Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Preparation of Silk Fibroin Microspheres and Its Application to Protein Adsorption

Yaowalak Srisuwan[a]; Prasong Srihanam[a]; Yodthong Baimark[a]
[a] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham, Thailand

To cite this Article Srisuwan, Yaowalak , Srihanam, Prasong and Baimark, Yodthong(2009) 'Preparation of Silk Fibroin Microspheres and Its Application to Protein Adsorption', Journal of Macromolecular Science, Part A, 46: 5, 521 — 525
To link to this Article: DOI: 10.1080/10601320902797780
URL: http://dx.doi.org/10.1080/10601320902797780

Journal of Macromolecular Science®, Part A: Pure and Applied Chemistry (2009) **46**, 521–525

ISSN: 1060-1325 print / 1520-5738 online
DOI: 10.1080/10601320902797780

Taylor & Francis
Taylor & Francis Group

Preparation of Silk Fibroin Microspheres and Its Application to Protein Adsorption

YAOWALAK SRISUWAN, PRASONG SRIHANAM and YODTHONG BAIMARK*

Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand

Received October 2008, Accepted November 2008

Silk fibroin (SF) microspheres were prepared by a water-in-oil emulsion solvent evaporation method without any surfactants. The water and oil phases were SF aqueous solution and paraffin, respectively. The SF microspheres with spherical shape and smooth surface were separated into the size ranges of <80 μm, 80–150 μm and >150 μm. SF conformation of the microspheres was investigated by Fourier transform infrared spectroscopy while scanning electron microscopy used for morphological observation. After being treated with methanol, the SF conformation was changed from random coil to β-sheet form. Finally, the influence of particle size on protein adsorption efficiency was studied. Bovine serum albumin (BSA) was chosen as a model protein to carry out an immobilization test onto the SF microspheres by non-covalent adsorption. Efficiency of BSA adsorption was significantly increased with decreasing the SF microsphere size.

Keywords: Silk fibroin, microspheres water-in-oil emulsion solvent evaporation, bovine serum albumin, protein adsorption

1 Introduction

Silk fibroin (SF) of *Bombyx mori* is a natural protein polymer with very good biocompatibility and biodegradability (1) that has been widely investigated as biomaterials for tissue engineering (2), enzyme immobilization (3, 4) and controlled release drug delivery (5). For these purposes, the SF devices such as fibers (6, 7), films (8, 9), nanoparticles (10) and microparticles (11) have been prepared and reported. SF devices have been found to be excellent enzyme immobilization materials (12–14). Although SF microspheres have been prepared by a spray drying method (15, 16), however, the preparation of SF mirospheres by water-in-oil (W/O) emulsion solvent evaporation and its protein adsorption have not been reported.

In this work, we report the preparation of SF microspheres by W/O emulsification solvent evaporation method, where the insoluble treatment of the microspheres was performed by methanol treatment and its application to protein adsorption. Bovine serum albumin (BSA) was used as a model protein. Influence of microsphere sizes on BSA adsorption was also investigated.

2 Experimental

2.1 Materials

Silk fibroin (SF) aqueous solution was prepared by a chemical degummed method and dissolved before dialysis, respectively as follows. Cocoons of *Bombyx mori* were removed sericin by boiling twice in a 0.5% Na_2CO_3 solution at 90°C for 30 min, then rinsed with distilled water and air dried at room temperature. Degummed SF was dissolved in the $CaCl_2$-ethanol-water system (mole ratio = 1:2:8), by stirring at 80°C for 2 h. The resulting SF solution was then dialyzed against distilled water with cellulose tube for 3 days. The distilled water was refreshed every 3 h on the first day and then sequentially refreshed every 24 h. The final concentration after dialysis was adjusted to 4% (w/v) against distilled water. Bovine serum albumin (BSA, 97% Fluka) was used as a model protein without further purification.

2.2 Preparation of SF Microspheres

The SF microspheres were prepared by water-in-oil (W/O) emulsion solvent evaporation of SF aqueous solution in paraffin with slight heat to evaporate the water for 24 h.

*Address correspondence to: Y. Baimark, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand. E-mail: yodthong.b@msu.ac.th, yodthongb@gmail.com

The obtained microspheres were washed with n-hexane for several times to remove residue paraffin before treating with 90% (v/v) methanol aqueous solution for 1 h and dried in a vacuum oven at room temperature for a week. The SF microspheres were sieved into three particle size ranges of <80 μm, 80–150 μm and >150 μm.

