

Evaluation of Incorporation Efficiency of Drugs in Sericin/Alginate Particles

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Sericin is a water-soluble globular protein present in the silkworm cocoons (*Bombyx mori*), usually discharged in the wastewater of degumming process. Sodium alginate is a natural polysaccharide extracted from brown seaweed that has an abundant use in drug delivery systems. The blend of sericin/alginate may provide the most suitable characteristics for improvement of the drug encapsulation. The modification of the form of drug presentation becomes desirable for improved patient compliance and decreased side effects. The objective of this work is to evaluate the incorporation efficiency of quetiapine fumarate, acetylsalicylic acid, ibuprofen and ketoprofen in sericin/alginate particles. These particles were prepared by the ionic gelation technique using a blend of sericin and sodium alginate, with the drug embedded, and then, dripping the blend in calcium solution. The efficiency of incorporation was investigated for all formulations. The two best formulations were characterized by SEM (surface morphology) and by FTIR (drug polymer interaction). The results showed that the efficiency of incorporation reached values close to 18 % for quetiapine fumarate, 32 % for acetylsalicylic acid, 77 % for ibuprofen, and 81 % for ketoprofen. The FTIR and SEM analysis proved the incorporation of the drugs in the blend matrix.

1. Introduction

Silk is a fibrous protein produced by a variety of animals belonging to arthropods phylum. Among these animals, silkworm (*Bombyx mori*) highlights due its former domestication for silk production (Matsumoto et al., 2007). Silk fiber is mainly composed of two layers based on different proteins: fibroin in the inner layer, coated with an outer layer of sericin (Ude et al., 2014). In textile industry, sericin is discarded as residue, once it causes silk fiber hard and tough (Lamboni et al., 2015).

Sericin is a macromolecular globular protein, composed by 18 kinds of amino acids. Most of them possess strong side polar groups, making sericin water soluble (Zhang, 2002). The side groups enable sericin cross-linking, polymerization and blending with other proteins, such as sodium alginate, improving its properties and application (Lamboni et al., 2015). Sericin structure mainly involves an amorphous spiral form and to a lesser extent an organized β -sheet structure (Padamwar and Pawar, 2004). Properties of sericin as resistance to oxidation and ultraviolet radiation, antibacterial action, tyrosine and kinase inhibitory activity, biocompatibility, biodegradability, and anti carcinogenic effects make it interesting for several applications (Mondal et al., 2007).

Alginate is obtained from the intracellular matrix from brown algae (Gombotz and Wee, 2012). It is a linear polysaccharide polymer composed of M (β -d-mannuronate) and G (α -l-guluronate) blocks organized as consecutive G (G_n), consecutive M (M_n), and alternating M and G (MG_n) blocks. Alginate presents the ability of forming gels by contacting bivalent cation, as Ca^{2+} , producing an “egg box” cross-linking model (Lee and Mooney, 2012). Some alginate properties involve immunogenicity, bioadhesion, low toxicity, and biocompatibility, making possible its application in biomedical and pharmaceutical fields (Gombotz and Wee, 2012).

Although alginate has been extensively evaluated for drug incorporation such as theophylline (Miyazaki et al., 2000) and metronidazole (Murata et al., 2000), alginate and sericin blend has been evaluated for drug incorporation to a lesser extent. Zhang et al. (2015) observed blend potential for incorporation, as Khandai et al. (2010) and Vidart et al. (2016) evaluated aceclofenac and diclofenac sodium incorporation, respectively.

Ibuprofen, ketoprofen, and acetylsalicylic acid are nonsteroidal anti-inflammatory drugs (NSAIDs), usually employed at rheumatoid disease treatment. By inhibiting cyclooxygenases synthesis, these drugs act as anti-inflammatory, analgesic, and antipyretic agents. Side effect associated to NSAIDs is gastric discomfort. Also, they may present short life time, leading to peaks of concentration (Lakkis, 2007). Quetiapine is an antipsychotic drug indicated for schizophrenia and bipolar disorder treatment, acting as an antagonist at serotonin and dopamine receptors. As side effect, quetiapine act as a sedating agent at patients (Sanford and Keating, 2012).

In the present work, sericin and alginate blend was applied to incorporate ibuprofen (Ibu), ketoprofen (Keto), acetylsalicylic acid (ASA), and quetiapine fumarate (QTP) for controlled release system. Incorporating efficiency was evaluated, as well as the surface morphology, obtained by Scanning Electron Microscopy (SEM), and functional groups, obtained by Fourier Transformed Infrared Spectroscopy (FTIR).

