Journal of Materials Chemistry B

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/materialsB

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Preparation of Cobalt Mono-substituted Silicotungstic Acid Doped with Aniline for the Selective Adsorption of Ovalbumin

Dan-Dan Zhang[a], Qing Chen[a], Lin-Lin Hu[a], Xu-Wei Chen*[a] and Jian-Hua Wang*[a,b]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

A Keggin-type cobalt mono-substituted silicotungstic acid doped with aniline, ($SiW_{11}Co$/PANI composite, where PANI denotes polyaniline) is prepared by a liquid phase method at room temperature. The obtained $SiW_{11}Co$/PANI composite possesses porous framework structure and has proven to be a promising adsorbent for the retention of protein, which exhibits favorable selectivity toward the adsorption of ovalbumin from egg white. 5.0 mg of $SiW_{11}Co$/PANI composite gives rise to an adsorption efficiency of >70% for 100 mg L^{-1} ovalbumin in 1.0 mL of sample solution within a wide range of pH 3-9, and a maximum adsorption efficiency of 92% is achieved at pH 9. The adsorption behavior of ovalbumin onto $SiW_{11}Co$/PANI composite fits *Langmuir* adsorption model, corresponding to a sorption capacity of 200.0 mg g^{-1}. The retained ovalbumin could be readily recovered by using a 0.1 mol L^{-1} phosphate buffer at pH 5.6 as stripping reagent, providing a recovery of 84.4%. Circular dichroism (CD) spectra illustrate virtually no change on the conformation of ovalbumin after the process of adsorption/desorption. The $SiW_{11}Co$/PANI composite has been applied for the selective adsorption of ovalbumin from chicken egg white, and SDS-PAGE assay demonstrates that high purity of ovalbumin is obtained.

1. Introduction

Polyoxometalates (POMs) have been known for almost two centuries since the discovery of the first member of this class (the ammonium salt of $PMo_{12}O_{40}^{3-}$).[1, 2] These species are generally metal-oxo anionic clusters, consisting of transition metals in high oxidation states (mainly V(V), Mo(VI) and W(VI)) and oxo ligands. [3-6] A lot of POMs structures have been described so far, among which the most well known include Lindqvist, Anderson, Keggin and Dawson types. [1, 2, 7] The unique surface charge distribution, diverse compositional/structural varieties and favorable stability of POMs have been demonstrated in the fields of catalysis, medicine, materials science, surface chemistry, biology, photochromism and electrochromism. [8-13] In particular, POMs exhibit well-documented biological activities, and thus have found promising applications as anti-tumor, -viral and -bacterial inorganic medicinal agents.[14-16] The antibacterial activity of POMs could be controlled by regulating their structures or morphologies. [11,17] Nanocomposites based on Keggin-type polyoxometalate and porous bamboo charcoal can provide excellent antibacterial performance. [18] The application of polyoxometalate-based materials in biological fields is mostly based on their following features, e.g., polarity, morphology, acidity, redox property, electron donating and accepting capability, which are ease of modulation. [17] On the other hand, the surface of POMs is generally suitable to be modified by grafting various multifunctional groups or moieties. [17,19]

In the development of proteomics, extensive interests have been directed to the exploitation of highly selective schemes for the adsorption/isolation/purification of specific protein species from biological sample matrixes.[20,21] Conventionally, a large variety of approaches have been used for the isolation and purification of proteins, including precipitation ,[22] affinity chromatography ,[23,24] liquid-liquid extraction, [25] and solid phase extraction. [26-28] Among these protocols, solid phase extraction is widely employed, and the development of appropriate adsorbents is the key issue for the success in selective sorption of specific protein. However, polyoxometalate-based materials have rarely been reported for the purpose of protein adsorption.

In the present work, a Keggin-type cobalt mono-substituted silicotungstic acid doped with aniline ($SiW_{11}Co$/PANI composite, where PANI denotes polyaniline) is prepared by a liquid phase method at room temperature, and the composite has been characterized by means of TGA, SEM, TEM, FT-IR, BET, EDS and XRD. The $SiW_{11}Co$/PANI composite possesses porous structure, and it is used for the first time as adsorbent for the sorption of proteins. It exhibits obvious selectivity toward the adsorption of ovalbumin (Ova) in complex biological sample matrixes, and provides favorable biocompatibility.

2. Materials and Methods

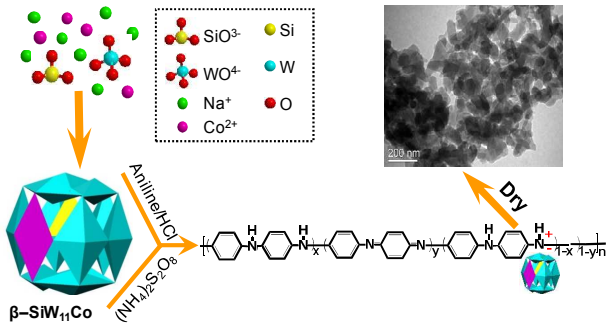
2.1. Materials and reagents

Lysozyme from chicken egg white (Lys, L2879, pI 11.0), bovine serum albumin (BSA, A 3311, pI 4.9), transferrin (TRF, 90190, pI 5.9) and ovalbumin (Ova, A5503, pI 4.7) are purchased from Sigma (St Louis, MO,USA) and used without further purification. Protein molecular weight marker (low, D532A, Takara Biotechnology, Dalian, China) is a mixture of six purified

