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/CET1543138

Evaluation of Drug Delivery of Diclofenac Sodium in Simulated Gastric and Enteric Systems by Mucoadhesive Sericin-Alginate Particles

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

[a]University of Campinas, Dept. of Products and Processes Design, Rua Albert Einstein, nº 500, 13083-852, Campinas – São Paulo – Brazil; tel. +55 19 35213928.
[b]State University of Maringá, Dept. of Chemical Engineering, Avenida Colombo, nº 5790, 87020-900, Maringá – Paraná – Brazil, tel. +55 44 3011476.
meuris@feq.unicamp.br

Diclofenac sodium (DS) is a very common non-steroidal anti-inflammatory drug used in the treatment of rheumatoid disorders and others inflammatory diseases. The required repeated daily dosages can cause damages to gastrointestinal, hepatic, cardiac systems, and others. Besides, DS is unstable in acid condition, what can decrease the therapeutic effect due the degradation of the drug. Sodium alginate is a natural polysaccharide extracted from brown seaweed that has an abundant use in drug delivery systems. The bioconjugation of sericin, a hydrophilic protein present in silkworm cocoons (Bombyx mori), with polymers has provided new drug delivery methods with reduced immunogenicity and increased drug stability. In this work particles of sericin-alginate blend with sodium diclofenac incorporated were produced by ionic gelation technique (dripping the blend in calcium solution). The release kinetic study was investigated at pH 1.2 and 6.8, simulating the pH of gastric and enteric systems and the obtained data were evaluated by different release mechanisms models. The zero order, first order, second order, Weibull, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, Baker-Londale, quadratic, logistic and Gompertz mathematical models were evaluated. The kinetic study showed that the amount of DS release versus time in gastric pH was almost negligible when compared with the enteric system. The drug release mechanism that best fit the enteric system was Gompertz model and to gastric system, the Logistic model fit the data with high value of R^2, both values close to 1. It was concluded that the particles produced with sericin/alginate with DS incorporated can protect the DS in acid condition and improve the DS release in enteric simulated condition.

1. Introduction

Diclofenac sodium is a non-steroidal anti-inflammatory drug widely used in the treatment of rheumatoid disorders and other chronic inflammatory diseases (Dutta and Sahu, 2012). The rapid metabolism and elimination of DS, and its short half-life (1 - 2 h) requires many dosages daily, what can cause damages in cardiac, gastrointestinal, hepatic and renal systems (Dutta and Sahu, 2012), furthermore, the incidence of side effects with the use of diclofenac reaches about 20% of patients (Santos et al., 2007). An alternative to avoid the irritation of gastric mucosa is use substances gastric resistant capable of protecting the stomach through the action of the drug (Santos et al., 2007).
Sericin is a family of silk protein of silkworms that glue fibroin fibers together to form the silkworm`s cocoons (Teramoto et al., 2012). The sericin protein is a hydrophilic water-soluble protein made of 18 amino acids most of which have strongly polar side groups such hydroxyl, carboxyl, and amino groups and its molecular weight ranges from 10 (Zhang, 2002) to over 400 kDa (Silva et al. 2014a) and all its properties enable to protein easy cross-linking, copolymerization, and blending with other polymers to form improved biodegradable materials

Please cite this article as: da Silva T.L., da Silva Junior A.C., Martins J.M., Vieira M.G.A., Gimenes M.L., da Silva M.G.C., 2015, Evaluation of drug delivery of diclofenac sodium in simulated gastric and enteric systems by mucoadhesive sericin-alginate particles, Chemical Engineering Transactions, 43, 823-828 DOI: 10.3303/CET1543138

(Turbiani et al., 2011). Bioconjugation with natural or synthetic polymers provides methods of delivering drugs such as peptides, enzymes, and oligonucleotides and it is advantageous because it leads to reduced immunogenicity and improved stability (Kundu et al., 2008). Sericin can be conjugated due to the presence of surface-active groups (–OH, –COOH, and $–NH_2$), which can form covalent links with the conjugate (Kundu et al., 2008).

