PREPARATION AND CHARACTERIZATION OF PARTICLES PRODUCED FROM SERICIN AND ALGINATE BLEND WITH INCORPORATED DRUG

A. C. da SILVA JUNIOR[1], T. L. da SILVA[1], J. O. DE LIMA, M. L. GIMENES[2], A. F. de ALMEIDA NETO[1], M. G. A. VIEIRA[1] and M. G. C. da SILVA[1]

[1] University of Campinas, School of Chemical Engineering
[2] State University of Maringá, Department of Chemical Engineering
E-mail: meuris@feq.unicamp.br

ABSTRACT – The use of biodegradable polymers for controlled release drug delivery systems are being widely studied, because the therapeutic benefits that can be achieved. Studies have shown that exposure of diclofenac sodium (DS) in acid medium, such as occurring after oral ingestion of the drug, decreases the anti-inflammatory activity. Thus, the incorporation of the drug into biopolymer particles becomes an alternative and the therapeutic effect is maintained due to the protection offered by the biomaterials. The objective of this study is to evaluate the incorporation of diclofenac sodium in particles formed from the blend of sericin and alginate subjected to gelation process with $CaCl_2$. Incorporation efficiency measurement, interaction and morphology of the drug with mixtures of biopolymers were made. Also, the particle characterization was carried out to analyze the physical-chemical parameters, such as the determination of the property pH_{ZPC}.

1. INTRODUCTION

Many of the drugs that are used in conventional treatment of diseases are usually administered in high doses to achieve the necessary therapeutic effect, although it can lead to various adverse effects. Usually this fact is related to the pharmacokinetic (poor biological half-life) and unfavorable profile or because of its inactivation in acid medium.

Diclofenac sodium (2-[(2,6-dichlorophenyl) amino] benzenacetic acid monosodium salt) is a synthetic non steroidal anti-inflammatory drug, widely employed for relief of pain, fever, inflammation associated to various infirmity processes, such as chronic arthritis, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and is sometimes used postoperatively (Alok *et al.*, 2013). This drug shows short biological half-life (1–2h), attributed to a very rapid metabolism and elimination, and has high percentage capacity to bind with plasma protein (Dutta and Sahu, 2012). It is needed repeated daily dosing of drug due to its short half-life which could lead to severe dose limiting side effects, including cardiac, gastrointestinal, hepatic and renal adverse effects (Dutta and Sahu, 2012). Furthermore, it is an unstable drug in acidic medium, with its use limited by the high incidence of undesirable effects on the gastrointestinal tract (Santos *et al.*, 2007).

In the case of diclofenac sodium, it is important to develop a substance capable of withstanding the attack of gastric juice without degrading the drug before it reaches the duodenum or jejunum. Other research has been done in the development of drug carriers systems that are absorbed only in the small intestine or confined in a segment of intestine. According to Kumari et al.(2010) biodegradable nanoparticles have been used frequently as drug delivery vehicles due to its grand bioavailability, better control release and less toxic properties. According to. Vroman and Tighzert (2009), mixing proteins or biodegradable polymers with each other can improve their intrinsic properties.

Among the most biomaterials used in the drug delivery systems the more commonly used is the alginate. Alginate is a naturally occurring anionic polysaccharide obtained from *Macrocystis pyrifera*, brown algae. It is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate and its epimer α-L-guluronate residues, respectively, covalently linked together in different sequences or blocks (Wantanasiri *et al.*, 2014). Alginate microparticles have excellent bioadhesive properties, especially in presence of other polysaccharides or proteins, which showed strong affinity for gastric mucosa (Jeon *et al.*, 2014; Al-Kahtani and Sherigara, 2014). The gelation process occurs through divalent cation binds to two carboxyl groups on the adjacent alginate molecules (Finotelli *et al.*, 2008).

The sericin is an extracted protein during the degumming process for the production of silk. According to Fabiani *et al.* (1996) the sericin is a useless by-product of the textile industry and presents high oxygen demand for its degradation by microbes. Usually, the sericin is discarded to the environment. Due to this fact several studies have sought to provide a greater amount of sericin aggregation such as the use of sericin in the drug delivery system. Its properties find uses in cosmetics and fabrics and show a considerable promise for protein adsorption, drug delivery and tissue engineering (Chen *et al.*, 2011). 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 (Turbiani *et al.*, 2011; Dash *et al.*, 2009).