proteins (Mr, in kDa: phosphorylase b, 97.2; serum albumin, 66.4; ovalbumin, 44.3; carbonic anhydrase, 29.0; trypsin inhibitor, 20.1; lysozyme, 14.3). Coomassie Brilliant Blue G-250 and R-250, NaCl, NaOH, NaH_2PO_4, Na_2HPO_4, $Na_2WO_3{\cdot}2H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, and imidazole are acquired from Sinopharm Chemical Reagent (Shanghai, China). These reagents are at least of analytical reagent grade unless otherwise specified. Ammonium peroxydisulfate, hydrochloric acid, ethanol, methanol, glycerin (Bodi Chemical Holding, Tianjin, China), aniline, KCl (Damao Chemical Holding, Tianjin, China) and sodium silicate (Second Chemical Holding, Shenyang, China) are used as received. De-ionized water of 18MΩ cm is used throughout the experiments.

2.2. Preparation of the $SiW_{11}Co$/PANI composite framework

The protocol for the preparation of $SiW_{11}Co$/PANI composite is illustrated in Scheme 1. Vacant silicotungstate is firstly prepared by substitution of tungsten (W) atom with cobalt (Co) atom. Then, vacant silicotungstate is inserted into the polyaniline structure at the –N= position to obtain the cobalt mono-substituted silicotungstic acid-aniline composite ($SiW_{11}Co$/PANI).



Scheme 1. The schematic illustration for the preparation of the cobalt mono-substituted silicotungstic acid doped with aniline ($SiW_{11}Co$/PANI) composite.

The details for the preparation of $SiW_{11}Co$/PANI composite are given in the following. Hydrochloric acid (4.0 mol L^{-1}) is drop-wisely added into 45 mL of sodium tungstate solution (3.0 mol L^{-1}) under vigorous stirring and during this process tungstic acid is produced. Hydrochloric acid is continuously added until the dissolution of the formed tungstic acid. 25 mL of sodium silicate (0.39 mol L^{-1}) is then added to the mixture followed by adjusting to pH 5.2 with dilute HCl. The reaction mixture is set to stand for 10 min, and 25 g of KCl is afterwards added under magnetic stirring for 25 min. After filtration, the obtained β-SiW_{11} white powder is washed with 1 mol L^{-1} KCl solution followed by drying under vacuum.

6.4 g of the β-SiW_{11} powder is added into 30 mL of de-ionized water under vigorous stirring to make a homogeneous suspension at 40°C (in a water-bath). 4 mL of cobalt nitrate solution (0.5 mol L^{-1}) is thereafter added to the suspension, which is allowed to react for 70 min. After filtration, 2.0 g of KCl is dissolved into the filtrate and the solution is placed in a 5°C refrigerator for the crystallization of the vacant silicotungstate. The obtained crystal is re-dissolved into 30 mL of HCl (1.0 mol L^{-1}), followed by adding 0.93 g of aniline and 2.3 g of ammonium peroxydisulfate, and the reaction mixture is vigorously stirred for 24 h. The final product, i.e., the cobalt mono-substituted silicotungstic acid doped with aniline ($SiW_{11}Co$/PANI), is collected by filtration. It is washed alternately with methanol and de-ionized water followed by drying under vacuum for 48 h.

2.3. Characterization of the $SiW_{11}Co$/PANI composite framework

FT-IR spectra are recorded on a Nicolet 6700 spectrophotometer (Thermo Electron, USA) using a KBr disk from 400 to 2500cm^{-1} with a resolution of 2.0 cm^{-1}. X-ray diffraction (XRD) patterns are taken on a Rigaku D/max-a X-ray diffractometer (Rigaku, Japan) with CuK_α radiation (k=1.54056 Å) with a step size of 0.02°. The thermal stability of the product is analyzed by using a TG-DSC simultaneous thermal analyzer (Mettler Toledo, Switzerland) within a temperature range of 25-900°C. SEM images and energy-dispersive X-ray spectroscopy (EDXS) are recorded on a LEO1430VP scanning electron microscope (LEO, Germany), and TEM images are recorded by using an H-7650 transmission electronic microscope (H-7650, Hitachi, Japan). Nitrogen sorption-desorption isotherms are measured by a Micromeritcs Tristar 3000 analyzer (USA).

2.4. Proteins adsorption with $SiW_{11}Co$/PANI composite framework

In the present study, Ova, BSA and Lys are employed as model proteins to evaluate the performance of $SiW_{11}Co$/PANI composite for protein adsorption. 5.0 mg of $SiW_{11}Co$/PANI composite is used to sorb the model proteins in 1.0 mL of sample solution by vigorously shaking the mixture for 10 min to facilitate the adsorption process. After centrifugation at 8000 rpm for 6 min, the supernatant is collected for the determination of residual protein contents with Bradford method by measuring the characteristic absorption at 595 nm. The adsorption efficiency of proteins was then calculated.

