Organic-Inorganic Layer-by-Layer Self-Assembled Multilayer Films:
Preparation, Characterization and Applications

by

Li-Rong CAI
M.Phil in Chemistry, Xiamen University, China, 1999

A Thesis Submitted to
The Hong Kong University of Science and Technology
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The Degree of Doctor of Philosophy
in Chemistry

September 2004, Hong Kong
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This is to certify that I have examined the above PhD thesis
and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by
the thesis examination committee have been made.

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September 2004
Dedication

to my family
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<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>ascorbic acid</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscope</td>
</tr>
<tr>
<td>Au_n</td>
<td>gold nanoparticles</td>
</tr>
<tr>
<td>CoP</td>
<td>cobalt porphyrin</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammogram</td>
</tr>
<tr>
<td>CyV</td>
<td>cycloviologen</td>
</tr>
<tr>
<td>FeP</td>
<td>iron porphyrin</td>
</tr>
<tr>
<td>FT-IR</td>
<td>fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>GC</td>
<td>glassy carbon</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>inductively coupled plasma - mass spectrometry</td>
</tr>
<tr>
<td>ITO</td>
<td>indium-tin-oxide coated glass</td>
</tr>
<tr>
<td>LB</td>
<td>Langmuir-Blodgett</td>
</tr>
<tr>
<td>LbL</td>
<td>layer-by-layer</td>
</tr>
<tr>
<td>MA</td>
<td>methylacridinium</td>
</tr>
<tr>
<td>MV</td>
<td>methyl viologen</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffer solution</td>
</tr>
<tr>
<td>PDDA</td>
<td>poly (diallydimethyl-ammonium chloride)</td>
</tr>
<tr>
<td>Pd_n</td>
<td>palladium nanoparticles</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>-------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>PMo</td>
<td>phosphomolybdic acid</td>
</tr>
<tr>
<td>POM</td>
<td>polyoxometalates</td>
</tr>
<tr>
<td>PorV</td>
<td>porphyrin viologen</td>
</tr>
<tr>
<td>PSS</td>
<td>poly (sodium 4-styrenesulfonate)</td>
</tr>
<tr>
<td>Pt\textsubscript{n}</td>
<td>platinum nanoparticles</td>
</tr>
<tr>
<td>PW</td>
<td>phosphotungstate</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive-oxygen-species</td>
</tr>
<tr>
<td>RNS</td>
<td>reactive-nitrogen-species</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
<tr>
<td>SiW</td>
<td>silicotungstate</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>ultraviolet-visible absorption spectroscopy</td>
</tr>
<tr>
<td>VT</td>
<td>viologen tetramer</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
</tbody>
</table>
Structure of Compounds

Methyl Viologen: (MV)

\[ \text{H}_3\text{C}^+\text{N}^+\text{C}_\text{H}_3 \]

2Cl⁻

Methylacridinium: (MA)

2Cl⁻

Porphyrin Viologen: (PorV)

Br⁻ 2I⁻

Cycloviologen: (CyV)

4Br⁻

Viologen Tetramer: (VT)

4Br⁻ 4I⁻
Phosphomolybdic: 
(\text{PMO}_{12}\text{O}_{40}^{3-})

Silicotungstate: 
(\text{SiW}_{12}\text{O}_{40}^{4+})

Phosphotungstate: 
(\text{P}_{2}\text{W}_{18}\text{O}_{62}^{6-})

PDDA:

PSS:
ABSTRACT

Several organic-inorganic hybrid layer-by-layer self-assembled multilayer films and their conversion to functional films by various methods were developed for two application objectives: high performance multi-purpose electrochemical sensing to detect biologically important redox-active species and electrochromic coating materials.

Chapter 1 is a brief introduction to the background and the current hotspots of layer-by-layer self-assembly method closely related to the main objectives of this thesis, including the assembly principle, characterization methods and specific applications.

Chapter 2 describes the self-assembly of layer-by-layer molecular films with viologen (and viologen-derivatives) and heteropoly oxometalate on various substrates (e.g. glassy carbon, ITO) by alternatively dipping the substrates in anionic and cationic species. Electrochemical method, UV-Vis and FT-IR spectroscopies were employed to characterize the multilayer films. The successful applications of newly developed films in bifunctional electrochemical sensing of H$_2$O$_2$/ascorbic acid and O$_2$/NO are demonstrated.

Chapter 3 presents the fabrication of layer-by-layer self-assembled multilayer molecular films with viologen (and viologen-derivatives) and heteropoly oxometalate and their application in electrochromism. The assembly process was monitored by
electrochemical method, UV-Vis and FT-IR spectroscopies. Spectroelectrochemistry, which couples the UV-Vis absorption with electrochemistry, was employed to study the reversible electrochromic behavior of the multilayer films.

Chapter 4 reports the preparation, characterization of hybrid films containing metalloporphyrins and metal nanoparticles converted from self-assembled multilayer films and their applications in biosensing. UV-Vis spectroscopy, AFM, XPS and electrochemical method were employed to characterize the films. The electrocatalytic behaviors of the films were studied in detail, including catalytic reduction of oxygen and catalytic oxidation of nitric oxide.

Chapter 5 focuses on a novel method to fabricate hybrid films containing metalloporphyrins and bimetallic nanoparticles by electrochemical or photochemical conversion of layer-by-layer assembled multilayer films. The assembling process and hybrid films were characterized by UV-Vis spectroscopy, electrochemical method, XPS, SEM and ICP-MS. The bimetallic nanoparticles can serve as catalysts in bifunctional sensing of O₂ and NO.

Chapter 6 demonstrates a novel method to prepare three-dimensional fractal Au nanostructure by its electrodeposition on polycation/polyanion multilayer films fabricated by layer-by-layer assembly. The fractal nanostructures were characterized by UV-Vis spectroscopy, SEM, AFM, XPS and electrochemical methods. The electrode modified with Au fractal nanostructures exhibits very high electrocatalytic activity towards the oxidation of NO.
Chapter 7 summarizes the major findings of this thesis and outlines my personal perspective for this project on the basis of my research experiences and thinkings.
CHAPTER 1

General Introduction
1.1 Layer-by-layer assembly on electroactive substrates

1.1.1 Surface modification techniques

The nanoscopic organization of matter exhibits special properties that are not possessed by matters with normal scale dimension [1]. One of the most active fields in the exploration of nano-scale materials is the specific surface modification with layers composed of well-defined nano-structure [2].

By specific surface modification with highly tailored layers, material with unique optical, electronic and catalytic properties can be developed. In the last century, various techniques for the modification of material surface were developed, including Langmuir-Blodgett (LB) technique, self-assembled monolayer (SAM) and the layer-by-layer (LbL) assembly technique.

LB technique, which was firstly introduced by I. Langmuir in 1920 [3] and applied extensively by K. Blodgett [4], involves the vertical movement of a solid substrate through the monolayer/air interface. The driving force for formation of LB films is the hydrophobic interaction between amphiphilic molecules on a gas-liquid interface. Fig. 1.1 shows the commonest form of LB film deposition [5]. Despite the advantages associated with LB films, such as high order of the building block and capacity to form multilayers, the LB is inevitably restricted to amphiphilic molecules.
Fig. 1.1 Langmuir–Blodgett film deposition [5].

Self-assembled monolayers are ordered molecular assemblies that are formed spontaneously by the adsorption of a surfactant with a specific affinity of its headgroup to a substrate (Fig. 1.2) [6, 7]. Such films have been used in the design of chemical sensors [8, 9], nonlinear optical materials [8, 10], optically sensitive interfaces [11], and high density memory devices [8, 12]. Thus, SAMs of a wide range of adsorbate functional groups have been developed on various substrates, including thiols [13-17], disulfides [18, 19], sulfides [20] and phosphines [21] on
metal surfaces (Au, Pt, Cu and Ag) and semiconductor surfaces (GaAs, CdSe and CdS), carboxylic acids [22, 23] on metal oxides and silanes [6, 24] on oxide surfaces. However, despite its obvious advantages such as the ease of preparation and tenability of surface properties, SAM technique also severely suffers from strict limitation of both the surface property of substrate and nature of the adsorbates. Only certain types of species, mainly sulfides, disulfides and silanes, are applicable for SAM preparation.

![Diagram of self-assembly monolayer technique and schematic structure of the resulting SAM film](image)

Fig. 1.2 Illustrative presentation of self-assembly monolayer technique and schematic structure of the resulting SAM film [6].

1.1.2 Layer-by-layer assembly

In the past ten years, scientists from various areas have been attracted to a versatile new method for the construction of highly tailored films. It was first
introduced by Decher in 1991, the so-called layer-by-layer (LbL) assembly technique in which it has experienced an initial period of exponential growth [2]. The LbL technique, which is based on alternating adsorption of cationic and anionic species, is a low-cost, environmentally friendly, wet-bench technique that allows one to fabricate films with controlled layer arrangement and film thickness. Typically, a LbL film is fabricated by alternatively dipping of the pretreated substrate in solutions of cationic and anionic species, as shown schematically in Fig. 1.3 [25].

Fig. 1.3 A. Schematic of the LbL film assembly process using slides and beakers. Step 1 and 3 represent the adsorption of a polyanion and polycation, respectively, and step 2 and 4 are washing steps. The four steps are the basis build-up sequence for
the simplest film architecture. B. Simplified molecular picture of the first two adsorption steps [25].

The desirable features of LbL technique lie in not only the simplicity and versatility of this technique, but also the high quality of coatings and uniform distribution of the assembled species. Various materials have been used as building blocks in the fabrication of LbL films, including small organic/inorganic molecules [26-30], macromolecules [31-33], biomacromolecules [34-35] such as proteins or DNA and colloids. Given accessibility to solvent, almost any kind of surface, including that of microcapsules, colloids and biological cells [36-38], can be used as substrate for LbL assembly, regardless of its shape.

Table 1.1 Comparison of LB, SAM and LbL techniques

<table>
<thead>
<tr>
<th></th>
<th>LB</th>
<th>SAM</th>
<th>LbL</th>
</tr>
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<tbody>
<tr>
<td><strong>Driving force</strong></td>
<td>Hydrophobicity</td>
<td>Covalent bonding</td>
<td>Electrostatic and secondary interactions</td>
</tr>
<tr>
<td><strong>Building block</strong></td>
<td>Amphiphilic molecules</td>
<td>Special ad-atoms: thiols, disulfides, sulfides, phosphines and silanes</td>
<td>Oppositely charged species</td>
</tr>
<tr>
<td><strong>Equipment</strong></td>
<td>Special equipment</td>
<td>Simple glassware</td>
<td>Simple glassware</td>
</tr>
<tr>
<td><strong>Substrate</strong></td>
<td>Flat substrate</td>
<td>Au, SiO₂, Al₂O₃, Ag, CdS, GaAs, CdSe</td>
<td>Almost any surface of any shape</td>
</tr>
<tr>
<td><strong>Stability</strong></td>
<td>Less stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td><strong>3D-capability</strong></td>
<td>Poor</td>
<td>Poor</td>
<td>Good</td>
</tr>
</tbody>
</table>
As a comparison, the main features of three thin film fabrication techniques mentioned above are summarized in Table 1.1.

1.1.3 Methods employed in the characterization of LbL films

UV-Vis spectroscopy

UV-Vis spectroscopy is probably the easiest way to monitor the multilayer growth of LbL films on optically transparent substrates such as glass and ITO. This technique can be employed in most of the systems with colored building blocks. Owing to the availability of the instrument in almost every chemistry lab and easy operation, UV-Vis spectrophotometer is commonly employed as a research tool in various LbL fabrication systems, such as that of polyelectrolytes [26, 39, 40], low molecular weight chromophores [41, 42], polymer-ion complexation [43], semiconductor nanoparticles [44], metallic nanoparticles [45-47]. In most successful LbL fabrications, a linear increase of the characteristic absorbance of the assembled species with the alternating adsorption cycles was observed, a nice indication that same amount of materials is deposited on the substrate in each cycle. Moreover, the amount of the species deposited on the substrate in each step can be quantified if the molar absorption coefficient is available.

Electrochemical methods

Electrochemical methods such as cyclic voltammetry have been applied as a tool for the characterization of the LbL films containing electroactive species. The growth of the LbL films can be monitored by cyclic voltammetry and additional information
can be obtained from the characteristic redox peaks in cyclic voltammogram. Willner and co-workers used this method to characterize the assembly of colloidal gold and organic oligo-cations, a crosslinker, on ITO electrodes [47]. The anodic and cathodic currents increase with the number of assembled layer. Information about the nanoparticle and the crosslinker densities as well as the lattice conductivity could also be obtained. Layer growth of myoglobin [48, 49] and glucose oxidase [50, 51] on electrode has also been investigated using this method.

**Infrared spectroscopy (IR)**

Similar to UV-Vis spectroscopy, Infrared spectroscopy is also a widely available and straightforward tool for monitoring the growth of LbL films. Additional information associated with the state of the assembled species in the film can be read by the fingerprints of the IR spectra. IR spectroscopy has been employed as a tool to monitor the assembly of Au nanoparticles, polyelectrolyte, and azo dye and among many others [51-57]. However, most common substrates are not IR transparent without interferences, the application of IR spectroscopy is therefore limited to a few IR transparent substrates such as KRS-5.

**Ellipsometry**

Ellipsometric measurement is the common optical technique for the determination of the thickness and refractive index of thin homogeneous films [5]. When a plane-polarized light interacts with a surface at some angle, it is resolved into its parallel and perpendicular components, which are reflected from the surface with changed amplitudes and phases. When the two component light beams are
combined, the result is elliptically polarized light. This phenomenon can be applied in ellipsometry to estimate the thickness of a transition region between the surface and air by measuring the ratio between the reflection coefficients of the two polarized light beams. The linear growth of film thickness of the multilayers built on silicon wafers was found via ellipsometry for the systems such as polycation/polyanion [58] and Au colloid/polyelectrolyte [59].

It should be pointed out that all the methods mentioned above are ex-situ methods, which require interruption of the deposition process in order to take the measurement. Apart from the interruption, the ellipsometry measurement has to carry out in dry atmosphere, which may not be desirable in certain cases.

**Quartz crystal microbalance (QCM)**

Quartz crystal microbalance, which is based on piezoelectric effect to measure the change in surface mass up to nanogram scale, is ideally suitable for screening the adsorption kinetics for new components and for optimizing the adsorption conditions. However, as a prerequisite, the multilayer being deposited should be rather rigid in order to evaluate the frequency displacements as adsorbed mass using the Sauerbrey equation. The linear increase of QCM frequency shifts with the increase in numbers of assembled layer has been observed in the LbL assembly of protein/inorganic nanoparticles [60], protein/polyelectrolyte [61] and inorganic nanoparticles/polyelectrolyte pairs [62].

**Microscopic methods**
The morphology of the assembled multilayer films is an essential issue in some cases, as the homogeneity and the surface coverage of assembled substances have important effects on the catalytic or optical properties of the resulting films.

*Scanning electron microscope (SEM)* is commonly used to view the morphology of the film both from the top and of the cross section. The latter one can only be obtained using SEM method. As the optimal magnification of SEM is normally below 50,000, it is more applicable to use SEM to investigate thicker multilayer films, which consist of metal oxide or semiconductor nanoparticles [63].

*Atomic force microscope (AFM)* can gives images with atomic resolution on both conductors and nonconductors [64]. It is easy to get three-dimensional profiles of surface observed with AFM.

*Other methods*

Other useful methods for characterization of the multilayer film include *X-ray photoelectron spectroscopy (XPS), inductively coupled plasma - mass spectrometry (ICP-MS), small angle X-ray measurement, surface plasmon resonance (SPR)* and *conductivity measurement*, depending on the specific properties the assembling species have.

### 1.1.4 Building blocks for LbL assembly

As mentioned previously, assembled layers in LbL films are simply constructed on the basis of the electrostatic interaction between oppositely charged species. This simplicity leads to the availability of large number of building blocks for
construction of LbL films. A large range of materials, from polymers and macromolecules to small ions, can be assembled with carefully selected conditions. In addition to the traditionally used polymer building blocks, several classes of building blocks such as viologens, porphyrins, heteropoly oxometalates, halide-metallic complexes and Werner-type metal-ammonia complexes are recently introduced in the fabrication of LbL films.

*Polyoxometalates (POMs)*

Polyoxometalates, which include the compounds historically known as "heteropoly acids", have been investigated for well over a century. POMs structures resemble discrete fragments of metal oxide structures with definite sizes and shapes [65]. Typically, a heteropoly complex contains a high atomic proportion of one kind of atom in positive oxidation state (addenda atoms) and much smaller proportion(s) of the other kind(s) of atom(s) in positive oxidation state(s) (heteroatoms) [66]. Due to the intrinsic nature of POMs, virtually, almost all molecular properties of this class of compound could be extensively modified, including molecular composition, size, shape, charge density, redox potentials, acidity and solubility [67]. The flexibility and diversified properties of POMs have drawn an increasing attention to the worldwide applications of POMs in heterogeneous and homogeneous catalysis [68-70], medicine [71, 72], molecular conductivity [73], molecular materials [74], magnetism [75] and photo- and electrochromism [76].

Polyoxometalates have been found to be extremely versatile inorganic building blocks for the construction of molecule-based materials. Organic/Inorganic Films can be obtained by Langmuir-Blodgett technique. Fig. 1.4 shows a well-organized layers
of polyoxoanion fabricated by Langmuir-Blodgett technique, using the adsorption properties of Keggin heteropolyanions along a positively charged monolayer of dimethyldioctadecylammonium (DODA) cation. Unique magnetic property was observed in the resultant composite film.

Fig. 1.4 Idealized structure of Langmuir-Blodgett films of Keggin anions and DODA [77].

The polyoxometalates can be used as electrocatalysts to minimize the activation energy, thus allowing the electrode reaction to occur at high current density close to the equilibrium potential or considerably below it. They can be attached onto electrode via interaction between the polyoxometalates and electrode surface. Three methods have been commonly used to immobilize polyoxometalates onto electrode surface. The first method is the adsorption of polyoxometalates on electrode surface
by dip coating [78]. The second method is to entrap polyoxometalates into polymers on electrode surface [78]. The third method is the electrodeposition of polyoxometalates onto electrode surface in a polyoxometalate solution under a constant potential of -1.2 V [79]. The POMs coated electrodes can actively mediate and catalyze the reduction of bromate [80], chromate, H₂O₂ [78], nitrite, as well as the oxidation of alkenes [81] and alcohols [82, 83].

Recently, with the development of LbL technology, much attention has been paid to self-assembly on electrode surfaces. Faulkner’s group [84] reported the first successful fabrication of LbL films of α-SiMo₁₂O₄₀⁴⁻ and α-PMo₁₂O₄₀³⁻, based on the electrostatic attraction between POMs anions and the positively charged component species. The LbL technique is a breakthrough for the immobilization of POMs and the combination of catalytic activity with other building blocks. For example, POMs have been assembled with various materials including metalloporphyrin [85], polymer [86-87], dendrimer [88-89], metal complexes [90]. Based on the diversified properties of POMs, LbL film of POMs were applied for various purposes, such as sensors for different analyte [86, 90-93] and electrochromism [93-94].

**Metal halides and metal ammonia complexes of precious metals**

Among the large selections of LbL building blocks, many are complicated macromolecules or even nanoparticles. To the best of our knowledge, the number of reported works on assembly of charged simple molecules is limited [95-97]. Recently, our group reported the successful preparation of LbL film with some simple metal complexes (AuCl₄⁻, PtCl₄²⁻, PtCl₆²⁻) and surfactant/polymer [97]. With
simple photochemical, sonochemical or electrochemical conversion of the metal complexes, gold or platinum nanoparticles were evenly distributed in the film.

*Porphyrians and phthalocyanines*

Porphyrians, phthalocyanines, and related compounds have been extensively studied due to their potential utility in optical [98], photoelectrochemical [99-102], and sensor [103-108] applications. Porphyrians and metalloporphyrins show catalytic activities in variety of reactions [109-111], and they display strong photoelectrochemical activity as well [112-114]. In addition, they tend to adsorb strongly on many surfaces, retaining many of their solution properties. However, the preparation of suitably tailored solid state assembly with the desired physical, chemical, and photophysical properties for the envisioned device applications has proven to be a difficult task.

A number of techniques have been developed to assemble thin homogeneous porphyrin films [115], but precise control of composition and thickness is challenging. Thermoevaporation [116] is the most widely used technique to obtain homogeneous films. Indeed, very well defined layered materials can be obtained by molecular beam epitaxy and sublimation in high vacuum. However, this technique is limited to volatile and thermally durable materials. The Langmuir-Blodgett (LB) method is another useful technique but is generally restricted to amphiphiles that are able to form stable monolayers on a liquid surface [117]. Beside this, films of porphyrians have also been prepared by dip-coating, spin-coating, and electropolymerization methods [119-121].
Making use of the advantages of availability and versatility of the LbL technique, porphyrin and its derivatives have been successfully assembled on various substrates [122-139]. The first successful work on layer-by-layer assembly of porphyrin was reported by Wrighton [140], in which film of tetraruthenated zinc porphyrin, $[\text{ZnTPyPBpy}]^{4+}$, and meso-tetraphenylporphyrin sulfonate, $[\text{M-TPPS}]^{4-}$ was constructed. Electrocatalytic activity of the component species was remained and photocatalytical activity toward the reduction of $O_2$ could be observed. These findings provide the background knowledge to the fabrication of compositionally modulated porphyrin films of arbitrary thickness with rationally tailored catalytic and photophysical properties.

1.2 Electrochromism

Electrochromism involves electroactive species (i.e., electron donor or acceptor, in the redox sense) and is the coloration of an electroactive surface film or of an electroactive solute, resulting from an electron-transfer reaction at an electrode [141, 142]. Examples of their applications in both prototype and commercial electrochromic devices included car mirrors, windows and sun-roofs of cars, windows of buildings, displays, printing, and frozen-food monitoring. Because of their fascinating spectroelectrochemical properties and their commercial applications, electrochromic materials are currently attracting much interest in academia and industry. Various electrochromic materials, including metal oxides, phthalocyanine compounds, prussian blue, viologens, electroactive conducting polymers, and even fullerene [143-145], have been investigated. The progress and remaining problems
have been summarized in some reviews papers [146-151] and monographs [141, 142].

Viologens are one class of electrochromes which have been extensively studied. One-electron reduction of viologens produces intense coloration of either solid or solution-state species, whereas a two-electron uptake yields a less intensely colored product. The specific properties like color and solubility of viologens can be adjusted chemically by suitable choice of attached substituents. Campus, F. reported that an interesting cell comprising a variety of viologens attached to ITO, with a prussian blue complementary electrode, exhibited huge absorbance [152].

It was found that the α-Keggin tungstate $H_3[\text{PW}_{12}\text{O}_{40}]$ was reduced photochemically to yield a blue-colored species which was reoxidized by air and by various other oxidizing agents such as $\text{Fe}^{3+}$, $\text{AgNO}_3$, and $\text{H}_2\text{O}_2$ [153, 154]. The reduced polyoxometalates, which are the so-called "heteropoly blues", have been used for the colorimetric analysis of the elements P, Si, As, and Ge and for the determination of uric acid, sugar, and other biological compounds [155, 156]. Tell and co-workers found that $H_3\text{PMo}_{12}\text{O}_{40}\cdot29\text{H}_2\text{O}$ electrochromic cell showed a useful response time of 50 ms [157, 158]. However, this electrochromic cell was suffered from a slow bleaching when it was intensely colored. Besides, the cell's life was also limited by an increase in cell impedance over time [159]. Moriguchi and Fendler used electrostatic assembly techniques to fabricate electrochromic films containing $[\text{W}_{10}\text{O}_{28}]^{4+}$ polyanion and poly(diallyldimethylammonium) polycation. This film showed a slow response time and a low optical contrast [160]. Recently, Kurth and co-workers reported the preparation of multilayer films containing the polyoxometalate cluster of $[\text{Eu}-(\text{H}_2\text{O})_3\text{PW}_{30}\text{O}_{110}]^{12-}$ using a layer-by-layer self-
assembly method. It was believed that the film had potential in the application of
polyoxometalates as electrochromic materials because it displayed strong
electrochromic property [161].

1.3 Electrochemical detection of reactive-nitrogen-species (RNS)
and reactive-oxygen-species (ROS)

The term RNS refers to redox derivatives of NO, including the parent radical
nitric oxide (NO) and an array of redox-active derivatives such as nitrogen dioxide
radical (NO$_2^-$) and peroxynitrite ion (ONOO'). The ROS cascade includes the parent
molecule superoxide (O$_2^-$), hydroxyl radicals (OH), hydroperoxyl radicals (HOO)
and oxygen derivatives that are not free radicals, i.e., hydrogen peroxide (H$_2$O$_2$).

Endogenous ROS and RNS are difficult to measure in biological systems because
they are produced in small quantities and are relatively unstable. In vivo, ROS and
RNS are rapidly degraded by interaction with endogenous antioxidants (e.g.,
superoxide dismutase, catalase, glutathione) and cellular components that include
thiol residues, molecular oxygen, metalloproteins, and other ROS or RNS. Some of
the interactions related to ROS and RNS are listed in Table 1.2 [162, 163].

Due to the fact that ROS and RNS species are inter-coupled, bifunctional or even
multi-functional sensing methods with sufficient sensitivity and selectivity for the
detection of ROS and RNS species are desirable.
Table 1.2 Some relevant ROS/RNS reactions and interactions [163].

<table>
<thead>
<tr>
<th>Reaction</th>
</tr>
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<tbody>
<tr>
<td>Spontaneous</td>
</tr>
<tr>
<td>$\text{O}_2^+ + \text{H}^+ \rightarrow \text{HOO}^-$</td>
</tr>
<tr>
<td>$\text{O}_2^+ + \text{O}_2^+ + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$</td>
</tr>
<tr>
<td>$\text{O}_2^+ + \text{Fe}^{3+} \rightarrow \text{Fe}^{2+} + \text{O}_2 + \text{H}_2\text{O}_2$</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{OH} + \text{OH}^-$</td>
</tr>
<tr>
<td>$\text{O}_2^- + \text{NO} \rightarrow \text{ONOO}^-$</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}_2 + \text{NO} \rightarrow \text{HO} + \text{HNO}_2$</td>
</tr>
<tr>
<td>$\text{ONOO}^- + \text{H}^+ \rightarrow \text{ONOOH}$</td>
</tr>
<tr>
<td>$\text{ONOOH} \rightarrow \text{OH} + \text{NO}_2$</td>
</tr>
<tr>
<td>$2\text{NO} + \text{O}_2 + \text{H}_2\text{O} \rightarrow 2\text{NO}_2$</td>
</tr>
<tr>
<td>Enzymatic</td>
</tr>
<tr>
<td>$2\text{O}_2^- + 2\text{H}^+ \overset{\text{SOD}}{\rightarrow} \text{H}_2\text{O}_2 + \text{O}_2$</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}_2 \overset{\text{CAT}}{\rightarrow} 2\text{H}_2\text{O} + \text{O}_2$</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}_2 + 2\text{GSH} \overset{\text{GPX}}{\rightarrow} \text{GSSG} + 2\text{H}_2\text{O}$</td>
</tr>
</tbody>
</table>

* $\text{O}_2^-$, superoxide; $\text{H}_2\text{O}_2$, hydrogen peroxide; OH, hydroxyl radical; HOO$^-$, hydroperoxyl radical; NO, nitric oxide; ONOO$^-$, peroxynitrite; NO$_2$, nitrogen dioxide; ONOOH, peroxynitrous acid; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; GPX, glutathione peroxidase; GSSG, oxidized glutathione.

Detection of nitric oxide (NO)

Due to the diversity of its physiological functions and general ubiquity, NO has become a species of extreme biological interest and has been a subject of significant interest to inorganic, organometallic, and environmental chemists for many years preceding its discovery as an endogenously generated species in mammalian systems. NO is one of the smallest biologically active molecular messengers. It is also one of the gaseous biological messengers which has a wide range of physiological and pathophysiological actions. There has been an ever-increasing number of reports demonstrating many important functions of NO in the control of the cardiovascular, central and peripheral nervous, and immune systems [164-170]. In 1992, NO was picked as the “molecule of the year” by Science [171].
NO can freely diffuse in and out of cells and tissues. At room temperature and pressure, it is a colorless gas (bp: -151.8°C; mp: -163.6°C). Its liquid and solid states are also colorless when the material is pure. Its solubility in water at 25 °C and 1 atmosphere pressure is $1.8 \times 10^{-3}$ mol dm$^{-3}$, which is unchanged within the pH range 2-13. It is one of the simplest odd-electron species in which the presence of an unpaired electron reduces the bond order to 2.5 (whereas it is around 3 in NO$^+$) [172-173]. Due to these chemical properties, NO is reluctant to be dimerized, has paramagnetic properties, and can react with molecular oxygen [174-177], superoxide [178-182], oxyhemoglobin and oxymyoglobin [184, 185], heme proteins and metals [186-192], and amines [193-196].

The physicochemical properties of NO including high diffusibility and reactivity result in a short biological half-life and make detection and quantification of NO difficult under physiological conditions [197,198]. Many techniques have been developed for the challenging task of the measurement of NO in single cells, tissues, and biological fluids, such as spectroscopic, spectrometric and electrochemical methods. The commonly used instrumental techniques for NO detection are spectroscopic and electrochemical methods. Mass spectrometry and gas chromatography have been used occasionally for NO evaluation but they are much less sensitive [172]. Spectroscopic methods include UV-visible spectroscopy, electron spin resonance (EPS), and chemiluminescence spectrometry. All spectroscopic methods detect NO indirectly and generate a product following a reaction with NO or one of its oxidation products [198]. Among all the methods mentioned above, the electrochemical method is the only protocol available that can be used to monitor concentrations of NO directly and continuously. Moreover, the
low nanomolar sensitivity, the fast response time, the availability of miniature electrodes, the minimal maintenance requirement and the low cost of electrochemical NO sensors have led to their utilization for NO detection in many research fields [199]. Therefore, electrochemical techniques are more suited for in situ, in vitro, and in vivo monitoring of kinetics of NO release.

NO has a complex redox chemistry, which is involved with numerous equilibrium, many of which are pH dependent [200]. NO can be readily oxidized by one electron to give nitrosonium ion (NO\(^+\)) or three electrons to give NO\(_3^-\), or reduced by one electron to give nitroxyl anion (NO\(^\cdot\)) [201]. These chemical properties make it possible to measure NO by electrochemical approaches, which include voltammetry, amperometry and coulometry. In the amperometric mode, current is measured while the potential is kept constant. In the voltammetric mode, the current is measured with the potential is scanned through the region that includes NO oxidation or reduction. Although the amperometric method is faster than the voltammetric method, voltammetry provides not only quantitative but also qualitative information that can prove that the current measured is in fact due to the oxidation or reduction of nitric oxide.

Several types of electrochemical sensors were reported for the direct measurement of NO in vitro and/or in biological media, including intact tissues and single cells. These methods is based either on the direct or catalytic oxidation of NO, or on the direct or catalytic reduction of NO. The electrochemical assay was firstly reported by Shibuki, which is based on direct oxidation of NO on a platinum electrode [202]. Meanwhile, Ichimori et al. reported the development of a NO-selective electrode made from Pt-Ir alloy coated with a three-layered membrane.
A similar assay was reported by Parniente et al., which is based on the direct oxidation of NO on a polycrystalline platinum electrode modified with a thin film of Nafion® and cellulose acetate [204]. However, the poor sensitivity makes it useless for the application in real biological system. Malinski and Taha reported in 1992 [205], a very interesting modification of carbon fiber electrode with electropolymerized nickel(II) tetrakis(3-methoxy-4hydroxyphenyl) porphyrin (Ni – TMPP) and Nafion®. The microsensor could be operated in either the amperometric or voltammetric mode, and was characterized by a linear response up to 300 μM of NO and a claimed detection limit of 10 nM. It was successfully employed to measure in situ NO released from a single cell with a response time of less than 10 ms. Bedioui’s group described electrochemical NO sensors based on nickel porphyrin and phthalocyanine [206, 207]. Jin’ group [208] described an electropolymerized film of metal ethylenebis (salicylideneiminate) [(M(salen), M = Co, Fe, Cu and Mn], which was utilized as material for development of an electrochemical sensor for the determination of NO in solution. It was proved that the use of chemically modified electrodes provides an elegant way to build up multi-layer structures that result in NO sensors with high performance characteristics.

Detection of hydrogen peroxide (H₂O₂)

Hydrogen peroxide in the atmosphere and in environmental water has become of interest as the terminal product of hydroperoxy radicals in photochemical reactions. Hydrogen peroxide functions as an oxidant in conversions of sulfur oxides to sulfuric acid, and also nitrogen oxides to nitric acid, in the atmosphere and rainwater, and
such conversions sometimes lead to undesirable effects for biological systems [209-213].

Because of such strongly oxidative functions, hydrogen peroxide is often used as a preservative or a bleaching agent in food manufacturing. However, since it was pointed out in 1980 that hydrogen peroxide appeared to exhibit carcinogenic activity, the amount of hydrogen peroxide allowed to remain in final food products has been officially regulated.

The determination of hydrogen peroxide is regarded as significant in clinical assays, because a variety of significant physiological components, such as glucose, uric acid, or cholesterol, are quantitatively oxidized with the corresponding oxidase enzymes, and the resulting hydrogen peroxide should be determined in high sensitivity.

In the past two decades, some analytical methods have been reported for such measurements, including spectrophotometry [214], fluorimetry [215], chemiluminescence [216], amperometry [217, 218] and voltammetry [219]. Electrochemical determinations of hydrogen peroxide are generally performed by oxidation at a platinum electrode [220]. Depending on the pH of the solution, a quite high positive potential must be applied for the oxidation of hydrogen peroxide and typical values are in the range of 0.7 to 0.9 V versus SCE [221]. As a consequence, many substances can interfere in the measurements. The use of biosensors with immobilized enzymes, such as peroxidase, is another approach commonly used. The detection is performed by spectrophotometry [222], fluorimetry [223], chemiluminescence [224] and electrochemical [225] techniques.
In physiological system the detection of hydrogen peroxide is challenging because of the complicated interactions between hydrogen peroxide and other coexisting species such as antioxidants and other RNS and ROS.

It should be noted that in the oxidative detection of H$_2$O$_2$ by electrochemical method, ascorbic acid (*vide infra*) often presents a serious interference.

**Detection of ascorbic acid (AA)**

Ascorbic acid known as vitamin C is the nutritional requirement of scurvy-prone animals incapable of synthesizing this compound [226, 227]. It is well known that AA exists extensively in fruit and joins in many biological reactions [228, 229]. Chemists have considerable interest in determining ascorbic acid in biological organization and food etc. Various methods and techniques, such as spectrometry [230], titrimetry [231], HPLC [232] and electrochemical methods [228, 229, 233-235], have been employed in its measurement.

**1.4 The objectives of this thesis**

The main aim of this thesis is to design and fabricate LbL films with building blocks of various desired properties, and to explore the potential applications of these films in electrochemical sensing and electrochromic coating.

A current development in the general area of material science is to design, from a wise choice of the constituent molecules, new materials that combine properties not normally associated with a single material. The technique of layer-by-layer assembly opens the door for the preparation of nanostructure films with high variability in
components, control of film thickness and spatial arrangement of layers. Despite its exponential expansion in the last ten years, the exploration of potentiality of LbL technique is much far from extensive. Therefore, the first objective of this thesis is to design and develop a series of nanostructure films with various component species, including heteropoly oxometalates, metalloporphyrins, viologens, polymers and metal halides complexes.

These films as well as their conversion by electrochemical and photochemical methods are examined for two application purposes: electrochemical sensing and electrochromic coating.

Due to the unique optical, electronic and catalytic properties of metal nanoparticles, fabrication of nanoparticles is of much interest. For the purpose of analytical application especially in electroanalysis, it is desirable to fabricate metal nanoparticles on electrode surface. Some synthetic methods for fabrication of nanoparticle on polymer matrix have been developed but all of them suffer from the complexity of the fabrication. A new approach, involving electrochemical reduction of LbL films assembled from PdCl$_4^{2-}$, PtCl$_4^{2-}$, and AuCl$_4^-$ anions and metalloporphyrin cations, have been designed and tested. Moreover, by LbL assembly with complexes of two metals with cationic species, this novel approach is used in fabrication of bimetallic hybrid nanoparticles of noble metals. The atomic ratio of two metals in bimetallic nanoparticles is conveniently adjusted by altering the relative number of the two noble metal complex layers.

Electrochemical sensors are ideal for in vivo monitoring of biomolecules since the sensor is simple, inexpensive and miniaturizable. As mentioned in the preceding section, levels of many redox-active biomolecules such as NO are coupled to other
redox-active species such as H₂O₂ and oxygen in physiological system. Therefore, it is essential to measure inter-coupled biomolecules with a single sensing head. LbL technique provides a convenient way to combine materials with different functions into one film, thus bi-functional or even multi-functional electrocatalytic films can be fabricated. Metalloporphyrins, which are electrocatalytically active towards many redox reactions involving ROS and RNS, can be fabricated with cationic species such as polyoxometalate (electro-mediators) and noble metal complexes (excellent electrocatalysts). The multifold sensing functions of the composite films are evaluated in the detection of NO, ascorbic acid, H₂O₂ and oxygen.

For electrochromism, it is difficult to adjust color change in a wide range of a single electrochromic species. Moreover, the fabrication of the electrochromic film usually requires costly process, which makes the exploration of ideal electrochromic materials more difficult. Therefore, using the LbL technique, the color of the film can be enriched. Composite films containing two electrochromic species, polyoxometalates and viologens can be easily fabricated and their electrochromic performance such as coloration and response time are evaluated.

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CHAPTER 2

Layer-by-Layer Self-Assembled Multilayer Molecular Film
from Viologen and Heteropoly Oxometalate: Preparation,
Characterization and Application in Electrochemical Sensing
2.1 Layer-by-layer Self-Assembled Multilayer Molecular Film from Methyl Viologen and Phosphomolybdic Acid: Preparation, Characterization and Application in Multi-Purpose Electrochemical Sensing

Abstract

Layer-by-layer self-assembled multilayer molecular films with methyl viologen cation and phosphomolybdic acid anion on various substrates (e.g. glassy carbon, ITO) were prepared by alternating dipping the substrates in anionic and cationic species. The assembly process was monitored by electrochemical method, UV-Vis and FT-IR spectroscopies. Together with results of electrochemical and UV-Vis measurements molecular level information obtained by FT-IR spectroscopy show that, equal amount of building blocks was assembled in each fabrication process. A successful application of such newly developed film as electrochemical sensing of H₂O₂ and ascorbic acid without interference to each other was illustrated. A straight calibration curve was obtained in concentration range of 8.0×10⁻⁶ mol L⁻¹ to 1.6×10⁻⁴ mol L⁻¹ for H₂O₂ and 1.0×10⁻⁷ mol L⁻¹ to 1.6×10⁻⁶ mol L⁻¹ for ascorbic acid, with detection limit of 1.0×10⁻⁶ mol L⁻¹ and 1.0×10⁻⁸ mol L⁻¹, respectively.
2.1.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the microstructure and spatial arrangement can be easily modified according to the aim of the study [10-12].

The detection of trace amounts of H$_2$O$_2$ and ascorbic acid is of practical importance in clinical and biological studies [13-16]. Analysis of biological systems, such as blood and brain fluid, is probably its main area of application, but a problem with such analyses is that both H$_2$O$_2$ and ascorbic acid are electroactive species, which may reduce or oxidize directly on the electrode surface and mix up the current. So it is essential to eliminate the interference between each other when we detect H$_2$O$_2$ and ascorbic acid respectively. To our knowledge, no paper has reported the directly simultaneous detection of H$_2$O$_2$ and ascorbic acid on one electrode, which is very important for in situ in vivo detection.
In this study, we describe a new self-assembled molecular film with negatively charged phosphomolybdic acid (PMo, a kind of heteropoly oxometalate) and positively charged methyl viologen (MV, a kind of viologen). We studied in detail the applications of such film in multi-purpose electrochemical sensing of both \( \text{H}_2\text{O}_2 \) and ascorbic acid.

