Smart Organometallic Polymer Platforms for Redox Sensing and as Metal Nanoparticle Foundry

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**SMART ORGANOMETALLIC POLYMER
PLATFORMS FOR REDOX SENSING AND
AS METAL NANOPARTICLE FOUNDRY**

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SMART ORGANOMETALLIC POLYMER PLATFORMS FOR REDOX SENSING AND AS METAL NANOPARTICLE FOUNDRY

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Chapter 1

General Introduction

This Chapter presents a short, general introduction to the redox-active, organometallic polymer poly(ferrocenylsilane) (PFS). We focus our research on the development of novel PFSs, containing functionalities that allow us to immobilize these polymers on electrode surfaces, or to form networks by crosslinking reactions. The resulting redox-active films and gels will be used in electrochemical sensing applications and as reductive encapsulant for the fabrication of metallic nanoparticles. In this Chapter, an overview of the Thesis is provided.

1.1 Introduction

Organometallic polymers refer to a wide range of metal-containing polymers, which have attracted rapidly expanding interest due to their unique chemical and physical properties.^{1–3} The presence of metals in their main chain or in side groups enables organometallic polymers to play a pivotal role in modern high-tech applications in many areas. In most cases, organometallic polymers have intrinsic redox and luminescent properties inherited from the metal centers and hence they are often regarded as stimulus responsive or smart macromolecular materials which exhibit abrupt conformational or chemical changes in response to small variations of external stimuli.^{4–8}

Poly(ferrocenylsilane)s (PFSs) are a novel type of metal-containing macromolecules with a backbone consisting of alternating ferrocene and organosilane units.^{9, 10} The presence of redox-active ferrocene units along the polymer main chain provides unique redox-responsive properties to PFSs, showing a complex multiple redox process.¹¹ At the same time, the presence of silane groups offers many opportunities for further functionalization of the polymer.

Figure 1.1 captures on the left a PFS chain with asymmetric substitution. Group R linked to Si can display various structures as shown on the right. By altering the

Figure 1.1: Redox active organometallic polymer: Poly(ferrocenylsilane)s.

chemical composition of R, the properties of the polymer can be varied in a broad range. When exposed to redox stimuli (electrochemical or chemical), PFS chains bound to surfaces (top) or in the bulk (bottom) can be reversibly oxidized and reduced. Typical electrochemical cycles show double-wave voltammograms as presented, related to a stepwise oxidation (first statistically every second ferrocene, and at higher potentials the remaining centers become oxidized). In the process the material changes color (from the orange-amber appearance in the neutral state, to dark green in the oxidized states, respectively).

1.2 Concept of this Thesis

In this Thesis the preparation and characterization of various PFSs are described, including virtually unexplored structures. We aimed at developing novel tailored architectures and exploring rationally designed systems as redox active platforms for specific functions. First we embarked on the quest to enhance the range of applications of surface bound PFS for sensing. To this end, we developed new strategies to immobilize the polymer by electrografting, and by layer-by-layer deposition in combination with covalent coupling. As we primarily aimed at biomedical applications, we mainly focused on water soluble PFS systems, including polyionic liquids and hydrogels. Inherent to the redox responsive behavior, PFS hydrogels have the ability to reduce metal ions that exhibit oxidation potentials exceeding the value typical for ferrocene. We tackled the question of making various PFS hydrogel structures, which could be swollen by electrolytes including the metal ions of interest. In the reduction process without the use of any external reducing agents, metal nanoparticles form upon exposing the salt solutions to the PFS hydrogel. These particles can be further used in sensing, in catalysis and for antimicrobial surfaces. This PFS hydrogel platform that we call “metal nanoparticle foundry” was established in bulk gels and gel films, and some applications were illustrated.

In particular, In **Chapter 2** we review the use of organometallic polymers as electrochemical sensors. The recent developments and some milestones related to designing electrochemical chemo/biosensors are covered.

Chapter 3 discusses the various approaches for fabricating polymer thin films on substrate surfaces. A description of chemical modification techniques for thin film preparation is provided.

Chapter 4 demonstrates a novel electrografting method to directly immobilize PFS chains to Au surfaces from ionic liquids. Using this simple and efficient approach, redox active PFS thin films were formed within 5 minutes and showed

excellent stability and redox properties. An electrochemical sensor for ascorbic acid is fabricated based on these PFS grafts.

In **Chapter 5** we develop a “grafting to” approach to tether PFS chains onto silicon (or gold) surfaces with an amine alkylation reaction. The electrochemical properties of the grafts are studied thoroughly, both in water and in organic media. The influence of the redox states of the grafts on the properties of the substrate surfaces were investigated by adherence measurements with in-situ electrochemical AFM. The PFS grafts could serve as an electrochemical sensor with high sensitivity and stability.

Chapter 6 displays a redox-active multilayer film obtained by covalent layer-by-layer assembly with redox-active PFS and a redox-inert polymer, poly(ethylene imine). Due to the formation of covalent bonds between the layers, the multilayered films showed high stability and were employed as electrochemical sensors for ascorbic acid and hydrogen peroxide. By tuning the number of layers, the sensitivity of the film response to the analyte could be optimized.

In **Chapter 7** we investigate the fabrication of a biosensor with cross-linkable PFS and glucose oxidase (GOx) in a multilayer form in which PFS serves as redox mediator. For this purpose, cationic PFS bearing methacrylate side groups was synthesized. The construction of multilayers and sensor characterization are presented.

Chapter 8 shows a clean and facile method to generate metal nanoparticles with redox active PFS hydrogel. The in-situ formation of various metal nanoparticles inside the hydrogel network *via* reduction of a number of metal salt precursors is discussed.

Chapter 9 reports the fabrication of stimuli-responsive hydrogel films based on PFSs. These PFS hydrogel thin films showed redox responsive properties and have promising applications in catalysis and sensing.

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Chapter 2

Organometallic Polymers for Electrode Decoration in Sensing Applications

Macromolecules containing metals combine the processing advantages of polymers with the functionality offered by the metal centers. This Chapter reviews the progress and developments in the area of electrochemical chemo/biosensors that are based on organometallic polymers. We focus on materials in which the metal centers provide function, allowing these materials to be used in electrochemical sensing applications through various transduction mechanisms. Examples of chemo/biosensors based on organometallic polymers possessing Fe, Os, Co and Ru metal centers are discussed.

2.1 Introduction

Organometallic polymers or metallopolymers refer to a wide range of metal-containing polymers, which have attracted rapidly expanding interest due to their unique chemical and physical properties and potential applications.^{1–9} Different metallic centers can adopt various coordination numbers, oxidation states and different coordination geometries. Additionally, different chain geometries, degrees of polymerization, types of bonding (covalent or supramolecular) and variation of other parameters of the primary chemical structure provide access to new and versatile classes of functional materials.¹⁰ The presence of metals in their main chain or in side groups enables organometallic polymers to play an unprecedented role in modern high-tech applications in the areas including nanomanufacturing,^{11, 12} ceramics precursors,¹³ ferromagnetic materials,¹⁴ separation, drug delivery,¹⁵ molecular motors¹⁶ and actuators,¹⁷ photovoltaic devices,¹⁸ catalysis,¹⁹ sensing, energy conversion and storage, etc.

The development of new, easily processible materials that feature metal centers in synthetic polymer chains motivated scientists to tackle the synthesis of poly(vinylferrocene) by radical-polymerization.²⁰ Since then, many effective synthetic approaches have been developed including polycondensation,^{21, 22} controlled radical polymerization, living ionic polymerization, ring-opening polymerization,^{23–25} and electropolymerization^{26, 27} to form organometallic polymers with main-chain or side-chain metal centers. Synthetic advances have also expanded from those that make use of traditional covalent bonds to incorporate metal centers in polymers, to approaches which use potentially reversible, “dynamic” binding by non-covalent coordination interactions that yield organometallic supramolecular polymers.^{1, 28} Many comprehensive reviews and books highlight and summarize the developments in the organometallic polymer area in detail, focusing on the synthetic accomplishments.^{25, 28–36}

Stimulus responsive, or smart macromolecular materials which exhibit abrupt conformational and chemical changes in response to small variations of external stimuli, are of intense current interest.^{37–39} The incorporation of metal centers in organometallic polymers offers many unique opportunities in the area of stimuli responsive materials. In most cases, organometallic polymers have intrinsic redox and luminescent properties inherited from the presence of metal centers and have been explored, e.g. as potential sensing materials, as they are capable to respond to an external stimulus and convert it into a signal which can be measured or recorded.^{40, 41}

Chemo/biosensors are a very important research area due to their impact in numerous fields, such as in industrial process management, clinical diagnostics,

food quality control, chemical threat detection and environmental monitoring.⁴² A chemo/biosensor is a device that detects the existence of particular chemical substances, a class of chemicals or a certain chemical reaction qualitatively or quantitatively.⁴⁰ Chemo/biosensors have been developed for cations, anions, acids, vapors, volatile organics, biomolecules and for many more molecular systems.^{42–44} Usually, the sensor contains a receptor which can selectively respond to a particular analyte or register chemical or biological changes, a transducer which converts this into a measurable signal and a signal processor which collects, amplifies, and allows one to read-out the signal.⁴¹ Existing transduction mechanisms include electrochemical, optical, calorimetric (thermal), gravimetric (mass) and so on.

