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Sericin and Alginate Blend as Matrix for Incorporation of Diclofenac Sodium

Jacyara M. M. Vidart[a], Mariana Nakashima[a],Thiago L. da Silva[a], Paulo C. P. Rosa[b], Marcelino L. Gimenes[c], Melissa G. A. Vieira[a], Meuris G. C. da Silva[a]*

[a]School of Chemical Engineering, University of Campinas, UNICAMP, 13083-825, Campinas – SP, Brazil
[b]School of Pharmaceutical Sciences, University of Campinas, UNICAMP, 13083-859, Campinas – SP, Brazil
[c]Department of Chemical Engineering, State University of Maringá, UEM, 87020-900, Maringá – PR, Brazil
meuris@feq.unicamp.br

Diclofenac sodium (DS) is a non-steroidal anti-inflammatory widely used clinically and has recurring side effects in gastrointestinal system. In addition, it requires multiple daily doses to maintain its therapeutic drug-blood level. Therefore, the modification of the form of presentation of the DS becomes desirable to improve patient compliance and decrease side effects. Sericin is a globular protein that offers many desirable characteristics for the incorporation of drugs. It is presented in the silkworm cocoons (*Bombyx mori*) and is usually discarded in the wastewater of degumming silk processing. Sodium alginate is a natural polysaccharide extracted from brown seaweed that has an abundant use in drug delivery systems. The blend of sericin and alginate may provide characteristics more suitable for improving the encapsulation. The aim of the current work is to evaluate the content of DS incorporated in sericin/alginate particles. The sericin solution was obtained by degumming process in autoclave (1 kgf/cm^2, 40 min) and the sodium alginate used was of analytical grade (Sigma-Aldrich). Blends of sericin and alginate with DS incorporated were produced in various formulations. These blends were dripped in $CaCl_2$ solution, in the ionic gelation technique, in order to produce the particles. The different formulations used were compared by evaluating the efficiency of incorporation of DS in the sericin/alginate particles. Additionally, the surface morphology was analyzed by SEM - Scanning Electron Microscopy, and the size of the particles was evaluated by OM - Optical Microscopy. The results showed that the efficiency of incorporation reached values in the range of 91.1 ± 2.4 % to 75.5 ± 2.1 %, and the SEM analysis proved the incorporation of the drug in the blend matrix.

1. Introduction

Sericin is a water-soluble globular protein that is easily soluble in hot or boiling water and is extracted from the silkworm *Bombyx mori* cocoons (Cao and Zhang, 2016). Most of the sericin is removed during the silk processing and it is usually discarded in the wastewater (Zhang, 2002).This fact leads to environmental contamination due to the high oxygen demand for its degradation by microbes (Aramwit et al., 2012). Therefore, find viable means to recover sericin would bring environmental and economic benefits (Aramwit et al., 2012). Sericin is a biocompatible and biodegradable protein, besides being digestible because of its susceptibility to the action of proteolytic enzymes present in the human organism (Padamwar and Pawar, 2004). To improve its properties, sericin can be cross-linked, copolymerized and blended with other polymers due to its polar side chain made of hydroxyl, carboxyl and amino groups (Dash et al., 2009). Because of its characteristics, sericin has wide applications in medical, pharmaceutical and cosmetic industries (Padamwar and Panwar, 2004).

Alginate is a nontoxic, biocompatible and biodegradable polysaccharide polymer (Dalaty et al., 2016). Alginate has muco and bioadhesive properties, is resistant to acidic pH and has chemical versatility that makes possible modifications in its properties (Sosnik, 2014). Thanks to these aspects, alginate has been widely used in many pharmaceutical applications, such as development of mucoadhesive and controlled release

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delivery systems for drugs and proteins (Dalaty et al., 2016). Therefore, the sericin/alginate blend can provide good conditions for drug incorporation (Khandai et al., 2010).
Diclofenac sodium (DS) is a non-steroidal anti-inflammatory drug widely prescribed for its analgesic and anti-inflammatory properties (Das and Subuddhi, 2015). This drug is used in the treatment of rheumatoid arthritis and osteoarthritis (Sinha et al., 2015). The biological half-life of DS is around 1 - 2 h and requires multiples dosing to maintain the therapeutic drug-blood level, what can cause adverse effects as gastritis, peptic ulceration and depression of renal functions (Nayak and Pal, 2011). A new matrix for DS can enable a sustained drug release with a minimal toxicity to the organism, which can reduce the drug side effects (Dutta and Sahu, 2012).
In the present work, sericin/alginate particles with diclofenac sodium were prepared by ionic gelation method and the incorporation efficiency of DS was evaluated. The surface morphology was analyzed by Scanning Electron Microscopy (SEM) and the particles size was examined by Optical Microscopy (OM).