The protein species adsorbed onto $SiW_{11}Co$/PANI composite are then recovered by use of a 0.1 mol L^{-1} phosphate buffer (at pH 5.6) as stripping reagent. For this purpose, 1.0 mL of phosphate buffer solution is used to mix with $SiW_{11}Co$/PANI composite and the mixture is oscillated for 10 min to facilitate the recovery of the adsorbed protein from the surface of $SiW_{11}Co$/PANI composite. The supernatant after centrifugation at 8000 rpm for 6 min is collected for the evaluation of the recovery or elution efficiency.

3. Results and Discussion

3.1. Preparation and characterization of the $SiW_{11}Co$/PANI composite framework

FT-IR spectra of β-SiW_{11} and $SiW_{11}Co$/PANI composite are shown in Figure 1. Four characteristic absorption bands are identified for the POMs framework within the 500-1200cm^{-1} fingerprint area of FT-IR spectra ,[29] i.e., asymmetric stretching vibrations of Si-O, W-O_d, W-O_b-W and W-O_c-W. [30] This observation indicates that β-SiW_{11} and the final product $SiW_{11}Co$/PANI retain the Keggin structure and β-SiW_{11} has been inserted into the polyaniline framework. Meanwhile, the following absorptions are clearly identified demonstrating the formation of $SiW_{11}Co$/PANI composite, these include vibration of benzoquinone at 1160 cm^{-1}, stretching vibrations of C-N and

phenyl ring modes at 1303 cm^{-1} and 1500 cm^{-1} respectively, as well as the absorption of quinoid structure at 1590 cm^{-1}.

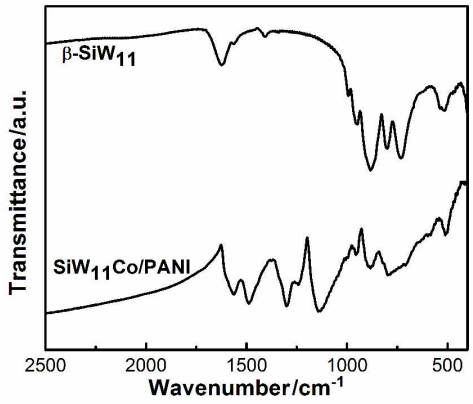


Figure 1. FT-IR spectra of β-SiW_{11} and SiW_{11}Co/PANI composite.

The thermogravimetric analysis result for SiW_{11}Co/PANI composite is shown in Figure 2. Three weight loss stages are observed along with the increase of temperature from 25 to 900°C. The first weight loss is obtained at a temperature of <100 °C, which is certainly attributed to the evaporation of crystal water in SiW_{11}Co/PANI composite. An obvious weight loss is afterwards observed as the temperature is >350 °C, this should be attributed to the decomposition of polyaniline molecules in the composite. When further increasing the temperature to 570°C, decomposition of the polyoxometalate contributes to a slight weight loss, e.g., at this stage the Keggin framework of the polyoxometalate has collapsed. The above results indicate that SiW_{11}Co/PANI composite is stable at a temperature of <350 °C.

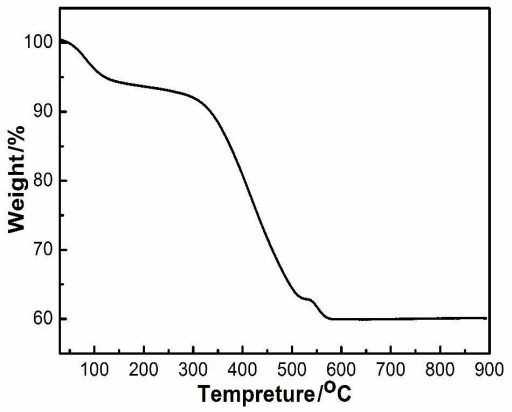


Figure 2. TG analysis result for SiW_{11}Co/PANI composite.

Figure 3 shows the X-ray diffraction patterns for the obtained β-SiW_{11} and the SiW_{11}Co/PANI composite, which provide some important information. It is clearly seen that except for the appearance of a diffraction peak at 2θ=7.5°, no other peaks in the XRD pattern of β-SiW_{11} are observed in the XRD pattern for SiW_{11}Co/PANI composite. In addition, the peak shape and intensity are completely different in the two XRD patterns. The wide and weak diffraction peak for SiW_{11}Co/PANI composite clearly indicates that the insertion of POMs into the polymer matrix produces a new material, i.e., an amorphous SiW_{11}Co/PANI composite.

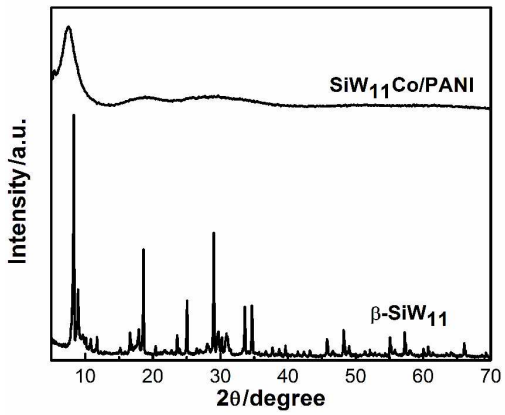


Figure 3. XRD patterns for β-SiW_{11} and SiW_{11}Co/PANI composite.

