economical and environmental friendly characteristics (Nit̃a et al., 2007). In addition, sodium alginate has a property of ionic cross-linking (gelation) in the presence of multivalent cations (Daemi and Barikani, 2012). The most common divalent ion used to cross-link sodium alginate is the divalent calcium ion that is able to

complex with G–G sequences, binding G-blocks of adjacent polymers chains to form ionic inter-chain bridges (Khandai et al., 2010), also known as 'egg box junctions' (Finotelli et al., 2008).

Still about the cross-linking processes, polymers like silk sericin and alginate can undergo a sol–gel transition (Gasperini et al., 2014) by thermal cross-linking (by heating) (Mohammed et al., 2000), photo-cross-linking and with pH variation, depending on β -sheet content in case of sericin (Gasperini et al., 2014).

The affinity of sericin and alginate for metals have been studied in recent years. Kwak et al. (2013) utilized sericin beads fabricated using a 1 M LiCl/DMSO solvent and used as adsorbent to Chromium(VI) and the maximum adsorption capacity found was 33.76 mg g^{−1} at pH 2. Chen et al. (2011) using powder of sericin and chitosan observed that both biosorbents displayed good capacity for gold adsorption of 1.0 and 3.3 mmol g^{−1} of gold, respectively. Nit̃a et al. (2007) utilized calcium alginate micro particles to evaluate adsorption of Pb⁺² and Cd⁺² ions and concluded that this kind of particle can be a suitable alternative to remove heavy metal from industrial contaminated wastewater.

Thus, the objective of this study is to evaluate the production of particle from sericin and alginate blend with ionic and thermal cross-linking. Different proportions in sericin-alginate blend formulation were investigated and all one were dripped in alcoholic (ethanol) and aqueous solutions of CaCl₂. Posteriorly, particles were dried at 40, 100, 125 and 150 °C. The particles were analyzed for the water solubility and its ability to adsorb metals (Zn⁺² and Cu⁺²) in aqueous solution, by metallic affinity tests.

A cleaner procedure to produce particles was proposed resulting in a darkening degree in the order of the increase of the thermal-crosslinking temperature. An increase in the efficiency of the metal affinity to zinc and copper in synthetic effluent, and lower water solubility of the particles was observed in particles thermal cross-linked in 40 and 100 °C.

2. Materials and methods

2.1. Materials

The *B. mori* silkworm's cocoons were provided by Bratac Silk Mills Company, located in the State of Paraná, the main State that produces silk in Brazil. Sodium Alginate was obtained from Sigma–Aldrich (United Kingdom). The ultrapure water used in experiments was delivery by Milli-Q reference water purification system (Millipore Merck). The metallic salts were analytical grade reagents (VETEC – Sigma–Aldrich, Brazil). All other reagents were also analytical grade.

2.2. Preparation of silkworm cocoons

The cocoons were manually cleaned to remove the impurities and dirty parts, and cut into small pieces (about 1 cm²). Subsequently, it was abundantly washed with tap water and rinsed for three times with deionized water. After washing, the cocoons were dried at 50 °C in a laboratory continuous flow oven to reach constant weight, in its temperature, and then it was ready for use in degumming process.

2.3. Degumming process and cryo-concentration of sericin

The removal of the protein from cocoons, degumming process, was carried in a ratio of 4.0 g of cocoons to 100 mL of ultrapure water (4:100 w/V). Because the extraction of sericin provides a very dilute solution of protein it is necessary an increase of the amount of sericin in solution which can be obtained by freezing/thawing procedure. This is a clean process that promotes the protein

precipitation what enables the separation by filtration, without chemical addition. Due to the formation of agglomerates, it is necessary that the solution be again heated in order to obtain a homogeneous sericin solution. In the development of this work, the procedure described by Silva et al. (2014a; 2014b) and Turbiani et al. (2011) was adopted.

The aqueous sericin solution (SS) was extracted using an autoclave at 120 °C (1 kgf/cm²) for 40 min. The processing period started to be measured after the system had reached the desired temperature and pressure. The solution, while hot, was filtered to remove the fibroin fibers, and then SS was stored in a sealed container at room temperature for 12 h to stabilize the hydrogel. Posteriorly, the aqueous liquor was frozen in a conventional freezer (−4 °C) for a minimum of 24 h and then it was thawed at room temperature. The precipitated sericin was filtered, heated in autoclave (120 °C, 10 min) to solubilize the protein again, and the concentrated protein solution was adjusted to 2.5% w/V, by dilution.