2. Materials and Methods

2.1 Materials

The cocoons of silkworm were ceded by Bratac Silk Mills Company, Paraná, Brazil. Diclofenac sodium was provided by Pharmanostra, São Paulo, Brazil. Sodium alginate was purchased from Sigma-Aldrich, United Kingdom. Calcium chloride ($CaCl_2$) was supplied by Neon, Brazil. Sodium phosphate tribasic, sodium hydroxide (NaOH) and hydrochloric acid (HCl) were provided by Dinâmica, Brazil. All the reagents were of analytical reagent grade purity. Deionized water (Milli Q) was utilized throughout the study.
In order to investigate the DS efficiency incorporation, a simulated intestinal fluid (phosphate buffer pH 6.8) was freshly prepared by dissolving 38 g sodium phosphate tribasic in 500 mL of deionized water, followed by the addition of 1,500 mL HCl 0.1 mol/L. The pH was finally adjusted to 6.8 with NaOH 1 M or HCl 1 mol/L.

2.2 Extraction sericin from cocoon of silkworm

Sericin was extracted from silkworm cocoons employing the method presented in Silva et al. (2014). Firstly, 40 g of cocoons, previously cleaned and cut, were added to 1 L of deionized water, and then, subjected to 120 °C and gauge pressure of 1 kgf/cm^2 for 40 min in autoclave (AV-18, Phoenix, Brazil). The sericin solution extracted was stored in a closed container at room temperature for 12 h, and thereafter, was frozen for 24 h. After this period, the solution was thawed at room temperature and 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 70 °C in autoclave for 10 min. The sodium alginate was added to the sericin solution at 55 °C, and the mixture was stirred at 4,000 rpm. Then, DS was added to the sericin/alginate blend and dispersed with an Ultraturrax® (T18, IKA, USA) at 8,000 rpm until homogeneity was obtained. As shown in Table 1, particles at four different formulations were prepared using the ionic gelation method. The mixture of sericin, alginate and DS was added dropwise to a calcium chloride solution (3 % w/v), stirred continuously. 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.

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

Formulation	Sericin (g)	Alginate (g)	DS (g)
F1	2.5	1.25	2.0
F2	2.5	2.60	2.0
F3	2.5	3.30	2.0
F4	-	4.00	2.0

2.4 Determination of incorporation efficiency

Accurately weighed 0.1 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 through a 0.45 µm filter. The DS content in the filtrate was determined by spectrophotometer (UVmini1240, Shimadzu, Japan) at 276 nm. All determinations were carried out in triplicate. The incorporation efficiency was calculated by Eq(1).

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

2.5 Morphological examination and particles size

The surface morphology of the formulated particles was analyzed by scanning electron microscopy (SEM). The samples were metalized with a thin layer of gold. The photomicrographs were taken at an acceleration voltage of 10 kV in electron microscope (440i, Electron Microscope LEO, England).

The particle size of the prepared formulations was determined by optical microscopy (DC4-456H, National, EUA) using the software ImageJ (Dhirhi et al., 2016). The diameter of 500 particles was measured and the mean diameter was calculated for each formulation. Besides, the particle size distribution was investigated and the Gaussian model was adjusted.

3. Results and Discussion

3.1 Incorporation efficiency

Figure 1 shows the effect of sericin and alginate concentration in the DS incorporation efficiency of the formulations prepared in accordance with Table 1. The incorporation efficiencies found were 91.1 ± 2.4 % to F1, 82.5 ± 3.6 % to F2, 77.9 ± 3.7 % to F3 and 75.5 ± 2.1 % to F4. It was noticed that the incorporation efficiency increased by increasing sericin proportion in the blend; therefore, sericin significantly contributes to the incorporations of the DS.