2.3 Characterization of SF Microspheres

FTIR spectra were collected by FTIR spectroscopy using a Perkin-Elmer Spectrum GX FTIR spectrometer with air as the reference. The resolution of 4 cm^{-1} and 32 scans were chosen. The KBr dish method was used for this experiment.

Microsphere morphology was investigated by scanning electron microscopy (SEM) using a JEOL JSM-6460LV SEM. The microspheres were coated with gold for enhancing conductivity before scan.

Particle size distributions of the microspheres were determined by light scattering analysis using a Coulter LS230 light scattering particle size analyzer at 25°C.

2.4 Protein Adsorption Test

Protein adsorption test was determined on the methanol treated SF microspheres. BSA was used as a model protein. Prior to adsorption test, the 10 mg of SF microspheres were immersed in the PBS solution for overnight to reach an equilibrium hydration. Fresh BSA solution was prepared in PBS at pH 7.4 to give a concentration of 1 mg/mL. The hydrated SF microspheres were incubated in BSA solution at 37°C with shaking at 200 rpm for 2 h. The BSA-adsorbed SF microspheres were recovered after ultra centrifugation and washing with 4×1 mL PBS to remove reversibly adsorbed BSA. The irreversibly adsorbed BSA was removed by soaking with 1 mL of 2% (w/v) sodium dodecyl sulfate for 1 h at room temperature. The amount of adsorbed BSA onto SF microspheres was measured by Lowry protein assay along with a calibration curve.

3 Results and Discussion

3.1 FTIR Spectra of SF Microspheres

The SF conformation of microspheres was determined from FTIR spectra. The position of absorption bands, especially amide bands were considered. Figures 1(a) and 1(b) show the FTIR spectra of non-methanol and methanol treated SF microspheres with particle size range of 80–150 μm, respectively. The absorption bands of SF microspheres in Figure 1(a) at 1654 (amide I) and 1560 (amide II) were assigned to the random coil conformation (17) suggested that the heat during solvent evaporation process did not completely induce the transitional conformation of SF from random coil to β-sheet. Yeo et al. (15) and Hino et al. (16)

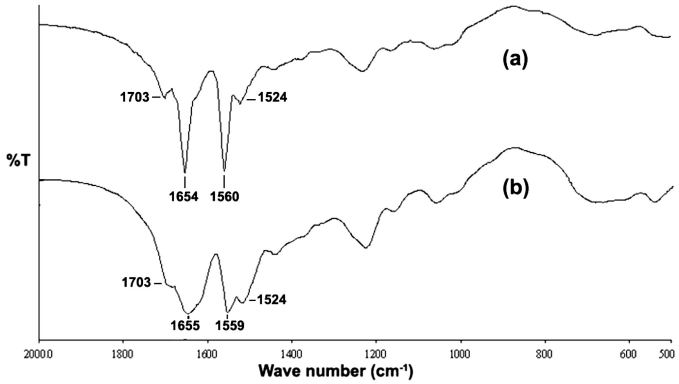

Fig. 1. FTIR spectra of (a) non-methanol and (b) methanol treated SF microspheres with the size range of 80–150 μm.

have been reported the conformation of SF microsphres constructed by spray drying at temperature about 85 and 120°C, respectively can be changed SF conformation from random coil to β-sheet conformation. This may suggest that the heat during the solvent evaporation step is only used to evaporate the water and had no affect on SF conformation.

However, the bands of β-sheet characteristics can also be observed at 1703 and 1524 cm^{-1} (17, 18) indicated that the non-methanol treated SF microspheres consisted of the both random coil and β-sheet forms with predominantly random coil conformation. The SF microparticles with <80 and >150 μm in size range show similar evidence. The FTIR results indicated that the particle size did not affect the SF conformation change during the solvent evaporation stage.

Methanol treatment is performed in order to obtain the water-insoluble SF microspheres. The FTIR spectrum of SF microspheres increased of the β-sheet characteristic bands after methanol treatment (Fig. 1(b)). The result indicated that the conformation changes from the random coil to the β-sheet form partially occurred in the microspheres.

