CHEMICAL ENGINEERING TRANSACTIONS
VOL. 43, 2015
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Chief Editors: Sauro Pierucci, Jiří J. Klemeš

ISBN 978-88-95608-34-1; **ISSN** 2283-9216
DOI: 10.3303/CET1543139

Evaluation of Incorporation of Diclofenac Sodium in Dried Sericin-Alginate Particles Prepared by Ionic Gelation Technique

Thiago L. da Silva[a]; Jacyara M. M. Vidart[a], Absolon C. da Silva Jr.[a]; Marcelino L. Gimenes[b]; Melissa G. A. Vieira[a]; Meuris G. C. da Silva*[,a]

[a]School of Chemical Engineering, State University of Campinas, UNICAMP, 13083-825, Campinas – SP, Brazil
[b]Department of Chemical Engineering, State University of Maringá, UEM, 87020-900, Maringá – PR, Brazil
meuris@feq.unicamp.br

The objective of the current work is to evaluate the incorporation of diclofenac sodium (DS) in particles produced from sericin and sodium alginate blend. The ionic gelation technique was used in order to obtain particles by dripping the blend with DS in ionic calcium solution. Different formulations among sericin, alginate and DS were investigated and the efficiency of incorporation, the components in particles composition analyzed by Fourier transform infrared spectroscopy (FTIR), surface morphology by SEM and zeta potential were evaluated. Sericin is a highly hydrophilic globular protein present in the silkworm cocoons (Bombyx mori) and usually it is discharged during the silk manufacturing. Sodium alginate is a linear polysaccharide extracted from brown seaweed and considered a good mucoadhesive agent which has abundant use in drug delivery systems. Both biopolymers are easy available, cheap, biodegradable and their chemical and physical characteristics enable their use in a wide range of materials. DS is a non-steroidal anti-inflammatory drug widely used and it is characterized by short biological half-life in organism. The use of polymers can increase the therapeutic efficacy due the drug release promoted. In this work the sericin solution was obtained by degumming process in autoclave (1 kgf/cm^2, 40 min) and the sodium alginate had analytical grade (Sigma-Aldrich). The results showed that the efficiency of incorporation reached values close to 90 % and the FTIR analyses indicated that the incorporated drug did not change its chemical structure in the particles.

1. Introduction

Sericin is a water soluble globular protein present in the cocoon of silkworm (Bombyx mori), usually discarded in wastewater processes of degumming and spinning of silk, which can adversely impact the environment (Silva et al., 2014a). This protein has excellent antioxidant, antibacterial and resistant to UV radiation and moisture properties (Mondal et al.,2007), attracting the attention of researchers for various uses currently being exploited in applications such as cosmetics (Zhang, 2002), supports for immobilized enzymes, dietary supplements, medical supplies and pharmaceuticals, and functional fibres (Kongdee et al., 2005).
Sodium Alginate is a biomaterial widely used in drug delivery systems (Khandai et al., 2010). Alginate is a naturally occurring polysaccharide found in all species of brown (Macrocystispyrifera) algae and some species of bacteria (Chan et al., 2001). It is a linear block copolymer linked homopolymer of (1-4) -β-D-mannuronate and its epimer α-L-guluronate residues are covalently linked together in different sequences or blocks (Wantanasiri et al., 2014). Alginate microparticles have excellent bioadhesive properties (Jeon et al., 2014), especially in the presence of other polysaccharides or proteins that have strong affinity for the gastric mucosa (Al-Kahtani and Sherigara, 2014). The gelation process occurs through the divalent cation that binds two carboxyl groups at adjacent alginate molecules (Finotelli et al., 2008).
The diclofenac sodium (2 - [(2,6-dichlorophenyl) amino] benzene acetate sodium) is a synthetic non-steroidal anti-inflammatory drug widely used for the relief of pain, fever, inflammation associated with many disease