SEM and TEM images as illustrated in Figure 4A and 4B reveal that many tiny particles aggregate together to form irregular particulate SiW_{11}Co/PANI composite. It can be seen that SiW_{11}Co/PANI composite possesses porous and reticular sheet structure. In addition, the size of SiW_{11}Co/PANI composite falls into sub-micrometer range. The EDS image in Figure 4C indicates that the composite mainly consists of C, N, O, W, Si and Co. BET result in Figure 4D further confirms that there exists porous structure for the composite, with an average pore width of 18nm.

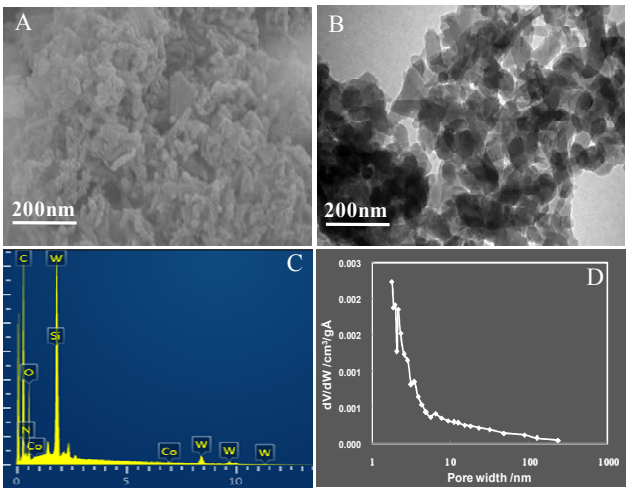


Figure 4. SEM (A) and TEM (B) images in addition to EDS(C) and BET(D) analysis results of the SiW_{11}Co/PANI composite.

3.2. Protein adsorption behavior by the SiW_{11}Co/PANI composite framework

In the present case, Ova and Lys represent acidic and basic proteins for the evaluation of adsorption behaviors by SiW_{11}Co/PANI composite as adsorbent. For the purpose of approving the real selectivity of the composite, another protein with similar isoelectric point (pI) value to Ova, i.e., BSA in this particular case, is also investigated. Considering that SiW_{11}Co/PANI composite is not stable at pH >9, adsorption experiments are conducted within a range of pH 3-9 adjusted by using dilute HCl and/or NaOH solutions. The results are

illustrated in Figure 5. It is seen that 5.0 mg of $SiW_{11}Co$/PANI composite gives rise to an adsorption efficiency of >70% for 100 mg L^{-1} Ova in 1.0 mL of sample solution within pH 3-9, and a maximum adsorption efficiency of 92% is achieved at pH 9. On the other hand, however, virtually no adsorption of Lys is observed within the whole pH range studied, and very low adsorption of BSA, i.e., at the level of <10%, is obtained within the same pH range. At pH 5, the adsorption of BSA is well controlled at <3%, while there is still 74% Ova adsorbed by the $SiW_{11}Co$/PANI composite. This observation well illustrates a favorable selectivity toward Ova against BSA and Lys.

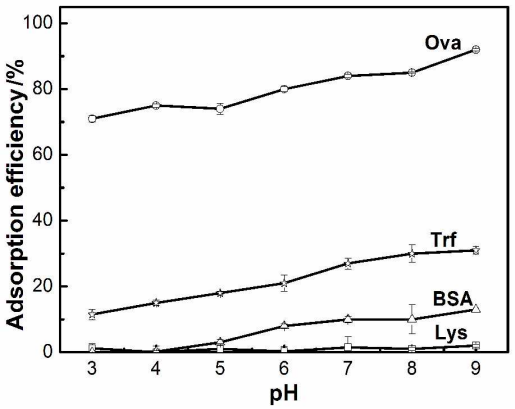


Figure 5. pH-dependent adsorption behaviors for Ova, Trf, BSA and Lys with the $SiW_{11}Co$/PANI composite as adsorbent. Protein solution: 100 mg L^{-1}, 1.0 mL; $SiW_{11}Co$/PANI composite: 5.0 mg; adsorption time: 10 min.

For further elucidation of the adsorption selectivity by the $SiW_{11}Co$/PANI towards Ova against BSA, SDS-PAGE assays have been performed for the Ova/BSA mixture before and after adsorption, Ova and BSA standard solutions, and Ova recovered from the composite with elution by phosphate (0.1 mol L^{-1}, pH 5.6). The results are given in Figure S1. It clearly illustrates obvious adsorption of Ova, while at the same time the adsorption of BSA is virtually not observed. In addition, significant amount of Ova is recovered from the composite by elution with phosphate buffer, while no BSA is recovered at all. These observations clearly demonstrated the selectivity of adsorption to Ova against BSA. In general, it would be interesting to evaluate the quantitative selectivity to Ova and BSA. For this purpose, adsorption of Ova/BSA mixture at 200 μg mL^{-1} each protein by $SiW_{11}Co$/PANI is performed. We have intended to separate and quantify Ova and BSA in both the supernatant and the recovered solution by HPLC with UV detection. However, it turns out that the commercial C18 column is unable to separate these two proteins, resulting in almost same retention times for them.