Figure 2.1: A schematic of electrochemical chemo/biosensors based on organometallic polymer modified electrodes.

Many commercial sensors have been developed based on inorganic-semiconductor or organic-polymeric films that react with analyte molecules.^{42, 45} The changes in chemical or physical properties of these films are monitored. Typically the concentration, chemical and physical characteristics of the analytes would determine the magnitudes of the changes. Although a variety of chemo/biosensors have been successfully commercialized, there is still a strong need for improvement in sensor fabrication with new materials and transduction mechanisms to enhance the sensing sensitivity, selectivity and reliability. Several new chemosensory systems based on organometallic polymers

have been explored. Owing to the intrinsic luminescent properties of some metals, organometallic polymers featuring such metallic centers were widely used as luminescence sensors by monitoring the fluorescence or phosphorescence change of the sensing system due to the presence of analytes. Several reviews and articles discuss the development of luminescence chemo/biosensors based on organometallic polymers.^{9, 40, 46–50} Organometallic polymers are also used as mechanical probes,⁵¹ to fabricate sensitive membranes in surface acoustic wave devices for humidity sensing,^{52–55} or in quartz crystal microbalance (QCM) sensors to measure organic vapors.⁵⁶ Owing to the intrinsic redox and affinity properties of the metal, organometallic polymers were also employed in a variety of electrochemical sensors by detecting the current, redox potential or resistance changes of the sensing system.

In this Chapter we survey the recent developments and highlight some milestones related to designing electrochemical chemo/biosensors with organometallic polymers.

Figure 2.2: Examples of organometallic polymers. (1) Pd(II)-containing fluorene-based polymetallaynes,⁵⁷ (2) poly(ferrocenylsilanes),²⁵ (3) side chain Co(I) polymers featuring cyclopentadienyl-cobalt-cyclobutadiene (CpCoCb) units⁵⁸ and (4) Ru(II)-containing star-shaped polymer.⁵⁹

2.2 Scope of organometallic polymers and metal-organic structures

Organometallic polymers can contain a variety of metal centers including main group (p-block) metals such as Sn and Pb,⁵⁷ transition metals such as Fe, Ir, Ru,⁵⁹ Cr, Os,⁶⁰ Pt,⁵⁷ Ag, Co,⁵⁸ or lanthanides and actinides such as Eu.²

The position of the metal centers in the polymers and the nature of the linkages between them define the various structural types.¹ Based on the location of the metal centers, organometallic macromolecules can be divided into polymers with metal moieties embedded within the polymer backbone (Figure 2.2, polymer 1, 2.) and in the pendant side groups (Figure 2.2, polymer 3).⁵⁸ Considering the

Figure 2.3: Dendrimer with redox-active $\text{Cp}^*\text{Fe}^{\text{II}}(\text{dppe})$ -alkynyl centres ($\text{Cp}^* = \eta^5\text{-C}_5\text{Me}_5$, $\text{dppe} = 1,2\text{-bis(diphenylphosphino)ethane}$). Reproduced with permission from *ref.* 61. Copyright (2014) Nature Publishing Group.

geometrical structure of macromolecules, the organometallic polymers may be linear (Figure 2.2, polymer 2), star-shaped (Figure 2.2, polymer 4) or dendritic (Figure 2.3).

The linkages binding the metals can be covalent, or non-covalent. Covalent linkages enable irreversible or “static” binding of the metal while non-covalent coordination can allow potentially reversible “dynamic” binding forming organometallic supramolecular polymers (Figure 2.4).¹ Metal-organic frameworks (MOFs), also known as coordination polymers, are a special kind of organometallic polymers which represent an interesting class of crystalline molecular materials synthesized by combining metal-connecting points and bridging ligands with one-, two-, or three-dimensional structures.⁶²

Figure 2.4: Organometallic polymer obtained by “dynamic binding” using M^{2+} complexation by the tritopic bis-terpyridine cyclam ligand. Reprinted with permission from *ref.* 63. Copyright (2013) Elsevier Inc.

2.3 Electrochemical sensors

Development of electrochemical chemo/biosensors has a practical significance as these sensors possess high selectivity, excellent sensitivity, low cost, ease of use, portability and simplicity of construction.⁶⁴ The analytes and reactions being monitored by electrochemical methods typically cause a measurable current (amperometry), a measurable charge accumulation or potential (potentiometry) or alter the conductive properties of the medium between electrodes (conduc-

tometry).⁴¹ Many signal transduction schemes require a physical interface which generally involves chemically modified electrodes⁶⁵ to tailor electrochemical responses to analytes and improve detection sensitivity, selectivity and device stability. When preparing chemically modified electrodes, a thin film with a particular chemical composition and certain architecture is coated onto or chemically bound to the electrode surface in a rationally designed manner, providing desirable properties to the electrode.⁶⁶

Major basic designs of thin polymer films include end-tethered polymer chains, films from functional particles, layer-by-layer assembled films,⁶⁷ block-copolymer films, crosslinked thin polymer films or hydrogel thin films, porous films, etc.^{68–70} The techniques involved to obtain these films include drop-casting and spin-coating, inkjet printing, doctor blading, layer-by-layer assembly, “grafting to” and “grafting from” methods, electropolymerization, etc.

Electrodes, modified with organometallic polymers, possess many interesting features that can be exploited for electroanalytical and sensor applications. With the organometallic polymers, a variety of metal centers can be introduced to the sensing systems. The properties of the electrode and the abilities in sensing are easily controlled by carefully choosing the proper metal, the ligands and the decoration architectures. For example, by changing the ligands for a certain metal (e.g. iron, cobalt), the redox properties can be tuned as the standard electrode potential is influenced by the ligands (Table 2.1). Additionally, organometallic polymer decorated electrodes often have a large surface with high redox-active center loadings.

Table 2.1: Standard electrode potentials of common half-reactions in aqueous solution, measured relative to the standard hydrogen electrode at 25 °C with all species at unit activity.⁷¹

Half-reactions	E^0 / V
$\text{Fe}^{3+} + \text{e}^- \longrightarrow \text{Fe}^{2+}$	+0.77
$\text{Fe}(\text{phen})_3^{3+} + \text{e}^- \longrightarrow \text{Fe}(\text{phen})_3^{2+}$	+1.14
$\text{Fe}(\text{CN})_6^{3-} + \text{e}^- \longrightarrow \text{Fe}(\text{CN})_6^{4-}$	+0.36
$[\text{Ferrocenium}]^+ + \text{e}^- \longrightarrow \text{Ferrocene}$	+0.40
$\text{Co}^{3+} + \text{e}^- \longrightarrow \text{Co}^{2+}$	+1.92
$\text{Co}(\text{NH}_3)_6^{3+} + \text{e}^- \longrightarrow \text{Co}(\text{NH}_3)_6^{2+}$	+0.06
$\text{Co}(\text{phen})_3^{3+} + \text{e}^- \longrightarrow \text{Co}(\text{phen})_3^{2+}$	+0.33
$\text{Co}(\text{C}_2\text{O}_4)_3^{3-} + \text{e}^- \longrightarrow \text{Co}(\text{C}_2\text{O}_4)_3^{4-}$	+0.57

2.3.1 Sensors based on ferrocene-containing organometallic polymers

Metallocenes exhibit remarkable electronic and optical properties which make them versatile building blocks for incorporation into polymer systems. Ferrocene (Fc) and its derivatives represent the most common metallocenes applied in organometallic polymers. Discovered in 1951,⁷² ferrocene has a “sandwich” like structure with two cyclopentadienyl (Cp) rings coordinated to one Fe(II) cation as a neutral complex. The complex is small with dimensions of $4.1 \times 3.3 \text{ \AA}$, while ferrocenium ion, the oxidized form of ferrocene, has dimensions of $4.1 \times 3.5 \text{ \AA}$.⁷³ Because of the excellent electrochemical characteristics, such as low oxidation potential (pH-independent), fast electron-transfer rate, high levels of stability in its two redox states, low cost, and well-defined synthetic procedures for many derivatives, ferrocene has proved to be a popular building block in electrocatalysis and electrochemical sensing materials.^{74, 75}

Polymers with ferrocene side groups

As mentioned, organometallic polymers with metal in the side groups are often utilized in electrochemical sensing systems. For example, the organometallic polymer poly(vinylanthracene-*co*-vinylferrocene) containing pendent ferrocene groups has been synthesized to form a dual pH/sulfide electrochemical sensor.⁷⁶ The electrode decoration was conducted by abrasively immobilizing the organometallic polymer onto the surface of a polished basal plane pyrolytic graphite (BPPG) electrode by gently rubbing the material onto the electrode surface. The oxidation of the ferrocene moiety involves an electrocatalytic reaction with sulfide (Scheme 2.1), showing an enhancement in the oxidative peak current: the ferrocene moiety is oxidized at the electrode surface while the sulfide reduces the ferrocenium ion back to ferrocene, which is then re-oxidized at the electrode surface. The current increased linearly with the sulfide concentration over the range of 0.2-2 mM at pH values above 6.88 (Figure 2.5 A). By monitoring the current changes, sulfide could be electrochemically detected by using poly(vinylanthracene-*co*-vinylferrocene) decorated electrodes.