2.1.2 Experimental

2.1.2.1 Reagents and materials.

Phosphomolybdic acid and methyl viologen were obtained from Aldrich and used as received. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolytes. 99.5% ethanol was obtained from Panreac (Spain). Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.) and KRS-5 polished disc windows (13mm x 1mm) from Wilmad (U.S.A.).

2.1.2.2 Instrumentation

(i) Electrochemical measurements

The cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA)
controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, Φ3mm, MF-2012, BAS, U.S.A.) as the working electrode. Prior to each experiment, GC electrode was polished by using alumina powder of size 1.0, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.

(ii) **UV-visible spectroscopy**

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the transparent ITO substrate. Bare ITO was used as the reference.

(iii) **Fourier transfer infrared spectroscopy (FTIR)**

The FTIR spectroscopic measurements were carried out on a Perkin Elmer Spectrum One spectrometer. The sample film was on KRS-5 disc.

2.1.2.3 Assembling of [PMo|MV]$_n$ film.
The self-assembled films on different substrates were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 5 mM PMO_{12}O_{40}^{3-} (dissolved in pH=3 PBS or 99.5% ethanol) for 5 minutes, then a layer of PMO_{12}O_{40}^{3-} spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of PMO_{12}O_{40}^{3-} were transferred into 5 mM methyl viologen (dissolved in pH=6 PBS) for 5 minutes, then a layer of methyl viologen spontaneously adsorbed onto the PMO_{12}O_{40}^{3-}-coated substrates. Finally, the substrates were washed with water. Thus the self-assembled film named [PMo|MV]_n film was prepared successfully. The films with different thickness can be achieved by repeating the above assembly process. The whole process is demonstrated in Fig. 2.1.1.

2.1.3 Results and discussion

2.1.3.1 Electrochemical Characterization

Fabrication of self-assembled film of phosphomolybdic acid and methyl viologen ([PMo|MV]_n film) was monitored by electrochemical method. Phosphomolybdic acid anions which have Keggin structure are unstable in neutral and basic aqueous solution and undergo a series of hydrolysis processes [17,18]. Therefore, electrochemical studies of [PMo|MV]_n film with phosphomolybdic acid anion were carried out in acidic aqueous solutions. Each cyclic voltammogram was recorded in a pH = 3 phosphate buffer solution after assembly of a layer of anion species and a
layer of cation on electrode (glassy carbon, GC). A serial of such recorded cyclic voltammograms is shown in Fig. 2.1.2. As can be seen, in the range from -0.25 to 0.50 V, three pairs of redox peaks are located at about 0.24 (I-I'), 0.14 (II-II') and -0.02 V (III-III'). These peaks of assembled layers are similar to that of 1 mM phosphomolybdic acid solution (inset A of Fig. 2.1.2) except that the peaks in former curves are sharper and also slightly shift. This character is also similar to which reported in other paper [18,19]. A cyclic voltammetric study of GC shows that no peak can be observed in the same potential range (curve a in Fig. 2.1.2). Based on the above results and reported study of electrochemical behavior of phosphomolybdic acid, electrochemical behavior shown in Fig. 2.1.2 was ascribed to redox of phosphomolybdic acid and the three pairs of peaks correspond to reduction and oxidation through two-, four-, and six-electron processes, respectively [20]. Another characteristic can be noted is that peak current of all the three pairs of peaks increased with increase of number of bilayers. Peak current ($I_p$) of the redox pair located at about 0.15 V was plotted versus number of bilayer and shown in inset B, it is obvious that $I_p$ increases proportionally with increase of number of bilayers. This indicates that the successive fabrication of the layer-by-layer self-assembled films on electrode and the amount of the anionic species assembled on electrode surface in each bilayer is almost the same.

Methyl viologen is also a kind of electroactive species and its redox peaks appear when the potential is lower than -0.40 V [21]. But a lower potential can cause irreversible reactions of phosphomolybdic acid and thus destroyed the self-assembled film. So, electrochemical character of methyl viologen can not be observed in such
potential region between -0.25 and 0.50 V in Fig. 2.1.2. In other words, assembly of methyl viologen can not be directly probed by electrochemical method.

2.1.3.2 Characterization of [PMo|MV]ₙ film by UV-Vis spectroscopy

Methyl viologen was found to absorb UV radiation at 254 nm, which provides possibility to monitor assembly of methyl viologen in [PMo|MV]ₙ film by UV-visible spectroscopy. For this purpose, [PMo|MV]ₙ films were fabricated on ITO-coated quartz substrate under the same conditions and their UV-visible spectra were collected and shown in Fig. 2.1.3. It can be seen in the figure that the intensity of the absorption peaks at 254 nm increased with number of bilayers. Furthermore, the inset depicts a linear correlation between the absorption intensity and the number of bilayers, which indicating that, similar to the anionic species, methyl viologen is assembled layer-by-layer in [PMo|MV]ₙ film and the amount of methyl viologen assembled after phosphomolybdic anion in each bilayer is almost the same.

2.1.3.3 Characterization of [PMo|MV]ₙ film by FT-IR spectroscopy

FT-IR is capable of providing quantitative and structural information of molecular level and is regarded as a powerful tool for both qualitative and quantitative study. Therefore, FT-IR spectroscopic studies were conducted to monitor growth of [PMo|MV]ₙ film and investigated the state of assembled species.

[PMo|MV]ₙ film was fabricated on a KRS-5 disc which is capable of transmitting IR radiation. During the course of alternatively assembling of phosphomolybdic acid
and methyl viologen, spectrum was collected after either an anionic or cationic layer was assembled onto the substrate. Fig. 2.1.4 shows a serial of IR spectra recorded after assembly of phosphomolybdic acid. Compared with IR spectra of pure phosphomolybdic acid and dication form of methyl viologen in aqueous, each of the spectra can be divided into two parts (400 – 1100 cm\(^{-1}\) and 1100 – 1800 cm\(^{-1}\)). The peaks at 400 – 1100 cm\(^{-1}\) can also be observed in the spectrum of pure phosphomolybdic acid and the locations of the peaks are almost the same. This comparison indicates that after assembly process, molecular structure of phosphomolybdic acid is retained. For the part of spectrum of 1100 – 1800 cm\(^{-1}\), it is much similar with the spectrum of pure dication form of methyl viologen in aqueous both in peak location and relative intensity of the peaks. Since IR spectra of both radical cation and di-reduced forms are substantially different with that of dication form, it is evident that viologen assembled on the substrate remains a dication form and the positive charges are retained. Based on the above results, no substantial change of structure of both anionic and cationic species can be observed, indicating that no substantial reaction occurred in the process of assembly. This can be evidence to show that the layers are assembled by electrostatic attraction.

Fig. 2.1.4 shows IR spectra of [PMo\(\text{MV}\)]\(_n\) film collected after phosphomolybdic acid was assembled on KRS-5 disc and Fig. 2.1.5 shows IR spectra of [PMo\(\text{MV}\)]\(_n\) film collected after methyl viologen was assembled on the disc. Peaks in spectra corresponding to phosphomolybdic acid and methyl viologen can easily identified. In the first spectrum (a) of the film in Fig. 2.1.4, some peaks appear in 400-1100 cm\(^{-1}\). As we know, the only compound exists in the first layer of the film is phosphomolybdic acid, so the peaks can be assign to IR absorption of
phosphomolybdic acid. From the second spectrum (b) to the last spectrum (f) in Fig. 2.1.3, we noticed two main points: one is that some new peaks appear at the location between 1100 and 1800 cm\(^{-1}\); the other is that the intensity of all the peaks increases with number of bilayers. Based on these evidences, we can say that the peaks between 1100 and 1800 cm\(^{-1}\) belong to methyl viologen and layer-by-layer molecular film was fabricated successfully.

Fig. 2.1.5 shows a serial of spectra collected after assembly of methyl viologen. In the spectrum collected after first layer of methyl viologen was assembled (Fig. 2.1.5a), some peaks, which are absent in Fig. 2.1.4a, although little but can be clearly observed in 1100 – 1800 cm\(^{-1}\). When observed in a quantitative point of view, the intensity of the peaks in this figure also increases with number of bilayers. In other words, the characters of Fig. 2.1.5 are consistent with Fig 2.1.4.

After comparing the spectra in Fig. 2.1.4 and Fig. 2.1.5 carefully, the growth of the peaks in 400-1100 cm\(^{-1}\), which belong to phosphomolybdic acid, can be only observed after each layer phosphomolybdic acid is assembled on the substrate. And the growth of the peaks in 1100-1800 cm\(^{-1}\), which belong to methyl viologen, can be only observed after each layer methyl viologen is assembled on the substrate. The peaks located at 1063 cm\(^{-1}\), belonging to phosphomolybdic acid, and the peaks at 1642 cm\(^{-1}\), belonging to methyl viologen, were integrated respectively in Fig. 2.1.4 and Fig. 2.1.5. The result is shown in Fig. 2.1.6. The area of the peaks is proportional to number of bilayers. This result is consistent with results of electrochemical and UV-Vis spectroscopic studies, indicating that both phosphomolybdic acid and methyl viologen are assembled on substrate layer-by-layer. It also testifies that a constant
amount of either anionic and cationic species was assembled in the bilayer of \([\text{PMo}][\text{MV}]_n\) film.

Combining electrochemical, UV-visible and IR characteristics of \([\text{PMo}][\text{MV}]_n\) film prepared in this study, some interesting features can be found. First, for a specific substrate, all the results prove that the amount of either anionic or cationic species assembled in the according layer is constant. Our investigation also found that amount of building block was limited by a certain value even extra dipping time was applied. This is reasonable for a layer-by-layer structure constructed by electrostatic attraction, i.e., equal amount of oppositely charged sites are generated with consumption of charge sites by electronic neutralization accompanied self-assembly. Second, molecular level information provided by FT-IR study indicates that both of the building block species remain their structure after the assembly process. For methyl viologen it is obvious that it exists in the film in a form of dication. Generally, a layer-by-layer film constructed by electrostatic attraction was successfully fabricated with our strategy.

2.1.3.4 Application in electrochemical sensing

2.1.3.4.1 The catalytic reduction of \(\text{H}_2\text{O}_2\)

Fig. 2.1.7A (b) shows cyclic voltammogram of 5 mM \(\text{H}_2\text{O}_2\) recorded on GC electrode modified with 4-bilayer \([\text{PMo}][\text{MV}]_n\) film in pH = 3 phosphous buffer solution. Comparing with cyclic voltammogram of 4-bilayer \([\text{PMo}][\text{MV}]_n\) film in pH=3 phosphate buffer solution (Fig. 2.1.7A (a)), the modified electrode gives
obvious current in 5 mM H$_2$O$_2$ when the potential is lower than 0.10 V, which is from electrochemical reduction of H$_2$O$_2$. Under the same condition, no discernible reduction peak can be observed on bare GC, which indicates that [PMo|MV]$_n$ film is catalytically active towards electrochemical reduction of H$_2$O$_2$. Since H$_2$O$_2$ gave evident reduction current on the [PMo|MV]$_n$ modified electrode, this electrode possesses the potential to be developed to a sensitive sensor for H$_2$O$_2$.

Since [PMo|MV]$_n$ film acts as an electrocatalyst in the reduction of H$_2$O$_2$, we showed great interest in whether anion or cation is more important in the reduction of H$_2$O$_2$. Fig. 2.1.9A (b) and (c) show cyclic voltammograms of 2-bilayer [PMo|MV]$_n$ film in 5 mM H$_2$O$_2$ with the outmost layer phosphomolybdic acid and methyl viologen respectively. It is obviously noted that the reduction current of H$_2$O$_2$ in Fig. 2.1.9A(c) is larger than that in Fig. 2.1.9A(b). This fact indicates that methyl viologen is more effective than phosphomolybdic acid in the reduction of H$_2$O$_2$. Some cyclic voltammograms under the same condition as Fig. 2.1.9A were recorded on 4-bilayer [PMo|MV]$_n$ film (Fig. 2.1.9B). In Fig. 2.1.9B, there is almost no difference between curve (b) and (c). Comparing with these figures, we notice that the difference of the effect between different outmost layers is smaller and smaller with the increase of number of bilayers.

2.1.3.4.2 The catalytic oxidation of ascorbic acid

Cyclic voltammogram of 0.5 mM ascorbic acid on GC electrode modified with 4-bilayer [PMo|MV]$_n$ film is shown in Fig. 2.1.7B(b), a broad anodic peak at 0.38 V can be observed, which can be assigned to electrochemical oxidation of ascorbic acid.
Except peaks of redox reaction of \([\text{PMo}[\text{MV}]]_n\) film, no additional cathodic peak exists. It shows that the electrochemical behavior of ascorbic acid on the electrode is a totally irreversible process. Since the peak current of ascorbic acid oxidation on modified electrode is just a little higher than that on a bare GC electrode, such \([\text{PMo}[\text{MV}]]_n\) film is proposed to be inactive to oxidation reaction of ascorbic acid and just act as an electron transferrer. Based on these facts, \([\text{PMo}[\text{MV}]]_n\) film also possesses the potential to be developed to a sensitive sensor for ascorbic acid.

### 2.1.3.4.3 The bifunctional sensing of \(H_2O_2\) and ascorbic acid

In most cases of biological and medical applications of \(H_2O_2\) sensing, ascorbic acid coexists with \(H_2O_2\) and interferes with the detection of \(H_2O_2\), moreover, as an medically important molecule, precise detection of ascorbic acid can provide valuable information for medical judgment. Thus, a sensor with selective sensitivity toward both \(H_2O_2\) and ascorbic acid will be desirable.

In Fig. 2.1.7, it is noted worthily that in the higher potential range the electrochemical behavior of the modified electrode remained almost identical when \(H_2O_2\) was present or absent in the solution. In contrast, in the lower potential range, no substantial change upon addition of ascorbic acid can be observed. These features imply that \(H_2O_2\) is inactive at higher potential while ascorbic acid at lower potential, thus one \([\text{PMo}[\text{MV}]]_n\) film modified electrode can be used to detect \(H_2O_2\) at lower potential and ascorbic acid at higher potential. So it is rational that on \([\text{PMo}[\text{MV}]]_n\) film there is no interference between \(H_2O_2\) and ascorbic acid and we can use such film to detect \(H_2O_2\) and ascorbic acid respectively in their mixture.
Fig. 2.1.8 (b) shows cyclic voltammogram of 5 mM H$_2$O$_2$ and 0.5 mM ascorbic acid recorded on 4-bilayer [PMo|MV]$_n$ film in pH = 3 phosphate buffer solution. It should be noticed that in the lower potential range the curve is almost same as that recorded in 5 mM H$_2$O$_2$ (Fig. 2.1.7A(b)), while in the higher potential range the curve is almost same as that recorded in 0.5 mM ascorbic acid (Fig. 2.1.7B(b)). It tests that these two molecules can be distinguished successfully from each other on such [PMo|MV]$_n$ film.

Amperometric measurements of H$_2$O$_2$ solution with various concentrations were conducted at $-0.15$ V on 4-bilayer [PMo|MV]$_n$ film. I-t curve of H$_2$O$_2$ with concentration increasing from $8\times10^{-6}$ mol L$^{-1}$ to $1.6\times10^{-4}$ mol L$^{-1}$ was shown in Fig. 2.1.10, the corresponding calibration curve shown in the inset shows a direct proportional relation between the current and concentration of H$_2$O$_2$. Linear regression performed on the calibration curve gave the value of coefficient correlation ($R^2$) as 0.9996. The relative standard deviation (R.S.D) was less than 5%. The detection limit calculated for S/N of 3 was $1\times10^{-6}$ mol L$^{-1}$.

Amperometric response of the modified electrode to ascorbic acid with various concentration range from $1.0\times10^{-7}$ mol L$^{-1}$ to $1.6\times10^{-6}$ mol L$^{-1}$ was tested at 0.38 V and the result was shown in Fig. 2.1.11. Current of the electrode increased in a proportional mode when concentration of ascorbic acid increases and gave a straight calibration curve with a regression correlation ($R^2$) of 0.9998. The relative standard deviation (R.S.D) was less than 5%. The detection limit of the modified electrode to ascorbic acid is determined as $1\times10^{-8}$ mol L$^{-1}$. This study illustrated a successful application of the newly developed electrode in electrochemical sensing of ascorbic acid.
The results show that \([\text{PMo}[\text{MV}]_n]\) film exhibits excellent selectivity and high sensitivity to both \(\text{H}_2\text{O}_2\) and ascorbic acid. The sensitivity of this electrochemical sensing system to these two molecules is comparable to the most sensitive sensors in the published works [22-24].

2.1.4 Conclusions

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged phosphomolybdic acid and positively charged methyl viologen on various substrates including glassy carbon, ITO and KRS-5 disc. The thickness of such film can be controlled easily in the assembly process. We have systematically characterized our self-assembled film and have monitored the growth of the film by using electrochemical method, UV-Vis and FT-IR spectroscopies. The results showed that both phosphomolybdic acid and methyl viologen assembled layer-by-layer on substrate.

The \([\text{PMo}[\text{MV}]_n]\) film exhibits excellent selectivity and high sensitivity to two medically important molecules, \(\text{H}_2\text{O}_2\) and ascorbic acid. A straight calibration curve was obtained in concentration range of \(8.0\times10^{-6}\ \text{mol L}^{-1}\) to \(1.6\times10^{-4}\ \text{mol L}^{-1}\) for \(\text{H}_2\text{O}_2\) and \(1.0\times10^{-7}\ \text{mol L}^{-1}\) to \(1.6\times10^{-6}\ \text{mol L}^{-1}\) for ascorbic acid, with detection limit of \(1.0\times10^{-6}\ \text{mol L}^{-1}\) and \(1.0\times10^{-8}\ \text{mol L}^{-1}\) respectively.
2.1.5 References


Legends and Figures

Fig. 2.1.1 Scheme of the preparation of layer-by-layer self-assembled molecular film.

Fig. 2.1.2 Cyclic voltammograms of (a) glassy carbon, (b-m) [PMo\(\text{MV}_n\)] films (from one up to twelve bilayers) in pH=3 phosphate buffer solution. Scan rate: 100 mV s\(^{-1}\).
Inset A: cyclic voltammogram of glassy carbon in 1mM phosphomolybdic acid. Inset B: the relationship between peak current and No. of bilayers.

Fig. 2.1.3 UV-Vis spectra of [PMo\(\text{MV}_n\)] film on ITO-coated quartz substrate. (a) two bilayers, (b) four bilayers, (c) six bilayers, (d) eight bilayers, (e) ten bilayers. The inset depicts the relationship between absorbance and No. of bilayers.

Fig. 2.1.4 IR spectra of [PMo\(\text{MV}_n\)] film on KRS-5 disk. Each spectrum was collected after phosphomolybdic acid was assembled on KRS-5 disk. (a) 0.5, (b) 1.5, (c) 2.5, (d) 3.5, (e) 4.5 bilayers. R = 4 cm\(^{-1}\). The spectra between 1100-1800 cm\(^{-1}\) were amplified 4 times.

Fig. 2.1.5 IR spectra of [PMo\(\text{MV}_n\)] film on KRS-5 disk. Each spectrum was collected after methyl viologen was assembled on KRS-5 disk. (a) 1, (b) 2, (c) 3, (d) 4, (e) 5 bilayers. R = 4 cm\(^{-1}\). The spectra between 1100-1800 cm\(^{-1}\) were amplified 4 times.

Fig. 2.1.6 A. The relationship between the area of the peaks at 1063 cm\(^{-1}\) and No. of bilayers in Fig. 3(■) and Fig. 4(●). B. The relationship between the area of the peaks at 1642 cm\(^{-1}\) and No. of bilayers in Fig. 3(■) and Fig. 4(●).

Fig. 2.1.7 A. Cyclic voltammograms of 4-bilayer [PMo\(\text{MV}_n\)] film in (a) pH=3 phosphate buffer solution (solid line), (b) 5 mM H\(_2\)O\(_2\) (in pH=3 phosphate buffer solution) (dash line). B. Cyclic voltammograms of 4-bilayer [PMo\(\text{MV}_n\)] film in (a)
pH=3 phosphate buffer solution (solid line), (b) 0.5 mM ascorbic acid (in pH=3 phosphate buffer solution) (dash line). Scan rate: 100 mV s⁻¹.

Fig. 2.1.8 Cyclic voltammograms of 4-bilayer [PMo|MV]ₙ film in (a) pH=3 phosphate buffer solution (solid line) and (b) 5 mM H₂O₂ + 0.5 mM ascorbic acid (in pH=3 phosphate buffer solution) (dash line). Scan rate: 100 mV s⁻¹.

Fig. 2.1.9 A. Cyclic voltammograms of 2-bilayer [PMo|MV]ₙ film (a) in pH=3 phosphate buffer solution (dot line), (b) with the outmost layer phosphomolybdic acid (dash line) and (c) with the outmost layer methyl viologen (solid line) in 5 mM H₂O₂ (in pH=3 phosphate buffer solution). B. Cyclic voltammograms of 4-bilayer [PMo|MV]ₙ film (a) in pH=3 phosphate buffer solution (dot line), (b) with the outmost layer phosphomolybdic acid (dash line) and (c) with the outmost layer methyl viologen (solid line) in 5 mM H₂O₂ (in pH=3 phosphate buffer solution). Scan rate: 100 mV s⁻¹.

Fig. 2.1.10 Changes of current in i-t curve of [PMo|MV]ₙ film in pH=3 phosphate buffer solution when various amount of hydrogen peroxide was added. Potential was held at −0.15V. The inset depicts the relationship between current and concentration of hydrogen peroxide.

Fig. 2.1.11 Changes of current in i-t curve of [PMo|MV]ₙ film in pH=3 phosphate buffer solution when various amount of ascorbic acid was added. Potential was held at 0.38V. The inset depicts the relationship between current and concentration of ascorbic acid.
Fig. 2.1.1

Heteropoly Oxometalate

Viologen or Viologen analog
Fig. 2.1.2
Fig. 2.1.4

![Graph showing wavenumber vs. reflection percentage for different bilayer samples.](image-url)
Fig. 2.1.5
Fig. 2.1.6

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**A**

Peak at 1063 cm\(^{-1}\)

No. of bilayers

Peak area / cm\(^{-1}\)

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**B**

Peak at 1642 cm\(^{-1}\)

No. of bilayers

Peak area / cm\(^{-1}\)
Fig. 2.1.7

Graph A

Graph B

I / μA vs. E / V (vs. SCE)
Fig. 2.1.8
Fig. 2.1.9

A

\[ I / \mu A \]

\[ E / V \text{ (vs. SCE)} \]

B

\[ I / \mu A \]

\[ E / V \text{ (vs. SCE)} \]
Fig. 2.1.10
Fig. 2.1.11
2.2 Layer-by-Layer Self-Assembled Multilayer Molecular Film from Methylacridinium and Phosphomolybdic Acid: Fabrication, Characterization and Application in Electrochemical Bifunctional Sensing of Ascorbic Acid and Hydrogen Peroxide

Abstract

Layer-by-layer self-assembled multilayer molecular films with methylacridinium cation and phosphomolybdic acid anion on various substrates (e.g. glassy carbon, ITO) were prepared by alternating dipping the substrates in anionic and cationic species. The assembly process was monitored by electrochemical method, UV-Vis and FT-IR spectroscopies. Together with results of electrochemical and UV-Vis measurements molecular level information obtained by FT-IR spectroscopy show that, equal amount of building blocks was assembled in each fabrication process. A successful application of such newly developed film as electrochemical sensing for H₂O₂ and ascorbic acid without interference to each other was illustrated. A straight calibration curve was obtained in concentration range of 2.0×10⁻⁶ mol L⁻¹ to 1.6×10⁻⁵ mol L⁻¹ for H₂O₂ and 1.0×10⁻⁷ mol L⁻¹ to 1.28×10⁻⁵ mol L⁻¹ for ascorbic acid, with detection limit of 2.0×10⁻⁷ mol L⁻¹ and 1.0×10⁻⁸ mol L⁻¹ respectively.
2.2.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the microstructure and spatial arrangement can be easily modified according to the aim of the study [10-12].

The detection of trace amounts of \( \text{H}_2\text{O}_2 \) and ascorbic acid is of practical importance in clinical and biological studies [13-16]. Analysis of biological systems, such as blood and brain fluid, is probably its main area of application, but a problem with such analyses is that both \( \text{H}_2\text{O}_2 \) and ascorbic acid are electroactive species, which may reduce or oxidize directly on the electrode surface and mix up the current. So it is essential to eliminate the interference between each other when we detect \( \text{H}_2\text{O}_2 \) and ascorbic acid respectively. To our knowledge, no paper has reported the directly simultaneous detection of \( \text{H}_2\text{O}_2 \) and ascorbic acid on one electrode, which is very important for in situ in vivo detection.
In this study, we describe a new self-assembled molecular film with negatively charged phosphomolybdic acid (PMo, a kind of heteropoly oxometalate) and positively charged methylacridinium (MA, a kind of viologen analog). We studied in detail the applications of such film as a multi-purpose bioelectrochemical sensing for both H₂O₂ and ascorbic acid.

2.2.2 Experimental

2.2.2.1 Reagents and materials.

Phosphomolybdic acid and methylacridinium were obtained from Aldrich and used as received. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolytes. 99.5% ethanol was obtained from Panreac(Spain). Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.) and KRS-5 polished disc windows (13mm x 1mm) from Wilmad (U.S.A.).

2.2.2.2 Instrumentation.

(i) Electrochemical measurements

The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West
Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, Φ3mm, MF-2012, BAS, U.S.A.) as the working electrode. Prior to each experiment, GC electrode was polished by using alumina powder of size 1, 0.3, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.

(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the transparent ITO substrate. Bare ITO electrode was used as the reference.

(iii) Fourier transfer infra spectroscopy (FTIR)

The FTIR spectroscopic measurements were carried out on a Perkin Elmer Spectrum One spectrometer. The sample film was on KRS-5 disc.

(iv) Atomic force microscopy (AFM)

AFM images were obtained on a multimode Scanning Probe Microscope with
Nanoscope IIIa control unit by Digital instrument Inc. Tapping mode was applied for the film morphology study. The sample film was on glassy carbon substrate.

2.2.2.3 Film fabrication.

The self-assembled films on different substrates were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 5 mM PMo$_{12}$O$_{40}^{3-}$ (dissolved in pH=3 PBS or 99.5% ethanol) for 5 minutes, then a layer of PMo$_{12}$O$_{40}^{3-}$ spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of PMo$_{12}$O$_{40}^{3-}$ were transferred into 5 mM methylacridinium (dissolved in pH=6 PBS) for 5 minutes, then a layer of methylacridinium spontaneously adsorbed onto the PMo$_{12}$O$_{40}^{3-}$-coated substrates. Finally, the substrates were washed with water. Thus the self-assembled film named [PMo|MA]$_n$ was prepared successfully. The films with different thickness can be achieved by repeating the above process. The whole process is demonstrated in Fig. 2.1.1.

2.2.3 Results and discussion

2.2.3.1. Electrochemical characterization

The formation of [PMo|MA]$_n$ film was monitored by cyclic voltammetry. Phosphomolybdic acid anions which have Keggin structure are unstable in neutral and basic aqueous solution and undergo a series of hydrolysis processes [17,18].
Therefore, electrochemical studies of [PMo\textsubscript{6}MA\textsubscript{4}]\textsubscript{n} film with Phosphomolybdic acid anion were carried out in acidic aqueous solutions. Fig. 2.2.1 shows cyclic voltammograms of [PMo\textsubscript{6}MA\textsubscript{4}]\textsubscript{n} film recorded in pH=3 phosphate buffer solution after phosphomolybdic acid was assembled on electrode (GC). Characteristic cyclic voltammogram of phosphomolybdic acid, three pairs of redox peaks about 0.24 (I-I'), 0.14 (II-II') and -0.02 V (III-III'), which correspond to reduction and oxidation through two-, four-, and six-electron processes, respectively [19], is observed comparing with cyclic voltammogram of GC in 1mM phosphomolybdic acid (Fig. 2.2.1 inset A). Such character is also similar to that reported in other papers [18, 20]. It is obvious that the current of each peak increases with number of bilayers. Inset B depicts the linear relationship between the current of redox peaks II-II' and number of bilayers. The facts indicate the successive fabrication of the layer-by-layer self-assembled films on electrode and same amount of phosphomolybdic acid assembled in each bilayer.

Methylacridinium is a kind of electroactive species, but its electrochemical character can not be observed in such potential region between ~0.25 and 0.60 V. So, we can not observe any electrochemical character of methylacridinium in Fig. 2.2.1. In other words, there is no evidence in such cyclic voltammograms to directly confirm that methylacridinium was assembled in each bilayer.

2.2.3.2. Characterization by UV-Vis spectroscopy

Methylacridinium has characteristic absorption in UV-Vis spectrometry, so we studied the growth of methylacridinium in [PMo\textsubscript{6}MA\textsubscript{4}]\textsubscript{n} film using UV-Vis
spectrometry. Fig. 2.2.2 shows UV-Vis spectra of [PMo|MA]ₙ film assembled on ITO-coated glass. The characteristic methylacridinium absorbance around 370 and 450 nm can be observed and the intensity of each peak increases with number of bilayers. The inset depicts the linear relationship between peak intensity and number of bilayers. It testifies that methylacridinium is assembled layer-by-layer in [PMo|MA]ₙ film and same amount of methylacridinium assembled after phosphomolybdic acid in each bilayer.

2.2.3.3. Characterization by FT-IR spectroscopy

FT-IR spectra of phosphomolybdic acid and methylacridinium show that both of these two compounds have characteristic vibrational peaks, therefore, FT-IR is very possible to serve to measure the layers growth of both phosphomolybdic acid and methylacridinium.

Fig. 2.2.3 shows IR spectra of [PMo|MA]ₙ film collected after phosphomolybdic acid was assembled on KRS-5 disc and Fig. 2.2.4 shows IR spectra of [PMo|MA]ₙ film collected after methylacridinium was assembled on the disc. Peaks in spectra corresponding to phosphomolybdic acid and methylacridinium can easily identified. In the first spectrum (a) of the film in Fig. 2.2.3, some peaks appear in 400-1100 cm⁻¹. As we know, the only compound exists in the first layer of the film is phosphomolybdic acid, so the peaks can be assign to IR absorption of phosphomolybdic acid. Some peaks at the same locations can also be observed in spectrum of pure phosphomolybdic acid. From the second spectrum (b) to the last spectrum (f) in Fig. 2.2.3, we noticed two main points: one is that some new peaks
appear at the location between 1100 and 1800 cm\(^{-1}\); the other is that the intensity of all the peaks increases with number of bilayers. Based on these evidences, we can say that the peaks between 1100 and 1800 cm\(^{-1}\) belong to methylacridinium and layer-by-layer molecular film was fabricated successfully.

In Fig. 2.2.4, the outmost layer of each bilayer is methylacridinium, so it is reasonable to observe the IR absorption peaks of both anion and cation in the first spectrum (a). The intensity of the peaks in this figure also increases with number of bilayers. In other words, the characters of Fig. 2.2.4 are consistent with Fig 2.2.3.

After comparing the spectra in Fig. 2.2.3 and Fig. 2.2.4 carefully, the growth of the peaks in 400-1100 cm\(^{-1}\), which belong to phosphomolybdic acid, can be only observed after each layer phosphomolybdic acid is assembled on the substrate. And the growth of the peaks in 1100-1800 cm\(^{-1}\), which belong to methylacridinium, can be only observed after each layer methylacridinium is assembled on the substrate. The peaks located at 1062 cm\(^{-1}\), belonging to phosphomolybdic acid, and the peaks at 1268 and 1550 cm\(^{-1}\), belonging to methylacridinium, were integrated respectively in Fig. 2.2.3 and Fig. 2.2.4. The result is shown in Fig. 2.2.5. The area of the peaks is proportional to number of bilayers. It testifies our conclusion that both phosphomolybdic acid and methylacridinium are assembled on substrate layer-by-layer. It also testifies that same amount of phosphomolybdic acid is assembled in each bilayer of the film and also same amount of methylacridinium is assembled in each bilayer of the film.

2.2.3.3. Characterization by AFM
Atomic force microscopy (AFM) gives some information about the surface morphology of [PMo|MA]_n film (Fig. 2.2.6) and especially the size of particles in [PMo|MA]_n film. It is reasonable that the surface of glassy carbon is not smooth enough (Fig. 2.2.6 A) and many scribing grid is found on the surface since the surface of glassy carbon was polished by micro powder. After 2-bilayer [PMo|MA]_n film was assembled on glassy carbon (Fig. 2.2.6 B), some particles are found on the surface and the size of the particles is about 70 nm and the thickness of the film is about 30 nm. When the number of bilayers is increasing, both particle size and the thickness of the film are increasing. Fig. 2.2.6 C shows the AFM image of 10-bilayer [PMo|MA]_n film on glassy carbon. The size of the particles increases to about 110 nm and the thickness of the film becomes about 200 nm. The AFM results show that the thickness of the assembled film can be controlled flexibly by adjusting the number of bilayers.

2.2.3.5 Application in electrochemical bifunctional sensing

2.2.3.5.1 The catalytic reduction of \( H_2O_2 \)

Fig. 2.2.7 (c) shows cyclic voltammogram of 5 mM \( H_2O_2 \) recorded on GC electrode modified with 10-bilayer [PMo|MA]_n film in pH = 3 phosphous buffer solution. As seen, the modified electrode gives large current when the potential is lower than 0.10 V, which is origin from electrochemical reduction of \( H_2O_2 \). Under the same condition, no discernible reduction peak can be observed on bare GC (Fig.
2.2.7 (a)), which indicates that [PMo|MA]_n film is catalytically active towards electrochemical reduction of H_2O_2.

In order to improve the sensitivity of the modified electrode, we thought of increasing the total amount of electrocatalytical molecules on the electrode surface. In our fabrication method, it is flexible to control the amount by changing different bilayers of [PMo|MA]_n film. Fig. 2.2.8 shows cyclic voltammograms of 5 mM H_2O_2 recorded on two, five and ten bilayers [PMo|MA]_n films in pH = 3 phosphous buffer solution. The reduction current of H_2O_2 increases with the increase of number of bilayers. According to this result, we can choose a thick [PMo|MA]_n film to improve the sensitivity in the reduction of H_2O_2. On the other hand, if the film is too thick, the response time will be delayed. So we use 10-bilayer [PMo|MA]_n film as our optimal electrode in our experiments.

Since H_2O_2 gave a evident reduction current on the [PMo|MA]_n modified electrode, this electrode possess the potential to be developed as a sensitive sensor for H_2O_2. Amperometric measurements of H_2O_2 solution with various concentrations were conducted at -0.15 V on 10-bilayer [PMo|MA]_n film. I-t curve of H_2O_2 with concentration increasing from 2×10^-6 mol L^-1 to 1.6×10^-5 mol L^-1 was shown in Fig. 2.2.9, the corresponding calibration curve shown in the inset shows a direct proportional relation between the current and concentration of H_2O_2. Linear regression performed on the calibration curve gave the value of coefficient correlation (R^2) as 0.9997. The relative standard deviation (R.S.D) was less than 5%. The detection limit calculated for S/N of 3 was 2×10^-7 mol L^-1.
2.2.3.5.2 The catalytic oxidation of ascorbic acid

Cyclic voltammogram of 1 mM ascorbic acid on GC electrode modified with 10-bilayer [PMo|MA]ₙ film is shown in Fig. 2.2.10, a broad anodic peak at 0.45 V can be observed, which can be assigned to electrochemical oxidation of ascorbic acid. Except peaks of redox reaction of [PMo|MA]ₙ film, no additional cathodic peak exists. It shows that the electrochemical behavior of ascorbic acid on the electrode is a totally irreversible process. The oxidation potential of ascorbic acid on the modified electrode is about 0.45 V, higher than that on bare GC carbon electrode. The peak current of ascorbic acid oxidation on modified electrode is just a little higher than that on a bare GC electrode. Based on this comparison, [PMo|MA]ₙ film is proposed to be inactive to oxidation reaction of ascorbic acid and act as an electron transferrer.

Amperometric response of the modified electrode to ascorbic acid with various concentration ranging from 1.0×10⁻⁷ mol L⁻¹ to 1.28×10⁻⁵ mol L⁻¹ was tested at 0.45 V and the result was shown in Fig. 2.2.11. Current of the electrode increased in a proportional mode when concentration of ascorbic acid increase and gave a straight calibration curve with a regression correlation (R²) of 0.9986. The relative standard deviation (R.S.D) was less than 5%. The detection limit of the modified electrode to ascorbic acid is determined as 1×10⁻⁸ mol L⁻¹. This study illustrated a successful application of the newly developed electrode as an electrochemical sensor for ascorbic acid.
2.2.3.5.3 The catalytic bifunctional sensing of H$_2$O$_2$ and ascorbic acid

In most cases of biological and medical applications of H$_2$O$_2$ sensing, ascorbic acid coexists with H$_2$O$_2$ and interferes with the detection of H$_2$O$_2$, moreover, as a medically important molecule, precise detection of ascorbic acid can provide valuable information for medical judgement. Thus, a sensor with selective sensitivity toward both H$_2$O$_2$ and ascorbic acid will be desirable.

In Fig. 2.2.7, it is noteworthy that in the higher potential range the electrochemical behavior of the modified electrode remained almost identical when H$_2$O$_2$ was present or absent in the solution. In contrast, in the lower potential range, no substantial change upon addition of ascorbic acid can be observed (Fig. 2.2.10). These features imply that H$_2$O$_2$ is inactive at higher potential while ascorbic acid at lower potential, thus one [PMolMA]$_n$ film modified electrode can be used to detect H$_2$O$_2$ at lower potential and ascorbic acid at higher potential. So it is rational that on [PMolMA]$_n$ film there is no interference between H$_2$O$_2$ and ascorbic acid.

The results show that [PMolMA]$_n$ film exhibits high sensitivity and excellent selectivity to both H$_2$O$_2$ and ascorbic acid. The sensitivity and selectivity of this electrochemical sensing system to these two molecules is comparable to the most sensitive sensors in the published works [21-23].

2.2.4 Conclusions

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged phosphomolybdic acid and
positively charged methylacridinium on various substrates including glassy carbon, ITO and KRS-5 disc. The thickness of such film can be controlled easily in the assembly process. We have systematically characterized our self-assembled film and have monitored the growth of the film by using electrochemical method, UV-Vis and FT-IR spectroscopies. The results showed that both phosphomolybdic acid and methylacridinium assembled layer-by-layer on substrate. The [PMo\[MA\]ₙ film exhibits high sensitivity and excellent selectivity to two medically important molecules, H₂O₂ and ascorbic acid.

2.2.5 References


Chapter 2

Legends and Figures

Fig. 2.2.1 Cyclic voltammograms of (a) glassy carbon (dot line), (b-k) [PMo|MA]ₙ films (from one up to ten bilayers) in pH=3 phosphate buffer solution. Scan rate: 100 mV s⁻¹. Inset A: cyclic voltammogram of glassy carbon in 1mM phosphomolybdic acid. Inset B: the relationship between peak current and No. of bilayers.

Fig. 2.2.2 UV-Vis spectra of [PMo|MA]ₙ film on ITO-coated quartz substrate. (a) two bilayers, (b) four bilayers, (c) six bilayers, (d) eight bilayers, (e) ten bilayers. The inset depicts the relationship between absorbance and No. of bilayers.

Fig. 2.2.3 IR spectra of [PMo|MA]ₙ film on KRS-5 disk. Each spectrum was collected after phosphomolybdic acid was assembled on KRS-5 disk. (a) 0.5, (b) 1.5, (c) 2.5, (d) 3.5, (e) 4.5, (f) 5.5 bilayers. R = 4 cm⁻¹. The spectra between 1100-1800 cm⁻¹ were amplified 3 times.

