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Development of Niacinamide Loaded-Sericin Nanoparticle Using Water in Silicone Technique

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Abstract. Sericin is a water soluble protein from silk cocoon which is widely used in cosmetic industry and biomedical field to delivery many active compounds into biological tissues. Several reports indicated that particles can be prepared from sericin by several methods. The purpose of this study is to develop the method for prepare niacinamide loaded-sericin nanoparticle by using water in silicone technique. In this study, sericin nanoparticles were prepared using water in silicone emulsification technique. Niacinamide was used as a model compound due to cosmetic benefit. However, water soluble property of niacinaimde makes it difficult to enter the skin. The sericin nanoparticles were prepared with various concentration of $CaCl_2$ (2%, 4%, 6% and 8%) as a cross-link reagent. The size of nanoparticles were evaluated by particle size analyzer. Entrapment efficiency was determined using HPLC. The morphology and shape of nanoparticles were observed by scanning electron microscope (SEM). The results showed that shape of sericin nanoparticles were spherical. The optimum condition was 2% sericin concentration, 8% $CaCl_2$ concentration, 15 minutes and 1000 rpm homogenizing time and speed, respectively. The entrapment of niacinamide in sericin nanparticles was 58.690%

Keywords: Sericin, Nanoparticle, Silicone emulsion

1. Introduction

Sericin is a second type of silk protein, which contains 18 amino acids including essential amino acids and is characterized by the presence of 32 percent of serine [1]. Sericin has polar side chains made of hydroxyl, carboxyl and amino groups that enable easy cross-linking, copolymerization or blending with other polymers to form improved biodegradable materials [2]. Several methods have been developed for the preparation of sericin nanoparticles such as method based on polymeric micelles. Kwang Yong Cho *et al*. reported the successful of preparing sericin nanoparticles conjugated with poly (ethylene glycol)[3]. Biman B. Mandal and S.C. Kundu reported that fabrication of self-assembled sericin-poloxamer were stable in aqueous solution, smaller in size and rapidly taken up by cells [2]. Water in silicone emulsion for chitosan particles preparation has been developed in our group [4]. We are now further investigating if other biodegradable materials can be used with the similar technique. Therefore, the sericin was used in this study.

2. Materials and Methods

2.1. HPLC Method Validation

The samples were detected using reverse-phase Platinum™ C18-EPS (53x7mm, 3 um). The injection volume was 20 µl and the temperature of column was maintained at 35^0C. The mobile phase consisted of 50

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mM Potassium phosphate buffer (pH 7.0) and Acetonitrile (99:1). The flow rate was 1.0 ml/min and time of analysis was 10 min. The absorbance was measured at 220 nm.

2.2. Silk Sericin Nanoparticle Preparation

The sericin nanoparticles were prepared using water in silicone emulsion technique. Sericin solution (2%w/v) containing 20% niacinamide was emulsified in to silicone oil (DC 345) containing 5% silicone emulsifier (DC5225C) while stirring at 6500 rpm for 60 min. Thereafter, the ionic gelation of sericin nanoparticles was achieved by adding 2% $CaCl_2$ solution with equal volume of sericin solution. After 24 hrs, the sericin nanoparticles were isolated by filtration. Some parameters that influenced the particles formation were also studied such as $CaCl_2$ concentration (2%, 4%, 6% and 8%), sericin concentration (0.5%, 1% and 2%), homogenizing time (15, 30 and 60 min) and speeds of homogenizer (6500, 9500 and 13500 rpm). Morphology of particles was observed using electron scanning microscope (SEM).

2.3. Determination of Particle Size

The sizes of sericin particles were evaluated by ZetaPALS® (Zeta Potential and Particle Size Analyzer) using distilled water as a liquid medium.

2.4. Determination of Entrapment Efficiency

Twenty milligrams of niacinamide-loaded sericin nanoparticles were suspended in 1 ml of phosphate buffer (pH 7) for 48 hrs at room temperature. Then, the suspension was centrifugated at 12000 rpm for 10 minutes and the supernatant was then filtered (0.2 um Nylon syringe filters, Whatman,UK). The filtrate was analyzed using HPLC. Entrapment efficiency was calculated from the following expression

Entrapment efficiency (%) = (amount of niacinamide in particles/amount of niacinamide loaded) x100

3. Results and discussions

HPLC method validation. The calibration data of standard niacinamide was showed in Table 1.