Scheme 2.1: The electrocatalytic reaction of sulfide with ferrocene moieties in a pH/sulfide electrochemical sensor.⁷⁶

Figure 2.5: (A) Dual pH/sulfide electrochemical sensor. Calibration plot of the normalized peak current of the ferrocene vs. sulfide concentration, at various pH values. (B) Calibration plot of the variation in the peak potential of the anthracene units with respect to the ferrocene units, as a function of pH. Reprinted with permission from *ref.* 76. Copyright (2006) Wiley-VCH.

Furthermore, electrodes covered with this organometallic polymer are also pH sensitive. The two-electron oxidation potential of the anthracene moiety was linearly related to the pH value (Figure 2.5 B), followed a Nernstian response with the protons, while the redox-active but pH-insensitive ferrocene moiety acted as the reference species (Figure 2.6). Additionally, the pH response was found to be temperature independent, showing an insignificant variation (<10 mV) over a range of temperatures. The ferrocene units in this instance had a dual role, as an internal calibrant for the system and as an electrocatalyst involved in the sensing mechanism. Owing to the support of the polymer chain, the signal of the ferrocene group in this organometallic polymer showed a superior stability at elevated temperatures compared to that of ferrocene in solutions.⁷⁷

With the same kind of organometallic polymers, the pH sensing ability was enhanced by associating the polymers with carbon nanotubes (CNTs).⁷⁸ The plot of the anthracene moiety peak potential against pH was linear up to at least pH 11.6, showing a wider pH sensing range.

Zhang et al. reported the fabrication of cationic poly(allylamine)ferrocene grafts on the surface of a gold electrode modified with negatively charged alkanethiols by electrostatic interaction.⁷⁹ The modified electrode was used as an ascorbic acid sensor. The cyclic voltammogram of the decorated electrode showed, upon addition of ascorbic acid, an increase of catalytic current and a decrease in overpotential of ascorbic acid, which provides evidence for excellent electrocatalytic performance of the ferrocene-containing polymer in ascorbic acid oxidation. The modified electrode has many advantages as it is simple to

Figure 2.6: The proposed electrochemical pathway for anthracene and ferrocene moieties in a pH/sulfide electrochemical sensor.⁷⁶

fabricate, has a fast response and good chemical and mechanical stability, which are important for building high performance sensors.

Many organometallic polymers containing ferrocene moieties are designed and synthesized to construct amperometric biosensors with enzymes in which the Fc moieties act as mediators to enable the shuttling of electrons between enzymes and electrode.⁸⁰ In most cases, the electrode surfaces were prepared by drop casting using mixtures of organometallic polymers and enzymes. Examples of redox ferrocene-containing organometallic polymers used in enzymatic sensing include poly(vinylferrocene-*co*-hydroxyethyl methacrylate),⁸¹ ferrocene-containing polyallylamine,⁸² poly(glycidylmethacrylate-*co*-vinylferrocene),⁸³ ferrocene-branched chitosan derivatives⁸⁴ etc.

The first generation of oxidase-based amperometric biosensors was based on the immobilization of oxidase enzymes on the surface of various electrodes. For these systems, the efficiency of electron transfer from enzymes to the electrode has been found to be poor in the absence of a mediator. Taking the glucose biosensors as a model, the electron transfer between glucose oxidase (GOx) active sites and the electrode surface is the limiting factor in the performance of amperometric glucose biosensors. Because of the thick protein layer surrounding its flavin adenine dinucleotide (FAD) redox center as an inherent barrier, glucose oxidase does not directly transfer electrons to conventional electrodes.⁸⁰ In GOx biosensors employing organometallic redox mediators, the metal center shuttles electrons between the FAD center and the electrode surface, thus significantly improving the performance of the sensors. The mediation cycle is shown in Figure 2.7, and the reactions involved are as follows (Scheme 2.2):

$M_{(ox)}$ and $M_{(red)}$ are the oxidized and reduced forms of the mediator. Two electrons are transferred from glucose to the redox centers of the GOx. These

Scheme 2.2: Redox reactions in mediator-based glucose biosensors.⁸⁰

electrons are then transferred to the mediator, forming the reduced form of the mediator. The reduced form is re-oxidized at the electrode, giving a current signal proportional to the glucose concentration as the oxidized form of the mediator is regenerated.⁸⁵

Figure 2.7: Sequence of events occurring in mediator-based glucose biosensors. Reprinted with permission from *ref. 80*. Copyright (2008) American Chemical Society.

Hydrogel films were obtained by crosslinking the drop casted films of enzymes and organometallic polymers to improve the stability and performance of the related biosensors. New materials were developed, with the aim of tailoring the interaction between the redox polymer and the enzyme and optimize the electron transfer between them. Polymer flexibility or segmental mobility, degree of functional density and hydration properties would all have impact on the performance of the sensor.⁸⁶ For example, redox polymer hydrogel films with glucose oxidase were formed by photoinitiated free-radical polymerization of poly(ethylene glycol) and vinylferrocene with a film thickness of $\sim 100 \mu\text{m}$.⁸⁵ Electrodes decorated with a crosslinked thin film of ferrocene-bearing poly(ethyleneimine) (PEI) and glucose oxidase hydrogel have also been utilized as glucose sensors.^{86, 87} The demand for reducing the film thickness emerged, as this was believed to enhance the sensing ability. By using crosslinkable polymers, it is possible to generate polymer coatings with varying thickness. Rhe and co-workers described the synthesis of poly(dimethylacrylamide) polymers containing both electroactive ferrocene moieties and photoreactive benzophenone groups which reacted as crosslinkers.⁸⁸ The ferrocene containing polymer was mixed with GOx and deposited as a thin

Figure 2.8: Fabrication of a covalently bound PNIPAM-ferrocene thin film on a gold electrode by a simple grafting to method. Adapted with permission from *ref. 89*. Copyright (2007) American Chemical Society.

film on the electrode surface. The polymer layer cross-linked and became firmly adhered to the electrode as a hydrogel thin film upon brief irradiation with UV light. Glucose-oxidizing electrodes with very high catalytic current responses were obtained.

In another study, a thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAM)-ferrocene polymer was synthesized and attached to a cysteamine-modified gold electrode by a simple grafting to method, forming a thin hydrophilic organometallic polymer film (Figure 2.8).⁸⁹ The organometallic polymer acted as a covalently bound mediator. The flexible, brush-like redox polymer thin layers allowed an efficient interaction with the enzyme [soluble glucose dehydrogenase (sGDH)] and enabled electrical communication between the cofactor pyrroloquinoline quinone (PQQ) of sGDH in the presence of glucose. At elevated temperature, the polymer shrank and the brush-like structure disappeared. Thus, the electron transfer between the electrode and sGDH could be controlled.

Polymer brushes containing ferrocene groups have been explored for decorating electrodes for electrochemical sensing. For example, Kang and co-workers developed an enzyme-mediated amperometric biosensor on an ITO electrode via surface-initiated atom-transfer radical polymerization (ATRP) of ferrocenylmethyl methacrylate (FMMA) and glycidyl methacrylate (GMA) (Figure 2.9) in a controlled approach.⁹⁰ By ATRP, a ferrocene-containing organometallic polymer brush film was introduced on the electrode surface. Glucose oxidase was subsequently immobilized via coupling reactions to the glycidyl group in GMA segments. With the introduction of a redox-P(FMMA) block as the electron-transfer mediator, the enzyme-mediated ITO electrode exhibited high sensitivity.