Hence, the present work focuses on the preparation and characterization of the sericin and alginate blend incorporated with diclofenac sodium (DS). The incorporation of drug to this biopolymer would enhance their therapeutic activity because of the controlled delivery to the targeted region and the reduction of severe side effects. The characterization was performed by measuring the incorporation and interaction of the drug with the biopolymer mixture. In addition, the morphology, swelling property and pHzpc were also determined and analyzed.

2. MATERIALS AND METHODS

2.1. Raw Materials

The particles were manufactured with sodium alginate (Sigma-Aldrich, United Kinddom), calcium chloride dihydrate (Vetec, Brazil), Diclofenac sodium (Henan Dongtai, China) and sericin extracted from the *Bombyx mori* silkworm´s cocoons (Bratac Silk Mills Company, Brazil).

2.2. Extraction of Sericin from Cocoon of Silk Worm

The silkworm cocoons were provided by Bratac Silk Mills Company and were checked for any impurity. The cocoons were cleaned, cut into small pieces and washed with deionized water. A solution of sericin (SS) was obtained after extraction in an autoclave during 40 minutes at 120 ºC. The SS was vacuum filtered to remove the fibers from the solution, stored in a sealed container, and then it was kept at room temperature for at least 12 hours to stabilize the hydrogel. After this period the SS was frozen in a conventional freezer for at least 24 h and then it was thawed at room temperature. The precipitated sericin was vacuum filtered and dried overnight at 50 °C.

2.3. Preparation of Particles

All the particles were prepared by gelation method similar to that applied by Khandai *et al.* (2010) with some modifications. Table 1 shows three different formulations of sericin/alginate blend incorporated with diclofenac sodium (DS). Sericin solutions of different concentrations were prepared by dissolving with ultra pure water in autoclave during 10 minutes at 100 ºC. This solution was added to Na-alginate and kept at 40 ºC until complete dissolved. After dissolved the Na-alginate, DS was added under gentle agitation at 40 ºC during 2h. The bubble free solution was added drop wise to a calcium chloride solution (3% w/V) using syringe pump with 24 G needle and continuously stirred at 100 rpm. After 30 minutes of stirring the particles were collected by filtration, washed with ultra pure water and dried overnight at room temperature.

Table 1 – Preparation of sericin/alginate particles incorporated with DS.

Formulation	Sericin (g/100mL)	Na-alginate (g)	DS (g)
A	2.5	1.0	-
B	-	1.0	-
F1	1.0	1.0	0.05
F2	1.5	1.0	0.05
F3	2.0	1.0	0.05

2.3. Incorporation efficiency

Approximately 100 mg of dried particles were pulverized. A quantity of 25 mg of powder was transferred to a 25 mL volumetric flask. 15 mL of methanol were added and then sonicated for 30 min (Brason1510 - USA) and added to the mark and waited for 1h sedimentation. 400 µL solutions were taken and diluted in a volumetric flask of 10 mL. The concentration of the drug solution was determined spectrophotometrically at 285 nm (UVmini1240 Shimadzu - Japan). Each determination was made in triplicate. The efficiency of incorporation was calculated by the Equation 1:

$$Incorporation\ efficiency = \frac{pratical\ drug\ content}{Theoretical\ drug\ content} \times 100 \quad (1)$$

2.4. Examination of Drug Interaction

Fourier transform infrared (FTIR) analysis of pure drug, sericin, alginate, blend sericin/alginate and optimized formulation was obtained using a FTIR analyzer (Nicolet 6700 FTIR, Thermo Scientific) to study the possible interactions between the pure drug and blend polymer. The samples were prepared on a KBr-press under a hydraulic pressure of 7 t. All analyses were performed with an average of 32 repeated scans and 4 cm^{-1} scan resolution taken between 400 and 4000 cm^{-1}.

2.5. Morphological Property

The surface of the morphology for both sericin/alginate and sericin/alginate/DS particles were examined by scanning electron microscopy (SEM), using a LEO 440i, MOD. 6070 (Cambridge - England) scanning microscope at an operating voltage of 15 kV.