3.2 Particle Size Distribution of SF Microspheres

Particle size distribution of the SF microspheres was determined from % weight fraction of microsphere with different sizes after sieving. The particle size distributions of both non-methanol and methanol treated SF microspheres obtained from sieving method are presented in Figure 2. It was found that the both SF microspheres show similar particle size distribution suggested that the methanol treatment did not effect microsphere size distribution. Almost SF microspheres had a particle size in the range of 80–150 μm according to the result of particle size distribution obtained from light scattering analysis, as shown in Figure 3.

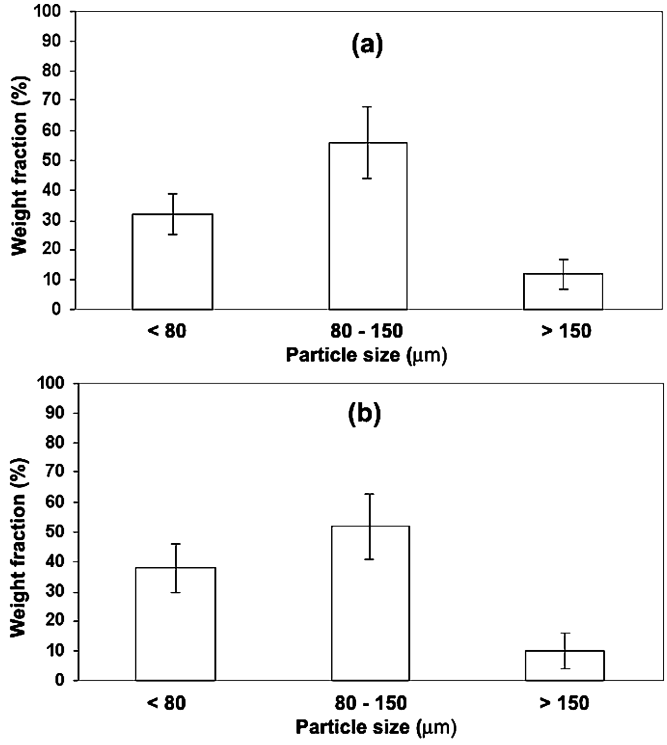

Fig. 2. Particle size distributions obtained from sieving method of (a) non-methanol and (b) methanol treated SF microspheres.

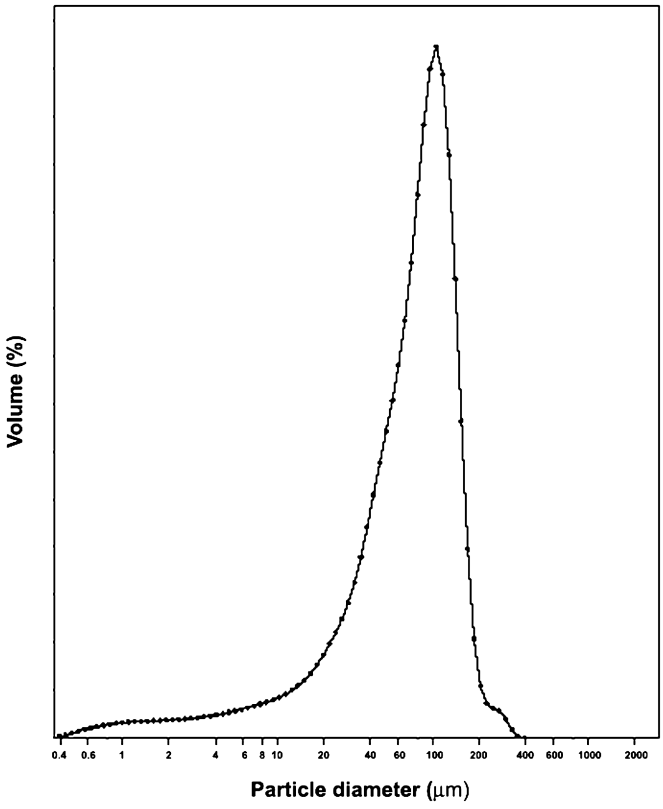

Fig. 3. Particle size distribution obtained from light scattering analysis of methanol treated SF microspheres.