In the above case, the ferrocene moieties of PFMMA segments in the polymer brush provide redox-active properties to the polymer while the GMA segments

Figure 2.9: Ferrocene containing polymer brushes by SI-ATRP and the immobilization of Glucose oxidase on the thin film. Adapted with permission from *ref. 90*. Copyright (2009) Elsevier.

offer possible sites for coupling with functional groups, e.g. GOx. Liu et al. used the same organometallic polymer brushes obtained by consecutive SI-ATRP of FMMA and GMA as label-free electrochemical immunosensors for sensitive detection of tumor necrosis factor- α antigen (TNF- α).⁹¹ The redox-active ferrocene moieties in the PFMMA segment were introduced on the Au electrode surface to generate the redox responsive signal, while the abundant epoxy groups in PGMA segments offered plentiful possibilities for coupling TNF- α antibodies by an aqueous carbodiimide coupling reaction. The antibody-coated electrode was used to detect target antigen by capturing TNF- α onto the electrode surface through immunoreaction which would cause a drop of redox currents of the film (Figure 2.10). The oxidation peak currents decreased linearly with TNF- α concentration in the range of 0.01 ng/mL to 1 μ g/mL with a detection limit of 3.94 pg/mL. By monitoring the oxidation peak current of the electrode, an electrochemical biosensor for certain antigens with good sensitivity was realized.

Garrido and co-workers prepared poly(methacrylic acid) brushes on a diamond electrode which was dual-functionalized with the redox enzyme glucose oxidase and aminomethyl ferrocene by the same chemistry and demonstrated the

Figure 2.10: Label-free electrochemical immunosensors based on ferrocene-containing polymer brushes. Adapted with permission from *ref.* 91. Copyright (2012) Elsevier.

amperometric detection of glucose by these organometallic polymer brushes.⁹² The GOx and ferrocene moieties were well-distributed within the polymer brushes. This attempt offers an interesting strategy for the fabrication of smart electrodes for biosensors by electrical wiring of enzymes with a redox-responsive polymer.

A signal amplification strategy for electrochemical detection of DNA and proteins, based on ferrocene containing polymer brushes, was also reported.⁹³ The DNA capture probe (thiolated ssDNA) with a complementary sequence to the target DNA was immobilized on the Au surface. After the formation of sandwiched DNA duplexes with probe DNA, target DNA and the initiator-labeled detection probe DNA, poly(2-hydroxyethyl methacrylate) (PHEMA) brushes were grown from the duplexes in a controlled manner. The growth of long chain polymeric material provided abundant sites for subsequent coupling of electrochemically active ferrocene moieties. These ferrocene-containing polymer brushes in turn significantly enhanced the electrochemical signal output. The measured redox current of ferrocene was proportional to the logarithm of DNA concentration from 0.1 to 1000 nM.

The electrostatic layer-by-layer (LbL) assembly technique has been broadly employed as a simple and convenient approach in fabricating nanostructures with precise control of film structure and composition.^{94–96} The LbL assembly is usually based on the alternative adsorption of oppositely charged polyelectrolytes

via electrostatic interaction. Furthermore, in addition to electrostatics, various other approaches for film assembly have been utilized to construct covalently bonded layers. For example, by covalent LbL assembly of periodate-oxidized glucose oxidase and the redox polymer poly(allylamine)ferrocene on cystamine modified Au electrode surfaces, highly stable glucose oxidase multilayer films were prepared as biosensing interfaces (Figure 2.11).⁹⁷ The electrode modified with the multilayer displayed excellent catalytic activity for the oxidation of glucose. Also, the sensitivity of the sensor depended on the number of bilayers. The catalytic current with a certain glucose concentration was linearly related to the number of assembled layers. By controlling the number of bilayers on the Au electrode, the sensor sensitivity could be tuned.

Figure 2.11: Layer-by-layer construction of GOx/PAA-Fc multilayer films on a Au electrode surface. Reprinted with permission from *ref.* 97. Copyright (2004) Elsevier.

Electropolymerization is another suitable deposition approach for the formation of ferrocene-containing polymeric systems to obtain directly coupled layers at the electrode surface.²⁶ In this method, electropolymerizable monomers functionalized with ferrocene units were used, e.g. thiophene, pyrrole, aniline,

or vinyl groups. Organometallic polymer films could be formed by this simple and reproducible process with controllable thickness and morphology. For example, Surinder et al. prepared a copolymer film of pyrrole and ferrocene carboxylate modified pyrrole (P(Py-FcPy)) on indium-tin-oxide (ITO) substrates by electrochemical polymerization. Glucose oxidase (GOx), the model enzyme, was entrapped during deposition in the fabrication of an electrochemical biosensor.⁹⁸ The redox properties of the pyrrole copolymer, enhanced by the presence of ferrocene moieties, showed a favorable electron transfer with an improved electrochemical signal for electrochemical biosensors. This example indicates the feasibility of fabricating sensitive electrochemical biosensors using ferrocene modified polypyrrole films.

Polymers containing ferrocene in the main chain

Redox responsive poly(ferrocenylsilane)s (PFSs) were also used to fabricate chemo/biosensors. PFSs^{4, 25} belong to the class of organometallic polymers. These polymers are composed of alternating ferrocene and alkylsilane units in the main chain and can be reversibly oxidized and reduced by chemical or electrochemical means.^{99–101} With the development of thermally induced, catalytic, living anionic and living photo-polymerization of silicon-bridged ferrocenophanes, well-defined PFSs showing a wide range of chain-substituted forms have become accessible.^{23, 102}

Figure 2.12: Fabrication process of electrodes comprised of GOx and PI-PFS mediators. Adapted with permission from *ref. 103*. Copyright (2012) American Chemical Society.

A PFS based glucose sensor was fabricated by decorating the porous carbon electrode with a layer of glucose oxidase and a film of polyisoprene-*b*-poly(ferrocenyldimethylsilane) (PI-*b*-PFDMS) by drop-coating followed by chemical crosslinking with OsO₄.¹⁰³ It was found that the morphology of the film could be controlled by varying the block ratio of the copolymer and the composition of the casting solvent. By treatment with OsO₄, a cross-linked and

stable film was obtained. Glucose oxidase was employed as model enzyme and PI-*b*-PFDMS was used as electron mediator (Figure 2.12). The role of block copolymer morphology on the mediation of electron transport between the electrode and reaction center was investigated. The Fc moieties packed within the self-assembled structures were very useful to improve the electron transfer rate between the GOx and the electrode. The utilization of a bicontinuous microphase-separated block copolymer structure revealed a remarkable enhancement in catalytic currents and good glucose sensitivities at low glucose concentrations.

Dendrimers containing ferrocene termini

Dendrimers are well-defined, highly branched, star-shaped macromolecules bearing a large number of functional end groups at the periphery of the molecule. Metallo-dendrimers have been prepared.¹⁰⁴ Dendrimers bearing ferrocene moieties belong to the family of redox-active organometallic polymers and may be useful in sensing applications. For example, redox active dendrimers consisting of flexible poly(propyleneimine) dendrimer cores with octamethylferrocenyl units were deposited onto a platinum electrode and the system was applied as hydrogen peroxide and glucose sensor.¹⁰⁵ The dendrimer modified electrodes acted as electrocatalysts in the sensing application and the structural characteristics of the dendrimers influenced the sensor's behavior.

Figure 2.13: Structure of the ferrocene-containing dendrimers and the redox sensing of both oxo anions (A^-) and metal cations (M^{n+}) by poly-1,2,3-triazolylferrocenyl dendrimers: cyclic voltammograms of dendrimers a) without and b) in the presence of $(n\text{-Bu}_4\text{N})(\text{H}_2\text{PO}_4)$; c) in the presence of $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)_2$. Adapted with permission from ref. 106. Copyright (2007) Wiley-VCH.

Astruc and co-workers synthesized a series of ferrocenyl dendrimers suitable for electrochemical sensing.^{106–111} 1,2,3-Triazolylferrocenyl dendrimers prepared with click chemistry are selective electrochemical sensors for both transition-metal cations and oxo anions (H_2PO_4^- and ATP^{2-}) with dramatic dendritic effects.¹⁰⁶ The ferrocenyl termini, which were directly attached to the triazole ring in the dendrimers, served as a redox monitor, showing a single, fully reversible CV wave (Figure 2.13 a). When an oxo anion (H_2PO_4^- or ATP^{2-} , but not HSO_4^-) or a transition-metal cation (Cu^+ , Cu^{2+} , Pd^{2+} , or Pt^{2+}) salt was added, the redox peak position of the ferrocenyl dendrimers shifted when they recognized certain oxo anions or transition metals. According to the Echegoyen-Kaifer model,¹¹² the phenomenon is a sign of a relatively “strong redox recognition”, indicated by only a shift of the initial CV wave.

For oxo anions, the peak position shifted to a less positive potential (Figure 2.13 b), showing that the dendrimer-oxo anion assembly is easier to oxidize than the dendrimer itself. For metal cations, the oxidation peak appeared at a more positive potential (Figure 2.13 c) than the initial peak, indicating that the cation-dendrimer assembly is more difficult to oxidize than the dendrimer. In this way, the metallodendrimers containing ferrocene termini served as redox sensors for selective recognition of anions and cations.