2.4. Sericin-alginate particles preparation

The sodium alginate was added in proportions 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 a peristaltic pump, in aqueous and alcoholic (ethanol) solution of CaCl₂ (3% w/V) and it was magnetic stirred for 24 h in its respective solution. After crosslinking process the sodium ions were ionic changed for calcium and the particles of sericin/calcium alginate were formed. The percentage of solubilized matter (water solubility – item 2.4.1) and other analyses were evaluated for wet and dried particles.

The particles with composition: 2.5% w/V of sericin and 2.0% w/V of alginate, produced in aqueous and alcoholic (ethanol) CaCl₂ solutions, were subjected to the tests of thermal crosslinking. First, the particles were dried at room temperature and, posteriorly, in order to perform the crosslinking process, the particles were heated (in a continuous flow oven) at 40, 100, 125 and 150 °C for 24 h. After this period, the wet and dried particles were analysed by water solubility and their affinity to Cu²⁺ and Zn²⁺ through adsorption tests.

2.5. Metal affinity test – single component

Metal speciation diagrams as a function of pH were simulated using the Hydra and Medusa software (Puigdomenech, 2004) to identify the different species in aqueous metals solutions. The diagrams were used to choose the pH interval in which chemical precipitation does not occur, that is, the pH range in which only the adsorption process is responsible for the decrease of metals in solution.

The metal solutions of 1 mmol L^{−1} of Cu²⁺ and Zn²⁺ were prepared by dissolving the nitrate salts Cu(NO₃)₂·3H₂O and Zn(NO₃)₂·6H₂O in ultrapure water (Mili-Q Millipore System). Samples of 0.5 g of each particle were immersed in 50 mL of metal solution (zinc and copper solutions) using Erlenmeyer flasks of 125 mL and then these recipes were maintained under agitation (200 rpm) at 25 °C for 24 h (Incubated Shaker, SI 600R, Lab Companion Jeio Tech, Korea). The pHs in these solutions were adjusted to desired values with nitric acid (HNO₃). During the metal affinity experiments the pH was monitored to avoid the increase in its values, what could promote the metallic precipitation affecting this way the adsorption evaluation. The affinities tests were performed with wet and dried particles thermally cross-linked at 40, 100, 125 and 150 °C. The sample of 0.5 g of wet particles was weighed discounting the water content (humidity percentage) of particle.

After 24 h of agitation, a sample of liquid fase was centrifuged for 10 min at 4000 rpm (Centurion Scientific, C2 Series). All metal concentrations were measured at atomic absorption spectroscopy (AAS) – Shimadzu Atomic Absorption Spectrophotometer GFA 7000A. The adsorption capacity of the particles (q_e), in the evaluated conditions, and the percentage removal (%R) were determined by Equations (1) and (2), respectively.

$$q_e = \frac{(C_0 - C_e) \cdot V}{m_p} \quad (1)$$

where, C_0 is the initial concentration, C_e is the equilibrium concentration (after 24 h), V is the volume in each Erlenmeyer flask (50 mL), and m_p is the dry mass of alginate-sericin particles (0.5 g).

$$\%R = \frac{(C_0 - C_e)}{C_0} \cdot 100 \quad (2)$$

2.6. Analyses

2.6.1. Percentage of humidity (ω) and solubility in water (S_w and S_D)

After stirring period of particles dripped in CaCl₂ aqueous and alcoholic solutions, the particles were collected and washed with deionized water for 10 min in magnetic stirring and rinsed, abundantly, with deionized water. For the calculus of ω , samples of wet particles (about 4 g of wet particles) were placed on absorbent filter paper to remove the surface moisture and the initial mass was determined ($m_{0\omega}$). The percentages of humidity (ω) were obtained by Eq. (3), where $m_{F\omega}$ is the mass of dry particles after 24 h (105 °C) in continuous flow oven.

$$\omega = (m_{0\omega} - m_{F\omega}) / m_{0\omega} \quad (3)$$

The percentage of solubilized matter of wet particles (S_w) were determined by Eq. (4), adapting the procedure adopted by Turbiani et al. (2011). Similarly the determination of percentage of humidity, samples of wet particles (3 ~ 4 g of wet particles) were placed on absorbent filter paper to remove the superficial moisture and then its samples were weighed and the initial mass was determined (m_{0S_w}). Posteriorly, the samples of wet particles were immersed in 100 mL of ultrapure water (Milli-Q), on Erlenmeyer of 125 mL, and the flask was agitated (200 rpm) at 25 °C for 24 h (Incubated Shaker – SI 600R, Lab Companion – Jeio Tech, Korea). After this period, the particles were filtered and dried for 24 h at 105 °C, in continuous flow oven. The dried samples were cooled to room temperature on silica gel desiccator and its final mass determined (m_{FS_w}).