Table 1: Calibration data of the standard niacinamide

Linearity range (ug/ml)	200 -3.0
Regression equation	y = 99784x-17660
Correlation coefficient	0.998
Limit of detection (ng/ml)	15.0
Limit of quantification (ug/ml)	3.0

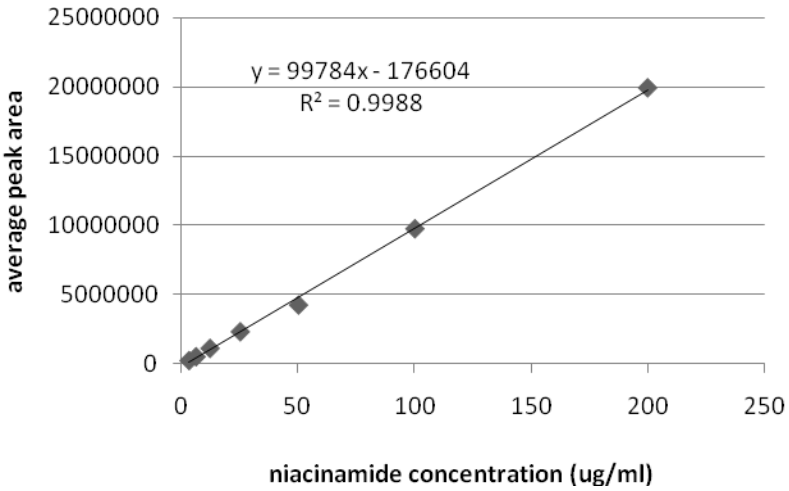

Fig. 1: Calibration curve of niacinamide

Sericin nanoparticle preparation. Sericin nanoparticles produced with various concentrations of sericin had different sizes. The smallest sericin nanoparticle was obtained from 2% sericin and 2% $CaCl_2$. The

different concentrations of $CaCl_2$ also gave different sizes of sericin nanoparticles. Their sizes trend to larger when increase the concentration of $CaCl_2$.

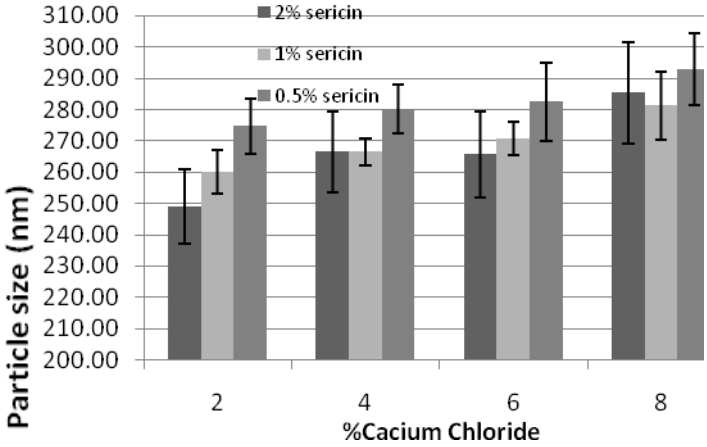

Fig. 2: Sizes of sericin nanoparicles produced with various conditions

Times for homogenization were varied at 15, 30 and 60 minutes while all other parameters such as sericin concentration, $CaCl_2$ concentration and speed of homogenizer were fixed at 2%, 2% and 6500 rpm, respectively. The sizes of particles obtained from any length of time were comparable.

The last varied parameters is a speed of homogenizer (6500, 9500 and 13500 rpm) while all other parameters were fixed at 2% sericin, 2% $CaCl_2$ and 15 minutes of homogenizing time. The size of particles decreased when increased the speed of homogenizer.

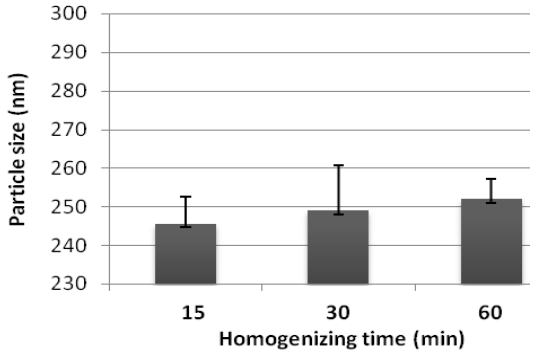

Fig. 3: Effect of homogenizing time on particle size

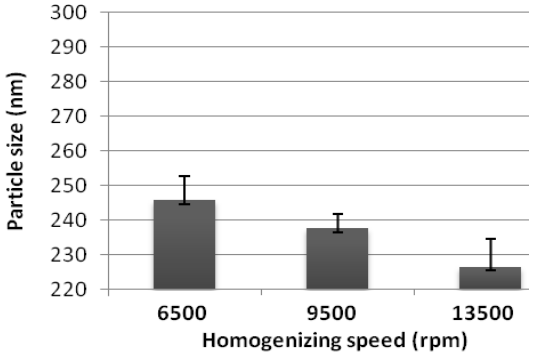

Fig. 4: Effect of homogenizing speed on particle size

In an attempt to reduce an energy using in the process, the speed of homogenizer was decreased to 1000 rpm, sericin and calcium chloride concentration were fixed (2% sericin, 8% $CaCl_2$). The homogenizing time was varied.

