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Preparation of micro-porous fibroin membrane by emulsion method

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Abstract

This research aims to provide a method for preparing micro-porous fibroin membrane by using emulsion method developed and to study the properties of the micro-porous fibroin membranes. Colloidal solution of ethyl acetate in medium phase of water and sodium dodecyl sulfate (SDS) emulsifier was used in this research. The membranes preparation were carried out by using the fraction (ω) of [oil]/[SDS] in the range of 10-50 and stabilized by using the 4% PEGDE (polyethylene glycol diglycidyl ether) crosslinking agent or 95% ethanol solution treatment and the followed by oven drying or freeze-drying. From the results, it found that the fibroin colloid solution used short times for gelation than fibroin solution in fibroin membrane preparation. The fraction of colloid solution using ω equal 30 was suitable for porous fibroin membrane preparation as possible. By the way, the freeze-drying provided more micro-porous level structure exhibited in the membrane more than oven drying. The fibroin membrane which stabilized by PEGDE adding has less water solubility than the fibroin membrane which stabilized by EtOH treatment and the fibroin membrane without treatment, respectively. This was affected by the conformation changing of the secondary structure of fibroin membrane from the Silk I (random coil and α helix) to the Silk II (β-sheet plate). In addition, the preparation of fibroin membrane by emulsion method with PEGDE agent adding made the membrane soft and flexible than the others methods.

Keywords: Micro-Porous; Fibroin Membrane; Emulsion Method; Colloid Solution

1. Introduction

Silk from domesticated silkworm, *Bombyx mori,* is a natural biopolymer that mainly consists of fibroin protein (about 70%wt) and sericin protein (about 30%wt). The fibroin protein has high content of Gly-Ala repeating units and the molecular weight about 25 – 325 kDa [1]. In commercial the fibrous silk fibroin (SF) mostly used as a textile material. Now a day, the SF derived from silk fibrous and silk waste can be used as a raw material to prepare SF solution for producing other smart materials such as SF film, SF membrane, SF microsphere, and SF nanofiber [2, 3].

These SF materials exhibit numerous advantages, including biocompatibility, high oxygen permeability, microbial resistance, and minimal inflammatory. Then the SF materials can be used in enzyme immobilization, cosmetics, tissue cultural, biosensor, drug delivery material, and bio-engineering [4, 5]. The porous SF membrane is a smart material which mostly used for wound protection, substrate for cell culture, enzyme immobilizing, drug releasing agent, cell culture, and bio electric membrane [6, 7]. The porous SF membrane can be prepared by using freeze-drying method, blending with inorganic substances, and colloidal method [8, 9]. Oil in water (o/w) or water in oil (w/o) micro-emulsion system is used to prepare colloidal solution for porous fibroin membrane preparation [12] via the colloidal method. Generally, stabilization of the SF membrane can be carried out by methanol treatment, but it becomes rigid and brittle when exposed to ambient air. To overcome this problems, it has been modified with chemical agents such as polyvinyl alcohol, polyethylene glycol diglycidyl ether (PEGDE), and genipin [10 – 12]. In this work, the microporous SF membranes were prepared by colloidal method using oil in water emulsion and then the microporous SF membrane properties were characterized.

2. Materials and methods

Materials

Silk waste was collected from Jun Mai Thai Co., Thailand. The PEGDE was purchased from Sigma-Aldrich Co. All chemicals were reagent grade and distilled water was used throughout this study. Silk waste was degummed 3 times using a 5 g L^{-1} Na_2CO_3 solution at 98 – 100 °C for 30 min each, rinsed with water and dried at 60 °C. The SF solution was prepared by dissolving a 10 g of the degummed silk in 100 mL of $CaCl_2$-ethanol-water (1:2:8 by mole ratio) solution at 110±5 °C for 2 hours. After that it was filtered and dialyzed against distilled water for 3 days by using dialysis tubes (molecular cutoff = 10,000), then 3% wt V^{-1} SF solution was obtained [11]. A Colloidal solution of ethyl acetate in medium phase of water and 10 mM of sodium dodecyl sulfate (SDS) emulsifier was prepared by shaking the mixed solution at 200 rpm 15 °C for 10 min by using incubator shaker and used it immediately.