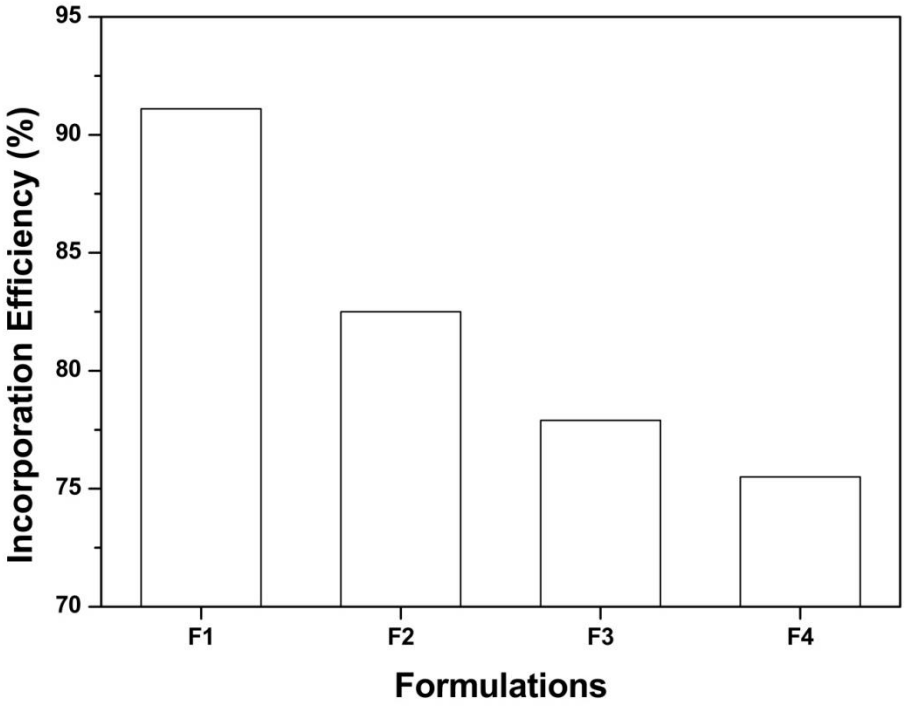

Figure 1: Effect of blend composition in the DS incorporation efficiency

3.2 Morphological examination (SEM)

The surface morphology of the sericin/alginate/DS particles (F1, F2 and F3) and alginate/DS particles (F4) was visualized by SEM and is presented in Figure 2. By analyzing the F1 micrograph with magnification of 150×, it was found that it has no clear-cut boundaries and has poorly defined shape, what does not favor the reproducibility of these particles. Spherical particles with a rough and rugged surface were observed in F2 and F3 micrographs with magnification of 150×. F4 micrographs indicated oval particles and a very rough and rugged surface too. Thus, it was evident from the SEM micrographs that balanced concentrations of sericin and alginate, as in F2 and F3, favor the sphericity of the particles. Drug crystals of DS were seen on the cross-section of the particles at magnification of 3,000× for all formulations. Besides, it is verified that roughness increased by increasing alginate proportion in the blend. It can be inferred that the F4 particles (alginate/DS) possibly release the drug in its dissolution medium (gastric or enteric) more rapidly when compared to particles containing sericin in its composition, since the surface has greater roughness and therefore, its contact surface is higher. Consequently, particles containing sericin can contribute to the sustained release of the DS present in the matrix and maybe reduce the drug side effects.

