

Adsorption Behavior of Heavy Metal Ions from Aqueous Solution by Soy Protein Hollow Microspheres

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ABSTRACT: Heavy metals have become ecotoxicological hazards owing to their tendency to not degrade but accumulate in the vital organs of biological bodies. Biosorption is now an efficient method to purify industrial wastewater containing toxic heavy metal ions by using biomass as sorbents. In this work, mimicking the fabricating process of “Tofu”, soy protein was heat-denatured and transformed into soy protein hollow microspheres (SPMs) with a diameter of about 4–45 μm , which were then used as biosorbents to remove heavy metal ions in the water system. The trace amount of remaining metal ions was tested by atomic absorption spectroscopy, and the sorption kinetic and isotherm models were calculated and set up to describe the adsorption behavior. The results showed adsorption capacities of Zn(II), Cr(III), Cd(II), Cu(II), Pb(II), and Ni(II) by SPMs at 70 °C of 254.95, 52.94, 120.83, 115.01, 235.56, and 177.11 mg/g, respectively, which are much higher than that of many other natural polymeric sorbents. The pseudofirst-order kinetic model and Freundlich isotherm model were well correlated to the experimental data. Overall, SPMs were efficient sorbents for binding heavy metal ions, and their sorption capacities were dependent on factors like denaturation content, temperature, time, pH, and initial ionic concentration.

1. INTRODUCTION

Water pollution caused by heavy metal ions had been attracting great attention in the modern industrial society, because heavy metal ions even in trace amount are hazardous to human health and the ecosystem and are difficult to remove even at low concentration.^{1,2} Various methods for the removal of toxic metals from an aqueous system have been developed for a long period,³ such as ion exchange, reverse osmosis, membrane filtration, complexation precipitation, and adsorption.⁴ Normally, adsorption is a highly effective, environmentally friendly, inexpensive, and easy way to operate among those physicochemical treatment processes,⁵ especially method of biosorption. Biosorption was used to describe the ability of biological materials like biopolymers to concentrate or remove organic or inorganic pollutants like heavy metals by the passive binding to nonliving biomass from an aqueous solution.⁶ Many macromolecules extracted from a biological body, such as chitosan,⁷ cellulose,^{8,9} starch,¹⁰ alginate,¹¹ and so on, have great potentials of adsorbing heavy metal ions from wastewater because they bear high contents of hydroxyl, amino, and other active functional groups on molecular chains. However, the adsorption capacity of these unmodified biopolymers was limited. To improve adsorption performance, micro- and nanotechnology applied on biopolymer absorbents were still under development.

Recently, biopolymer microspheres^{12,13} have been paid attention due to the good performance of such microspheres, such as low toxicity, good biocompatibility, low cost, minimization of secondary wastes and biodegradability, large surface area, and high mechanical stability. Among the most used biopolymers, modified chitosan beads showed a high capability of absorbing Cu(II) ions up to 64.62 mg/g.¹⁴ Yang et al.¹⁵ prepared neutral starch microspheres cross-linked by epichlorohydrin using an inverse microemulsion method. The reported anionic starch microspheres with average diameter of 75 μm had good sphericity, fine dispersibility, and good ability

of sorption heavy metals. The adsorbed amounts of starch microspheres were 75.99–83.33 mg/g for Cu(II) and 62.40–66.67 mg/g for Pb(II) ions, respectively. Meanwhile, chitin and alginate microspheres have also been explored for heavy metal ion treatment.

Soy protein is composed of a mixture of globular proteins, which can be divided into 2S, 7S, 11S, or 15S fractions on the basis of molecular weight and sedimentation coefficient.¹⁶ Among those fractions, 7S and 11S globulin are two main globular proteins, with amounts up to 37% and 31%, respectively.¹⁷ Globular proteins are composed of segments of polypeptides connected with hydrogen bonds, electrostatic interactions, disulfide bonds, and hydrophobic interactions.¹⁸ Once exposed to pH, ionic potential, heat, and other factors, conformational changes of unfolding globular proteins through a physical/chemical process would cause the denaturation of native globular proteins, i.e., converting into unfolded polypeptide chains, which are connected with interchanging of disulfide bonds. It has been widely reported that soy protein can be precipitated by adding many Ca(II) or Mg(II) ions in order to make a food “Tofu” as a result of metal ion-induced association. On the basis of the “salt-out” theory, we could deduce that heavy metal ions can be combined to soy protein and then taken away from an aqueous system. Soy protein hydrogel modified by ethylenediaminetetraacetic acid dianhydride was also reported to have good metal-chelating properties.^{19,20} In this work, soy protein microsphere would be fabricated and used to uptake heavy metals ions; simultaneously, various parameters like contact time, ionic concentration, and adsorption temperature were investigated to

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determine adsorption behavior of soy protein hollow microspheres (SPMs) in an aqueous system. We aim to set up a meaningful sorption model about this novel biosorbent for future application of heavy metal environmental treatments.

2. MATERIALS AND METHODS

2.1. Materials. Commercial soy protein isolate (SPI) purchased from Dupont-Yunmeng Protein Technology Co. Ltd. (Yunmeng, China) was used as pure soy protein. The original moisture and protein content of SPI (dry basis) were 5.0% and 92.3%, respectively. Cadmium(II) nitrate tetrahydrate, lead(II) nitrate, nickel(II) nitrate hexahydrate, cupric(II) sulfate anhydrous, chromium(III) chloride hexahydrate, and zinc(II) sulfate heptahydrate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All these chemicals were analytical grade and used as received without further purification.