2.6. pH_{ZPC} Analyses

The surface charge density was calculated using the potentiometric titration data for a blank electrolyte solution and optimized formulation suspensions ($C_{particle}$=0.6 wt.%), at a constant salinity of 1M $C_2H_7NO_2$. According to the difference of acid or base volume used to obtain the same pH value, this parameter was calculated by the Equation 2:

$$\sigma = \frac{\Delta V \times c \times F}{m \times S_{BET}} \tag{2}$$

where $\Delta V = V_s - V_e$ is the difference between the base(acid) volumes added to the electrolyte solution V_e and suspension V_s to achieve the same pH; F the Faraday constant (9.64853399(24) x 10^4 C mol^{-1}); S_{BET} the specific surface area; c the concentration of base (acid); and m the weight of the optimized particles.

3. RESULTS AND DISCUSSION

3.1. Incorporation efficiency

The effect of biomaterial (sericin/alginate blend) concentration on the incorporation efficiency of the prepared particles is shown in Figure 1. Diclofenac sodium containing particles had incorporation efficiency in the range of 71.16 ± 2.15% to 88.24 ± 3.13%. It has been found that increasing sericin concentration in the sericin/alginate blend decreased significantly the effect of the drug incorporation in the particles. At higher concentration of sericin in the polymer blend, the viscosity of the medium increases and there is lesser availability of water. As a result, it decreases the solubility of the drug which shall increase the loss of drug in the curing medium, thus also decreasing the incorporation efficiency of the prepared formulations.

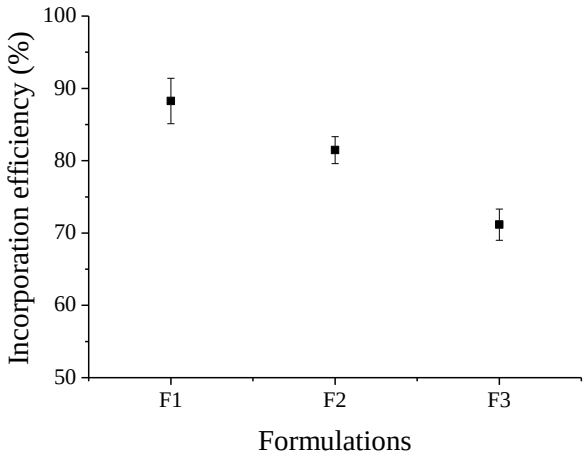

Figure 1 – Effect of biomaterial concentration on incorporation efficiency of particles.

3.2. Examination of drug interaction

Figure 2 shows the infrared spectrums of pure diclofenac sodium (DS), sericin (Ser), alginate (Alg), sericin/alginate blend (Ser/Alg) and optimized formulation (OF). The infrared spectrum of pure DS showed that the peaks between 1000 cm^{-1} and 1350 cm^{-1} occurred from C–N stretching; whereas peaks at 1506 cm^{-1} and 1575 cm^{-1} resulted from C═C stretching and C═O stretching of carboxyl group, respectively. In the FTIR spectrum of sodium alginate, the characteristic peaks appeared at 1411 cm^{-1} and 1600 cm^{-1}, for symmetric $–COO^-$ stretching vibration, asymmetric $–COO^-$ stretching vibration, respectively.

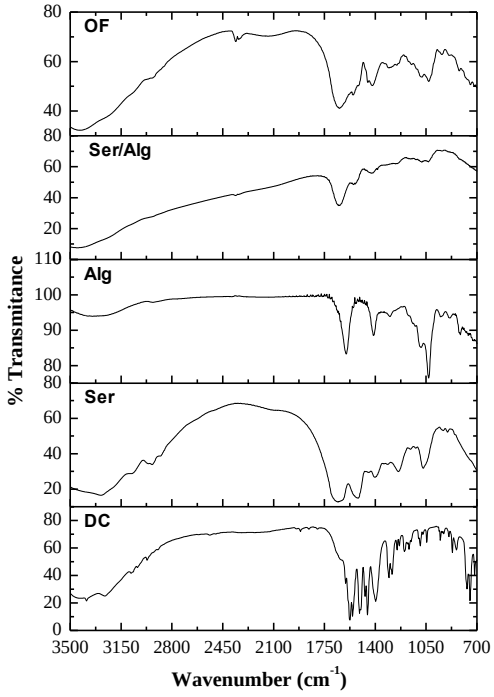

Figure 2 – Infrared spectrum of pure diclofenac sodium (DS), sericin (Ser), alginate (Alg), blend sericin/alginate (Ser/Alg) and optimized formulation (OF).

It was observed from the FTIR study that all the major peaks of diclofenac sodium were almost intact in the particle formulation. The FTIR spectra indicate the stable nature of diclofenac sodium in the particle formulation.