Fig. 2.2.4 IR spectra of [PMo|MA]ₙ film on KRS-5 disk. Each spectrum was collected after methylacridinium was assembled on KRS-5 disk. (a) 1, (b) 2, (c) 3, (d) 4, (e) 5, (f) 6 bilayers. R = 4 cm⁻¹. The spectra between 1100-1800 cm⁻¹ were amplified 3 times.

Fig. 2.2.5 The relationship between the area of the peaks at 1062 cm⁻¹, 1268 cm⁻¹ and 1550 cm⁻¹ and No. of bilayers in Fig. 3(■) and Fig. 4(○).

Fig. 2.2.6 A. AFM image of glassy carbon surface. B. AFM image of 2-bilayer [PMo|MA]ₙ. C. AFM image of 10-bilayer [PMo|MA]ₙ.

Fig. 2.2.7 Cyclic voltammograms of (a) glassy carbon in 5 mM H₂O₂ (in pH=3 phosphate buffer solution) (dot line), (b) 10-bilayer [PMo|MA]ₙ film in pH=3
phosphate buffer solution (dash line), and (c) 10-bilayer $[\text{PMo}[\text{MA}]_n$ film in 5 mM $\text{H}_2\text{O}_2$ (in pH=3 phosphate buffer solution) (solid line).

Fig. 2.2.8 Cyclic voltammograms of (a) glassy carbon (dot line), (b) 2-bilayer $[\text{PMo}[\text{MA}]_n$ film (dash line), (c) 5-bilayer $[\text{PMo}[\text{MA}]_n$ film (dash dot line), and (d) 10-bilayer $[\text{PMo}[\text{MA}]_n$ film (solid line) in 5 mM $\text{H}_2\text{O}_2$ (in pH=3 phosphate buffer solution). Scan rate: 100 mV s$^{-1}$. 

Fig. 2.2.9 Changes of current in i-t curve of $[\text{PMo}[\text{MA}]_n$ film in pH=3 phosphate buffer solution when various amount of hydrogen peroxide was added. Potential was held at $-0.150\text{V}$. The inset depicts the relationship between current and concentration of hydrogen peroxide.

Fig. 2.2.10 Cyclic voltammograms of 10-bilayer $[\text{PMo}[\text{MA}]_n$ film in (a) pH=3 phosphate buffer solution (dot line), and (b) 1 mM ascorbic acid (in pH=3 phosphate buffer solution) (solid line). Scan rate: 100 mV s$^{-1}$.

Fig. 2.2.11 Changes of current in i-t curve of $[\text{PMo}[\text{MA}]_n$ film in pH=3 phosphate buffer solution when various amount of ascorbic acid was added. Potential was held at 0.450V. The inset depicts the relationship between current and concentration of ascorbic acid.
Fig. 2.2.1
Fig. 2.2.2

[Graph showing absorbance vs. wavelength and absorbance vs. number of bilayers, with a peak at 372 nm]
Fig. 2.2.3
Fig. 2.2.4

[Diagram showing wavenumber in cm\(^{-1}\) with peaks at 1590, 1288, and 1062 for different bilayer samples labelled a to f. Bars indicate T% = 7% and T% = 20% for comparison.]
Fig. 2.2.5

(A) Peak at 1062 cm\(^{-1}\)

(B) Peak at 1550 cm\(^{-1}\)
Fig. 2.2.6

A

B

C
Fig. 2.2.9
Fig. 2.2.10
Fig. 2.2.11
2.3 Layer-by-Layer Self-Assembled Multilayer Molecular Film from Porphyrin Viologen and Phosphomolybdic Acid: Fabrication, Characterization and Application in Bifunctional Sensing of Nitric Oxide and Oxygen

Abstract

Layer-by-layer self-assembled multilayer molecular films with porphyrin viologen cation and phosphomolybdic acid anion on various substrates (e.g. glassy carbon, ITO) were prepared by alternating dipping the substrates in anionic and cationic species. The assembly process was monitored by electrochemical method and UV-Vis spectroscopy. Results of electrochemical and UV-Vis show that equal amount of building blocks were assembled in each fabrication process. Different metal cations were substituted successfully into the film to achieve the different functions of the film. A successful application of such newly developed film in electrochemical sensing of O$_2$ and NO was illustrated.

2.3.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].
The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the microstructure and spatial arrangement can be easily modified according to the aim of the study [10-12].

NO has attracted intense interests due to its important roles in cellular communication and in host defense mechanisms of eukaryotes [13]. Detection of trace amount of NO in biological systems is quite challenging because of various interference from other coexisting species [14].

Oxygen is a definitely important molecule in not only biological systems but also fuel cell application. There is a long history of studies of oxygen [15-17] and today there are still many studies on it [18,19].

In this study, we describe a new self-assembled molecular film with negatively charged phosphomolybdic acid (PMo, a kind of heteropoly oxometalate) and positively charged porphyrin viologen (PorV, a kind of viologen). We studied in detail the applications of such film in multi-purpose electrochemical sensing of both O₂ and NO.

2.3.2 Experimental
2.3.2.1 Reagents and materials.

Phosphomolybdic acid, cobalt(II) perchlorate hexahydrate and nickel(II) perchlorate hexahydrate were obtained from Aldrich and used as received. Porphyrin viologen was synthesized and supplied by Prof. Okura (Japan). Phosphate buffer solution (PBS) was obtained from Riedel–de Haen (Germany) and employed as the supporting electrolytes. 99.5% ethanol was obtained from Panreac (Spain). Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.) and KRS-5 polished disc windows (13mm x 1mm) from Wilmad (U.S.A.). Standard saturated NO solutions were prepared by bubbling a high purity NO gas through milli-Q pure water for 40 minutes after it was bubbled with high purity N2 gas for 30mins [20-22]. The saturated aqueous NO solution has a NO concentration of $1.8 \times 10^{-3} \text{ mol L}^{-1}$ at 20°C, $P(\text{NO}) = 1 \text{ atm}$ [23, 24]. The O2 concentration of air-saturated water is $2.6 \times 10^{-4} \text{ mol L}^{-1}$ calculated from its saturated solubility [25].

2.3.2.2 Instrumentation.

(i) Electrochemical measurements

The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was
employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, φ3mm, MF-2012, BAS, U.S.A.) as the working electrode. Prior to each experiment, GC electrode was polished by using alumina powder of size 1, 0.3, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.

(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the transparent ITO substrate. Bare ITO electrode was used as the reference.

2.3.2.3 Fabrication of [PMo|PorV]ₙ film.

The self-assembled films on different substrates were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 5 mM PMo₁₂O₄₀³⁻ (dissolved in pH=3 PBS) for 5 minutes, then a layer of PMo₁₂O₄₀³⁻ spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with corresponding solvent. Then the substrates with a layer of PMo₁₂O₄₀³⁻ were transferred into saturated porphyrin viologen (dissolved in pH=1 PBS) for 5 minutes, then a layer of porphyrin viologen spontaneously adsorbed onto the PMo₁₂O₄₀³⁻-coated substrates. Finally, the
substrates were washed with solvent. Thus the self-assembled film named [PMo|PorV]ₙ was prepared successfully. The films with different thickness can be achieved by repeating the above assembly process. The whole process is demonstrated in Fig. 2.1.1.

2.3.3 Results and discussion

2.3.3.1. Characterization of [PMo|PorV]ₙ film by UV-Vis spectroscopy

Porphyrin viologen was found to absorb UV radiation and the absorption peaks appear at 225, 262, 422, 520, 562, 591, 648 nm (Fig. 2.3.1 inset A), which provides possibility to monitor assembly of porphyrin viologen in [PMo|PorV]ₙ film by UV-visible spectroscopy. For this purpose, [PMo|PorV]ₙ films were fabricated on ITO-coated glass substrate under the same conditions and their UV-visible spectra were collected and shown in Fig. 2.3.1. It can be seen in the figure that the shape of each spectrum is almost the same as that of pure porphyrin viologen solution while the position of each corresponding peak is shifted to higher wavelength. It may be due to the difference between solution environment and film formation. The intensity of the absorption peaks increased with number of bilayers. Furthermore, the inset B in Fig. 2.3.1 depicts a linear correlation between the absorption intensity of the absorption peaks at 442 nm and the number of bilayers, which indicating that porphyrin viologen is assembled layer-by-layer in [PMo|PorV]ₙ film and the amount of porphyrin viologen assembled after phosphomolybdic anion in each bilayer is almost the same.
Since phosphomolybdic acid gives no contribution to the absorption in Fig. 2.3.1, the assembly of phosphomolybdic acid can not be directly probed by UV-Vis spectroscopy. Electrochemical method is a useful method to study heteropoly oxometalate, so that the assembly of phosphomolybdic acid can be monitored by electrochemical method.

2.3.3.2. Electrochemical characterization

Fabrication of self-assembled film of phosphomolybdic acid and porphyrin viologen ([PMo|PorV]n) was monitored by electrochemical method. Phosphomolybdic acid anions that have Keggin structure are unstable in neutral and basic aqueous solution and undergo a series of hydrolysis processes [26, 27]. Therefore, electrochemical studies of [PMo|PorV]n film with phosphomolybdic anion were carried out in acidic aqueous solutions. Each cyclic voltammogram was recorded in pH = 3 phosphate buffer solution after the assembly of a layer of anion species and a layer of cation species on glassy carbon. A serial of such recorded cyclic voltammograms is shown in Fig. 2.3.2. As can be seen, three pairs of redox peaks are located at about 0.24, 0.14 and -0.02 V in the range from -0.20 to 0.60 V. These peaks of assembled bilayers are similar to that of 1 mM phosphomolybdic acid solution (Fig. 2.3.2 inset A) except that the peaks in former curves are sharper and also slightly shift. This character is also similar to those reported in other papers [27, 28]. A cyclic voltammetric study of glassy carbon shows that no peak can be observed in the same potential range. Based on the above results and reported study of electrochemical behavior of phosphomolybdic acid, electrochemical behavior
shown in Fig. 2.3.2 was ascribed to redox of phosphomolybdic acid and the three pairs of peaks correspond to reduction and oxidation through two-, four-, and six-electron processes respectively [29]. Another characteristic can be noted is that peak current of all the three pairs of peaks increased with increase of number of bilayers. Peak current ($I_p$) of the redox pair located at -0.009 and -0.041 V was plotted versus number of bilayers and shown in Fig. 2.3.2 inset B. It is obvious that $I_p$ increases proportionally with number of bilayers. This indicates that the successive fabrication of the layer-by-layer self-assembled films on electrode and the amount of the anionic species assembled on electrode surface in each bilayer is almost the same.

Combining electrochemical and UV-visible characteristics of [PMoPorV]$_n$ film prepared in this study, some interesting features can be found. For a specific substrate, all the results prove that the amount of either anionic or cationic species assembled in the according bilayer is constant. Our investigation also found that amount of building block was limited by a certain value even extra dipping time was applied. This is reasonable for a layer-by-layer structure constructed by electrostatic attraction, i.e., equal amount of oppositely charged sites are generated with consumption of charge sites by electronic neutralization accompanied self-assembly. Generally, a layer-by-layer film constructed by electrostatic attraction was successfully fabricated with our strategy.

2.3.3.3 Application in electrochemical sensing

2.3.3.3.1 The catalytic bifunctional detection of both $O_2$ and NO
In most cases of biological and medical system, O$_2$ coexists with NO, moreover, as an important molecule, precise detection of NO can provide valuable information for medical judgment. Thus, a bifunctional sensor with selective sensitivity toward both O$_2$ and NO should be desirable.

Fig. 2.3.3(b) displays the cyclic voltammograms of 12-bilayer [PMo|PorV]$_n$ modified GC electrode in air-saturated pH=7 phosphate buffer solution with 2.0×10$^{-4}$ mol L$^{-1}$ NO. Compared with the CV characterization of 12-bilayer [PMo|PorV]$_n$ in pH=7 phosphate buffer solution without O$_2$ (Fig. 2.3.3(a)), not only the marked reduction current appears when the potential is below -0.30 V, but also the large oxidation current is observed when the potential is over 0.60 V in Fig. 2.3.3(b). The large oxidation current can be assigned to electrochemical oxidation of NO and the anodic peak appears at 0.79 V, while the reduction current can be assigned to electrochemical reduction of O$_2$. It shows that the electrochemical behaviors of NO and O$_2$ on the electrode are totally irreversible process. These features imply that [PMo|PorV]$_n$ modified GC electrode has electrocatalytical properties toward both NO and O$_2$. In other words, [PMo|PorV]$_n$ film has potential to be a good sensor for the detection of NO and O$_2$. Since O$_2$ is reduced while NO is oxidized on [PMo|PorV]$_n$ modified GC electrode, one [PMo|PorV]$_n$ film modified electrode can be used to detect O$_2$ at lower potential and NO at higher potential. So it is rational that [PMo|PorV]$_n$ film can be used in bifunctional bioelectrochemical sensing of O$_2$ and NO.

Since metalloporphyrin is a more attractive redox catalyst or mediator than free base porphyrin in many electrochemical reactions for the reduction or oxidation of
substrates such as O$_2$, H$_2$O$_2$ and NO, some metal ions were considered to dope in [PMo|PorV]$_n$ film to achieve more sensitive electrochemical sensing.

2.3.3.3.2 The film doped with Co$^{2+}$ for the enhancement of O$_2$ reduction

It is well known that cobalt porphyrin is a good catalyst for the reduction of oxygen and many researchers show interest in it [30, 31], so Co$^{2+}$ was chosen to dope in porphyrin viologen in [PMo|PorV]$_n$ film to form cobalt-substituted porphyrin viologen.

[PMo|PorV]$_n$ film was immersed in stirred 2.0 $\times$ 10$^{-2}$ mol L$^{-1}$ Co(ClO$_4$)$_2$ aquatic solution for five hours, followed by extensive rinse with water. Fig. 2.3.4 shows UV-Vis spectra of 12-bilayer [PMo|PorV]$_n$ film before and after immersion in 2.0 $\times$ 10$^{-2}$ mol L$^{-1}$ Co(ClO$_4$)$_2$ solution. After immersion, the peak strength of porphyrin viologen has negligible changes, while the peak at 444 is blue shifted to 439, which is consistent with the difference of the absorption between free base porphyrin and cobalt porphyrin [32]. These evidences show that the amount of porphyrin viologen in the film is almost same and the Co$^{2+}$ is doped successfully in porphyrin viologen in the film after 5-hour immersion in Co(ClO$_4$)$_2$ solution. Fig. 2.3.5 shows cyclic voltammograms of 12-bilayer [PMo|PorV]$_n$ film in N$_2$-saturated pH=3 phosphate buffer solution before and after immersion in Co(ClO$_4$)$_2$ solution. Since the two curves have no significant difference about the peak current or the peak position or the curve shape and all the characteristics are assigned to phosphomolybdic anion, it can be confirmed that the amount of phosphomolybdic anion in the film has almost no change, as porphyrin viologen does, after 5-hour immersion in Co(ClO$_4$)$_2$ solution.
These figures convince that the method of immersion is useful to induce Co$^{2+}$ into porphyrin viologen in [PMoPorV]$_n$ film.

Fig. 2.3.6 (b) and (c) show cyclic voltammogram of 12-bilayer [PMoPorV]$_n$ film before and after doped with Co$^{2+}$ recorded in air-saturated pH=3 phosphate buffer solution. Comparing with cyclic voltammogram of the film recorded in N$_2$-saturated pH=3 phosphate buffer solution (Fig. 2.3.6(a)), there is obvious current in Fig. 2.3.6 (b) when the potential is lower than -0.35 V, which is from electrochemical reduction of O$_2$. Under the same condition, Co$^{2+}$-substituted [PMoPorV]$_n$ film not only gives larger current in Fig. 2.3.6 (c), but also the start reduction potential increases 0.05 V to -0.30 V, which indicates that Co$^{2+}$ is doped in the film successfully and such Co$^{2+}$-substituted [PMoPorV]$_n$ film is more catalytically active towards electrochemical reduction of O$_2$. Since O$_2$ gives more evident reduction current on the Co$^{2+}$-substituted [PMoPorV]$_n$ modified electrode than on the [PMoPorV]$_n$ film without Co$^{2+}$, such Co$^{2+}$-substituted [PMoPorV]$_n$ film possesses the potential to be developed to a sensitive sensor for O$_2$.

2.3.3.3.3 The film doped with Ni$^{2+}$ for the enhancement of NO oxidation

It is known that nickel porphyrin is a good catalyst for the oxidation of nitric oxide [33], so Ni$^{2+}$ was chosen to dope in porphyrin viologen in [PMoPorV]$_n$ film to form nickel-substituted porphyrin viologen.

[PMoPorV]$_n$ film was immersed in stirred 2.0 × 10$^{-2}$ mol L$^{-1}$ Ni(ClO$_4$)$_2$ aquatic solution for five hours, followed by extensive rinse with water. Fig. 2.3.7 shows UV-Vis spectra of 12-bilayer [PMoPorV]$_n$ film before and after immersion in 2.0 × 10$^{-2}$
mol L\(^{-1}\) Ni(ClO\(_4\))\(_2\) solution. After immersion, the peak strength of porphyrin viologen has negligible changes, while the peak at 444 is blue shifted to 437, which is consistent with the difference of the absorption between free base porphyrin and nickel porphyrin [32]. These evidences show that the amount of porphyrin viologen in the film is almost same and the Ni\(^{2+}\) is doped successfully in porphyrin viologen in the film after 5-hour immersion in Ni(ClO\(_4\))\(_2\) solution. Cyclic voltammograms of 12-bilayer [PMo\(]\text{PorV}\)\(_n\) film in N\(_2\)-saturated pH=3 phosphate buffer solution before and after immersion in Ni(ClO\(_4\))\(_2\) solution shows that the amount of phosphomolybdic anion in the film has also almost no change after 5-hour immersion in Ni(ClO\(_4\))\(_2\) solution. These convince that the method of immersion is also useful to induce Ni\(^{2+}\) into porphyrin viologen in [PMo\(]\text{PorV}\)\(_n\) film. The evidences indicate that various metal ions can be doped conveniently into such film by the method of immersion according to our different purposes.

Fig. 2.3.8 (b) shows cyclic voltammogram of 12-bilayer [PMo\(]\text{PorV}\)\(_n\) film before and after doped with Ni\(^{2+}\) recorded in air-saturated pH=3 phosphate buffer solution with 2.0 \(\times\) 10\(^{-4}\) mol L\(^{-1}\) NO. Comparing with cyclic voltammogram of the film before doped with Ni\(^{2+}\) recorded in the same solution (Fig. 2.3.8(a)), there is obvious NO oxidation current increase in Fig. 2.3.8 (b) when the potential is higher than 0.65 V. The increase of the oxidation current indicates that Ni\(^{2+}\) is doped in the film successfully and such Ni\(^{2+}\)-substituted [PMo\(]\text{PorV}\)\(_n\) film is more catalytically active towards electrochemical oxidation of NO. Since NO gives more evident oxidation current on the Ni\(^{2+}\)-substituted [PMo\(]\text{PorV}\)\(_n\) modified electrode than on the [PMo\(]\text{PorV}\)\(_n\) film without Ni\(^{2+}\), such Ni\(^{2+}\)-substituted [PMo\(]\text{PorV}\)\(_n\) film possesses the potential to be developed to a sensitive sensor for NO.
2.3.4 Conclusions

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged phosphomolybdic acid and positively charged porphyrin viologen on various substrates including glassy carbon and ITO. The thickness of such film can be controlled easily in the assembly process. We have systematically characterized our self-assembled film and have monitored the growth of the film by using electrochemical method and UV-Vis spectroscopies. The results showed that both phosphomolybdic acid and porphyrin viologen assembled layer-by-layer on substrate. The $[\text{PMo}[\text{PorV}]_n$ film exhibits excellent selectivity and high sensitivity to two medically important molecules, $\text{O}_2$ and $\text{NO}$. The $\text{Co}^{2+}$-substituted $[\text{PMo}[\text{PorV}]_n$ film or the $\text{Ni}^{2+}$-substituted $[\text{PMo}[\text{PorV}]_n$ film was fabricated successfully to achieve the enhancement of the electrochemical reduction of $\text{O}_2$ or to achieve the enhancement of the electrochemical oxidation of $\text{NO}$, respectively.

2.3.5 References


Legends and Figures

Fig. 2.3.1 UV-Vis spectra of [PMo]PorV\_n film (from one up to nine bilayers) on ITO-coated glass substrate. Inset A: UV-Vis spectra of Porpyrin-Viologen solution. Inset B: the relationship between absorbance and No. of bilayers.

Fig. 2.3.2 Cyclic voltammograms of [PMo]PorV\_n film (from one up to eight bilayers) in pH=3 phosphate buffer solution. Scan rate: 50 mV s\(^{-1}\). Inset A: cyclic voltammogram of glassy carbon in 1mM phosphomolybdic acid in pH=3 phosphate buffer solution. Inset B: the relationship between peak current and No. of bilayers.

Fig. 2.3.3 Cyclic voltammograms of 12-bilayer [PMo]PorV\_n film in (a) N\(_2\)-saturated PH=3 phosphate buffer and (b) air-saturated PH=3 phosphate buffer solution with 2.0 \times 10^{-4} \text{ mol L}^{-1} \text{ NO}. Scan rate: 50 mV s\(^{-1}\).

Fig. 2.3.4 UV-Vis spectra of 12-bilayer [PMo]PorV\_n film before (a) and after (b) immersion in 2.0 \times 10^{-2} \text{ mol L}^{-1} \text{ Co(ClO}_4\text{)}_2.

Fig. 2.3.5 Cyclic voltammograms of 12-bilayer [PMo]PorV\_n film before (a) and after (b) immersion in 2.0 \times 10^{-2} \text{ mol L}^{-1} \text{ Co(ClO}_4\text{)}_2 in N\(_2\)-saturated PH=3 phosphate buffer solution.

Fig. 2.3.6 Cyclic voltammograms of (a) 12-bilayer [PMo]PorV\_n film in pH=3 phosphate buffer solution without O\(_2\) (dash line) (b) 12-bilayer [PMo]PorV\_n film in air-saturated PH=3 phosphate buffer solution (dot line), and (c) 12-bilayer [PMo]PorV\_n film after doped with Co\(^{2+}\) in air-saturated pH=3 phosphate buffer solution (solid line). Scan rate: 50 mV s\(^{-1}\).
Fig. 2.3.7 UV-Vis spectra of 12-bilayer [PMo|PorV]ₙ film before (a) and after (b) immersion in $2.0 \times 10^{-2}$ mol L⁻¹ Ni(ClO₄)₂.

Fig. 2.3.8 Cyclic voltammograms of (a) 12-bilayer [PMo|PorV]ₙ film in air-saturated pH=3 phosphate buffer solution with $2.0 \times 10^{-4}$ mol L⁻¹ NO (dot line), and (b) 12-bilayer [PMo|PorV]ₙ film after doped with Ni²⁺ in air-saturated pH=3 phosphate buffer solution with $2.0 \times 10^{-4}$ mol L⁻¹ NO (solid line). Scan rate: 50 mV s⁻¹.
Fig. 2.3.1
Fig. 2.3.2
Fig. 2.3.4
Fig. 2.3.5

![Graph representing electrochemical data with two curves labeled a and b. The x-axis represents E / V (vs. SCE) ranging from -0.6 to 0.6, and the y-axis represents I / µA ranging from -6 to 4. The graph shows multiple peaks and troughs for each curve.]
Fig. 2.3.6
Fig. 2.3.7
Fig. 2.3.8

![Graph showing current (I) vs. voltage (E) with two curves labeled 'a' and 'b'.]
CHAPTER 3

Layer-by-Layer Self-Assembled Multilayer Molecular Films
from Viologen-Derivatives and Heteropoly Oxometalates:
Preparation, Characterization and Application in
Electrochromic Coating
3.1 Layer-by-Layer Self-Assembled Multilayer Film from Cycloviologen and Phosphotungstate

Abstract

Layer-by-layer self-assembled multilayer molecular films with cycloviologen cation and phosphotungstate anion on various substrates (e.g. glassy carbon, ITO, quartz) were prepared by alternating dipping the substrates in anionic and cationic species. The assembly process was monitored by electrochemical method and UV-Vis spectroscopy. The results of electrochemical and UV-Vis measurements show that equal amount of building blocks was assembled in each fabrication process. Such newly developed film has a successful application in electrochromic coating.

3.1.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate
adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be easily modified according to the aim of the study [10-12].

An electroactive species often exhibits new optical absorption bands, i.e. shows a new colour, in accompaniment with an electron-transfer or ‘redox’ reaction in which it either gains or loses an electron; that is to say, it undergoes reduction or oxidation. Such colouration was first termed ‘electrochromism’ in 1961 by Platt [13]. Now electrochromism is defined as the electrochemical generation of colour in accompaniment with an electron transfer (or ‘redox’) reaction. Although electrochromic systems as displays need to compete with both cathode ray tube (CRT) and liquid crystal display (LCD) for commercial viability, they possess many advantages over both [14]. In most cases, only one redox active species is used as electrochromic material in one system [15, 16]. To the best of our knowledge, few studies focus on two kinds of redox active species, especially combined organic and inorganic species, in the application of electrochromic system.

Here we describe a new self-assembled homogeneous molecular film with negatively charged phosphotungstate (PW, a kind of heteropoly oxometalate) and positively charged cycloviologen (CyV, a kind of viologen). We studied in detail its application in electrochromic coating.

3.1.2 Experimental
3.1.2.1 Reagents and materials.

Phosphotungstate (K₆P₂W₁₈O₆₂) and cycloviolegen were synthesized and isolated according to reference [17, 18]. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolyte. Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.).

3.1.2.2 Instrumentation.

(i) Electrochemical measurements

The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, φ3mm, MF-2012, BAS, U.S.A.) as the working electrode. Prior to each experiment, GC electrode was polished by using alumina powder of size 1.0, 0.3, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.
(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the transparent ITO or quartz substrate. Bare ITO or quartz was used as the reference.

(iii) In situ UV-visible spectroscopy

Potential applied on the electrode was controlled by a CHI 750A electrochemical workstation (CH Instruments Inc, USA). Platinum wire was used as a counter electrode and Ag/AgCl electrode was used as a reference electrode. Phosphate buffer solution was employed as supporting electrolyte. Modified ITO electrode (30 x 30 x 0.5 mm float glass slide, ITO-coated two sides, Della Technologies, Limited-Stillwater, U.S.A) was used as a working electrode.

3.1.2.3 Fabrication of [PW(CyV)]<s> film.

The self-assembled films on different substrates were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 5 mM P₂W₁₈O₆₂⁶⁻ (dissolved in pH=3 PBS) for 5 minutes, then a layer of P₂W₁₈O₆₂⁶⁻ spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of P₂W₁₈O₆₂⁶⁻ were transferred into 2 mM cycloviologen (dissolved in pH=6 PBS) for 5
minutes, then a layer of cycloviologen spontaneously adsorbed onto the $\text{P}_2\text{W}_{18}\text{O}_{62}^{6-}$-coated substrates. Finally, the substrates were washed with water. Thus the self-assembled film named $[\text{PW}\{\text{CyV}\}]_n$ was prepared successfully. The films with different thickness can be achieved by repeating the above assembly process. The whole process is demonstrated in Fig. 2.1.1.

### 3.1.3 Results and discussion

#### 3.1.3.1 Electrochemical characterization

Fabrication of self-assembled film of phosphotungstate and cycloviologen ($[\text{PW}\{\text{CyV}\}]_n$ film) was monitored by electrochemical method. Phosphotungstate anions that have Dawson structures are unstable in neutral and basic aqueous solution and may undergo rapid decomposition processes [19, 20]. Therefore, electrochemical studies of $[\text{PW}\{\text{CyV}\}]_n$ film with phosphotungstate anion were carried out in acidic aqueous solutions. Each cyclic voltammogram was recorded in a pH = 3 phosphate buffer solution after assembly of a layer of anion species and a layer of cation on electrode (glassy carbon, GC). A serial of such recorded cyclic voltammograms is shown in Fig. 3.1.1. As can be seen, in the range from $-0.65$ to $0.30$ V, three pairs of redox peaks are located at about $0.02$ V (I-I'), $-0.27$ V (II-II') and $-0.56$ V (III-III'). These peaks of assembled layers are similar to that of $1 \text{ mM}$ phosphotungstate in pH=3 PBS (inset A of Fig. 3.1.1) except that the peaks in former curves are sharper and also slightly shifted. This character is also similar to those reported in other papers [21, 22]. A cyclic voltammetric study of GC shows that no
peak can be observed in the same potential range. Based on the above results and reported study of electrochemical behavior of phosphotungstate, electrochemical behavior shown in Fig. 3.1.1 was ascribed to redox of phosphotungstate and the three pairs of peaks correspond to reduction and oxidation through one-, one-, and two-electron processes respectively [19]. Another characteristic can be noted is that peak current of all the three pairs of peaks increased with increase of number of bilayers. Peak current ($I_p$) of the redox pair located at about -0.57 V was plotted versus number of bilayer and shown in inset B, it is obvious that $I_p$ increases proportionally with number of bilayers. This indicates that the successive fabrication of the layer-by-layer self-assembled films on electrode and the amount of the anionic species assembled on electrode surface in each bilayer is almost the same.

Fig. 3.1.2 shows the cyclic voltammograms obtained for the [PW(CyV)$_n$] film at five different pH values, pH 1 up to pH 7. As can be seen, the three redox processes are all clearly visible in Fig. 3.1.2 (a) to (f). The first two monoelectronic waves are seen to be pH independent while the third bielectronic wave is seen to be pH dependent, negative shift about 56 mV/pH with the increase of pH value. This agrees with solution behavior for this heteropoly oxometalate [23].

$$P_2W_{18}O_{62}^{6-} + e^- \rightarrow P_2W_{18}O_{62}^{7-} \quad (3.1.1)$$

$$P_2W_{18}O_{62}^{7-} + e^- \rightarrow P_2W_{18}O_{62}^{8-} \quad (3.1.2)$$

$$P_2W_{18}O_{62}^{8-} + 2e^- + 2H^+ \rightarrow H_2P_2W_{18}O_{62}^{8-} \quad (3.1.3)$$

As the pH of the solution is increased to pH 6, the third two-electron redox peaks split into two reversible one-electron peaks and (3.1.3) is replaced by (3.1.4) and (3.1.5).

$$P_2W_{18}O_{62}^{8-} + e^- \rightarrow P_2W_{18}O_{62}^{9-} \quad (3.1.4)$$
\[ P_2W_{18}O_{62}^{9-} + e^- \rightarrow P_2W_{18}O_{62}^{10-} \]  \hspace{1cm} (3.1.5)

So that it is reasonable that four redox peaks are visible in Fig. 3.1.2 (g).

No electrochemical character of cycloviologen can be observed in cyclic voltammogram obtained on the [PW|CyV] \(_n\) film. In other words, assembly of cycloviologen cannot be directly probed by electrochemical method.

### 3.1.3.2 Characterization of [PW|CyV] \(_n\) film by UV-Vis spectroscopy

Cycloviologen was found to absorb UV radiation at 266 nm, which provides possibility to monitor assembly of cycloviologen in [PW|CyV] \(_n\) film by UV-visible spectroscopy. For this purpose, [PW|CyV] \(_n\) films were fabricated on ITO-coated quartz substrate under the same conditions and their UV-visible spectrum were collected and shown in Fig. 3.1.3. It can be seen in the figure that the intensity of the absorption peaks increased with number of bilayers. Furthermore the inset in Fig. 3.1.3 depicts a linear correlation between the absorption intensity at 266 nm and the number of bilayers, which indicating that, similar to the anionic species, cycloviologen is assembled layer-by-layer in [PW|CyV] \(_n\) film and the amount of cycloviologen assembled after phosphotungstate anion in each bilayer is almost the same.

Combining electrochemical and UV-visible characteristics of [PW|CyV] \(_n\) film prepared in this study, some interesting features can be found. For a specific substrate, all the results prove that the amount of either anionic or cationic species assembled in the according bilayer is constant. Our investigation also found that amount of building block was limited by a certain value even extra dipping time was
applied. This is reasonable for a layer-by-layer structure constructed by electrostatic attraction, i.e., equal amount of oppositely charged sites are generated with consumption of charge sites by electronic neutralization accompanied self-assembly. Generally, a layer-by-layer film constructed by electrostatic attraction was successfully fabricated with our strategy.

3.1.3.3 Application in electrochromic coating

In general, the redox of POM is accompanied by the visible colour change during the cyclic voltammetry of POM in an aqueous acidic solution originates in the presence of an intervalence charge-transfer band ($M^{V}-O-M^{VI}$ $\leftrightarrow$ $M^{VI}-O-M^{V}$, here $M$ = Mo or W) which is readily affected by an applied voltage and/or light and hence it manifests itself in electrochromism and/or photochromism [24, 25]. For viologen, in general, a solution of viologen is colourless, but upon one-electron reduction, an intensely coloured insoluble film is formed, adhering to the electrode surface. It is the ability to form a coloured image on a conducting substrate (‘write mode’) or remove the coloured image by reversing the polarity of the power source (‘bleach mode’) re-oxidizing the coloured radical cation electrolytically [26]. The electrochromism accessible in viologen systems arises from the reactions:

\[
\begin{align*}
\text{R}^S\text{N} \leftrightarrow \text{R}^S\text{N} + e^- & \quad \text{(usually colourless)} \quad \text{R}^S\text{N} \leftrightarrow \text{R}^S\text{N} + e^- \quad \text{colour 1} \\
\text{R}^S\text{N} \leftrightarrow \text{R}^S\text{N} + e^- & \quad \text{(colour 1)} \quad \text{R}^S\text{N} \leftrightarrow \text{R}^S\text{N} + e^- \quad \text{colour 2}
\end{align*}
\]
Electrochromic coating of [PW|CyV]$_n$ multilayer films was investigated using *in situ* spectroelectrochemical technique by absorption spectrometric measurements at different applied potentials. Since one of phosphotungstate redox process is pH dependent in our study range, the effect of pH value in this electrochromic system is also investigated. The reversibility and the stability of the electrochromic properties associated with the oxidation and the reduction of [PW|CyV]$_n$ film were examined by spectroelectrochemical switch-potential experiments.

In situ UV-Vis spectra of 30-bilayer [PW|CyV]$_n$ film on ITO substrate in pH=3 phosphate buffer solution (PBS) at various applied potentials from 0.00, -0.20, -0.40, -0.50, -0.60, -0.65, down to -0.70 V and from -0.70, -0.60, -0.50, -0.40, -0.20, back to 0.00 V are shown in Fig. 3.1.6 and Fig. 3.1.7 respectively. As can be seen, at the open circuit potential, about 0.00 V in this system, the main absorption appears in 380 ~ 600 nm though the absorption is very weak. So the freshly prepared 30-bilayer [PW|CyV]$_n$ film should be pale orange, according to the textbook chart of approximate wavelength ranges of reflected colours [27]. As the potential is applied on the film from 0.00 V step down to -0.70 V, the absorption increases gradually first from 550 nm, which can be assigned to the electrochromism of phosphotungstate, followed by the new absorption gradual increase in the range of 350 ~ 450 and 450 ~ 580 nm, which can be assigned to the electrochromism of cycloioiologen. So that the film should turn blue first, then a little violet colour should appear after the potential down to -0.50 V, but the film should be still in blue since the main absorption is in the range of 600 ~ 850 nm. When the step potential is applied from -0.70 V back to 0.00 V, the open circuit potential, the absorption decreases correspondingly to the original (Fig. 3.1.7). Therefore, the coloured film
bleaches gradually in the potential modulation process. It means that the whole process from pale colour to colouration followed by bleaching is reversible.

Since the third redox process of phosphotungstate is pH dependent, the electrochromic behavior of the film should be studied in various pH values. Some similar characteristics can be observed between the UV-Vis spectra on the \([PW(CyV)]_n\) film in pH 1 (Fig. 3.1.4 and Fig. 3.1.5), pH 5 (Fig. 3.1.8 and Fig. 3.1.9) and pH 7 (Fig. 3.1.10 and Fig. 3.1.11) buffer solutions under different applied potentials. These similar characteristics in the figures testify that the whole process between pale colour and colouration is also reversible in pH 1, pH 5 and pH 7 systems.

Some new characteristics can be observed by comparing the spectra between different pH values under various applied potentials. The absorbance in the range of over 600 nm, which belong to the electrochromic change of phosphotungstate, arrives at a certain maximum (around 0.35 on this film) at different potential, for example, -0.60 V in pH 1, -0.70 V in pH 3, -0.75 V in pH 5 and -0.85 V in pH 7. Such results are reasonable and consistent with the electrochemical study results shown in Fig. 3.1.2 because the third redox process of phosphotungstate is pH dependent. The absorbance in the range of 350 ~ 580 nm, which belong to the electrochromic change of cyclovioiologen, is independent of pH.

All the above results in electrochromic coating show that a wide range wavelength is involved in electrochromism on \([PW(CyV)]_n\) film and many different kinds of electrochromic spectra can be achieved flexibly by adjusting pH value and applied potential. These features should be very useful in practice.
Although the film can be used in neutral electrolyte, it is the critical condition to the film due to the possibility of decomposition of the film. So the electrochromic application of the film in neutral electrolyte is not suggested.

To study the electrochromic behavior of \([\text{PW}|\text{CyV}]_n\) film in detail, the combined electrochemical and UV-Vis spectroscopic methods was developed. Fig. 3.1.12 and Fig. 3.1.13 show UV-Vis absorbance response of 30-bilayer \([\text{PW}|\text{CyV}]_n\) film on ITO substrate in pH=5 phosphate buffer solution under potential switched between 0.00 and -0.70 V at 380 nm and at 650 nm respectively. The result that the process is reversible is testified again. In other words, \([\text{PW}|\text{CyV}]_n\) film has good write-erase efficiency in electrochromic coating. Fig. 3.1.13 also shows the potential of the film to be a long-life electrochromic material. In fact, such experiments can be taken at any wavelength between 350 and 900 nm, which covering the whole visible light region.

3.1.4 Conclusions

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged phosphotungstate and positively charged cycloviologen on various substrates including glassy carbon, ITO and quartz. The thickness of such film can be controlled easily in the assembly process. We have systematically characterized our self-assembled film and have monitored the growth of the film by using electrochemical method and UV-Vis spectroscopies. The results show that both phosphotungstate and cycloviologen are assembled layer-by-layer on substrate. The \([\text{PW}|\text{CyV}]_n\) film exhibits excellent electrochromic behavior and good
stability. Such film may become one of the most promising electrochromic materials in practice.

3.1.5 References


Legends and Figures

Fig. 3.1.1 Cyclic voltammograms of [PW|CyV]_n films in pH=3 phosphate buffer solution. From inside to outside: two, four, six, eight, ten, twelve bilayers. Scan rate: 100 mV s\(^{-1}\). Inset A: cyclic voltammogram of glassy carbon in 1mM phoshotungstate in pH=3 phosphate buffer solution. Inset B: the relationship between peak current at -0.570 V and No. of bilayers.

Fig. 3.1.2 Cyclic voltammograms of 20-bilayer [PW|CyV]_n film in (a) pH=1, (b) pH=2, (c) pH=3, (d) pH=4, (e) pH=5, (f) pH=6 and (g) pH=7 buffer solution. Scan rate: 100 mV s\(^{-1}\).

Fig. 3.1.3 UV-Vis spectra of [PW|CyV]_n film on a quartz slide. From bottom to top: two, four, six, eight, ten, twelve bilayers. The inset depicts the relationship between absorbance at 266 nm and No. of bilayers.

Fig. 3.1.4 In situ UV-Vis spectra of 30-bilayer [PW|CyV]_n film on ITO substrate in pH=1 buffer solution at open circuit potential and applied potentials from 0.00, -0.20, -0.40, -0.50, down to -0.60 V.

