

Chromium(VI) Adsorption Behavior of Silk Sericin Beads

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Abstract

Silk sericin (SS) has been fabricated into beads using a 1 M LiCl/DMSO solvent and utilized as a heavy metal adsorbent. Among the various heavy metals, we targeted Cr(VI) for adsorption using SS beads and found that its adsorption depended on the coagulant used for the fabrication of the SS beads. When methanol was used as a coagulant, the beads had a better adsorption capacity than when ethanol was used except at pH 1. The adsorption behavior of Cr(VI) on the SS beads followed the BET isotherm. The maximum adsorption capacity was 33.76 mg/g at pH 2. The adsorption of Cr(VI) was confirmed by FT-IR and EDS analyses. Finally, the desorption was carried out using NaOH solution, and it was found that 73.19% of the adsorbed Cr(VI) could be detached.

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Introduction

The recovery and utilization of silk sericin (SS) have attracted much interest among scientists in the field of sericulture, since they can provide an additional benefit to the sericulture industry. SS can be recovered from degumming waste using membrane techniques (Capar *et al.*, 2004). However, without an appropriate application of the recovered SS, this technique will not be applied in the industry. Therefore, there has been much research on new applications of SS besides in cosmetics, where SS has been used widely for a long time. We have previously

reported that SS can be fabricated into beads (Oh *et al.*, 2007) and microspheres (Oh *et al.*, 2011) using a 1 M LiCl/DMSO solvent. As one potential application, the SS beads and microspheres were used as drug carriers. However, there has been some debate on the safety of SS biomaterials, and there have been several reports questioning the biocompatibility of SS (Liu *et al.*, 2006; B. Panilaitis *et al.*, 2003). Although the use of SS in the biomedical field is attractive and many studies have been reported on such applications (Kundu *et al.*, 2008; Kim *et al.*, 2012; Seo *et al.*, 2011), they cannot be realized in the near future unless these safety concerns are solved. Outside the

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biomedical field, there have been attempts to use SS in materials fields as fibers (Zhang *et al.*, 2012; Oh *et al.*, 2008) and films (Teramoto *et al.*, 2005). However, the pure SS and various polymer blends with SS could not achieve the mechanical strength needed for practical applications. Therefore, more effort is required to find an appropriate application of SS.

Heavy metal pollution is a serious problem that has a severe impact on the environment and human health. Lead, cadmium, mercury and chromium are the major toxic heavy metals, and their levels in any human commodities are strictly regulated by the government. Among these toxic metal ions, we focused on the removal of chromium using SS. Chromium and its compounds are widely used in various industrial processes such as tanning, electroplating, and synthesis of dyes and pigments. Chromium can exist in eleven valence states, but Cr(III) and Cr(VI) have the largest impact on the environment because of their stability (Kotaś *et al.*, 2000). Cr(VI) has a higher toxicity than Cr(III) because of its high uptake by cells and the formation of radicals inside the cell (Costa, 2003). Therefore, there have been many studies on the removal of Cr(VI), and various materials and techniques have been applied (Owlad *et al.*, 2009). Conventionally, the precipitation technique has been widely used for Cr(VI) removal, but the disposal of the solid waste remains a problem. Adsorption techniques have been widely used recently, where activated carbon is used as an adsorbent. However, because of the high cost of the activated carbon, there is a need to find cheaper alternatives. Various agricultural by-products have been investigated as substitutes, and many were found to be effective for the removal of Cr(VI) (Sud *et al.*, 2008). Mudhoo *et al.* (Mudhoo *et al.*, 2012) suggested that the biomass for the metal adsorption should come in large quantities from industrial wastes. SS meets these requirements, and previously, Song *et al.* (Song *et al.*, 2007) has reported on Cr(III) adsorption using SS. They prepared amphiphilic core-shell poly(methyl acrylate)/SS nanospheres, and found that 4.876 mg of Cr(III) can be adsorbed per gram of nanospheres.

In this study, we have prepared SS macrobeads using a previously reported method (Oh *et al.*, 2007) and investigated their Cr(VI) adsorption ability. The removal of Cr(VI) was measured using colorimetric methods, and the adsorption of Cr(VI) was confirmed by FT-IR and EDS analyses. In addition, we predicted the mechanism of adsorption of Cr(VI) onto SS using isothermal models.

Materials and Methods

Materials

Silkworm cocoons were kindly provided by Heung Jin Co. Ltd. All chemicals were purchased from Sigma-Aldrich (USA).

