Production of Silk Fibroin Membrane for Heavy Metal Removal in Water

Suntorn Sudsandee (✉ suntorn.sud@mfu.ac.th)

School of Health Science Mae Fah Luang University <https://orcid.org/0000-0002-4557-189X>

Pannipha Dokmaingam

Mae Fah Luang University School of Health Science

Rachaneekorn Mingkhwan

: Mahidol University Faculty of Tropical Medicine

Nuttapohn Kiangkoo

Mahidol University Faculty of Tropical Medicine

Suwalee Worakhunpiset

Mahidol University Faculty of Tropical Medicine

Patharawadee Boonying

National Nanotechnology Center

Chien-Chieh Hu

National Taiwan Institute of Technology: National Taiwan University of Science and Technology

Sawanya Laohaprapanon

WU: Walailak University

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Abstract

Thermodynamically driven salts and annealing processes developed the pure silk fibroin (SF) membranes from *Bombyx mori* cocoons. For more generation, the silk fibroin solution 5 wt% mixed the sodium chloride (NaCl) and polyethylene glycol (PEG). The top surface and cross-section of silk fibroin mixed with NaCl (SF/NaCl) showed pore in the surface membrane and hydrophilic property. The β -sheet content in the SF/NaCl membrane is higher than pure silk fibroin (SF) and the silk fibroin mixed with PEG (SF/PEG) membranes. Then cadmium (Cd), lead (Pb), and mercury (Hg) metals in water filtered pass through only SF/NaCl experimented. The pressure, pH, and temperature were effect by the flow rate efficiency of SF/NaCl membranes. The results showed that the highest % removal of Cd, Pb, and Hg were 45.36%, 61.43%, and 86.87%, respectively. The best condition of absorption capacity was 8.50, 6.42, and 41.14 mg/g for Cd, Pb, and Hg. As a natural biopolymer, SF/NaCl possibly apply for Hg removal for water treatment, its nontoxicity, excellent biocompatibility, and mechanical toughness.

1. Introduction

Silk cocoons are biopolymers, the long filament to protect larvae from humidity, bacteria, molds, and ultraviolet from sunlight during completed metamorphosis (Floren et al. 2016). Silk fibers are consist of two types of protein microfilaments that the fibroin coated by the sericin (Altman et al. 2003; Hardy et al. 2010; Chung et al. 2011; Putthanarat et al. 2000). The silk fiber of *B. mori* cocoon, there is fibroin about 70–80%, sericin 20–30%, carbohydrates 1.2–1.6%, and other components (Rockwood et al. 2011). The crystalline region is composed of a repeated protein sequence of Gly-Ala-Gly-Ala-X, which form β -sheet structures packed by hydrogen bonds and protein chains (Floren et al. 2016; Chung et al. 2011; Inoue et al. 2000). The thermodynamically driven salts and annealing processes were applied to produce silk fibroin. Inorganic salt or lithium bromide (LiBr) has been used to dissolve silk fiber's degumming (Kim et al. 2015; Rockwood et al. 2011; Zhang et al. 2015).

In previous studies, there are many methods for silk fibroin membranes and films and applied to remove the heavy metals—silk fibroin modified to natural biomaterial membrane for heavy metal removals (Gao et al. 2017). Gao et al., 2017, used silk fibroin membrane to remove lead (Pb^{2+}) and cadmium (Cd^{2+}) ions, effective between 82% and 56%, respectively. Furthermore, Silk fibroin/chitosan (SF/CS) blend membranes which result shown that the permeability of SF/CS blend membranes was in the sequence $\text{K}^+ > \text{Ca}^{2+} > \text{Cd}^{2+} > \text{Pb}^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+}$ (Du et al. 2006). In addition, Rastogi et al. 2020 produced silk fibroin blended with different biomaterials for metals adsorption, which they found efficiencies equivalent to 81.1% for Cd and 93.75% for Pb ions. There were sever studies that produced film and membrane to remove and adsorb metals ions from water by using silk fibroin combined with biomaterial hybrids (Aslani et al. 1988; Ishikawa et al. 2002; Chang et al. 2007; Zhou et al. 2001; Ramya and Sudha 2013; Sato et al. 2019).

