Preparation of Dipalmitoyl Phosphatidylcholine-Modified Chitosan Nanostructures and Its Application for Enhancing Chromium Adsorption

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Abstract Chitosan (CTS) nanospheres are successfully modified dipalmitoyl phosphatidylcholine (DPPC) through by solvent removal. The physicochemical properties are characterized by dynamic light scattering (DLS), transmission electron microscope (TEM) and fourier transform infrared spectroscopy (FT-IR). Furthermore, the CTS-DPPC nanospheres are used as a novel biosorbent for the application in Cr(VI) adsorption, and the effect of pH and additional anions on the Cr(VI) adsorption performance is evaluated. Additionally, CTS-DPPC nanofibers are produced by electrospinning to improve adsorption capacity. The results show that the order of Cr(VI) adsorption performance is CTS-DPPC nanofibers, CTS-DPPC nanospheres and CTS nanospheres, which suggests DPPC is helpful for CTS to remove metal ion. Moreover, X-ray photoelectron spectrophotometer (XPS) analyses indicate that both amino and hydroxyl groups of CTS-DPPC nanospheres are engaged in the adsorption.

Keywords chitosan, dipalmitoyl phosphatidylcholine, nanosphere, nanofiber, Cr(VI) adsorption

Introduction

The pulmonary surfactant (PS) is a basic component in lung, which plays an important role during many physiological processes. Additionally, PS can increase lung compliance, reduce the surface tension, anti-oxidation, anti-infection, promote discharge of intrapulmonary foreign particles and protect the lung again injury. For example, Paola^[1] reported PS had the positive effect on the bronchopulmonary dysplasia. Recently, some research on the interaction between PS and PM 2.5 is reported. For example, Kendall^[2] reported that PS can resolve the toxic PM2.5 particles by pimeiosis. Specially, PS can act on heavy metal ions from PM2.5 particles, which can alleviate the lung disease, even the whole body harm by blood circulation. Concretely, there are a lot of heavy metal elements in PM2.5 particles, which can penetrate alveolar and then deposit on the surface epithelial cell after the interaction with PS.^[3] During the process, the endoplasmic reticulum will secrete more fatty acid and acetyl coenzyme A carboxylase, which would lead to the transformation from acetyl coenzyme A to malonyl coenzyme A. What's more, diglyceride acyltransferase and hormone-sensitive lipase will also promote the increase of triglycerides.^[4,5] All of immune response will promote to absorb the toxic PM2.5 particles. However, the interaction mechanism between PS and heavy metal ions is still unclear.

The treatment technologies of heavy metal ions have been well developed, including precipitation,^[6] adsorption,^[7] ion exchange,^[8] reverse osmosis,^[9] electrodialysis,^[10] and so on. Among them, the biosorbent is popular. For example, chitosan (CTS) has received considerable interest and been extensively reported because of the presence of an amino group of the 2-amino-2-deoxy-glucose (glucosamine) unit.^[11] Sag^[12] used natural CTS to remove metal ions, and Ahmad^[13] used CTS to remove the oil of waste water. Furthermore, many modified

CTS have been obtained so as to win the optimized operation conditions and absorption property. For example, Wang^[14] used CTS molecules and collagen fiber to prepare a new type of adsorbent to remove heavy metal ions of waste water. Nalini^[15] showed the crosslinking chitosan microspheres to remove the Cr(VI) in water. However, the active biology constituent has rarely been combined with CTS to improve adsorption performance.

In order to understand the interaction mechanism between PS and heavy metal ions and to develop a novel biosorbent, CTS is modified by PS in present experiment to absorb Cr(VI), a main ingredient of PM2.5. The effect of pH and additional anions on the Cr(VI) adsorption performance was evaluated, and their adsorption mechanism was proved by XPS. The research provides a potential method for shielding PM2.5 in biomedical applications.