Extraction of silk sericin

SS was extracted by boiling 25 g of Bombyx mori silkworm cocoons with 1 L of distilled water using an autoclave for 1 h at 120°C. The extracted solution was filtered with a nonwoven filter in order to remove the remaining cocoons. The SS solution was frozen at -70°C for 4 h and lyophilized.

Preparation of silk sericin beads

The lyophilized SS was dissolved in 1M LiCl/DMSO solution for 2 h at 50°C to prepare a dope solution of 25% (w/v). The dope solution was dropped into alcohol coagulants through a 26G syringe using a syringe pump (KD scientific, USA). Methanol and ethanol were used as coagulants, and the obtained beads are designated as SS-M and SS-E, respectively. The SS beads were left in the coagulant bath for another 1 h. They were then filtered with a nonwoven filter and washed with the same coagulant to remove the residual LiCl and DMSO. To enhance the water stability and mechanical strength of the SS beads, the beads were immersed in a crosslinking reagent. The crosslinking was performed with 2%

(v/v) glutaraldehyde (GA) in the same coagulant. The reaction was carried out for 1 h at room temperature. Finally, the SS beads were washed with the same coagulant followed by distilled water to remove the excess GA.

Batch adsorption and desorption studies

The stock solution of Cr(VI) (1000 mg/L) was prepared by dissolving 2.827 g of potassium dichromate, $K_2Cr_2O_7$, in 1 L of distilled water. Different concentrations of Cr(VI) solution were prepared by diluting the stock solution with distilled water. Each adsorption experiment was conducted in a 250 mL Erlenmeyer flask containing 100 mL of Cr(VI) solution. The mixtures were stirred continuously on a multi-stirrer (JEIO TECH, Korea) at 180 rpm for 24 h at 25°C. In order to find the optimum pH, batch adsorption experiments were conducted in a pH range from 1.0 to 5.0 for the Cr(VI) solution (100 mg/L). The adsorption isotherm was studied by varying the initial Cr(VI) concentrations from 25 to 300 mg/L. The concentration of Cr(VI) in the solution was determined using a standard colorimetric method (Snell *et al.*, 1959). After each batch experiment, the Cr(VI) solution was filtered with a nonwoven to remove SS beads and reacted with 1,5-diphenylcarbazide in acid solution. The formation of a red-violet complex measured spectrophotometrically at 540 nm using microplate reader (Synergy H1, Biotek, USA). The equilibrium adsorption capacity, q_e , was determined using the following equation:

$$q_e = \frac{(C_0 - C_e)}{M} \times V$$

where C_0 and C_e are the initial and the equilibrium concentration of the Cr(VI) in the testing solution (mg/L), V is the volume of the testing solution (L), and M is the weight of the SS beads (g).

The desorption of Cr(VI) was performed by using 0.1 M NaOH solution. After the adsorption experiment, the SS beads were recovered from the

Cr(VI) solution and washed gently with distilled water three times to remove the residual Cr(VI) on the surface. The beads were immersed into 100 mL of 0.1 M NaOH and stirred overnight. The desorption efficiency was calculated as

desorption efficiency(%) =

$$\frac{\text{Mass of Cr(VI) ions desorbed}}{\text{Mass of Cr(VI) ions adsorbed}} \times 100$$

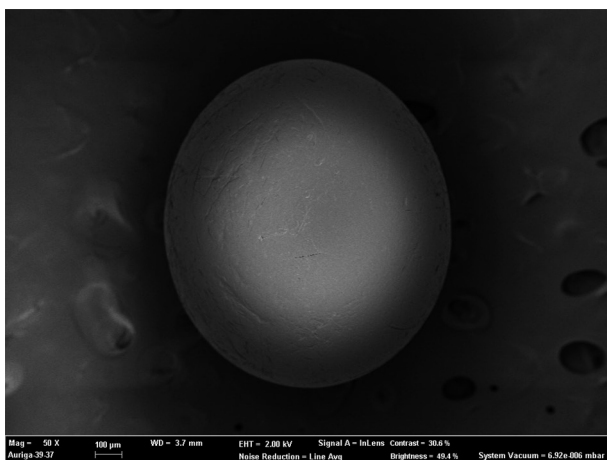
Characterization of sericin beads

ATR-FTIR spectroscopy (Thermo Scientific, USA) was used to identify the Cr(VI) adsorbed onto the SS beads. The samples were examined within the wavenumber range 700–4000 cm^{-1} , and 32 scans with 8 cm^{-1} resolution were used to obtain the spectra. The morphology and EDS spectra of the SS beads were evaluated using a field-emission scanning electron microscope (FE-SEM) (SUPRA 55VP, Carl Zeiss, Germany).