Ova is a glycoprotein with isoelectric point at pI 4.7. [31] Its carbohydrate chain is linked to Asn-292, which is solvent exposed sugar moiety bound. [32, 33]The porous structure of $SiW_{11}Co$/PANI composite might provide a suitable residence for the accommodation of the carbohydrate chain in Ova. At pH<5, hydrogen bonds are formed between the hydroxyl groups on the carbohydrate chain and the oxygen atoms in $SiW_{11}Co$/PANI composite, which serve as a major driving force for the adsorption of Ova. This has been demonstrated by the fact that a gradual decline of adsorption is observed with the increase of temperature within 20-60°C. The adsorption efficiencies at 20 °C, 25 °C, 30 °C, 40 °C and 60 °C are 100%, 95%, 84%, 73% and 63%. The increase of pH value causes a decrease on the protonation of oxygen atoms in $SiW_{11}Co$/PANI composite, thus hydrogen bonding interaction contributes more to the adsorption of Ova. As pH exceeds pI of the protein, i.e., pH>5 in this particular case, the protein turns to negatively charged. According to the hard and soft acids and bases (HSAB) theory, [34] oxygen atoms with negative charge (RO^-) in Ova coordinate with cobalt cation in $SiW_{11}Co$/PANI composite, and thus the adsorption of Ova takes place. The negative charge and coordination interaction get strengthened when increasing the pH value, which further enhance the adsorption of Ova.

The trend of adsorption for another glycoprotein, i.e., transferrin, looks similar to that for Ova, while its adsorption efficiency is much lower. This observation is consistent with the structure differences in the two protein species. Human transferrin consists of a single polypeptide chain of 679 amino acids and two N-linked glycan chains, the latter consists of sialic acid residues. [35]On the other hand, carbohydrate moiety in Ova consists of 4–6 mannose residues and 2–4 N-acetyl- b-D-glucosamine residues.[33] That is transferrin possesses less active functional sites for the interaction to $SiW_{11}Co$/PANI composite with respect to Ova, and thus results in low adsorption efficiency.

Unlike Ova and transferrin, Lys is not a glycoprotein, it contains no oligosaccharide chain and there are less hydroxyl groups on its surface. Thus, the above mentioned driving forces or interactions governing the adsorption of protein are not found or very weak for the case of Lys-$SiW_{11}Co$/PANI adsorption system. Therefore, the adsorption of Lys by $SiW_{11}Co$/PANI composite is virtually not observed under the experimental conditions of the present study.

3.3 Adsorption of Ova on the $SiW_{11}Co$/PANI composite

The above discussions reveal a clear discrimination between Ova and Lys for their adsorption by $SiW_{11}Co$/PANI composite. This provides a promising potential for the selective isolation of Ova from biological samples in the presence of Lys and complex sample matrix components. In the treatment of real biological samples, the difference in sample matrix generally results in a large variation of ionic strength in the final solution. This tends to pose great effect on the protein-surface interaction and is thus critical for the adsorption of proteins .[36] In this respect, the dependence of adsorption behaviour of Ova should be carefully investigated.

The effect of ionic strength on the adsorption is studied by addition of various amount of NaCl into the Ova solution. As illustrated in Figure 6A, an increase of ionic strength from 0-0.3 mol L^{-1} results in a slight improvement on the adsorption efficiency of Ova, while a very small decline is observed by further increasing the ionic strength to 0.4 mol L^{-1}. This observation clearly demonstrates that electrostatic interaction is out of the driving forces for governing the adsorption of Ova onto the $SiW_{11}Co$/PANI composite. It is seen that the variation of ionic strength within a certain range causes very limited change on the adsorption of Ova, and that the adsorption is better performed in an aqueous medium of low ionic strength. In practice no adjustment on the ionic strength is performed for the treatment of

real biological samples in the ensuing investigations.

The effect of adsorption time is investigated within 2-20 min and the results are given in Figure 6B. It is seen that a maximum adsorption is achieved at a sorption time of 10 min, and afterwards the curve is leveled off with further increasing the sorption time to 20 min. For the ensuing studies, a sorption time of 10 min is adopted.

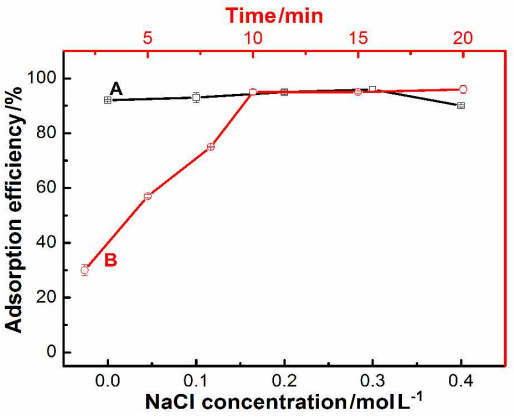


Figure 6. The effect of (A) ionic strength (NaCl concentration) and (B) adsorption time on the adsorption of Ova. (A) Ova solution: 100 mg L^{-1}, 1.0 mL; pH 9.0; $SiW_{11}Co$/PANI: 5.0 mg; adsorption time: 10 min. (B) Ova solution: 100 mg L^{-1}, 1.0 mL; pH 9.0; $SiW_{11}Co$/PANI: 5.0 mg.