Fig. 3.1.5 In situ UV-Vis spectra of 30-bilayer [PW|CyV]_n film on ITO substrate in pH=1 buffer solution at applied potentials from -0.60, -0.50, -0.40, -0.20, up to 0.00 V.

Fig. 3.1.6 In situ UV-Vis spectra of 30-bilayer [PW|CyV]_n film on ITO substrate in pH=3 phosphate buffer solution at applied potentials from 0.00, -0.20, -0.40, -0.50, -0.60, -0.65, down to -0.70 V.

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Fig. 3.1.7 In situ UV-Vis spectra of 30-bilayer [PW|CyV]ₙ film on ITO substrate in pH=3 phosphate buffer solution at applied potentials from -0.70, -0.60, -0.50, -0.40, -0.20, up to 0.00 V.

Fig. 3.1.8 In situ UV-Vis spectra of 30-bilayer [PW|CyV]ₙ film on ITO substrate in pH=5 phosphate buffer solution at applied potentials from 0.00, -0.20, -0.40, -0.50, -0.60, -0.70, down to -0.75 V.

Fig. 3.1.9 In situ UV-Vis spectra of 30-bilayer [PW|CyV]ₙ film on ITO substrate in pH=5 phosphate buffer solution at applied potentials from -0.75, -0.60, -0.40, -0.20, up to 0.00 V.

Fig. 3.1.10 In situ UV-Vis spectra of 30-bilayer [PW|CyV]ₙ film on ITO substrate in pH=7 phosphate buffer solution at applied potentials from 0.00, -0.20, -0.40, -0.60, -0.70, -0.75, -0.80, down to -0.85 V.

Fig. 3.1.11 In situ UV-Vis spectra of 30-bilayer [PW|CyV]ₙ film on ITO substrate in pH=7 phosphate buffer solution at applied potentials from -0.85, -0.75, -0.70, -0.60, -0.40, up to 0.00 V.

Fig. 3.1.12 UV-Vis absorbance response of 30-bilayer [PW|CyV]ₙ film on ITO substrate in pH=5 phosphate buffer solution at potential switched between 0.00 and -0.70 V. Wavelength: 380 nm.

Fig. 3.1.13 UV-Vis absorbance response of 30-bilayer [PW|CyV]ₙ film on ITO substrate in pH=5 phosphate buffer solution at potential switched between 0.00 and -0.70 V. Wavelength: 600 nm.
Fig. 3.1.1
Fig. 3.1.3

![Graph showing absorbance vs. wavelength and number of bilayers. The inset graph shows a linear relationship at 266 nm.](image)
Fig. 3.1.4

Absorbance

Wavelength (nm)

E = -0.60 V
-0.50 V
-0.40 V
-0.20 V
0.00 V
open circuit

CyV
PW
Fig. 3.1.7

[Graph showing absorbance vs. wavelength with annotations for CyV and PW at different voltages.]
Fig. 3.1.8
Fig. 3.1.9

![Absorbance vs. Wavelength Graph]

- Absorbance
- Wavelength (nm)

- CyV
- CyV
- CyV + PW
- PW

E = -0.75 V
-0.60 V
-0.40 V
-0.20 V
0.00 V
Fig. 3.1.10
Fig. 3.1.11
Fig. 3.1.12
Fig. 3.1.13
3.2 Layer-by-Layer Self-Assembled Multilayer Molecular Film from Viologen Tetramer and Phosphotungstate

Abstract

Layer-by-layer self-assembled multilayer molecular films with viologen tetramer cation and phosphotungstate anion on various substrates (e.g. glassy carbon, ITO, KRS-5) were prepared by alternating dipping the substrates in anionic and cationic species. The assembly process was monitored by electrochemical method, UV-Vis and FT-IR spectroscopies. Together with results of electrochemical, UV-Vis measurements and molecular level information obtained by FT-IR spectroscopy shows that, equal amount of building blocks was assembled in each fabrication process. Such newly developed film has a successful application in electrochromic coating.

3.2.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable
attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be easily modified according to the aim of the study [10-12].

An electroactive species often exhibits new optical absorption bands, i.e. shows a new colour, in accompaniment with an electron-transfer or ‘redox’ reaction in which it either gains or loses an electron; that is to say, it undergoes reduction or oxidation. Such colouration was first termed ‘electrochromism’ in 1961 by Platt [13]. Now electrochromism is defined as the electrochemical generation of colour in accompaniment with an electron transfer (or ‘redox’) reaction [14]. Although electrochromic systems as displays need to compete with both cathode ray tube (CRT) and liquid crystal display (LCD) for commercial viability, they possess many advantages over both [14]. In most cases, only one redox active species is used as electrochromic material in one system [15, 16]. To the best of our knowledge, few studies focus on two kinds of redox active species, especially combined organic and inorganic species, in the application of electrochromic system.

Here we describe a new self-assembled homogeneous molecular film with negatively charged phosphotungstate (PW, a kind of heteropoly oxometalate) and positively charged viologen tetramer (VT, a kind of viologen). We studied in detail its application in electrochromic coating.
3.2.2 Experimental

3.2.2.1 Reagents and materials.

Phosphotungstate (K₆P₂W₁₈O₆₂) and viologen tetramer were synthesized and isolated according to reference [17, 18]. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolyte. Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.) and KRS-5 polished disc windows (13mm x 1mm) from Wilmad (U.S.A.).

3.2.2.2 Instrumentation.

(i) Electrochemical measurements

The cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, Φ3mm, MF-2012, BAS, U.S.A.) as the working electrode. Prior to each experiment, GC electrode
was polished by using alumina powder of size 1, 0.3, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.

(ii) *UV-visible spectroscopy and in situ UV-visible spectroscopy*

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the transparent ITO or quartz substrate. Bare ITO or quartz was used as the reference.

For *in situ* UV-Vis experiments in electrochromism, potential applied on the electrode was controlled by a CHI 750A electrochemical workstation (CH Instruments Inc, USA). Platinum wire was used as counter electrode and Ag/AgCl electrode was used as reference electrode. Phosphate buffer solution was employed as supporting electrolyte. Modified ITO electrode (30 x 30 x 0.5 mm float glass slide, ITO-coated two sides, Della Technologies, Limited-Stillwater, U.S.A) was used as a working electrode.

(iii) *Fourier Transfer Infrared spectroscopy (FTIR)*

The FTIR spectroscopic measurements were carried out on a Perkin Elmer Spectrum One spectrometer. The sample film was on KRS-5 disc.
3.2.2.3 Fabrication of [PW|VT]_n film.

The self-assembled films on different substrates were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 5 mM P_2W_18O_62^{6-} (dissolved in pH=3 PBS) for 5 minutes, then a layer of P_2W_18O_62^{6-} spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of P_2W_18O_62^{6-} were transferred into 2 mM viologen tetramer (dissolved in pH=6 PBS) for 5 minutes, then a layer of viologen tetramer spontaneously adsorbed onto the P_2W_18O_62^{6-}-coated substrates. Finally, the substrates were washed with water. Thus the self-assembled film named [PW|VT]_n was prepared successfully. The films with different thickness can be achieved by repeating the above assembly process. The whole process is demonstrated in Fig. 2.1.1.

3.2.3 Results and discussion

3.2.3.1 Electrochemical characterization

Fabrication of self-assembled film of phosphotungstate and viologen tetramer ([PW|VT]_n) was monitored by electrochemical method. Phosphotungstate anions that have Dawson structures are unstable in neutral and basic aqueous solution and may undergo rapid decomposition processes [19, 20]. Therefore, electrochemical studies of [PW|VT]_n film with phosphotungstate anion were carried out in acidic aqueous solutions. Each cyclic voltammogram was recorded in a pH = 3 phosphate buffer
solution after assembly of a layer of anion species and a layer of cation on electrode (glassy carbon, GC). A serial of such recorded cyclic voltammograms is shown in Fig. 3.2.1. As can be seen, in the range from -0.65 to 0.25 V, three pairs of redox peaks are located at about 0.02 V (I-I’), -0.27 V (II-II’) and -0.56 V (III-III’). These peaks of assembled layers are similar to that of 1 mM phosphotungstate in pH=3 PBS (inset A of Fig. 3.2.1) except that the peaks in former curves are sharper and also slightly shifted. This character is also similar to those reported in other papers [21, 22]. A cyclic voltammetric study of GC shows that no peak can be observed in the same potential range. Based on the above results and reported study of electrochemical behavior of phosphotungstate, electrochemical behavior shown in Fig. 3.2.1 was ascribed to redox of phosphotungstate and the three pairs of peaks correspond to reduction and oxidation through one-, one-, and two-electron processes respectively [19]. Another characteristic can be noted is that peak current of all the three pairs of peaks increased with increase of number of bilayers. Peak current ($I_p$) of the redox pair located at about -0.58 V was plotted versus number of bilayer and shown in inset B, it is obvious that $I_p$ increases proportionally with number of bilayers. This indicates that the successive fabrication of the layer-by-layer self-assembled films on electrode and the amount of the anionic species assembled on electrode surface in each bilayer is almost the same.

Fig. 3.2.2 shows the cyclic voltammograms obtained for the [PW|VT]$_n$ film at five different pH values, pH 1 up to pH 5. As can be seen, the three redox processes are all clearly visible in the five curves. The first two monoelectronic waves are seen to be pH independent while the third bielectronic wave is seen to be pH dependent,
negative shift about 56 mV/pH with the increase of pH value. This agrees with solution behavior for this heteropoly oxometalate [23].

\[
P_2W_{18}O_{62}^{6-} + e^- \rightarrow P_2W_{18}O_{62}^{7-} \quad (3.2.1)
\]

\[
P_2W_{18}O_{62}^{7-} + e^- \rightarrow P_2W_{18}O_{62}^{8-} \quad (3.2.2)
\]

\[
P_2W_{18}O_{62}^{8-} + 2e^- + 2H^+ \rightarrow H_2P_2W_{18}O_{62}^{8-} \quad (3.2.3)
\]

No electrochemical character of viologen tetramer can be observed in cyclic voltammogram obtained on the [PW|VT]_n film. In other words, assembly of viologen tetramer can not be directly probed by electrochemical method.

### 3.2.3.2 Characterization of [PW|VT]_n film by UV-Vis spectroscopy

Viologen tetramer was found to absorb UV radiation at 264 nm, which provides possibility to monitor assembly of viologen tetramer in [PW|VT]_n film by UV-visible spectroscopy. For this purpose, [PW|VT]_n films were fabricated on ITO-coated quartz substrate under the same conditions and their UV-visible spectrum were collected and shown in Fig. 3.2.3. It can be seen in the figure that the intensity of the absorption peaks increased with number of bilayers. Furthermore the inset in Fig. 3.2.3 depicts a linear correlation between the absorption intensity at 264 nm and the number of bilayers, which indicating that, similar to the anionic species, viologen tetramer is assembled layer-by-layer in [PW|VT]_n film and the amount of viologen tetramer assembled after phosphotungstate anion in each bilayer is almost the same.

### 3.2.3.3 Characterization of [PW|VT]_n film by FTIR spectroscopy
FTIR is capable of providing quantitative and structural information of molecular level and is regarded as a powerful tool for both qualitative and quantitative study. Therefore, FT-IR spectroscopic studies were conducted to monitor growth of [PW\VT]_n film and investigated the state of assembled species.

[PW\VT]_n film was fabricated on a KRS-5 disc which is capable of transmitting IR radiation. During the course of alternatively assembling of phosphotungstate and viologen tetramer, spectrum was collected after either an anionic or cationic layer was assembled onto the substrate.

In the first spectrum of the film (Fig. 3.2.4 (a)), some peaks appear in 400-1150 cm\(^{-1}\). As we know, the only compound exists in the first layer of the film is phosphotungstate, so the peaks can be assign to IR absorption of phosphotungstate. In the spectrum collected after first layer of viologen tetramer following the first layer assembly of phosphotungstate was assembled (Fig. 3.2.5 (a)), some new peaks, which are absent in Fig. 3.2.4 (a), can be clearly observed in 1150 – 1800 cm\(^{-1}\). So the new peaks can be assign to IR absorption of viologen tetramer.

Fig. 3.2.4 shows a serial of IR spectra recorded after each assembly of phosphotungstate on KRS-5 disc. From the second spectrum (b) to the last spectrum (e) in Fig. 3.2.4, we noticed two main points: one is that some new peaks appear at the location between 1150 and 1800 cm\(^{-1}\); the other is that the intensity of all the peaks increases with number of bilayers. Based on these evidences, we can say that the peaks between 1150 and 1800 cm\(^{-1}\) belong to viologen tetramer and layer-by-layer molecular film was fabricated successfully.

Fig. 3.2.5 shows IR spectra of [PW\VT]_n film collected after viologen tetramer was assembled on the disc. When observed in a quantitative point of view, the
intensity of the peaks in Fig. 3.2.5 also increases with number of bilayers. In other words, the characters of Fig. 3.2.5 are consistent with Fig 3.2.4.

Such assignment of the peaks can be easily identified when the IR spectra in Fig 3.2.4 and Fig. 3.2.5 are compared with IR spectra of pure phosphotungstate and viologen tetramer in aqueous. Each of the spectra can be divided into two parts (400 – 1150 cm\(^{-1}\) and 1150 – 1800 cm\(^{-1}\)). The peaks at 400 – 1150 cm\(^{-1}\) can also be observed in the spectrum of pure phosphotungstate and the locations of the peaks are almost the same. This comparison indicates that after assembly process, molecular structure of phosphotungstate is retained. For the part of spectrum of 1150 – 1800 cm\(^{-1}\), it is much similar with the spectrum of pure viologen tetramer in aqueous both in peak location and relative intensity of the peaks. Based on the above results, no substantial change of structure of both anionic and cationic species can be observed, indicating that no substantial reaction occurred in the process of assembly. This can be evidence to show that the bilayers are assembled by electrostatic attraction.

After comparing the spectra in Fig. 3.2.4 and Fig. 3.2.5 carefully, the growth of the peaks in 400-1150 cm\(^{-1}\), which belong to phosphotungstate, can be only observed after each layer phosphotungstate is assembled on the substrate. And the growth of the peaks in 1150-1800 cm\(^{-1}\), which belong to viologen tetramer, can be only observed after each layer viologen tetramer is assembled on the substrate. The peaks located at 1090 cm\(^{-1}\), belonging to phosphotungstate, and the peaks at 1638 cm\(^{-1}\), belonging to viologen tetramer, were integrated respectively in Fig. 3.2.4 and Fig. 3.2.5. The result is shown in Fig. 3.2.6. The area of the peaks is proportional to number of bilayers. This result is consistent with results of electrochemical and UV-Vis spectroscopic studies, indicating that both phosphotungstate and viologen
tetramer are assembled on substrate layer-by-layer. It also testifies that a constant amount of either anionic or cationic species was assembled in the bilayer of [PW[VT]$_n$] film.

Combining electrochemical, UV-visible and IR characteristics of [PW[VT]$_n$] film prepared in this study, some interesting features can be found. First, for a specific substrate, all the results prove that the amount of either anionic or cationic species assembled in the according bilayer is constant. Our investigation also found that amount of building block was limited by a certain value even extra dipping time was applied. This is reasonable for a layer-by-layer structure constructed by electrostatic attraction, i.e., equal amount of oppositely charged sites are generated with consumption of charge sites by electronic neutralization accompanied self-assembly. Second, molecular level information provided by FT-IR study indicates that both of the building block species remain their structure after the assembly process. For viologen tetramer it is obvious that it exist in the film in a form of cation. Generally, a layer-by-layer film constructed by electrostatic attraction was successfully fabricated with our strategy.

3.2.3.4 Application in electrochromic coating

In general, the redox of POM is accompanied by the visible colour change during the cyclic voltammetry of POM in an aqueous acidic solution originates in the presence of an intervalence charge-transfer band ($M^{V^-}\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\
manifests itself in electrochromism and/or photochromism [24, 25]. For viologen, in general, a solution of viologen is colourless, but upon one-electron reduction, an intensely coloured insoluble film is formed, adhering to the electrode surface. It is the ability to form a coloured image on a conducting substrate (‘write mode’) or remove the coloured image by reversing the polarity of the power source (‘bleach mode’) re-oxidizing the coloured radical cation electrolytically [26]. The electrochromism accessible in viologen systems arises from the reactions:

\[
R_1^\text{N} - \text{N} - R + e^- \rightleftharpoons R_2^\text{N} - \text{N} - R \quad (3.2.4)
\]

(usually colourless) \hspace{1cm} (colour 1)

\[
R_1^\text{N} - \text{N} - R + e^- \rightleftharpoons R_2^\text{N} - \text{N} - R \quad (3.2.5)
\]

(colour 1) \hspace{1cm} (colour 2)

Electrochromic coating of \([PW\vert VT]_n\) multilayer films was investigated using \textit{in situ} spectroelectrochemical technique by absorption spectrometric measurements at different applied potentials. Since one of phosphotungstate redox process is pH dependent in our study range, the effect of pH value in this electrochromic system is also investigated. The reversibility and the stability of the electrochromic properties associated with the oxidation and the reduction of \([PW\vert VT]_n\) film were examined by spectroelectrochemical switch-potential experiments.

In situ UV-Vis spectra of 30-bilayer \([PW\vert VT]_n\) film on ITO substrate in pH=1 buffer solution at various applied potentials from 0.00, -0.20, -0.40, -0.50, -0.55, down to -0.60 V and from -0.60, -0.55, -0.50, -0.40, -0.20, back to 0.00 V are shown in Fig. 3.2.7 and Fig. 3.2.8 respectively. As can be seen, at the open circuit potential, about 0.20 V in this system, the main absorption appears in 380 – 600 nm though the
absorption is very weak. So the freshly prepared 30-bilayer [PW|VT]ₙ film should be pale orange, in fact it is almost colourless, according to the textbook chart of approximate wavelength ranges of reflected colours [27]. As the potential is applied on the film from 0.00 V step down to −0.60 V, the absorption increases gradually first from 465 nm, which can be assigned to the electrochromism of phosphotungstate, followed by the new absorption gradual increase in the range of 350 ~ 580 nm, which can be assigned to the electrochromism of viologen tetramer. So that the film should turn bluer and bluer first when the potential is below 0.00 V, then a little violet colour should appear after the potential down to −0.50 V, but the film should be still in blue since the main absorption is in the range of 600 ~ 850 nm. When the step potential is applied from -0.60 V to 0.00 V, even to the open circuit potential, the absorption decreases correspondingly to the original (Fig. 3.2.8). Therefore, the coloured film bleaches gradually in the potential modulation. It means that the whole process from pale colour to colouration followed by bleaching is reversible.

Since the third redox process of phosphotungstate is pH dependent, the electrochromic behavior of the film should be studied in various pH values. Some similar characteristics can be observed between the UV-Vis spectra on the [PW|VT]ₙ film in pH=3 PBS (Fig. 3.2.9 and Fig. 3.2.10) and in pH=5 PBS (Fig. 3.2.11 and Fig. 3.2.12) under different applied potential. These similar characteristics in the figures testify that the whole process between pale colour and colouration is also reversible in pH=3 and pH=5 systems. Some new characteristics can be observed by comparing the spectra between different pH values under various applied potentials. The absorbance in the range of over 600 nm, which belong to the electrochromic change
of phosphotungstate, arrives at a certain maximum (around 0.4 on this film) at
different potential, for example, -0.60 V in pH=1, -0.70 V in pH=3, -0.75 V in pH=5.
Such results are reasonable and consistent with the electrochemical study results
shown in Fig. 3.2.2 because the third redox process of phosphotungstate is pH
dependent. The absorbance in the range of 350 – 580 nm, which belong to the
electrochromic change of viologen tetramer, is independent of pH.

In fact the film can be used in neutral electrolyte, but it is the critical condition to
the film due to the decomposition of the film. So the electrochromic application of
the film in neutral electrolyte is not suggested.

All the above results in electrochromic coating show that a wide range
wavelength is involved in electrochromism on [PW|VT]$_n$ film and many different
kinds of electrochromic spectra can be achieved flexibly by adjusting pH value and
applied potential. These features should be very useful in practice.

Fig. 3.2.13 shows the pictures of 30-bilayer [PW|VT]$_n$ film on ITO substrate in
pH=3 phosphate buffer solution at applied step potentials from 0.20, -0.20, -0.40, -
0.55, -0.65, -0.60, -0.55, -0.40, -0.20, 0.00, back to 0.20 V. It is obvious that the film
exhibits uniform colour changes in electrochromic coating and the reversible process
of colouration and bleach can be controlled flexibly by simply applying certain
potential value on the film.

To study the electrochromic behavior of [PW|VT]$_n$ film in detail, some combined
electrochemical and UV-Vis spectroscopic methods were developed. Fig. 3.2.14
shows UV-Vis absorbance response at 550 nm of 30-bilayer [PW|VT]$_n$ film on ITO
substrate in pH=5 phosphate buffer solution at applied cyclic potential from 0.00 to –
0.75 V. The absorbance response curve in each cycle is symmetric so the result that
the process is reversible is testified again. In other words, \([\text{PW|VT}]_n\) film has good write-erase efficiency in electrochromic coating.

Such information also can be concluded from the following results in Fig. 3.2.15 and Fig. 3.2.16, which shows UV-Vis absorbance response of 30-bilayer \([\text{PW|VT}]_n\) film on ITO substrate in pH=5 phosphate buffer solution under potential switched between 0.00 and -0.75 V at 380 nm and at 650 nm respectively. Fig. 3.2.16 shows the potential of the film to be a long-life electrochromic material. In fact, such experiments can be taken at any wavelength between 350 and 850 nm, which covering the whole visible light region.

### 3.2.4 Conclusions

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged phosphotungstate and positively charged viologen tetramer on various substrates including glassy carbon, ITO and KRS-5 disc. The thickness of such film can be controlled easily in the assembly process. We have systematically characterized our self-assembled film and have monitored the growth of the film by using electrochemical method, UV-Vis and FT-IR spectroscopies. The results show that both phosphotungstate and viologen tetramer assembled layer-by-layer on substrate. The \([\text{PW|VT}]_n\) film exhibits excellent electrochromic behavior and good stability. Such film may become one of the most promising electrochromic materials in practice.
3.2.5 References


Legends and Figures

Fig. 3.2.1 Cyclic voltammograms of [PW|VT]$_n$ films in pH=3 phosphate buffer solution. From inside to outside: two, four, six, eight, ten, twelve, fourteen, sixteen, eighteen bilayers. Scan rate: 100 mV s$^{-1}$. Inset A: cyclic voltammogram of glassy carbon in 1mM phosphotungstic acid in pH=3 phosphate buffer solution. Inset B: the relationship between peak current at $-0.580$ V and No. of bilayers.

Fig. 3.2.2 Cyclic voltammograms of 18-bilayer [PW|VT]$_n$ film in (a) pH=1, (b) pH=2, (c) pH=3, (d) pH=4 and (e) pH=5 phosphate buffer solution. Scan rate: 100 mV s$^{-1}$.

Fig. 3.2.3 UV-Vis spectra of [PW|VT]$_n$ film on ITO-coated quartz substrate. (a) one bilayer, (b) two bilayers, (c) three bilayers, (d) four bilayers, (e) five bilayers and (f) six bilayers. The inset depicts the relationship between absorbance at 264 nm and No. of bilayers.

Fig. 3.2.4 FTIR spectra of [PW|VT]$_n$ film on KRS-5 disc. Each spectrum was collected after phosphotungstate was assembled on KRS-5 disc. (a) 0.5, (b) 1.5, (c) 2.5, (d) 3.5, (e) 4.5 bilayers. $R = 4$ cm$^{-1}$. The spectra between 1150 and 1800 cm$^{-1}$ were amplified 2 times.

Fig. 3.2.5 FTIR spectra of [PW|VT]$_n$ film on KRS-5 disc. Each spectrum was collected after viologen tetramer was assembled on KRS-5 disc. (a) 1, (b) 2, (c) 3, (d) 4, (e) 5 bilayers. $R = 4$ cm$^{-1}$. The spectra between 1150 and 1800 cm$^{-1}$ were amplified 2 times.

Fig. 3.2.6 The relationship between the area of the peaks at 1090 cm$^{-1}$ and 1638 cm$^{-1}$ and No. of bilayers in Fig. 3(■) and Fig. 4(○).
Fig. 3.2.7 In situ UV-Vis spectra of 30-bilayer [PW\VT]_n film on ITO substrate in pH=1 phosphate buffer solution at applied potentials from 0.00, -0.20, -0.40, -0.50, -0.55, down to -0.60 V.

Fig. 3.2.8 In situ UV-Vis spectra of 30-bilayer [PW\VT]_n film on ITO substrate in pH=1 phosphate buffer solution at applied potentials from -0.60, -0.55, -0.50, -0.40, -0.20, up to 0.00 V.

Fig. 3.2.9 In situ UV-Vis spectra of 30-bilayer [PW\VT]_n film on ITO substrate in pH=3 phosphate buffer solution at applied potentials from 0.00, -0.20, -0.50, -0.55, -0.60, -0.65, down to -0.70 V.

Fig. 3.2.10 In situ UV-Vis spectra of 30-bilayer [PW\VT]_n film on ITO substrate in pH=3 phosphate buffer solution at applied potentials from -0.70, -0.60, -0.50, -0.40, -0.20, up to 0.00 V.

Fig. 3.2.11 In situ UV-Vis spectra of 30-bilayer [PW\VT]_n film on ITO substrate in pH=5 phosphate buffer solution at applied potentials from 0.00, -0.20, -0.40, -0.50, -0.60, -0.65, -0.70, down to -0.75 V.

Fig. 3.2.12 In situ UV-Vis spectra of 30-bilayer [PW\VT]_n film on ITO substrate in pH=5 phosphate buffer solution at applied potentials from -0.75, -0.65, -0.50, -0.40, -0.20, up to 0.00 V.

Fig. 3.2.13 The pictures of 30-bilayer [PW\VT]_n film on ITO substrate in pH=3 phosphate buffer solution at applied step potentials from 0.20, -0.20, -0.40, -0.55, -0.65, -0.60, -0.55, -0.40, -0.20, 0.00, back to 0.20 V.

Fig. 3.2.14 UV-Vis absorbance response of 30-bilayer [PW\VT]_n film on ITO substrate in pH=5 phosphate buffer solution at applied cyclic potential from 0.00 to -0.75 V. Scan rate: 10 mV s⁻¹. 2 Cycles. Wavelength: 550 nm.
Fig. 3.2.15 UV-Vis absorbance response of 30-bilayer [PW|VT]ₙ film on ITO substrate in pH=5 phosphate buffer solution at potential switched between 0.00 and -0.75 V. Wavelength: 380 nm.

Fig. 3.2.16 UV-Vis absorbance response of 30-bilayer [PW|VT]ₙ film on ITO substrate in pH=5 phosphate buffer solution at potential switched between 0.00 and -0.75 V. Wavelength: 650 nm.
Fig. 3.2.1
Fig. 3.2.2
Fig. 3.2.4
Fig. 3.2.5
Fig. 3.2.6

- Peak at 1090 cm$^{-1}$
- Peak at 1638 cm$^{-1}$

Peak area vs. No. of bilayers
Fig. 3.2.7
Fig. 3.2.8
Fig. 3.2.9
Fig. 3.2.10
Fig. 3.2.11
Fig. 3.2.12
Fig. 3.2.14
Fig. 3.2.15

Absorbance

0.00 V

-0.75 V

Time (s)

0 50 100 150 200 250

0.05
Fig. 3.2.16
3.3 Layer-by-Layer Self-Assembled Multilayer Molecular Film from Viologen Tetramer and Silicotungstate

Abstract

Layer-by-layer self-assembled multilayer molecular films with viologen tetramer cation and silicotungstate anion on various substrates (e.g. glassy carbon, ITO, quartz) were prepared by alternating dipping the substrates in anionic and cationic species. The assembly process was monitored by electrochemical method and UV-Vis spectroscopy. The results of electrochemical and UV-Vis measurements show that equal amount of building blocks was assembled in each fabrication process. Such newly developed film has a successful application in electrochromic coating.

3.3.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate
adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be easily modified according to the aim of the study [10-12].

An electroactive species often exhibits new optical absorption bands, i.e. shows a new colour, in accompaniment with an electron-transfer or ‘redox’ reaction in which it either gains or loses an electron; that is to say, it undergoes reduction or oxidation. Such colouration was first termed ‘electrochromism’ in 1961 by Platt [13]. Now electrochromism is defined as the electrochemical generation of colour in accompaniment with an electron transfer (or ‘redox’) reaction. Although electrochromic systems as displays need to compete with both cathode ray tube (CRT) and liquid crystal display (LCD) for commercial viability, they possess many advantages over both [14]. In most cases, only one redox active species is used as electrochromic material in one system [15, 16]. To the best of our knowledge, few studies focus on two kinds of redox active species, especially combined organic and inorganic species, in the application of electrochromic system.

Here we describe a new self-assembled homogeneous molecular film with negatively charged silicotungstate (SiW, a kind of heteropoly oxometalate) and positively charged viologen tetramer (VT, a kind of viologen). We studied in detail its application in electrochromic coating.

3.3.2 Experimental
3.3.2.1 Reagents and materials.

Viologen tetramer was synthesized and isolated according to reference [17]. Silicotungstate (K₄SiW₁₂O₄₀) was purchased from Aldrich and used as received. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolyte. Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.).

3.3.2.2 Instrumentation.

(i) Electrochemical measurements

The cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, Φ3mm, MF-2012, BAS, U.S.A.) as the working electrode. Prior to each experiment, GC electrode was polished by using alumina powder of size 1, 0.3, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.
(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the transparent ITO or quartz substrate. Bare ITO or quartz was used as the reference.

(iii) In situ UV-visible spectroscopy

Potential applied on the electrode was controlled by a CHI 750A electrochemical workstation (CH Instruments Inc, USA). Platinum wire was used as a counter electrode and Ag/AgCl electrode was used as a reference electrode. Phosphate buffer solution was employed as supporting electrolyte. Modified ITO electrode (30 x 30 x 0.5 mm float glass slide, ITO-coated two sides, Della Technologies, Limited-Stillwater, U.S.A) was used as a working electrode.

3.3.2.3 Fabrication of [SiW|VT]_n film.

The self-assembled films on different substrates were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 5 mM SiW_{12}O_{40}^{4-} (dissolved in pH=3 PBS) for 5 minutes, then a layer of SiW_{12}O_{40}^{4-} spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of SiW_{12}O_{40}^{4-} were transferred into 2 mM viologen tetramer (dissolved in pH=6 PBS)
for 5 minutes, then a layer of viologen tetramer spontaneously adsorbed onto the SiW$_{12}$O$_{40}^{4-}$-coated substrates. Finally, the substrates were washed with water. Thus the self-assembled film named [SiW|VT]$_n$ was prepared successfully. The films with different thickness can be achieved by repeating the above assembly process. The whole process is demonstrated in Fig. 2.1.1.

### 3.3.3 Results and discussion

#### 3.3.3.1 Electrochemical characterization

Fabrication of self-assembled film of silicotungstate and viologen tetramer ([SiW|VT]$_n$ film) was monitored by electrochemical method. Silicotungstate anions that have Keggin structures are unstable in neutral and basic aqueous solution and may undergo rapid decomposition processes [18, 19]. Therefore, electrochemical studies of [SiW|VT]$_n$ film with silicotungstate anion were carried out in acidic aqueous solutions. Each cyclic voltammogram was recorded in a pH = 3 phosphate buffer solution after assembly of a layer of anion species and a layer of cation on electrode (glassy carbon, GC). A serial of such recorded cyclic voltammograms is shown in Fig. 3.3.1. As can be seen, in the range from −0.90 to 0.10 V, three pairs of redox peaks are located at about -0.22 V (I-I′), -0.61 V (II-II′) and -0.82 V (III-III′). These peaks of assembled layers are similar to that of 1 mM silicotungstate in pH=3 PBS except that the peaks in former curves are sharper and also slightly shifted. This character is also similar to those reported in other papers [20, 21]. A cyclic voltammetric study of GC shows that no peak can be observed in the same potential
range. Based on the above results and reported study of electrochemical behavior of silicotungstate, electrochemical behavior shown in Fig. 3.3.1 was ascribed to redox of silicotungstate and the three pairs of peaks correspond to reduction and oxidation through one-, one-, and two-electron processes respectively [18]. The first two monoelectronic waves are seen to be pH independent while the third bielectronic wave is seen to be pH dependent, negative shift about 56 mV/pH with the increase of pH value. This agrees with solution behavior for this heteropoly oxometalate [22].

\[
\begin{align*}
\text{SiW}_{12}\text{O}_{40}^{4+} + e^- & \rightarrow \text{SiW}_{12}\text{O}_{40}^{5-} & (3.3.1) \\
\text{SiW}_{12}\text{O}_{40}^{5-} + e^- & \rightarrow \text{SiW}_{12}\text{O}_{40}^{6-} & (3.3.2) \\
\text{SiW}_{12}\text{O}_{40}^{6-} + 2e^- + 2H^+ & \rightarrow H_2 \text{SiW}_{12}\text{O}_{40}^{6-} & (3.3.3)
\end{align*}
\]

Another characteristic can be noted is that peak current of all the three pairs of peaks increased with increase of number of bilayers. Peak current \((I_p)\) of the redox pair located at about -0.85 V was plotted versus number of bilayer and shown in inset, it is obvious that \(I_p\) increases proportionally with increase of number of bilayers. This indicates that the successive fabrication of the layer-by-layer self-assembled films on electrode and the amount of the anionic species assembled on electrode surface in each bilayer is almost the same.

No electrochemical character of viologen tetramer can be observed in cyclic voltammogram obtained on the \([\text{SiW[VT]}_n\) film. In other words, assembly of viologen tetramer can not be directly probed by electrochemical method.
3.3.3.2 Characterization of $[\text{SiW}_2\text{VT}_6]$ film by UV-Vis spectroscopy

Viologen tetramer was found to absorb UV radiation at 268 nm, which provides possibility to monitor assembly of viologen tetramer in $[\text{SiW}_2\text{VT}_6]$ film by UV-visible spectroscopy. For this purpose, $[\text{SiW}_2\text{VT}_6]$ films were fabricated on quartz substrate under the same conditions and their UV-visible spectrum were collected and shown in Fig. 3.3.2. It can be seen in the figure that the intensity of the absorption peaks increased with number of bilayers. Furthermore the inset in Fig. 3.3.2 depicts a linear correlation between the absorption intensity at 268 nm and the number of bilayers, which indicating that, similar to the anionic species, viologen tetramer is assembled layer-by-layer in $[\text{SiW}_2\text{VT}_6]$ film and the amount of viologen tetramer assembled after silicotungstate anion in each bilayer is almost the same.

The UV-Vis spectra of the assembly of $[\text{SiW}_2\text{VT}_6]$ film on the ITO substrate are shown in Fig. 3.3.3. The broad band in the range of 350 ~ 650 nm is another characteristic absorption of viologen tetramer. The inset in Fig. 3.3.3 depicts a linear correlation between the absorption intensity at 402 nm and the number of bilayers, which also indicating that viologen tetramer is assembled layer-by-layer in $[\text{SiW}_2\text{VT}_6]$ film and the amount of viologen tetramer assembled after silicotungstate anion in each bilayer is almost the same.

Combining electrochemical and UV-visible characteristics of $[\text{SiW}_2\text{VT}_6]$ film prepared in this study, some interesting features can be found. For a specific substrate, all the results prove that the amount of either anionic or cationic species assembled in the according bilayer is constant. Our investigation also found that amount of building block was limited by a certain value even extra dipping time was
applied. This is reasonable for a layer-by-layer structure constructed by electrostatic attraction, i.e., equal amount of oppositely charged sites are generated with consumption of charge sites by electronic neutralization accompanied self-assembly. Generally, a layer-by-layer film constructed by electrostatic attraction was successfully fabricated with our strategy.

3.3.3.3 Application in electrochromic coating

In general, the redox of POM is accompanied by the visible colour change during the cyclic voltammetry of POM in an aqueous acidic solution originates in the presence of an intervalence charge-transfer band (\(M^{V-O-M^{VI}} \leftrightarrow M^{VI-O-M^{V}}\), here \(M = Mo\) or \(W\)) which is readily affected by an applied voltage and/or light and hence it manifests itself in electrochromism and/or photochromism [23, 24]. For viologen, in general, a solution of viologen is colourless, but upon one-electron reduction, an intensely coloured insoluble film is formed, adhering to the electrode surface. It is the ability to form a coloured image on a conducting substrate (‘write mode’) or remove the coloured image by reversing the polarity of the power source (‘bleach mode’) re-oxidizing the coloured radical cation electrolytically [25]. The electrochromism accessible in viologen systems arises from the reactions:

\[
\begin{align*}
    &\text{(usually colourless)} \quad \text{N-N} + e^- &\quad \text{(colour 1)} \\
    &\text{(colour 1)} \quad \text{N-N} + e^- &\quad \text{(colour 2)}
\end{align*}
\]
Electrochromic coating of [SiW\(\text{VT}\)\(_n\)] multilayer films was investigated using \textit{in situ} spectroelectrochemical technique by absorption spectrometric measurements at different applied potentials. Since one of silicotungstate redox process is pH dependent, the effect of pH value in this electrochromic system is also investigated. The reversibility and the stability of the electrochromic properties associated with the oxidation and the reduction of [SiW\(\text{VT}\)\(_n\)] film were examined by spectroelectrochemical switch-potential experiments.

In situ UV-Vis spectra of 30-bilayer [SiW\(\text{VT}\)\(_n\)] film on ITO substrate in pH=3 phosphate buffer solution (PBS) at various applied potentials from 0.00, -0.20, -0.50, -0.60, -0.65, -0.70, down to -0.75 V and from -0.75, -0.60, -0.50, -0.40, back to -0.20 are shown in Fig. 3.2.6 and Fig. 3.2.7 respectively. As can be seen, at the open circuit potential, about 0.00 V in this system, the main absorption appears in 450 ~ 550 nm though the absorption is weak. So the freshly prepared 30-bilayer [SiW\(\text{VT}\)\(_n\)] film should be pale red according to the textbook chart of approximate wavelength ranges of reflected colours [26]. As the potential is applied on the film from 0.00 V step down to -0.75 V, the absorption increases gradually first from 465 nm, which can be assigned to the electrochromism of silicotungstate, followed by the new absorption gradual increase in the range of 350 ~ 580 nm, which can be assigned to the electrochromism of viologen tetramer. When the step potential is applied from -0.75 V back to -0.20 V, the absorption decreases correspondingly to the original (Fig. 3.2.7). Therefore, the coloured film bleaches gradually in the potential modulation process. It means that the whole process from pale colour to colouration followed by bleaching is reversible.
Since the third redox process of silicotungstate is pH dependent, the
electrochromic behavior of the film should be studied in various pH values. Some
similar characteristics can be observed between the UV-Vis spectra on the
\([\text{SiW}\{\text{VT}\}]_n\) film in pH 1 (Fig. 3.2.4 and Fig. 3.2.5), pH 5 (Fig. 3.2.8 and Fig. 3.2.9)
and pH 7 (Fig. 3.2.10 and Fig. 3.2.11) buffer solutions under different applied
potentials. These similar characteristics in the figures testify that the whole process
between colourless and colouration is also reversible in pH 1, pH 5 and pH 7 systems.

Some new characteristics can be observed by comparing the spectra between
different pH values under various applied potentials. The absorbance in the range of
over 600 nm, which belong to the electrochromic change of silicotungstate, arrives at
a certain maximum at different potential, for example, -0.60 V in pH 1, -0.75 V in
pH 3, -0.80 V in pH 5 and -0.85 V in pH 7. The absorbance in the range of 350 ~ 580
nm, which belong to the electrochromic change of viologen tetramer, is independent
of pH.