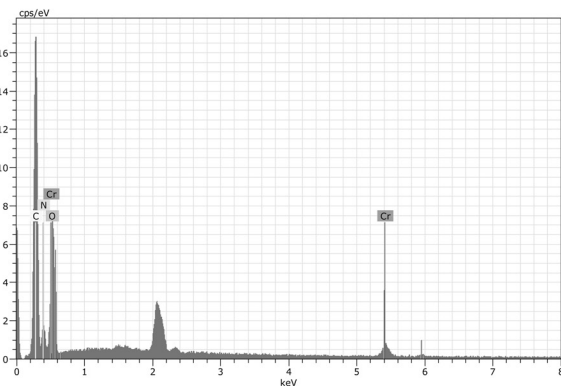
Results and Discussion

Evidence of Cr(VI) adsorption onto SS beads

We prepared SS beads following the same procedure reported previously by our group (Oh *et al.*, 2007). For their application as a bioadsorbent, a further crosslinking procedure using glutaraldehyde is employed in order to make the beads insoluble. Fig. 1a shows an SEM image of an SS bead after Cr(VI) adsorption. The bead maintained its shape even after it was subjected to pH 2. EDS analysis of the surface of an SS bead after Cr(VI) adsorption revealed that Cr(VI) is deposited on its surface (Fig. 1b). Besides those for carbon, nitrogen, and oxygen, two peaks for chromium could be observed. Fig. 2 shows the ATR-FTIR spectra of the SS bead before and after Cr(VI) adsorption. After Cr(VI) adsorption, new peaks due to Cr(VI)-O stretching vibration could be found at 897 and 933 cm^{-1} (Bolte *et al.*, 2005). This



(a)



(b)

Fig. 1. SEM image of an SS bead (a) and EDS analysis of the surface of the SS bead (b).

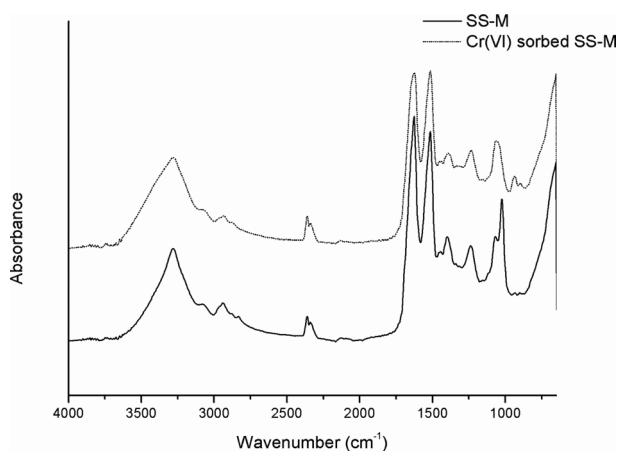


Fig. 2. ATR-FTIR spectra of SS beads before and after Cr(VI) adsorption.

also demonstrates that Cr(VI) is adsorbed on the SS bead. There have been some reports that metal ions

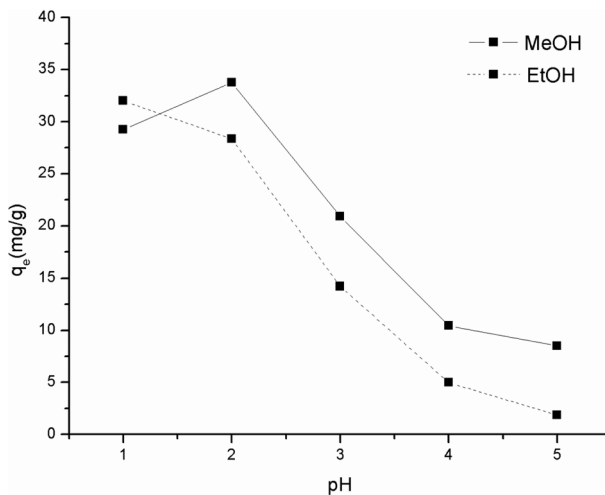


Fig. 3. Effect of coagulant and pH on the adsorption of Cr(VI) onto SS beads.

can induce structural deformation of proteins (Zhou *et al.*, 2004; Ahern and Garrell, 1991). However, there was no change in the amide I ($1600\text{-}1700\text{ cm}^{-1}$) peak after Cr(VI) adsorption, indicating that there were no structural changes. With these results, we confirm that SS beads can be used as a bioadsorbent of Cr(VI).