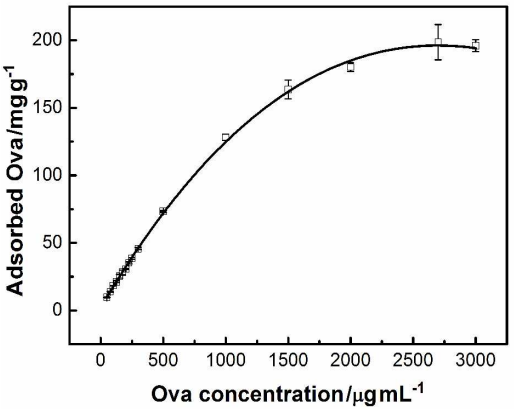


Figure 7. The adsorption isotherm of Ova on $SiW_{11}Co$/PANI composite. Ova solution: 0-3000 μg mL^{-1}, 1.0 mL; $SiW_{11}Co$/PANI: 5.0 mg; pH 9.0; adsorption time: 10 min.

To evaluate the adsorption capacity for the Ova by $SiW_{11}Co$/PANI composite, a series of Ova solutions within 0-3000 μg mL^{-1} are treated with 5.0 mg of the composite, by following the adsorption procedure as described in the *experimental* section. After adsorption, the residual Ova content in the supernatant is determined, and thereafter the adsorption isotherm is achieved by plotting Ova concentration *versus* the adsorbed amount of protein, as illustrated in Figure 7. It is obvious that the adsorption behavior of Ova by the $SiW_{11}Co$/PANI composite fits *Langmuir* adsorption model, where C_e (μg mL^{-1}) is the equilibrium concentration, Q_e (mg g^{-1}) denotes the adsorption capacity, Q_{max} (mg g^{-1}) represents the theoretical maximum adsorption capacity, and K_d (μg mL^{-1}) is the adsorption equilibrium constant.

$$\frac{1}{Q_e} = \frac{K_d}{Q_{max} \cdot C_e} + \frac{1}{Q_{max}}$$

By fitting the experimental data to this equation, the maximum adsorption capacity for Ova by the $SiW_{11}Co$/PAN composite is derived to be 200.0 mg g^{-1}.

3.4. The recovery of retained Ova from the $SiW_{11}Co$/PANI composite

For further biological investigations, it is necessary to transfer the adsorbed protein into aqueous medium. Therefore, the recovery of the retained Ova from the $SiW_{11}Co$/PANI surface is highly desirable. The performances of a few potential stripping reagents for the collection of Ova from the $SiW_{11}Co$/PANI surface are evaluated, including NaCl (2 mol L^{-1}), Tris-HCl (0.1 mol L^{-1}, pH 7), SDS (0.05 mol L^{-1}), phosphate buffer (0.1 mol L^{-1}, pH 5.6) and imidazole (0.1 mol L^{-1}). The results indicate that phosphate buffer (0.1 mol L^{-1}, pH 5.6) and imidazole (0.1 mol L^{-1}) offer favorable recoveries of 84.4% and 73.0% for Ova, while the other stripping reagents mentioned herein give rise to virtually no recovery of the adsorbed Ova by the $SiW_{11}Co$/PANI composite. Considering that biological investigations are mostly conducted in a buffer medium and imidazole tends to cause conformational change for protein (as demonstrated in Figure 8), phosphate is thus preferential for the elution of adsorbed Ova in this case.

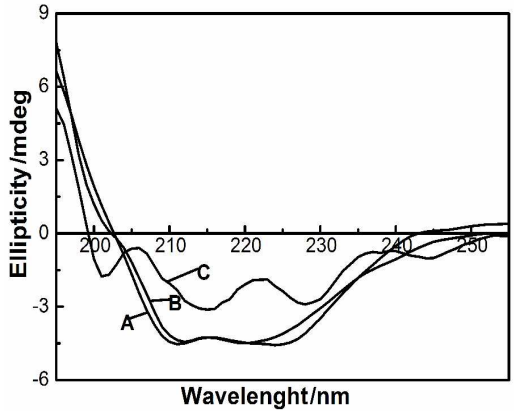


Figure 8. CD spectra of Ova. (A) Ova standard solution prepared in de-ionized water; (B) Ova in the eluate after removal of phosphate buffer by dialysis; (C) Ova in the eluate after removal of imidazole (0.1 mol L^{-1}) by dialysis.

It is generally important to evaluate whether there is denaturation or conformational change for the recovered protein after the process of adsorption and desorption. For this purpose, circular dichroism (CD) spectra for Ova are recorded in a standard solution prepared in de-ionized water and in the eluate after removal of phosphate buffer and imidazole (0.1 mol L^{-1}) by dialysis . The results are given in Figure 8. It is obvious that the CD spectrum for the recovered Ova after the process of adsorption/desorption by phosphate buffer (Figure 8B) is the same as that for the standard Ova solution (Figure 8A), both spectra exhibit two negative peaks at 210 nm and 222nm, respectively. These peaks are the characteristics of the α-helical structure of proteins, and are attributed to n-π* transition of the α-helix peptide bond. [37] This observation clearly indicates favourable biocompatibility for the $SiW_{11}Co$/PANI composite,

which helps to maintain the conformation/structure of Ova during the adsorption-desorption process. It is noted that obvious conformational change of the protein is observed when imidazole is adopted as the stripping reagent (Figure 8C).