2.2. Preparation of SPMs and Removal of Heavy Metal Ions. A target amount of SPI was vacuum-dried at 100 °C for 2 h, and then, 1.0 g of dried SPI was poured into a flask containing 200 mL of deionized water to prepare soy protein slurry under mechanical agitation at 50 rpm for half an hour at 70 °C.

Heavy metal ion adsorption experiments were then performed according to the batch method. A target amount of zinc sulfate dehydrated at 70 °C was added into the prepared soy protein suspension, thus composing simulated wastewater with different concentrations of Zn(II). Adsorption of Zn(II) ions by soy protein was achieved at 70 °C under mechanical agitation at 50 rpm for 4 h. After adsorption, soy protein binding Zn(II) ions were separated from the mixed suspension by centrifugation at 12 000 rpm (HC-2061 centrifuge, Anhui USTC Zonkia Scientific Instruments Co., Ltd.). Finally, the supernatant was diluted for concentration analysis using atomic absorption spectroscopy (AAS, 3510, Shanghai branch, Agilent technologies Inc.). Other ions like Cd(II), Pb(II), Ni(II), Cu(II), and Cr(III) ions with different concentrations were adsorbed, separated, and analyzed in the same way as Zn(II). In addition, Cd(II), Zn(II), and Cr(III) ions were poured into soy protein suspension and stirred for 4 h at 30, 40, 50, 60, 70, and 80 °C, respectively. After centrifugation, the separated supernatants were subsequently used for AAS testing to analyze the adsorption capacity of heavy metals by soy protein under varying temperature. Contact time dependent adsorption experiments were carried out as follows. A target amount of dehydrated zinc sulfate was poured into soy protein aqueous suspension (2.5 g/L) at 70 °C. During the adsorption process, a 25 mL suspension was sampled by pipet (25 mL) at particular intervals, and then, the corresponding supernatant was immediately centrifuged, collected, and diluted for concentration analysis by AAS. Similarly, the dependence of contact time on adsorption of other ions like Cd(II), Pb(II), Ni(II), Cu(II), and Cr(III) was tested. During our experiments, we tested the pH of solutions before and after adsorption and found that pH varied within 0.05, so the influence could be neglected. Furthermore, no additional competitive ions from buffer solution would affect the sorption amount and sorption behavior of heavy metal ions. Therefore, without any buffer solution, nitric acid (0.1 M) was dropped into soy protein suspension containing heavy metal salts to adjust systemic pH at 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, respectively; thus, pH dependent adsorption of heavy metals

was tested by AAS. Adsorption capacity of soy protein q_t (mg/g) above-mentioned was expressed as the following equation:

$$q_t = \frac{m_1 - V_i \times C_i}{m} \quad (1)$$

where C_i represents the final metal ion concentrations of the solution after the adsorption processes (mg/L), V_i represents the final solution volume (L), m represents adsorbent mass (g), and m_1 represents the initial metal ion mass (mg).

2.3. Morphological Characterization of SPMs. The above obtained sediment after centrifugation was baked at 40 °C in a drying oven for 12 h. The obtained baking “cakes” were cut into small pieces, one of which was further sintered at 350 °C for 1 h, and then, the baking “cake” and calcinated “cake” piece were coated with gold for scanning electron microscopy (SEM, Hitachi S-3600N VP SEM, Hitachi, Japan) observation at 20 kV. The specific surface areas of sorbents were measured by the nitrogen adsorption/desorption isotherm method at liquid nitrogen temperature using the Automated Gas Sorption (Autosorb-iQ-AG-MP, Quantachrome Co., U.S.A.). The Brunauer–Emmett–Teller (BET) model was applied to calculate the apparent surface area.

3. RESULTS AND DISCUSSION

3.1. Appearance and Morphology of SPMs. The Brunauer–Emmett–Teller (BET) surface area of soy protein

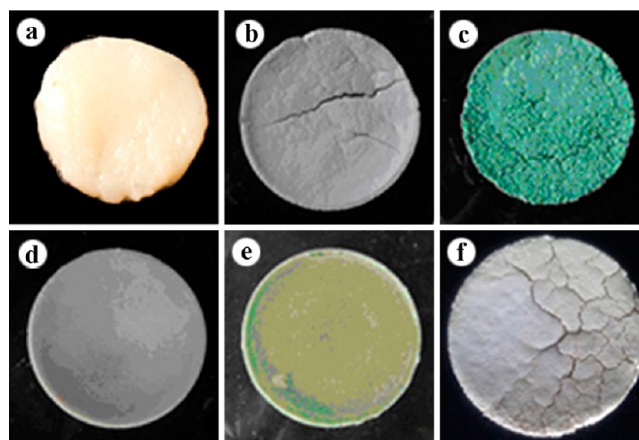


Figure 1. Photographs of SPMs (a) and SPMs binding Cd(II) (b), Cu(II) (c), Pb(II) (d), Ni(II) (e), and Zn(II) (f) ions, respectively.

and thermally denatured soy protein was 6.20 and 9.76 m²/g, respectively, indicating a higher surface area of the sorbent. Photographs of baking “cake” were displayed in Figure 1, in which panels a–f represent SPMs and SPMs adsorbed Cd(II), Cu(II), Pb(II), Ni(II), and Zn(II), respectively. Soy protein was easily thermo-heated into weak gel (Figure 1a). When heavy metal ions were adsorbed into soy protein, the gel was solidified and could be baked into “cake” which had a solid form and special color originated from the nature of ions, e.g., blue cake with Cu(II) ion. Interestingly, the “cake” containing Pb(II) and Ni(II) ions is very strong and likely solidified from a gelation sediments; that is, these two heavy metal ions were tightly bound onto or into soy protein polypeptides. In the case of other “cakes” with Cu(II), Zn(II), and Cd(II) ions, many cracks exhibited on their surfaces. It is known that soy protein can be heat-denatured, which means unfolding chains and functional groups such as disulfide groups and hydrophobic