$$S_w = [m_{0S_w} \cdot (1 - \omega) - m_{FS_w}] / [m_{0S_w} \cdot (1 - \omega)] \quad (4)$$

The percentages of solubilized matter of dried particles (S_D) were measured by Eq. (5). The particles produced in aqueous and alcoholic solutions were washed and rinsed with deionized water and posteriorly dried at 40 °C. Posteriorly, depending on the desired thermal crosslinking temperature, the particles were subjected for 24 h period at temperatures of 40, 100, 125 and 150 °C, in a continuous flow oven.

The particles were dried first at 40 °C temperature because when beads were directly dried at higher temperatures (100, 125, and 150 °C), they become more brittle due to the fact that the water evaporation occurs so quick that it promotes some cracks on the particle during the drying process. After this period, the particles were cooled at room temperature (on silica gel desiccator) and an initial sample was weighted (m_{SOD}) and immersed in 100 mL in ultrapure water (in Erlenmeyers of 125 mL). After agitation period

(200 rpm, 24 h, 25 °C), the particles were filtered and dried again (24 h, 105 °C) and the final mass was measured (m_{SFD}). All analyses were performed in triplicate.

$$S_D = (m_{\text{SOD}} - m_{\text{SFD}}) / m_{\text{SOD}} \quad (5)$$

2.6.2. Fourier transformed infrared – FTIR – spectroscopy

The FTIR spectra of particles, produced in aqueous and alcoholic solutions, were collected using a Transformed Infrared Spectrophotometer (Nicolet 6700 FTIR, Thermo Scientific – Madison/USA) in order to obtain information about the functional groups and chemical properties of the particles. KBr pellet method was used in this analyze. The KBr pellets were prepared by compression at 7 ton for 7 min. All analyses were performed with an average of 32 repeated scans and 4 cm^{-1} scan resolution recorded in the region of 400 and 4000 cm^{-1} .

2.6.3. SEM – Scanning Electron Microscopy

The surface morphology of sericin-alginate particles were observed by a Scanning Electron Microscope with Energy Detector (LEO Electron Microscopy, LEO 440i, MOD. 6070, Cambridge–England). The particle's surfaces were gold sputter coated before the scanning. It was used a Polaron Sputter Coater (VG Microtech, MOD. SC7620, Uckfield – England) which provides an estimated thickness of 92 Å of gold on the surfaces of specimen. The particles were coated twice. The beam current and the accelerating voltage for SE imaging was 50 pA and 10 kV, respectively. The images were captured with a magnification of 1500 \times .

3. Results and discussion

Silkworm cocoons were degummed in autoclave and cryo-concentrated by freezing the solution in conventional refrigerator and thawing the solution at room temperature. The concentrated sericin solution, obtained by filtration of thawed solution, was adjusted by dilution and the blend with different proportions of sodium alginate (1%, 2% and 3% w/V) and sericin (2.5% w/V) was produced and dripped in alcoholic and aqueous CaCl_2 solutions (3% w/V) in order to produce particles that were dried at room temperature and posteriorly cross-linked at 100 °C for 24 h. In this step of formulations evaluation, the temperature of drying process was fixed at 100 °C because in this temperature all water content of particles evaporate and the investigation can be done in dry base.

3.1. Effect of the composition of particles and the CaCl_2 solution for ionic crosslinking

The percentage of humidity of wet particles and the water solubility of wet and dried particles were compared on Table 1, where,

Table 1

Humidity of wet particles (ω), Solubility in water of wet particles (S_W), Solubility in water of dried particles (S_D) 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).

Composition	Alginate (w/V)	CaCl_2 solution (3% w/V)	ω (%)	S_W (%)	S_D (%)
A	1%	Aqueous	94.44 \pm 0.01	22.20 \pm 6.36	18.13 \pm 2.26
B	1%	Alcoholic	93.83 \pm 0.02	13.59 \pm 2.05	10.59 \pm 0.06
C	2%	Aqueous	94.21 \pm 0.03	21.85 \pm 0.91	18.47 \pm 1.08
D	2%	Alcoholic	92.98 \pm 0.03	11.51 \pm 2.23	10.02 \pm 0.56
E	3%	Aqueous	93.10 \pm 0.03	19.29 \pm 0.54	16.50 \pm 0.26
F	3%	Alcoholic	92.44 \pm 0.12	10.58 \pm 2.21	16.45 \pm 0.61

the different formulations produced and the results obtained in the tests are presented.