Gelation study of the SF colloidal solution

The SF solution mixed with each the colloidal solution which have fraction (ω) of [oil]/[SDS] in the range of 10 – 50 and then poured the mixed solution into beaker size 100 mL and measured viscosity of the mixed colloid solution until gelation occurred by using viscosity meter (model RI:2:M-H, Shannon, Ireland).

Fibroin membrane preparation and stabilization

The fibroin membranes were prepared by colloidal method which carried out by using a 10 mL of each mixed solution of the SF solution and the colloidal solution which have ω of [oil]/[SDS] in the range of 10 – 50 and then poured the mixed solution into a polystyrene dish (54 cm^2) and leaved it dry at room temperature for 24 hours. After that pulled out the membrane from the dish and then soaked in 95% ethanol for 5 min followed by soaked in water and oven drying at 60 °C for 9 hours or freeze-drying at low temperature for 3 hours. For PEGDE stabilized SF membrane was prepared as described above but used 4% PEGDE in the mixed solution and no have ethanol treatment in the

process. The SF membrane preparing with general method was prepared as above without colloidal solution adding and not has 95% ethanol treatment or the PEGDE adding in the stabilization process.

Membrane investigation

Membrane morphology and conformation structure were characterized by scanning electron microscopy (SEM, model J6336F, JEOL, Japan) and the Fourier transform infrared spectroscopy (FTIR, model Tensor 27, Bruker, Germany), respectively. Water solubility of the fibroin membrane was determined by shaking a 10 mL (V, mL) of aqueous solution pH 7 in contact with 50 mg of fibroin membrane (G, mg) at room temperature for 60 min and then leaved it for 24 hours. The decanted solution was measured its absorbance (A, Abs) at 276 nm by a UV-Vis spectrometer (model UV-1700, Shimadzu, Japan) and calculated the percent water solubility of the membrane (D) by using the equation (1) ;

$$D = \left(K.V.A \times 100 \,/\, G \right) \tag{1}$$

Where K is the water solubility constant equal 0.983 mg mL^{-1} Abs^{-1}. The mechanical properties of the membranes were analysed using a universal tester (model LRX, LLOYD, UK).

3. Results and Discussion

Gelation of SF colloidal solution

Gelation of the silk fibroin colloid (SFC) solution and the SFC solution with the 4% PEGDE modified SFC solution were studied by viscosity measurement of the mixed SFC solution over times and the result is shown in Fig. 1. The results show that all types of SFC solution with PEGDE have duration times to gel formation less than one hour, with maximum viscosity in the range of 47 – 57 Pa s. Obviously, the SF solution and the SF solution with PEGDE have viscosity equal 0.21 Pa s and 0.22 Pa s, respectively. The rapid gelation of the SFC solution caused by SDS emulsifier in the colloid solution [9]. In addition, the PEGDE modification into the SFC solutions exhibit more rapid gelation than the SFC solution only. This rapid gelation property effect to rapid fibroin membrane preparation further.