Figure 2.14: Molecular structures of osmium-based polymers Os1 to Os6.¹¹³

2.3.2 Functionalization and applications with Os-containing compounds

Osmium is a transition metal in the platinum group which can form compounds with oxidation states ranging from -2 to $+8$. Os(II), Os(III) and Os(IV) complexes are the most widely used in electrochemical studies.⁷³ Osmium-based redox organometallic polymers have attracted a lot of interest as efficient redox platforms for catalysis and biosensing because of their facile and reversible electron-transfer capability, and the possibility to tune the redox potential by changing ligand and backbone structure.¹¹⁴ Figure 2.14 and Table 2.2 summarize the structures of several osmium-based polymers possessing redox centers dispersed along the backbone,¹¹³ such as poly(vinylimidazole) (PVI), poly(4-vinylpyridine) (P4VP), or polypyrrole and others¹¹⁵ and their redox potential under certain conditions. Unlike the ferrocene moieties which are neutral groups within the polymer, Os complexes with ligands often introduce charges into the polymer.

Table 2.2: Redox potential (vs. Ag/AgCl /V) of osmium-based organometallic polymers.

Compound	Redox potential	Ref.	Compound	Redox potential	Ref.
Os1	+ 0.55, pH 5	116	Os2	+ 0.35, pH 7.4	117
Os3	+ 0.10, pH 5	118	Os4	− 0.16, pH 7.4	119
Os5	− 0.069, pH 7.4	120	Os6	− 0.19, pH 7.2	121

Osmium-based polymers are excellent candidates as effective mediators for shuttling electrons between electrode and analytes and have been applied in biosensors for measuring ascorbic acid,¹²² lactate,¹²³ H_2O_2 ,¹²⁴ dopamine,¹²⁵ etc.

There are many examples where osmium-containing organometallic polymers are used to “wire” enzymes in order to create amperometric biosensors. In enzyme electrodes, the polymer structure on the electrode is one of the key factors that influences the electron transfer rate, surface coverage of redox active centers, charge transport and propagation. Diffusion and permeation of soluble species through the polymer thus affect the performance of polymer-decorated electrodes in sensing.¹²⁶

Like ferrocene-containing organometallic polymers, Os-containing polymers can also form stable hydrogels in aqueous solution and provide excellent matrices for immobilizing enzymes on electrode surfaces. When enzymes and mediators are co-immobilized in the film, they are concentrated and closely connected which leads to strong bioelectrocatalytic activities. Much effort has been made to enhance the conductivity and performance of osmium-polymer-hydrogel-based biosensors.^{115, 127–131} For example, new linkers were introduced between the

osmium complex and the polymer backbone,¹³² co-electrodeposition techniques were employed to form crosslinked thin films from enzymes and polymers,¹³³ and carbon nanotubes or graphene were integrated with the polymers.¹³⁴

Zafar et al. assembled FAD-dependent, glucose dehydrogenase (GcGDH) based hydrogel thin films with different Os polymers on graphite electrodes for glucose sensing.¹³¹ Six different Os-containing polymers with PVI or P4VP backbone, whose redox potentials were tuned by the ligands, were employed in the immobilization of the enzyme. The type of Os-containing polymer and enzyme/Os polymer ratio significantly affect the performance of the biosensors.

Figure 2.15: Preparation of HPC-g-P4VP-Os(bpy). Reprinted with permission from *ref.* 126. Copyright (2012) American Chemical Society.

A thermo-, pH-, and electrochemical-sensitive hydroxypropylcellulose-g-poly(4-vinylpyridine)-Os(bipyridine) (HPC-g-P4VP-Os(bpy)) graft copolymer (Figure 2.15) was synthesized by Huang et al.¹²⁶ A biosensor for glucose detection was fabricated by immobilizing GOx on the graft copolymer-decorated electrode. The water-soluble HPC backbone with excellent swelling ability provided an excellent environment for enzyme activity while the Os complex served as the redox mediator. The sensor showed an enhanced sensitivity for glucose detection up to 0.2 mM.

Stable and porous films were formed by drop-coating electrodes with PVP-Os/chitosan and enzyme composites, showing an enhanced electrocatalytic activity for glucose sensing.⁶⁰ The porous structures (Figure 2.16 B) resulted from random inter and intra polymeric cross-linking between two positively charged polymers, PVP-Os and chitosan, by glutaraldehyde, while the PVP-Os film had a homogeneous and smooth morphology (Figure 2.16 A). When testing the GOx/PVP-Os- and GOx/PVP-Os/Chitosan- modified electrodes, the latter was found to exhibit a more than three times higher catalytic current. The enhanced

Figure 2.16: SEM images of (A) the PVP-Os polymer and (B) PVP-Os/chitosan composite. (C) Molecular structure of osmium polymer/chitosan composite (PVP-Os/chitosan). Adapted with permission from *ref. 60*. Copyright (2013) Wiley-VCH.

catalytic conversion rate of the chitosan composite for glucose oxidation is a result of the stable incorporation of the enzyme into the porous and highly hydrophilic hydrogel. The porous structure enables the fast movement of chemicals involved in the glucose oxidation reaction.

Minko, Katz et al. developed a smart sensing system based on an organometallic polymer containing Os centers in the side chains.^{135, 136} A poly(4-vinylpyridine) (P4VP) brush functionalized with $\text{Os}(\text{dmo-bpy})_2^{2+}$ (dmo-bpy = 4,4'-dimethoxy-2,2'-bipyridine) redox groups was grafted to an ITO electrode. The electron exchange between the polymer-bound Os complex and the electrode was tuned by the swelling degree of the polymer chain. At $\text{pH} < 4.5$, due to the protonation of the pyridine groups, the film swelled, allowing electron exchange (Figure 2.17). At $\text{pH} > 6$, the polymer was in a collapsed state and the electrochemical process was inhibited because of frozen polymer chain motion.¹³⁶

The structural changes of the polymer enabled the reversible transformation of the electrode surface between the active and inactive state. The electrochemical activity of the Os-containing polymer modified electrode was combined with a biocatalytic reaction of glucose in the presence of soluble glucose oxidase (GOx), showing reversible activation of the bioelectrocatalytic process. The pH-controlled switchable redox activity enabled the modified electrode to serve as a “smart” interface for a new generation of electrochemical biosensors with a signal controlled activity.¹³⁵

Os-containing polymers also have potential uses in gene detection ar-

Figure 2.17: Reversible pH-controlled transformation of the Os-containing organometallic polymer on the electrode surface between electrochemically active and inactive states. Adapted with permission from *ref. 135*. Copyright (2008) American Chemical Society.

Figure 2.18: A DNA base-pair mismatch detection system based on an Os-containing polymer. Reprinted with permission from *ref. 137*. Copyright (1999) American Chemical Society.

rays.^{137–140} For example, an Os-containing polymer in combination with the enzyme soybean peroxidase (SBP) was used to detect a single base pair mismatch in an 18-base oligonucleotide.¹³⁷ A single-stranded 18-base probe oligonucleotide was covalently attached to an Os-containing redox polymer film on a microelectrode, while the target single-stranded 18-base oligonucleotides were bound to the enzyme. Hybridization of the probe and target oligonucleotides (Figure 2.18) brought the enzyme close to the modified electrode which switched on the electrocatalytic reduction of H_2O_2 to water. By monitoring the current enhancement, the single base mismatch in an oligonucleotide could be amperometrically sensed

Figure 2.19: Illustration of the nucleic acid electrochemical activator bilayer detection platform. Adapted with permission from *ref. 140*. Copyright (2004) American Chemical Society.

with the organometallic polymer-coated electrode.

Employing a similar mechanism, an enzyme-amplified amperometric nucleic acid biosensor was proposed by Gao et al. based on sandwich-type assays.¹⁴⁰ The capture probe, sample DNA and detection probe with GOx formed a sandwich structure on the electrode by hybridization. The Os-containing organometallic polymer was introduced on the electrode surface by electrostatic interaction, activating and mediating the enzymatic reactions of the enzyme labels (Figure 2.19). With high electron mobility and good kinetics provided by the organometallic polymer, the nucleic acid molecules were amperometrically measured at femtomolar levels.