2. Materials and Methods

2.1 Materials

As the source of sericin it was employed cocoon of silkworm (*Bombyx mori*) provided by Bratac Silk Mills Company (Paraná - Brazil). All drugs were granted by Geolab[®], Brazil. The chemical reagents of analytical grade purity were purchased as follows: sodium alginate from Sigma-Aldrich, United Kingdom; calcium chloride (CaCl₂) from Neon, Brazil; sodium phosphate tribasic, sodium hydroxide (NaOH) and hydrochloric acid (HCl) from Dinâmica, Brazil. Ultrapure water (Reverse osmosis, Gehaka, Brazil) was used throughout the study.

2.2 Extraction of sericin from cocoon of silkworm

The methodology for sericin extraction was obtained from Silva et al. (2014). Cocoons were previously cleaned, cut and washed. 40 g of cocoons were added to 1 L of ultrapure water, and then, in autoclave (AV-18, Phoenix, Brazil), it was subjected to 120 °C and gauge pressure of 0.98 bar for 40 min. After this period, the sericin solution was stored in a closed container at room temperature for 12 h, and after that, it was frozen for 24 h. After this time, the solution was thawed at room temperature and then filtered. The concentration of the sericin solution was adjusted to 2.5 % (w/v).

2.3 Drug incorporation and preparation of particles

In order to prepare the sericin/alginate blend, the sericin solution 2.5 % (w/v) was heated at 120 °C in autoclave for 15 min. 2.8 g of sodium alginate was added to 100 mL of sericin solution at 55 °C, and the mixture was stirred at 4,000 rpm until solution gets homogeneous. Then, 2.0 g of drug was added to the sericin/alginate blend and dispersed with an Ultraturrax[®] (T18, IKA, USA) at 8,000 rpm until homogeneity was achieved. As shown in Table 1, particles at four different drugs were prepared using the ionic gelation method, which is based on the ability of alginates to form a gel in the presence of multivalent ions, like Ca²⁺ (Silva et al., 2016). Formulations with sericin, alginate and drug, and blank formulations (without sericin) were prepared to compare the sericin influence on the particles characteristics.

Table 1: Mass composition of sericin/alginate particles with drug incorporated (dry weight basis).

Formulations	Drug	Sericin (%)	Alginate (%)	Drug content (%)
S1	Quetiapine fumarate	34.2	38.4	27.4
B1	Quetiapine fumarate	-	58.3	41.7
S2	Acetylsalicylic acid	34.2	38.4	27.4
B2	Acetylsalicylic acid	-	58.3	41.7
S3	Ibuprofen	34.2	38.4	27.4
B3	Ibuprofen	-	58.3	41.7
S4	Ketoprofen	34.2	38.4	27.4
B4	Ketoprofen	-	58.3	41.7

The mixture of sericin, alginate and drug was added dropwise to a calcium chloride solution (3 % w/v), stirred continuously. This solution concentration aims to keep calcium ions excess, allowing cross-linking of alginate

(exchange with sodium of alginate). Once the solution is saturated with sodium, it is replaced by a new CaCl_2 solution. The saturation is evidenced by the decrease in cross-linking efficiency in particle formation process. After dripping, the particles were kept under stirring at 100 rpm for 30 min, and then washed with deionized water and dried at room temperature.

2.4 Determination of incorporation efficiency

Accurately weighed 0.100 g of dried particles was added to 500 mL of phosphate buffer (pH 6.8), and kept overnight. Hence, the suspension was subjected to sonication for 15 min in a sonicator (1510RMTH, Branson, USA) and filtered. The drug content in the filtrate was determined by spectrophotometer (UVmini1240, Shimadzu, Japan) at 288 nm to quetiapine fumarate (S1 and B1 formulations), 265 nm to acetylsalicylic acid (S2 and B2 formulations), 222 nm to ibuprofen (S3 and B3 formulations) and 258 nm to ketoprofen (S4 and B4 formulation). All determinations were carried out in triplicate. The incorporation efficiency was calculated by Eq(1).

$$\text{Incorporation} = \frac{\text{Practical DS Content}}{\text{Theoretical DS Content}} \times 100 \quad (1)$$

2.5 Morphological examination (SEM)

The surface morphology of all formulations developed and pure drugs was analyzed by scanning electron microscopy (SEM) in electron microscope (440i, Electron Microscope LEO, England).

2.6 Drug polymer interaction study (FTIR)

To evaluate the possible interactions between the drugs and the sericin/alginate blend, Fourier Transform Infrared analysis (FTIR) was performed using a FTIR spectroscope (Nicolet 6700, Thermo Scientific, USA). The measurements were performed in transmittance mode, using the snap-in baseplate attachment, range $4,000 - 400 \text{ cm}^{-1}$ with resolution of 4 cm^{-1} and 32 scans.