Alginate is a water-soluble linear polysaccharide (biopolymer) extracted from brown seaweed that has abundant applications in drug delivery systems, cell encapsulation, food industry, cosmetics, etc (Khandai et al., 2010). As well as sericin, this polysaccharide are easy available, cheap and biodegradable. Alginates can be considered as block polymers, which mainly consist of mannuronic acid (M), guluronic acid (G) and mannuronic-guluronic (MG) blocks (Das and Senapati, 2008). Alginate gel beads are commonly formed by dripping sodium alginate solution into an aqueous solution of calcium ions typically made from calcium chloride (ionic gelation technique) (Kuo and Ma, 2001). The dried alginate beads have the property of reswelling and thus they can act as controlled release system (Das and Senapati, 2008).

In the present work, particles of sericin-alginate blend with sodium diclofenac incorporated was produced by ionic gelation technique and then the drug released in system at pH 1.2 (simulated gastric system) and 6.8 (simulated enteric systems) were investigated. The obtained data were evaluated in different mathematical release models in order to determine the best one that describe the drug release in gastrointestinal conditions. In this work zero order, first order, second order, Weibull, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, Baker-Lonsdale, quadratic, logistic and Gompertz mathematical models were investigated.

2. Materials and Methods

The Bombyx mori silkworm´s cocoons were provided by Bratac Silk Mills Company, Paraná – Brazil. The cocoons were manually cleaned and cut into small pieces (about 1 cm^2). The pieces were washed in tap water and rinsed, abundantly, with deionized water. The cleaned cocoons were dried overnight at 50 ºC. The sericin solution was obtained degumming silkworm cocoons with ultrapure water (4.5 g of dried cocoon: 100 mL of ultrapure water), using an autoclave at 120 ºC (1 kgf/cm^2) for 40 min (Silva et al., 2014b). The sericin solution was stored in sealed container at room temperature for 12 h to stabilize the hydrogel. After this period, the solution was frozen in conventional refrigerator for a minimum of 24 h, and then the solution was thawed at room temperature. This procedure promotes the precipitation of protein that was separated by filtration. The solution was again heated in autoclave (1 kgf/cm^2, 10 min) to solubilize the precipitated protein and then the solution concentration was adjusted to desired values (Silva et al., 2014b).

2.1 Preparation of particles

The sodium alginate was added in concentration of 1.0 g/L in the adjusted sericin solution (2.5 g/L) at 40 ºC. Posteriorly, DS (0.05 g of DS/100mL of blend) was added at the blend, between sericin and alginate, and the mixture was gently stirred at 150 rpm, in order to avoid the bubble air formation, for 2h (mechanical stirrer – Fisatom, mod 715). Particles without DS were also produced and evaluated (formulation of control).

The particles were prepared by ionic gelation process. The blend was dripped by a Syringe Pump (Samtronic Infusion Systems, mod 670, 50 mL syringe, 40 mm X 1.2 mm needle) in aqueous $CaCl_2$ solution (3% w/V). The particles were cross-linked in calcium solution for 30 min, then, they were washed in deionized water and dried at room temperature.

2.1 Cal ibration Curves

Buffer DS solutions with concentrations of 0.0255, 0.0510, 0.1020, 0.1530, 0.2040 and 0.2550 mg/mL were prepared in pH 1.2 and 6.8. According to Kleinubing et al. (2014), the pHs 1.2 and 6.8 simulate the gastric and enteric system. The calibration curves were determined in a spectrophotometer (UV mini 1240 Shimadzu – Japan) at λ = 285 nm. The curves were used to determine the amount of DS in solution in drug release experiments at pH 1.2 and 6.8.