3.5. Isolation of Ova from chicken egg white

The practicability of $SiW_{11}Co$/PANI composite in the adsorption of Ova in real biological sample matrixes is demonstrated by the selective separation of Ova from chicken egg white. In practice, chicken egg white from fresh eggs is 100-fold diluted and adjusted to pH 9, the mixture is then gently stirred to form a homogeneous suspension. The suspension is centrifuged at 5000 rpm for 5 min at 4°C, and the supernatant is then collected and mixed with 5.0 mg of $SiW_{11}Co$/PANI composite for performing adsorption by following the same procedure as described in the *experimental* section. The adsorbed Ova is recovered by phosphate buffer followed by SDS-PAGE assay, and the results are given in Figure 9. For SDS-PAGE assay, the sample solution is mixed with loading buffer and boiling for 5 min, electrophoresis is then performed on 5% polyacrylamide stacking particles at 80 V and 12% polyacrylamide resolving particles at 180 V. The protein bands are visualized by staining with 0.1% (w/v) Coomassie Brilliant Blue R250, and de-staining with a solution containing 7.5% (v/v) acetic acid and 5% (v/v) methanol. The protein bands in chicken egg white (Lane b) are attributed mainly to conalbumin (77.7 kDa), Ova (44.3 kDa) and Lys (14.3 kDa). After adsorption, the band intensity for Ova at 44.3 kDa (Lane c) becomes much weaker than that in Lane b, while the other protein bands remain virtually unchanged. After recovering the retained protein from the $SiW_{11}Co$/PANI composite, only a single band is observed at 44.3 kDa (Lane d). As a comparison, the band for a 200 μg mL^{-1} standard Ova solution is given in Lane e. The above observations indicate that Ova could be effectively isolated from chicken egg-white in the presence of Lys and coexisting sample matrix components with $SiW_{11}Co$/PANI composite as adsorbent.

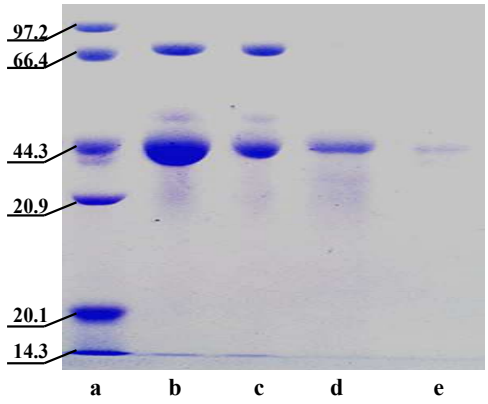


Figure 9. SDS-PAGE assay results. Lane a: protein Marker (kDa); Lane b: 100-fold diluted egg white without pretreatment; Lane c: 100-fold diluted egg white after adsorption by $SiW_{11}Co$/PANI composite; Lane d: Ova recovered from $SiW_{11}Co$/PANI composite surface; Lane e: Ova standard solution of 200 μg mL^{-1}.

4. Conclusions

A biocompatible Keggin-type cobalt mono-substituted silicotungstic acid doped with aniline has been prepared, shortly as $SiW_{11}Co$/PANI composite. The composite exhibits favorable selectivity toward the adsorption of ovalbumin from egg white in the presence of other proteins and the complex sample matrix components. In addition, a high sorption capacity of 200.0 mg g^{-1} is achieved. This study provides an alternative approach for the development of polyoxometalates (POMs)-based hybrid materials for highly selective adsorption of biomacromolecules from complex biological samples. It certainly expands the scope of applications of POMs in the field of life science separations.

Acknowledgements

The authors appreciate financial supports from the Natural Science Foundation of China (21275027, 21235001 and 21475017), the Program of New Century Excellent Talents in University (NCET-11-0071), the SRFDP program (20120042110020), Liaoning Provincial Natural Science Foundation (2014020041), and Fundamental Research Funds for the Central Universities (N140505003, N141008001 and N130105002).

Notes and references

[a] *Research Center for Analytical Sciences, College of Sciences, Northeastern University, Shenyang, China.* [b] *Collaborative Innovation Center of Chemical Science and Engineering, Tianjin 300071, China. Fax: +86 24 83676698; Tel: +86 24 83688944; E-mail:chenxuwei@mail.neu.edu.cn (X.-W. Chen); jianhuajrz@mail.neu.edu.cn (J.-H Wang).*

