

A novel biological active multilayer film based on polyoxometalate with pendant support-ligand

Huiyuan Ma^{a,b}, Jun Peng^{a,*}, Zhangang Han^a, Xia Yu^a, Baoxia Dong^a

^aFaculty of Chemistry, Northeast Normal University, Changchun 130024, PR China

^bDepartment of Chemistry, Harbin Normal University, Harbin 150080, PR China

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Abstract

A novel nanosized biological active multilayer film composed of polyoxometalate (POM) anion α -[SiW₁₁O₃₉Co(H₂PO₄)]⁷⁻ (abbr. SiW₁₁Co-PO₄) and poly(diallyldimethylammonium chloride) (abbr. PDDA) was fabricated by layer-by-layer self-assembly (LBL). The composition and growth processes of the films have been determined by X-ray photoelectron spectra (XPS) and ultraviolet-visible absorption spectra (UV). The composite film was formed by the alternate adsorption of SiW₁₁Co-PO₄ and PDDA, and the deposition process was quantitative and highly reproducible from layer to layer. The morphology of the film was studied by atomic force microscopy (AFM), which showed that the film was relatively uniform and smooth, and POM anions aggregated into nanoclusters distributing on the surface uniformly. The film exhibited favorable electrochemical behavior of POM indicated by cyclic voltammetry (CV). The film can immobilize the DNA molecules via Mg²⁺-bridging medium.

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Keywords: Layer-by-layer self-assembly; Polyoxometalate; Film; DNA chip

1. Introduction

The studies on hybrid materials of polyoxometalates (POMs) with organic or biochemical substances have led to great achievements in recent years, as they combine both inorganic and organic merits, possessing new synergy properties and diverse applications in photo- and electrochromism, magnetism, catalysis and medicine [1–7]. Covalent surface modification of POMs is of great interest in view of its importance in functionalization and in immobilization [8–13].

Deoxyribonucleic acids (DNA) are important biological materials [14]. Recently, much attention has been paid to DNA due to their physicochemical stability, topology of molecular structure, mechanical rigidity, biocompatibility and specific complementary recognition ability [15]. However, DNA is highly water-soluble and biochemically unstable. Stabilizing DNA by immobilization on a solid

support is important for the utilization of DNA as functional materials [16–18]. Physically adsorbed biomolecules can be readily lost and are often prone to denaturation and thereby to lose their biocatalytic or biorecognition activities. Many techniques are being developed to overcome those problems. For example, the functionalization of nanoparticles with biomolecules is realized through electrostatic adsorption, by covalent binding, through bifunctional linkers, by specific affinity interactions, and so on. Thompson et al. reported the label-free DNA hybridization probe based on a conducting polymer polypyrrole (PPy) film, which was coated on electrode [19]. After being modified with a layer of poly(2,5-dithienylpyrrole) with a phosphonic acid group, pTPTC3-PO₃H₂, the polymer film electrode was used for binding the DNA probe via its phosphoric acid residues with the help of magnesium cations. With this DNA probe for voltammetric observation, noncomplementary and complementary interactions of can be clearly distinguished.

Inspired by the excellent work Liz and co-workers, in this work we use covalent surface modification of POM

*Corresponding author. Fax: +86 431 568 4009.

E-mail address: jpeng@nenu.edu.cn (J. Peng).

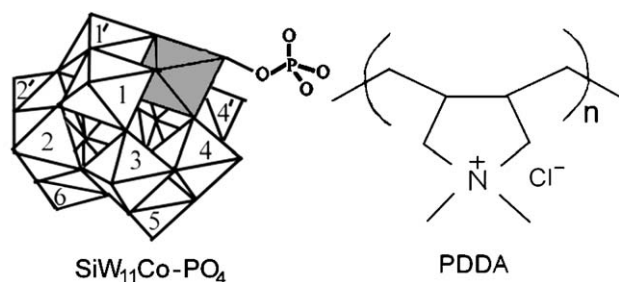


Fig. 1. Structures of the anionic $\text{SiW}_{11}\text{Co-PO}_4$ and cationic PDDA.

$\alpha\text{-}[\text{SiW}_{11}\text{O}_{39}\text{Co}(\text{H}_2\text{PO}_4)]^{7-}$ ($\text{SiW}_{11}\text{Co-PO}_4$) [20] and poly (diallyldimethylammonium chloride (PDDA) (their structural scheme is shown in Fig. 1) to fabricate multilayer composite films by LBL method, which is a simple and versatile technique to fabricate thin films [21–25], and further to bind DNA by mediated Mg^{2+} ions. A combination of the unique properties of POM films and biomaterials such as DNA will provide a unique opportunity in nanobiotechnology area and wonderful prospect of applications in target syntheses, drug-delivery, biosensors, bioelectronic devices, and so forth, even though these areas are still in an embryonic phase and require additional fundamental research.