All the above results in electrochromic coating show that a wide range
wavelength is involved in electrochromism on \([\text{SiW}\{\text{VT}\}]_n\) film and many different
kinds of electrochromic spectra can be achieved flexibly by adjusting pH value and
applied potential. These features should be very useful in practice.

Although the film can be used in neutral electrolyte, it is the critical condition to
the film due to the decomposition of the film. So the electrochromic application of
the film in neutral electrolyte is not suggested.

To study the electrochromic behavior of \([\text{SiW}\{\text{VT}\}]_n\) film in detail, the combined
electrochemical and UV-Vis spectroscopic methods was developed. Fig. 3.2.12 and
Fig. 3.2.13 show UV-Vis absorbance response of 30-bilayer \([\text{SiW}\{\text{VT}\}]_n\) film on ITO
substrate in pH=5 phosphate buffer solution under potential switched between 0.00 and -0.75 V at 550 nm and at 360 nm respectively. The result that the process is reversible is testified again. In other words, [SiW|VT]$_n$ film has good write-erase efficiency in electrochromic coating. Fig. 3.2.13 also shows the potential of the film to be a long-life electrochromic material. In fact, such experiments can be taken at any wavelength between 350 and 900 nm, which covering the whole visible light region.

3.3.4 Conclusions

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged silicotungstate and positively charged viologen tetramer on various substrates including glassy carbon, ITO and quartz. The thickness of such film can be controlled easily in the assembly process. We have systematically characterized our self-assembled film and have monitored the growth of the film by using electrochemical method and UV-Vis spectroscopies. The results show that both silicotungstate and viologen tetramer are assembled layer-by-layer on substrate. The [SiW|VT]$_n$ film exhibits good electrochromic behavior and good stability.

3.3.5 References


25. Monk, P. M. S. *The Viologens*, John Wiley and Sons, **1998**.

Legends and Figures

Fig. 3.3.1 Cyclic voltammograms of [SiW|VT]_n films in pH=3 phosphate buffer solution. From inside to outside: two, three, four, five, six, seven, eight bilayers. Scan rate: 100 mV s\(^{-1}\). Scan rate: 100 mV s\(^{-1}\). Inset: the relationship between peak current at -0.580 V and No. of bilayers.

Fig. 3.3.2 UV-Vis spectra of [SiW|VT]_n film on quartz substrate. From bottom to top: two, four, six, eight, ten, twelve bilayers. The inset depicts the relationship between absorbance at 268 nm and No. of bilayers.

Fig. 3.3.3 UV-Vis spectra of [SiW|VT]_n film on ITO-coated glass substrate. From bottom to top: one, two, three, four, five, six, seven, eight, nine bilayers. The inset depicts the relationship between absorbance at 475 nm and No. of bilayers.

Fig. 3.3.4 In situ UV-Vis spectra of 30-bilayer [SiW|VT]_n film on ITO substrate in pH=1 phosphate buffer solution at applied potentials from -0.20, -0.40, -0.50, -0.60, down to -0.65 V.

Fig. 3.3.5 In situ UV-Vis spectra of 30-bilayer [SiW|VT]_n film on ITO substrate in pH=1 phosphate buffer solution at applied potentials from -0.65, -0.60, -0.50, -0.40, up to -0.20 V.

Fig. 3.3.6 In situ UV-Vis spectra of 30-bilayer [SiW|VT]_n film on ITO substrate in pH=3 phosphate buffer solution at applied potentials from -0.20, -0.50, -0.60, -0.65, -0.70, down to -0.75 V.

Fig. 3.3.7 In situ UV-Vis spectra of 30-bilayer [SiW|VT]_n film on ITO substrate in pH=3 phosphate buffer solution at applied potentials from -0.75, -0.60, -0.50, -0.40, up to -0.20 V.
Fig. 3.3.8 In situ UV-Vis spectra of 30-bilayer [SiWVT]$_n$ film on ITO substrate in pH=5 phosphate buffer solution at applied potentials from -0.20, -0.40, -0.50, -0.60, down to -0.80 V.

Fig. 3.3.9 In situ UV-Vis spectra of 30-bilayer [SiWVT]$_n$ film on ITO substrate in pH=5 phosphate buffer solution at applied potentials from -0.80, -0.70, -0.60, -0.50, -0.40, up to -0.20 V.

Fig. 3.3.10 In situ UV-Vis spectra of 30-bilayer [SiWVT]$_n$ film on ITO substrate in pH=7 phosphate buffer solution at applied potentials from -0.20, -0.50, -0.60, -0.65, down to -0.85 V.

Fig. 3.3.11 In situ UV-Vis spectra of 30-bilayer [SiWVT]$_n$ film on ITO substrate in pH=7 phosphate buffer solution at applied potentials from -0.85, -0.70, -0.60, -0.40, up to -0.20 V.

Fig. 3.3.12 UV-Vis absorbance response of 30-bilayer [SiWVT]$_n$ film on ITO substrate in pH=5 phosphate buffer solution at potential switched between 0.00 and -0.75 V. Wavelength: 550 nm.

Fig. 3.3.13 UV-Vis absorbance response of 30-bilayer [SiWVT]$_n$ film on ITO substrate in pH=5 phosphate buffer solution at potential switched between 0.00 and -0.75 V. Wavelength: 365 nm.
Fig. 3.3.1
Fig. 3.3.3
Fig. 3.3.4
Fig. 3.3.5
Fig. 3.3.6
Fig. 3.3.7
Fig. 3.3.8
Fig. 3.3.10
Fig. 3.3.11
Fig. 3.3.12
CHAPTER 4

Hybrid Films Containing Metal Nanoparticles: Preparation, Characterization and Applications in Electrochemical Sensing
4.1 A Novel Method to Fabricate Hybrid Film Containing Metalloporphyrin and Palladium Nanoparticles from Self-Assembled Multilayer Film and its Application in Bifunctional Sensing of Nitric Oxide and Oxygen

Abstract

We demonstrate the fabrication of a hybrid multilayer molecular film by alternative self-assembling of cobalt porphyrin cation (CoP^4+) and tetrachloropalladate anion (PdCl_4^2-) in a layer-by-layer manner on glassy carbon and ITO. The assembling process was monitored by UV-Vis spectroscopy, which shows that equal amount of building blocks were deposited in each assembling step. Such multilayer film was electrochemically reduced to yield nanocomposite of Pd nanoparticles. The process was characterized by UV-Vis spectroscopy, electrochemical method, XPS and AFM. The film can serve as a bifunctional sensor for electro-reductive detection of O_2 and electro-oxidative detection of NO. Such bifunctional sensor for NO and O_2 is highly desirable in quantitation of NO levels in biological and medical systems, since it is known that NO level is highly regulated by various reactive oxygen species. Our bifunctional sensor provides a means to detect NO and O_2 at the same site. A straight calibration curve was obtained in concentration range of 4.0×10^{-8} mol L^{-1} to 1.28×10^{-6} mol L^{-1} for NO and 2.5×10^{-7} mol L^{-1} to 6.0×10^{-6} mol L^{-1} for O_2, with detection limit of 4.0×10^{-9} mol L^{-1} and 5.0×10^{-8} mol L^{-1} respectively.
4.1.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be modified according to the aim of study [10-12]. However, to the best of our knowledge, there are few reports that combine such technique and electrochemical method for the formation of metal nanoparticles.

NO has attracted intense interests due to its important roles in cellular communication and in host defense mechanisms of eukaryotes [13]. Detection of trace amount of NO in biological systems is challenging because of various interference from other coexisting species [14]. NO level is highly regulated by reactive oxygen species, and unfortunately the existence of O₂ is indispensable for in vivo detection of NO, therefore, measurement of NO concentration is only valuable in the existence of O₂.
Herein we describe a new Pd nanoparticles film that was reduced from a novel self-assembled molecular film with negatively charged tetrachloropalladate (PdCl$_4$$^{2-}$) and positively charged cobalt porphyrin (CoP$^{4+}$). Such new film was characterized and confirmed by UV-Vis spectroscopy, electrochemical method, XPS and AFM. We studied in detail the applications of such film in bifunctional bioelectrochemical sensing of both NO and O$_2$.

4.1.2 Experimental

4.1.2.1 Reagents and materials.

Potassium tetrachloropalladate and cobalt-tetra (N-methyl-4-pyridyl)-porphyrin chloride were obtained from Aldrich and used as received. Sulfuric acid, reagent ACS, was obtained from Acros. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolytes. Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.). Standard saturated NO solutions were prepared by bubbling a high purity NO gas through milli-Q pure water for 40 minutes after it was bubbled with high purity N$_2$ gas for 30mins [15-17]. The saturated aqueous NO solution has a NO concentration of 1.8×10$^{-3}$ mol L$^{-1}$ at 20$^\circ$C, P$_{NO}$ = 1 atm [18, 19]. The concentration of air-saturated water is 2.6×10$^{-4}$ mol L$^{-1}$ calculated from its saturated solubility [20].
4.1.2.2 Instrumentation.

(i) Electrochemical measurements

The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, Ф3mm, MF-2012, BAS, U.S.A.), or ITO electrode as the working electrode. Prior to each experiment, GC electrode was polished by using alumina powder of size 1, 0.3, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.

(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the ITO substrate. Bare ITO electrode was used as the reference.

(iii) Atomic force microscopy (AFM)
AFM images were obtained on a multimode Scanning Probe Microscope with Nanoscope IIIa control unit by Digital instrument Inc. Tapping mode was applied for the film morphology study with scan area of 2.5×2.5μm. The sample film was on the ITO substrate.

(iv) *X-ray Photoelectron Spectroscopy (XPS)*

XPS measurement was performed on PHI 5600 (Physical Electronics, USA). Aluminum anode was used as X-ray monochromatic source. Pass energy 58.7 eV is used for narrow scan. The sample film was on the ITO substrate.

4.1.2.3 *Fabrication of [CoP|Pd(II)]<sub>n</sub> film and its electrochemical conversion.*

The self-assembled films on different substrates (glassy carbon and ITO) were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 2×10⁻³ mol L⁻¹ CoP<sup>4+</sup> (dissolved in water) for 5 minutes, then a layer of cobalt porphyrin spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of CoP<sup>4+</sup> were transferred into 5×10⁻³ mol L⁻¹ PdCl<sub>4</sub><sup>2-</sup> (dissolved in water) for 5 minutes, then a layer of PdCl<sub>4</sub><sup>2-</sup> spontaneously adsorbed onto the CoP<sup>4+</sup>-coated substrates by electrostatic attraction. Then, the substrates were washed with water. The film with different thickness can be achieved by repeating the above process. Finally, the film named [CoP|Pd(II)]<sub>n</sub> was transferred into 0.5 mol L⁻¹ H₂SO₄ and PdCl<sub>4</sub><sup>2-</sup> in such film was electrochemically reduced to yield Pd
nanoparticles. Thus the self-assembled film named [Pd\textsubscript{n}/CoP] was prepared successfully. The process is demonstrated in Fig. 4.1.1.

### 4.1.3 Results and discussion

#### 4.1.3.1 Characterization by UV-Vis spectroscopy

The growth of [CoP\textsubscript{2}|Pd(II)]\textsubscript{n} film was monitored by UV-Vis spectrometry. Fig. 4.1.2 shows UV-Vis spectra of [CoP|Pd(II)]\textsubscript{n} film assembled on ITO-coated glass. The characteristic cobalt porphyrin absorbance around 300 and 440 nm can be observed and the intensity of each peak increases with number of CoP-PdCl\textsubscript{4}\textsuperscript{2-} bilayers. The inset depicts the linear relationship between peak intensity and number of bilayers. It testifies that same amount of cobalt porphyrin is assembled layer-by-layer in each bilayer [CoP|Pd(II)]\textsubscript{n} film.

After the film was assembled successfully, [CoP|Pd(II)]\textsubscript{n} film was transferred into 0.5 mol L\textsuperscript{-1} H\textsubscript{2}SO\textsubscript{4} PdCl\textsubscript{4}\textsuperscript{2-} in the film was reduced by cyclic voltammetry under potential between −0.2 ~ 0.2 V (vs. SCE) (Fig. 4.1.3 inset). The color of the film was turned from yellow to gray. The typical metal deposition peak between -0.2 ~ 0 V can be observed in inset, which indicates the formation of Pd metal. Fig. 4.1.3 shows UV-Vis spectra of the film before and after electrochemical reduction. After reduction, the characteristic cobalt porphyrin absorbance around 300 and 440 nm still remains and the absorption is almost unchanged. Since the color was changed to gray after reduction, it is reasonable that the absorbance in Fig. 4.1.3(b) is not equal to
zero in the whole range. Comparing these two UV-Vis spectra, the reduction of PdCl$_4^{2-}$ and the formation of Pd(0) in the film can be confirmed.

4.1.3.2 Electrochemical characterization

$[\text{CoP}][\text{Pd(II)}]_n$ film can also be prepared on glassy carbon (GC) according to layer-by-layer self-assembly process mentioned above. After it was reduced to form $[\text{Pd}_n/\text{CoP}]$ film in 0.5 mol L$^{-1}$ H$_2$SO$_4$ by cyclic voltammetry under potential between $-0.2 \sim 0.2$ V, cyclic voltammogram (CV) of this film was recorded in 0.5 mol L$^{-1}$ H$_2$SO$_4$ (Fig. 4.1.4A). Characteristic cyclic voltammogram of Pd nanoparticle, a pair of redox peaks at $-0.09$ V and $-0.13$ V, which correspond to adsorption and desorption of hydrogen on Pd nanoparticle respectively [21], can be observed. It is very similar to cyclic voltammogram of Pd thin film on glassy carbon in 0.5 mol L$^{-1}$ H$_2$SO$_4$ (Fig. 4.1.4B). It testifies the formation of Pd metal in $[\text{Pd}_n/\text{CoP}]$ film.

4.1.3.3 Characterization by XPS

X-ray photoelectron spectroscopy (XPS) is a good method to determine the valence of elements. Fig. 4.1.5 shows the XPS results of the as-prepared film before electrochemical reduction ($[\text{CoP}][\text{Pd(II)}]_n$ film) and that after reduction ($[\text{Pd}_n/\text{CoP}]$ film) on ITO-coated glass. The Pd(3d$_{5/2}$) and Pd(3d$_{3/2}$) peaks are present at 337.3 and 342.7 eV respectively, which is depicted in Fig. 4.1.5A, before reduction. After electrochemical reduction, both of the peaks shift to 335.4 and 340.7 respectively, which is shown in Fig. 4.1.5B. These results indicates that the palladium element
exists in an oxidation form Pd(II) in [CoP|Pd(II)]$_n$ film while it exits in the form of metal Pd(0) in [Pd$_n$/CoP] film [22]. The Co(2p$_{3/2}$) and Co(2p$_{1/2}$) peaks are present at 780.5 and 795.8 eV respectively in both Fig. 4.1.4C, before reduction, and Fig. 4.1.5D, after reduction. These clarify that cobalt element exists in the form of oxidation Co(II) in [CoP|Pd(II)]$_n$ film and its valence is not changed when the film is reduced to form [Pd$_n$/CoP] film [22].

The XPS experiment also gives some information about chlorine element. The peak strength becomes greatly weak after reduction though its position doesn’t change any. It shows that most of Cl(-1) in PdCl$_4^{2-}$ are released and move out during the reduction process from the film. It is reasonable that part of the Cl(-1) ions are in the reduced film to obtain electronic equilibrium in the film.

### 4.1.3.4 Characterization by AFM

From UV-Vis spectroscopy, XPS and electrochemical results, we can confirm that Pd(0) is formed during the electrochemical reduction process, but we can not tell directly if Pd(0) is nanoparticle in [Pd$_n$/CoP] film. Atomic force microscopy (AFM) gives some information about the surface morphology of [CoP|Pd(II)]$_n$ and [Pd$_n$/CoP] films (Fig. 4.1.6) and especially the size of Pd particles in [Pd$_n$/CoP] film. The surface of [CoP|Pd(II)]$_n$ film displays poor morphology before it is reduced (Fig. 4.1.6A). After electrochemical reduction, distributed Pd nanoparticles can be observed clearly in Fig. 4.1.6B. It is obvious that the surface of [Pd$_n$/CoP] film displays orderly homogeneous morphology. Fig. 4.1.7 shows AFM images of the various bilayer numbers. The size of Pd nanoparticles becomes bigger and bigger
when the assembly number of the film increases. Different amount and size of Pd nanoparticles in [Pdₙ/CoP] film can be controlled by changing the bilayer number of [CoP|Pd(II)]ₙ film when assembly.

4.1.3.5 Application in bifunctional electrochemical sensing of NO and O₂

In most cases of biological and medical system, O₂ coexists with NO and interferes with the detection of NO. Moreover, as an important molecule, precise detection of NO can provide valuable information for medical judgment. Thus, a bifunctional sensor with selective sensitivity toward both O₂ and NO should be desirable.

Fig. 4.1.8 displays the cyclic voltammograms of 10-bilayer [Pdₙ/CoP] modified GC electrode (Fig. 4.1.7(b)) and bare GC electrode (Fig. 4.1.7(a)), respectively, in air-saturated pH=7 phosphate buffer solution with 1.0×10⁻⁴ mol L⁻¹ NO. In Fig. 4.1.7(a), only oxidation current is observed when the potential is higher than 0.65 V and the anodic peak is present at 0.99 V, which is assigned to the oxidation of NO on GC electrode. There is no evidently reductive current on GC, so that O₂ is inactive on GC. Compared with the CV characterization on the bare GC electrode, not only an enhancement of oxidation current is observed on [Pdₙ/CoP] modified GC electrode and the peak potential decreases by 0.18 V, but also the large reduction current appears when the potential is below 0.10 V (Fig. 4.1.7(b)). On [Pdₙ/CoP] modified electrode, the anodic peak at 0.81 V, which can be assigned to electrochemical oxidation of NO, and the cathodic peak at −0.05 V, which can be assigned to electrochemical reduction of O₂, can be observed. It shows that the electrochemical
behaviors of NO and O₂ on the electrode are totally irreversible process. These features imply that [Pdₙ/CoP] modified GC electrode has electrocatalytical properties toward both NO and O₂. In other words, [Pdₙ/CoP] film has potential to be a good sensor for the detection of NO and O₂. Since O₂ is reduced while NO is oxidized on [Pdₙ/CoP] modified GC electrode, one [Pdₙ/CoP] film modified electrode can be used to detect O₂ at lower potential and NO at higher potential. So it is rational that [Pdₙ/CoP] film can be used in bifunctional bioelectrochemical sensing of O₂ and NO.

Amperometric response of 10-bilayer [Pdₙ/CoP] modified GC electrode to NO with various concentration range from 4.0×10⁻⁸ mol L⁻¹ to 1.28×10⁻⁶ mol L⁻¹ was tested at 0.75 V and the result was shown in Fig. 4.1.9. As can be seen from the figure, the modified electrode shows fast, sensitive and reproducible properties. Current of the electrode increases in a proportional mode when concentration of NO increases and it gives a straight calibration curve with a regression correlation (R²) of 0.9958. The relative standard deviation (R.S.D) is less than 5%. The detection limit of the modified electrode to NO is determined as 4.0×10⁻⁹ mol L⁻¹. This study illustrates a successful application of the newly developed electrode in electrochemical sensing of NO.

Amperometric measurements of O₂ solution with various concentrations were conducted at −0.05 V on 10-bilayer [Pdₙ/CoP] film. I-t curve of O₂ with concentration increasing from 2.5×10⁻⁷ mol L⁻¹ to 6.0×10⁻⁶ mol L⁻¹ was shown in Fig. 4.1.10, the corresponding calibration curve shown in the inset shows a direct proportional relation between the current and concentration of O₂. Linear regression performed on the calibration curve gave the value of coefficient correlation (R²) as
0.9928. The detection limit was $5.0 \times 10^{-8}$ mol L$^{-1}$, which is calculated from the signal
to noise ratio of 3.

4.1.4 Conclusion

In this work, a layer-by-layer self-assembled molecular film has been
successfully prepared with negatively charged tetrachloropalladate and
positively charged cobalt porphyrin on glassy carbon and ITO. Further, a
novel film containing Pd nanoparticles was formed from the self-assembled
molecular film by electrochemical reduction. The thickness of such film and
the size of Pd nanoparticles can be controlled easily in the assembly process.
We have systematically characterized our self-assembled film by using
electrochemical method, AFM, UV-Vis and XPS spectroscopies and have
monitored the growth of the film by UV-Vis spectroscopy. The
electrochemical results showed that [Pd$_n$/CoP] film exhibited high sensitivity and
excellent selectivity to both O$_2$ and NO. In other words, the novel molecular film
can be used in bifunctional bioelectrochemical sensing of the detection of NO
and O$_2$.

4.1.5 Reference

1. Murray, R. W. in *Electroanalytical Chemistry*, Bard, A. J. (Ed.), Marcel Dekker:


Legends and Figures

Fig. 4.1.1  Scheme illustrating the LbL assembling of \([\text{CoP}|\text{Pd(II)}]_n\) film and its electrochemical conversion to \([\text{Pd}_n/\text{CoP}]\) film.

Fig. 4.1.2 UV-Vis spectra of the assembly of \([\text{CoP}|\text{Pd(II)}]_n\) film on ITO-coated glass substrate. (a) two bilayers, (b) four bilayers, (c) six bilayers, (d) eight bilayers, (e) ten bilayers, (f) twelve bilayers, (g) fourteen bilayers. The inset depicts the relationship between absorbance and No. of bilayers.

Fig. 4.1.3 UV-Vis spectra of 10-bilayer \([\text{CoP}|\text{Pd(II)}]_n\) film on ITO-coated glass substrate (a) before and (b) after electrochemical reductive process. The inset is the cyclic voltammogram of electrochemical reduction. Scan rate: 50 mV s\(^{-1}\).

Fig. 4.1.4 Cyclic voltammograms of (A) 10-bilayer \([\text{Pd}_n/\text{CoP}]\) film on glassy carbon and (B) Pd thin film on glassy carbon recorded in 0.5 mol L\(^{-1}\) H\(_2\)SO\(_4\). Scan rate: 50 mV s\(^{-1}\).

Fig. 4.1.5 XPS spectra of (A) palladium element in 10-bilayer \([\text{CoP}|\text{Pd(II)}]_n\) film, (B) palladium element in 10-bilayer \([\text{Pd}_n/\text{CoP}]\) film, (C) cobalt element in 10-bilayer \([\text{CoP}|\text{Pd(II)}]_n\) film and (D) cobalt element in 10-bilayer \([\text{Pd}_n/\text{CoP}]\) film on ITO-coated glass substrate.

Fig. 4.1.6 AFM images of (A) 10-bilayer \([\text{CoP}|\text{Pd(II)}]_n\) film on ITO-coated glass substrate and (B) 10-bilayer \([\text{Pd}_n/\text{CoP}]\) film on ITO-coated glass substrate.

Fig. 4.1.7 AFM images of (A) 16-bilayer \([\text{Pd}_n/\text{CoP}]\) film, (B) 32-bilayer \([\text{Pd}_n/\text{CoP}]\) film, (C) 48-bilayer \([\text{Pd}_n/\text{CoP}]\) film on ITO-coated glass substrate.
Fig. 4.1.8 A. Cyclic voltammograms of (a) glassy carbon (dot line) and (b) 10-bilayer [Pd₆/CoP] film modified on glassy carbon (solid line) in air-saturated pH=7 phosphate buffer solution with 1.0×10⁻⁴ mol L⁻¹ NO. Scan rate: 100 mV s⁻¹.

Fig. 4.1.9 Changes of current in i-t curve of 10-bilayer [Pd₆/CoP] film modified on glassy carbon in pH=7 phosphate buffer solution when various amount of NO was added. Potential was held at 0.75 V. The inset depicts the relationship between current and concentration of NO.

Fig. 4.1.10 Changes of current in i-t curve of 10-bilayer [Pd₆/CoP] film modified on glassy carbon in pH=7 phosphate buffer solution when various amount of O₂ was added. Potential was held at -0.05 V. The inset depicts the relationship between current and concentration of O₂.
Cobalt-tetra(N-methyl-4-pyridyl)-porphyrin cation

PdCl$_4^{2-}$, tetrachloropalladate anion

Pd nanoparticle
Fig. 4.1.2
Fig. 4.1.3
Fig. 4.1.4

Graph A:
- I / µA vs. E / V (vs. SCE)
- Peak at approximately 0.1 V

Graph B:
- I / µA vs. E / V (vs. SCE)
- Peak at approximately -0.2 V
Fig. 4.1.5
Fig. 4.1.6

A

B
Fig. 4.1.7
Fig. 4.1.8
Fig. 4.1.9
Fig. 4.1.10

[Graph showing a plot with time (t/s) on the x-axis and current (I/µA) on the y-axis. The graph includes markers for different concentrations of [O₂]/µM, including 0.25 µM, 0.5 µM, 1.0 µM, 2.0 µM, 4.0 µM, and 6.0 µM.]
4.2 Hybrid Film Containing Metalloporphyrin and Platinum Nanoparticles

Abstract

We demonstrate the fabrication of a hybrid molecular film by alternatively self-assembling of iron porphyrin cation (FeP⁺) and tetrachloroplatinate anion (PtCl₄⁻) in a layer-by-layer manner on glassy carbon and ITO. The assembling process was monitored by UV-Vis spectroscopy, which shows that equal amount of building blocks were deposited in each assembling step. Such multilayer film was electrochemically reduced to yield nanocomposite of iron porphyrin and Pt nanoparticles. The process was characterized by UV-Vis spectroscopy, electrochemical method and XPS. The film can serve as a bifunctional sensor for electro-reductive detection of O₂ and electro-oxidative detection of H₂O₂. Such bifunctional sensor of H₂O₂ and O₂ is highly desirable in quantitation of H₂O₂ levels in biological and medical systems, since it is known that H₂O₂ level is highly regulated by various reactive oxygen species. Our bifunctional sensing system provides a means to detect H₂O₂ and O₂ at the same electrode. A straight calibration curve was obtained in concentration range of 2.0×10⁻⁷ mol L⁻¹ to 9.6×10⁻⁶ mol L⁻¹ for H₂O₂ and 1.0×10⁻⁷ mol L⁻¹ to 6.0×10⁻⁶ mol L⁻¹ for O₂, with detection limit of 1.0×10⁻⁷ mol L⁻¹ and 5.0×10⁻⁸ mol L⁻¹ respectively.
4.2.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be modified according to the aim of study [10-12]. However, to the best of our knowledge, there are few reports that combine such technique and electrochemical method for the formation of metal nanoparticles.

Nanoscale particles of transition metals are of considerable interest as catalysts in organic and inorganic transformations, as electrocatalysts in fuel cells and as materials with novel electronic, optical and magnetic properties [13]. Platinum is arguably one of the most important catalytically active metals.

The detection of trace amounts of H₂O₂ is of practical importance in clinical and biological studies [14, 15]. Analysis of biological systems, such as blood and brain fluid, is probably its main area of application, but a problem with such analyses is that detection of trace amount of H₂O₂ in biological systems is quite challenging.
because of various interference from other coexisting electroactive species, for example O$_2$. So it is essential to eliminate the interference between each other when we detect H$_2$O$_2$ and O$_2$ respectively. The simultaneous detection of H$_2$O$_2$ and O$_2$ on one electrode is very important for *in situ in vivo* detection.

Herein we describe a new Pt nanoparticles film which was reduced from a novel self-assembled molecular film with negatively charged tetrachloroplatinate (PtCl$_4^{2-}$) and positively charged iron porphyrin (FeP$^{4+}$). Such new film was characterized and confirmed by UV-Vis spectroscopy, electrochemical method and XPS. We studied in detail the applications of such film in bifunctional bioelectrochemical sensing of both H$_2$O$_2$ and O$_2$.

### 4.2.2 Experimental

#### 4.2.2.1 Reagents and materials

Potassium tetrachloroplatinate and iron-tetra (N-methyl-4-pyridyl)-porphyrin chloride were obtained from Aldrich and used as received. Sulfuric acid, reagent ACS, was obtained from Acros. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolytes. Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.). The concentration of air-saturated water is $2.6\times10^{-4}$ mol L$^{-1}$ calculated from its saturated solubility [16].

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4.2.2.2 Instrumentation

(i) Electrochemical measurements

The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, Φ3mm, MF-2012, BAS, U.S.A.), or ITO electrode as the working electrode. Prior to each experiment, GC electrode was polished by using alumina powder of size 1, 0.3, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.

(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the ITO substrate. Bare ITO electrode was used as the reference.
(iii) Atomic force microscopy (AFM)

AFM images were obtained on a multimode Scanning Probe Microscope with Nanoscope IIIa control unit by Digital instrument Inc. Tapping mode was applied for the film morphology study with scan area of 2.5×2.5μm. The sample film was on the ITO substrate.

(iv) X-ray Photoelectron Spectroscopy (XPS)

XPS measurement was performed on PHI 5600 (Physical Electronics, USA). Aluminum anode was used as X-ray monochromatic source. Pass energy 58.7 eV is used for narrow scan. The sample film was on the ITO substrate.

4.2.2.3 Fabrication of [FeP|Pt(II)]ₙ film and its electrochemical conversion.

The self-assembled films on different substrates (glassy carbon and ITO) were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 2×10⁻³ mol L⁻¹ FeP⁴⁺ (dissolved in water) for 5 minutes, then a layer of iron porphyrin spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of FeP⁴⁺ were transferred into 5×10⁻³ mol L⁻¹ PtCl₄²⁻ (dissolved in water) for 5 minutes, then a layer of PtCl₄²⁻ spontaneously adsorbed onto the FeP⁴⁺-coated substrates by electrostatic attraction. Then, the substrates were washed with water. The film with different thickness can be achieved by repeating
the above process. Finally, the film named [FeP|Pt(II)]ₙ was transferred into 0.5 mol L⁻¹ H₂SO₄ and PtCl₄²⁻ in such film was electrochemically reduced to yield Pt nanoparticles. Thus the self-assembled film named [Pt₅/FeP] was prepared successfully. The process is demonstrated in Fig. 4.2.1.

4.2.3 Results and discussion

4.2.3.1 Characterization by UV-Vis spectroscopy

The growth of [FeP|Pt(II)]ₙ film was monitored by UV-Vis spectrometry. Fig. 4.2.2 shows UV-Vis spectra of [FeP|Pt(II)]ₙ film assembled on ITO-coated glass. The characteristic iron porphyrin absorbance around 310 and 400 nm can be observed and the intensity of each peak increases with number of FeP-PtCl₄²⁻ bilayers. The inset depicts the linear relationship between peak intensity and number of bilayers. It testify that same amount of iron porphyrin is assembled layer-by-layer in each bilayer [FeP|Pt(II)]ₙ film.

After the film was assembled successfully, [FeP|Pt(II)]ₙ film was transferred into 0.5 mol L⁻¹ H₂SO₄. PtCl₄²⁻ in the film was reduced by cyclic voltammetry under potential between –0.2 ~ 0.2 V (vs. SCE). The color of the film was turned from yellow to gray. Fig. 4.2.3 shows UV-Vis spectra of the film before and after electrochemical reduction. After reduction, the characteristic iron porphyrin absorbance around 400 nm still remains and the absorption is almost unchanged. Since the color was changed to gray after reduction, it is reasonable that the absorbance in Fig. 4.2.3(b) is not equal to zero in the whole range. Comparing these
two UV-Vis spectra, the reduction of PtCl$_4^{2-}$ and the formation of Pt(0) in the film can be confirmed.

4.2.3.2 Electrochemical characterization

[FeP|Pt(II)]$_n$ film can also be prepared on glassy carbon (GC) according to layer-by-layer self-assembly process mentioned above. The typical metal deposition peak between -0.2 ~ 0 V can be observed when [FeP|Pt(II)]$_n$ film was reduced, which indicates the formation of Pt metal. After it was reduced to form [Pt$_n$/FeP] film, cyclic voltammogram of this film was recorded in 0.5 mol L$^{-1}$ H$_2$SO$_4$ (Fig. 4.2.4). Characteristic cyclic voltammogram of Pt nanoparticle, a pair of redox peaks around -0.10 V, which correspond to adsorption and desorption of hydrogen on Pt nanoparticles respectively [17], can be observed. It is very similar to cyclic voltammogram of Pt thin film on glassy carbon in 0.5 mol L$^{-1}$ H$_2$SO$_4$ [17]. It testifies the formation of metal Pt in [Pt$_n$/FeP] film.

4.2.3.3 Characterization by XPS

X-ray photoelectron spectroscopy (XPS) is a good method to determine the valence of elements. Fig. 4.2.5 shows the XPS results of the as-prepared film before electrochemical reduction ([FeP|Pt(II)]$_n$ film) and that after reduction ([Pt$_n$/FeP] film) on ITO-coated glass. The Pt(4f$_{7/2}$) and Pt(4f$_{5/2}$) peaks are present at 72.6 and 76.0 eV respectively, which is depicted in Fig. 4.2.5A, before reduction. After electrochemical reduction, both of the peaks shift to 71.4 and 74.7 respectively,
which is shown in Fig. 4.2.5B. These results indicates that the palladium element exists in an oxidation form Pt(II) in \([\text{FeP}\vert \text{Pt(II)}]_n\) film while it exits in the form of metal Pt(0) in \([\text{Pt}_n\vert \text{FeP}]\) film [18]. The Fe(3p) peaks are present at 56.1 in both Fig. 4.2.5C, before reduction, and Fig. 4.2.5D, after reduction. These clarify that iron element exists in the form of oxidation Fe(III) in \([\text{FeP}\vert \text{Pt(II)}]_n\) film and its valence is not changed when the film is reduced to form \([\text{Pt}_n\vert \text{FeP}]\) film [18].

The XPS experiment also gives some information about chlorine element. The peak strength becomes greatly weak after reduction though its position doesn’t change any. It shows that most of Cl(-1) in PtCl$_4^{2-}$ are released and move out during the reduction process from the film. It is reasonable that part of the Cl(-1) ions are in the reduced film to obtain electronic equilibrium in the film.

### 4.2.3.4 Applications in bifunctional electrochemical sensing of H$_2$O$_2$ and O$_2$

In most cases of biological and medical system, O$_2$ coexists with H$_2$O$_2$ and interferes with the detection of H$_2$O$_2$, moreover, as an important molecule, precise detection of H$_2$O$_2$ can provide valuable information for medical judgement. Thus, a bifunctional sensor with selective sensitivity toward both O$_2$ and H$_2$O$_2$ should be desirable.

Fig. 4.2.6 displays the cyclic voltammograms of 10-bilayer \([\text{Pt}_n\vert \text{FeP}]\) modified GC electrode (Fig. 4.2.6(b)) and bare GC electrode (Fig. 4.2.6(a)), respectively, in air-saturated pH=7 phosphate buffer solution with $1.0 \times 10^{-3}$ mol L$^{-1}$ H$_2$O$_2$. In Fig. 4.2.6(a), neither evident oxidation current nor evident reduction current is observed when the potential range is from $-0.20$ V to $0.60$ V, so that both O$_2$ and H$_2$O$_2$ are
inactive on GC in such potential range. Compared with the CV characterization on the bare GC electrode, not only an enhancement of oxidation current is observed on [Pt₄/FeP] modified GC electrode when the potential is higher than 0.20 V, but also the large reduction current appears when the potential is below 0.20 V and the cathodic peak at 0.0 V (Fig. 4.2.6(b)). These features imply that [Pt₄/FeP] modified GC electrode has electrocatalytical properties toward H₂O₂ or O₂ or both H₂O₂ and O₂. In other words, [Pt₄/FeP] film has potential to be a good sensor for the detection of H₂O₂ or O₂ or both.

Fig. 4.2.7 displays cyclic voltammograms of 10-bilayer [Pt₄/FeP] film modified on glassy carbon in pH=7 phosphate buffer solution (O₂ free) (Fig. 4.2.7(a)), in air-saturated pH=7 phosphate buffer solution (Fig. 4.2.7(b)), in pH=7 phosphate buffer solution (O₂ free) with 1.0×10⁻³ mol L⁻¹ H₂O₂ (Fig. 4.2.7(c)) and in air-saturated pH=7 phosphate buffer solution with 1.0×10⁻³ mol L⁻¹ H₂O₂ (Fig. 4.2.7(c)), respectively. Comparing with the four curves, the anodic current in Fig. 4.2.6b can be assigned to electrochemical oxidation of H₂O₂ and the cathodic current can be assigned to electrochemical reduction of both H₂O₂ and O₂.

Since only H₂O₂ is oxidized on [Pt₄/FeP] modified GC electrode, there is no doubt that [Pt₄/FeP] film modified electrode can be well used to detect H₂O₂ at higher potential. Amperometric response of 10-bilayer [Pt₄/FeP] modified GC electrode to H₂O₂ with various concentration to H₂O₂ range from 2.0×10⁻⁷ mol L⁻¹ to 9.6×10⁻⁶ mol L⁻¹ was tested at 0.40 V and the result was shown in Fig. 4.2.7. As can be seen from the figure, the modified electrode shows fast, sensitive and reproducible properties. Current of the electrode increased in a proportional mode when concentration of NO increased and gave a straight calibration curve with a regression
correlation ($R^2$) of 0.999. The relative standard deviation (R.S.D) was less than 5%.
The detection limit of the modified electrode to $H_2O_2$ is determined as $1.0 \times 10^{-7}$ mol L$^{-1}$. This study illustrated a successful application of the newly developed electrode in electrochemical sensing of $H_2O_2$.

If the sample is $O_2$ free, $H_2O_2$ can also be detected at lower potential by reduction method. Amperometric measurements of $H_2O_2$ solution with various concentrations were conducted at 0.11 V on 10-bilayer [Pt$_{np}$/FeP] film. I-t curve of $H_2O_2$ with concentration increasing from $2.0 \times 10^{-7}$ mol L$^{-1}$ to $6.0 \times 10^{-6}$ mol L$^{-1}$ was shown in Fig. 4.2.9, the corresponding calibration curve shown in the inset shows a direct proportional relation between the current and concentration of $H_2O_2$. Linear regression performed on the calibration curve gave the value of coefficient correlation ($R^2$) as 0.995. The relative standard deviation (R.S.D) was less than 5%. The detection limit was $1.0 \times 10^{-7}$ mol L$^{-1}$, which is calculated from the signal to noise ratio of 3.

As for $O_2$, if the sample is $H_2O_2$ free, $O_2$ also can be detected easily at lower potential since $O_2$ is reduced on [Pt$_{np}$/FeP] modified GC electrode. Amperometric measurements of $O_2$ solution with various concentrations were conducted at 0.11 V on 10-bilayer [Pt$_{np}$/FeP] film. I-t curve of $O_2$ with concentration increasing from $1.0 \times 10^{-7}$ mol L$^{-1}$ to $6.0 \times 10^{-6}$ mol L$^{-1}$ was shown in Fig. 4.2.10, the corresponding calibration curve shown in the inset shows a direct proportional relation between the current and concentration of $O_2$. Linear regression performed on the calibration curve gave the value of coefficient correlation ($R^2$) as 0.996. The detection limit was $5.0 \times 10^{-8}$ mol L$^{-1}$. 
If the sample has both H$_2$O$_2$ and O$_2$, the detection of O$_2$ should be a little complicated. At lower potential, the reductive current of both H$_2$O$_2$ and O$_2$ can be directly detected. At higher potential, the oxidative current of H$_2$O$_2$ can be undoubtedly detected. Then we can get corresponding reductive current of H$_2$O$_2$ by using the calibration curves of both oxidation (Fig. 4.2.8) and reduction (Fig. 4.2.9). So the reductive current of O$_2$ can be calculated and finally the detection of O$_2$ can be completed by using its calibration curve (Fig. 4.2.10).

So it is rational that [Pt$_n$/FeP] film can be used in bifunctional bioelectrochemical sensing of O$_2$ and H$_2$O$_2$.