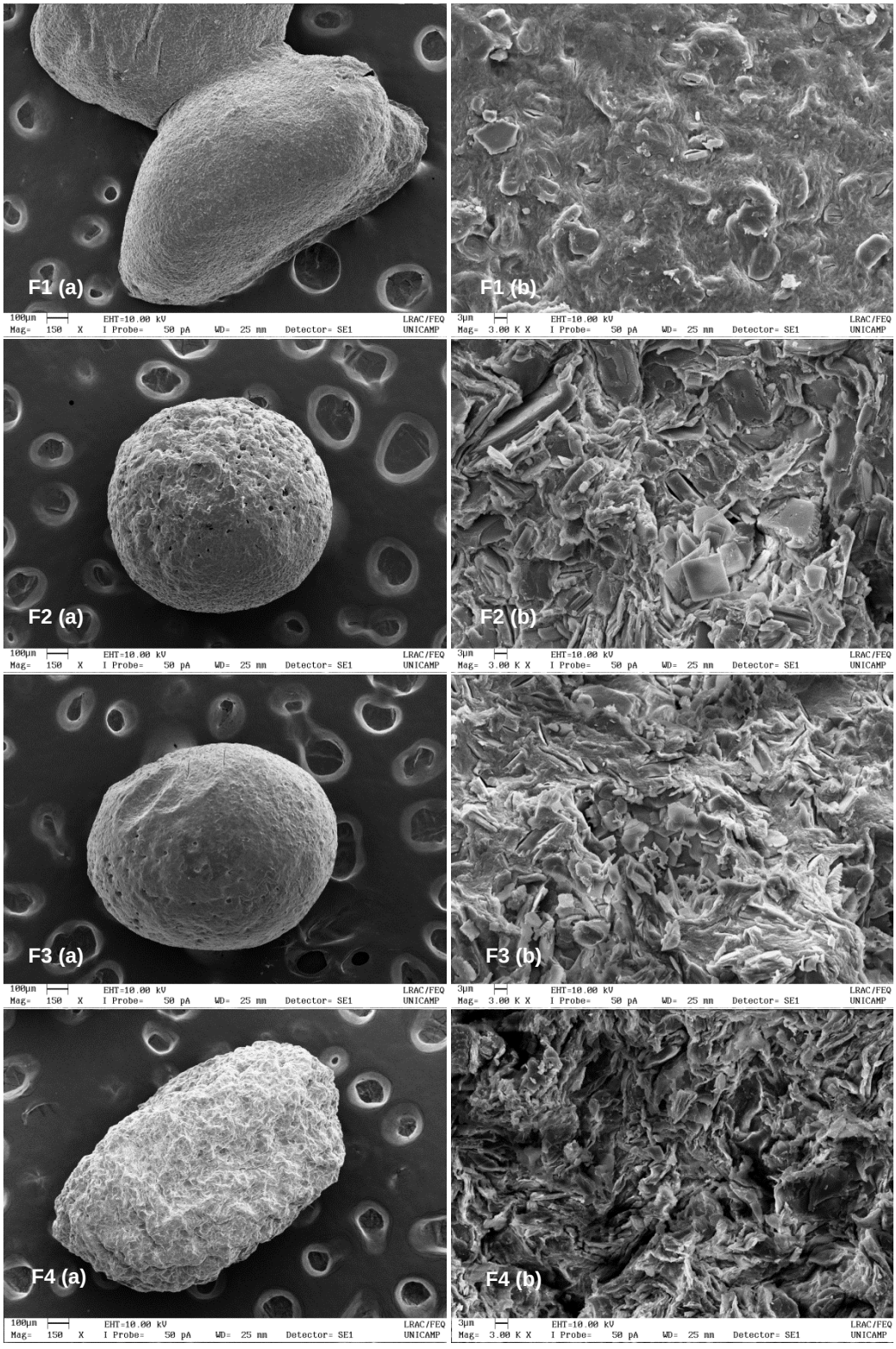

Figure 2: Micrographs of formulations evaluated (as shown in table 1). (a) Particle with DS incorporated (Magnification of 150x). (b) Cross-section of the particle (Magnification of 3,000x)

3.3 Particles size

Figure 3 shows the effect of blend composition on particle size distribution and Table 2 presents the mean diameter of particles, in conjunction with the adjusted coefficient of determination for normal (Gaussian)

distribution fitting. By analyzing Figure 3, it can be seen that the size distribution of particles obtained for all formulations, except for F1, follow the normal distribution. The values of the mean diameters obtained for each formulation and the adjusted coefficient of determination (R_a^2) are shown in Table 2, from which it is verified that the best fit is given to particles F2 with $R_a^2 = 0.9885$. This result may indicate a better reproducibility of F2 particles when compared to the others, once its distribution is symmetrical.