2. Experimental section

2.1. Materials and instruments

Polyelectrolyte PDDA (MW. 400,000) was commercially obtained from Aldrich and used without further purification. Sodium salt of DNA and ethidium bromide (EB) were commercially obtained from Takara biotechnology CO., LTD. $\text{SiW}_{11}\text{Co-PO}_4$ was synthesized according to the literature method [20]. The product was identified by elemental analysis and NMR spectrum. The water used in all experiments was deionized to a resistivity of 16–18 $\text{M}\Omega/\text{cm}$. All other reagents were of reagent grade.

UV-vis spectra were recorded on a 756 CRT UV-vis spectrophotometer made in Shanghai, China. X-ray photoelectron spectra (XPS) were performed on an Escalab MKII photoelectronic spectrometer with $\text{MgK}\alpha$ (1253.6 eV) as X-ray source. Atomic force microscopy (AFM) image was obtained on Digital Nanoscope IIIa instrument operating in the tapping mode with silicon nitride tips. A CHI 600 electrochemical workstation connected to digital-586 personal computer was used for control of the electrochemical measurements and for data collection. A conventional three-electrode system was used, with an ITO electrode or a $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n\}$ multilayer films coated ITO electrode as a working electrode, platinum foil as a counter electrode, and Ag/AgCl as a reference electrode, in pH 3.5 buffer solution. Electron spin resonance (ESR) experiment was carried out using Japanese JES-FE3AX spectrometer at 293 K.

2.2. Fabrication of the film

2.2.1. Layer-by-layer assembly

The substrates of silica, quartz glass slide and ITO glass used were cleaned by immersing into a $\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2 = 7:3$ (V:V) solution for a few minutes followed by washing copiously with deionized (DI) water. Further purification for the substrates of silica and quartz glass slide was carried out with a $\text{NH}_3\text{H}_2\text{O}:\text{H}_2\text{O}_2:\text{H}_2\text{O} = 1:1:5$ (V:V:V) solution for a few minutes, rinsed again in DI water. After this cleaning procedure, the substrates became hydrophilic. The multilayer film was prepared by immersing the dry slide into 3×10^{-2} mol/L PDDA solution (the concentration was calculated based on the repeating unit of PDDA shown in Fig. 1) for 20 min, followed by washing with DI water and drying with a nitrogen stream. After these steps of treatment, the positively charged polycation PDDA was anchored to the surface of the substrate by electrostatic attraction. The PDDA-coated substrates then were alternately dipped into 1×10^{-3} mol/L $\text{SiW}_{11}\text{Co-PO}_4$ solution and 1×10^{-2} mol/L PDDA for 20 min, also rinsed in DI water, and dried under a nitrogen stream after each dipping.

2.2.2. Immobilization of DNA

Two as-fabricated quartz slides with $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n \text{SiW}_{11}\text{Co-PO}_4\}$ film were used for DNA binding tests. Slide 1 was immersed into 5×10^{-2} mol/L MgCl_2 aqueous solution for 15 min, followed by washing with DI water and drying with nitrogen stream. A drop of EB solution and a drop of DNA + EB solution were trickled onto different positions on the surface of the slide, respectively. Then the film was dried with nitrogen stream. Afterward, it was rinsed with 0.1 mol/L Tris-HCl buffer, pH 7.3 (Tris: trihydroxymethyl aminomethane), followed by blowing over with a nitrogen stream to remove the adhered rinsing solution. Slide 2 was treated by the same method as Slide 1 except for the absence of MgCl_2 . EB is a fluorescence label molecule to intercalate into base pairs of DNA [26]. When DNA molecules are attached onto the film, fluorescence can be observed lent a helping hand of EB under UV irradiation. Fluorescence intensities for the as-treated slides were observed under UV irradiation and recorded by camera.