Effect of coagulant and pH on Cr(VI) adsorption

The SS beads were prepared by adding the dope solution dropwise into a coagulant. We have previously shown that both methanol and ethanol can be used as coagulants (Oh *et al.*, 2007). Fig. 3 shows the effect of the coagulant and the pH on the Cr(VI) adsorption. Overall, SS beads prepared with the methanol coagulant (SS-M) had better adsorption capacities than those prepared with the ethanol coagulant (SS-E), except at pH 1. The adsorption of Cr(VI) is poorer at higher pH in both SS-M and SS-E. It is well known that Cr(VI) exists as a chromate ion in solution, and its exact chemical formula depends on the pH. Above pH 6, Cr(VI) forms CrO_4^{2-} ; between pH 2 and 6, it forms HCrO_4^- and $\text{Cr}_2\text{O}_7^{2-}$; and below pH 1, H_2CrO_4 is predominant. Because of this ionic character, Cr(VI) is adsorbed onto positive surfaces instead of negative surfaces. SS has a high portion of charged amino acids compared to silk fibroin, and

negatively charged amino acids are more predominant than positively charged ones. For this reason, the isoelectric point (pI) of SS is slightly acidic, between 4 and 5. This means that the net charge of SS below pH 4 is positive, whereas it is negative when the pH is higher than 5. Therefore, chromate anions could not be adsorbed at high pH because of the charge repulsion. The maximum adsorption occurred at pH 2 for SS-M and at pH 1 for SS-E. Interestingly, SS-M had a better adsorption capacity than SS-E. This might be due to the slight differences between the hydrophobicities of the coagulants. When the SS solution is dripped into the coagulant, mass transfer occurs between the solvent (1 M LiCl/DMSO) and the coagulant (methanol or ethanol). In particular, the solvent diffuses out and the coagulant diffuses in, which results in solidification of the SS. During this event, SS will come into contact with the coagulant, and its surface characteristics will depend on the hydrophobicity or hydrophilicity of the coagulant. If the coagulant is hydrophobic, then the hydrophobic domain of the SS will face toward the surface of the bead. In the opposite case, where the coagulant is hydrophilic, now the hydrophilic domain of SS will be located on the surface of the bead. Because methanol is more hydrophilic than ethanol, the surface of the SS-M would be more hydrophilic than that of the SS-E. In general, charged amino acids make up the hydrophilic domains of proteins. Therefore, there will be more charged amino acids on the surfaces of the SS-M beads than on the SS-E surfaces, which will result in higher adsorption capacities in all pH ranges. Since SS-M has a better adsorption capacity for Cr(VI) and shows the highest adsorption capacity at pH 2, SS-M was used as the adsorbent and the pH of the testing Cr(VI) solution was fixed at 2 for further experiments.

Effect of initial concentration of Cr(VI)

The initial concentration of Cr(VI), C_0 , is important, since it is the driving force for the Cr(VI) adsorption. As shown in Fig. 4, for C_0 values up to 100 mg/L, the equilibrium adsorption capacity, q_e , increases

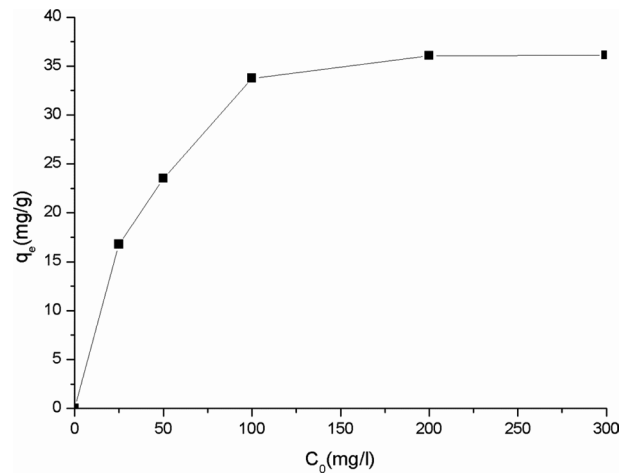


Fig. 4. Effect of initial concentration of Cr(VI) on the adsorption capacity of SS beads.