In Table 1, the presented comparative study on the effect of alginate proportion in formation of particles and the effect of crosslinking process in alcoholic and aqueous solution indicate that these parameters affect the properties of the produced particles. Close values to water solubility in wet particles dripped in alcoholic solution were obtained (19.29 ~ 22.20%), and the same behaviour was obtained in particles dripped in aqueous solution of CaCl_2 , whose values range between 10.58 and 13.59%. In general, the water solubility of particles decreases when the blend was dripped in alcoholic solutions. Since sericin and alginate are less soluble in alcohol (ethanol) than in water, it seems to contribute to the cross-linking process of sodium alginate in CaCl_2 solution due to the fact the alcohol maintain the structure of the hydrogel beads during stirring period decreasing this way the solubility of the protein and polysaccharide. Also, according to Aramwit 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 100 °C the water solubility decrease. The thermal drying has improved the cross-linking process of sericin and alginate blend in particles. As related by Aramwit et al. (2012), heating favours crosslinking by disrupting the protein structure what exposes the sulfhydryl 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 this sericin membrane study, one of the targets was to produce flexible membrane.

Excepting for F particles, for all others it was found a smaller S_D value in comparison with S_W . 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 allow the droplets formation. In addition, it was observed that a large part of the particles cross-linked in alcoholic CaCl_2 solution (F particles) were broken during the stirring time. It was most likely due to the fact that the blend of F was too viscous, which impaired the crosslinking process of the alginate by calcium.

3.2. Microscopic studies

SEM micrographs of dried sericin-alginate particles (formulations: A, B, C, D, E and F) are shown in Fig. 1.

It can be seen in Fig. 1 that the particles present homogeneous composition. When the frosted sericin solution was thawed, the sericin precipitation that occurs in this process impaired 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).