For past decades, heavy metals, such as cadmium (Cd), lead (Pb), and mercury (Hg), have been highly toxic and hazardous pollutants from industry, agriculture, and community activities. Therefore, Cd, Pb, Hg

are toxicity, persistency, and bioaccumulation in water bodies has brought great harm to aquatic living. It is complicated to eliminate low concentration heavy metals from the wastewater. The wastewater must take the new methods to remove heavy metals from effluent water before discharge to the environment. The membrane technology is processed to treat the ability of silk fibroin to adsorb heavy metals from wastewater. Water treatment technology requires safe polymers and materials to focus on natural materials. Natural silk fibroin has become used for water treatment because of its nontoxicity, good biocompatibility, biodegradability, and mechanical toughness (Yazawa et al. 2016; Shome et al. 2021; Yao et al. 2021, Chen et al. 2021). Membrane technology has been used as an effective technology for removing water contaminants. A variety of membrane materials have been developed for specific purposes and to improve purification efficiency. A nontoxic natural biopolymer, biocompatibility, biodegradability, and toughness, all is the advantage of silk fibroin can apply for water treatment. This experiment aimed to produce an alternative membrane from natural material to remove heavy metals from water.

2. Experiment

2.1. Chemical and materials

All chemicals used in this study are in analytical grade. *Bombyx mori* cocoon was purchased from Buriram province, Thailand, and it was used without any purification. Polyethylene glycol (PEG), sodium chloride (NaCl), and lithium bromide (LiBr) were purchased from Union Science, Ltd. co (Chiang Mai, Thailand). The metal salts of Cd, Pb, and Hg ions were obtained from Loba Chemie Pvt. Ltd (Mumbai, India).

2.2. Silk fibroin preparation

The Silk fibroins (SF) were prepared in the laboratory following standard procedures reported in the literature (Rockwood et al. 2011, Sudsandee et al. 2019). Briefly, *B. mori* cocoon was boiled for 30 min in 0.02 M Na₂CO₃ aqueous solution to extract the sericin protein. The fibers were rinsed several times with distilled water and air-dried at room temperature overnight. LiBr aqueous solution at 9.3 M poured on the degummed silk fibers and oven at 60 °C for 4 h. The silk fibroin solution was dialyzed against distilled water for 72 h using a dialysis tube (MWCO 3,500). The purified solution was centrifuged at 6000 rpm for 30 min to eliminate the insoluble fibrous. The final SF concentration was measured by weight balance, and it was diluted with distilled water to obtain an SF concentration of 5 to 8 wt.% for further use in this study.

2.3. Synthesis of Silk Membrane

In this study, the silk fibroin membranes were prepared by a drop-casting method. Ten grams of sodium chloride (NaCl) and 4 mL of polyethylene glycol (PEG) were separately mixed with 50 mL of 5.0 wt% silk fibroin solution to generate a blended silk fibroin/sodium chloride (SF/NaCl) and silk fibroin/polyethylene glycol (SF/PEG) membrane. The mixed solutions were poured into glass plates with a diameter of 80

mm, and the solution was dried in an oven at 60 °C for 24 h. The dried membrane was soaked in the distilled water until uses. New distilled water is changed daily.

2.4. Characterization

The morphology of surface and cross-sectional of pure and blended SF membranes were studied by using a scanning electron microscope (LEO Electron Company, USA) with a magnification of 100k and a voltage of 3.0 kV. All infrared membranes were collected using a Fourier transform infrared spectroscopy (ThermoFisher SCIENCETIFIC, Waltham, Massachusetts, United States). For each measurement, the scan was recorded with a resolution of 4 cm⁻¹, and the measurement range was between 400 and 4000 cm⁻¹. The secondary structure of SF, including beta sheets and alpha helices, was evaluated using a software peak fit. The confirmation of silk II structure (β -sheet) analyzed a distinct shift in the β -sheet peak at 1612–1624 cm⁻¹ (Litvinov et al. 2012). The surface hydrophilicity properties of the membranes were inspected by water contact angle (FACE, Automatic Interfacial Tensiometer, PD-VP) measurement.

Pure water flux (flow rate) analyses for the prepared membranes were carried out in a dead-end mode. The newly prepared pristine or blended SF membranes were placed on the membrane filtration cell, which has a diameter of 5 cm and a volume capacity of 1,000 mL. The membrane filtration cell was connected to the vacuum pump, as shown in Fig. 1. Pure water flux of every membrane sample was monitored at the operating pressure between 100 - 500 mbars.