Please cite this article as: da Silva T.L., Martins J.M., da Silva Junior A.C., Gimenes M.L., Vieira M.G.A., da Silva M.G.C., 2015, Evaluation of incorporation of diclofenac sodium in dried sericin-alginate particles prepared by ionic gelation technique, Chemical Engineering Transactions, 43, 829-834 DOI: 10.3303/CET1543139

processes, such as chronic arthritis, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and is sometimes used postoperatively (Alok et al., 2013). This drug shows a short biological half-life (1-2 h) assigned to a very fast metabolism and elimination, and has a high ability to bind with plasma proteins (Dutta and Sahu, 2012). Repeated daily dosing of drug which could consequently lead to severe dose-limiting side effects, including cardiac, gastrointestinal, hepatic and renal adverse effects (Dutta and Sahu, 2012). Gastric resistant formulations allow gastric disorder reduction, protect active ingredients from instability at acidic pH, and facilitate gastrointestinal tract's distal regions absorption (Kleinubing et al., 2014).
In the present work, mucoadhesive particles of sericin, sodium alginate and diclofenac sodium drug (DS) were prepared by ionic gelation technique and the incorporation of the drug in these dried particles was investigated for different formulations. The different formulations were evaluated by efficiency of drug incorporation, drug polymer interaction study (by FTIR analyses), morphological examination (SEM) and zeta potential.

2. Experimental

2.1 Materials

The materials used for the development of this study were: cocoons of silkworm *Bombyx mori*, provided by the silk spinning BRATAC Company, located in the state Paraná - Brazil; pharmaceutical grade diclofenac sodium (Henan Dongtai brand); HPLC grade methanol (JT Baker brand); sodium alginate (Sigma-Aldrich brand); analytical standard anhydrous calcium chloride - $CaCl_2$ (Vetec brand).
The equipments used were syringe infusion pump (model ST670, Santronic brand); ultrasonic bath (Brason Utrasonic, model 1510); Spectrophotometer UV - VIS Spectrophotometers (Shimadzu, model UV-1240 mini).

2.2 Sericin extraction from cocoon of silkworm

The cocoons of silkworm, previously cleaned and cut, were added to the water in the weight ratio of 4:100 (g of cocoons:mL of water), and taken to the autoclave for degumming process pressure of 1 kgf/cm^2 for 40 min.
The mixture of cocoons and water of degumming process, still hot, was filtered in a vacuum filtration system with a filter paper with porosity of 14 µm in order to separate the silk fibers (fibroin) from extracted sericin solution. The sericin solution obtained was stored in a plastic bottle and placed at room temperature for 12 h to form a stable hydrogel solution of sericin and then this solution was frozen for 24 h (Silva et al., 2014b Gimenes et al., 2014). After this period, the solution was thawed at room temperature. The precipitated sericin was vacuum filtered and the concentrated sericin solution obtained in previous step was heated in autoclave (120 ºC, 10 min) to solubilize the precipitated protein. The solution was adjusted by dilution (Silva et al., 2014b).

2.3 Preparation of particles

The sericin solutions of different concentrations were prepared with deionized water and placed in an autoclave for 10 min at 1kgf/cm^2. Then the solution was removed from the autoclave and cooled to reach a temperature of 40 °C, then sodium alginate was added and mechanically stirred (150 rpm). After dissolving all the sodium alginate (Alg-Na), the diclofenac sodium (DS) was added to the solution and mechanically stirred for 2 h (150 rpm). The mixture sericin/Alg-Na/ DS was dripped to a calcium chloride solution (3 % w/v) using a 8 G needle (40 mm X 1.2 mm needle) with continuously stirred. The stirring was continued for 30 minutes for complete reaction, and then the particles was collected by filtration, washed extensively with deionized water and dried at room temperature. Table 1 shows all formulations evaluated.

Table 1: Formulations of sericin – alginate particles with diclofenac sodium (DS) incorporated.

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

2.4 Evaluation of particles

2.4.1 Determination of incorporation efficiency

The efficiency of incorporation was calculated by Eq(1). Therefore, approximately 100 mg of dried particles were ground in a porcelain mortar and 25 mg of the milled particles was transferred to a 25 mL volumetric flask and then 15 mL of methanol was added. The flask was placed in an ultrasound (Brason, model 1510R-MTH, USA) for 30 min. Then, the flask volume was completed with methanol. After 1h of sedimentation of the entire suspension, an aliquot of 400 µL of supernatant was diluted in a volumetric flask of 10 mL. The

concentration was determined by spectrophotometer (Shimadzu model UVmini1240 – Japan) with wavelength of 285 nm. All determinations were carried out in triplicate.