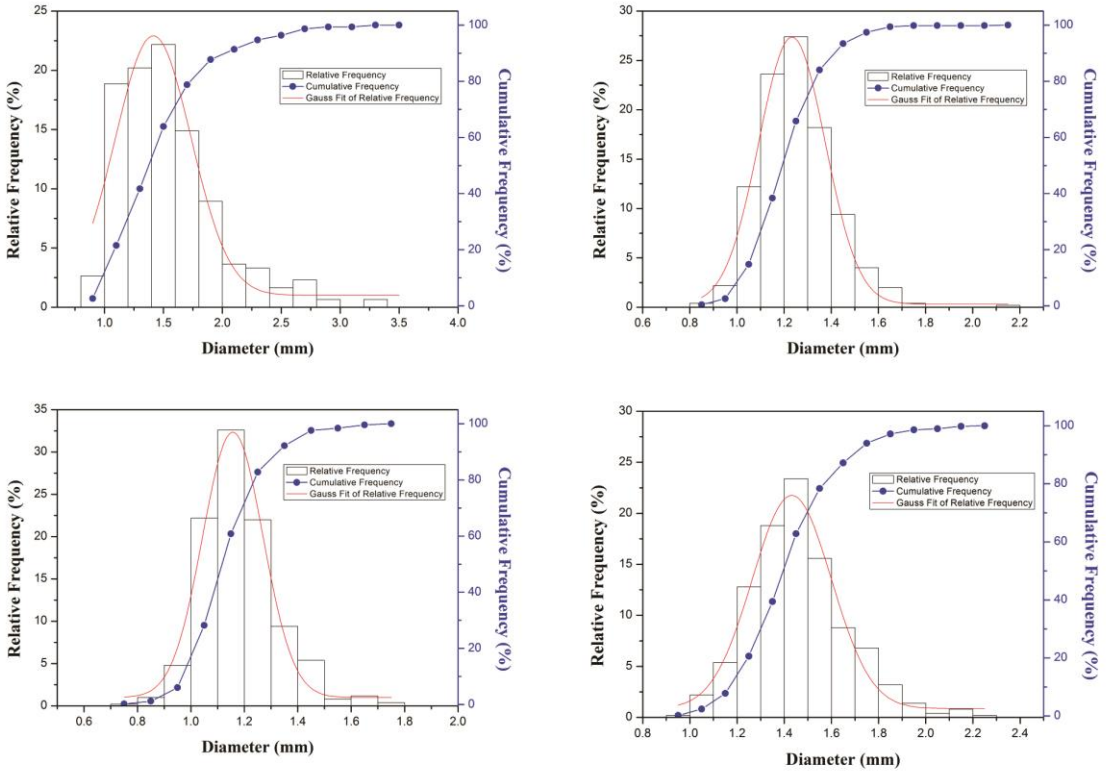

Figure 3: Effect of blend composition on particle size distribution.

Table 2: Particles size and distribution fitting.

Formulation	Mean Diameter (mm)	R_a^2
F1	1.55 ± 0.42	0.9274
F2	1.26 ± 0.15	0.9885
F3	1.18 ± 0.14	0.9759
F4	1.46 ± 0.20	0.9704

By the evaluation of all the results presented, it was found that F2 and F3 particles showed the best properties for drug incorporation. These formulations exhibited good incorporation efficiency values, spherical particles with well-defined shape and size distribution according to normal distribution indicating increased reproducibility.

Although F1 incorporation efficiency is the highest obtained from all the formulations developed, it was found from SEM analysis that it does not present a well-defined shape, which may limit the reproducibility of these particles, just as was observed by the size distribution curve and higher standard deviation of the mean diameter.

F4 particles presented lower incorporation efficiency among all the other formulations, indicating the influence of sericin in the DS incorporation. The SEM micrographs showed an oval shape and higher roughness, which can negatively influence the drug release. The particle size distribution following a normal distribution model indicates that the particles are reproductive.

4. Conclusions

In this study, sericin/alginate particles containing diclofenac sodium were successfully prepared employing ionic gelation method. The presence of sericin in the particles composition favors the drug incorporation, as verified by the increasing incorporation efficiency with the increasing sericin proportion in the blend. The SEM analysis showed that balanced concentrations of sericin and alginate, as in F2 and F3, favor the sphericity of the particles, and that the roughness surface increased by the increasing alginate proportion in the blend. Therefore, particles containing sericin can contribute to the sustained release of the DS present in the matrix and can possibly reduce the drug's side effects. For all formulations, except F1, the values of adjusted coefficient of determination were close to unity to normal fit modeling, indicating that the particle size distribution follows normal distribution and that the particles are reproductive. F2 and F3 particles showed the best properties for drug incorporation by the evaluation of all results presented.

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