3. Results and discussion

3.1. Ultraviolet-visible absorption spectra

The multilayer film assembly process was monitored by UV-Vis spectroscopy after each bilayer-deposition cycle. Fig. 2 shows the UV-Vis spectra of the multilayer $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n\}$ ($n = 0-8$) films assembled on a quartz glass slide. The existence of the strong characteristic absorption peaks at 206 and 258 nm for $\text{SiW}_{11}\text{Co-PO}_4$, attributed to the charge-transfer transitions of $\text{O}_d \rightarrow \text{W}$ and $\text{O}_b(\text{O}_c) \rightarrow \text{W}$ (O_d = terminal oxygen, O_b and O_c = bridge

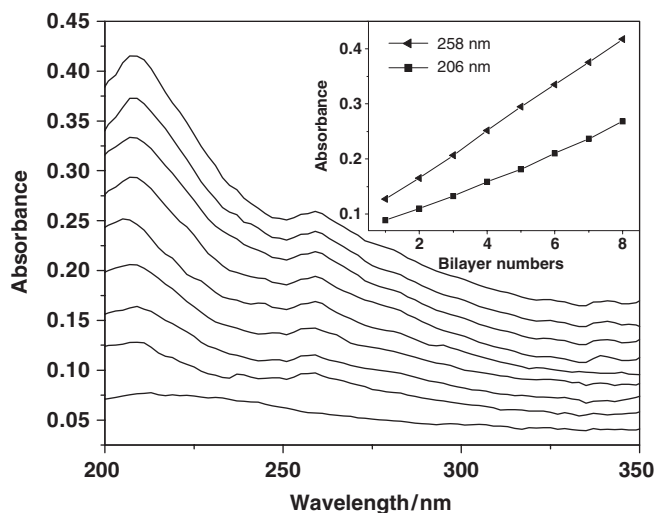


Fig. 2. UV-vis absorption spectra of $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n\}$ multilayer films with $n = 0$ – 8 on quartz glass slide. From the lower to the upper curves, the number of $\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA}$ layers is 0, 1, 2, 3, ..., 7 and 8, respectively.

oxygen), respectively, indicating that the $\text{SiW}_{11}\text{Co-PO}_4$ anion was incorporated into the composite film. The plot of absorbance values at 206 and 258 nm vs. the numbers of bilayer was shown in the inset. The absorbance values increase linearly with the bilayer numbers, which gives evidence of the quantitative and reproducible growth of the multilayer film from layer to layer.

3.2. Atomic force microscopy

The morphology of the film was observed by AFM. Fig. 3 shows the AFM image of the thin film $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_3 \text{SiW}_{11}\text{Co-PO}_4\}$. The surface of the film assembled on silica slide is uniform and smooth. The mean interface roughness is ca. 1.7 nm, calculated from an area of $0.5 \times 0.5 \mu\text{m}^2$. The vertical grain topography of the film surface with a lot of protuberant peaks is observed in the three-dimensional AFM image, indicating that the $\text{SiW}_{11}\text{Co-PO}_4$ anions aggregate into clusters with a mean grain size of ca. 23 nm, and furthermore, distributing uniformly on the film surface.

3.3. X-ray photoelectron spectrum

The further evidence that $\text{SiW}_{11}\text{Co-PO}_4$ and PDDA are indeed incorporated into the LBL film comes from XPS of the $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n (\text{SiW}_{11}\text{Co-PO}_4)\}$ ($n = 8$) film, shown in Fig. S1. The film exhibited peaks corresponding to $\text{C}1s$ (BE = 284.9 eV), $\text{N}1s$ (BE = 400.9 eV), $\text{O}1s$ (BE = 530.8 eV), $\text{P}2p$ (BE = 133.3 eV), $\text{Si}2p$ (BE = 101.5 eV), $\text{Co}2p$ (BE = 786.8), $\text{W}4f\ 5/2$ (BE = 34.9 eV), and $\text{W}4f\ 7/2$ (BE = 36.8 eV). The $\text{C}1s$ and $\text{N}1s$ signals can be assigned to the PDDA. The $\text{O}1s$, $\text{P}2p$, $\text{Si}2p$, $\text{Co}2p$, and $\text{W}4f$ signals can be assigned to the $\text{SiW}_{11}\text{Co-PO}_4$ polyanion.