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

2.4.2 Drug polymer interaction study (FTIR)

To evaluated the possible interactions between the drug post-incorporation with the mixture of alginate and sericin Fourier Transform Infrared analysis (FTIR) with pure drug, pure sericin, sodium alginate and the best incorporation of diclofenac sodium formulation obtained was performed using a FTIR analyzer (Nicolet ThermoScientific - 6700FTIR). Samples were ground and mixed with KBr (potassium bromide) which was pressed with a hydraulic rush (7 tons, 15 seconds). All analyzes were conducted on average 32 repetitions readings to scan 4 in 4 cm^{-1} resolution of between 400 cm^{-1} to 4,000 cm^{-1}.

2.4.3 Morphological examination (SEM) and diameter measuring

The morphology of the particle surfaces, prepared according to Table 1, was examined by scanning electron microscopy (SEM). The samples were metallized with a thin layer of gold. The images were obtained in electron microscope (Electron Microscopy LEO, 440i model, England) with 15 kV voltage operating conditions (Mag. 150 X and 5.0 KX). The diameters of particles were determined analyzing micro images (taken from optical microscope) by the program Motic Images Advanced 3.2. The mean diameter of particles (F1, F2 and F3) was determined by the average of 150 particles with the same formulation.

2.4.4 Zeta potential

The surface charge density was determined by the potentiometric titration with magnetic stirring. First, the electrolyte solution of ammonium acetate (with constant salinity – 1 M) without the presence of particles was titrated with acetic acid and ammonium hydroxide solutions. Then, another titration with the particles suspended in electrolyte solution was performed with acetic acid and ammonium hydroxide solutions. Potentiometric titration of the electrolyte solution with and without suspended particles uses different volumes to achieve the same pH, so by volume differences between the two types of titration it is possible to calculate the potential of surface charges by Eq(2).

$$\sigma = \frac{\Delta V \times C \times F}{m \times S_{BET}} \quad (2)$$

Where ΔV is the difference between the volumes added (by titration of the suspension with and without particles in suspension) to obtain the same pH; F is the Faraday constant (9.64853399 x 10^4 $C.mol^{-1}$); S_{BET} is the specific surface area; C is the concentration of base (or acid) and m is the weight of particles used.

3. Result and Discussion

3.1 Determination of incorporation efficiency

The effect of alginate and sericin concentration in the efficiency of incorporation of the particles prepared in accordance with Table 1 is shown in Figure 1. Particles containing diclofenac sodium showed incorporation efficiency in the range of 71.16 ±2.15% to 88.24 ±3.13%. The F1 was the optimum formulation (OF); this formulation had the highest incorporation efficiency (88.24%). It was found that increasing the concentration of sericin in the blend significantly reduced the effect of drug incorporation into particles.

3.2 Drug polymer interaction study (FTIR)

Figure 2 shows the infrared spectra of diclofenac sodium pure (DS) sericin (Ser), alginate (Alg), blend sericin/alginate (Ser/Alg) and the optimum formulation (OF). The infrared spectrum of pure DS showed that the peaks between 1,000 cm^{-1} and 1,350 cm^{-1} correspond to the CN group; while the peaks at 1,506 cm^{-1} and 1,575 cm^{-1} corresponding to C═C group and the carboxyl group C═O. In the spectrum of sodium alginate, characteristic peaks appeared at 1,411 cm^{-1} and 1,600 cm^{-1}, which corresponds to the symmetric group COO vibration and vibration of the asymmetrical -COO- group. It was observed that all major diclofenac sodium peaks remained almost unchanged in formulated particle. The FTIR spectra indicated the stable nature of the diclofenac sodium formulated particle.

.