### 4.2.4 Conclusions

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged tetrachloroplatinate and positively charged iron porphyrin on glassy carbon and ITO. Further, a novel film containing Pt nanoparticles was formed from the self-assembled molecular film by electrochemical reduction. The thickness of such film and the size of Pt nanoparticles can be controlled easily in the assembly process. We have systematically characterized our self-assembled film by using electrochemical method, UV-Vis and XPS spectroscopies and have monitored the growth of the film by UV-Vis spectroscopy. The electrochemical results showed that [Pt$_n$/FeP] film exhibited high sensitivity to both O$_2$ and H$_2$O$_2$. In other words, the novel molecular film can be used in bifunctional bioelectrochemical sensing of the detection of H$_2$O$_2$ and O$_2$. 

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4.2.5 References


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Legends and Figures

Fig. 4.2.1 Scheme illustrating the LbL assembling of \([\text{FeP}\vert\text{Pt(II)}]_n\) film and its electrochemical conversion to \([\text{Pt}_n\vert\text{FeP}]\) film.

Fig. 4.2.2 UV-Vis spectra of the assembly of \([\text{FeP}\vert\text{Pt(II)}]_n\) film on ITO-coated glass substrate. (a) one bilayer, (b) two bilayers, (c) three bilayers, (d) four bilayers, (e) five bilayers, (f) six bilayers, (g) seven bilayers. The inset depicts the relationship between absorbance and No. of bilayers.

Fig. 4.2.3 UV-Vis spectra of 10-bilayer \([\text{FeP}\vert\text{Pt(II)}]_n\) film on ITO-coated glass substrate (a) before and (b) after electrochemical reductive process.

Fig. 4.2.4 Cyclic voltammograms of 10-bilayer \([\text{Pt}_n\vert\text{FeP}]\) film on glassy carbon recorded in 0.5 mol L\(^{-1}\) H\(_2\)SO\(_4\). Scan rate: 50 mV s\(^{-1}\).

Fig. 4.2.5 XPS spectra of (A) platinum element in 10-bilayer \([\text{FeP}\vert\text{Pt(II)}]_n\) film, (B) platinum element in 10-bilayer \([\text{Pt}_n\vert\text{FeP}]\) film, (C) iron element in 10-bilayer \([\text{FeP}\vert\text{Pt(II)}]_n\) film and (D) iron element in 10-bilayer \([\text{Pt}_n\vert\text{FeP}]\) film on ITO-coated glass substrate.

Fig. 4.2.6 Cyclic voltammograms of (a) glassy carbon (dot line) and (b) 10-bilayer \([\text{Pt}_n\vert\text{FeP}]\) film modified on glassy carbon (solid line) in air-saturated pH=7 phosphate buffer solution with 1.0×10\(^{-3}\) mol L\(^{-1}\) H\(_2\)O\(_2\). Scan rate: 50 mV s\(^{-1}\).

Fig. 4.2.7 Cyclic voltammograms of 10-bilayer \([\text{Pt}_n\vert\text{FeP}]\) film modified on glassy carbon (a) in pH=7 phosphate buffer solution (O\(_2\) free), (b) in air-saturated pH=7 phosphate buffer solution, (c) in pH=7 phosphate buffer solution (O\(_2\) free) with 1.0×10\(^{-3}\) mol L\(^{-1}\) H\(_2\)O\(_2\) and (d) in air-saturated pH=7 phosphate buffer solution with 1.0×10\(^{-3}\) mol L\(^{-1}\) H\(_2\)O\(_2\). Scan rate: 50 mV s\(^{-1}\).
Fig. 4.2.8 Changes of current in i-t curve of 10-bilayer [Pt₆/FeP] film modified on glassy carbon in pH=7 phosphate buffer solution when various amount of H₂O₂ was added. Potential was held at 0.40 V. The inset depicts the relationship between oxidative current and concentration of H₂O₂.

Fig. 4.2.9 Changes of current in i-t curve of 10-bilayer [Pt₆/FeP] film modified on glassy carbon in pH=7 phosphate buffer solution when various amount of H₂O₂ was added. Potential was held at 0.11 V. The inset depicts the relationship between reductive current and concentration of H₂O₂.

Fig. 4.2.10 Changes of current in i-t curve of 10-bilayer [Pt₆/FeP] film modified on glassy carbon in pH=7 phosphate buffer solution when various amount of O₂ was added. Potential was held at 0.11 V. The inset depicts the relationship between current and concentration of O₂.
Iron-tetra(N-methyl-4-pyridyl)-porphyrin cation

PtCl$_4^{2-}$, tetrachloroplmatinate anion

Pt particle
Fig. 4.2.2

[Graph showing absorbance against wavelength with an inset graph showing absorbance at 427 nm against the number of bilayers.]
Fig. 4.2.3
Fig. 4.2.4
Fig. 4.2.5

Diagram showing binding energy in eV and intensity in a.u. for different samples labeled A, B, C, and D.
Fig. 4.2.7
Fig. 4.2.8
Fig. 4.2.9

![Graph showing current I vs. time t for different concentrations of \( [H_2O_2] \) in \( \mu \text{M} \). The graph indicates a decrease in current with increasing time and concentration.](image-url)
4.3 Fabrication of Gold Nanoparticles by in-situ Electrochemical or Photochemical Conversion of a Layer-by-Layer Self-Assembled Multilayer Film and its Application in Nitric Oxide Sensing

Abstract

We demonstrate the fabrication of a hybrid molecular film by alternatively self-assembling of polycation (PDDA) and tetrachloroaurate anion (AuCl₄⁻) in a layer-by-layer manner on glassy carbon and ITO. The assembling process was monitored by UV-Vis spectroscopy, which shows that equal amount of building blocks were deposited in each assembling step. Such multilayer film was electrochemically or photochemically reduced to yield nanocomposite of Au nanoparticles. The process was characterized by UV-Vis spectroscopy, electrochemical method, XPS and AFM. The film can serve as sensor for electro-oxidative detection of NO. Such sensor for NO is highly desirable in quantitative NO levels in biological and medical systems.

4.3.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].
The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be modified according to the aim of study [10-12].

Nanoscale particles of transition metals are of considerable interest as catalysts in organic and inorganic transformations, as electrocatalysts in fuel cells and as materials with novel electronic, optical and magnetic properties [13]. Au nanoparticles as attractive candidates of inorganic building blocks have been intensively investigated in recent years because of their wide applications in microelectronic devices, spectroscopy and sensor technology, etc [14, 15]. However, to the best of our knowledge, there are few reports that combine layer-by-layer self-assembled technique and electrochemical method for the formation of metal nanoparticles.

NO has attracted intense interests due to its important roles in cellular communication and in host defense mechanisms of eukaryotes [16]. Detection of trace amount of NO in biological systems is quite challenging because of various interference from other coexisting species [17].

Herein we describe two kinds of new Au nanoparticles films that were photochemically or electrochemically reduced from novel self-assembled molecular films with negatively charged tetrachloroaurate (AuCl₄⁻) and positively charged
polymer (PDDA). Such new films were characterized and confirmed by UV-Vis spectroscopy, XPS and SEM. We studied in detail the applications of such films in bioelectrochemical sensing of NO.

4.3.2 Experimental

4.3.2.1 Reagents and materials.

Poly(diallyldimethyl-ammonium chloride) (PDDA) (20 wt.% in water, Mw ca. 200,000-350,000) and potassium tetrachloroaurate (KAuCl₄) were purchased from Aldrich and used as received. Sulfuric acid, reagent ACS, was obtained from Acros. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolytes. Milli-Q pure water was used throughout this work. Quartz and ITO-coated glass (both sides) was purchased from Hong Kong Labware Company and Delta Technologies (U.S.A.), respectively. Quartz was used to monitor self-assembly process by UV-Vis spectroscopy and ITO glass was for the other characterizations. Standard saturated NO solutions were prepared by bubbling a high purity NO gas through milli-Q pure water for 40 minutes after it was bubbled with high purity N₂ gas for 30mins [18-20]. The saturated aqueous NO solution has a NO concentration of 1.8×10⁻³ mol L⁻¹ at 20°C, P(NO) = 1atm [21,22].
4.3.2.2 Instrumentation.

(i) Electrochemical measurements

The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified ITO electrode (9 x 9 x 0.5 mm float glass slide, ITO-coated two sides, Della Technologies, Limited-Stillwater, U.S.A) as the working electrode.

(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was deposited on quartz or ITO.

(iii) Scanning Electron Microscopy (SEM)
Scanning electron microscopy (SEM) images were obtained on a JEOL 6300F ultra-high resolution scanning electron microscope operated at 15 KV. The sample film was on the ITO substrate.

(iv) X-ray Photoelectron Spectroscopy (XPS)

XPS measurement was performed on PHI 5600 (Physical Electronics, USA). Aluminum anode was used as X-ray monochromatic source. Pass energy 58.7 eV is used for narrow scan. The sample film was on the ITO substrate.

(v) UV-Light Source

The UV-light source used for the sample exposure was an ORIEL 66011 500 W Xe/HgXe arc lamp (ORIEL corporation, Stratford, USA).

4.3.2.3 Fabrication of \([\text{PDDA}|\text{Au(III)}]\), film and its conversion by electrochemical or photochemical method.

The self-assembled films on different substrates were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 1.0 g L\(^{-1}\) PDDA (dissolved in water) for 5 minutes, then a layer of PDDA spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water and dried by N\(_2\). Then the substrates with a layer of PDDA were transferred into \(3\times10^{-3}\) mol L\(^{-1}\) \(\text{AuCl}_4^-\) (dissolved in water) for 5
minutes, then a layer of AuCl₄⁻ spontaneously adsorbed onto the PDDA-coated substrates by electrostatic attraction. Then, the substrates were washed with water and dried by N₂. The film with different thickness can be achieved by repeating the above process. Thus the film named [PDDA|Au(III)]ₙ was formed.

After that, the film can be reduced by either electrochemical or photochemical method to form Au nanoparticles. If [PDDA|Au(III)]ₙ was transferred into 0.5 mol L⁻¹ H₂SO₄ and in such film AuCl₄⁻ was electrochemically reduced to yield Au nanoparticles, the electrochemically reduced film named [Auₙ^E/PDDA] was prepared successfully. If [PDDA|Au(III)]ₙ was exposed in ultraviolet light for 30 minutes and AuCl₄⁻ was converted to Au nanoparticles, the photochemically reduced film named [Auₙ^D/PDDA] was prepared successfully. All the above processes are demonstrated in Fig. 4.3.1.

4.3.3 Results and discussion

4.3.3.1 Characterization by UV-Vis spectroscopy

The process of the layer-by-layer self-assembly of KAuCl₄ and PDDA was monitored by UV-Vis spectroscopy. Fig. 4.3.2 shows the spectra of the film assembled on a quartz substrate. The peak appearing at 231 nm can be assigned to a ligand-to-metal charge-transfer (LMCT) transition of AuCl₄⁻ ion [23]. From the change of absorbance at 231 with the number of bilayers in inset, it is clear that the film of KAuCl₄ and PDDA have been successfully fabricated in the assembly
process and the same amount of PDDA is assembled layer-by-layer in each bilayer [PDDA|Au(III)]ₙ film.

The photochemical reduction of [PDDA|Au(III)]ₙ was carried on by UV irradiation for 30 minutes. The resulting UV spectra are shown in Fig. 4.3.3c. In this case, the absorption peak of AuCl₄⁻ vanished, while a new brand arising from the Au nanoparticles plasma was occurred around 520 nm. If [PDDA|Au(III)]ₙ was kept in dark, AuCl₄⁻ could not change to Au nanoparticles after 15 hours. Since the spectrum (Fig. 4.3.3b) almost did not change any and the peak at 231 nm still remained, comparing with the UV spectrum of [PDDA|Au(III)]ₙ just assembled (Fig. 4.3.3a). The figure suggests that photochemical reduction is effective to change the AuCl₄⁻ of the multilayer film into Au nanoparticles. Fig. 4.3.4 shows the UV spectra of [Auₙ⁵/PDDA] with three different explosive time. It testifies that 30 minutes for UV irradiation is enough to change [PDDA|Au(III)]ₙ to [Auₙ⁵/PDDA].

The UV-Vis spectra of the assembly of [PDDA|Au(III)]ₙ film on the ITO substrate are shown in Fig. 4.3.5. The broad brand around 402 nm is another characteristic absorption of AuCl₄⁻. Its absorbance linear growth with number of bilayers (Fig. 4.3.5 inset) confirms the formation of KAuCl₄ and PDDA multilayer film. It could be found that the absorbance value at 402 nm on the ITO substrate is bigger than that on the quartz (Fig. 4.3.2). This should arise from the substrate effect, for the ITO is consisted of nanoparticles, having larger surface area than quartz.

The electrochemical reduction of [PDDA|Au(III)]ₙ on ITO substrate was carried on by cyclic voltammetry in 0.5 mol L⁻¹ H₂SO₄ under potential –0.2 ~ 0.21 V with 9 cycles. Fig. 4.3.6 shows UV-Vis spectra of the film before and after electrochemical reduction. After reduction, absorption peak of AuCl₄⁻ vanished, while a new brand
arising from the Au nanoparticles plasma was occurred around 520 nm. So that the reduction of AuCl₄⁻ and the formation of Au(0) in the film can be confirmed.

Comparing Fig. 4.3.3 and Fig. 4.3.6, both photochemical and electrochemical methods are effective to change the AuCl₄⁻ in the multilayer film into Au nanoparticles.

4.3.3.2 Characterization by XPS

In order to confirm the result of the transition from AuCl₄⁻ to Au, the films before reduction and after either photochemical or electrochemical reduction are measured by XPS (Fig. 4.3.7). Before reduction, the Au(4f½) and Au(4f¾) peaks are present at 88.9 and 85.2 eV respectively, which is depicted in Fig. 4.3.7A. After photochemical reduction, both of the peaks shift to 87.4 and 83.9 respectively, which is shown in Fig. 4.3.7B. After electrochemical reduction, both of the peaks shift to 87.6 and 84.0 respectively (Fig. 4.3.7C), which is almost the same as Fig. 4.3.7B. These results indicate that the gold element exists in an oxidation form Au(III) in [PDDA/Au(III)]ₙ film while it exits in the form of metal Au(0) in both [Au₅⁹/PDDA] and [Au₉⁸/PDDA] films [24]. In other words, the original Au(III) in ion form in the film has been alternated into metal form Au(0) successfully by either photochemical or electrochemical method.

4.3.3.3 Characterization by SEM
Fig. 4.3.8A shows the SEM image of the film after photochemical reduction, which indicates the Au nanoparticles are dispersed homogeneously all over the surface. After electrochemical reduction, the SEM image (Fig. 4.3.8B) shows that Au nanoparticles consist in [Au\textsubscript{n}\textsuperscript{E}/PDDA] film. It is in agreement with the observation in XPS and UV-Vis spectra that the Au nanoparticles are formed by either photochemical or electrochemical reduction of [PDDA|Au(III)]\textsubscript{n} film. The difference between these two images is that the Au nanoparticle size in Fig. 4.3.8B is larger than that in Fig. 4.3.8A. It gives evidence that the electrochemical method is of advantage to the aggregation of Au nanoparticles during the reduction process while the photochemical method is of advantage to formatting uniform Au nanoparticles on substrate during the reduction process.

### 4.3.3.4 Application in electrochemical sensing of NO

Catalysis and analysis are the most important applications of metal Nanostructure [25]. Herein, the application of the Au nanoparticles in NO electrochemical sensing was investigated. Owing to NO important physiological roles, the measurement of NO has attracted extensive attentions [26].

Fig. 4.3.9 displays the cyclic voltammograms of 13-bilayer [Au\textsubscript{n}\textsuperscript{E}/PDDA] modified ITO in absence and presence of 4.0×10\textsuperscript{-6} mol L\textsuperscript{-1} NO in pH=7 phosphate buffer solution. In Fig. 4.3.9a, a pair of oxidation and reduction peaks at 0.90 and 0.44 V appears, which corresponds to the gold oxide formation and sequent reduction respectively. Compared with Fig. 4.3.9a, after addition of NO, not only a new anodic peak appeared at around 0.98 V can be observed, but also the large oxidation current
appears when the potential is over 0.62 V (Fig. 4.3.9b). This new anodic peak and the large oxidation current can be assigned to the electrochemical oxidation of NO on [Au$_n$E/PDDA] modified ITO electrode. It shows that the electrochemical behavior of NO on the electrode is a totally irreversible process. This indicates that [Au$_n$E/PDDA] modified ITO electrode has electrocatalytical properties toward NO oxidation. In other words, [Au$_n$E/PDDA] film has potential to be a good sensor for the detection of NO.

Amperometric response of 13-bilayer [Au$_n$E/PDDA] modified ITO electrode to NO with various concentration range from 1.0×10$^{-7}$ mol L$^{-1}$ to 2.0×10$^{-6}$ mol L$^{-1}$ was tested at 0.80 V and the result was shown in Fig. 4.3.10. Current of the electrode increases in a proportional mode when concentration of NO increases and it gives a straight calibration curve with a regression correlation (R$^2$) of 0.993. The relative standard deviation (R.S.D) is less than 5%. This study illustrates a successful application of the newly developed electrode in electrochemical sensing of NO.

Some similar results were also found on 13-bilayer [Au$_n$P/PDDA] modified ITO. In Fig. 4.3.11, the oxidation and reduction peaks of Au nanoparticles are also at 0.90 and 0.43 V respectively, but the peak of NO oxidation appears at 0.86 V, which is a little lower than that on [Au$_n$E/PDDA] film. Amperometric response of 13-bilayer [Au$_n$P/PDDA] modified ITO electrode to NO with various concentration range from 1.0×10$^{-7}$ mol L$^{-1}$ to 1.0×10$^{-5}$ mol L$^{-1}$ was tested at 0.80 V and the result was shown in Fig. 4.3.12. Current of the electrode increases in a proportional mode when concentration of NO increases and it gives a straight calibration curve with a regression correlation (R$^2$) of 0.990.
The above results illustrate the fast respond, high sensitivity and reproducibility of both [Au$_n$P/PDDA] and [Au$_n$E/PDDA] films. The study reveals the great potential of such fabricated Au nanoparticles in the field of electrochemistry.

4.3.4 Conclusions

In this work, a novel layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged tetrachloroaurate and positively charged polymer PDDA on quartz and ITO. Further, a new film containing Au nanoparticles was formed from the self-assembled molecular film by either photochemical or electrochemical reduction. We have systematically characterized our self-assembled film by using UV-Vis, XPS, and SEM spectroscopies and have monitored the growth of the film by UV-Vis spectroscopy. The electrochemical results showed that both [Au$_n$P/PDDA] and [Au$_n$E/PDDA] film exhibited high sensitivity and excellent selectivity to NO. In other words, the novel molecular films can be used in bioelectrochemical sensing of NO.

4.3.5 References


Legends and Figures

Fig. 4.3.1 Scheme of the preparation of $[\text{Au}^E_n\\text{PDDA}]$ and $[\text{Au}^P_n\\text{PDDA}]$ film.

Fig. 4.3.2 UV-Vis spectra of the assembly of $[\text{PDDA}\vert\text{Au(III)}]_n$ film on a quartz substrate with two, four, six, eight, ten, twelve and fourteen bilayers. The inset depicts the relationship between absorbance at 231 nm and number of bilayers.

Fig. 4.3.3 UV-Vis spectra of 13-bilayer $[\text{PDDA}\vert\text{Au(III)}]_n$ film on a quartz substrate (a) before and (c) after UV irradiation for 30 minutes. (b) UV-Vis spectra of 13-bilayer $[\text{PDDA}\vert\text{Au(III)}]_n$ film on a quartz substrate after 15 hours in dark.

Fig. 4.3.4 UV-Vis spectra of 13-bilayer $[\text{PDDA}\vert\text{Au(III)}]_n$ film on a quartz substrate (a) before UV irradiation and after UV irradiation for (b) 20 minutes, (c) 30 minutes, (d) 60 minutes.

Fig. 4.3.5 UV-Vis spectra of the assembly of $[\text{PDDA}\vert\text{Au(III)}]_n$ film on a quartz substrate with two, four, six, eight, ten, twelve, fourteen, sixteen and eighteen bilayers. The inset depicts the relationship between absorbance at 402 nm and number of bilayers.

Fig. 4.3.6 UV-Vis spectra of 18-bilayer $[\text{PDDA}\vert\text{Au(III)}]_n$ film on ITO-coated glass substrate (a) before and (b) after electrochemical reductive process.

Fig. 4.3.7 Au 4f XPS spectra of 13-bilayer $[\text{PDDA}\vert\text{Au(III)}]_n$ films after UV irradiation (dot line) and electro-reduction (solid line).

Fig. 4.3.8 A. SEM micrograph of 13-bilayer $[\text{PDDA}\vert\text{Au(III)}]_n$ films after UV irradiation.
B. SEM micrograph of 13-bilayer \([\text{PDDA}|\text{Au(III)}]_n\) films after electrochemical reduction.

Fig. 4.3.9 Cyclic voltammograms of 13-bilayer \([\text{Au}_n^E/\text{PDDA}]\) film on ITO in pH=7.0 PBS solution in the absence (a) and presence (b) of 4.0\(\times\)10\(^{-6}\) mol L\(^{-1}\) NO. Scan rate: 50 mV s\(^{-1}\). Area: 0.9 cm\(\times\)0.9 cm.

Fig. 4.3.10 Changes of current in i-t curve of 13-bilayer \([\text{Au}_n^E/\text{PDDA}]\) film modified on ITO in pH=7 phosphate buffer solution when various amount of NO was added. Potential was held at 0.80 V. The inset depicts the relationship between current and concentration of NO. Area: 0.9 cm\(\times\)0.9 cm.

Fig. 4.3.11 Cyclic voltammograms of 13-bilayer \([\text{Au}_n^P/\text{PDDA}]\) film on ITO in pH=7.0 PBS solution in the absence (a) and presence (b) of 4.0\(\times\)10\(^{-6}\) mol L\(^{-1}\) NO. Scan rate: 50 mV s\(^{-1}\). Area: 0.9 cm\(\times\)0.9 cm.

Fig. 4.3.12 Changes of current in i-t curve of 13-bilayer \([\text{Au}_n^P/\text{PDDA}]\) film modified on ITO in pH=7 phosphate buffer solution when various amount of NO was added. Potential was held at 0.80 V. The inset depicts the relationship between current and concentration of NO. Area: 0.9 cm\(\times\)0.9 cm.
Fig. 4.3.1

PDDA, poly(diallyldimethylammonium) cation

AuCl₄⁻, tetrachloroaurate anion

Au particle
Fig. 4.3.2
Fig. 4.3.3
Fig. 4.3.6
Fig. 4.3.7

A

\[ \text{Intensity / a.u.} \]

\[ \text{Binding Energy (eV)} \]

\[ \text{Au}_{4f} 85.2 \]

\[ 88.9 \]

B

\[ \text{Intensity / a.u.} \]

\[ \text{Binding Energy (eV)} \]

\[ \text{Au}_{4f} 83.9 \]

\[ 87.4 \]

C

\[ \text{Intensity / a.u.} \]

\[ \text{Binding Energy (eV)} \]

\[ \text{Au}_{4f} 84.0 \]

\[ 87.6 \]
Fig. 4.3.9
Fig. 4.3.10
Fig. 4.3.11
Fig. 4.3.12
CHAPTER 5

Novel Methods to Fabricate Hybrid Films Containing
Bimetallic Nanoparticles and the Application in Bifunctional
Electrochemical Sensing
5.1 Pd-Pt Bimetallic Nanoparticles with Controlled Atomic Ratio and Their Application in Bifunctional Electrochemical Sensing

Abstract

We demonstrate the fabrication of a hybrid molecular film by alternatively self-assembling of cobalt porphyrin cation (CoP$^{4+}$), tetrachloropalladate anion (PdCl$_4^{2-}$) and tetrachloroplatinate anion (PtCl$_4^{2-}$) in a layer-by-layer manner on glassy carbon and ITO. The assembling process was monitored by UV-Vis spectroscopy, which showed that equal amount of building blocks were deposited in each assembling step. Such multilayer film was electrochemically reduced to yield nanocomposite of cobalt porphyrin and Pd-Pt bimetallic nanoparticles. This is a novel method to fabricate bimetallic nanoparticles. The process was characterized by UV-Vis spectroscopy, electrochemical method, XPS, SEM and ICP-MS. Through adjusting the assembled layer number of the different anions or the concentration of the anions, various ratios between two metals can be controlled flexibly. The film can serve as a bifunctional sensor for electro-reductive detection of O$_2$ and electro-oxidative detection of NO. Such bifunctional sensor of NO and O$_2$ is highly desirable in quantitation of NO levels in biological and medical systems, since it is known that NO level is highly regulated by various reactive oxygen species. Our bifunctional sensing system provides a means to detect NO and O$_2$ at the same site. A straight calibration curve was obtained in concentration range of 4.0×10$^{-8}$ to 1.28×10$^{-6}$ mol L$^{-1}$ for NO and 5.0×10$^{-8}$ to 1.6×10$^{-6}$ mol L$^{-1}$ for O$_2$, with detection limit of 4.0×10$^{-9}$ mol L$^{-1}$ and
5.0 \times 10^{-8} \text{ mol L}^{-1} \text{ respectively.}

\textbf{5.1.1 Introduction}

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be modified according to the aim of study [10-12]. However, to the best of our knowledge, there are few reports that combine such technique and electrochemical method for the formation of bimetallic nanoparticles.

Nanoscale particles of transition metals are of considerable interest as catalysts in organic and inorganic transformations, as electrocatalysts in fuel cells and as materials with novel electronic, optical and magnetic properties [13]. It is well known that bimetallic catalysts often exhibit superior catalytic properties than pure metals [14]. Platinum and palladium are arguably the most important catalytically active metals and they belong to the same group of the periodic table. Particularly,
palladium adlayers on single crystal platinum is well characterized [15-17]. Therefore Platinum and palladium are considered as ideal candidates for an electrochemical study of bimetals. The composition of bimetals is an important part in the study of their properties and catalytical activities [18, 19]. For example, palladium in silver has a narrower d-band than in pure palladium. In consequence, from a certain dilution up (about 60% silver), the whole narrowed band of states localized around palladium atoms falls below the Fermi level. The narrowing of the d-band results from the diminished overlap of the palladium orbitals, and from the suppressing of the d-d electron repulsion by dilution [20].

NO has attracted intense interests due to its important roles in cellular communication and in host defense mechanisms of eukaryotes [21]. Detection of trace amount of NO in biological systems is quite challenging because of various interference from other coexisting species [22]. NO level is highly regulated by reactive oxygen species, and unfortunately the existence of O₂ is indispensable for in vivo detection of NO, therefore, measurement of NO concentration is only valuable in the existence of O₂.

Herein we describe a new Pd-Pt bimetallic nanoparticles film that was reduced from a novel self-assembled molecular film with negatively charged tetrachloropalladate (PdCl₄²⁻), tetrachloroplatinate (PtCl₄²⁻) and positively charged cobalt porphyrin (CoP⁴⁺). Such new film was characterized and confirmed by UV-Vis spectroscopy, electrochemical method, XPS and SEM. We also introduce a flexible method to control the ratio of metals in such film by adjusting the assembled layer number of the different anions or the concentration of the anions. It was characterized and confirmed by UV-Vis spectroscopy and ICP-MS. We studied in
detail the applications of such film in bifunctional bioelectrochemical sensing of both NO and O₂.

5.1.2 Experimental

5.1.2.1 Reagents and materials.

Potassium tetrachloropalladate, potassium tetrachloroplatinate and cobalt-tetra(N-methyl-4-pyridyl)-porphyrin chloride were obtained from Aldrich and used as received. Sulfuric acid, reagent ACS, was obtained from Acros. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolytes. Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.). Standard saturated NO solutions were prepared by bubbling a high purity NO gas through milli-Q pure water for 40 minutes after it was bubbled with high purity N₂ gas for 30mins [23-25]. The saturated aqueous NO solution has a NO concentration of 1.8×10⁻³ mol L⁻¹ at 20°C, P(NO) = 1atm [26, 27]. The concentration of air-saturated water is 2.6×10⁻⁴ mol L⁻¹ calculated from its saturated solubility [28].

5.1.2.2 Instrumentation.

(i) Electrochemical measurements
The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, Ø3mm, MF-2012, BAS, U.S.A.), or ITO electrode as the working electrode. Prior to each experiment, GC electrode was polished by using alumina powder of size 1, 0.3, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.

(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the transparent ITO substrate. Bare ITO electrode was used as the reference.

(iii) X-ray photoelectron spectroscopy (XPS)

XPS measurement was performed on PHI 5600 (Physical Electronics, USA). Aluminum anode was used as X-ray monochromatic source. Pass energy 58.7 eV is used for narrow scan. The sample film was on the ITO substrate.
(iv) Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) images were obtained on a JEOL 6300F ultra-high resolution scanning electron microscope operated at 15 KV. The sample film was on the ITO substrate.

(v) Inductively coupled plasma - mass spectrometry (ICP-MS)

Inductively coupled plasma - mass spectrometry (ICP-MS) measurement was performed on an Elan 6000 ICP-Mass spectrometer.

5.1.2.3 Fabrication of [CoP|Pd(II)-Pt(II)]ₙ film and its electrochemical conversion.

The self-assembled films on different substrates were prepared by alternatively dipping the substrates in anionic and cationic species. We use two kinds of analogous processes to fabricate the films.

1. The clean substrates were immersed in 2×10⁻³ mol L⁻¹ CoP⁴⁺ (dissolved in water) for 5 minutes, then a layer of cobalt porphyrin spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of CoP⁴⁺ were transferred into 5×10⁻³ mol L⁻¹ PdCl₄⁻² (dissolved in water) for 5 minutes, then a layer of PdCl₄⁻² spontaneously adsorbed onto the CoP⁴⁺-coated substrates by electrostatic attraction. Then, the substrates were washed with water. After this process, the substrates were immersed
in $2 \times 10^{-3}$ mol L$^{-1}$ CoP$^{4+}$ again for 5 minutes. Then the substrates were transferred into $5 \times 10^{-3}$ mol L$^{-1}$ PtCl$_4^{2-}$ (dissolved in water) for 5 minutes. After these four steps, the first quadri-layer unit was formed (Fig. 5.1.1A). The film with different thickness can be achieved by repeating the above process.

2. The clean substrates were immersed in $2 \times 10^{-3}$ mol L$^{-1}$ CoP$^{4+}$ (dissolved in water) for 5 minutes as process 1. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of CoP$^{4+}$ were transferred into 5 mol L$^{-1}$ PdCl$_4^{2-}$ + 5 mol L$^{-1}$ PtCl$_4^{2-}$ (dissolved in water) for 5 minutes, then a layer of PdCl$_4^{2-}$ and PtCl$_4^{2-}$ spontaneously adsorbed onto the CoP$^{4+}$-coated substrates by electrostatic attraction. Then, the substrates were washed with water. After these two steps, the first bi-layer was formed (Fig. 5.1.1B). The film with different thickness can be achieved by repeating the above process.

Finally, the film named [CoP|Pd(II)-Pt(II)]$_n$ was transferred into 0.5 mol L$^{-1}$ H$_2$SO$_4$. Then Pd(II) and Pt(II) in such film was electrochemically reduced to yield Pd-Pt bimetallic nanoparticles. Thus the self-assembled film named [(Pd-Pt)$_n$/CoP] was prepared successfully. The process is demonstrated in Fig. 5.1.1.

5.1.3 Results and discussion

5.1.3.1 Characterization

5.1.3.1.1 UV-Vis spectroscopy study
Using layer-by-layer self-assembled method (Fig. 5.1.1A), [CoP|Pd(II)-Pt(II)]ₙ film was fabricated with PdCl₄²⁻, PtCl₄²⁻ and cobalt porphyrin cation. The growth of [CoP|Pd(II)-Pt(II)]ₙ film was monitored by UV-Vis spectrometry. Fig. 5.1.2 shows UV-Vis spectra of [CoP|Pd(II)-Pt(II)]ₙ film assembled on ITO-coated glass. The characteristic cobalt porphyrin absorbance around 300, 440 and 540 nm can be observed and the intensity of each peak increases with number of as-prepared quadri-layers. Additionally the absorption of potassium tetrachloropalladate and potassium tetrachloroplatinate contributed to the intensity of the absorbance around 300 nm. The inset depicts the linear relationship between peak intensity and number of quadri-layers. It testify that same amount of cobalt porphyrin is assembled layer-by-layer in each quadri-layer [CoP|Pd(II)-Pt(II)]ₙ film.

After the film was assembled successfully, [CoP|Pd(II)-Pt(II)]ₙ film was transferred into 0.5 mol L⁻¹ H₂SO₄. PdCl₄²⁻ and PtCl₄²⁻ in the film was reduced by cyclic voltammetry under potential between -0.2 ~ 0.2 V (vs. SCE). The color of the film was turned from yellow to gray. Fig. 5.1.3 shows UV-Vis spectra of the film before and after electrochemical reduction. After reduction, the characteristic cobalt porphyrin absorbance around 440 and 540 still remains and the intensity is almost unchanged, while the intensity of the absorbance around 300 nm is decreased due to the reduction of PdCl₄²⁻ and PtCl₄²⁻. Since the color was changed to gray after reduction, it is reasonable that the absorbance in Fig. 5.1.3 (b) is not equal to zero in the whole range. Comparing these two UV-Vis spectra, the reduction of PdCl₄²⁻ and PtCl₄²⁻ and the formation of Pd(0)-Pt(0) in the film can be confirmed.
5.1.3.1.2 Electrochemical study

[CoP]Pd(II)-Pt(II)]ₙ film can also be prepared on glassy carbon (GC) according to layer-by-layer self-assembly process mentioned above. When it was reduced in 0.5 mol L⁻¹ H₂SO₄ by cyclic voltammetry under potential between -0.2 ~ 0.2 V, the typical metal deposition peak between -0.2 ~ 0.1 V can be observed in Fig. 5.1.4A, which indicates the formation of Pd-Pt bimetal. After the electrochemical reductive process, cyclic voltammogram (CV) of this film was recorded in 0.5 mol L⁻¹ H₂SO₄ (Fig. 5.1.4B). Characteristic cyclic voltammogram of Pd-Pt bimetallic particles, a pair of redox peaks at ~0.13 V, which can be assigned to adsorption and desorption of hydrogen on Pd-Pt bimetallic nanoparticles respectively, can be observed.

5.1.3.1.3 X-ray photoelectron spectroscopy (XPS) study

X-ray photoelectron spectroscopy (XPS) is a good method to determine the valence of elements. Fig. 5.1.5 shows the XPS results of the as-prepared film before electrochemical reduction ([CoP]Pd(II)-Pt(II)]ₙ) and that after reduction ([Pd-Pt]ₙ/CoP) on ITO-coated glass.

The Pd(3d₅/₂) and Pd(3d₃/₂) peaks are present at 337.3 and 342.7 eV respectively, which is depicted in Fig. 5.1.5A, before reduction. After electrochemical reduction, the peaks shift to 335.4 and 340.7 respectively, which is shown in Fig. 5.1.5B. These results indicate that the palladium element exists in an oxidation form Pd(II) in [CoP]Pd(II)-Pt(II)]ₙ while it exits in the form of metal Pd(0) in [(Pd-Pt)ₙ/CoP] [29].
The Pt(4f\(_{7/2}\)) and Pt(4f\(_{5/2}\)) peaks are present at 72.5 and 75.8 eV respectively, which is depicted in Fig. 5.1.5C, before reduction. After electrochemical reduction, the peaks shift to 71.0 and 74.4 eV respectively, which is shown in Fig. 5.1.5D. These results indicate that platinum element exists in an oxidation form Pt(II) in [CoP]Pd(II)-Pt(II)\(_n\) while it exits in the form of metal Pt(0) in [(Pd-Pt)\(_n\)/CoP] [29].

The Co(2p\(_{3/2}\)) and Co(2p\(_{1/2}\)) peaks are present at 780.5 and 795.8 eV respectively in XPS figures of the as-prepared films before reduction and after reduction. These clarify that cobalt element exists in the form of oxidation Co(II) in [CoP]Pd(II)-Pt(II)\(_n\) and its valence is not changed when the film is reduced to form [(Pd-Pt)\(_n\)/CoP] [29].

The XPS experiment also gives some information about chlorine element. The peak strength becomes greatly weak after reduction though its position doesn’t change any. It shows that most of Cl(-1) in PdCl\(_4^2^-\) and PtCl\(_4^2^-\) are released and move out during the reduction process from the film. It is reasonable that part of the Cl(-1) ions are in the reduced film to obtain electronic equilibrium in the film.

5.1.3.1.4 Scanning electron microscopy (SEM) study

From UV-Vis spectroscopy, XPS and electrochemical results, we can confirm that Pd(0) and Pt(0) are formed during the electrochemical reduction process, but we can not tell directly if Pd(0) or Pt(0) is nanoparticle in [(Pd-Pt)\(_n\)/CoP]. Scanning electron microscopy (SEM) gives some information about the surface morphology of [(Pd-Pt)\(_n\)/CoP] films (Fig. 5.1.6) and especially the size of Pd-Pt bimetallic particles in the film. Distributed Pd-Pt bimetallic nanoparticles can be observed clearly in Fig.
5.1.6. It is obvious that the surface of [(Pd-Pt)$_n$/CoP] film displays orderly homogeneous morphology. Different amount and size of Pd-Pt nanoparticles in [(Pd-Pt)$_n$/CoP] film can be achieved by changing the bilayer number of [CoP|Pd(II)-Pt(II)]$_n$ film when assembly.

5.1.3.2 Control the Pd/Pt ratio in fabricated film

5.1.3.2.1 UV-Vis spectroscopy study

The layer-by-layer self-assembled growth of [CoP|Pd(II)-Pt(II)]$_n$ film was monitored by UV-Vis spectrometry. The characteristic cobalt porphyrin absorbance around 300, 440 and 540 nm can be observed and the intensity of each peak increases with number of as-prepared quadri-layers (Fig. 5.1.2). The inset depicts the linear relationship between peak intensity and number of quadri-layers. It testify that same amount of cobalt porphyrin is assembled layer-by-layer in each quadri-layer [CoP|Pd(II)-Pt(II)]$_n$ film.

If we look into the UV-Vis spectra of [CoP|Pd(II)-Pt(II)]$_n$ film collected after each bi-layer was assembled (Fig. 5.1.7), some new characterization was found. According to Beer-Lambert law, $A = \varepsilon \cdot c \cdot l$ and $\Gamma = \frac{c \cdot l}{2} = \frac{A}{2\varepsilon}$, where $A$ is absorbance, $\varepsilon$ is molar absorptivity, $c$ is molar concentration, $l$ is the length of light path, and $\Gamma$ is the calculated molecular coverage of chromophore on surface. The molecular absorptivity of cobalt porphyrin at 440 nm is $5.44 \times 10^4$ M$^{-1}$ cm$^{-1}$, the average increase in absorbance when cobalt porphyrin was assembled after PdCl$_4^{2-}$,
$\Delta A_{CoP-Pd}$ at 440nm, is 0.023, while the average increase when cobalt porphyrin was assembled after PtCl$_4^{2-}$, $\Delta A_{CoP-Pt}$ at 440nm, is 0.012. Then,

$$\Gamma_{CoP-Pd} = \frac{\Delta A_{CoP-Pd}}{2\varepsilon} = \frac{0.023}{2 \times 5.44 \times 10^4 M^{-1} cm^{-1}} = 2.1 \times 10^{-7} M \cdot cm = 2.1 \times 10^{-10} mol \cdot cm^{-2}$$

$$\Gamma_{CoP-Pt} = \frac{\Delta A_{CoP-Pt}}{2\varepsilon} = \frac{0.012}{2 \times 5.44 \times 10^4 M^{-1} cm^{-1}} = 1.1 \times 10^{-7} M \cdot cm = 1.1 \times 10^{-10} mol \cdot cm^{-2}$$

These results show that more cobalt porphyrin was assembled after PdCl$_4^{2-}$ than after PtCl$_4^{2-}$. These indicate that more PdCl$_4^{2-}$ than PtCl$_4^{2-}$ can be attracted to cobalt porphyrin.