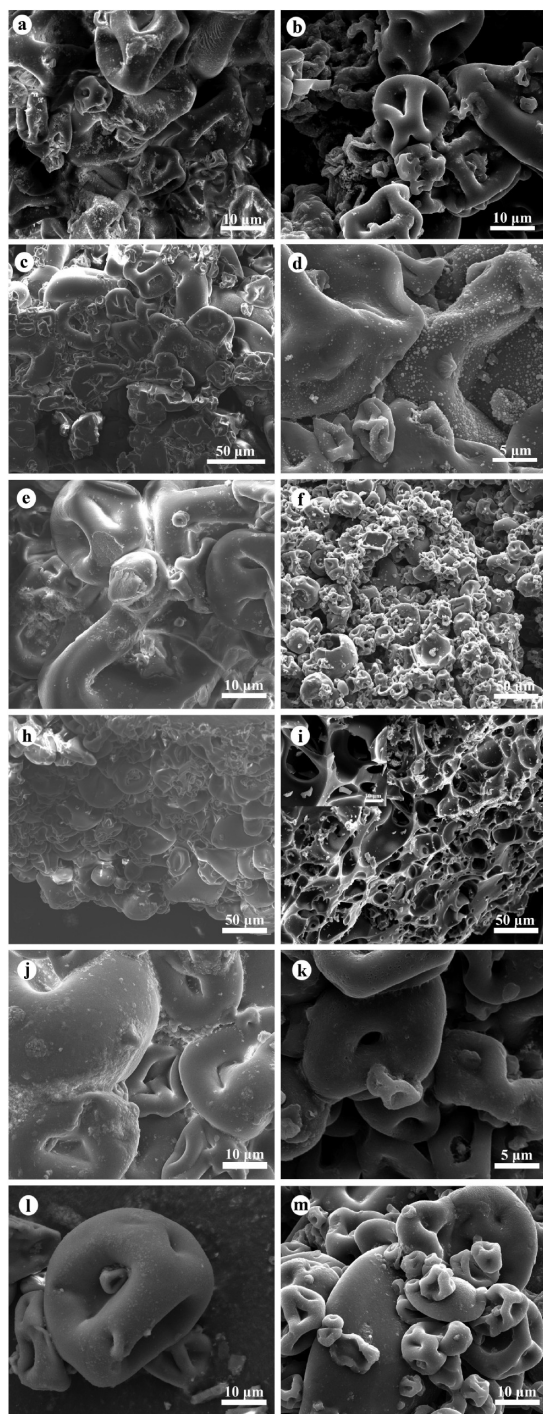


Figure 2. SEM micrographs of SPM adsorbed Cd(II) (a), Cr(III) (c), Cu(II) (e), Ni(II) (h), Pb(II) (j), and Zn(II) (l) and sinter of SPM adsorbed Cd(II) (b), Cr(III) (d), Cu(II) (f), Ni(II) (i), Pb(II) (k), and Zn(II) (m), calcinated at 350 °C.

groups become exposed and immediately interact with each other, leading to reversible polypeptide aggregation and formation of gelation network during the heating process. When denatured protein encountered heavy metal ions, active sites in the hydrophilic region were exposed to chelate ions and oxidation reactions simultaneously would break the intermolecular disulfide bonding bridge via thiol-disulfide interchange.²¹ That is why Pb(II) ion is most known as a breaker of disulfide bonding in killing blood protein. Thus, it could be

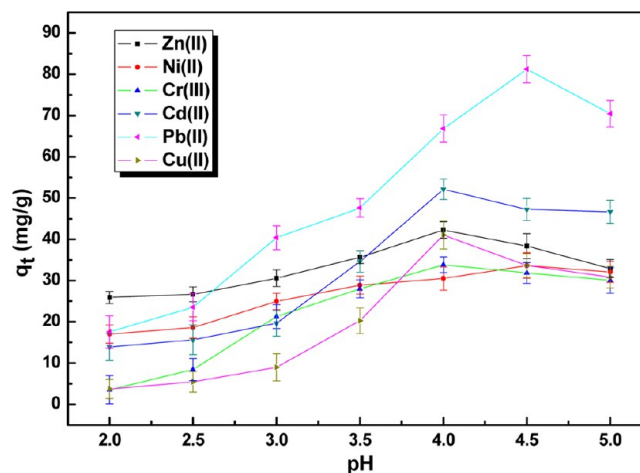


Figure 3. Effects of pH on adsorption of metal ions by SPMs (initial concentration of the heavy metals: 200 mg/L; stirring: 150 rpm; pH: 2.0–5.0; temperature: 20 °C; adsorption time: 4 h).

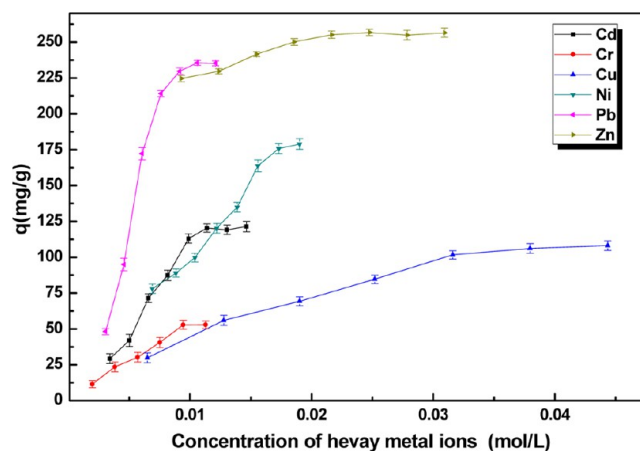


Figure 4. Dependence of adsorption capacity of SPMs on the initial concentration of Cd(II), Cr(III), Cu(II), Pb(II), Ni(II), and Zn(II) ions (initial concentration of the heavy metals: 0–0.045 mol/L; stirring: 150 rpm; pH: 5.5; temperature: 20 °C; adsorption time: 4 h).