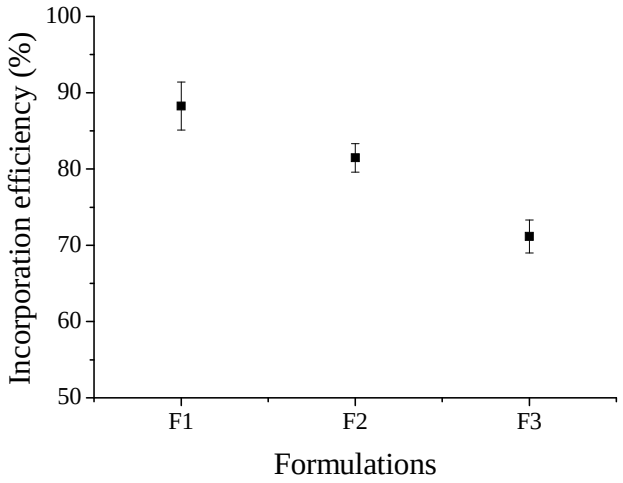

Figure 1: Effect of concentration of blend in the DS incorporation efficiency.

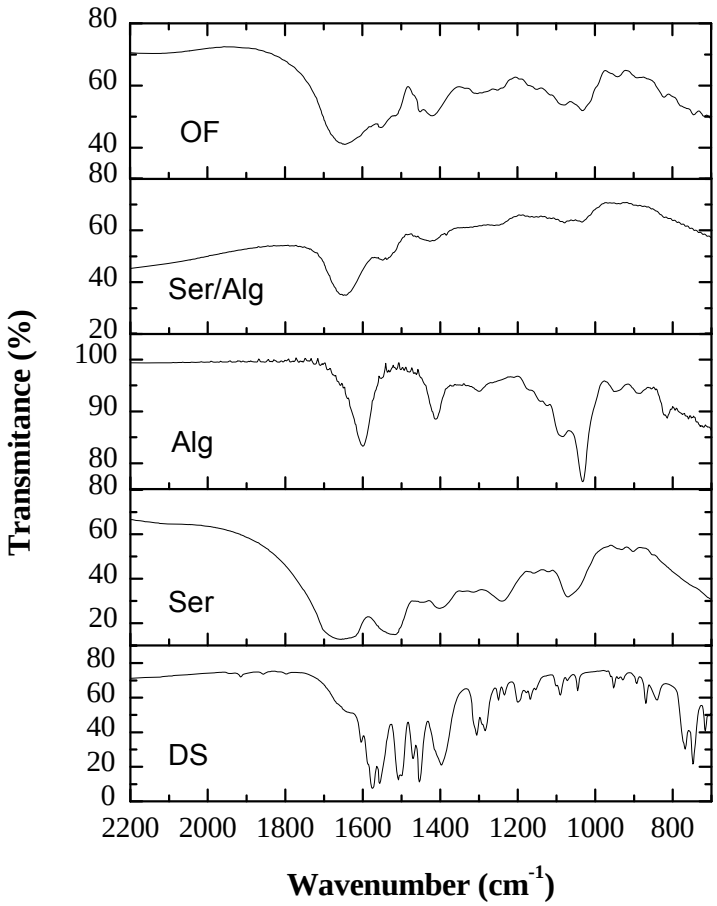

Figure 2: Infrared spectrum of pure diclofenac sodium (DS), sericin (Ser), alginate (Alg), mixture of sericin / alginate (Ser / Alg) and optimized formulation (OF).

3.3 Morphological Examination (SEM)

The morphology of the particles sericin/alginate and sericin/alginate/DS can be seen in the micrographs shown in Figure 3. Spheroidal particles were observed in all formulations tested. However, there were no differences in the characteristics of the particle surface, depending on the ratio of drug used. By comparing the images of the particles formed from sericin/alginate (A) with a mixture of sericin, alginate and DS (F1, F2, and F3), there is a significant roughness of all the samples. Nevertheless, the particles formed with the incorporated drug (F1, F2 and F3) are similar to a surface mineral deposit and this can be attributed to the adsorbed drug crystals on the surface of the particle. According to the bottom micrographs of Figure 3, crystals of F3 are larger than those of F1, while in F2 they have a poorly defined shape. These facts are probably related to the increase of sericin in formulation which can result in particles with small structural differences.