Experimental

Materials and chemicals

Chitosan powder, sodium tripolyphosphate (TPP), potassium dichromate, ethyl alcohol, acetic acid, sodium chloride, sodium nitrate and sodium sulfate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Dipalmitoyl phosphatidylcholine (DPPC) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Synthesis of CTS nanospheres

CTS nanospheres were prepared by ionic gelation according to the previous reports as the following:^[16] 50 mg of CTS powder was dissolved in 4 mL of 1% acetic acid solution at room temperature. Furthermore, the pH value of the resultant solution was adjusted to 3 using 0.1 mol·L⁻¹ NaOH solution after being filtered by 0.44 and 0.22 μm of filter paper in order. After that, 5 mg of sodium TPP was dissolved in 5 mL of dou-

ble-distilled water at room temperature and the resultant solution (1 mg/mL) was filtered as described above. Then, 1.5 mL of TPP solution was added to 4 mL of CTS solution under continuous stirring for 30 min. The resulting products were collected by centrifuging, washing with water three times and then lyophilized.

Preparation of CTS-DPPC nanospheres

CTS-DPPC nanospheres were prepared as following steps: CTS nanospheres were dissolved in 1% acetic acid solution. Then 50 mg of DPPC powder was dissolved in ethyl alcohol and stirred for 2 h at 40 °C. Next, the DPPC solution was slowly added into the CTS nanospheres solution and stirred at 40 °C with constant speed until the ethyl alcohol was totally evaporated. The resulting products were collected and lyophilized.

Preparation of CTS-DPPC nanofibers

CTS-DPPC nanofibers were prepared by electrospinning. 100 mg of CTS and 50 mg of DPPC powders were dissolved in acetic acid. Then the CTS-DPPC solution was drawn into a 5 mL syringe with a metal capillary tip of 0.7 mm inner diameter. Finally, the solution was ejected from the syringe using a feed rate of 0.1 mm/min, an applied voltage varying from 20 kV, and the tip-to-collector distance varying from 8 cm. The products were collected on an aluminum foil continuously.

Characterization

Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire UK) based on dynamic light scattering was used to characterize the nanosphere size. The morphology of the samples was obtained by using a field-emission scanning electron microscope (FESEM, Hitachi, S-4800). Transmission electron microscope (TEM) images were recorded on a JEOL-2100F instrument using an accelerating voltage of 200 kV. Fourier transform infrared spectroscopy (FT-IR) (Nicolet 6700 FT-IR Spectrometer) was used to characterize the chemical change of the particles before and after Cr(VI) adsorption. Surface information was detected by X-ray photoelectron spectrophotometer (XPS), and C, N, O and Cr(VI) on each sample were scanned. Determination of the chromium concentration in a sample was performed by Varian ICP 710 Inductively coupled Plasma-Mass Spectrometry (ICP-MS) analysis.

Cr(VI) adsorption

Potassium dichromate was used to prepare Cr(VI) stock solution, and a series of solutions with 50–500 mg/L Cr(VI) were prepared. For adsorption experiments, 0.05 g of adsorbent was weighed and put into 50 mL Cr(VI)-containing solution in a reagent bottle. The bottle was then put on a shaking plate (150 rpm) mounted in a water bath for temperature control (25 °C) till the adsorption reached equilibrium. After that, 10 mL solution was taken from the bottle and put in a centrifuge tube, then transferred to a centrifuge to separate the solid and the liquid for further analysis. The rotation rate of the centrifuge was set at 9000 rpm, and 5 min was allowed to guarantee complete separation. The supernatant was taken to determine the remaining chromium concentration. The adsorption capacity (Q) of the sorbent was calculated using the following expression:

$$Q = \frac{(C_0 - C_f)V}{M}$$

where Q is the amount adsorbed (mg/g), C_0 and C_f are the initial and final metal ion concentrations (mg/L), respectively; V is the solution volume (L) and M is the amount of adsorbent (g) used.

Results and Discussion

DPPC is the most important content in PS and has been

used to absorb many metal ions.^[17] Therefore, to evaluate the interaction between PS and PM2.5 particles, the absorption of metal ions by DPPC was performed.