2.5 Membrane performance of metal removal

The separation performance of the blended SF membrane was analyzed using metal solutions of Cadmium (Cd), Lead (Pb), and Mercury (Hg) as a feed solution at the concentration of 10 ppm. The membrane filtration of the metal solution was carried out at a pressure between 100 - 500 mbars. The permeate was collected over declined intervals in the sample container, and the solution were tested for metal concentration using atomic absorption spectrophotometer, AAS (Hitachi High Technologies, Tokyo, Japan) for Cd and Pb. At the same time, the Hg was measured using an MA-3000 mercury analyzer (Nippon Instruments, Tokyo, Japan). Each experiment was carried duplicated while the removal rate of studied metals can be calculated using **equation 1** (Gao et al., 2017).

$$\% \text{ removal} = (C_0 - C_t) / C_0 \times 100 \text{ ————(1)}$$

Where C_0 is initially concentration of metal (mg/L)

C_t is final concentration of metal after pass membrane (mg/L)

The effect of pH on membrane performance for metal removal was tested between 7 and 13. The effect of temperature (5, 10, 20, 30, and 40 °C) on membrane flow rate was also measured.

The adsorption capacity (q) was measured by Equation 2 (Ouyang et al.2919; Rastogi et al. 2020):

$$q \text{ (mg/g)} = (C_0 - C_t) V_s / m \text{ ————(2)}$$

Where C_0 is metal concentration in stock solution (mg/L)

C_t is metal concentration after filtration test (mg/L)

V_s is metal solution volume (L)

m is membrane mass (g)

3. Results And Discussion

3.1 Morphology of silk fibroin membranes

Pure SF membranes and blended SF membranes of NaCl and PEG were simply prepared by the drop-casting method. The physical characteristic (i.e., color) of pure SF membrane slightly differed from the blended membranes as presented in Fig. 2(a), (b), and (c). The blended SF membrane had high turbidity, while the membrane surface of SF/PEG was the cloudy white color. The obtained membranes are homogenous.

Figure 2(d), (e), and (f) show the morphology of the surface and cross-sectional picture of all prepared membranes in this study. As observed, the SF/NaCl has high porosity compared with the SF/PEG and SF membranes, respectively. For the SF/PEG membrane, many wrinkles were found on the membrane surface. For porosity from the natural polymer by using crystalline sodium chloride (NaCl) salt and mixed with a solution mixture main component is polyamide. Then formed by baking, NaCl crystals are dissolved with water. This crystal causes the missing portion of the sodium chloride to become porous. The sodium chloride was applied to control scaffolds with a pore size (Park et al. 2010; Yan et al. 2012). The influence of NaCl on silk fibroin solution is show in Figure 2(e) and Figure 2(h). Moreover, in contrast with Pacharawan et al. 2013, this study can improve the porosity of silk fibroin membrane using polyethylene glycol.

The FTIR spectra of SF, SF/NaCl, and SF/PEG membranes were collected to investigate the change of surface chemistry of the prepared silk membranes. As presented in Fig. 3, the characteristic structure of silk II at the adsorption peak of 1620 cm^{-1} , were found in all membranes. This peak refers to the formation of β -sheet structure in the silk membrane.

The amount of β -sheet crystals found on SF/NaCl membrane was higher than that on SF and SF/PEG membranes. Silk fibroin is consists of β -sheet crystallites and amorphous domains, and these can building blocks (Yuan et al. 2014). Keten et al. 2010 indicated β -sheet crystallites within a few nanometers, much higher stiffness and strength must be achieved (Keten et al., 2010). Water significantly impacts the mechanical properties, making the membrane more feasible (Fu C, 2009). Meanwhile, Yuan et al. found that water molecules play a weakening forming hydrogen bonds, β -chains, and the instability of the crystallite (Cheng et al. 2014).

The hydrophilicity of the studied SF membranes was also investigated using the water contact angle method. As shown in Fig. 4, the contact angle of the SF/NaCl and SF/PEG blended membranes was decreased due to the addition of NaCl and PEG to SF matrix. Among the studied membranes, SF/NaCl blended membranes have the lowest contact angle of 22.25° followed by 57.85° for SF/PEG membrane and 72.06° for pure SF membrane. The difference in hydrophilicity in the membrane may come from the surface roughness and membrane pores (Chan and Ng, 2016; Mohammad et al. 2019). Increased surface hydrophilicity can make the membrane fouling resistant due to the water's easy diffusion through the membrane thickness. Hence, the SF/NaCl blended membrane has better chances of antifouling ability and higher water flux.

3.2 Pure water flux (flow rate)

The deionized water was treated by keeping the temperature (20°C) and flow rate varying the pressure as 100 mbars, 200 mbars, 300bars, 400 mbars, and 500 mbars. As shown in Fig. 5, the flow rate of membrane permeation was increased from 120 mL/min to 1033.3 mL/min when pressure was increased from 100 mbars to 500 mbars. The increasing pressure during the membrane contact with water can reduce the number and strength of hydrogen bonds between β -chains, thus dramatically weakening the strength of silk fibroin (Cheng et al. 2014; Love et al. 2019; Yang et al. 2019; Kook et al. 2019).