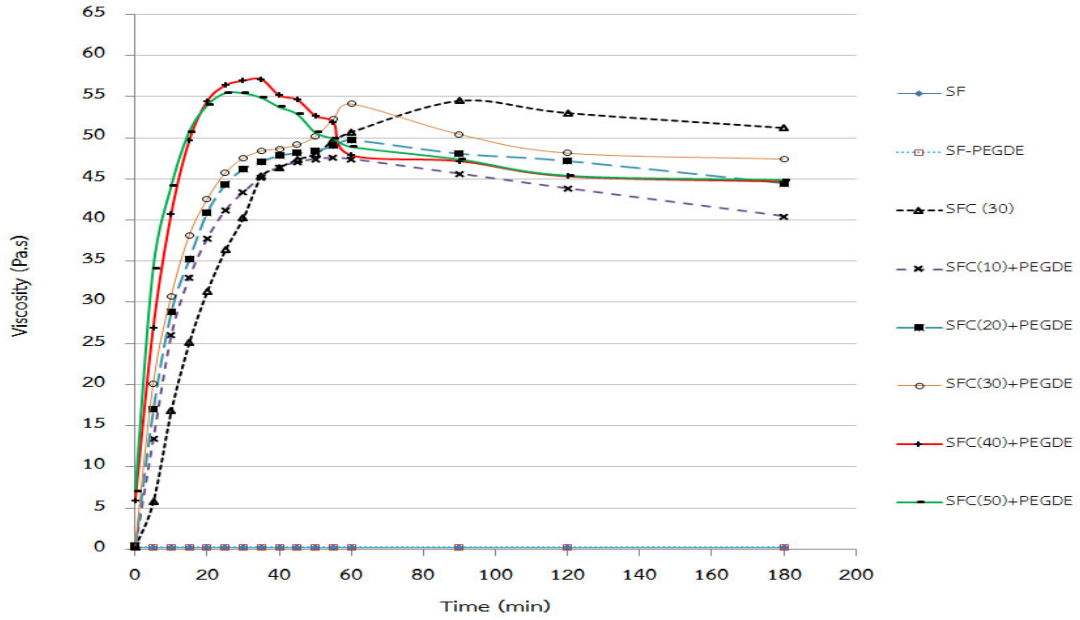


Fig. 1 The relationship of viscosity (Pa.s) and of SF solution (SF), SF solution with PEGDE (SF-PEGDE), SF colloid solution (SFC), and SF colloid solution with PEGDE in various **ω** fractions (SFC+PEGDE).

Membrane water solubility

The percent water solubility of the SFC, SFC-EtOH, and SFC-PEGDE membranes of oven drying and freeze-drying are shown in Fig. 2. The SFC membrane without stabilized by ethanol treatment or PEGDE modification shows high water solubility than the SFC membrane which stabilized by EtOH treatment and PEGDE adding, respectively. These results suggest that the PEGDE greatly stabilized the SFC membrane against solubilization [10, 11].

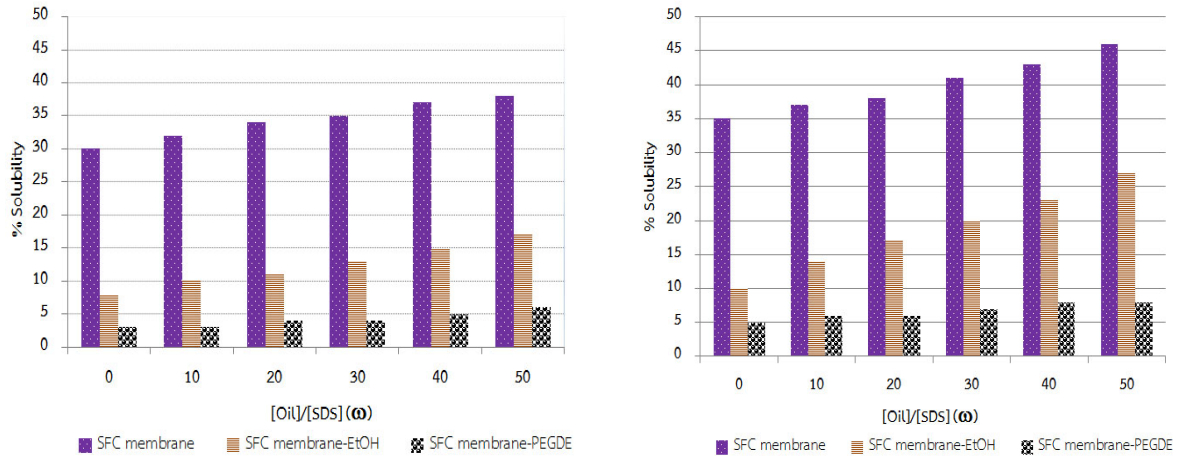
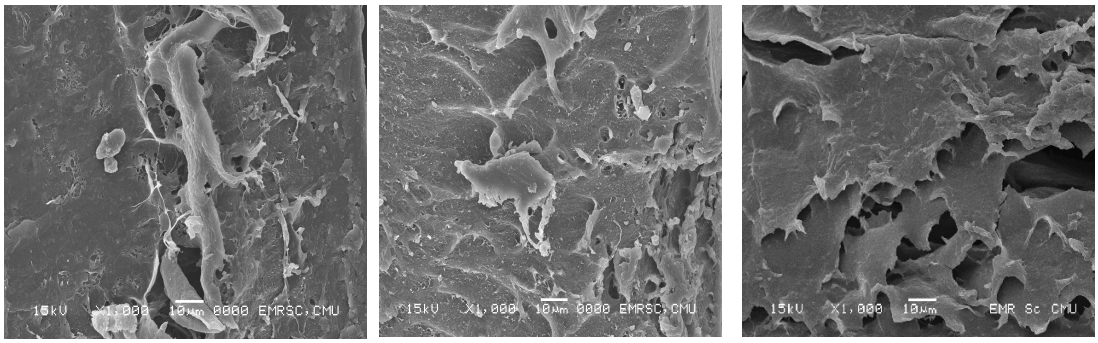