2.2 Buffer solutions (pH 1.2 – gastric system; pH 6.8 – enteric system)

Buffer solutions were used in solutions of calibration curve and in drug release experiments (concentration *versus* time). Buffer solution of pH 1.2 was prepared as follow: 13.8 g of sodium phosphate monobasic salt were solubilized in 1000 mL of ultrapure water (Mili-Q) and then the pH was adjusted with sodium phosphate dibasic salt (10 mmol/L). Buffer solution of pH 6.8 was prepared as follow: 13.8 g of sodium phosphate monobasic salt were solubilized in 1000 mL of ultrapure water (Mili-Q) and then the pH was adjusted with concentrated phosphoric acid. All reagents were of analytical grade.

2.3 In vitro drug released experiments.

The profile of the release of diclofenac sodium (DS) was performed in an environment that simulates the gastric and enteric (intestinal) conditions. The concentration of DS released from particles of sericin/alginate/DS in

buffer solutions of pH 6.8 (System 1) and pH 1.2 (System 2) were determined by spectrophotometer Shimadzu model UVmini1240 (Japan), λ = 285 nm.

- System 1: DS release from alginate/sericin/DS particles in buffered solution of pH 6.8 (enteric system).
- System 2: DS release from alginate/sericin/DS particles in buffered solution of pH 1.2 (gastric system).

The release kinetic study was carried out with the control formulation and formulation F, described in item 2.1, according to Khandai et al. (2013) and Kleinubing et al. (2014), with some modifications. Approximately 50 mg of each formulation was added into 100 mL of pH 6.8 and in pH 1.2 buffer solutions in a Erlenmeyer flask and then stirred in an orbital shaker water bath (Orbital Shaker Bath -Model: 3540 - USA). The speed of the bath and the temperature were maintained at 100 rpm and 37 ± 0.5 °C, for 24 h. Aliquots of 1 mL were taken every 30 min interval during the first 5 h, then the penultimate aliquot were taken at 10 h and the last one at 24 h, according the procedure adopted by Kleinubing et al. (2014). For each aliquot removed the same volume of fresh medium was added at 37 ± 0.5 °C. The removed samples were diluted 1:4 and filtered with Millipore Millex-GV filter (0.22 microns) and then the DS concentration were determined using the spectrophotometer.

2.4 Mathematical Modeling

In order to evaluate the drug release in gastric and enteric conditions, different mathematical models were applied to represent the data obtained in the release experiments in systems 1 and 2. Table 1 presents the mathematical models applied in this work. More information about these mathematical models used to describe the dissolution profile of drugs are present in Polli et al. (1997), Costa and Lobo (2001) and Kleinubing et al. (2014).

Table 1: Mathematical models used to describe the drug dissolution curve.

Model	Equation	Parameters
Zero order	$Q_t = Q_0 + K_0 t$	Q_t: amount of drug dissolved in time t, Q_0: initial amount of drug in the solution (most times, Q_0=0), K_0: zero order release constant
First order	$lnQ_t = lnQ_0 + K_1 t$	Q_t: amount of drug released in time t, Q_0: initial amount of drug in the solution, K_1: first order release constant.
Second order	$Q_t/Q_\infty(Q_\infty - Q_t) = K_2\, t$	Q_t: amount of drug released at time t, Q_∞: total amount of drug in the solution, K_2: second-order constant release.
Weibull	$\log[-ln(1-m)] = b.\log(t - T_i) - \log a$	m: cumulative fraction of drug, a: time scale of the process, T_i: time interval before the beginning of the dissolution or release process, b: shape parameter characterized as exponential curve (b = 1), sigmoidal (b> 1) or parabolic (b <1).
Higuchi	$Q_t = K_H\sqrt{t}$	K_H: Higuchi dissolution constant
Hixson-Crowell	${W_0}^{1/3} - {W_t}^{1/3} = K_S t$	W_0: initial amount of drug in the pharmaceutical dosage form, W_t: remaining amount of drug in the pharmaceutical dosage form at time t, K_S: constant incorporating the surface–volume relation.
Korsmeyer-Peppas	$\frac{M_t}{M_\infty} = a.t^n$	a: constant incorporating structural and geometric characteristics of the drug dosage form, n: release exponent indicative of the drug release mechanism, M_t/M_∞ (fractional release of drug).
Baker-Londale	$\frac{3}{2}\left[1 - \left(1 - \left(\frac{Q_t}{Q_\infty}\right)\right)^{2/3}\right] - \frac{Q_t}{Q_\infty} = k.t$	Q_t: drug released amount at time t, Q_∞: amount of drug released at an infinite time, k: release constant that corresponds to the slope.
Quadratic	$Q_t = 100\,(K_1 t^2 + K_2 t)$	The parameters K_1 and K_2 are parameters of the quadratic model.
Logistic	$Q_t = A/\left[1 + e^{-K(t-\gamma)}\right]$	The parameters A, K and γ are parameters of the logistic model.
Gompertz	$Q_t = A.e^{-e-K(t-\gamma)}$	The parameters A, K and γ are parameters of the Gompertz model.