3.3 Morphology of SF Microspheres

Morphology of non-methanol and methanol treated SF microspheres is observed from SEM images as shown in Figures 4 and 5, respectively. The non-methanol treated microspheres were spherical shape with smooth surface compared to SF microspheres prepared by spray drying and with being deflated (15). This may be due to the fact that water molecules were rapidly evaporated out. It showed that the water evaporation rate of W/O emulsion solvent

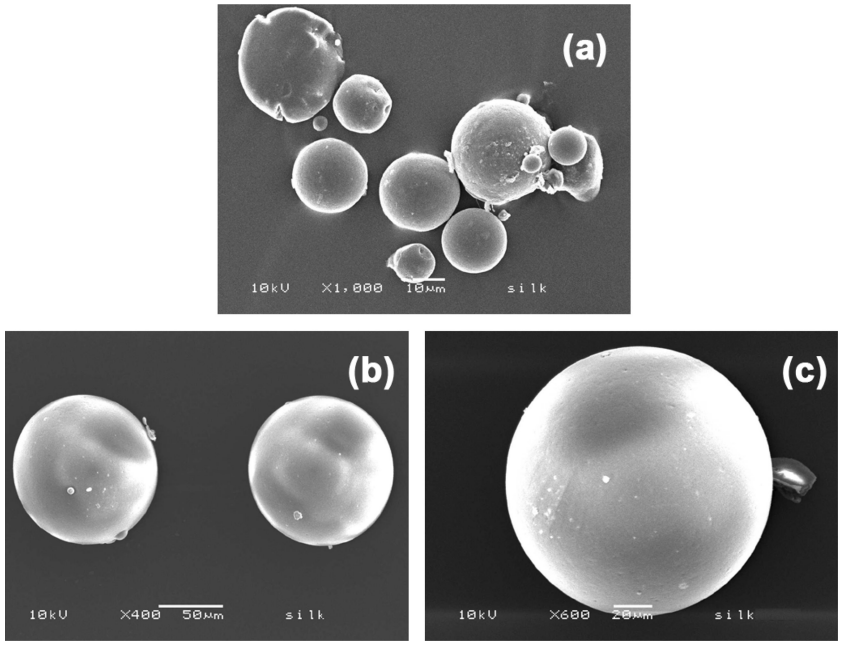

Fig. 4. SEM images of non-methanol treated SF microspheres with the size ranges of (a) <80, (b) 80–150 and (c) >150 μm.

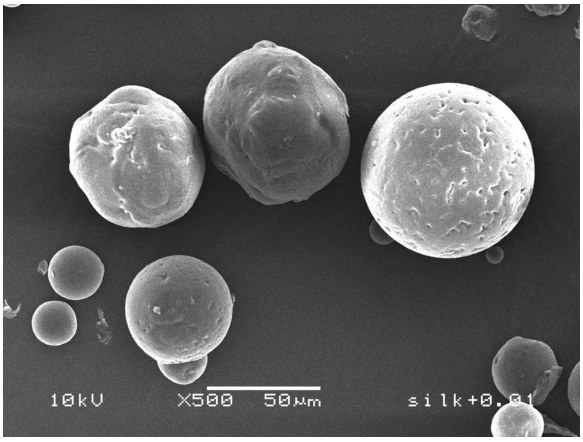

Fig. 5. SEM images of methanol treated SF microspheres with the size of <80 μm.

evaporation technique used in this work was slower. The results suggested that the W/O emulsification solvent evaporation is a suitable technique for preparing the SF microspheres with a smooth surface.

The microsphere surface of methanol treatment appears rougher than the non-methanol treatment, due to the fact that the SF phase was induced to crystallization or β-sheet formation (9).

Although the alcohol treatment must be used for conformational transition from random coil (water-soluble) form to β-sheet (water-insoluble) form, however, the SF microspheres prepared by the W/O emulsion solvent evaporation technique show more consistent spherical shape.

3.4 BSA Adsorption Test

Adsorption of enzyme onto the matrix is still interesting approach for high density protein organization aimed to improve the enzyme stability. In this work, BSA, model protein was used to adsorption test. Percentages of BSA adsorption onto the different sizes of methanol treated SF microspheres are illustrated in Figure 6. The % BSA adsorption was dramatically increased when the particle size was decreased due to the increasing surface area of SF microspheres. The microspheres with higher BSA adsorption will have a faster released rate of BSA. Thus, the BSA release rate from SF microspheres could be adjusted by varying the microsphere sizes.