3.3. Morphological Property

The morphology of the particles of sericin/alginate blend, sericin/alginate/DC and diclofenac sodium (DC) can be seen in the micrographs by scanning electron microscopy (SEM) shown in Figure 3. Spherical particles were observed in all tested formulations. However, there were differences in the surface characteristics of the particles, depending on the ratio drug used. Comparing the images of the particles formed from the sericin/alginate (A) with the mixture of sericin, alginate and DS (F1, F2, and F3), it is noticed a significant roughness for all the samples. However, the particles formed with the incorporated drug (F1, F2 and F3) are similar to sandpaper. The appearance of sandpaper on all formulations (F1, F2, F3) is characterized by drug crystals adsorbed on the particle surface.

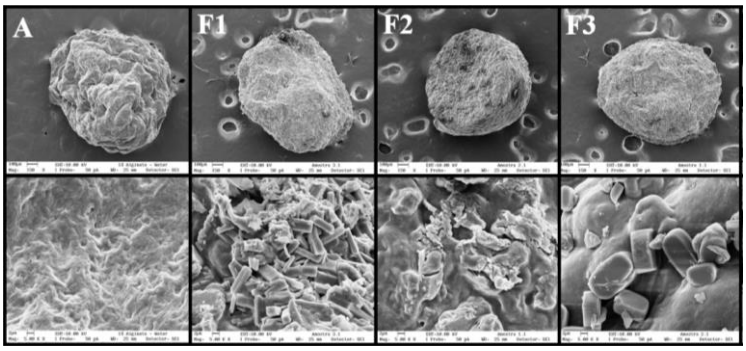

Figure 3– Scanning electron microscopy images of blend sericin/alginate with drug and without incorporated. (A) Particle with sericin/alginate ratio of 2.5:1.0 wt.%. (F1) Particle with sericin/alginate/DC ratio 1.0:1.0:1.0 wt.%. (F2) Particle with sericin/alginate/DC ratio 1.5:1.0:0.5 wt.%. (F3) Particle with sericin/alginate/DC ratio 2.0:1.0:0.5 wt.%. For all images in the row first magnification bar represents 100 μm, while for others images magnification bar represents 2 μm.

It was also verified that the granularity on the surface was not uniform. Khandai *et al.* (2010) performed the incorporation of the drug with vigorous stirring, while in this work the particles were heated at 40 °C and the medium was gently stirred. This procedure was adopted to prevent bubble formation. Probably, the method of incorporation needs to be optimized to reduce the irregularity in the granularity observed on the particles surface.

3.4. pH_{ZPC} Analyses

Figure 4 shows the surface charge density as a function of pH for the aqueous suspensions of sericin/alginate particles with incorporated DS. According to Khandai *et al.* (2010) pH close to neutral for sericin/alginate particles forms a gel that promotes the mucoadhesion cell surface for a fairly long time. The pH_{ZPC} of the sericin/alginate particles with DS incorporated was 7.12. According to the results found for pH_{ZPC} the particles should

exhibit good mucoadhesive property. This favorable characteristic of mucoadhesion was also verified in similar particles produced by Khandai *et al.* (2010).

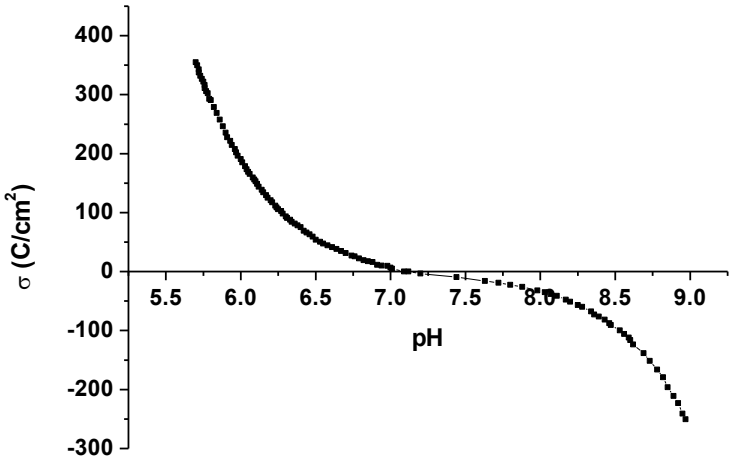

Figure 4 - Surface charge density as a function of pH for the aqueous suspensions of sericin/alginate particles with DS incorporated.