5.1.3.2.2 ICP-MS study

Since UV-Vis spectroscopy results are not the direct evidence to illustrate that there are more PdCl$_4^{2-}$ than PtCl$_4^{2-}$ assembled in the film and the ratio between PdCl$_4^{2-}$ and PtCl$_4^{2-}$ cannot be figured out by using UV-Vis spectroscopy method, a more effective method should be used to study such film. Inductively coupled plasma-mass spectrometry (ICP-MS) is a good technique to measure elements ratio.

From ICP-MS result of the film which is the same as that in UV-Vis spectroscopy study, the ratio between Pd and Pt is about 2.4. It directly shows that more PdCl$_4^{2-}$ than PtCl$_4^{2-}$ can be attracted to cobalt porphyrin. This is consistent with the UV-Vis spectroscopy result.

We found that the molar ratio between Pd and Pt can be well and easily controlled by changing the number of assembled PdCl$_4^{2-}$ and PtCl$_4^{2-}$ layers. It can be simplified that more layers of PtCl$_4^{2-}$ should be assembled in the film if we would
increase Pt content in the film. Some ICP-MS results about the relationship between molar ratio of Pd and Pt in Pd-Pt bimetallic nanoparticles and ratio of the number of the assembled layer between PdCl$_4^{2-}$ and PtCl$_4^{2-}$ were shown in table 1. If Pd/Pt = 1 in Pd-Pt bimetallic nanoparticles is needed to be fabricated, two layers PtCl$_4^{2-}$ should be assembled while one layer PdCl$_4^{2-}$ is assembled in the film. Similarly, if four layers PtCl$_4^{2-}$ are assembled in the film while one layer PdCl$_4^{2-}$ is assembled, Pd-Pt bimetallic nanoparticles with Pd/Pt = 0.5 should be formed. The rule of the relationship between molar ratio of Pd and Pt in Pd-Pt bimetallic nanoparticles and ratio of the number of the assembled layer between PdCl$_4^{2-}$ and PtCl$_4^{2-}$ is obvious in the results (Table 5.1.1.).

Since PdCl$_4^{2-}$ and PtCl$_4^{2-}$ can both adsorb on cobalt porphyrin, we think about using PdCl$_4^{2-}$ + PtCl$_4^{2-}$ solution instead to simplify the method. The designed preparation method was introduced in detail in film fabrication (Fig. 5.1.1B). Briefly, the substrate with a first layer of CoP$_4^{4+}$ were transferred into 5 mol L$^{-1}$ PdCl$_4^{2-}$ + 5 mol L$^{-1}$ PtCl$_4^{2-}$ (dissolved in water), then a layer of PdCl$_4^{2-}$ and PtCl$_4^{2-}$ spontaneously adsorbed onto the CoP$_4^{4+}$-coated substrates. By repeating the above process, the film with different thickness can be achieved. ICP-MS result showed that Pd/Pt in such film was about 13. It testified that much more PdCl$_4^{2-}$ than PtCl$_4^{2-}$ adsorbed on CoP$_4^{4+}$ due to the result of the surface competition between PdCl$_4^{2-}$ and PtCl$_4^{2-}$. Some ICP-MS results about the relationship between molar ratio of Pd and Pt in Pd-Pt bimetallic nanoparticles and ratio of the original solution concentration ratio of PdCl$_4^{2-}$ and PtCl$_4^{2-}$ were shown in table 5.1.2. Although this method can also be applied to prepare various ratio Pd-Pt nanoparticles, it is not a good method to fabricate ratio controlled Pd-Pt nanoparticles. In another word, the method which
was illustrated in Fig. 5.1.1A is a better and flexible one to prepare ratio controlled Pd-Pt bimetallic nanoparticles.

5.1.3.3 Applications in bifunctional electrochemical sensing of NO and O$_2$

In most cases of biological and medical system, O$_2$ coexists with NO and interferes with the detection of NO, moreover, as an important molecule, precise detection of NO can provide valuable information for medical judgement. Thus, a bifunctional sensor with selective sensitivity toward both O$_2$ and NO should be desirable.

Fig. 5.1.8 displays the cyclic voltammograms of 6-quadri-layer [(Pd-Pt)$_n$/CoP] modified GC electrode (Fig. 5.1.8(b)) and bare GC electrode (Fig. 5.1.8(a)), respectively, in air-saturated pH=7 phosphate buffer solution with 1.0×10$^{-4}$ mol L$^{-1}$ NO. In Fig. 5.1.8(a), only oxidation current is observed when the potential is higher than 0.65 V and the anodic peak is present at 0.99 V, which is assigned to the oxidation of NO on GC electrode. There is no evidently reductive current on GC, so that O$_2$ is inactive on GC. Compared with the CV characterization on the bare GC electrode, not only an enhancement of oxidation current is observed on [(Pd-Pt)$_n$/CoP] modified GC electrode and the peak potential decreases by 0.20 V, but also the large reduction current appears when the potential is below 0.40 V (Fig. 8(b)). On [(Pd-Pt)$_n$/CoP] modified electrode, the anodic peak at 0.78 V, which can be assigned to electrochemical oxidation of NO, and the cathodic peak at 0.16 V, which can be assigned to electrochemical reduction of O$_2$, can be observed. It shows that the electrochemical behaviors of NO and O$_2$ on the electrode are totally irreversible.
process. These features imply that [(Pd-Pt)ₙ/CoP] modified GC electrode has electrocatalytical properties toward both NO and O₂. In other words, [(Pd-Pt)ₙ/CoP] film has potential to be a good sensor for the detection of NO and O₂. Since O₂ is reduced while NO is oxidized on [(Pd-Pt)ₙ/CoP] modified GC electrode, one [(Pd-Pt)ₙ/CoP] film modified electrode can be used to detect O₂ at lower potential and NO at higher potential. So it is rational that [(Pd-Pt)ₙ/CoP] film can be used in bifunctional bioelectrochemical sensing of O₂ and NO.

Amperometric response of 6-quadri-layer [(Pd-Pt)ₙ/CoP] modified GC electrode to NO with various concentration to NO range from 4.0×10^{-8} mol L⁻¹ to 1.28×10^{-6} mol L⁻¹ was tested at 0.70 V and the result was shown in Fig. 5.1.9. As can be seen from the figure, the modified electrode shows fast, sensitive and reproducible properties. Current of the electrode increased in a proportional mode when concentration of NO increased and gave a straight calibration curve with a regression correlation (R²) of 0.9999. The relative standard deviation (R.S.D) was less than 5%. The detection limit of the modified electrode to NO is determined as 4.0×10^{-9} mol L⁻¹.

This study illustrated a successful application of the newly developed electrode in electrochemical sensing of NO.

Amperometric measurements of O₂ solution with various concentrations were conducted at 0.05 V on 6-quadri-layer [(Pd-Pt)ₙ/CoP] film. I-t curve of O₂ with concentration increasing from 5.0×10^{-8} mol L⁻¹ to 1.6×10^{-6} mol L⁻¹ was shown in Fig. 5.1.10, the corresponding calibration curve shown in the inset shows a direct proportional relation between the current and concentration of O₂. Linear regression performed on the calibration curve gave the value of coefficient correlation (R²) as
0.9991. The relative standard deviation (R.S.D) was less than 5%. The detection limit was $5.0 \times 10^{-8}$ mol L$^{-1}$, which is calculated from the signal to noise ratio of 3.

5.1.4 Conclusions

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged tetrachloropalladate, tetrachloroplatinate and positively charged cobalt porphyrin on glassy carbon and ITO. Further, a novel film containing Pd-Pt bimetallic nanoparticles was formed from the self-assembled molecular film by electrochemical reduction. The size of Pd-Pt bimetallic nanoparticles and the ratio of Pd and Pt can be controlled easily in the assembly process. We have systematically characterized our self-assembled film by using electrochemical method, SEM, UV-Vis and XPS spectroscopies and have monitored the growth of the film by UV-Vis spectroscopy and various Pd/Pt ratio bimetallic nanoparticles were fabricated and characterization successfully. The electrochemical results showed that [(Pd-Pt)$_n$/CoP] film exhibited high sensitivity and excellent selectivity to both O$_2$ and NO. In other words, the novel molecular film can be used in bifunctional bioelectrochemical sensing of NO and O$_2$.

5.1.5 References


Chapter 5

Legends and Figures

Fig. 5.1.1 Scheme illustrating the assembling of \([\text{CoP}\mid\text{Pd(II)}\text{-Pt(II)}]_n\) films and their electrochemical conversion to \([(\text{Pd-Pt})_n/\text{CoP}]\) films. (A) \([\text{CoP}\mid\text{Pd(II)}\mid\text{CoP}\mid\text{Pt(II)}]\) quadri-layer as the basic assembling unit; (B) \([\text{CoP}\times\text{Pd(II)} + y\text{Pt(II)}]\) bilayer as the basic assembling unit.

Fig. 5.1.2 UV-Vis spectra of the assembly of \([\text{CoP}\mid\text{Pd(II)}\text{-Pt(II)}]_n\) film on ITO-coated glass substrate. (a) one quadri-layer, (b) two quadri-layers, (c) three quadri-layers, (d) four quadri-layers, (e) five quadri-layers, (f) six quadri-layers. The inset depicts the relationship between absorbance and No. of quadri-layers.

Fig. 5.1.3 UV-Vis spectra of 6-quadri-layer \([\text{CoP}\mid\text{Pd(II)}\text{-Pt(II)}]_n\) film on ITO-coated glass substrate (a) before and (b) after electrochemical reductive process.

Fig. 5.1.4 Cyclic voltammograms of (A) 6-quadri-layer \([\text{CoP}\mid\text{Pd(II)}\text{-Pt(II)}]_n\) film on glassy carbon and (B) 6-quadri-layer \([(\text{Pd-Pt})_n/\text{CoP}]\) film on glassy carbon recorded in 0.5 mol L\(^{-1}\) H\(_2\)SO\(_4\). Scan rate: 50 mV s\(^{-1}\).

Fig. 5.1.5 XPS spectra of (A) palladium element in 6-quadri-layer \([\text{CoP}\mid\text{Pd(II)}\text{-Pt(II)}]_n\) film, (B) palladium element in 6-quadri-layer \([(\text{Pd-Pt})_n/\text{CoP}]\) film, (C) platinum element in 6-quadri-layer \([\text{CoP}\mid\text{Pd(II)}\text{-Pt(II)}]_n\) film and (D) platinum element in 6-quadri-layer \([(\text{Pd-Pt})_n/\text{CoP}]\) film on ITO-coated glass substrate.

Fig. 5.1.6 SEM images of 6-quadri-layer \([(\text{Pd-Pt})_n/\text{CoP}]\) film on ITO-coated glass substrate.

Fig. 5.1.7 UV-Vis spectra of the assembly of \([\text{CoP}\mid\text{Pd(II)}\text{-Pt(II)}]_n\) film on ITO-coated glass substrate. (a) two bi-layers (one quadri-layer), (a’) three bi-layers, (b) four bi-layers (two quadri-layers), (b’) five bi-layers, (c) six bi-layers, (c’) seven bi-layers, (d)
eight bi-layers, (d’) nine bi-layers, (e) ten bi-layers, (e’) eleven bi-layers, (f) twelve bi-layers.

Fig. 5.1.8 Cyclic voltammograms of (a) glassy carbon (dot line) and (b) 6-quadri-layer [(Pd-Pt)_n/CoP] film modified on glassy carbon (solid line) in air-saturated pH=7 phosphate buffer solution with 1.0×10^{-4} mol L^{-1} NO. Scan rate: 100 mV s^{-1}.

Fig. 5.1.9 Changes of current in i-t curve of 6-quadri-layer [(Pd-Pt)_n/CoP] film modified on glassy carbon in pH=7 phosphate buffer solution when various amount of NO was added. Potential was held at 0.70 V. The inset depicts the relationship between current and concentration of NO.

Fig. 5.1.10 Changes of current in i-t curve of 6-quadri-layer [(Pd-Pt)_n/CoP] film modified on glassy carbon in pH=7 phosphate buffer solution when various amount of O_2 was added. Potential was held at 0.05 V. The inset depicts the relationship between current and concentration of O_2.

Table 5.1.1 The relationship between molar ratio of Pd and Pt in Pd-Pt bimetallic nanoparticles and ratio of the number of the assembled layer between PdCl_4^{2-} and PtCl_4^{2-}.

Table 5.1.2 The relationship between molar ratio of Pd and Pt in Pd-Pt bimetallic nanoparticles and ratio of the concentration of PdCl_4^{2-} and PtCl_4^{2-}.
Fig. 5.1.1A

Cobalt-tetra(N-methyl-4-pyridyl)-porphyrin cation
PdCl$_4^{2-}$, tetrachloropalladate anion
PtCl$_4^{2-}$, tetrachloroplatinate anion
Pd-Pt bimetallic nanoparticle
Cobalt-tetra(N-methyl-4-pyridyl)-porphyrin cation

\[ \text{PdCl}_4^{2-} + \text{PtCl}_4^{2-} \]

Pd-Pt bimetallic nanoparticle
Fig. 5.1.2
Fig. 5.1.4
Fig. 5.1.5

![Graphs showing binding energy and intensity](image)

(A) Pd peak at 337.3 eV, 342.7 eV

(B) Pd peak at 335.4 eV, 340.7 eV

(C) Pt peak at 72.5 eV, 75.8 eV

(D) Pt peak at 71.0 eV, 74.4 eV
Fig. 5.1.6
Fig. 5.1.8

![Graph showing two curves labeled 'a' and 'b'.]
Fig. 5.1.9
Fig. 5.1.10
### Table 5.1.1

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<th>((\text{CoP}{\text{PdCl}_4^{2-}})_n(\text{CoP}{\text{PtCl}_4^{2-}})_m) (n:m)</th>
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### Table 5.1.2

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<th>((\text{CoP}{\text{xPdCl}_4^{2-}\text{yPtCl}_4^{2-}})_n) (x:y)</th>
<th>Molar ratio Pd/Pt assembled</th>
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<td>~13:1</td>
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5.2 Hybrid Films Containing Pd-Au Bimetallic Nanoparticles and Their Application in Bifunctional Sensing

Abstract

We demonstrate the fabrication of a hybrid molecular film by alternatively self-assembling of cobalt porphyrin cation (CoP^4+), tetrachloropalladate anion (PdCl_4^{2-}) and tetrachloroaurate anion (AuCl_4^-) in a layer-by-layer manner on glassy carbon and ITO. The assembling process was monitored by UV-Vis spectroscopy, which showed that different building blocks were deposited successfully in each assembling step. Such multilayer film was electrochemically reduced to yield nanocomposite of cobalt porphyrin and Pd-Au bimetallic nanoparticles. This is a novel method to fabricate bimetallic nanoparticles. The process was characterized by UV-Vis spectroscopy, electrochemical method, XPS and AFM. The film can serve as a bifunctional sensor for electro-reductive detection of O_2 and electro-oxidative detection of NO. Such bifunctional sensor for NO and O_2 is highly desirable in quantitation of NO levels in biological and medical systems, since it is known that NO level is highly regulated by various reactive oxygen species. Our bifunctional sensing system provides a means to detect NO and O_2 at the same site. A straight calibration curve was obtained in concentration range of 4.0×10^{-8} to 1.28×10^{-6} mol L^{-1} for NO and 5.0×10^{-8} to 1.2×10^{-6} mol L^{-1} for O_2, with detection limit of 4.0×10^{-9} mol L^{-1} and 5.0×10^{-8} mol L^{-1} respectively.
5.2.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be modified according to the aim of study [10-12]. However, to the best of our knowledge, there are few reports that combine such technique and electrochemical method for the formation of bimetallic nanoparticles.

Nanoscale particles of transition metals are of considerable interest as catalysts in organic and inorganic transformations, as electrocatalysts in fuel cells and as materials with novel electronic, optical and magnetic properties [13]. It is well known that bimetallic catalysts often exhibit superior catalytic properties than pure metals [14]. Palladium adlayers on single crystal platinum is well characterized [15-17]. Therefore, palladium are considered as an ideal candidate for an electrochemical study of bimetals. Au nanoparticles as attractive candidates of inorganic building blocks have been intensively investigated in recent years because of their wide
applications in microelectronic devices, spectroscopy and sensor technology, etc [18, 19].

NO has attracted intense interests due to its important roles in cellular communication and in host defense mechanisms of eukaryotes [20]. Detection of trace amount of NO in biological systems is quite challenging because of various interference from other coexisting species [21]. NO level is highly regulated by reactive oxygen species, and unfortunately the existence of $O_2$ is indispensable for \textit{in vivo} detection of NO, therefore, measurement of NO concentration is only valuable in the existence of $O_2$.

Herein we describe a new Pd-Au bimetallic nanoparticles film that was reduced from a novel self-assembled molecular film with negatively charged tetrachloropalladate ($\text{PdCl}_4^{2-}$), tetrachloroaurate ($\text{AuCl}_4^-$) and positively charged cobalt porphyrin ($\text{CoP}^{4+}$). Such new film was characterized and confirmed by UV-Vis spectroscopy, electrochemical method, XPS and AFM. We studied in detail the applications of such film in bifunctional bioelectrochemical sensing of both NO and $O_2$.

\subsubsection*{5.2.2 Experimental}

\subsubsection*{5.2.2.1 Reagents and materials.}

Potassium tetrachloropalladate, potassium tetrachloroaurate and cobalt-tetra (N-methyl-4-pyridyl)-porphyrin chloride were obtained from Aldrich and used as received. Sulfuric acid, reagent ACS, was obtained from Acros. Phosphate buffer
solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolytes. Milli-Q pure water was used throughout this work. Glassy carbon (GC) electrodes were purchased from BAS, ITO-coated glass (both sides) from Delta Technologies (U.S.A.). Standard saturated NO solutions were prepared by bubbling a high purity NO gas through milli-Q pure water for 40 minutes after it was bubbled with high purity N₂ gas for 30mins [22-24]. The saturated aqueous NO solution has a NO concentration of 1.8×10⁻³ mol L⁻¹ at 20°C, P(NO) = 1 atm [25, 26]. The concentration of air-saturated water is 2.6×10⁻⁴ mol L⁻¹ calculated from its saturated solubility [27].

5.2.2.2 Instrumentation.

(i) Electrochemical measurements

The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified glassy carbon (GC, φ3mm, MF-2012, BAS, U.S.A.), or ITO electrode as the working electrode. Prior to each experiment, GC electrode was polished by using alumina powder of size 1, 0.3, down to 0.05 μm and cleaned in an ultrasonic bath with pure water.
(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the transparent ITO substrate. Bare ITO electrode was used as the reference.

(iii) X-ray Photoelectron Spectroscopy (XPS)

XPS measurement was performed on PHI 5600 (Physical Electronics, USA). Aluminum anode was used as X-ray monochromatic source. Pass energy 58.7 eV is used for narrow scan. The sample film was on the ITO substrate.

(iv) Atomic force microscopy (AFM)

AFM images were obtained on a multimode Scanning Probe Microscope with Nanoscope IIIa control unit by Digital instrument Inc. Tapping mode was applied for the film morphology study with scan area of 2.5×2.5µm. The sample film was on the ITO substrate.

(v) Scanning electron microscopy (SEM)
Scanning electron microscopy (SEM) images were obtained on a JEOL 6700 ultra-high resolution scanning electron microscope operated at 5 KV. The sample film was on the ITO substrate.

5.2.2.3 Fabrication of [CoP|Pd(II)-Au(III)]\textsubscript{n} film and its electrochemical conversion.

The self-assembled films on different substrates were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in $2 \times 10^{-3}$ mol L$^{-1}$ CoP$^{4+}$ (dissolved in water) for 5 minutes, then a layer of cobalt porphyrin spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of CoP$^{4+}$ were transferred into $5 \times 10^{-3}$ mol L$^{-1}$ PdCl$_4^{2-}$ (dissolved in water) for 5 minutes, then a layer of PdCl$_4^{2-}$ spontaneously adsorbed onto the CoP$^{4+}$-coated substrates by electrostatic attraction. Then, the substrates were washed with water. After this process, the substrates were immersed in $2 \times 10^{-3}$ mol L$^{-1}$ CoP$^{4+}$ again for 5 minutes. Then the substrates were transferred into $5 \times 10^{-3}$ mol L$^{-1}$ AuCl$_4^{-}$ (dissolved in water) for 5 minutes. After these four steps, the first quadri-layer unit was formed. The film with different thickness can be achieved by repeating the above process. Finally, the film named [CoP|Pd(II)-Au(III)]\textsubscript{n} was formed.

After that, the film can be directly transferred into 0.5 mol L$^{-1}$ H$_2$SO$_4$ and Pd(II) and Au(III) in such film was electrochemically reduced to yield Pd-Au bimetallic nanoparticles. Thus the self-assembled film named [(Pd-Au)$_n$/CoP]$_{CV}$ was prepared successfully. All the processes are demonstrated in Fig. 5.2.1.
5.2.3 Results and discussion

5.2.3.1 Characterization by UV-Vis spectroscopy and electrochemistry

Using layer-by-layer self-assembled method, [CoP|Pd(II)-Au(III)]\textsubscript{n} film was fabricated with PdCl\textsubscript{4}\textsuperscript{2-}, AuCl\textsubscript{4}\textsuperscript{-} and cobalt porphyrin cation. The growth of [CoP|Pd(II)-Au(III)]\textsubscript{n} film was monitored by UV-Vis spectrometry. Fig. 5.2.2 shows UV-Vis spectra of [CoP|Pd(II)-Au(III)]\textsubscript{n} film assembled on ITO-coated glass. The characteristic cobalt porphyrin absorbance around 320, 435 and 550 nm can be observed and the intensity of each peak increases with number of as-prepared quadri-layers. The inset depicts the linear relationship between peak intensity and number of quadri-layers. It testify that same amount of cobalt porphyrin is assembled in each quadri-layer [CoP|Pd(II)-Au(III)]\textsubscript{n} film.

If we look into the UV-Vis spectra of [CoP|Pd(II)-Au(III)]\textsubscript{n} film collected after each bi-layer was assembled (Fig. 5.3.3), some new characterization was found. According to Beer-Lambert law, \( A = \varepsilon \cdot c \cdot l \) and \( \Gamma = \frac{c \cdot l}{2} = \frac{A}{2 \varepsilon} \), where \( A \) is absorbance, \( \varepsilon \) is molar absorptivity, \( c \) is molar concentration, \( l \) is the length of light path, and \( \Gamma \) is the calculated molecular coverage of chromophore on surface. The molecular absorptivity of cobalt porphyrin at 435nm is \( 5.44 \times 10^4 \) M\textsuperscript{-1} cm\textsuperscript{-1}, the average increase in absorbance when cobalt porphyrin was assembled after PdCl\textsubscript{4}\textsuperscript{2-}, \( \Delta A_{\text{CoP-Pd}} \) at 435nm, is 0.0133, while the average increase when cobalt porphyrin was assembled after AuCl\textsubscript{4}\textsuperscript{-}, \( \Delta A_{\text{CoP-Au}} \) at 435nm, is 0.00587. Then,
\[
\Gamma_{\text{CoP-Pd}} = \frac{\Delta A_{\text{CoP-Pd}}}{2e} = \frac{0.0133}{2 \times 5.44 \times 10^4 \text{M}^{-1}\text{cm}^{-1}} = 1.2 \times 10^{-7} \text{M} \cdot \text{cm} = 1.2 \times 10^{-10} \text{mol} \cdot \text{cm}^{-2}
\]

\[
\Gamma_{\text{CoP-Au}} = \frac{\Delta A_{\text{CoP-Au}}}{2e} = \frac{0.00587}{2 \times 5.44 \times 10^4 \text{M}^{-1}\text{cm}^{-1}} = 0.54 \times 10^{-7} \text{M} \cdot \text{cm} = 0.54 \times 10^{-10} \text{mol} \cdot \text{cm}^{-2}
\]

These results show that more cobalt porphyrin was assembled after \(\text{PdCl}_4^{2-}\) than after \(\text{AuCl}_4^{-}\). These indicate that more \(\text{PdCl}_4^{2-}\) than \(\text{AuCl}_4^{-}\) can be attracted to cobalt porphyrin.

After the film was assembled successfully, \([\text{CoP}]\text{Pd(II)}\cdot\text{Au(III)}\) film was transferred into 0.5 mol L\(^{-1}\) \(\text{H}_2\text{SO}_4\), \(\text{PdCl}_4^{2-}\) and \(\text{AuCl}_4^{-}\) in the film was reduced by cyclic voltammetry under potential between \(-0.2 \sim 0.2\) V (vs. SCE). The typical metal deposition peak between \(-0.2 \sim 0.1\) V can be observed, which indicates the formation of Pd-Au bimetal. The color of the film was turned from yellow to gray.

Fig. 5.2.4 shows UV-Vis spectra of the film before and after electrochemical reduction. After reduction, the characteristic cobalt porphyrin absorbance around 320, 435 and 550 still remains and the absorption is almost unchanged. Since the color was changed to gray after reduction, it is reasonable that the absorbance in Fig. 5.2.4b is not equal to zero in the whole range. Comparing these two UV-Vis spectra, the reduction of \(\text{PdCl}_4^{2-}\) and \(\text{AuCl}_4^{-}\) and the formation of \(\text{Pd(0)}\cdot\text{Au(0)}\) in the film can be confirmed.

### 5.2.3.2 X-ray photoelectron spectroscopy (XPS) study

X-ray photoelectron spectroscopy (XPS) is a good method to determine the valence of elements. Fig. 5.2.5 shows the XPS results of the as-prepared film before
electrochemical reduction ([CoP|Pd(II)-Au(III)]ₙ film) and that after reduction ([Pd-Au]ₙ/CoP]CV film) on ITO-coated glass. The Pd(3d₅/₂) and Pd(3d₃/₂) peaks are present at 337.3 and 342.7 eV respectively, which is depicted in Fig. 5.2.5A, before reduction. After electrochemical reduction, the peaks shift to 335.4 and 340.7 respectively, which is shown in Fig. 5.2.5C. These results indicates that the palladium element exists in an oxidation form Pd(II) in [CoP|Pd(II)-Au(III)]ₙ film while it exits in the form of metal Pd(0) in [(Pd-Au)ₙ/CoP]CV film [28].

After reductive process, Au(0) was also formed in [(Pd-Au)ₙ/CoP]CV film. It can be deduced from the fact that the Au(4f₇/₂) and Au(4f₅/₂) peaks are present at 83.5 and 87.1 eV respectively in Fig. 5.2.5D, after reduction. Before electrochemical reduction, the peaks should be at about 86.5 and 90 eV respectively according to the reference [28]. In Fig. 5.2.5B, before reduction, both signals of Au(III) and Au(0) are shown since Au(III) is easy to be reduced to Au(0) under illumination. We can conclude that gold element exists in an oxidation form Au(III) in [CoP|Pd(II)-Au(III)]ₙ film while it exits in the form of metal Au(0) in [(Pd-Au)ₙ/CoP]CV film [28].

The Co(2p₃/₂) and Co(2p₁/₂) peaks are present at 780.5 and 795.8 eV respectively in XPS figures of the as-prepared films before reduction and after reduction. These clarify that cobalt element exists in the form of oxidation Co(II) in [CoP|Pd(II)-Au(III)]ₙ film and its valence is not changed when the film is reduced to form [(Pd-Au)ₙ/CoP]CV film [28].

The XPS experiment also gives some information about chlorine element. The peak strength becomes greatly weak after reduction though its position doesn’t change any. It shows that most of Cl(-1) in PdCl₄²⁻ and AuCl₄⁻ are released and move
out during the reduction process from the film. It is reasonable that part of the Cl(-1) ions are in the reduced film to obtain electronic equilibrium in the film.

### 5.2.3.3 Atomic force microscopy (AFM) study

From UV-Vis spectroscopy, XPS and electrochemical results, we can confirm that Pd(0) and Au(0) are formed during the electrochemical reduction process, but we can not tell directly if Pd(0) or Au(0) is nanoparticle in [(Pd-Au)$_n$/CoP]$_{CV}$ film. Atomic force microscopy (AFM) gives some information about the surface morphology of [(Pd-Au)$_n$/CoP]$_{CV}$ films (Fig. 5.2.6) and especially the size of Pd-Au bimetallic particles in the film. Distributed Pd-Au bimetallic nanoparticles can be observed clearly in Fig. 5.2.6. It is obvious that the surface of [(Pd-Au)$_n$/CoP]$_{CV}$ film displays orderly homogeneous morphology. The size of every nanoparticle is about 70 nm and most of the nanoparticles are in the state of aggregation. Such nanoparticles are aggregated into a big particle, which size is about 100 ~ 200 nm. Different amount and size of Pd-Au nanoparticles in [(Pd-Au)$_n$/CoP]$_{CV}$ film can be achieved by changing the bilayer number of [CoP|Pd(II)-Au(III)]$_n$ film when assembly.

### 5.2.3.4 Scanning electron microscopy (SEM) study

The result of SEM study (Fig. 5.2.7) is consistent with AFM result. Most of the nanoparticles are in the state of aggregation.
5.2.3.5 Application in bifunctional electrochemical sensing of NO and O₂

In most cases of biological and medical system, O₂ coexists with NO and interferes with the detection of NO, moreover, as an important molecule, precise detection of NO can provide valuable information for medical judgement. Thus, a bifunctional sensor with selective sensitivity toward both O₂ and NO should be desirable.

Fig. 5.2.8 displays the cyclic voltammograms of 6-quadri-layer [(Pd-Au)n/CoP]CV modified GC electrode (Fig. 5.2.8(b)) and bare GC electrode (Fig. 5.2.8(a)), respectively, in air-saturated pH=7 phosphate buffer solution with 1.0×10⁻⁴ mol L⁻¹ NO. In Fig. 5.2.8(a), only oxidation current is observed when the potential is higher than 0.65 V and the anodic peak is present at 0.99 V, which is assigned to the oxidation of NO on GC electrode. There is no evidently reductive current on GC, so that O₂ is inactive on GC. Compared with the CV characterization on the bare GC electrode, not only an enhancement of oxidation current is observed on [(Pd-Au)n/CoP]CV modified GC electrode and the peak potential decreases by 0.13 V, but also the large reduction current appears when the potential is below 0.30 V (Fig. 5.2.8(b)). On [(Pd-Au)n/CoP]CV modified electrode, the anodic peak at 0.86 V, which can be assigned to electrochemical oxidation of NO, and the cathodic peak at −0.04 V, which can be assigned to electrochemical reduction of O₂, can be observed. It shows that the electrochemical behaviors of NO and O₂ on the electrode are totally irreversible process. These features imply that [(Pd-Au)n/CoP]CV modified GC electrode has electrocatalytical properties toward both NO and O₂. In other words, [(Pd-Au)n/CoP]CV film has potential to be a good sensor of the detection of NO and
O₂. Since O₂ is reduced while NO is oxidized on [(Pd-Au)ₙ/CoP]_{CV} modified GC electrode, one [(Pd-Au)ₙ/CoP]_{CV} film modified electrode can be used to detect O₂ at lower potential and NO at higher potential. So it is rational that [(Pd-Au)ₙ/CoP]_{CV} film can be used in bifunctional bioelectrochemical sensing of O₂ and NO.

Amperometric response of 6-quadri-layer [(Pd-Au)ₙ/CoP]_{CV} modified GC electrode to NO with various concentration to NO range from 4.0×10⁻⁸ mol L⁻¹ to 1.28×10⁻⁶ mol L⁻¹ was tested at 0.70 V and the result was shown in Fig. 5.2.9. As can be seen from the figure, the modified electrode shows fast, sensitive and reproducible properties. Current of the electrode increased in a proportional mode when concentration of NO increased and gave a straight calibration curve with a regression correlation (R²) of 0.998. The relative standard deviation (R.S.D) was less than 5%. The detection limit of the modified electrode to NO is determined as 4.0×10⁻⁹ mol L⁻¹. This study illustrated a successful application of the newly developed electrode in electrochemical sensing of NO.

Amperometric measurements of O₂ solution with various concentrations were conducted at -0.05 V on 6-quadri-layer [(Pd-Au)ₙ/CoP]_{CV} film. I-t curve of O₂ with concentration increasing from 5.0×10⁻⁸ mol L⁻¹ to 1.2×10⁻⁶ mol L⁻¹ was shown in Fig. 5.2.10, the corresponding calibration curve shown in the inset shows a direct proportional relation between the current and concentration of O₂. Linear regression performed on the calibration curve gave the value of coefficient correlation (R²) as 0.9998. The relative standard deviation (R.S.D) was less than 5%. The detection limit was 5.0×10⁻⁸ mol L⁻¹, which is calculated from the signal to noise ratio of 3.
5.2.4 Conclusions

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged tetrachloropalladate, tetrachloroaurate and positively charged cobalt porphyrin on glassy carbon and ITO. Further, a novel film containing Pd-Au bimetallic nanoparticles was formed from the self-assembled molecular film by electrochemical reduction. The thickness of such film and the size of Pd-Au bimetallic nanoparticles can be controlled easily in the assembly process. We have systematically characterized our self-assembled film by using electrochemical method, AFM, UV-Vis and XPS spectroscopies and have monitored the growth of the film by UV-Vis spectroscopy. The electrochemical results showed that [(Pd-Au)$_n$/CoP]$_{CV}$ film exhibited high sensitivity and excellent selectivity to both O$_2$ and NO. In other words, the novel molecular film can be used in bifunctional bioelectrochemical sensing of NO and O$_2$.

5.2.5 References


Legends and Figures

Fig. 5.2.1 Scheme illustrating the assembling of \([\text{CoP}]_{\text{Pd(II)-Au(III)}} \) film and its electrochemical conversion to \([(\text{Pd-Au})_n/\text{CoP}]_{\text{CV}} \) film

Fig. 5.2.2 UV-Vis spectra of the assembly of \([\text{CoP}]_{\text{Pd(II)-Au(III)}} \) film on ITO-coated glass substrate. (a) one quadri-layer, (b) two quadri-layers, (c) three quadri-layers, (d) four quadri-layers, (e) five quadri-layers, (f) six quadri-layers and (g) seven quadri-layers. The inset depicts the relationship between absorbance and No. of quadri-layers.

Fig. 5.2.3 UV-Vis spectra of the assembly of \([\text{CoP}]_{\text{Pd(II)-Au(III)}} \) film on ITO-coated glass substrate. (a) one bilayer, (a') two bilayers (one quadri-layer), (b) three bilayers, (b') four bilayers (two quadri-layers), (c) five bilayers, (c') six bilayers, (d) seven bilayers, (d') eight bilayers, (e) nine bilayers, (e') ten bilayers, (f) eleven bilayers, (f') twelve bilayers, (g) thirteen bilayers (g') fourteen bilayers.

Fig. 5.2.4 UV-Vis spectra of 6-quadri-layer \([\text{CoP}]_{\text{Pd(II)-Au(III)}} \) film on ITO-coated glass substrate (a) before and (b) after electrochemical reductive process.

Fig. 5.2.5 XPS spectra of (A) palladium element in 6-quadri-layer \([\text{CoP}]_{\text{Pd(II)-Au(III)}} \) film, (B) gold element in 6-quadri-layer \([\text{CoP}]_{\text{Pd(II)-Au(III)}} \) film, (C) palladium element in 6-quadri-layer \([(\text{Pd-Au})_n/\text{CoP}]_{\text{CV}} \) film and (D) gold element in 6-quadri-layer \([(\text{Pd-Au})_n/\text{CoP}]_{\text{CV}} \) film on ITO-coated glass substrate.

Fig. 5.2.6 AFM image of 6-quadri-layer \([(\text{Pd-Au})_n/\text{CoP}]_{\text{CV}} \) film on ITO-coated glass substrate.

Fig. 5.2.7 SEM image of 6-quadri-layer \([(\text{Pd-Au})_n/\text{CoP}]_{\text{CV}} \) film on ITO-coated glass substrate.
Fig. 5.2.8 Cyclic voltammograms of (a) glassy carbon (dot line) and (b) 6-quadri-layer [(Pd-Au)$_n$/CoP]$_{CV}$ film modified on glassy carbon (solid line) in air-saturated pH=7 phosphate buffer solution with $1.0 \times 10^{-4}$ mol L$^{-1}$ NO. Scan rate: 50 mV s$^{-1}$.

Fig. 5.2.9 Changes of current in i-t curve of 6-quadri-layer [(Pd-Au)$_n$/CoP]$_{CV}$ film modified on glassy carbon in pH=7 phosphate buffer solution when various amount of NO was added. Potential was held at 0.70 V. The inset depicts the relationship between current and concentration of NO.

Fig. 5.2.10 Changes of current in i-t curve of 6-quadri-layer [(Pd-Au)$_n$/CoP]$_{CV}$ film modified on glassy carbon in pH=7 phosphate buffer solution when various amount of O$_2$ was added. Potential was held at -0.05 V. The inset depicts the relationship between current and concentration of O$_2$. 
Cobalt-tetra(N-methyl-4-pyridyl)-porphyrin cation

PdCl$_4^{2-}$, tetrachloropalladate anion

AuCl$_4^-$, tetrachloroaurate anion

Pd-Au bimetallic nanoparticle
Fig. 5.2.2
Fig. 5.2.5
Fig. 5.2.6
Fig. 5.2.7
Fig. 5.2.8

![Graph showing electrochemical behavior with curves labeled a and b.](image-url)
Fig. 5.2.9
Fig. 5.2.10
5.3 Hybrid Film Containing Pd-Au Bimetallic Nanoparticles
Fabricated by Photochemical and Electrochemical Methods and
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Abstract

We demonstrate the fabrication of a hybrid molecular film by alternatively self-assembling of cobalt porphyrin cation (CoP$^{1+}$), tetrachloropalladate anion (PdCl$_4^{2-}$) and tetrachloroaurate anion (AuCl$_4^-$) in a layer-by-layer manner on glassy carbon and ITO. The assembling process was monitored by UV-Vis spectroscopy, which showed that different building blocks were deposited successfully in each assembling step. Such multilayer film was reduced to yield nanocomposite of cobalt porphyrin and Pd-Au bimetallic nanoparticles by using combined photochemical and electrochemical methods. This is a novel method to fabricate bimetallic nanoparticles. The process was characterized by UV-Vis spectroscopy, electrochemical method, XPS and SEM. The film can serve as a bifunctional sensor for electro-reductive detection of O$_2$ and electro-oxidative detection of NO. Such bifunctional sensor for NO and O$_2$ is highly desirable in quantitation of NO levels in biological and medical systems, since it is known that NO level is highly regulated by various reactive oxygen species. Our bifunctional sensing system provides a means to detect NO and O$_2$ at the same site. A straight calibration curve was obtained in concentration range of 4.0×10$^{-8}$ to 2.60×10$^{-6}$ mol L$^{-1}$ for NO and 5.0×10$^{-8}$ to 3.2×10$^{-6}$ mol L$^{-1}$ for O$_2$, with detection limit of 8.0×10$^{-9}$ mol L$^{-1}$ and 1.0×10$^{-8}$ mol L$^{-1}$ respectively.
5.3.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be modified according to the aim of study [10-12]. However, to the best of our knowledge, there are few reports that combine such technique and electrochemical method for the formation of bimetallic nanoparticles.

Nanoscale particles of transition metals are of considerable interest as catalysts in organic and inorganic transformations, as electrocatalysts in fuel cells and as materials with novel electronic, optical and magnetic properties [13]. It is well known that bimetallic catalysts often exhibit superior catalytic properties than pure metals [14]. Palladium adlayers on single crystal platinum is well characterized [15-17]. Therefore, palladium is considered as an ideal candidate for an electrochemical study of bimetals. Au nanoparticles as attractive candidates of inorganic building
blocks have been intensively investigated in recent years because of their wide applications in microelectronic devices, spectroscopy and sensor technology, etc [18, 19].