As shown in Figure 1, FT-IR spectra were used to characterize the change of DPPC before and after absorbing Cr(VI). In curve a of the spectra of DPPC, the typical absorption peaks at 3100–2800 cm^{-1} are associated with the C–H stretching vibration of phospholipid acyl chain, including the symmetrical and asymmetrical stretching vibrations of CH_2 . As shown in the characteristic peaks of the phosphatidyl polar group of DPPC in the range of 1800–700 cm^{-1} , the bands at 1238 and 1085 cm^{-1} are assigned to the asymmetrical and symmetrical stretching vibration of PO_2^- , the bands at 967 and 918 cm^{-1} are produced by the asymmetrical and symmetrical stretching vibration of $\text{N}^+(\text{CH}_3)_3$, and the bands at 815, 768 and 715 cm^{-1} are characteristic of the P–O–C anti-symmetrical and symmetrical stretching vibrations.^[18] As can be seen from curve b, when Cr(VI) was introduced into the DPPC molecule, the vibration of PO_2^- and $\text{N}^+(\text{CH}_3)_3$ moved to the low wavenumbers of 1223 and 1062 cm^{-1} , and 1223 and 1062 cm^{-1} , respectively. It is attributed to the presence of strong electrostatic interaction between metal ions and polar hydrophilic groups PO_2^- , which induces average of negative charge in $\text{O}=\text{P}-\text{O}^-$. As a result, the IR spectra showed a decreased vibration wavenumber of $\nu_{\text{as}}(\text{PO}_2^-)$.^[19] Thus, these results indicate that the presence of strong interaction between Cr(VI) and polar hydrophilic groups of DPPC.

Figure 1 FT-IR spectra of DPPC before (curve a) and after (curve b) absorbing Cr(VI).

Furthermore, the complexation between CTS and DPPC was prepared to absorb Cr(VI). The DPPC modified CTS nanospheres (DPPC-CTS) was synthesized by solvent removal. As shown in Figure 2a, TEM image shows DPPC-CTS nanospheres with core in gray and shell in dark are uniform nanospheres with an average diameter of about 60 nm, which suggests that DPPC molecules could be coated on the surface of CTS nanospheres. Moreover, Figure 2b shows that the average hydrodynamic size of the CTS and DPPC-CTS nanospheres in deionized water is about 190 and 220 nm, respectively, which reveals the DPPC addition has little influence on the size of the nanospheres. As shown in Figure 2c, the zeta potential values before and after introducing DPPC are 40.5 and 20.3 mV, respectively. Additionally, the addition of DPPC is further evidenced by FT-IR spectra (Figure 2d). In the spectra of CTS nanospheres, the typical peaks at 1635 and 1535 cm^{-1} are attributed to characteristic stretching vibrations of C=O and C–N, respectively, where the band at 3200, 1070 and 1020 cm^{-1} is the characteristic stretching and bending vibration of

N—H. Compared to the spectra of CTS nanospheres, the typical peaks at about 1200 and 960 cm^{-1} (labeled by asterisks) can be obviously found in the spectra of CTS-DPPC nanosphere, which shows the successful addition of DPPC on CTS nanospheres. The above results show that the object product is prepared successfully in our experiment.

Figure 2 (a) TEM image of DPPC-CTS nanospheres, (b) the average hydrodynamic size, (c) the zeta potential value and (d) FT-IR spectra of CTS and DPPC-CTS nanospheres.

To estimate the absorption ability of CTS-DPPC nanospheres, an absorption treatment of CTS and CTS-DPPC nanospheres to Cr(VI) was detected with the increasing adsorption time. As shown in Figure 3a, the adsorption capacity of CTS and CTS-DPPC nanospheres to Cr(VI) increases as a function of time at 0–2 h. About 33.8 and 44.6 mg/g of Cr(VI) are absorbed by CTS and CTS-DPPC nanospheres after 2 h exposure, respectively. The result proves that DPPC is helpful for CTS to remove metal ion. Moreover, there is a slight increase of the Cr(VI) adsorption capacity for CTS-DPPC and CTS nanospheres, which seems to reach adsorption equilibrium for both CTS and CTS-DPPC nanospheres. About 34.5 and 46.2 mg/g of Cr(VI) are absorbed by CTS and CTS-DPPC nanospheres at 7 h. The results display that the Cr(VI) adsorption capacity is improved with the addition of DPPC.