4. CONCLUSION

This study reports for the first time the successful preparation of sericin/alginate blend with incorporated diclofenac sodium by ionic gelation method. The result of incorporation in the diclofenac sodium (DS) in natural protein was more favorable for lower concentrations of sericin. Infrared spectra showed that the drug structure has not been changed after the incorporation mixture biomaterial (serinicin/alginate). The surface morphology study showed presence of crystals (diclofenac sodium) on particles surface in all formulations. Thus, the present study found that pH_{ZPC} analyzes indicate that the particles formed from alginate and sericin can be used for drug delivery systems.

5. ACKNOWLEDGEMENTS

The authors would like to thank Bratac Silk Mills Company for providing us silkworm cocoons, CNPq, FAPESP and Fundação Araucária-PR for the financial support.

6. REFERENCES

AIELO, P. B.; BORGES, F. A.; ROMEIRA, K. M.; MIRANDA, M. C. R.; ARRUDA, L. B.; LIMA FILHO, P. N.; DRAGO, B. C.; HERCULANO, R. D. Evaluation of sodium diclofenac release using natural rubber latex as carrier. *Mat. Research*, 2013.

AL-KAHTANI, A. A.; SHERIGARA, B.S. Controlled release of diclofenac sodium through acrylamide grafted hydroxyethyl cellulose and sodium alginate. *Carb. Pol.*, v. 104, p. 151-157, 2014.

ALOK, A.; ADHIKARI, J.S.; CHAUDHURY, N.K. Radioprotective role of clinical drug diclofenac sodium. *Mut. Research*, v. 755, p. 156-162, 2013.

CHEN, X.; LAM, K. F.; MAK, S. F.; YEUNG, K. L. Precious metal recovery by selective adsorption using biosorbents. *J. H. Mat.*, v. 186, p. 902-910, 2011.

DASH, B. C.; MANDAL, B. B.; KUNDU, S. C.; Silk gland sericin protein membranes: Fabrication and characterization for potential biotechnological applications. *J. Biot.*, v. 144, p. 321-329, 2009.

DUTTA, R. K.; SAHU, S. Development of diclofenac sodium loaded magnetic nanocarriers of pectin interacted with chitosan for targeted and sustained drug delivery. *Col. and Surf. B: Biointerfaces*, v. 97, p. 19-26, 2012.

FABIANI, C.; PIZZICHINI, M.; SPADONI, M.; ZEDDITA, G. Treatment of waste water from silk degumming processes for protein recovery and water reuse. *Des.* v. 105, p. 1-9, 1996.

FINOTELLI, P. V.; SAMPAIO, D. A.; MORALES, M. A.; ROSSI, A. M.; ROCHA-LEÃO, M. H. Ca alginate as scaffold for iron oxide nanoparticles synthesis. *Braz. J. Ch. Eng.* v. 25, p. 759-764, 2008.

LACHMAN, L.; LIBERMAN, H. A.; KANIG J. L. *The Theory and Practice of Industrial Pharmacy*, Bombay: Varghese Publishing House, 1991.

TURBIANI F. R. B.; TOMADON JUNIOR J.; SEIXAS F. L.; GIMENES M. L.; Properties and Structure of Sericin Films: Effect of the Crosslinking Degree. *Che. Eng. Trans.*, v. 24, p. 1489-1494, 2011.

WANTANASIRI, P.; RATANAVARAPORN, J.; YAMDECH, R.; ARAMWIT, P. Fabrication of silk sericin/alginate microparticles by electrohydrodynamic spraying technique for the controlled release of silk sericin. *J. Elect.*, v. 72, p. 22-27, 2014.

ZHANG, J.; WANG, Q.; WANG, A. In situ generation of sodium alginate/hydroxyapatite nanocomposite beads as drug-controlled release matrices. *Acta Biomaterialia*, v. 6, p. 445-454, 2010.

KUMARI, A.; YADAV, S. K.; YADAV, S. C. Biodegradable polymeric nanoparticles based drug delivery systems. *Coll. and Surf. B: Biointerfaces*, v. 75 p. 1-18, 2010.

JEON, O.; SAMOREZOV, J. E.; ALSBERG, E. Single and dual crosslinked oxidized methacrylated alginate/PEG hydrogels for bioadhesive applications. *Acta Biomaterialia*, v. 10 p. 47-55, 2014.