2.3.3 Immobilization and use of Co-containing molecules

Cobalt-based organometallic polymers are also well-suited for sensory applications as the coordination ability of the cobalt enables further bonding of specific analytes.¹⁴¹ Compared to other metal centers, cobalt is less sensitive to water and oxygen in ambient conditions.¹⁴² Swager et al. prepared a series of cobalt-containing conducting organometallic polymers and demonstrated that the communication between the metal center and polymer backbone could be tuned

Figure 2.20: (A) Fabrication of conducting organometallic polymer electrode devices by electropolymerization across interdigitated microelectrodes (IME). (B) Chemoresistive response to NO gas exposure in dry N₂. Unconditioned film shown in black, conditioned film at 0.262 V (vs Fc/Fc⁺) for 2 min shown in red, and poly-EDOT film shown in blue. Adapted with permission from *ref.* 142. Copyright (2006) American Chemical Society.

by the reversible binding of small molecules. The energy levels of the metal-based orbitals could be altered, which made these polymers highly suitable for small ligating molecules detection.³⁴

For instance, a selective and effective detection system for the physiologically important species nitric oxide has been developed based on the chemoresistive changes in a cobalt-containing conducting organometallic polymer film device.¹⁴² The corresponding metal-containing monomer, featuring polymerizable 3,4-(ethylenedioxy)thiophene (EDOT) groups, was electropolymerized onto the working electrode surfaces, forming a conducting organometallic film (Figure 2.20 A). The polymer film was highly conductive and the metal was intimately involved in the conduction pathway. When NO was exposed to the microelectrodes decorated with these cobalt-containing conducting polymer, coordination of the ligand occurred, which changed the orbital energies of the complex, resulting in an increase in electrical resistance (Figure 2.20 B). The cobalt metal center adopted a square pyramidal coordination arrangement to accommodate the addition of a bent NO ligand to form polymer(NO) complexes. The device was insensitive to gases such as CO₂, O₂ and CO while showing a large, irreversible resistance change when exposed to NO₂.

2.3.4 Electrode decoration with Ru-containing polymers

Organometallic polymers containing ruthenium are often used in photoelectrochemical sensors.^{143–146} The ruthenium moieties within the polymer serve as photoelectrochemically active materials. Take [Ru(bpy)₃]²⁺ (bpy=2,2'-bipyridine)

Figure 2.21: Schematic illustrations of (A) anodic and (B) cathodic photocurrent generation mechanisms by a ruthenium complex. Adapted with permission from *ref.* 147. Copyright (2014) American Chemical Society.

as an example, where the excited state of $Ru(II)$ is generated under irradiation. The $[Ru(bpy)_3]^{2+}$ can react as electron donor or acceptor, producing an anodic or cathodic photocurrent (Figure 2.21).¹⁴⁷

Based on this phenomenon, Cosnier et al. fabricated several photoelectrochemical immunosensors for the detection of biologically important species. For example, a biotinylated tri(bipyridyl) ruthenium(II) complex (Figure 2.22) with pyrrole groups was electropolymerized on the electrode to form a biotinylated Ru-containing polypyrrole film. A cathodic photocurrent could be generated under illumination in the presence of an oxidative quencher. The immunosensor platform was built by subsequently attaching avidin and biotinylated cholera toxin (the probe) to the Ru-containing organometallic polymer decorated electrode via the avidin-biotin reaction. The photocurrent of the layered system decreased as

Figure 2.22: Structure of the biotinylated tri(bipyridyl) ruthenium(II) complex.

the increase in steric hindrance thwarted the diffusion of quencher molecules to the underlying Ru-containing polymer film. When the analyte, cholera toxin antibodies (anti-CT), was introduced to the system, the photocurrent decreased further, due to the specific binding of antibodies onto the electrode. By monitoring the variation of photocurrent, detection of the corresponding antibody was realized from 0 to 200 $\mu\text{g/mL}$.¹⁴³

Similarly, a label-free photoelectrochemical immunosensor and aptasensor were fabricated based on another Ru(II) containing organometallic copolymer. The bifunctional copolymer¹⁴⁴ was electropolymerized on the electrode using pyrene-butrylic acid, *N,N'*-bis(carboxymethyl)-L-lysine amide (NTA-pyrene) and [tris-(2,2'-bipyridine)(4,4'-(bis(4-pyrenyl-1-yl)butyloxy)-2,2'-bipyridine)] ruthenium (II) hexafluorophosphate (Ru(II)-pyrene complex). The pyrene groups, present in both compounds, underwent oxidative electropolymerization on platinum electrodes.

Figure 2.23: Photoelectrochemical immunosensor and aptasensor. (A) Operating principle of the photoelectrochemical immunosensors. (B) Calibration curve for sensing anti-CT concentrations ranging from 0 to 8 $\mu\text{g/mL}$. (C) Photocurrent measurement for the electrode (a) before and (b) after thrombin binding aptamer anchoring and (c) after incubation with thrombin (12 pM). (D) Calibration curve for photoelectrochemical aptasensing for thrombin concentrations ranging from 0 to 10 pM. All measurements were recorded in deaerated 10 mM sodium ascorbate 0.1 M PBS solution. Reprinted with permission from ref. 144. Copyright (2013) Elsevier.

The resulting copolymer contained NTA moieties, which functioned as an immobilization system for biotin- and histidine-tagged biomolecules, while Ru(II)-pyrene served as the photoelectrochemical transducing molecule.

Upon illumination, the excited state of Ru(II) can be generated and further quenched by sacrificial electron donors or acceptors, generating photocurrent. To construct an immunosensor for cholera antitoxin antibodies (anti-CT) detection, the biotin-Cu(NTA) interactions were used to modify the electrode with biotin-conjugated cholera toxin molecules (CT) (Figure 2.23 A). The resulting copolymer-CT immunosensor was exposed to different anti-CT concentrations and the photocurrent responses were recorded. The normalized immunosensor response increased linearly with increasing antibody concentration (Figure 2.23 B). By immobilizing thrombin binding aptamer (TBA) to the Ru-containing copolymer film, a photoelectrochemical aptasensor for thrombin was also developed (Figure 2.23 C and D).

2.3.5 Electrochemical sensors with metal-organic coordination polymers

Metal-organic coordination polymers (MOCP), also known as metal-organic frameworks (MOFs) or coordination networks, are a special kind of organometallic polymers where the formation of metal-ligand bonds was used to build polymer backbones. The wide range of choices for the organic linkers and metal ions for MOFs construction have permitted the rational structural design of various MOFs with targeted properties.^{62, 148–150} Ultrahigh porosity, large accessible surface areas, tunable structure, open metal sites, and high thermal and chemical stability of MOFs make them promising candidates for potential applications in many fields. Here we focus on the applications of MOFs in electrochemical sensing.

Some MOFs or MOF complexes exhibited excellent electrocatalytic activity which is suitable for electrochemical sensor fabrication. For example, a two-dimensional Co-based metal-organic coordination polymer (Co-MOCP) was prepared by a simple solvothermal synthesis. 1,3,5-Tri(1-imidazolyl)benzene, a typical imidazole-containing tripodal ligand with N donors, was used for the construction of the 2-D coordination architectures with Co²⁺. The electrode decorated with Co-MOCP was used for the electrocatalytic oxidation of reduced glutathione (GSH).¹⁵¹ This electrochemical sensor showed a wide linear range (from 2.5 μ M to 0.95 mM), low detection limit (2.5 μ M), and high stability towards GSH, which renders it a good platform for GSH sensing.

Heterogeneous MOFs were also proposed for sensor fabrication. Hosseini et al. developed L-cysteine¹⁵² and hydrazine¹⁵³ electrochemical sensors with Au-

SH-SiO₂ nanoparticles immobilized on Cu-MOFs. Guo et al. demonstrated the electrocatalytic oxidation of NADH and reduction of H₂O₂ with macroporous carbon (MPC) supported Cu-based MOF hybrids.¹⁵⁴

Cu terephthalate MOFs were integrated with graphene oxide (GO) and deposited onto a glassy carbon electrode. The hybrid film was subjected to electro-reduction to convert GO in the composite to graphene, the highly conductive reduced form. Because of the synergistic effect from graphene's high conductivity and the unique electron mediating action of Cu-MOF, the decorated electrode showed a high sensitivity and low interference towards acetaminophen (ACOP) and dopamine (DA). By monitoring the oxidation peak current of the two drugs with differential pulse voltammetric (DPV) measurements, the concentrations of ACOP and DA could be determined.¹⁵⁵

Owing to the high porosity and impressive absorbability, MOFs could be used as novel and efficient immobilization matrices for enzymes. Glucose oxidase-based glucose biosensors and tyrosinase-based phenolic biosensors were fabricated with Au or Pt based organometallic polymers.¹⁵⁶ The coordinated organometallic polymer network can immobilize enzymes with high load/activity, showing improved mass-transfer efficiency, and the thus-prepared glucose and phenolic biosensors exhibited excellent performance with long-term stability. Figure 2.24 displays the one-pot fabrication process of the functional electrode and the biosensing mechanism. 2,5-Dimercapto-1,3,4-thiadiazole (DMcT) which enables coordination of two or more metal ions was chosen to react with Au ions to form a porous structure in the presence of tyrosinase. Chronoamperometric measurements were used to monitor the current variation under different phenolic concentrations. The decorated electrode showed enhanced enzyme catalysis efficiency and excellent sensing performance towards phenol, resulting from

Figure 2.24: Illustration of the fabrication of tyrosinase-based phenolic biosensors and the biosensing mechanism. Reprinted with permission from *ref. 156*. Copyright (2011) American Chemical Society.

the porous structure of the organometallic network which provided adequate space for enzyme entrapment and facilitated the mass transfer of the analytes and products.