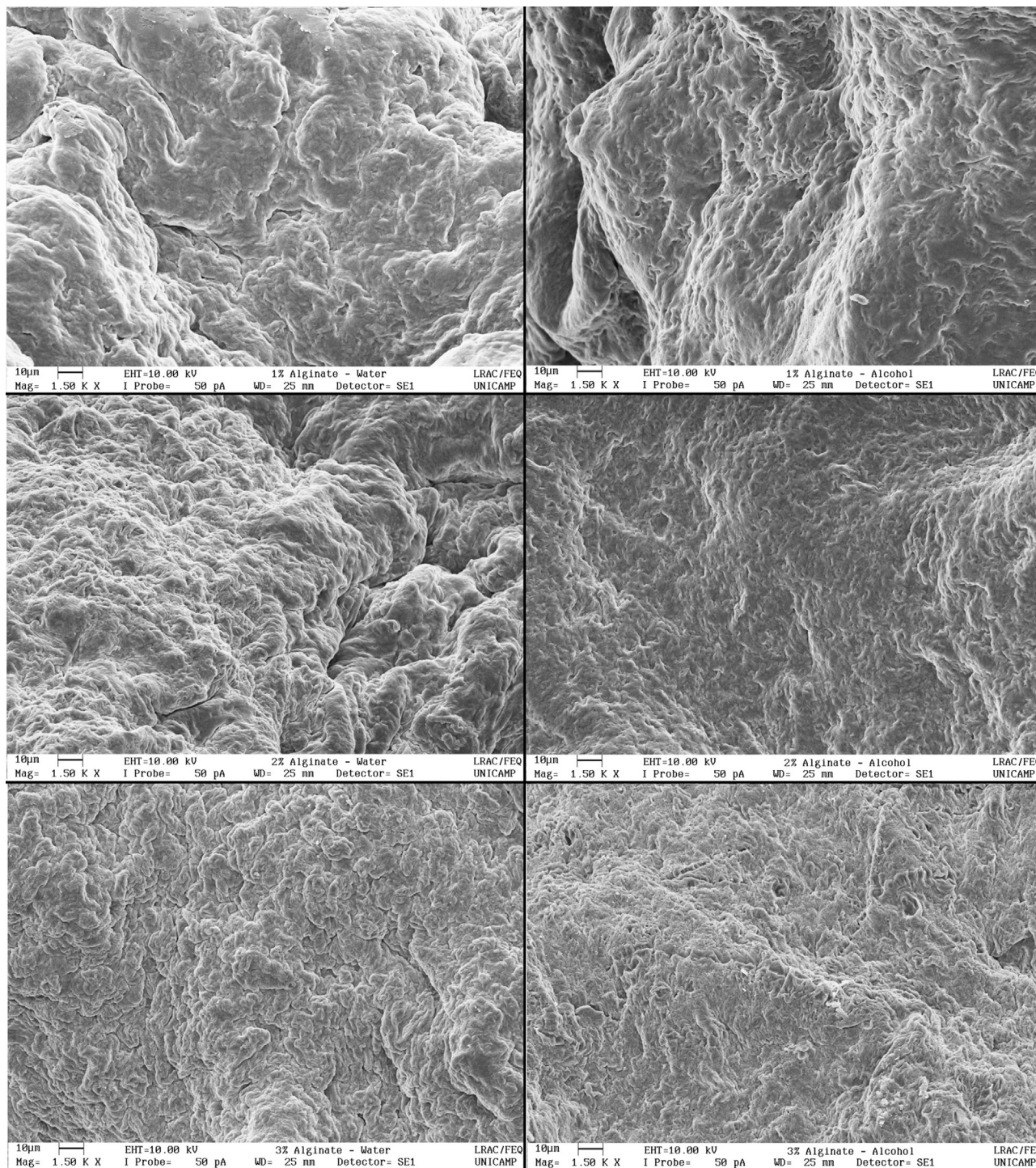


Fig. 1. SEM surface images of sericin (2.5% w/v) and alginate (1% – A,B; 2% – C,D; 3% w/v – E,F) particles dripped in aqueous (A, C, E) and alcoholic (B, D, F) CaCl_2 solutions (Mag. 1500X, 10 kV).

3.3. FTIR analysis

From FTIR spectra at Fig. 2, it was possible to observe that the containing of amide absorption bands of protein in the FTIR spectra. Characteristic amide absorption bands of protein are $1710\text{--}1590\text{ cm}^{-1}$ for amide I; $1570\text{--}1480\text{ cm}^{-1}$ for amide II and $1270\text{--}1200\text{ cm}^{-1}$ 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 $\text{C}=\text{O}$ 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

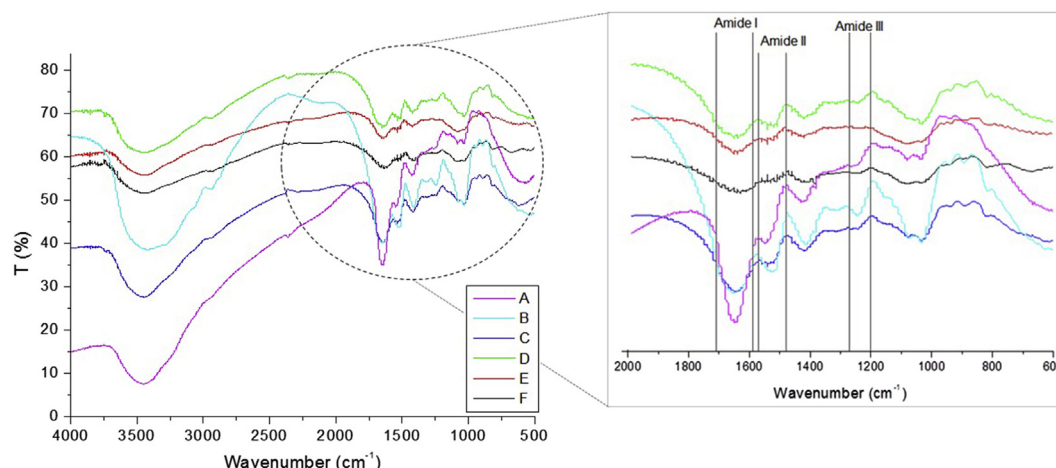


Fig. 2. FTIR spectra of sericin (2.5% w/V) and alginate (1% – A,B; 2% – C,D; 3% w/V – E,F) particles dripped in aqueous (A, C, E) and alcoholic (B, D, F) CaCl_2 solutions.

the produced particles. The β -sheet structure presents characteristic peaks at $1698\text{--}1623\text{ cm}^{-1}$ (Gil et al., 2011) and this kind of organized structure is related to the decreasing of water solubility, as previously discussed.