The most appropriate model selection criterion was based on the higher value of determination coefficient ($R^2_{Adjusted}$) calculated according to equation: $R^2_{Adjusted} = 1 - [(n-1)/(n-p)].(1-R^2)$, according to Costa and Lobo (2001). In this equation, *n* is the number of dissolution data points and *p* is the number of parameters in the model. Whereas the R^2 always increases or at least stays constant when adding new model parameters, $R^2_{adjusted}$ can actually decrease, thus giving an indication if the new parameter really improves the model or might lead to over fitting. In other words, the "best" model would be the one with the highest adjusted coefficient of determination (Costa and Lobo, 2001).

3. Results and Discussion

Table 2 shows the values of release constants, parameters and correlation coefficients (R^2 and $R^2_{Adjusted}$) obtained by mathematical modelling of zero order, second order, Weibull, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, Baker-Londale, quadratic, logistic and Gompertz models to System 1 (enteric condition) and System 2 (gastric condition).

Table 2: Parameters set of mathematical models and correlation coefficient to system 1 and 2.

Model	Release constant and parameters				R^2	$R^2_{Adjusted}$
Zero order	K_0					
System 1	0.0008	-	-	-	0.6642	0.6642
System 2	5.89x10^{-6}	-	-	-	0.5927	0.5927
First order	K_1					
System 1	0.0028	-	-	-	0.2003	0.2003
System 2	0.0014	-	-	-	0.4155	0.4155
Second order	K_2					
System 1	0.0038	-	-	-	0.0287	0.0287
System 2	0.0002	-	-	-	0.6247	0.6247
Weibull	-	B	A	-		
System 1	-	1.0542	12.77	-	0.9501	0.9455
System 2	-	1.0643	1223.5	-	0.9688	0.960
Higuchi	K_H					
System 1	0.0612	-	-	-	0.6642	0.6642
System 2	0.0005	-	-	-	0.6262	0.6262
Hixson-Crowell	K_S					
System 1	0.0102	-	-	-	0.6642	0.6642
System 2	9x10^{-5}	-	-	-	0.6262	0.6262
Korsmeyer-Peppas	n		A			
System 1	0.9173	-	0.0028	-	0.8824	0.8717
System 2	0.7855	-	0.000051	-	0.8808	0.8699
Baker-Londale	K					
System 1	0.004	-	-	-	0.9160	0.9160
System 2	2x10^{-8}	-	-	-	0.8988	0.8988
Quadratic	K_1	K_2				
System 1	-5.004	0.00102	-	-	0.9576	0.9537
System 2	-4.41x10^{-9}	8.74x10^{-6}	-	-	0.9472	0.9424
Logistic	K		A	γ		
System 1	0.01408	-	41.1030	215.1581	0.9902	0.9882
System 2	0.01426	-	0.33492	205.63	0.9912	0.9894
Gompertz	K		A	γ		
System 1	0.00845	-	42.0068	170.8656	0.9931	0.9917
System 2	0.00866	-	0.34152	161.0922	0.9887	0.9864