Figure 3 The effect of contact time on adsorption capacity of CTS and CTS-DPPC nanospheres.

Generally, the electrostatics is the popular adsorption mechanism between CTS and metal ions.^[20] In order to reveal the interaction between CTS-DPPC nanosphere and Cr(VI), the

effect of pH and additional anions on the Cr(VI) adsorption performance by CTS and CTS-DPPC nanospheres was evaluated. As shown in Figure 4a, the Cr(VI) adsorption capacity increased at the range of pH = 2–3, and then decreased at the range of pH = 3–6 for both CTS and CTS-DPPC nanospheres at the acidic solution condition. The maximum Cr(VI) adsorption capacity at the pH = 3 is 33.8 and 44.6 mg/g for CTS and CTS-DPPC nanospheres, respectively. As we well known, in acid solution of pH = 2–6, the amino groups from CTS and DPPC molecules can be easily protonated to NH_3^+ , but Cr(VI) always appears in the term of chromate, including $[\text{HCrO}_4]^-$, $[\text{CrO}_2]^{2-}$, $[\text{Cr}_2\text{O}_7]^{2-}$, $[\text{Cr}_3\text{O}_{10}]^{2-}$ and $[\text{CrO}_4]^{2-}$ with the increasing pH from 2 to 6.^[21] Thus, a strong interaction could exist between the oxy anions of Cr(VI) and the positively charged surface of CTS and CTS-DPPC nanospheres. Moreover, pH is smaller or the acidity is higher, the protonation is stronger, which means that more NH_3^+ could be existed at pH 2–3. However, the $[\text{HCrO}_4]^-$ of chromate at pH = 2 could weak the electrostatic interaction between Cr(VI) and CTS or CTS-DPPC nanospheres because of weak ionic charge. With the further increasing pH from 3 to 6, the ionic radius of chromate such as $[\text{CrO}_2]^{2-}$, $[\text{Cr}_2\text{O}_7]^{2-}$, $[\text{Cr}_3\text{O}_{10}]^{2-}$ and $[\text{CrO}_4]^{2-}$ could be enlarged, which may decrease the adsorption performance due to the steric hindrance. Therefore, these results may contribute to the maximum Cr(VI) adsorption capacity at the condition of pH = 3. Moreover, the more NH_3^+ coming from both CTS and DPPC molecules leads to the stronger adsorption ability of CTS-DPPC nanospheres than that of CTS nanospheres. Furthermore, the effect of additional ions on the Cr(VI) adsorption performance by CTS and CTS-DPPC nanospheres was performed. The different anions (Cl^- , NO_3^- and SO_4^{2-} and in the form of NaCl, NaNO_3 and Na_2SO_4) were added to the experimental system. As shown in Figure 4b, the inhibition of Cr(VI) adsorption by these anions are found with the order of $\text{SO}_4^{2-} > \text{NO}_3^- > \text{Cl}^-$, in which the adsorption capacity are decreased by 9.84%, 4.56%

Figure 4 (a) The effect of pH on adsorption capacity of CTS and CTS-DPPC nanospheres and (b) the effect of additional anions on adsorption capacity of CTS-DPPC nanospheres.

and 0.9%. The results could contribute to the competing effect of the additional anions to interact with protonated amino groups through decreasing the number of the binding sites on CTS-DPPC nanospheres. Furthermore, the adsorption capacity is greatly affected by SO_4^{2-} due to the crosslink of sulfate ions with protonated amino groups expect the strong competition between SO_4^{2-} and Cr(VI) .^[22] In a word, the protonation of CTS and DPPC molecule, the charge and steric stabilization of chromate and the competing additional anion can influence the Cr(VI) adsorption performance.