The temperature effect of flowing rate of SF/NaCl membrane was treated by keeping the pressure (200 mbars) constant and then varying the temperature at 5°C, 10°C, 20°C, 30°C, and 45°C of initial water. The average flow rate of membrane permeation was increased from 730.0 mL/min to 1035.3 mL/min when pressure was increased from 5 °C to 45 °C. Then the average flow rate increased to 951.7 mL/min at 30 C° but it was decreased to 1045.3 ml/min at 40 C°, as shown in Fig. 6. The water temperature between 5 – 20 °C showed flow rate stability to increase, but after 20 oC was the flow rate instability. The water molecules influence the glass transition of silk (Yazawa et al. 2016), the effect of thermal water can be changing the flow rate properties. In addition, heating solution cast fibroin can be converted to the silk II β -sheet structure commonly found in *B. mori* cocoon fibers (Drummy et al. 2005).

The water pH range was 7-13. The pH effect of the flowing rate of SF/NaCl membrane, the metal wastewater sample was treated by keeping the pressure (200 mbars) constant. The average flow rate of membrane permeation was increased from 180.0 mL/min to 597.3 mL/min when pH was increased from 7 to 13. Then it was decreased to 540.0 mL/min at pH 13, as shown in Fig. 7.

3.3 Heavy metal removal and efficiency.

The SF/NaCl membranes were applied to remove the three metals Cd, Pb, and Hg in deionized waters. It was the concentration 10 ppm. Various pH of metal waters of Cd, Pb, and Hg has shown the % removal in Fig. 8.

pH

Cd removal was found in the range of 10.76 – 45.36 %, as shown in Fig. 8. The highest % removal for Cd was 45.36% at pH 12. At the same time, Pb % removal was found to range from 10.80 – 61.43%. The highest % removal for Pb was 61.43% at pH 12. The highest % removal for Hg was 78.18% at pH 9. The mean concentration of Hg removal was range 70.20 – 78.18 % for pH 7-12. Comparing the % removals of Cd, Pb, and Hg found that Hg was high efficiency for metal removal by use SF/NaCl higher than Cd and Pb at pH 7-12.

Temperature

The various metal water temperature was range from 5 – 40 °C, as shown in Fig. 9. Cd removal was found in the range of 6.90 – 15.04 %. The highest % removal for Cd was 15.04% at 5 °C. At the same time, Pb % removal was found the range of 13.93 – 16.27%. The highest % removal for Pb was 16.27 % at 40 °C. The highest % removal for Hg was 72.72 % at 30 °C. The mean concentration of Hg removal was range 61.26 – 72.72 % for temperatures 5 – 40 °C. Comparing the % removals of Cd, Pb, and Hg found that Hg was high efficiency for metal removal by use SF/NaCl higher than Cd and Pb at temperature 5 – 40 °C.

Pressure

The various metal water pressure in the filtrated column was range 100 – 500 mbars as shown in Fig. 10. Cd removal was found to range from 2.90 – 16.34 %. The highest % removal for Cd was 16.34 % at pressure 500 mbars. At the same time, Pb % removal was found to the range to 2.50 – 16.13%. The highest % removal for Pb was 16.13% at pressure 200 mbars. The highest % removal for Hg was 86.87 % at pressure 100 mbars. The mean concentration of Hg removal was range to 62.71 – 86.87 % for pressure 100-500 mbars. Comparing the % removals of Cd, Pb, and Hg found that Hg was high efficiency for metal removal by use SF/NaCl higher than Cd, and Pb at pressure 100-500 mbars.

Comparison of the % of metal removal is shown in Table 1. This study showed that the highest % removal of Cd, Pb, and Hg were 45.36%, 61.43%, and 86.87%, respectively. This study, apply SF/NaCl shown that % Cd removal is lower than research studies by using the MWSF (modified water-insoluble silk fibroin) and chitosan/silk fibroin films (Ramya and Sudha 2013; Gao et al., 2017). In addition, the % Pb removal is lower than the studies of Gao et al., 2017 and Zhao et al., 2020. SF/NaCl membrane is excellent for removal Hg greater than Metallic molybdenum disulfide (MoS₂)/Silk nanofibril (Zhao et al., 2020).