Table 1. Adsorption Capacities (mg/g and mmol/g) of Various Heavy Metal Ions by SPMs at 50 and 70 °C

metal ions	maximum adsorption capacity at 50 °C		maximum adsorption capacity at 70 °C	
	mg/g	mmol/g	mg/g	mmol/g
Zn(II)	174.71	2.69	254.95	3.92
Cr(III)	42.60	0.82	52.94	1.02
Pb(II)	169.18	0.82	235.56	1.14
Cd(II)	106.37	0.94	120.83	1.08
Cu(II)	92.43	1.45	115.01	1.80
Ni(II)	130.05	2.22	177.11	3.02

deduced that heavy metal ions played an important role in denaturation, aggregation (especial disulfide bridges), network formation, and gel stiffening of soy protein. Adsorption of heavy metal ions was an irreversible denaturation process of soy protein.

Micrographs of soy protein adsorbed heavy metal ions and its deposits sintered at 350 °C were shown in Figure 2a, b (Cd²⁺); c, d (Cr³⁺); e, f (Cu²⁺); h, i (Ni²⁺); j, k (Pb²⁺); l, m (Zn²⁺), respectively. As it can be seen, denatured soy protein positively

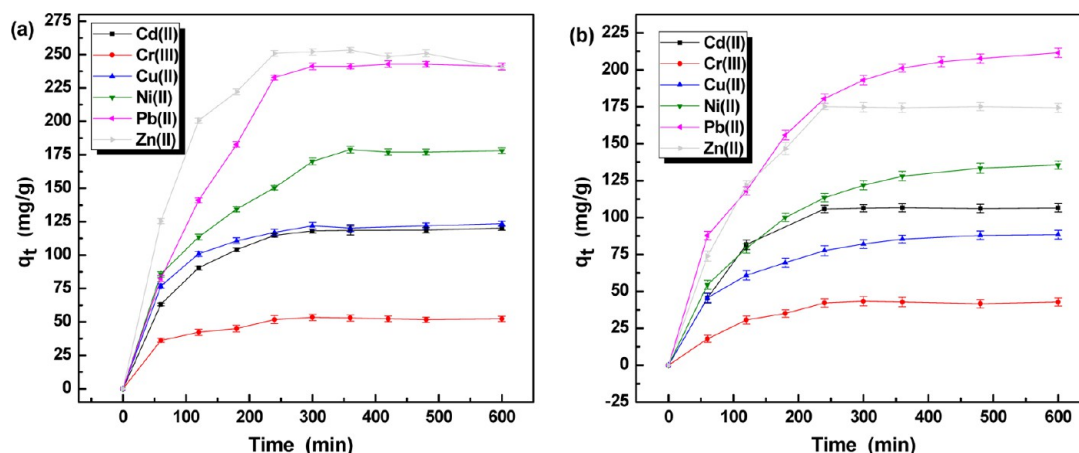


Figure 5. Effects of contact time on the adsorption capacity of heavy metal ions for SPMs at 70 °C (a) and 50 °C (b) (initial concentration of the heavy metals: 200 mg/L; stirring: 150 rpm; pH: 5.5; temperature: 70 and 50 °C; adsorption time: 0–600 min).

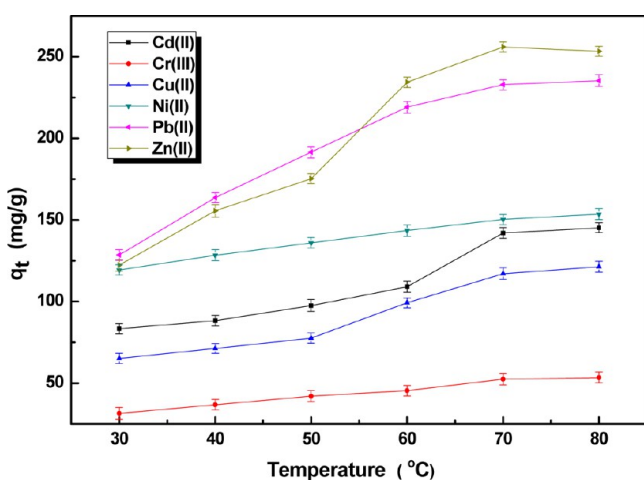


Figure 6. Effects of temperature on the adsorption capacity of Cd(II), Zn(II), and Cr(III) ions for SPMs (initial concentration of the heavy metals: 200 mg/L; stirring: 150 rpm; pH: 5.5; temperature: 30–80 °C; adsorption time: 4 h).