Mao et al. studied a series of zeolitic imidazolate frameworks (ZIFs) as a matrix for integrated dehydrogenase-based electrochemical biosensors. ZIFs with various pore sizes, surface areas and functional groups were investigated as matrix for co-immobilizing electrocatalysts (i.e., methylene green, MG) and dehydrogenases (i.e., glucose dehydrogenase, GDH).¹⁵⁷ ZIF-70 [$\text{Zn}(\text{Im})_{1.13}(\text{nIm})_{0.87}$, Im=imidazole, nIm=2-nitroimidazole] showed outstanding adsorption capacities toward MG and GDH and was used to construct a biosensor by drop-casting MG/ZIF-70 on a glassy carbon electrode, followed by coating GDH onto the MG/ZIF-70 composite. In a continuous-flow system, the biosensor was linearly responsive to glucose in the range of 0.1 - 2 mM.

Electrochemical sensors for the differential pulse anodic stripping voltammetric determination of lead based on multi-wall carbon nanotubes@ $\text{Cu}_3(\text{BTC})_2$ (BTC=benzene-1,3,5-tricarboxylate)¹⁵⁸ and amino-functionalized $\text{Cu}_3(\text{BTC})_2$ ¹⁵⁹ were also reported. The sensing systems showed excellent calibration responses towards lead at low concentrations, resulting from the absorbing effect of the MOFs.

MOFs also showed superior sorption properties towards small molecules. The high porosity and reversible sorption behavior suggests that the MOFs are suitable candidates for fabricating gas sensors. The absorption by, or desorption of molecules from the MOFs often induces changes in the dielectric properties of these materials.¹⁶⁰ By utilizing this characteristic, MOFs were applied as sensing materials for impedimetric gas sensors. For example, Achmann et al. constructed the first impedance sensor with Fe-1,3,5-benzenetricarboxylate-MOF (Fe-BTC) for humidity sensing which responded linearly in the range of 0 to 2.5 vol% water.¹⁶⁰

A Rubidium ion containing metal-organic framework CD-MOF has been shown as a candidate for CO_2 detection. The organometallic polymer CD-MOF showed an extended cubic structure comprising units of six γ -cyclodextrins (CD), linked by rubidium ions, which could react with gaseous CO_2 to form CO_2 -bound CD-MOF. The absorption process is reversible (Figure 2.25). The pristine CD-MOF exhibited a high ionic conductivity. When binding with CO_2 , a large drop in the conductivity (~ 550 -fold) was monitored by electrochemical impedance spectroscopy. The CO_2 sensors that were fabricated based on this principle were capable of measuring CO_2 concentrations quantitatively.¹⁶¹ Figure 2.25 also shows the cyclic change of conductivity of the CD-MOF with sequential CO_2 absorption and desorption. The plot of average conductivity value vs. CO_2 concentration shows that the sensitivity of the conductivity change is relatively high at low CO_2 concentrations.

Figure 2.25: Rubidium ion containing metal-organic framework CD-MOF based CO₂ sensor. Adapted with permission from *ref. 161*. Copyright (2014) American Chemical Society.

This example demonstrates that MOFs have a promising future in the field of quantitative sensing applications.

2.4 Conclusions

This Chapter reviewed the role of organometallic polymers as active components in electrochemical sensors. Strategies for immobilization of organometallic polymers on electrode surfaces and opportunities for the resulting decorated electrodes in sensing are discussed. As is illustrated in this Chapter, rational design of composition and structure of the organometallic components and improvements in fabrication techniques continuously advance the development of electrode surfaces towards greater sensing selectivity and lower limits of detection. Importantly, advances in the design and synthesis of organometallic polymers also enable scientists to introduce novel sensing principles to further enhance performance and broaden the applicability and scope of electrochemical sensors.

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Chapter 3

Polymer Thin Film Preparation Approaches

Modification of surfaces with polymer layers is of significant interest, as such layers provide functionality to these surfaces and act as interface between the underlying substrate and the outside world. Attachment of specific polymer layers can cause surfaces to become hydrophilic, hydrophobic, protein-repelling or -adsorbing, stimuli-responsive, redox-active, conducting, luminescent, etc., consequently supplying these substrates with enormous technological application potential. In this Chapter, approaches for the design and fabrication of polymer thin films on substrate surfaces are summarized.

3.1 Introduction

The design of smart surfaces with controlled interfacial properties has become an enabling approach in various areas of surface engineering for applications in nanotechnology, bio-nanotechnology, microfluidics, sensing, and other areas of advanced, high value added materials applications.^{1–4} Surfaces modified with stimuli-responsive polymers can adjust themselves to respond stimuli, which are employed in an intelligent way to control properties and functions. The use of smart polymers enables one to reversibly switch adhesiveness, friction, wettability, bioactivity, surface (visco)elasticity, color, morphology, and chemical composition.^{5, 6} The use of polymers for surface engineering is growing at a tremendous rate due to the development of surface attachment and polymerization techniques, as well as to the development of other surface attachment approaches. The ability to prepare thin films with thicknesses ranging from fractions of a nanometer (monolayer) to several micrometers and to tailor the surface properties of a material on the nanoscale offers exciting possibilities in molecular electronics, organic solar cells, biocompatible surfaces, drug delivery, sensor fabrication, and microfluidics.

Generally, the methods to fabricate thin polymer films on surfaces fall into two categories: physical modification and chemical modification. The former approach, physical modification, involves the deposition of functional surface structures via spin coating,⁷ thermal spraying, dip coating,⁸ precipitation, the Langmuir-Blodgett technique or polymer adsorption. Physical modification is a very simple, effective method and is widely employed in the manufacturing industry, for example in microelectronics, for optical projection lithography. Physical surface modification methods rely on physical interactions, for instance hydrophobic or electrostatic forces, between (parts of) the polymer with the surface for anchoring a polymer layer on a substrate. In case of chemical modification, polymer chains bearing suitable functional groups are attached to surfaces through covalent bonds by various synthetic approaches. Advantages of chemical attachment include controllable introduction of polymer chains to surfaces and the high stability of the resulting films.

In this Chapter, the approaches to fabricate polymer thin films on substrate surfaces are reviewed. We then narrow our focus to chemical modification techniques for thin film preparation on substrates.

3.2 Self-assembly technique

The spontaneous assembly of thiol-terminated molecules onto a gold surface is a well-established method for creating molecularly thin films with tailored head groups at the organic compound-air interface.⁹ Simply by exposing the gold surface to an environment containing thiol-ended molecules (e.g. solutions of alkanethiols, disulfides, etc.), the surface becomes coated with an organic layer known as self-assembled monolayer (SAM). Such layers have been around and used extensively for surface modification for some time. As in this Thesis we use thiol SAMs only in a very limited number of cases, and as this area has been extensively reviewed, we do not provide here an overview of this method; instead we refer to review papers from the literature *ref.* 9. In general, SAMs are nanostructures with a number of fascinating properties (Figure 3.1) which serve as a useful platform and basis for the construction of more complex 3-D structures.

Figure 3.1: Scheme of an ideal, single-crystalline SAM of alkanethiolates supported on a gold surface. The anatomy and characteristics of the SAM are highlighted. Reprinted with permission from *ref.* 9. Copyright (2005) American Chemical Society.

3.3 Polymer attachment by the “grafting to” approach

As mentioned, covalent attachment of polymer chains is a powerful tool to modify surfaces. In the “grafting to” method, a polymer is synthesized prior to surface attachment, which possesses either a reactive functionality at a chain end or reactive groups as pendant moieties. Subsequently, the polymer is grafted to a substrate by allowing the functional group(s) to react with a second, complementary functional group located at the surface to form covalently tethered

chains (Figure 3.2).^{10, 11} This technique is simple and effective. However, the polymer grafting density is often limited both thermodynamically and kinetically and the maximum surface coverage by the polymer is limited by the volume of the polymer chain in its coiled state.¹²

Figure 3.2: “Grafting to” approach to immobilize polymer chains on substrates.

Attachment of thiol moieties to polymer chains allows one to immobilize these polymers onto gold surfaces. For example, ethylenesulfide end-functionalized poly(ferrocenyldimethylsilane)s (ESPFSs) were prepared by our group and studied as redox active thin film coating. By immersing gold substrates into the polymer solution, a stable ESPFSs layer on gold substrates was formed with moderate coverage.^{13–15} This film allowed us to control the surface adhesion and friction by changing the redox state of the ESPFSs. Jones et al.¹⁶ investigated the grafting of carboxylic acid and triethoxysilane-terminated polystyrene onto silicon wafers. Polymers terminated with epoxide,¹⁷ amine,¹⁸ anhydride or hydroxide groups were also reported to form stable layers through the “grafting to” method. Wang et al.¹⁹ reported the immobilization of a polyfluorene derivative on an epoxy-terminated SAM on a glass surface. The amine group incorporated into the polymer side chain served as anchor group for immobilization. Minko and Katz et al.²⁰ functionalized an ITO surface with bromomethyldimethylchlorosilane to yield a Br-functionalized interface, then poly(4-vinyl pyridine) (P4VP) was grafted to the surface through quaternized pyridine groups, resulting in tethered polymer chains.