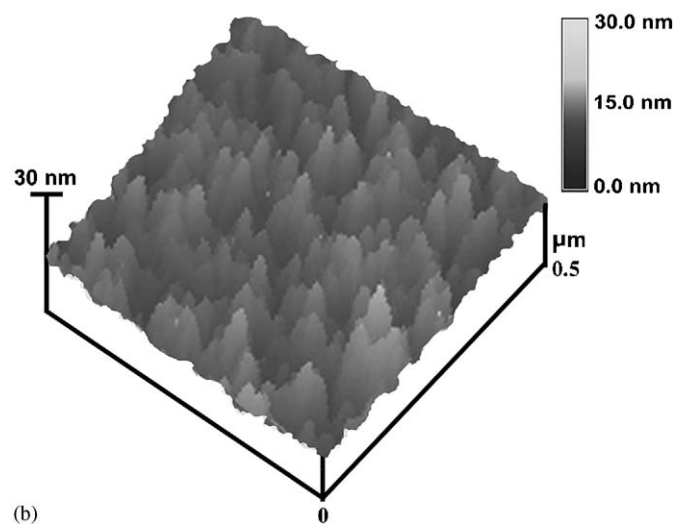
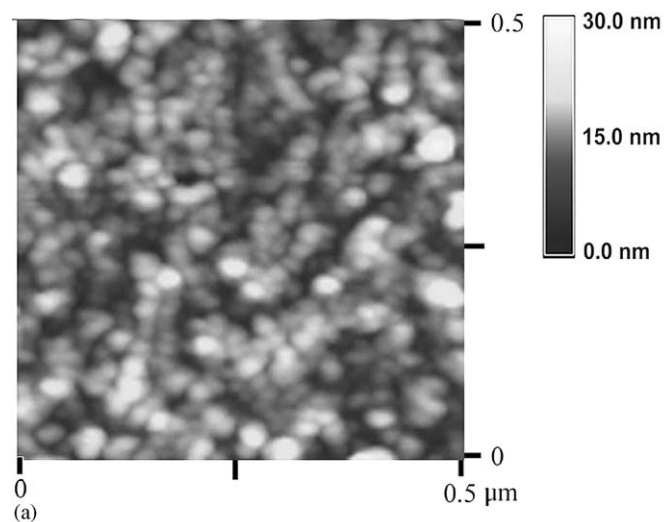


Fig. 3. AFM image of $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_3 \text{SiW}_{11}\text{Co-PO}_4\}$ multilayer film: (a) the planar image and (b) three dimensional image.

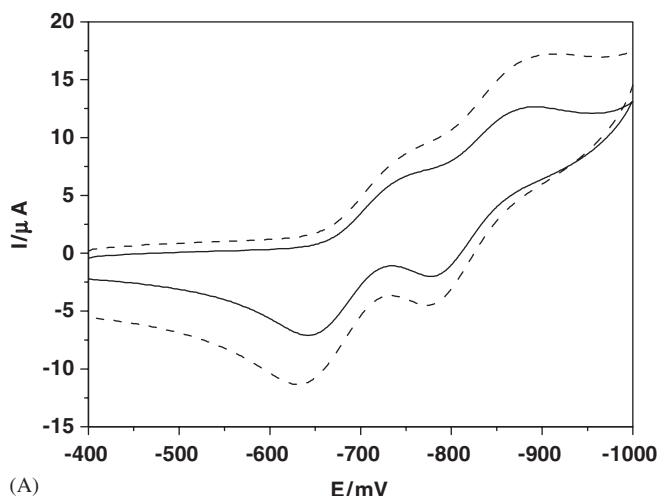
The XPS results can also confirm the existence of the polyelectrolyte PDDA and polyanion $\text{SiW}_{11}\text{Co-PO}_4$ in the multilayer film.

3.4. Electron spin resonance

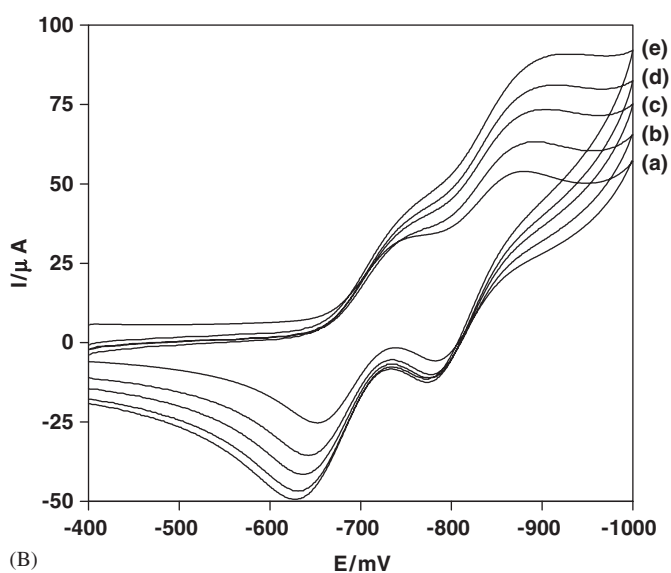
ESR of the thin film $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_{15} \text{SiW}_{11}\text{Co-PO}_4\}$ on a mica substrate shown in Fig. S2 exhibits a broad signal with g values of 2.0492, ascribed to Co^{2+} in the polyanion.