Furthermore, the adsorption mechanism of CTS-DPPC nanospheres to Cr(VI) was proved by XPS. The XPS spectrum provided in Figure 5a reveals carbon, oxygen, nitrogen, and chromium are the predominant elements observed on the surface from binding energies at 282 eV (C 1s), 530 eV (O 1s), 395 eV (N 1s), and 550 eV (Cr 2p). Chromium is magnified in the spectra, which shows that Cr 2p_{3/2} peak at 576.64 and 2p_{1/2} peak at 586.24 are observed (Figure 5b). According to the previous report, the higher binding energy belongs to Cr(VI) and the lower binding energy peak to Cr(III) .^[20] Thus, the presence of two chromium peaks reveals that Cr(VI) is reduced partially. Therefore, there are two adsorption models including the physical adsorption and chemical adsorption, which comes from the electrostatic interaction between Cr(VI) and CTS or CTS-DPPC nanospheres and from the reduce reaction of Cr(VI) and functional groups in CTS and DPPC molecules. Furthermore, N 1s and O 1s peaks of CTS-DPPC nanospheres before and after absorbing Cr(VI) are shown in Figures 5c and 5d. N 1s peak at 340 eV of CTS-DPPC nanospheres before absorbing Cr(VI) is split to two peaks at 399 and 402 eV. According to previous report, the two peaks assigned to NH_2 or NH in the lower binding energy and NH_3^+ in the higher binding energy, respectively.^[23] The significant change indicates the involvement of the amino group in the adsorption. Moreover, O 1s peak at 532 eV of CTS-DPPC nanospheres before absorbing Cr(VI) is shifted to 533 eV, which suggests that the hydroxyl groups are also involved in the adsorption. In other words, Cr ions of Cr(VI) and Cr(III) could be absorbed through their electrostatic interaction with NH_3^+ and complexation with amino and hydroxyl groups.

Figure 5 XPS spectra of (a) predominant elements and (b) Cr 2p for DPPC after absorbing Cr(VI) , (c) N 1s and (d) O 1s for DPPC before and after absorbing Cr(VI) .

As we well known, the increasing surface area is an effective route to improve the adsorption capacity. Thus, the electrospinning using the same DPPC-CTS content of

CTS-DPPC nanospheres in acetic acid as the spinning solution was performed. As shown in Figure 6a, the shape net-like composed by fibers with about 100 ± 30 nm in diameter is obtained with the 20 kV of spinning voltage and 8 cm of the collection distance. The product was used as a sorbent to remove Cr(VI) . As shown in Figure 6b, the adsorption capacity of CTS-DPPC nanofibers to Cr(VI) is a continuous increase as a function of time at 0–7 h. About 62.9 mg/g of Cr(VI) are absorbed after 7 h exposure, which is obvious higher than that of CTS-DPPC nanospheres. It is attributed to the increasing contact area, leading to the exposure of much more functional groups for acting with Cr(VI) .

Figure 6 (a) SEM image and (b) the effect of contact time on adsorption capacity of electrospun DPPC-CTS nanofibers.

Conclusions

CTS-DPPC nanospheres and nanofibers obtained through CTS modified by DPPC in our experiment are used as a novel biosorbent for the application in Cr(VI) absorption. The results show that DPPC is helpful for CTS to remove metal ion, and 44.6 and 62.9 mg/g of Cr(VI) is absorbed by CTS-DPPC nanospheres and nanofibers after 2 h exposure, respectively. The effect of pH and additional anions on the Cr(VI) adsorption performance is evaluated, which reveals that the maximum adsorption capacity of Cr(VI) is at pH = 3 and anions affect Cr(VI) adsorption on CTS-DPPC nanofibers in the order of $\text{SO}_4^{2-} > \text{NO}_3^- > \text{Cl}^-$. Furthermore, the adsorption mechanism of CTS-DPPC nanospheres to Cr(VI) was proved by XPS. The result shows that both amino and hydroxyl groups are involved in the adsorption of Cr(VI) ions on the CTS-DPPC nanospheres. All the results are indicative that CTS-DPPC nanospheres modified by DPPC can be used as a promising sorbents for Cr(VI) removal.

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