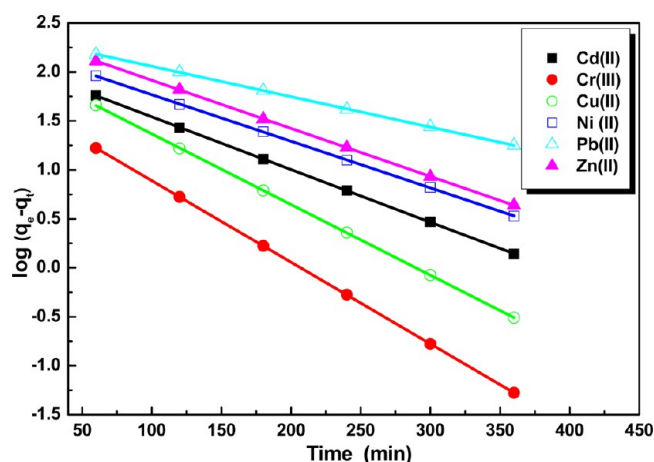


Figure 7. $\log(q_e - q_t)$ as a function of time for Cd(II), Cr(III), Cu(II), Ni(II), Pb(II), and Zn(II) ions, respectively.

existed in the form of microspheres with a diameter range of 4–45 μm . In most cases, the microspheres collapsed during the baking and sintering process. As can be observed from the cross-section of soy protein sintered in Figure 2i, microspheres were stacked together and presented a hollow structure. Many small particles occurred on the surface of SPMs as observed from Figure 2a–f, which were the heavy metal salts with a size of 5–50 μm . Some ionic salts were thought to be combined in the shell of microspheres instead of the outer surface, which could be revealed from Figure 2h–m. It was thought that adsorption of heavy metal ions by SPMs contributed to the

driving forces of electrostatic interactions, chelation, ionic bonding, etc. For instance, heavy metal ions like Zn(II), Ni(II), and Pb(II) could chemically bond to thiol or other groups of denatured protein leading to these ions binding the soy polypeptide network and penetrating into the microsphere shell.^{19,20} Therefore, it can be concluded that plenty of hydrophilic active sites located on/in the denatured SPMs played an important role in adsorption of heavy metals.

3.2. Sorption Behavior of SPMs. **3.2.1. Effects of pH of Suspension.** pH is known as an important factor affecting the association degree of metal ions on the biomass surface with multifunctional groups.²² When the systemic pH of suspension was adjusted between 2.0 and 7.0 by using HNO_3 , the sorption

Table 2. Freundlich and Langmuir Isotherm Constants for Metal Ions Binding to SPMs

metal ions	measured q_{max} (mg/g)	Langmuir			Freundlich		
		Q_{max} (mg/g)	b (L/mg)	R^2	$1/n$	K_F (mg/g)	R^2
Cd(II)	120.83	10000.0	−0.0084	0.0011	1.0517	0.061	0.9441
Cr(III)	52.94	250.0	0.4938	0.6189	0.8953	0.192	0.9872
Cu(II)	115.01	204.1	0.4414	0.9665	0.6807	0.539	0.9756
Ni(II)	177.11	1428.6	0.1321	0.2362	0.9052	0.320	0.9758
Pb(II)	235.56	1111.1	−0.0833	0.0842	1.1775	0.030	0.8995
Zn(II)	254.95	277.8	7.2000	0.9989	0.1225	102.849	0.9173

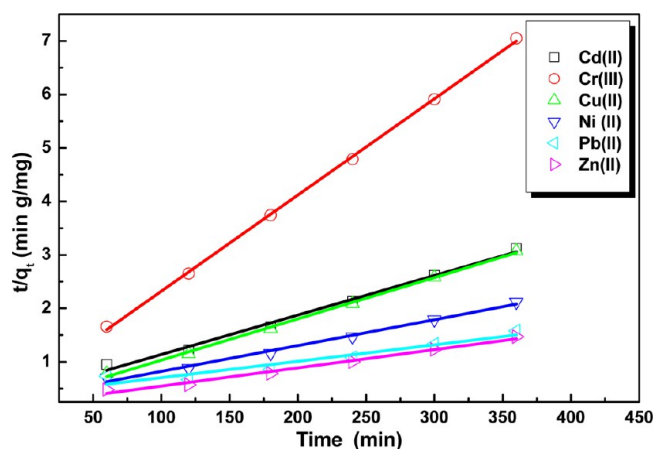


Figure 8. t/q_t as a function of time for Cd(II), Cr(III), Cu(II), Ni(II), Pb(II), and Zn(II) ions, respectively.

capacities varied with pH and are displayed in Figure 3. It can be observed that the adsorption of these heavy metals are highly pH dependent. The adsorption of Zn(II), Cr(III), Cd(II), Cu(II), Pb(II), and Ni(II) increased with pH increasing from 2 to 4 and decreased with pH increasing from 4 to 5. The highest value is at pH of 4.0 or 4.5. The facts were that dissociation degree of functional groups from sorbent surface increased with rising pH and, in consequence, ionic interactions increased; e.g., $-\text{NH}_2$ of soy protein can chelate with metal ions and also with H^+ , so there is an equilibrium between them. Consequently, the concentration of H^+ could affect the adsorption capacity of the active groups on soy protein microspheres. As the pH value was reduced, the equilibrium shifted toward the direction to generate $\text{R}-\text{NH}_3^+$, thus leading to dissociation of metal ions. It is well-known that the isoelectric point of soy protein is at about 4–5; in other words, the solubility is at a minimum, charges of protein are neutralized, and electrostatic repulsive forces among groups have disappeared. Our results indicated that adsorption capacity was consistent with the isoelectric point of soy protein and dramatically changed with the solubility of soy protein which varied with pH. Since secondary hydrolysis processes took place for those heavy metal salts, a slow decrease of adsorption efficiency is observed as pH values were higher than 4.5 or 5.0. Therefore, an initial pH at 4.5 or 5.0 was considered as an optimum sorption condition to remove heavy loading metal ions.