3.5. Electrochemistry of the $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n\}$ film assembled on ITO glass substrate

Cyclic voltammograms (CV) present in Fig. 4. The CV of a bare ITO electrode just after being immersed into 0.2 M HAc-NaAc solution (pH = 3.5) containing 0.5 mM $\text{SiW}_{11}\text{Co-PO}_4$ exhibits that in the range -1000 and 500 mV, the anion gives two two-electron redox waves of $\text{W(VI)}/\text{W(V)}$



(A)



(B)

Fig. 4. Cyclic voltammograms of (A) the bare ITO electrode in 0.5 mM $\text{SiW}_{11}\text{Co-PO}_4$ in 0.2 M HAc-NaAc (solid line) and the LBL film of $\text{SiW}_{11}\text{Co-PO}_4$ on ITO electrode (dot line) in 0.2 M blank HAc-NaAc, scan rate is 10 mV/S and (B) the LBL film of $\text{SiW}_{11}\text{Co-PO}_4$ on ITO electrode in 0.2 M blank HAc-NaAc at scan rates (a) 20, (b) 40, (c) 60, (d) 80 and (e) 100 mV/S.

and the midpoint potentials $E_{\text{mid}} = (E_{\text{pa}} + E_{\text{pc}})/2$ are -664 and -804 mV, respectively. The CV of the $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_7\}$ -modified ITO electrode in the same buffer solution shows that the surface formal potential E_{sur}° are -682 and -836 mV, respectively, which shift negatively perhaps due to $\text{SiW}_{11}\text{Co-PO}_4$ in different phases. The result demonstrate that the electrochemical property of $\text{SiW}_{11}\text{Co-PO}_4$ is fully maintained in the LBL film.

3.6. Bonding interaction of POM films with DNA

All the dots in slides **1** [$\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n \text{SiW}_{11}\text{Co-PO}_4/\text{Mg}^{2+}\}$] and **2** [$\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n \text{SiW}_{11}\text{Co-PO}_4\}$] emit red fluorescence

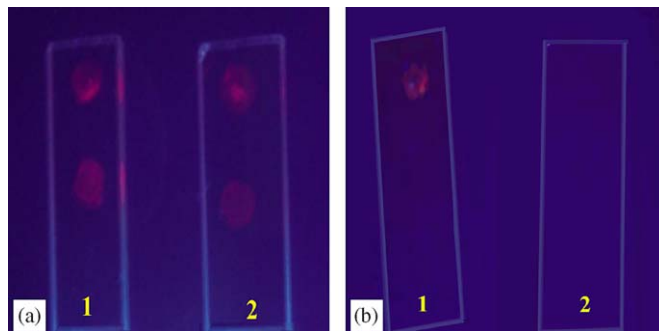


Fig. 5. Photos of the slides **1** and **2** under UV irradiation: slide **1**, $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n \text{SiW}_{11}\text{Co-PO}_4/\text{Mg}^{2+}\}$; slide **2**, $\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n \text{SiW}_{11}\text{Co-PO}_4\}$. The upper red dots arise from EB-stained DNA and lower ones only arise from EB; (a) before washing (b) after washing with 0.1 mol/L Tris-HCl buffer.

under UV irradiation after being treated as described in Section 2.2.2. The fluorescence of the two upper red dots in slides **1** and **2** arises from EB-stained DNA and that of lower ones arises only from EB without DNA [Fig. 5(a)]. Slides **1** and **2** then were rinsed with (HCl+Tris) buffer solution and dried, and were observed again under UV lamp [Fig. 5(b)]. We can see from the photo in Fig. 5(b) that fluorescence of both the lower dots from EB disappears owing to the removal of water-soluble EB through washing the slides with (HCl+Tris) buffer solution, while fluorescence of the upper dot from EB+DNA in slide **1** still exists. This result verifies the fact that DNA molecules are immobilized on the film [$\{\text{PDDA}/(\text{SiW}_{11}\text{Co-PO}_4/\text{PDDA})_n \text{SiW}_{11}\text{Co-PO}_4\}$], which has ability to bind DNA through coordination bonding of Mg ions. As is known, DNA backbones are composed of deoxyriboses and phosphate groups. We deduce that a main interaction exists between the phosphate groups of DNA and the pendant group $-\text{PO}_4$ of $\text{SiW}_{11}\text{Co-PO}_4$ via Mg^{2+} ion contact through a scheme shown in Fig. S3. The heteropolyanion $\text{SiW}_{11}\text{Co-PO}_4$ film should possess potential biological application. Further study on this is under way.

4. Conclusions

Novel thin multilayer films based on POMs with pendant surface ligands were fabricated by the layer-by-layer self-assembly method and characterized by various physical measurements. The film could be used to immobilize DNA molecules through metal coordination interaction, which possesses potential biological activity and application, and is worthy to study further.

Acknowledgments

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Appendix A. Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jssc.2005.09.031.

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