The average membrane mass (m: unit, gram (g)) was 1.76 g, and the metal solution volume (Vs: unit, liter(L)) was range 0.12 – 1.04 liters. The adsorption capacity (mg/g) of Cd, Pb, and Hg for SF/NaCl membrane is shown in Table 2. In this study, the highest adsorption capacity of Cd was 8.50 mg/g of metal water at 5 °C, at pH 7 and, pressure 200 mbars. For Pb found that the highest adsorption capacity was 6.42 mg/g of metal water at pH 12, at 20 °C, and pressure 200 mbars. Finally. The highest adsorption capacity of Hg was 41.14 mg/g of metal water at 100 mbars, at pH 7, and at 20 °C. The pH of

the metal solution found that Cd and Pb were high efficiencies of adsorption capacity at pH 12, and Hg was pH 9. In addition, the appropriate temperature of a metal solution of Cd, Pb, and Hg was at 5 °C, 40 °C, 30 °C, respectively. The best condition of absorption capacity was 8.50, 6.42, and 41.14 mg/g for Cd, Pb, and Hg, but the result was lower than the study of Cd and Pb absorption capacity of Rastogi et al. 2020.

Table 1
Comparison between the membranes in this study and others reported

Filtration Membrane	% Removal			References
	Cd	Pb	Hg	
SF/NaCl	45.36	61.43	86.87	This study
MWSF (modified water-insoluble silk fibroin)	65.00	82.00	-	Gao et al., 2017
Chitosan/silk fibroin	81.10	-	-	Ramya and Sudha 2013
Metallic molybdenum disulfide (MoS ₂)/Silk nanofibril	-	65.00	62.00	Zhao et al., 2020

Table 2
The adsorption capacity (mg/g) of Cd, Pb, and Hg for SF/NaCl membrane

Heavy metal	Adsorption capacity (mg/g) : highest efficiency		
	pH	Temperature (°C)	Pressure (mbar)
Cd	4.77	8.50	0.89
	(at pH 12)	(at 5 °C)	(at 500 mbars)
Pb	6.42	0.89	5.08
	(at pH 12)	(at 40 °C)	(at 200 mbars)
Hg	8.07	4.34	41.14
	(at pH 9)	(at 30 °C)	(at 100 mbars)

Conclusion

The possibility of silk fibroin membranes for heavy metal removal was evaluated in this work. SF membrane, SF/PEG membrane, and SF/NaCl were developed by casting 5 wt% of *Bombyx mori* cocoon. The SF/NaCl membrane produced pore size on membranes, water permeate and showed higher

crystalline formation (β -sheet) that enhanced the structured membrane. The contact angle ($^{\circ}$) of SF/NaCl was lower than SF and SF/PEG membranes. The SF/NaCl removed the Cd, Pb, and Hg. Deference of pressure, pH, and the temperature was effect by the flow rate efficiency of SF/NaCl membranes. The results showed that the highest % removal of Cd, Pb, and Hg were 45.36%, 61.43%, and 86.87%, respectively. The best condition of absorption capacity was 8.50, 6.42, and 41.14 mg/g for Cd, Pb, and Hg. As a natural biopolymer, SF/NaCl possibly apply for Hg removal for water treatment, its nontoxicity, excellent biocompatibility, and mechanical toughness.

Declarations

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Availability of data and materials

The data are available upon a reasonable request to only the corresponding author.

Authors Contributions

SS involved in conceptualization, laboratory analysis, writing - original draft, editing, and project administration. PD, PB, and HC-C are involved in conceptualization. RM, NK involved laboratory analysis. SW and SL involved final editing.

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Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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Competing Interests section

Not applicable.

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Figures

Figure 1

Membrane filtration experimental set-up

Figure 2

The morphology of the surface and cross-sectional images of the SF membranes. (a-c) Digital photo of the obtained membranes. (d-i) Surface and cross-sectional scanning electron microscope (SEM) images of the SF, SF/NaCl, and SF/ PEG membranes, respectively

Figure 3

FTIR spectra of pure SF and both blended membranes

Figure 4

Water contact angle of blended SF membranes compared with pure SF membrane

Figure 5

Relationship between flow rate and pressure of the filtrated column

Figure 6

Relationship between flow rate and temperature of the water

Figure 7

Relationship between flow rate and pH of the water

Figure 8

The % removal of metals (Cd, Pb, Hg) at pH 7-12.

Figure 9

The % removal of metals (Cd, Pb, Hg) at temperature 5 – 40 oC

Figure 10

The % removal of metals (Cd, Pb, Hg) at 100 – 500 mbars of pressures.