Fig. 2 Percent water solubility of the SFC membranes which prepared by oven drying (a) and freeze-drying (b).

Membrane morphology

The cross-section morphology of the SFC membranes surface was investigated. Different types of the SFC membranes were prepared by using the SF colloidal solution (ω = 30) and dried by freeze-drying method. From the observation it found that the SFC membrane prepared by using SF colloid solution with 4% PEGDE showed more porosity and opened surface than the SFC membrane prepared by using 95% EtOH treatment and the SFC membrane without stabilization as shown In Fig. 3.



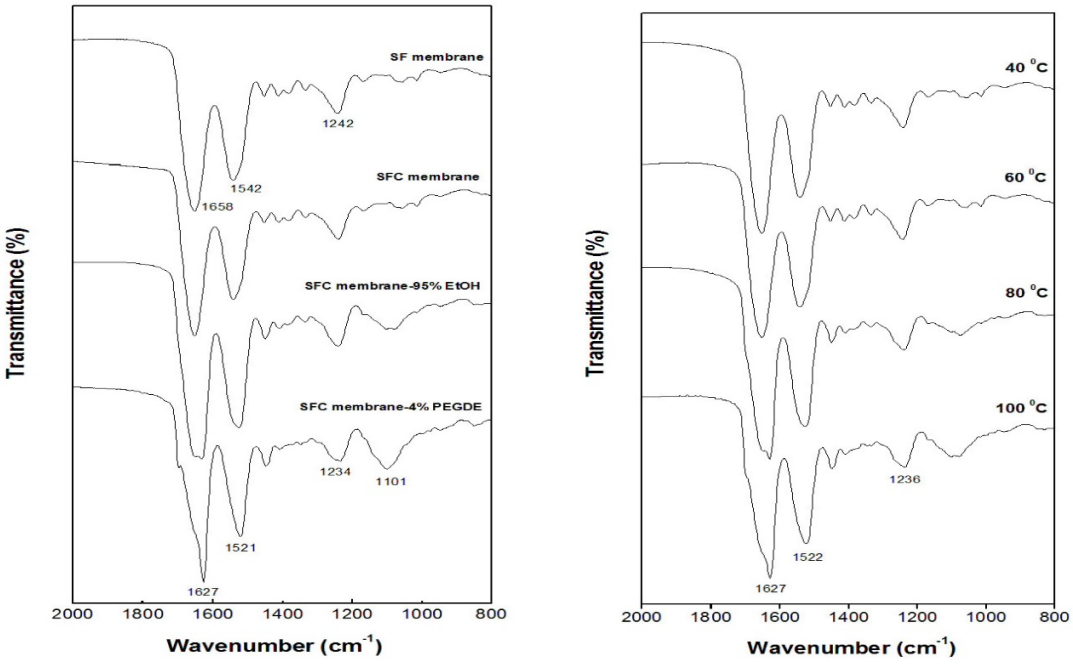
(a) (b) (c)

Fig. 3 SEM images of the SFC membrane (a), the SFC membrane with 95% EtOH treatment (b), and the SFC membrane with 4% PEGDE modifying (c).