In the particles C, D, E and F the amount of alginate becomes larger than in the A and B ones. This may be related to the different FTIR spectra observed for these particles, besides possible reactions that occur during the formation of the different proportions of the blends and crosslinking process heat.

3.4. Thermal cross-linking evaluation

The particles with composition of 2.5% w/V sericin and 2% alginate were produced by ionic cross-linking in aqueous and alcoholic CaCl_2 solutions. After being dried at 40°C , the particles were subject to thermal cross-linking process at temperatures of 40, 100, 125 and 150°C for 24 h in a continuous flow oven. New rounds of experiments were done in this step of study with a new batch of silkworm cocoons. Table 2 shows the results of percentage of solubilized matter (water solubility – S_D) of particles after the thermal cross-linking processes.

Lower S_D values were obtained when the particles were cross-linked at 100°C ; 7.3% and 5.0% to particles produced in aqueous and alcoholic CaCl_2 solutions, respectively. The thermal process of crosslinking decreases the percentage of solubility in particles dried at 100°C , but higher temperatures increase the solubility of particles. As related by Aramwit et al. (2012), heating favors cross-linking by disrupting the protein structure exposing the sulfhydryl and hydrophobic groups what result in the formation of disulphide linkages. The cross-linked particles at 125 and 150°C had a darker color than the ones cross-linked at 40 and 100°C . The increase of temperature of thermal cross-linked was followed by an increase of browning degree of the particles.

Table 2
Percentage of solubilized matter (S_D) of thermal cross-linked particles at 40, 100, 125 and 150°C .

Temperature of crosslinking process	S_D (%) of particles produced in aqueous CaCl_2 solution	S_D (%) of particles produced in alcoholic CaCl_2 solution
40°C	$12.5 (\pm 0.2)$	$10.8 (\pm 0.1)$
100°C	$7.3 (\pm 0.1)$	$5.0 (\pm 0.1)$
125°C	$10.2 (\pm 0.2)$	$7.3 (\pm 0.1)$
150°C	$30.7 (\pm 0.4)$	$24.8 (\pm 0.2)$

The changes observed in color and structure of particles dried at 125 and 150°C , probably are related with the Maillard reactions that occur with proteins and polysaccharides in heating processes. These reactions were responsible for greatest browning in protein and polysaccharides exposed to heating (Mohammed et al., 2000). The Maillard reaction reduces sugar, condensing with compounds possessing a free amino group (of an amino acid or in proteins mainly in ϵ -amino group and α -amino groups of terminal amino acids), and, in advanced stages a range of reactions takes place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations, which ultimately, in a final stage, leads to the formation of brown nitrogenous polymers and co-polymers (Martins et al., 2001). Maillard reactions probably are related to changes in particle exposed to heat for a long period of time and high temperature (24 h , 150°C) causing an increase in solubility observed due to the degradation of the protein and polysaccharide.

According to Mohammed et al. (2000), in general, there is a decrease in solubility with increasing temperature of heating, but most protein also showed an increase in solubility on extensive heating, what could be seen in this work. Also, it is important to notice that the metal solutions in which affinity tests of dried particles were executed, at 125 and 150°C , had a brown color after the period of 24 h, due to the solubilized matter of particles.

3.5. Affinity metallic tests to Cu^{2+} and Zn^{2+}

The speciation diagrams as function of pH, to zinc and copper, were performed by HYDRA software. In Fig. 3 these diagrams are presented.

From Fig. 3, it can be seen that the Cu^{2+} and Zn^{2+} fractions occurs in pH less than 5. Therefore, in order to evaluate the adsorption of these metals in affinity tests, the pH was adjusted and controlled during the experiments with HNO_3 solution to values of approximately 4.0–4.5. In this range of pH safely only the ionic species of copper and zinc (Cu^{2+} and Zn^{2+}) are present. This procedure was adopted to avoid the chemical precipitation that can occur in pH higher than, approximately, 5 to copper and to avoid the formation of ZnOH^+ specie to zinc.

Figs. 4 and 5 show the adsorption capacity and reduction of the concentration of Cu^{2+} and Zn^{2+} by particles produced from sericin and alginate in aqueous and alcoholic CaCl_2 solutions, respectively. Particles samples of 0.5g were immersed in 50 mL of solutions of zinc and copper and after 24 h period the bioadsorption of these particles were evaluated.