Khandai et al. (2010) performed the incorporation of the drug, with vigorous stirring and they observed the formation of a lot of roughness. In this work, the particles were heated to 40 °C and the medium was gently stirred, thus it was obtained a less rough surface than observed in Khandai's work. This procedure was adopted in present work to avoid the formation of bubbles. The mean diameters of particles were 1.176 mm (±0.158 mm), 1.225 mm (±0.142 mm) and 1.302 mm (±0.218 mm) to F1, F2 and F3.

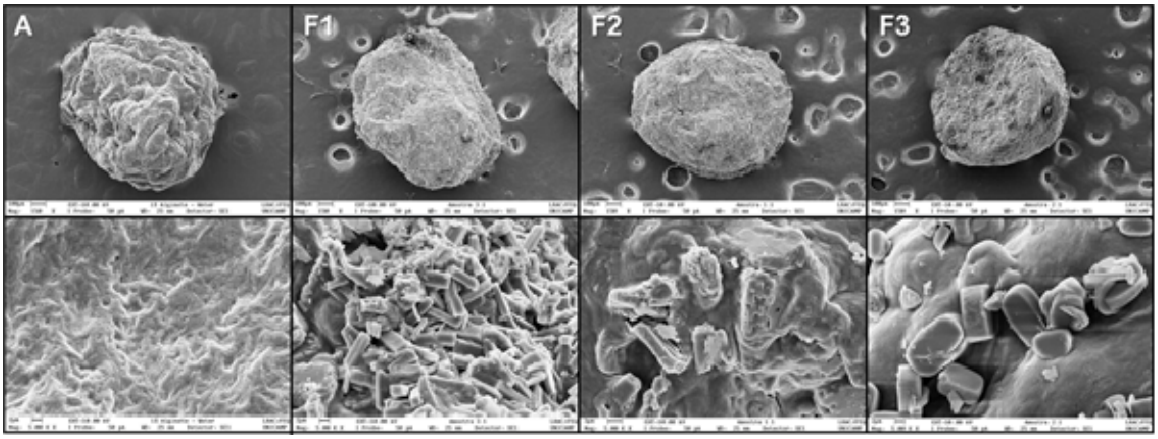

Figure 3: Micrographs of sericin/alginate mixture with and without drug incorporated. (A) Particles of sericin/alginate. (F1), (F2), (F3) sericin/alginate/DS particles (as shown in Table 1).

3.4 Determination by titration of the zeta potential of the particle with incorporated drug

Figure 4 shows the surface charge density as a function of pH of the aqueous suspensions containing particles of sericin/alginate/DS. The pH_{ZPC} sericin/alginate/DS particle was 7.12. According to the results for pH_{ZPC}, the particles must have a good mucoadhesive property. This favorable characteristic of mucoadhesion was also found in similar particles produced by Khandai et al. (2010) in which the particles near neutral pH form a gel promoting mucoadhesion on cell surface for a long time.

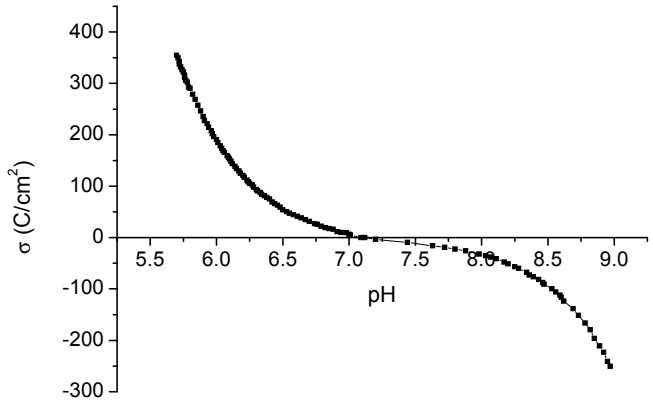

Figure 4: Surface charge density as a function of pH of the aqueous suspensions of F1 particles.