3.2.2. Effects of Initial Concentration of Metal Ions. Figure 4 shows the effects of initial ionic concentration of heavy metal ions on adsorption capacity of SPMs. The adsorption capacities increased with the increase of concentration of heavy metal ions and gradually achieved a maximum adsorption value, which is noted as the maximum adsorption capacity. The fact is that at low concentration the ratio of available binding sites to the total metal ions was high and all metal ions could be bound to the active sites of microspheres, whereas at high concentrations, the ratio was lower and consequently the binding was dependent on the initial concentrations. As listed in Table 1, the maximum adsorption capacities of Cr(III), Cu(II), Cd(II), Ni(II), Pb(II), and Zn(II) ions by AAS at 70 °C were 52.94, 115.01, 120.83, 177.11, 235.56, and 254.95 mg/g or 1.02, 1.80, 1.08, 3.02, 1.14, and 3.92 mmol/g, respectively, and decreased in the order: Zn(II) > Ni(II) > Cu(II) > Pb(II) > Cd(II) > Cr(III). In order to understand the level of adsorption degree, we made a

Table 3. Kinetic Parameters of Regression Equations of Sorption Model at 70 °C

metal ions	pseudo first-order				pseudo second-order				Elovich equation			Morris-Weber equation			
	R^2	k_1	calculated q_e	R^2	k_2	calculated q_e	α ($\text{mg g}^{-1} \text{min}^{-1}$)	β (g mg^{-1})	R^2	break point t_1 (min)	q_1 (%)	R_1^2	R_2^2		
Cd(II)	0.9999	0.0124	120.23	0.9906	0.0006	135.32	84.7338	0.0400	0.8884	240	95.41	0.9838	0.8938		
Cr(III)	1.0000	0.0192	55.21	0.9994	0.0015	55.59	17.6036	0.1308	0.8576	240	96.88	0.9681	0.9988		
Cu(II)	0.9999	0.0166	123.22	0.9977	0.0006	129.53	29.7230	0.0458	0.9285	300	98.70	0.9744	0.9987		
Ni(II)	0.9999	0.011	175.39	0.9903	0.0004	207.04	390.2682	0.0222	0.9511	300	95.00	0.9980	0.8784		
Pb(II)	0.9999	0.0072	233.71	0.9059	0.0003	325.73	1224.7328	0.0133	0.8948	240	95.88	0.9950	0.7976		
Zn(II)	0.9999	0.0113	254.68	0.9825	0.0003	294.12	149.0023	0.0198	0.7747	240	99.05	0.9581	0.7194		

Table 4. Pseudofirst-Order and Pseudosecond-Order Kinetic Parameters Obtained from the Linear Fits of Experimental Data for Cd(II), Cr(III), and Zn(II) at 50 °C

metal ions	measured q_e	pseudofirst-order			pseudosecond-order		
		R^2	k_1	calculated q_e	R^2	k_1	calculated q_e
Cd(II)	106.40	0.9999	0.0093	105.51	0.9327	0.0006	180.83
Cr(III)	42.27	0.9998	0.0091	42.66	0.9961	0.0016	44.98
Cu(II)	77.59	0.9912	0.0088	85.27	0.9989	0.0001	103.09
Ni(II)	113.46	0.9990	0.0076	141.64	0.9969	0.0001	163.93
Pb(II)	180.52	0.9919	0.0069	190.99	0.9935	0.0001	256.41
Zn(II)	175.13	0.9999	0.0097	173.78	0.8928	0.0004	268.10

Table 5. Activation Energy Calculated from the Pseudofirst-Order Kinetic Model Obtained from the Linear Fits of Experimental Data to the First-Order Rate Equation For Cd(II), Cr(III), and Zn(II) Ions

metal ions	temperature (K)	q_e (mg/g)	apparent rate constant k	E_a (kJ/mol)
Cd(II)	323.15	106.37	0.0093	13.11
	343.15	120.83	0.0124	
Cr(III)	323.15	42.60	0.0091	34.40
	343.15	52.94	0.0192	
Cu(II)	323.15	89.07	0.0088	29.26
	343.15	115.01	0.0166	
Ni(II)	323.15	136.95	0.0076	17.04
	343.15	177.11	0.0110	
Pb(II)	323.15	215.17	0.0069	19.62
	343.15	235.56	0.0072	
Zn(II)	323.15	174.71	0.0097	6.85
	343.15	254.95	0.0113	

comparison of soy protein with other effective biopolymer adsorbents, such as chitosan. Chitosan was reported to have saturated adsorption capacities of 0.5, 1.5–2.5, 0.6–1.3, 2.0, 0.2, and 0.94 mmol/g corresponding to Cr(III),²³ Cu(II),²⁴ Cd(II),²⁵ Ni(II),²⁶ Pb(II),²⁷ and Zn(II),²⁸ respectively. It is easy to find that SPMs had a higher capability of binding heavy metals than chitosan except the case of comparable adsorption for Cu(II) and Cd(II).

3.2.3. Effects of Contact Time and Temperature. The time profiles of metal ions sorption by SPMs at 70 and 50 °C are presented in Figure 5a,b, respectively. The amount of metal ions adsorbed did not increase with contact time until the plateau, implying equilibrium was reached. The amount of metal ions adsorbed rapidly in the initial stage was attributed to the availability of binding sites of the microspheres, but as time went by, the sorption slowed down before reaching equilibrium at about 4 h at 70 °C. The equilibrium values of Cd(II) 114.75 mg/g, Cr(III) 51.74 mg/g, Cu(II) 117.09 mg/g, Ni(II) 150.28 mg/g, Pb(II) 232.84 mg/g, and Zn(II) 250.86 mg/g could be achieved at a high level of adsorption efficiency. The time profile at 50 °C had a similar trend to reach equilibrium at about 4 h, and the equilibrium values were Cd(II) 106.40 mg/g, Cr(III) 42.27 mg/g, Cu(II) 77.59 mg/g, Ni(II) 113.46 mg/g, Pb(II) 180.52 mg/g, and Zn(II) 175.13 mg/g. Maximum sorption capacities of heavy metal ions by SPMs at 70 and 50 °C are summarized in Table 1, respectively. Obviously, the equilibrium sorption amount of heavy metal ions on SPMs at 70 °C was higher than that at 50 °C, which means the binding mechanism was dependent on the temperature and thereby heating denaturation of soy protein.