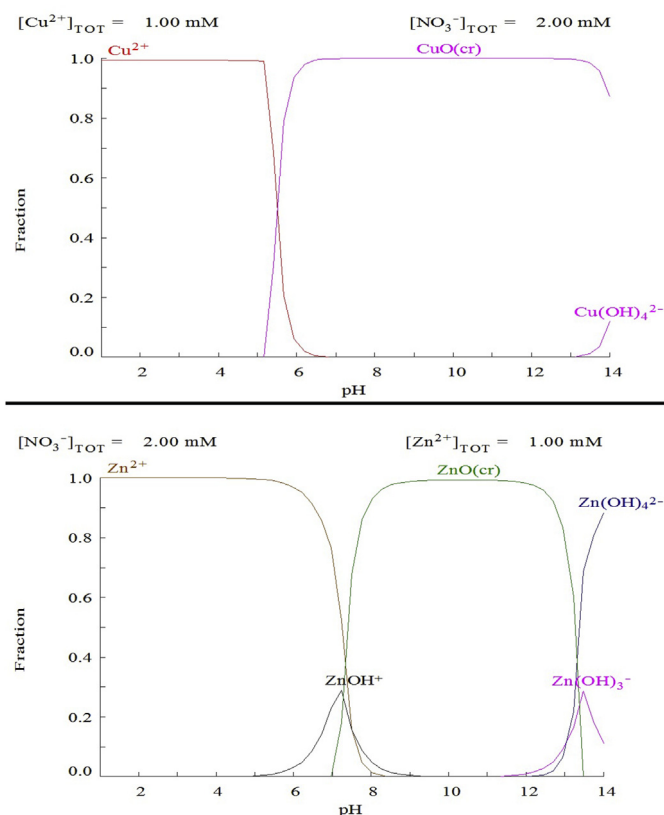


Fig. 3. Metal speciation diagram of copper and zinc as function of pH.

In Fig. 4, it can be observed that the wet particle and the particles cross-linked at 40 and 100 °C had similar results. When the particles were produced in alcoholic CaCl_2 solution, there is a little increase in the capacity of adsorption and an increase in the reduction of the concentration of Cu^{2+} , comparing the results obtained with particles cross-linked in aqueous CaCl_2 solution at the same temperature. The reduction of 63.3%, 32.2% (to particles produced in aqueous CaCl_2 solution) and 65.8%, 38.8% (to particles produced in alcoholic particles) observed in the concentration of Cu^{2+} for particles cross-linked at 125 and 150 °C, respectively, was smaller than the reductions promoted by particles cross-linked at

40, 100 °C and by wet particles. Probably, as discussed previously, the changes promoted by Maillard reactions in cross-linked particles at 125 and 150 °C are responsible for the decrease of adsorption capacity of particles.

Fig. 5 shows the results of affinity of the sericin/alginate particles by Zn^{2+} . Similarly seen in Fig. 4, in affinity tests with Zn^{2+} , the cross-linked particles at 40 and 100 °C presents a greater adsorption capacity than the ones cross-linked at 125 and 150 °C. The q_e values to particles cross-linked in 150 °C were very small to both particles: 15.2% and 12.1% to particles produced in aqueous and alcoholic solutions before drying process. Probably, it is due to the changes that the high temperature and the long period of time promoted during the crosslinking process (150 °C, 24 h) at these particles.

Comparing the results in Table 2 and the results showed in Figs. 4 and 5, it is possible to see that the best crosslinking temperature was 100 °C to produce particles. In this temperature, the S_D value was smaller than in the other temperatures, what is very important in continuous and batch adsorption processes. Furthermore, the adsorption capacity of particles cross-linked in this temperature presented good values, comparing with the adsorption capacity of the other particles.

In general, the adsorption capacities of Zn^{2+} by particles was slightly smaller than the ones of Cu^{2+} . The particles produced from the sericin and alginate blend had major affinity by Cu^{2+} than to Zn^{2+} . Comparing the particles cross-linked in the same temperature, all had greater affinity to Cu^{2+} than to Zn^{2+} .