4. Conclusions

Particles containing diclofenac sodium were successfully prepared employing ionic gelation technique. The result of the incorporation of diclofenac sodium (DS) in the natural protein was more favourable at lower sericin concentrations. Infrared spectrum (FTIR) showed that the drug structure was not changed after the incorporation process in biomaterial (serinicin/alginate). The surface morphology revealed the presence of crystals (diclofenac sodium) on the particle surface in all formulations. The result of pH_{ZPC} analysis (the value 7.12) is characteristic of a good mucoadhesive property which facilitates its use as a means of drug transport.

Acknowledgements

The authors thank the BRATAC Company for providing the cocoons silkworm, CNPq and FAPESP for financial support.

References

Al-KahtanI A. A.; Sherigara B.S., 2014, Controlled release of diclofenac sodium through acrylamide grafted hydroxyethyl cellulose and sodium alginate, Carbohydrate Polymers, 04, 151-157.

Alok A., Adhikari J.S., Chaudhury N.K, 2013, Radioprotective role of clinical drug diclofenac sodium, Mut. Research, 755, 156-162.

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

Finotelli P. V., Sampaio D. A., Morales M. A., Rossi A. M., Rocha-Leão M. H., 2008, Ca alginate as scaffold for iron oxide nanoparticles synthesis, Brazilian. Journal Chemistry Engineering, 25, 759-764.

Gimenes, M. L., Silva, V. R., Vieira, M. G. A., Silva, M. G. C., Scheer, A. P., 2014, High Molecular Sericin from Bombyx mori Cocoons: Extraction and Recovering by Ultrafiltration, International Journal of Chemical Engineering and Applications, 5, 266 - 271.

Jeon O., Samorezov J. E., Alsberg E., 2014, Single and dual crosslinked oxidized methacrylated alginate/PEG hydrogels for bioadhesive applications, Acta Biomaterialia, 10, 47-55.

Khandai M., Chakraborty S., Sharma A., Pattnaik S., Patra C. N., Dinda S. C., Sem K. K., 2010, Preparation and evaluation of algino-sericin mucoadhesive microspheres: An approach for sustained drug delivery, Journal of Advanced Pharmaceutical Research, 01, 48 - 60.

Kleinubing, S. A., Seraphim, D. C., Vieira, M. G. A., Canevesi, R. L. S., Silva, E. A., César, C. L., Mei, L. H. I., 2014, Gastro-resistant controlled release of OTC encapsulated in alginate/chitosan matrix coated with acryl-EZE® MP in fluidized bed, Journal of Applied Polymer Science, 131(12), 1 - 9.

Kongdee A., Bechtold T., Teufel L., 2005, Modification of cellulose fiber with silk sericina, Journal of Applied Polymer Science, 96, 1421-1428.

Mondal M., Trivedy K., Kumar, N, 2007, The silk proteins, sericin and fibroin in silkworm, *Bombyx mori* Linn. - A review, Caspian Journal of Environmental Sciences, 5, 63–76.

Silva T.L., Silva Jr A.C., Ribani M., Vieira M.G.A., Gimenes M.L., Silva M.G.C., 2014a, Evaluation of molecular weight distribution of sericin in solutions concentrated via precipitation by ethanol and precipitation by freezing/thawing, Chemical Engineering Transactions, 38, 103-108 DOI: 10.3303/CET1438018

Silva T.L., Silva Jr A.C., Vieira M.G.A., Gimenes M.L., Silva M.G.C., 2014b, Production and physicochemical characterization of microspheres made from sericin and alginate blend, Chemical Engineering Transactions, 39, 643-648 DOI:10.3303/CET1439108

Wantanasiri P., Ratanavaraporn J., Yamdech R., Aramwit P., 2014, Fabrication of silk sericin/alginate microparticles by electrohydrodynamic spraying technique for the controlled release of silk sericina, Journal of Electrostatics, 72, 22-27.

Zhang Y. Q., 2002, Applications of natural silk protein sericina in biomaterials, Biotechnology Advances, 20, 91-100.