When temperature was extended from 30 to 80 °C, temperature profiles of metal ion uptake of three special cases of Zn(II), Cr(III), and Cd(II) were shown in Figure 6. The adsorption capacities of Cd(II), Cr(III), Cu(II), Ni(II), Pb(II), and Zn(II) at 30 °C were 83.36, 31.43, 65.12, 119.32, 128.54, and 122.29 mg/g, respectively, whereas at 80 °C they were enhanced up to 145.18, 53.39, 121.34, 153.54, 235.34, and 253.34 mg/g, respectively. The adsorption capacity was increased with increasing temperature from 30 to 80 °C. A sharp elevation occurred between 50 and 70 °C which was caused by a critical denaturation of soy protein at this temperature range. Therefore, the active binding sites exposed on the microspheric surface were correlated with the degree of protein denaturation, and above 70 °C, the binding process was almost completed, thus leading to no significant changes of ionic uptake.

3.3. Sorption Isotherm. Two major isotherm equations, the Langmuir and Freundlich isotherms,^{29,30} are established to fit the experimental data and describe the isotherm constants of metal ion uptake by sorbents. The Langmuir adsorption isotherm can be expressed as:

$$\frac{C_e}{Q_e} = \frac{1}{Q_{\max}} C_e + \frac{1}{Q_{\max} b} \quad (2)$$

where C_e is the equilibrium concentration of metal ions (mg/L), Q_e is the adsorption amount at the equilibrium (mg/g), Q_{\max} is the maximum capacity (mg/g), and b is the Langmuir constant related to the affinity of binding sites (L/mg).

The linear form of the Freundlich isotherm is expressed as:

$$\log Q_e = \log K_F + \frac{1}{n} \log C_e \quad (3)$$

where K_F is roughly an indicator of the adsorption capacity and $1/n$ is the adsorption intensity. The magnitude of the exponent $1/n$ gives an indication of the favorability of adsorption. The value of $1/n$ is more than 1, which represents favorable adsorption condition.³¹

The parameters from the Langmuir and Freundlich model are calculated and listed in Table 2. The Freundlich equation represented a better fit of experimental data than the Langmuir equation for metal ions although it seemed that the model of Zn(II) ions was also suitable for the Langmuir equation. The $1/n$ value can be used to predict binding affinity of the sorbents toward metal ions; a smaller value of $1/n$ implied a stronger interaction between sorbent and metal ions. As presented in Table 2, the values of $1/n$ from application of the Freundlich model lying between 0 and 1.2 indicated that the metal ions were favorably adsorbed by the SPMs, and the $1/n$ values were in the decreasing order of Zn(II) < Cu(II) < Cr(III) < Ni(II) <

Cd(II) < Pb(II), suggesting the strongest binding interaction between Zn(II) and active sites of the SPMs.

3.4. Sorption Kinetics. **3.4.1. Pseudofirst-Order Kinetics and Pseudosecond-Order Kinetics.** Sorption rate is an important factor for determining the efficiency of a sorption process. Of all adsorption kinetic models, the pseudofirst-order kinetics and pseudosecond-order kinetics are the most widely used models to determine the rate constant and the controlling mechanism of the sorption process. The expression of the pseudofirst-order kinetics model is given by following:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (4)$$

The linear form of this model obtained by the integral could be expressed as eq 5:

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \quad (5)$$

where k_1 is the pseudofirst-order rate constant (min^{-1}), q_e is the amount of heavy metal ions adsorbed at equilibrium ($\text{mg}\cdot\text{g}^{-1}$), and q_t is the amount of the adsorption at any time t ($\text{mg}\cdot\text{g}^{-1}$). Such an equation should yield a straight line with intercept equal to $\log q_e$ and slope equal to $-(k_1/2.303)$.

As could be observed from the expression above, the pseudofirst-order kinetics model is based on the assumption that adsorption was controlled by diffusion steps, and the rate of adsorption is in direct proportion to the difference value of equilibrium adsorption capacity and the adsorption capacity at any time t . While the expression of pseudosecond-order kinetics model is given by following:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (6)$$

The linear form of this model obtained by the integral could be expressed as eq 7:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (7)$$

where k_2 is the pseudosecond-order rate constant ($\text{g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$), q_e is the amount of heavy metal ions adsorbed at equilibrium ($\text{mg}\cdot\text{g}^{-1}$), and q_t is the amount of the adsorption at any time t ($\text{mg}\cdot\text{g}^{-1}$).

On the basis of the time profiles above-mentioned, the pseudofirst-order and pseudosecond-order dynamic models were fitted and set up in Figures 7 and 8. The kinetic parameters obtained by the sorption of heavy metal ions on SPMs at 70 °C are summarized in Table 3. Linear correlation coefficients (R^2) from the pseudofirst-order dynamic model were closer to 1 than those from the pseudosecond-order dynamic model, and the experimental equilibrium sorption capacities determined from the contact time profiles correlated well to theoretical equilibrium sorption capacity calculated using the pseudofirst-order dynamic model. Thus, it is obviously deduced that the adsorption fits to the pseudofirst-order kinetic model better. Using the same method, the sorption kinetic at the temperature of 50 °C was also described by the pseudofirst-order and the pseudosecond-order dynamic model, respectively. The heavy metal sorption at 50 °C still followed the same model (the pseudofirst-order), which means the sorption mechanism does not change with the varying temperature at all. As shown in Table 4, the sorption at 50 °C had a lower sorption rate constant than that at high

temperature (70 °C) as well as less equilibrium uptake. Therefore, the higher temperature was favored for more effectively and faster adsorption of heavy metal ions by the SPMs.