1. A. Dolbecq, E. Dumas, C. d. R. Mayer and P. Mialane, *Chemical record*, 2010, **110**, 6009.
2. P. Putaj and F. Lefebvre, *Coordination Chemistry Reviews*, 2011, **255**, 1642.
3. B. Hasenknopf, K. Micoine, E. Lacôte, S. Thorimbert, M. Malacria and R. Thouvenot, *European Journal of Inorganic Chemistry*, 2008, **2008**, 5001.
4. H. Stephan, M. Kubeil, F. Emmerling and C. E. Müller, *European Journal of Inorganic Chemistry*, 2013, **2013**, 1585.
5. C. L. Hill, *Journal of Molecular Catalysis A: Chemical*, 2007, **262**, 2.
6. Y. F. Song, D. L. Long, C. Ritchie and L. Cronin, *Chemical record*, 2011, **11**, 158.
7. H. Tan, W. Chen, Y.-G. Li, D. Liu, L. Chen and E. Wang, *Journal of Solid State Chemistry*, 2009, **182**, 465.
8. G. Li, Y. Ding, J. Wang, X. Wang and J. Suo, *Journal of Molecular Catalysis A: Chemical*, 2007, **262**, 67.
9. J. Luo, J. Zhao, J. Yuan, Y. Li, L. Chen, P. Ma, J. Wang and J. Niu, *Inorganic Chemistry Communications*, 2013, **27**, 13.
10. S. Liu and Z. Tang, *Nano Today*, 2010, **5**, 267.
11. C. Li, J. Lu, F. Tu, J. Chen and Y. Li, *Inorganic Chemistry Communications*, 2011, **14**, 1192.
12. L. Y. Feng, Y. H. Wang, Y. J. Qi, C. W. Hu, Y. Xu and E. B. Wang, *Journal of Molecular Structure* 2003, **645**, 231.
13. H. Ma, Z. Zhang, H. Pang, S. Li, Y. Chen and W. Zhang, *Electrochimica Acta*, 2012, **69**, 379.
14. M. Inoue, T. Suzuki, Y. Fujita, M. Oda, N. Matsumoto and T. Yamase, *Journal of inorganic biochemistry*, 2006, **100**, 1225.
15. J.-Q. Sha, X. Li, Y.-H. Zhou, P.-F. Yan, G.-M. Li and C. Wang, *Solid State Sciences*, 2011, **13**, 1972.

16. S. Shigeta, S. Mori, T. Yamase, N. Yamamoto and N. Yamamoto, *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, 2006, **60**, 211.
17. J.-Q. Sha, L.-Y. Liang, X. Li, Y. Zhang, H. Yan and G. Chen, *Polyhedron*, 2011, **30**, 1657.
18. F.-C. Yang, K.-H. Wu, W.-P. Lin and M.-K. Hu, *Microporous and Mesoporous Materials*, 2009, **118**, 467.
19. J.-Q. Sha, L.-Y. Liang, P.-F. Yan, G.-M. Li, C. Wang and D.-Y. Ma, *Polyhedron*, 2012, **31**, 422.
20. S. A. i.-U. i, M. Ballauff and J. Dzubiella, *Chem. Soc. Rev.*, 2014, **10**, 7899.
21. Y. Li, X. Zhang and C. Deng, *Chemical Society reviews*, 2013, **42**, 8517.
22. M. N. Gupta, R. Kaul, D. Guoqiang, U. Dissing and B. Mattiasson, *J. Mol. Recognit.*, 1996, **9**, 356.
23. W.-C. Lee and K. H. Lee, *Analytical Biochemistry*, 2004, **324**, 1.
24. T. Burnouf and M. Radosevich, *J. Biochem. Biophys. Methods* 2001, **49**, 575.
25. Y. P. Tzeng, C. W. Shen and T. Yu, *Journal of chromatography. A*, 2008, **1193**, 1.
26. X. Chen, S. Chen, J. Liu and J. Wang, *Microchimica Acta*, 2008, **165**, 217.
27. G. Zhang, Q. Cao, N. Li, K. Li and F. Liu, *Talanta*, 2011, **83**, 1515.
28. M. Odabaşi, R. Say and A. Denizli, *Materials Science and Engineering: C*, 2007, **27**, 90.
29. Y. Kikukawa, K. Suzuki, K. Yamaguchi and N. Mizuno, *Inorganic chemistry*, 2013, **52**, 8644.
30. D. Julião, D. M. Fernandes, L. Cunha-Silva, D. Ananias, S. S. Balula and C. Freire, *Polyhedron*, 2013, **52**, 308.
31. J. A. Huntington and P. E. Stein, *Journal of Chromatography B*, 2001, **756**, 189.
32. L. Lv and Y. Chi, *European Food Research and Technology*, 2012, **235**, 981.
33. J. de Groot, H. A. Kosters and H. H. de Jongh, *Biotechnology and bioengineering*, 2007, **97**, 735.
34. R. M. Lopachin, T. Gavin, A. Decaprio and D. S. Barber, *Chemical research in toxicology*, 2012, **25**, 239.
35. M. Steven D. Rauch, *The Laryngoscope*, 2000, **110**, 545.
36. T. S. Tsapikouni and Y. F. Missirlis, *Colloids and surfaces. B, Biointerfaces*, 2007, **57**, 89.
37. L. Han, Y. Shu, X. Wang, X. Chen and J. Wang, *Analytical and bioanalytical chemistry*, 2013, **405**, 8799.

Graphical Abstract

A cobalt mono-substituted silicotungstic acid doped with aniline ($SiW_{11}Co$/PANI composite, PANI denotes polyaniline) possesses porous framework structure and exhibits favorable selectivity to ovalbumin adsorption.