4. Conclusions

The particles produced in this study showed lower water solubility when the blend was dripped in alcoholic (ethanol) CaCl_2 solution (ionic gelation process). The cross-linking process by heating also decreased the water solubility of particles. For environmental uses, like biosorption processes, the decrease of solubility is a fundamental factor to be accomplished. The solubilisation of the adsorbent turns the adsorption process infeasible for both batch and continuous processes because adsorbent is lost during the 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

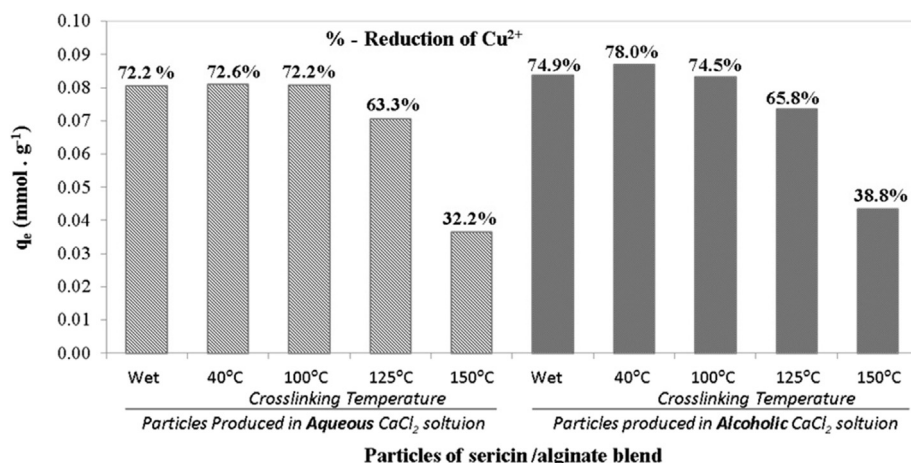


Fig. 4. Adsorption capacity (q_e) and reduction of the concentration (%) of Cu^{2+} in the copper tests affinity for wet particles and particles thermally cross-linked at 40, 100, 125 and 150 °C.

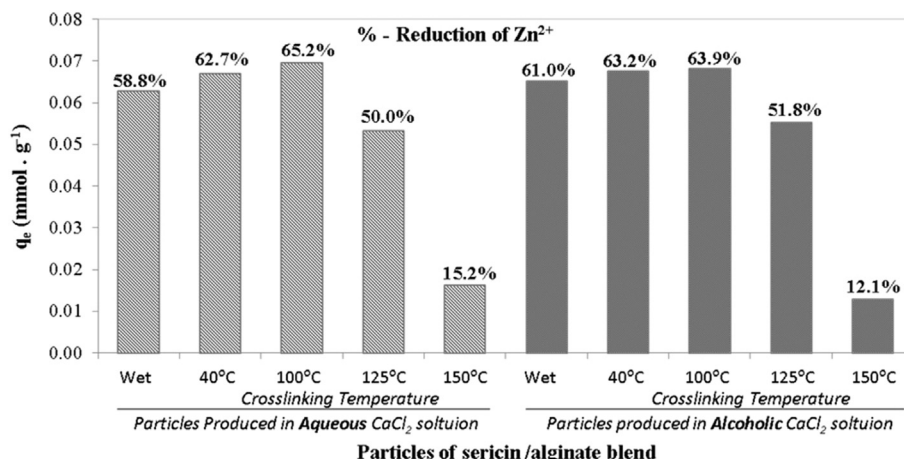


Fig. 5. Adsorption capacity (q_e) and reduction of the concentration (%) of Zn^{2+} in the zinc tests affinity for wet particles and particles thermally cross-linked at 40, 100, 125 and 150 °C.

processes) promoted changes in the secondary structure of the blend, what could be seen in FTIR spectra of the particles.

In evaluation of thermal cross-linked particles, the dried particles produced from sericin/alginate blend in alcoholic $CaCl_2$ solution showed smaller solubility in water than the ones produced in aqueous solution. In general, when particles were cross-linked at 100 °C, the S_D results reached the smaller values. The cross-linking process at 125 and 150 °C promotes changes in the structure of particles that increases the water solubility and decreases its adsorption capacity. The tests indicated that the particles of sericin/alginate had greater affinity to Cu^{2+} than Zn^{2+} , because comparing particles cross-linked at the same temperature, the reduction observed in concentration of Cu^{2+} were greater than the ones observed in Zn^{2+} solutions.

The results suggest the potential use of sericin-alginate particles, crosslinked by ionic gelation and by heat, to adsorption processes of toxic metals, zinc and copper. The use of these biomaterials is interesting because of their sources. Sericin is a waste protein of the silk spinning process, while the alginate comes from abundant and renewable sources of brown algae. Both materials are biodegradable, cheap and promising in the use for environmental purposes and industrial treatment of effluents.

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