3.4.2. Intraparticle Model (Morries-Weber Equation). When absorbates transmit from solution into solid phase like absorbents, pore and intraparticle diffusion are often rate-limiting in a batch reactor system. The intraparticle diffusion was explored by using the following equation suggested by Weber and Morries:³²

$$q_t = k_p t^{0.5} \quad (8)$$

where the parameter q_t is the amount adsorbed at time t ($\text{mg}\cdot\text{g}^{-1}$), k_p is the intraparticle diffusion equation constant ($\text{mg}\cdot\text{g}^{-1}\cdot\text{min}^{-0.5}$), and t is the time.

According to the Weber-Morries model, the plot of q_t against $t^{0.5}$, should give a straight line when diffusion plays a role in the sorption rate and should cross the origin if intraparticle diffusion is the rate determining step.^{32,33} The resulted parameters are listed in Table 3; the Weber-Morries model had two linear regions with one break point. The $q_1\%$ before break point at about 240–300 min was more than 95%, indicating that the adsorption rate of heavy metals are higher in the beginning owing to the large surface area of the adsorbent available for sorption. Then, adsorbate formed a thick layer in the exterior gradually due to the inter attraction and molecular association. This blocked the further adsorption, and the uptake rate was limited during transport from the exterior to the interior sites of the SPMs.

3.4.3. Elovich Equation. If the process is a chemisorption on highly heterogeneous sorbents, the sorption kinetics could be interpret by Elovich equation as follows:³⁴

$$q_t = \left(\frac{1}{\beta} \right) \ln(\alpha\beta) + \frac{1}{\beta} \ln(t) \quad (9)$$

where q_t is the sorption capacity ($\text{mg}\cdot\text{g}^{-1}$) at time t (min), α is the initial adsorption rate ($\text{mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$), and β is the desorption constant ($\text{g}\cdot\text{mg}^{-1}$). α and β can be obtained from the slopes and intercepts of q_t versus $\ln t$ plots. The Elovich equation is established if the process is based on diffusion or chemical reaction control. When the adsorption is on an energetically heterogeneous surface, the parameter α is related to the distribution of activation energies, and β is a function of both the particle structural–chemical characteristics and solute diffusion coefficient. On the basis of the above Elovich models, the calculated parameters are listed in Table 3. The Elovich model shows a preferable linear relationship ($R^2 > 0.85$, except Zn(II)) and high α , indicating quick and effective sorption.

3.5. Activation Energy of Sorption. The activation energy can be thought of as the minimum kinetic energy required for a particular reaction to overcome the energetic barrier that the adsorbate ions could be fixed by the adsorbents. The activation energy of the adsorption (E_a ; J/mol) can be calculated using the following equation:

$$\ln \frac{k(T_2)}{k(T_1)} = \frac{E_a}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \quad (10)$$

where k is apparent rate constant, E_a is activation energy, R is gas constant ($8.314 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$), and T is temperature (K). According to the parameters of the pseudofirst-order kinetic model, activation energies of Zn(II), Cr(III), and Cd(II)

adsorption by SPMs are calculated and summarized in Table 5. As seen from the table, the value of E_a related to adsorption of Zn(II) ion was lower than that of other metal ions, indicating that the energetic barrier against the adsorption of Zn(II) ion was easier to overcome; therefore, the adsorption reaction of Zn(II) was more facile to occur than that of other ions. Cr(III) ion was the most difficult to combine on soy protein among the three metal ions. Simultaneously, it is easy to find that the equilibrium adsorption capacity of three ions had the opposite order with E_a , which provides a reasonable mechanism of why soy protein had selective adsorption capacity for heavy metal ions.

4. CONCLUSIONS

In summary, SPM with diameter ranging from 4 to 45 μm was prepared by heat-induced denaturation and then utilized as biosorbent for heavy metal ions. The higher denaturation would lead to the more hydrophilic active groups exposed on the outside of microspheres and hence increased the binding sites for metal ions by increasing temperature and time. The maximum adsorption capacities of Cr(III), Cu(II), Cd(II), Ni(II), Pb(II), and Zn(II) ions at 70 °C were 52.94, 115.01, 120.83, 177.11, 235.56, and 254.95 mg/g or 1.02, 1.80, 1.08, 3.02, 1.14, and 3.92 mmol/g, respectively, and decreased in the order: Zn(II) > Ni(II) > Cu(II) > Pb(II) > Cd(II) > Cr(III). SPM displayed a great advantage over some other metal ion biosorbents since it has a high sorption capacity. The adsorption kinetic and isotherm models were calculated and fitted the pseudofirst-order kinetic model and Freundlich model, respectively. The selectivity of heavy metal ions binding onto microspheres resulted from sorption active energy; thus, the maximum adsorption capacity of Zn(II) ion was much higher than that of Cd(II) and Cr(III) ions because of the lower energetic barrier. Additionally, adsorption was dependent on pH value, and maximum sorption was reached at about the isoelectric point of soy protein. All in all, SPMs were facile to prepare and easily separated from an aqueous system by centrifugal settlement, which is anticipated to be widely used in heavy metal treatments in the future.

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Notes

The authors declare no competing financial interest.

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