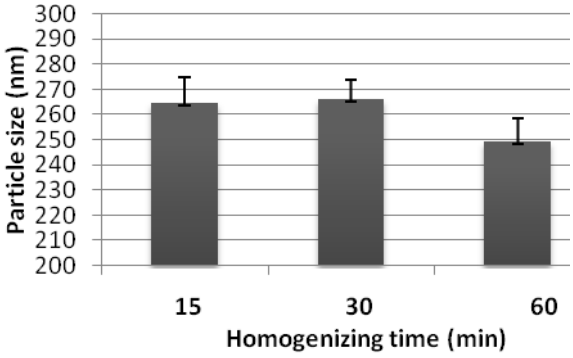

Fig. 5: Size of sericin naoparticles produced with homogenizing speed 1000 rpm at different of homogenizing times

The result showed that the size of sericin nanoparticles obtained from 1000 rpm of homogenizing speed was similar to that particle obtained from 6500 rpm. Therefore, the optimum conditions were 1000 rpm and 15 mins of homogenizing time because the particle can be produced gentlely.

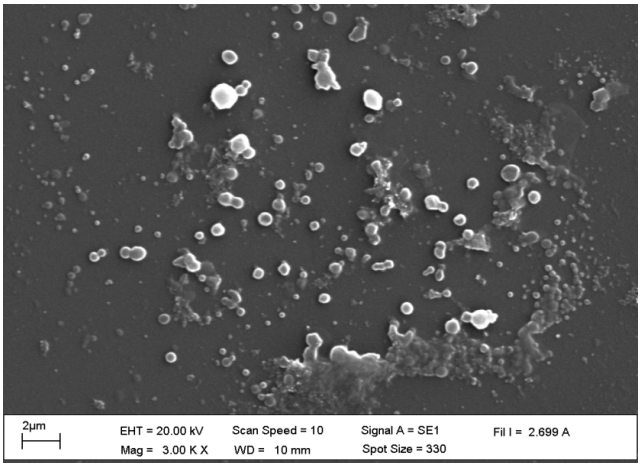

Fig. 6: Electron micrograph of sericin nanoparticle produced from water in silicone emulsion technique

Entrapment efficiency. Sericin nanoparticles produced with various concentrations of calcium chloride gave very low percent of entrapment. The experiment for improving the entrapment efficiency was concerned. Concentration of sericin, concentration of $CaCl_2$, time for homogenization and homogenizing speed were fixed at 2%, 8%, 15 mins and 1000 rpm, respectively. In preparation process, 15% acetone was added into sericin solution before emulsified to DC345. The addition of organic solvent may ease the solidification process. After 24 hrs of cross-linking time, the system was heated. The sericin nanoparticles preparing from these conditions were determine for entrapment efficiency. Various test conditions were shown in Table.2

Table 2: Percents of entrapment in various conditions

Preparing condition				%entrapment
%niacinamide	%acetone	Heating temperature (°C)	Heating time (hr)	
20	15	50	1	0.419
20	15	50	5	1.550
5	15	50	5	2.080
5	15	80	5	4.089
1	15	80	5	29.219
0.5	15	80	5	58.690

The percent of niacinamide entrapped in sericin nanoparticles was increased when decreased niacinamide loading amount. On the other hand, the percent of entrapment was increased when increased the heating temperature and heating time. Possible explanation for these finding is that there is water remaining after stop the cross-linking process. Therefore, niacinamide might be still solubilized in the water. Thus, heating the system and adding organic solvent could speed up the water removal time. Thus, particle's entrapment efficiency can be improved.

4. Conclusions

Sericin nanoparticles were successfully produced by water in silicone technique. Particle's properties were depended upon several influence parameters. The optimum condition was 2% sericin concentration, 8% $CaCl_2$ concentration, 15 minutes and 1000 rpm homogenizing time and speed, respectively. Entrapment efficiency of niacinamide in sericin nanoparticle crucially depends on an initial loading of niacinamide and the adding of organic solvent. The optimum concentration was 0.5% niacinamde and 15% acetone.

5. References

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