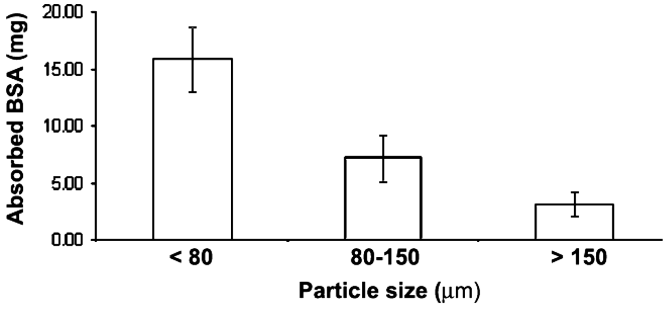

Fig. 6. Amount of adsorbed BSA onto per gram of methanol treated SF microspheres.

4 Conclusions

The SF microspheres with a spherical shape and smooth surface were successfully prepared by the W/O emulsion solvent evaporation method without any surfactants. The random coil form was predominantly SF conformation of the microspheres and changed to β-sheet form after alcohol treatment. The SF microspheres with 80–150 μm in size were the largest fraction. The SF microspheres show a high efficiency to adsorb the BSA protein which increased with the decreasing of microsphere size.

The SF microspheres prepared by the W/O emulsion solvent evaporation and then methanol treatment are useful for adsorption application of other proteins.

Acknowledgements

The authors thank the Faculty of Science, Mahasarakham University, fiscal year 2008 and the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Thailand for financial supports.

References

1. Altman, G.H., Diaz, F., Jakuba, C., Calabro, T., Horan, R.L., Chen, J., Lu, H., Richmond, J. and Kaplan, D.L. (2003) *Biomaterials*, 24, 401–416.
2. Tamada, Y. (2005) *Biomacromolecules*, 6, 3100–3106.
3. Yoshimizu, H. and Asakura, T. (1990) *J. Appl. Polym. Sci.*, 40, 127–134.
4. Liu, Y., Qian, J., Liu, H., Zhang, X., Deng, J. and Yu, T. (1996) *J. Appl. Polym. Sci.*, 61, 641–647.
5. Hofmann, S., Wong Po Foo, C.T., Rossetti, F., Textor, M., Vunjak-Novakovic, G., Kaplan, D.L., Merkle, H.P. and Meinel, L. (2006) *J. Controlled Release*, 111, 219–227.
6. Wang, M., Jin, H.J., Kaplan, D.L. and Rutledge, G.C. (2004) *Macromolecules*, 37, 6856–6864.
7. Li, C., Vepari, C., Jin, H.J., Kim, H.J. and Kaplan, D.L. (2006) *Biomaterials*, 27, 3115–3124.
8. Liu, Y., Liu, H., Qian, J., Deng, J. and Yu, T. (1996) *J. Macromol. Sci. A.*, 33, 209–219.
9. Jin, H.J., Park, J., Valluzzi, R., Cebe, P. and Kaplan, D.L. (2004) *Biomacromolecules*, 5, 711–717.
10. Zhang, Y.Q., Shen, W.D., Xiang, R.L., Zhuge, L.J., Gao, W.J. and Wang, W.B. (2007) *J. Nanopart. Res.*, 9, 885–900.
11. Wang, X., Wenk, E., Matsumoto, A., Meinel, L., Li, C. and Kaplan, D.L. (2007) *J. Controlled Release*, 117, 360–370.
12. Zhang, Y.-Q. (1998) *Biotechnol. Adv.,* 16: 961–971.

13. Zhang, Y.-Q., Zhu, J. and Gu, R.-A. (1998) *Appl. Biochem. Biotechnol.*, 75, 215–233.
14. Zhang, Y.-Q., Shen, W.-D., Gu, R.A., Zhu, J. and Xue, R.-Y. (1998) *Anal. Chim. Acta*., 369, 123–128.
15. Yeo, J.H., Lee, K.G., Lee, Y.W. and Kim, S.Y. (2003) *Eur. Polym. J*., 39, 1195–1199.
16. Hino, T., Tanimoto, M. and Shimabayashi, S. (2003) *J. Colloid. Interf. Sci*., 266, 68–73.
17. Zuo, B., Liu, L. and Wu, Z. (2007) *J. Appl. Polym. Sci*., 106, 53–59.
18. Lv, Q., Cao, C., Zhang, Y., Ma, X. and Zhu, H. (2005) *J. Appl. Polym. Sci*., 96, 2168–2173.