NO has attracted intense interests due to its important roles in cellular communication and in host defense mechanisms of eukaryotes [20]. Detection of trace amount of NO in biological systems is quite challenging because of various interference from other coexisting species [21]. NO level is highly regulated by reactive oxygen species, and unfortunately the existence of O₂ is indispensable for in vivo detection of NO, therefore, measurement of NO concentration is only valuable in the existence of O₂.

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5.3.2 Experimental

5.3.2.1 Reagents and materials.

Potassium tetrachloropalladate, potassium tetrachloroaurate and cobalt-tetra (N-methyl-4-pyridyl)-porphyrin chloride were obtained from Aldrich and used as
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5.3.2.2 Instrumentation.

(i) Electrochemical measurements

The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified ITO electrode (9 x 9 x 0.5 mm float glass slide, ITO-coated two sides, Della Technologies, Limited-Stillwater, U.S.A) as the working electrode.
(ii) UV-visible spectroscopy

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was on the transparent ITO substrate. Bare ITO electrode was used as the reference.

(iii) X-ray photoelectron spectroscopy (XPS)

XPS measurement was performed on PHI 5600 (Physical Electronics, USA). Aluminum anode was used as X-ray monochromatic source. Pass energy 58.7 eV is used for narrow scan. The sample film was on the ITO substrate.

(iv) Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) images were obtained on a JEOL 6300F ultra-high resolution scanning electron microscope operated at 15 KV and on a JEOL 6700 ultra-high resolution scanning electron microscope operated at 5 KV. The sample film was on the ITO substrate.

(v) UV-Light Source

The UV-light source used for the sample exposure was an ORIEL 66011 500 W Xe/HgXe arc lamp (ORIEL corporation, Stratford, USA).
5.3.2.3 Fabrication of [CoP|Pd(II)-Au(III)]\textsubscript{n} film and its conversion by photochemical and electrochemical methods

The self-assembled films on ITO were prepared by alternatively dipping the substrates in anionic and cationic species. The clean substrates were immersed in 2×10\textsuperscript{-3} mol L\textsuperscript{-1} CoP\textsuperscript{4+} (dissolved in water) for 5 minutes, then a layer of cobalt porphyrin spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water. Then the substrates with a layer of CoP\textsuperscript{4+} were transferred into 5×10\textsuperscript{-3} mol L\textsuperscript{-1} PdCl\textsubscript{4}\textsuperscript{2-} (dissolved in water) for 5 minutes, then a layer of PdCl\textsubscript{4}\textsuperscript{2-} spontaneously adsorbed onto the CoP\textsuperscript{4+}-coated substrates by electrostatic attraction. Then, the substrates were washed with water. After this process, the substrates were immersed in 2×10\textsuperscript{-3} mol L\textsuperscript{-1} CoP\textsuperscript{4+} again for 5 minutes. Then the substrates were transferred into 5×10\textsuperscript{-3} mol L\textsuperscript{-1} AuCl\textsubscript{4}\textsuperscript{-} (dissolved in water) for 5 minutes. After these four steps, the first quadri-layer unit was formed. The film with different thickness can be achieved by repeating the above process. Finally, the film named [CoP|Pd(II)-Au(III)]\textsubscript{n} was formed.

After that, the film can be treated via photochemical and electrochemical combining steps. Firstly, [CoP|Pd(II)-Au(III)]\textsubscript{n} film was exposed in ultraviolet light for 4 hours. Since Au(III) can be converted to Au(0) easily by a photoinduction under ultraviolet irradiation [28]. Au(III) was reduced to Au(0), but Pd(II) remained its valence after photoinduction. Then the film was transferred into 0.5 mol L\textsuperscript{-1} H\textsubscript{2}SO\textsubscript{4} and Pd(II) was electrochemically reduced to Pd(0) and [(Pd-Au)\textsubscript{0}/CoP]\textsubscript{PE} was formed. All the processes are demonstrated in Fig. 5.3.1.
5.3.3 Results and discussion

5.3.3.1 Characterization by UV-Vis spectroscopy and electrochemistry

Using layer-by-layer self-assembled method, [CoP|Pd(II)-Au(III)]ₙ film was fabricated with PdCl₄²⁻, AuCl₄⁻ and cobalt porphyrin cation. The growth of [CoP|Pd(II)-Au(III)]ₙ film was monitored by UV-Vis spectrometry. Fig. 5.3.2 shows UV-Vis spectra of [CoP|Pd(II)-Au(III)]ₙ film assembled on ITO-coated glass. The characteristic cobalt porphyrin absorbance around 320, 435 and 550 nm can be observed and the intensity of each peak increases with number of as-prepared quadri-layers. The absorbance of potassium tetrachloropalladate and potassium tetrachloroaurate contributed to the intensity of the absorbance around 320 nm. The inset depicts the linear relationship between peak intensity and number of quadri-layers. It testify that same amount of cobalt porphyrin is assembled in each quadri-layer [CoP|Pd(II)-Au(III)]ₙ film.

After the film was assembled successfully, it was exposed in ultraviolet light for four hours. Then AuCl₄⁻ in the film was reduced to Au(0) while PdCl₄²⁻ in the film did not change and [CoP|Pd(II)-Au(0)]ₙ was formed. Fig. 5.3.3 (b) shows UV-Vis spectra of the film after photochemical reduction. After reduction, the characteristic cobalt porphyrin absorbance around 435 and 550 nm still remains and the absorption is almost unchanged.

[CoP|Pd(II)-Au(0)]ₙ film was transferred into 0.5 mol L⁻¹ H₂SO₄. In the film, PdCl₄²⁻ was reduced by cyclic voltammetry under potential between −0.2 ~ 0.2 V (vs. SCE). The color of the film was turned from yellow to gray. Fig. 5.3.3 (c) shows
UV-Vis spectra of the film after electrochemical reduction. After reduction, the characteristic cobalt porphyrin absorbance around 435 and 550 nm still remains and the absorption is almost unchanged, while the intensity of the absorbance around 320 nm is decreased due to the reduction of PdCl$_2^-$, Since the color was changed to gray after reduction, it is reasonable that the absorbance is not equal to zero in the whole range.

Comparing these three UV-Vis spectra, the reduction of PdCl$_4^{2-}$ and AuCl$_4^-$ and the formation of Pd(0)-Au(0) in the film can be confirmed.

5.3.3.2 X-ray photoelectron spectroscopy (XPS) study

X-ray photoelectron spectroscopy (XPS) is a good method to determine the valence of elements. Fig. 5.3.4 shows the XPS results of the as-prepared film before conversion ([CoP|Pd(II)-Au(III)]$_n$ film), that after photochemical conversion ([CoP|Pd(II)-Au(0)]$_n$ film) and that after photochemical and electrochemical conversion ([[(Pd-Au)$_n$/CoP] film) on ITO-coated glass.

The Pd(3d$_{5/2}$) and Pd(3d$_{3/2}$) peaks are present at 337.3 and 342.7 eV respectively, which is depicted in Fig. 5.3.4A, before conversion. After photochemical conversion, the peaks remain there, which is shown in Fig. 5.3.4C. After electrochemical conversion, the peaks shift to 335.4 and 340.7 respectively, which is shown in Fig. 5.3.4E. These results indicates that the palladium element exists in an oxidation form Pd(II) in [CoP|Pd(II)-Au(III)]$_n$ film and [CoP|Pd(II)-Au(0)]$_n$ film while it exits in the form of metal Pd(0) in [(Pd-Au)$_n$/CoP]$_{PE}$ film [29].
The Au(4f\textsubscript{7/2}) and Au(4f\textsubscript{5/2}) peaks are present at 72.5 and 75.8 eV respectively, which is depicted in Fig. 5.3.4B, before reduction. After photochemical conversion, the peaks shift to 83.7 and 87.3 eV respectively, which is shown in Fig. 5.3.4D. After further electrochemical conversion, the peaks do not change, which is shown in Fig. 5.3.4F. These results indicates that gold element exists in an oxidation form Au(III) in [CoP|Pd(II)-Au(III)]\textsubscript{n} film while it exits in the form of metal Au(0) in both [CoP|Pd(II)-Au(0)]\textsubscript{n} film and [(Pd-Au)\textsubscript{n}/CoP]\textsubscript{PE} film [29].

The Co(2p\textsubscript{3/2}) and Co(2p\textsubscript{1/2}) peaks are present at 780.5 and 795.8 eV respectively in XPS figures of the as-prepared films before and after conversion. These clarify that cobalt element exists in the form of oxidation Co(II) in [CoP|Pd(II)-Au(III)]\textsubscript{n} film and its valence is not changed when the film is converted to form [(Pd-Au)\textsubscript{n}/CoP]\textsubscript{PE} film [29].

The XPS experiment also gives some information about chlorine element. The peak strength becomes greatly weak after reduction though its position doesn’t change any. It shows that most of Cl(-1) ions are released and move out during the reduction process from the film. It is reasonable that part of the Cl(-1) ions are in the reduced film to obtain electronic equilibrium in the film.

5.3.3.3 Scanning electron microscopy (SEM) study

From UV-Vis spectroscopy, XPS and electrochemical results, we can confirm that Pd(0) and Au(0) are formed after photochemical and electrochemical conversion, but we can not tell directly if Pd(0) or Au(0) is nanoparticle in [(Pd-Au)\textsubscript{n}/CoP]\textsubscript{PE} film. Scanning electron microscopy (SEM) gives some information about the surface
morphology of \([\text{CoP}][\text{Pd(II)}]+\text{Au(0)}\) film (Fig. 5.3.5A) and \([(\text{Pd-Au})_n/\text{CoP}]_{\text{PE}}\) film (Fig. 5.3.5B) and especially the size Pd-Au bimetallic particles in the film.

Au nanoparticles can be observed clearly in Fig. 5.3.5A. It is obvious that the surface of \([\text{CoP}][\text{Pd(II)}]+\text{Au(0)}\) film is homogeneous.

Distributed Pd-Au bimetallic nanoparticles can be observed clearly in Fig. 5.3.5B. It is obvious that the surface of \([(\text{Pd-Au})_n/\text{CoP}]_{\text{PE}}\) film displays orderly homogeneous morphology and the particle size is about 70 nm. Different amount and size of Pd-Au nanoparticles in \([(\text{Pd-Au})_n/\text{CoP}]_{\text{PE}}\) film can be achieved by changing the bilayer number of \([\text{CoP}][\text{Pd(II)}]+\text{Au(III)}\) film when assembly.

5.3.3.4 Application in bifunctional electrochemical sensing of NO and O2

In most cases of biological and medical system, O2 coexists with NO and interferes with the detection of NO, moreover, as an important molecule, precise detection of NO can provide valuable information for medical judgement. Thus, a bifunctional sensor with selective sensitivity toward both O2 and NO should be desirable.

Fig. 5.3.6 displays the cyclic voltammograms of 6-quadri-layer \([(\text{Pd-Au})_n/\text{CoP}]_{\text{PE}}\) modified ITO electrode (Fig. 5.3.6(b)) and bare ITO electrode (Fig. 5.3.6(a)), respectively, in air-saturated pH=7 phosphate buffer solution with \(1.0\times10^{-4}\) mol L\(^{-1}\) NO. In Fig. 5.3.6(a), neither oxidative current nor evidently reductive can be seen on ITO electrode, so that both NO and O2 are inactive on ITO electrode. Compared with the CV characterization on the bare ITO electrode, not only large oxidation current is observed on \([(\text{Pd-Au})_n/\text{CoP}]_{\text{PE}}\) modified ITO electrode when the potential is above...
0.70 V, but also the large reduction current appears when the potential is below 0.20 V (Fig. 5.3.6(b)). On [(Pd-Au)$_n$/CoP]$_{PE}$ modified electrode, the anodic peak at 0.86 V, which can be assigned to electrochemical oxidation of NO, and the cathodic peak at -0.08 V, which can be assigned to electrochemical reduction of O$_2$, can be observed. It shows that the electrochemical behaviors of NO and O$_2$ on the electrode are totally irreversible process. These features imply that [(Pd-Au)$_n$/CoP]$_{PE}$ modified ITO electrode has electrocatalytical properties toward both NO and O$_2$. In other words, [(Pd-Au)$_n$/CoP]$_{PE}$ film has potential to be a good sensor for the detection of NO and O$_2$. Since O$_2$ is reduced while NO is oxidized on [(Pd-Au)$_n$/CoP]$_{PE}$ modified ITO electrode, one [(Pd-Au)$_n$/CoP]$_{PE}$ film modified electrode can be used to detect O$_2$ at lower potential and NO at higher potential. So it is rational that [(Pd-Au)$_n$/CoP]$_{PE}$ film can be used in bifunctional bioelectrochemical sensing of O$_2$ and NO.

Amperometric response of 6-quadri-layer [(Pd-Au)$_n$/CoP]$_{PE}$ modified ITO electrode to NO with various concentration to NO range from 4.0×10$^{-8}$ mol L$^{-1}$ to 2.6×10$^{-6}$ mol L$^{-1}$ was tested at 0.75 V and the result was shown in Fig. 5.3.7. As can be seen from the figure, the modified electrode shows fast, sensitive and reproducible properties. Current of the electrode increased in a proportional mode when concentration of NO increased and gave a straight calibration curve with a regression correlation (R$^2$) of 0.998. The relative standard deviation (R.S.D) was less than 5%. The detection limit of the modified electrode to NO is determined as 8.0×10$^{-9}$ mol L$^{-1}$, which is calculated from the signal to noise ratio of 3. This study illustrated a successful application of the newly developed electrode in electrochemical sensing of NO.
Amperometric measurements of O$_2$ solution with various concentrations were conducted at -0.05 V on 6-quadri-layer [(Pd-Au)$_n$/CoP]$_{PE}$ film. I-t curve of O$_2$ with concentration increasing from $5.0 \times 10^{-8}$ mol L$^{-1}$ to $3.2 \times 10^{-6}$ mol L$^{-1}$ was shown in Fig. 5.3.8, the corresponding calibration curve shown in the inset shows a direct proportional relation between the current and concentration of O$_2$. Linear regression performed on the calibration curve gave the value of coefficient correlation ($R^2$) as 0.9995. The relative standard deviation (R.S.D) was less than 5%. The detection limit was $1.0 \times 10^{-8}$ mol L$^{-1}$.

**5.3.4 Conclusions**

In this work, a layer-by-layer self-assembled molecular film has been successfully prepared with negatively charged tetrachloropalladate, tetrachloroauration and positively charged cobalt porphyrin on glassy carbon and ITO. Further, a novel film containing Pd-Au bimetallic nanoparticles was formed from the self-assembled molecular film by both photochemical and electrochemical reduction. The thickness of such film and the size of Pd-Au bimetallic nanoparticles can be controlled easily in the assembly process. We have systematically characterized our self-assembled film by using electrochemical method, SEM, UV-Vis and XPS spectroscopies and have monitored the growth of the film by UV-Vis spectroscopy. The electrochemical results showed that [(Pd-Au)$_n$/CoP]$_{PE}$ film exhibited high sensitivity and excellent selectivity to both O$_2$ and NO. In other words, the novel
molecular film can be used in bifunctional bioelectrochemical sensing of NO and O₂.

5.3.5 References


Legends and Figures

Fig. 5.3.1 Scheme illustrating the assembling of [CoP\|Pd(II)-Au(III)]\text{PE} film and its photochemical and electrochemical conversion to [(Pd-Au)\text{n}/CoP]\text{PE} film.

Fig. 5.3.2 UV-Vis spectra of the assembly of [CoP\|Pd(II)-Au(III)]\text{n} film on ITO-coated glass substrate. (a) one quadri-layer, (b) two quadri-layers, (c) three quadri-layers, (d) four quadri-layers, (e) five quadri-layers, (f) six quadri-layers and (g) seven quadri-layers. The inset depicts the relationship between absorbance and No. of quadri-layers.

Fig. 5.3.3 UV-Vis spectra of 6-quadri-layer [CoP\|Pd(II)-Au(III)]\text{n} film on ITO-coated glass substrate (a) before any conversion, (b) after photochemical conversion and (c) after photochemical and electrochemical conversion.

Fig. 5.3.4 XPS spectra of (A) palladium element in 6-quadri-layer [CoP\|Pd(II)-Au(III)]\text{n} film, (B) gold element in 6-quadri-layer [CoP\|Pd(II)-Au(III)]\text{n} film, (C) palladium element in 6-quadri-layer [CoP\|Pd(II)-Au(0)]\text{n} film, (D) gold element in 6-quadri-layer [CoP\|Pd(II)-Au(0)]\text{n} film, (E) palladium element in 6-quadri-layer [(Pd-Au)\text{n}/CoP]\text{PE} film and (F) gold element in 6-quadri-layer [(Pd-Au)\text{n}/CoP]\text{PE} film on ITO-coated glass substrate.

Fig. 5.3.5 A. SEM image of 6-quadri-layer [CoP\|Pd(II)-Au(0)]\text{n} film on ITO-coated glass substrate. B. SEM image of 6-quadri-layer [(Pd-Au)\text{n}/CoP]\text{PE} film on ITO-coated glass substrate.

Fig. 5.3.6 Cyclic voltammograms of (a) bare ITO-coated glass electrode (dot line) and (b) 6-quadri-layer [(Pd-Au)\text{n}/CoP]\text{PE} film modified on ITO (solid line) in air-
saturated pH=7 phosphate buffer solution with $1.0 \times 10^{-4}$ mol L$^{-1}$ NO. Scan rate: 50 mV s$^{-1}$.

Fig. 5.3.7 Changes of current in i-t curve of 6-quadri-layer [(Pd-Au)$_n$/CoP]$_{PE}$ film modified on ITO-coated glass electrode in pH=7 phosphate buffer solution when various amount of NO was added. Potential was held at 0.75 V. The inset depicts the relationship between current and concentration of NO.

Fig. 5.3.8 Changes of current in i-t curve of 6-quadri-layer [(Pd-Au)$_n$/CoP]$_{PE}$ film modified on ITO-coated glass electrode in pH=7 phosphate buffer solution when various amount of O$_2$ was added. Potential was held at -0.05 V. The inset depicts the relationship between current and concentration of O$_2$. 
Fig. 5.3.1

Cobalt-tetra(N-methyl-4-pyridyl)-porphyrin cation

PdCl$_4^{2-}$, tetrachloropalladate anion

AuCl$_4^{-}$, tetrachloroaurate anion

Au nanoparticle

Pd-Au bimetallic nanoparticle
Fig. 5.3.2
Fig. 5.3.3
Fig. 5.3.4
Fig. 5.3.5 A
Fig. 5.3.5 B
Fig. 5.3.6

![Graph showing redox behavior with currents (I) in µA on the y-axis and electrode potentials (E) in V (vs. SCE) on the x-axis. Two curves are depicted: a dashed line labeled 'a' and a solid line labeled 'b'.]
Fig. 5.3.7
Fig. 5.3.8
CHAPTER 6

A Novel Method to Prepare Three-Dimensional Fractal Metal Nanostructures by Electrodeposition on Polyelectrolytes-Multilayer-Film Substrate
Abstract

We demonstrate the fabrication of a hybrid multilayer molecular film by alternatively self-assembling of polycation (poly(diallyldimethyl-ammonium chloride), PDDA) and polyanion (poly(sodium 4-styrenesulfonate), PSS) in a layer-by-layer manner on ITO. The assembling process was monitored by UV-Vis spectroscopy, which shows that equal amount of building blocks were deposited in each assembling step. Such polyelectrolytes film was used as a preformed matrix in electrochemical deposition to yield three-dimensional fractal Au nanostructure. The process was characterized by UV-Vis spectroscopy, electrochemical method, XPS, SEM and AFM. The film can be used in sensing of NO. Such sensor for NO is highly desirable in quantitation of NO levels in biological and medical systems. A straight calibration curve was obtained in concentration range of 4.0×10^{-8} mol L^{-1} to 4.0×10^{-6} mol L^{-1} with detection limit of 1.0×10^{-8} mol L^{-1}.

6.1 Introduction

Electrode surface modification with electroactive film remains the subject of intensive studies since its origin two decades ago. Areas such as electrocatalysis, electrochromic materials, optical materials and energy conversion continue to be actively explored as applications of surface modification [1, 2].

The techniques of layered architectures in a designed way, such as the Langmuir-Blodgett deposition, self-assembly and other methods have received considerable attraction in recent years [3-6]. Recently, a novel preparative technique
of molecular multilayer film, first introduced by Decher et al., has been developed via the alternate adsorption of anionic and cationic species [7-9]. This new technique is based on electrostatic interaction as the driving force. Molecular film with electrostatic linkage can be easily prepared and the micro-structure and spatial arrangement can be modified according to the aim of study [10-12].

The study of the structure and dynamics of deposition of metal on a solid substrate is both fundamental and technological importance [13,14]. Of particular interest is the electrochemically deposited nanostructure. Such efforts include under potential deposition on single crystal surfaces [15], electrochemical atomic layer epitaxy [16], electrodeposition on supported microporous film [17] and so on. However, to the best of our knowledge, few studies have focused on the preparation of metal nanostructure by electrodeposition on a surface covered by a layer-by-layer film.

NO has attracted intense interests due to its important roles in cellular communication and in host defense mechanisms of eukaryotes [18]. Detection of trace amount of NO in biological systems is quite challenging because of various interference from other coexisting species [19].

Herein we describe a novel three-dimensional fractal Au nanostructure by electrodeposition of Au onto an ITO substrate modified with layer-by-layer self-assembled polyelectrolytes (PDDA and PSS). Such new film was characterized and confirmed by UV-Vis spectroscopy, electrochemical method, XPS, AFM and SEM. We studied in detail the application of such film in bioelectrochemical sensing of NO.
6.2 Experimental

6.2.1 Reagents and materials.

Poly(diallyldimethyl-ammonium chloride) (PDDA) (20 wt.% in water, Mw ca. 200,000-350,000), poly(sodium 4-styrenesulfonate (PSS) (Mw ca. 70,000) and potassium tetrachloroaurate (KAuCl₄) were purchased from Aldrich and used as received. Sulfuric acid, reagent ACS, was obtained from Acros. Phosphate buffer solution (PBS) was obtained from Riedel – de Haen (Germany) and employed as the supporting electrolytes. Milli-Q pure water was used throughout this work. Quartz and ITO-coated glass (both sides) was purchased from Hong Kong Labware Company and Delta Technologies (U.S.A.), respectively. Standard saturated NO solutions were prepared by bubbling a high purity NO gas through milli-Q pure water for 40 minutes after it was bubbled with high purity N₂ gas for 30mins [20-22]. The saturated aqueous NO solution has a NO concentration of $1.8 \times 10^{-3}$ mol L⁻¹ at 20°C, $P(\text{NO}) = 1$ atm [23,24].

6.2.2 Instrumentation.

(i) Electrochemical measurements

The electrode modification and cyclic voltammetric measurements were carried out on a BAS 100B/W electrochemical workstation (Bioanalytical Systems, West Lafayette, USA) controlled by the BAS 100B/W software through a personal
computer. A single-compartment BAS Model VC-2 electrochemical cell was employed in all experiments and the three-electrode system, which includes a platinum wire (MW-1032, BAS, U.S.A.) as a counter electrode, a saturated calomel electrode (SCE) as a reference electrode, and a modified or unmodified ITO electrode (9 x 9 x 0.5 mm float glass slide, ITO-coated two sides, Della Technologies, Limited-Stillwater, U.S.A) as the working electrode.

**(ii) UV-visible spectroscopy**

Absorption spectra were recorded on a diode array spectrophotometer (V550 spectrophotometer, Jasco Ltd). Quartz rectangular cuvette (10mm light path, Aldrich, U.S.A.) was used. The sample film was deposited on quartz or ITO substrate.

**(iii) X-ray Photoelectron Spectroscopy (XPS)**

XPS measurement was performed on PHI 5600 (Physical Electronics, USA). Aluminum anode was used as X-ray monochromatic source. Pass energy 58.7 eV is used for narrow scan. The sample film was on the ITO substrate.

**(iv) Scanning Electron Microscopy (SEM)**

Scanning electron microscopy (SEM) images were obtained on a JEOL 6300F ultra-high resolution scanning electron microscope operated at 15 KV. The sample film was on the ITO substrate.
(v) **Atomic force microscopy (AFM)**

AFM images were obtained on a multimode Scanning Probe Microscope with Nanoscope IIIa control unit by Digital instrument Inc. Tapping mode was applied for the film morphology study with scan area of 2.5×2.5μm. The sample film was on the ITO substrate.

### 6.2.3 Three-dimensional fractal Au nanostructure fabrication

The self-assembled films on different substrates were prepared by alternatively dipping the substrates in cationic and anionic species. The clean substrates were immersed in 1.0 g L⁻¹ PDDA (dissolved in water) for 5 minutes, then a layer of PDDA spontaneously adsorbed onto the substrate surfaces. After that, the substrates were washed thoroughly with water and dried by N₂. Then the substrates with a layer of PDDA were transferred into 1.0 g L⁻¹ PSS (dissolved in water) for 5 minutes, then a layer of PSS spontaneously adsorbed onto the PDDA-coated substrates by electrostatic attraction. Then, the substrates were washed with water and dried by N₂. The film with different thickness can be achieved by repeating the above process. Thus the film named [PDDA|PSS]ₙ was formed.

After that, [PDDA|PSS]ₙ film was transferred into 3×10⁻³ mol L⁻¹ KAuCl₄ + 0.5 mol L⁻¹ H₂SO₄ solution and three-dimensional fractal Au nanostructure was yielded by electrochemical reduction on [PDDA|PSS]ₙ film, the film named Au/[PDDA|PSS]ₙ was prepared successfully. All the above processes are
demonstrated in Fig. 6.1.

6.3 Results and discussion

6.3.1 Characterization by UV-Vis spectroscopy

The process of the layer-by-layer self-assembly of two classical polyelectrolytes, PDDA and PSS, which were chosen for the great stability of the resulting film in aqueous solutions because of their strong electrostatic interaction, was monitored by UV-Vis spectroscopy. Fig. 6.2 shows the spectra of the film assembled on a quartz substrate. The peak appearing at 225 nm can be assigned to the phenyl group of PSS. From the change of absorbance at 225 with the number of bilayers in inset, it is clear that the film of PDDA and PSS have been successfully fabricated in the assembly process and the same amount of PSS is assembled in each bilayer [PDDA|PSS]_n film.

On an ITO glass modified with such fabricated 6.5-bilayer [PDDA|PSS]_n film, Au was electrodeposited from a 3×10^{-3} mol L^{-1} KAuCl_4 + 0.5 mol L^{-1} H_2SO_4 aqueous solution by cycling the potential from 200 to \(-210\) mV only once at 50 mV/s scan rate. Fig. 6.3 shows the UV-Vis spectrum of the film after deposition, in which a broad and continuous absorption band centered at about 640 nm is observed. Usually, such band, visibly different from that of the discrete Au nanoparticles, may be accounted for the aggregation or close pack of Au nanoparticles. Our following investigation shows that the UV-Vis spectrum herein is related with a novel fractal Au nanostructure.
6.3.2 Characterization by XPS

In order to confirm the result of the formation of Au on \([\text{PDDA}\text{PSS}]_n\) film, the surface after electrodeposition was detected by X-ray photoelectron spectroscopy (XPS) (Fig. 6.4). The \(\text{Au}(4f_{5/2})\) and \(\text{Au}(4f_{7/2})\) peaks appear at 87.5 and 83.9 eV respectively, which indicates that Au in metal form is deposited on the \([\text{PDDA}\text{PSS}]_n\) film [25].

6.3.3 Electrochemical characterization

Cyclic voltammogram shows a pair of oxidation and reduction peaks at 0.864 and 0.456 V (Fig. 6.5) respectively. Generally, corresponding to the gold oxide formation and sequent reduction, they could be regarded as characteristic peaks of Au nanostructure. The voltammetric result associated with XPS data demonstrates the formation of Au nanostructure by electrodeposition of Au on \([\text{PDDA}\text{PSS}]_n\) film.

6.3.4 Characterization by SEM

The morphology of thus formed Au nanostructure was observed by scanning electron spectroscopy (SEM). From Fig. 6.6A, in the large scale, a great number of Au islands with different diameters from several decades nanometers to one micrometer are distributed all over the surface. When the single island is zoomed in, a beautiful three-dimensional fractal Au nanostructure is exhibited (Fig. 6.6B), with the branches of 50-70 nm in diameter and several hundred nanometers in length. It is
clear that not a two-dimensional "snowflake", but a three-dimensional fractal cluster is fabricated. In contrast, Au electrodeposition on a naked ITO substrate in same condition doesn't product similar structures (Fig. 6.7).

The situation of Au electrodeposition on different bilayers \([\text{PDDA}\text{PSS}]_{n}\) films was investigated to study in detail the role of polyelectrolytes-multilayer-matrix film in the formation of fractal Au nanostructure. Fig. 6.8 shows four different SEM images of the fractal Au nanostructure formations on 1.5-bilayer (Fig. 6.8A), 3.5-bilayer (Fig. 6.8B), 6.5-bilayer (Fig. 6.8C) and 12.5-bilayer (Fig. 6.8D) \([\text{PDDA}\text{PSS}]_{n}\) films respectively. Some information can be concluded after the four images are compared with one another. The density of Au nanostructure is more and more higher and the average size of the Au fractal is more and more larger when the number of bilayer of \([\text{PDDA}\text{PSS}]_{n}\) film increases.

According to SEM results, it is believe that the layer-by-layer polyelectrolytes-multilayer-matrix film plays a key role in the formation of such Au nanostructure. The growth of a solid phase on a foreign substrate is a complex process that involves the initial formation of nuclei and their growth formation of cluster [26]. Due to the covering of an inconductive multilayer polymer film on the ITO surface, three possible influences on the formation of Au nanostructure could be presumed, although the fundamental basis of the formation of such structure is not yet fully understood. Firstly, the nuclei formation just could take place on the defective spot, i.e. the position on the ITO surface not occupied by the film. Therefore, the secrete Au islands on the surface are formed. Secondly, the growth of the Au nanostructure on the surface would be radiate, but vertically favored because of the film lateral barrier. Therefore, the structure is a three-dimensional, not a
two-dimensional snowflake [27]. Last but not less, the polymer film would be
dynamic on the surface during the Au electrodeposition, thus new deposition place
would appear with time. That is possibly why there are different-size islands
coexisting on the surface.

6.3.5 Characterization by AFM

Atomic force microscopy (AFM) was used to observe the surface morphology of
[PDDA:PSS]ₙ film (Fig. 6.9). The observed particles are the multilayer-film-coated
ITO particles. It indicates that a polymer multilayer film with a high surface
coverage is formed.

In order to obtain more information about the Au islands’ radius and heights, the
surface was characterized by AFM (Fig. 6.10). From the section analysis of AFM
image (Fig. 6.10B), the aspect ratio can be calculated, \( f = \frac{\text{maximum island height}}{\text{maximum island radius}} \), as more than 0.5, which confirms the resulting nanostructure
is three-dimensional.

6.3.6 Application in electrochemical sensing of NO

Catalysis and analysis are the most important applications of metal nanostructure
[28]. Herein, the application of the fractal Au nanostructure in NO electrochemical
sensing was investigated. Owing to NO important physiological roles, the
measurement of NO has attracted extensive attentions [29].

Fig. 6.11 displays the cyclic voltammograms of the Au nanostructure deposited on
a 6.5-bilayer [PDDA\textsc{PSS}]_n modified ITO in absence and presence of NO in pH=7 phosphate buffer solution. After addition of NO, not only a new anodic peak appeared at around 0.805 V can be observed, but also the large oxidation current appears when the potential is over 0.62 V. This new anodic peak and the large oxidation current can be assigned to the electrochemical oxidation of NO on such Au nanostructure modified ITO electrode. It shows that the electrochemical behavior of NO on the electrode is an irreversible process. The current of the oxidative peak at 0.805 V increases in a proportional mode when concentration of NO increases in the range from $2.0 \times 10^{-6}$ to $3.2 \times 10^{-5}$ mol L$^{-1}$ and it gives a straight calibration curve with a regression correlation ($R^2$) of 0.999. The relative standard deviation (R.S.D) is less than 5\%. In contrast, the [PDDA\textsc{PSS}]_n film before electrodeposition of Au doesn’t shows any electrochemical activity whether NO is present or not. This indicates that the resulting Au nanostructure has a good catalytic activity for NO oxidation. In other words, such Au nanostructure has potential to be a good sensor for the detection of NO.

For the trace amount detection, amperometric method should be used. Amperometric response of the Au nanostructure modified electrode to NO with different concentrations was tested at 0.78 V and the result was shown in Fig. 6.12. The steady-state oxidative current increases in a proportional mode when concentration of NO increases in the range from $4.0 \times 10^{-8}$ to $4.0 \times 10^{-6}$ mol L$^{-1}$ and it gives a straight calibration curve with a regression correlation ($R^2$) of 0.999. The relative standard deviation (R.S.D) is less than 5\%. The detection limit is $1.0 \times 10^{-8}$ mol L$^{-1}$, which is based on the signal-to-noise ratio of three. This study illustrates the fast respond, high sensitivity and reproducibility of such Au nanostructure modified
electrode. The above study reveals the great potential of such fabricated Au nanostructure in the field of electrochemistry.

6.4 Conclusions

In this work a new strategy was introduced to combine the layer-by-layer self-assembly technique with electrochemical deposition for controlling the density and morphology of gold nanostructure fabrication: electrodeposition of Au onto a surface covered by a layer-by-layer polyelectrolytes-multilayer-matrix film. Thus prepared three-dimensional Au nanostructure as well as its application in NO electroanalysis sensing was investigated. It is believed that the further research on it would be very meaningful in both theoretical and applied fields. Its potential applications in electrochemistry could be greatly anticipated. Such new method can be extended to apply in the preparation of other three-dimensional fractal metal nanostructure, for example, Ag and Cu.

6.5 References


Legends and Figures

Fig. 6.1 Scheme of the preparation of three-dimensional fractal Au nanostructure on [PDDA|PSS]ₙ film.

Fig. 6.2 (A) The UV-Vis spectra of assembly of [PDDA|PSS]ₙ layer-by-layer films on a quartz substrate. The insert shows the growth of the UV absorbance at 225 nm of the films as a function of the number of bilayers.

Fig. 6.3 The UV-Vis spectrum of a 6.5-bilayer [PDDA|PSS]ₙ film on an ITO glass after Au electrodeposition.

Fig. 6.4 XPS spectrum of a 6.5-bilayer [PDDA|PSS]ₙ film on an ITO glass after Au electrodeposition.

Fig. 6.5 Cyclic voltammograms of the Au nanostructure deposited on a 6.5-bilayer [PDDA|PSS]ₙ modified ITO in pH=7.0 PBS solution. Area: 0.9 cm×0.9 cm.

Fig. 6.6 SEM images of fractal Au nanostructure electrodeposited on a 6.5-bilayer [PDDA|PSS]ₙ film modified ITO.

Fig. 6.7 SEM images of Au nanostructure electrodeposited on a bare ITO.

Fig. 6.8 SEM images of fractal Au nanostructure electrodeposited on (a) 1.5-bilayer, (b) 3.5-bilayer, (c) 6.5-bilayer and (d) 12.5-bilayer [PDDA|PSS]ₙ film modified ITO.

Fig. 6.9 AFM height image (3 μ M×3 μ M) of a 6.5-bilayer [PDDA|PSS]ₙ film on a ITO glass.

Fig. 6.10 AFM height image (3 μ M×3 μ M) (A) and its cross section (B) of a
6.5-bilayer \([\text{PDDA\midPSS}]_{n}\) film on a ITO glass after Au electrodeposition.

Fig. 6.11 Cyclic voltammograms of the Au nanostructure deposited on a 6.5-bilayer \([\text{PDDA\midPSS}]_{n}\) modified ITO in a PBS solution (pH=7.0) in the (a) absence and presence of (b) 2.0\times10^{-6}, (c) 4.0\times10^{-6}, (d) 8.0\times10^{-6}, (e) 1.6\times10^{-5} and (f) 3.2\times10^{-5} mol L^{-1} NO. Scan rate: 50 mV/s. Area: 0.9 cm\times 0.9 cm.

Fig. 6.12 The amperometric response of the Au nanostructure deposited on a 6.5-bilayer \([\text{PDDA\midPSS}]_{n}\) modified ITO glass (0.9 cm\times 0.9 cm) in a PBS solution (pH=7.0) at the potential of 0.78 V with successive additions of NO at the concentrations of 0.04, 0.10, 0.24, 0.60, 1.40, 2.80 and 4.0 \(\mu\)M. The insert shows the current versus the concentrations of NO.
Fig. 6.1

Electrochemical reduction in KAuCl₄ solution

PDDA, poly(diallyldimethylammonium) cation

PSS, poly(sodium 4-styrenesulfonate) anion
Fig. 6.2

[Graph showing absorbance vs. wavelength with an inset showing absorbance vs. number of bilayers at 225 nm]
Fig. 6.3
Fig. 6.4
Fig. 6.5
Fig. 6.6
Fig. 6.9
Fig. 6.11
Fig. 6.12
CHAPTER 7

Summary and Perspective
The main objectives of this PhD thesis are: 1. The development of new organic-inorganic layer-by-layer self-assembled multilayer films and/or their conversion to functional films by electrochemical and photochemical methods; 2. To explore the applications of these films in electrocatalysis and electrochromism.

Chapter 2 reports the assembly of several new organic-inorganic layer-by-layer molecular films on various substrates. The layer-by-layer self-assembling process was monitored by electrochemical method, UV-Vis and FT-IR spectroscopies. The films exhibit good selectivity and high sensitivity to the catalytic sensing of several important small biomolecules such as NO, H₂O₂ and ascorbic acid.

Chapter 3 describes the successful preparation of layer-by-layer self-assembled multilayer films with heteropoly oxometalate and viologen for the purpose of electrochromic application. Electrochemical method, UV-Vis and FT-IR spectroscopies were employed to systematically characterize the new films. The films exhibit excellent electrochromic performance and good stability.

Chapter 4 introduces an easy method to fabricate layer-by-layer multilayer films and their conversion by photochemical and electrochemical methods to hybrid films containing metal nanoparticles. Electrochemical method, AFM, UV-Vis and XPS spectroscopies were used to monitor and confirm the formation of metal nanoparticles. These novel molecular films contain catalytically active metalloporphyrins and metal nanoparticles for application in bifunctional bioelectrochemical sensing.
Chapter 5 presents layer-by-layer self-assembly of multilayer films and their conversion to hybrid functional films by electrochemical and/or photochemical methods. The hybrid films generated contain bimetallic nanoparticles and metalloporphyrins. The size of bimetallic nanoparticles and the ratio between two metals can be controlled in the assembly process. The electrochemical results showed that the films thus prepared exhibit high sensitivity and excellent selectivity to the electrochemical detection of O₂ and NO.

Chapter 6 suggests a new strategy to couple the layer-by-layer self-assembly technique with electrochemical deposition for controlling the density and morphology of gold nanostructure fabrication: electrodeposition of Au onto a surface covered by layer-by-layer polyelectrolytes-multilayer-matrix film. As-prepared three-dimensional Au nanostructure as well as its application in electrocatalytic sensing of NO was investigated in detail. We believe that the further research on it will be very important in both theoretical and applied aspects. This novel method may be extended to the preparation of three-dimensional fractal nanostructure of other metals such as Ag and Cu.

On the basis of the findings made in this thesis, the following aspects should be further pursued in the future study:

1. To miniaturize the substrate size and to modify it by layer-by-layer self-assembled films and to evaluate their analytical performance in in vivo detection.

2. To explore the applications of electrochromic layer-by-layer multilayer films
in electrochromic devices.

3. To systematically study the novel three-dimensional fractal metal nanostructure, including their analytical performance and spectroscopic features (e.g., surface-enhance Raman scattering).