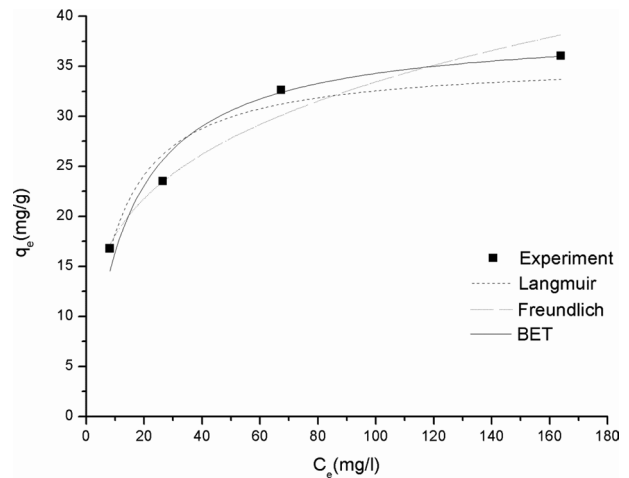


Fig. 5. Adsorption isotherm for Cr(VI) onto SS beads.

almost linearly. The q_e is 16.78 mg/g at $C_0 = 25$ mg/L but increases to 33.76 mg/g at $C_0 = 100$ mg/L. When C_0 exceeds 200 mg/L, q_e plateaus at about 36 mg/g, indicating saturation. Since the SS beads have only a limited number of binding sites, a higher concentration gradient is required in order to achieve saturation. This means that the removal ratio will decrease with increasing C_0 , as was observed in this experiment.

Adsorption isotherm

The adsorption isotherm provides useful information about what kind of interaction occurs between

Table 1. Isotherm model constants

Isotherm models	Langmuir isotherm			Freundlich isotherm			BET isotherm		
	Q	b	R ²	K	n	R ²	Q	B	R ²
Constants	35.68	0.10	0.938	9.81	3.75	0.957	39.0	8.90×10 ⁵	0.997

the metal ion and the adsorbent. Fig. 5 shows the adsorption isotherm for Cr(VI) adsorption onto SS-M, and Table 1 shows the Langmuir, Freundlich, and BET model constants. It was found that the BET model fits better than the Langmuir or Freundlich model. The differences between these models are that the Langmuir model assumes monolayer adsorption while the Freundlich and BET models assume multilayer adsorption. As shown in Table 1, the Langmuir model has the least R² value, indicating that the Cr(VI) adsorption onto SS-M occurs on multiple layers rather than one single layer. This is obvious, because although the SS bead has a compact inner structure, chromate ions can penetrate into the interior part of the SS beads because of swelling of the SS or the concentration gradient. Therefore, once the outermost layer of the SS adsorbs Cr(VI), the next layer will be ready for additional Cr(VI) adsorption. As a result, the Langmuir model is inadequate for explaining the results of this study. On the other hand, the Freundlich model assumes that the multilayers are heterogeneous, whereas the BET model is an extension of Langmuir model, in which each layer behaves according to the Langmuir model. Thus, the Freundlich model is also incapable of explaining the mechanism of Cr(VI) adsorption onto SS, because the affinity of Cr(VI) would be the same for all SS layers. The mechanism of Cr(VI) adsorption onto SS can therefore be explained as follows. At first, Cr(VI) is adsorbed onto the outermost layer of the SS bead, where the positively charged amino acids act as the binding sites for chromate anions. Once the outermost layer is fully occupied, the Cr(VI) will be adsorbed onto the next layer, and this process will be repeated until all accessible layers are saturated.

Table 2. Desorption efficiency for Cr(VI) from SS beads

Mass of Cr(VI) in SS-M (mg)		Cr(VI) desorption efficiency (%)
Before washing	After washing	
33.76	9.05	73.19

Removal of Cr(VI)

For reuse of the SS beads, the adsorbed Cr(VI) should be easily detached. There are many mechanisms for adsorption of Cr(VI) onto biosorbents. However, the fundamental adsorption mechanism is that the biosorbent should have positive charges in order to hold the negative chromate ions. As mentioned above, SS is a protein that has positive net charge at pH 2. If we increase the pH of the solution then the SS would have a negative net charge, resulting in desorption of the chromate ions. Therefore, we washed the Cr(VI)-adsorbed SS beads with NaOH solution. Table 2 shows the desorption results after NaOH washing. About 73.19% of the adsorbed Cr(VI) can be desorbed from the SS beads, which indicates that the SS beads can be continuously reused for Cr(VI) adsorption.

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