Membrane conformation

The membranes were prepared via different methods and used oven drying at temperature 60 °C. The secondary structure studying of different type membranes were investigated by using the Fourier Transform Infared Spectrophotometer (FT-IR) and the results shown in Fig. 4. It was found that the SF membrane and the SFC membrane without stabilized by ethanol treatment or PEGDE modification exhibit absorption bands of amide I (C=O stretching) at 1,658 cm^{-1}, amide II (N-H bending vibration) at 1,542 cm^{-1} and amide III (C-N stretching) at 1,242 cm^{-1}, respectively. These peaks assign to be Silk I structure (α-helix and random coil structure). Comparing to the SFC membrane with 95% EtOH treatment (SFC-95% EtOH) and the SFC membrane with 4% PEGDE modifying (SFC-4%PEGDE), all the amide peaks show the red-shift; amide I at 1,627 cm^{-1}, amide II at 1,521 cm^{-1} and amide III at 1,234 cm^{-1}, respectively. These positions assign to be Silk II structure (β-sheet structure) [10 – 13]. For the secondary structure of the SFC membranes which oven dried at different temperature at 40 – 100 °C for 3 hours are shown in Fig. 4(b). It found that the SFC membrane with oven drying at temperature 40 °C and 60 °C showed position of peaks similar at wave number around 1,658 cm^{-1}, 1,542 cm^{-1} and 1,242 cm^{-1} belong with the amide I, amide II and amide III, respectively. It indicates that these SFC membranes have silk I structure. Whereas the SFC membrane which oven drying at 80 °C and 100 °C show peak similar at 1,627 cm^{-1}, 1,522 cm^{-1} and 1,236 cm^{-1} of the amide I, amide II and amide III, respectively. These peaks inform that the membranes have Silk II structure. Then oven drying at temperature 80 °C and 100 °C may cause changed the secondary conformation structure of the membrane structure from Silk I structure to Silk II structure [14, 15]. Although the SFC membranes prepared by using oven dried at 80 °C and 100 °C cause the membrane more stable (from Silk II

structure) than the membranes which prepared by oven drying at temperature 40 °C and 60 °C, but it made the SFC membrane more hardness and brittle.



(a) (b)

Fig. 4 FTIR spectra of the SFC membranes prepared by different method and using oven drying at 60 °C (a) and the SFC membrane which oven dried at different temperature 40-100 °C for 3 hours (b).

Mechanical properties of membrane

From the mechanical studying of the membrane which prepared by using SF solution (SF membrane), the membrane which prepared by using SFC solution (SFC membrane), the membrane which prepared by using SFC solution and stabilized by EtOH treatment (SFC-EtOH), and the membrane which prepared by using SFC solution and stabilized by PEGDE modification (SFC-PEGDE), all types of the SF membranes were dried by oven drying at 60 °C for 3 hours. Then the tensile strength was determined by tensile tester and the results shown in Table 1. From the results it found that the tensile strength of the SFC-EtOH membrane is greater than the SFC membrane, the SF membrane, and the SFC-PEGDE membrane, respectively. While the ability to stretch (percent elongation) of the SFC-PEGDE membrane is greater than the SFC membrane, the SFC-EtOH membrane, and the SF membrane, respectively. Moreover the Young's modulus of the SFC-EtOH membrane shows higher than the SF membrane, the SFC membrane, and the SFC-PEGDE, respectively. Thus the SFC-EtOH membrane has high tensile strength and modulus, while the SFC-PEGDE membrane has stretch and flexibility.

Table 1 Mechanical properties of the membranes.

Mechanical properties	Membrane types			
	SF	SFC	SFC-EtOH	SFC-PEGDE
Tensile strength (MPa)	55.40±2.80	56±1.80	64.70±3.50	32.80±4.20
Elongation (%)	2.80±1.40	4.80±1.60	3.70±1.10	22.80±1.80
Young's modulus (MPa)	2,156.40±58.60	1,687.30±78.50	2,277.80±55.20	1,134.20±65.40

4. Conclusion

This study showed the preparation of porous silk fibroin membrane using emulsion method developed. It can be used to prepare micro-porous silk fibroin membrane with less water solubility by stabilized with EtOH treatment or PEGDE modification. The SFC solution used in this study is suitable for micro-porous fibroin membrane preparation. Because of the decreasing times in gelation compared with a general method. The SFC membrane with 4% PEGDE and drying with freeze-drying method provided more porosity in the membrane. Using EtOH treatment, PEGDE modification, and high temperature (80 – 100 °C) could be transformed the secondary structure of the membrane to β-sheet structure. In addition the membranes prepared by modification the SFC solution with 4% PEGDE, the prepared membrane exhibited more stabilized in water and highly flexible. Thus this method can be used to prepare the porous SF membrane for use in medical applications or in biological material applications further.

6. Acknowledgement

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7. References

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