For System 1, both R^2 values of Logistic and Gompertz models tested showed values close to unity. Thus, these two models can represent the dissolution profile (drug release) of the DS to System 1 (simulated enteric system). Also to System 1, for the Logistic and Gompertz models, it is observed that the $R^2_{Adjusted}$ decreases when compared to R^2). Greater values of R^2 of Gompertz and Logistic models are related with the number of

parameters of these models which is higher than the number of the other analysed models parameters in this study. According to Costa and Lobo (2001) the R^2 (coefficient of determination) is a common method to evaluate the "fit" of a model equation. Still according to the authors, usually, the R^2 value tends to get greater with the addition of model parameters, irrespective of the significance of the variable added to the model. However, for the same number of parameters the coefficient of determination can be used to obtain the best model equation to fit the profile of drug released. When evaluating models with different numbers of parameters the $R^2_{Adjusted}$ (adjusted correlation coefficient) is more appropriate. In this case, $R^2_{Adjusted}$ of both Logistic and Gompertz models have shown greater values than that of the other models. Comparing the best two models to System 1, it is observed Gompertz model values of R^2 and $R^2_{Adjusted}$ slightly higher than the ones of Logistic model.

Also, the same value of R^2 and $R^2_{Adjusted}$ observed in zero order, first order, second order, Higuchi, Hixson-Crowell and Baker-Lonsdale models are related with the number of the parameters of these mathematical models. In these models the number of parameters is 1 and by the $R^2_{Adjusted}$ equation (topic 2.4) it can be seen that this way $R^2_{Adjusted} = R^2$.

For System 2, as observed for the System 1, R^2 and $R^2_{Adjusted}$ values were higher when the data were adjusted by the Logistic and Gompertz models. Comparing all the models presented in Table 2, the Logistic and Gompertz models have shown higher values of correlation coefficient, closer to 1. To System 2, the Logistic model presents a correlation coefficient slightly higher than the one of Gompertz model.

The data present in Table 2 show that, with exception of Gompertz and Logistic models, all others have failed to fit the DS release data from sericin/Alginate/DS particles, in gastric and enteric conditions. In general, the models with one parameter presented the worst results of correlation coefficient. The models with two parameters presented better results than the one with one parameter, but, worst results than the ones obtained by Logistic and Gompertz models (with three parameters). This fact is according to Costa and Lobo (2001), previously discussed in this work.

Figure 1 shows a graphical representation of Logistic and Gompertz mathematical modelling of released data of DS from particles in System 1 and 2.

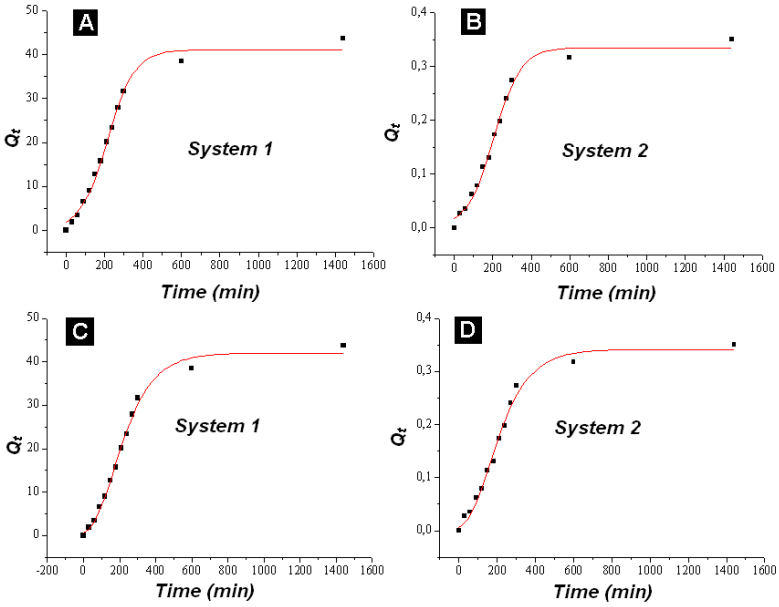

Figure 1: Profile of release of DS from particles of sericin/alginate/DS in Systems 1 and 2, fitted by Logistic model (A and B) and Gompertz model (C and D).