3. Results and Discussion

3.1 Incorporation efficiency

Figure 1 shows the effect of particle composition in the drug incorporation efficiency of the formulations prepared in accordance with Table 1. For all cases, it was found that sericin contributed to the increased incorporation efficiency of the drugs into the matrix. This is interesting due the reuse of sericin, a textile industry residue that negatively affects the environmental. Also, a greater amount of drug added will be effectively incorporated into the particle, enabling to save raw material. Furthermore, the ibuprofen and ketoprofen drugs presented higher affinity for the incorporation matrix, with incorporation efficiency of $76.0 \pm 2.1 \%$ for S3 and $82.9 \pm 1.5 \%$ for S4.

Quetiapine presented the lowest incorporation efficiency, maybe due its high pKa (ionization constant) value (6.8) when compared to other drugs (ASA 3.49, Ibu 4.91, Keto 4.45). Sericin/alginate blend and calcium chloride solution present pH 5.5 and 6.5, respectively. Quetiapine pKa is greater than both pH values, indicating it is predominantly in non ionized form during particles production. This characteristic does not favor quetiapine binding to the blend. Acetylsalicylic acid also presented low incorporation efficiency probably due its higher water solubility when compared to the other drugs. This higher water solubility of the ASA may favor the loss of the drug to the aqueous solution CaCl_2 at the time of dripping. Thus, ASA shows great affinity for water than the others, leading to low incorporation efficiency in protein blend.

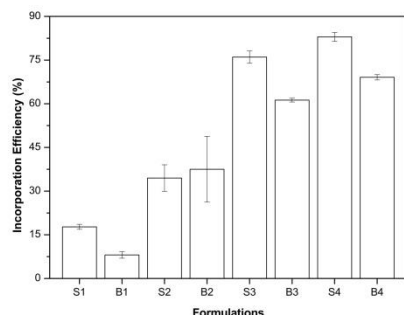


Figure 1: Effect of particle composition in the drug incorporation efficiency.

3.2 Morphological examination (SEM)

The surface morphology of the sericin/alginate/drugs particles (S3 and S4), alginate/drug particles (B3 and B4) and pure drugs (Ibu and Keto) was analyzed by SEM and the result is presented in Figure 2. Spherical particles with a rough surface were observed in B3 (a), S3 (a), B4 (a), and S4 (a) micrographs with magnification of 150x. Drug crystals of ibuprofen and ketoprofen were checked in the cross-section of the particles B3 (b) and S3 (b), for ibuprofen, and B4 (b) and S4 (b), for ketoprofen, at magnification of 3,000x. Thus, it was evident from the SEM micrographs that ibuprofen and ketoprofen were successfully incorporated into the sericin/alginate matrix.

SEM analysis also provided particle diameters for particles with ibuprofen, B3 (a) and S3 (a), and ketoprofen, B4 (a) and S4 (a), as 1.15, 1.26, 1.06, and 1.30 μm , respectively. Although the mean particle diameters are similar, lower values were obtained for alginate/drug particles.