As previously noted by the correlation coefficient (Table 2), in Figure 1 it is observed that the mathematical modelling curve produced by Logistic and Gompertz models showed a good fit of the data on System 1 and 2. Also, it is observed, comparing the profile of system 1 and system 2, that the amount of DS dissolved in System 2 are very lower than the amount dissolved in System 1. The amount of DS released in gastric medium was almost insignificant when compared with the amount released in enteric medium. This is a very important fact because it shows that in acid condition, the particle produced from sericin and alginate can retard the release of DS. The development of capsules, polymeric material or blends that protect the drug from degradation in acid

medium promotes an increasing of therapeutic effect because a greater amount of drugs can reach the intestine, where the substance is absorbed, and protects the gastric mucosa irritations characteristics of this drug (Santos *et al*. 2007). Also, according Palomo et al. (1999) diclofenac sodium undergoes an intramolecular cyclization under the acidic conditions, found in gastric juices, which can cause its inactivation. In this study, the particles produced from sericin and alginate blend were able to protect the DS in acid condition and release the drug in simulated enteric system.

4. Conclusions

In this study, the mathematical Logistic and Gompertz models fit, satisfactorily, the experimental data of release drug in gastric and enteric systems. Even in gastric simulated system, where the DS dissolution was very small, the Logistic and Gompertz model could describe the experimental data. To System 1 and 2, the values of correlation coefficients (R^2 and $R^2_{Adjusted}$) were close to unity to Logistic and Gompertz fit modelling. The other mathematical modelling studied in this work did not fit adequately the profiles of DS release. The results showed that the particles produced from sericin/alginate blend were able to protect the DS from acid degradation and was able to release the drug to enteric medium, which can cause a better anti-inflammatory effect of the drug.

Acknowledgements

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

References

Costa P., Lobo J.M.S., 2001, Modelling and Comparison of dissolution profiles. European Journal of Pharmaceutical Sciences, 13, 123-133.

Das M.K., Senapati P.C., 2008, Furosemide-loaded Alginate Microspheres Prepared by Ionic Cross-linking Technique: Morphology and Release Characteristics, Indian J Pharm Sci, 70, 77-84.

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, 97, 19-26.

Khandai M., Chakraborty S., Sharma A., Pattnaik S., Patra C. N., Dinda S. C., Sen K. K., 2010, Preparation and evaluation of algino-sericin microspheres: An approach for sustained drug delivery, Journal of Advanced Pharmaceutical Research, 1, 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, 1-9.

Kundu S.C., Dash B.C., Dash R., Kaplan D.L., 2008, Natural protective glue protein, sericin bioengineered by silkworms: Potential for biomedical and biotechnological applications, Prog. Polym. Sci., 33, 998-1012.

Kuo C.K., Ma P.X., 2001, Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties, Biomaterials, 22, 511-521.

Palomo M.E., Ballesteros M.P. and Frutos P., 1999, Analysis of diclofenac sodium and derivatives, J. Pharm. Biomed. Anal., 21, 83-94.

Polli J.E., Rekhi G.S., Augsburger L.L., Shah V.P., 1997, Methods to Compare Dissolution Profiles and a Rationale for Wide Dissolution Specifications for Metoprolol Tartrate Tablets, J.Pharm Sci, 85,691-00.

Santos L., Guterres S.S., Bergold A.M., 2007, Preparation and Evaluation of Gastro-resistant Capsules of Diclofenac Sodium, Latin American Journal of Pharmacy, 26, 355 – 361. (in Portuguese)

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

Teramoto H., Kameda T., Tamada Y., 2008, Preparation of gel film from Bombyx mori silk sericin and its characterization as a wound dressing, Biosci. Biotechnol. Biochem., 12, 3189-3196.

Turbiani F. R. B., Tomadon Junior J., Seixas F. L., Gimenes M. L., 2011, Properties and Structure of Sericin Films, Chemical Engineering Transactions, 24, 1489-1494, DOI: 10.3303/CET1124249.

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