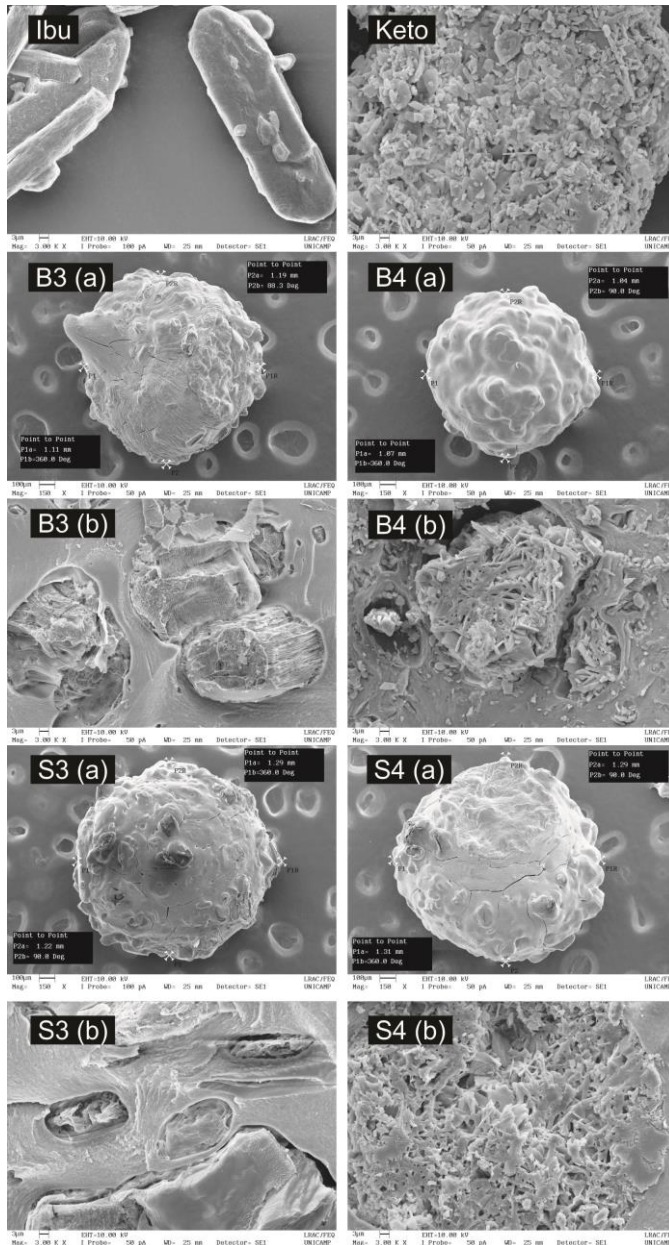


Figure 2: Micrographs of pure drugs ibuprofen (Ibu) and ketoprofen (Keto) (magnification of 3,000x), and formulations content ibuprofen and ketoprofen (S3, B3, S4, B4). (a) Particle with magnification of 150x. (b) Cross-section of the particle with magnification of 3,000x.

3.3 Fourier Transform Infrared analysis (FTIR)

Figure 3 presents the FTIR spectra of sericin/alginate particles without drug (SerAlg), pure drug, alginate/drug particles (B_i), and sericin/alginate/drug particles (S_i). In the spectrum, (a) is for ibuprofen and (b) is for ketoprofen. Pure ibuprofen (Ibu) presented several characteristics peaks. High intensity peaks at 1721 cm⁻¹ and 1231 cm⁻¹ are assigned to the C=O stretching and C-O stretching, respectively (Salmoria et al., 2016). The range of 1460 – 1550 cm⁻¹ corresponds to aromatic C=C bonds (Kamari and Ghiaci, 2016). The presence of an intense peak at 779 cm⁻¹ is specific to aromatic stretching bending vibration (Tihan et al., 2016). These peaks were also verified in the formulations B3 and S3, confirming the drug incorporation.

In the FTIR spectrum of pure ketoprofen (Keto), the characteristics peaks appeared at 1697 cm⁻¹ and 1655 cm⁻¹, for dimeric carboxylic acid carbonyl group stretching and the ketonic carbonyl stretching vibration, respectively (Khan et al., 2013). In addition, a peak at 1598 cm⁻¹ related to the aromatic C=C bonds and a peak at 1445 cm⁻¹ related to the C-C stretch in ring were observed (Das et al., 2016). The range of 860 – 640 cm⁻¹ is attributed to the presence of aromatic ring. Like ibuprofen, the drug incorporation is confirmed by similar peaks verified in the formulations B4 and S4.

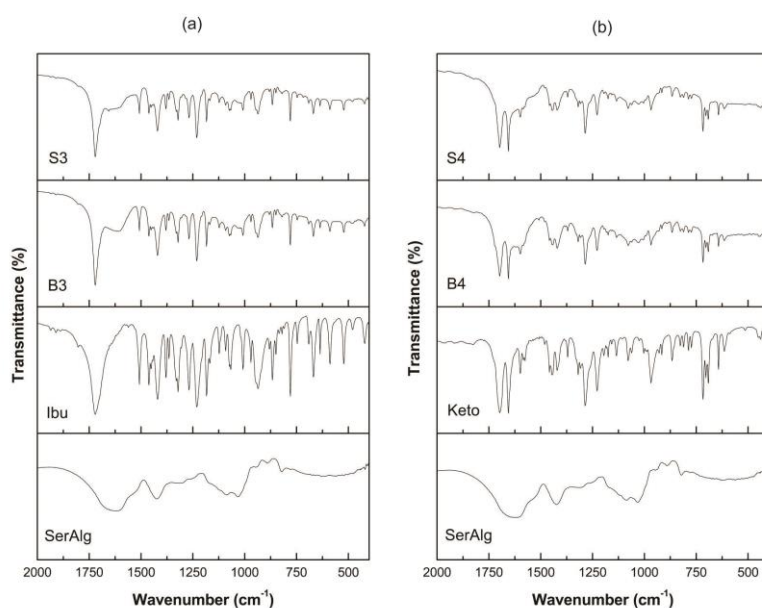


Figure 3: FTIR spectra of sericin/alginate particles without drug (SerAlg), pure drug, alginate/drug particles (B_i) and sericin/alginate/drug particles (S_i), for (a) ibuprofen and (b) ketoprofen.

4. Conclusions

In this work, particles containing drugs were successfully prepared employing ionic gelation technique. The sericin favours the drug incorporation, as verified by the increasing incorporation efficiency in the particles with sericin and alginate for all developed formulations. The ibuprofen and ketoprofen drugs presented higher affinity for the incorporation matrix when compared to the quetiapine fumarate and acetylsalicylic acid. Drug incorporation was confirmed by SEM and FTIR analyses considering the presence of drug crystals in the particle morphology and characteristics peaks, respectively.

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