3.4 The “grafting from” approach

The “grafting from” approach encompasses surface initiated polymerizations employing initiator molecules (covalently) attached to the substrate. Initiator

immobilization to the surface can be accomplished through e.g. chemisorption, followed by the reaction of this surface-bound species with monomers in solution, forming a densely grafted film (a so-called polymer brush) on the surface (Figure 3.3).²¹ (We note, that in the brush form the polymer chains are elongated and stretched due to steric crowding. This requires either high grafting density, or large chain lengths. If the surface crowding is moderate or low, the conformation of the surface grafted macromolecules does not change significantly with respect to the conformation in a good solvent. Such surface grafts are referred to as “mushrooms”).

Controlled/living polymerization methods^{22, 23} are especially well-suited for the preparation of polymer brushes, following the “grafting from” strategy. With surface-initiated controlled polymerization, the brush thickness, grafting density, chain length, film composition, and film architecture (e.g. gradients) can be precisely tailored (Figure 3.4).^{24–27} Anionic polymerization, cationic polymerization, ring-opening polymerization, and ring-opening metathesis polymerization have all been employed for the synthesis of polymer brushes.²¹ Radical-based polymerization reactions have several advantages and show compatibility with both aqueous and organic media and high tolerance toward a wide range of functional groups. Among the surface-initiated controlled radical polymerization techniques the attenuated free radical approach (atom-transfer radical polymerization, ATRP) is most frequently used for brush film preparation by the “grafting from” technique.²⁸

Surface-initiated ATRP is chemically extremely versatile, compatible with a large assortment of monomers and functional groups, and tolerates a relatively high degree of impurities. Examples of polymer brushes obtained by surface-initiated reversible-addition fragmentation chain transfer polymerization (SI-RAFT),^{29–31} surface-initiated nitroxide-mediated polymerization (SI-NMP)^{32, 33} and surface-initiated photoiniferter-mediated polymerization (SI-PIMP)^{34, 35} have also been reported.

Figure 3.3: “Grafting from” method to immobilize polymer chains on substrates.

Figure 3.4: Different polymer brush architectures that can be prepared via surface-initiated controlled radical polymerization. (A) block copolymer brushes; (B) random copolymer brushes; (C) cross-linked polymer brushes; (D) free-standing polymer brushes; (E) hyperbranched polymer brushes; (F) highly branched polymer brushes; (G) Y-shaped binary mixed polymer brushes; (H) standard binary mixed brushes; (I) molecular weight gradient polymer brushes; (J) grafting density gradient polymer brushes; (K, L) chemical composition gradient polymer brushes. Reprinted with permission from *ref. 21*. Copyright (2009) American Chemical Society.

3.5 Electropolymerization

Electropolymerization represents a well-established approach for the simple and reproducible formation of polymer structures on electrodes as a thin film.^{36, 37} The monomers used for electropolymerization can include heterocyclic compounds such as pyrrole, thiophene, furan, indole, thianaphthene, carbazole and polycyclic benzenoid and nonbenzenoid hydrocarbons such as fluorene, fluoranthene, triphenylene, and pyrene.³⁸ In these cases, the monomers undergo an oxidation polymerization at a given anodic potential. In case of poly(thiophene) thin films, for example, electropolymerization of thiophene involves an electrochemical process of oxidation and a chemical process of coupling and eliminating protons on the monomers, forming dimers and oligomers. Molar mass increase of oligomers through the same processes leads to the formation of poly(thiophene).^{39, 40} Depending upon the polymerization conditions, electropolymerized poly(thiophene) thin films may consist of long, flexible macromolecules or shorter, more crosslinked chains. When the polymer chains grow long enough, or when a cross-linked structure is formed, the poly(thiophene) chains become insoluble

and are deposited at the working electrode surface as a thin film.⁴¹ Different electrochemical techniques can be employed for electropolymerization, e.g. potentiostatic, galvanostatic and potentiodynamic methods. The morphology of the film is influenced significantly by the techniques, although the polymerization mechanism remains the same.³⁶ Electropolymerization with vinylics has also been reported.⁴² Lecayon and coworkers found that the electrochemical reduction of acrylonitrile on a metallic electrode resulted in the formation of a thin polymer layer.⁴³ The monomers underwent a reductive reaction and anionic polymerization during the electropolymerization process. The electropolymerization of vinylics often requires environments free of oxygen and water.⁴²

The electropolymerization method offers several advantages: solubility problems are overcome as the polymer itself is formed directly on the respective surface while only the corresponding monomers have to be dissolved; the thickness of the film can be finely controlled electrochemically; and it is possible to modify the material properties by parameter control of the electrodeposition process.

3.6 Layer-by-layer assembly

Layer-by-layer (LbL) assembly has become an established, highly versatile approach for the facile fabrication of nanostructured, organized, multilayered thin films.⁴⁴ The LbL method allows one to generate tailor-made thin films from a nearly unlimited range of functional components in a stepwise fashion. Therefore, LbL-fabricated films combine a controlled thickness with desired chemical and physical properties. Initial attempts in this area focused on the electrostatic LbL assembly of synthetic charged polymers (polyelectrolytes), first introduced by the group of Decher in the 1990s.^{45, 46} Typically, the oppositely charged polymer species are adsorbed alternately to a surface (planar⁴⁷ or colloidal^{48, 49}), forming a nanometer thin film. The procedure involves sequential dipping and rinsing steps to remove the excess and non-adsorbed material between each layer. These multilayer thin films consist of semi-interpenetrated alternating polyelectrolyte layers where the overall thickness depends on the number of dipping steps (Figure 3.5). Besides synthetic polyelectrolytes, the range of materials for electrostatic LbL deposition includes metal and inorganic nanoparticles, dyes, peptides, oligonucleotides, proteins, and enzymes,⁵⁰ providing these thin films with diverse chemical and biological functions. A key advantage offered by the LbL approach is that the thickness of the film can be controlled precisely by varying the specific materials being used, the number of layers and the specific adsorption conditions (salt concentration in the supporting electrolyte). By tuning the temperature,

Figure 3.5: Schematic representation of electrostatic layer-by-layer assembly with polyelectrolytes. Reprinted with permission from *ref.* 47. Copyright (1997) American Association for the Advancement of Science.

solvent polarity, the ionic strength of the adsorption and rinse solutions, the thickness of adsorbed layers can be controlled with sub-nanometer resolution.⁴⁴

Sparked by the landmark discovery of the electrostatic LbL assembly, subsequent work has demonstrated that, besides electrostatics, also other forms of interactions can be employed to form highly functionalized thin films, such as hydrogen bonding,^{51, 52} sequential chemical reactions,^{53, 54} DNA hybridization,⁵⁵ metal-ligand complexation, hydrophobic interactions, molecular recognition,^{56–58} charge transfer interactions,^{59, 60} etc.

Among these novel LbL assembly methods, the covalent LbL assembly technique should be mentioned. Covalent LbL assembly is a step-by-step construction process in which covalent bonds are formed during the sequential deposition steps. Usually, the polymers bearing complementary chemical groups are able to react spontaneously to form a covalent bond under mild conditions.⁶¹

The sequential covalent binding strategy not only provides precise control over the film thickness and composition, but also renders the multilayer films highly stable, which therefore do not disassemble with pH or ionic strength changes.⁴⁴

Caruso et. al reported an elegant approach to prepare ultrathin LbL films *via* click chemistry.⁶² By using azide- or alkyne- modified poly(acrylic acid) (PAA),

Figure 3.6: LbL assembly of multilayer films *via* stepwise click chemistry. Poly(acrylic acid) bearing alkyne or azide groups is reacted alternately in the presence of Cu(I). Reprinted with permission from *ref.* 62. Copyright (2006) American Chemical Society.

stepwise growth of LbL films was achieved in the presence of copper(I) and sodium ascorbate in aqueous solution. Using click chemistry, covalent bonds were formed under mild conditions with high yields (Figure 3.6). Besides the click chemistry mentioned, further examples include reactions of amine groups with activated esters or anhydrides to form amides⁶³ or imides,⁶⁴ thiol-ene reactions,⁶⁵ reactions of primary amines with azalactone groups, Michael additions,⁶⁶ etc.

3.7 Cross-linking strategies

Substrate-supported thin films of hydrogels reported in literature are often obtained by cross-linking strategies. Hydrogels or cross-linked polymer networks, which can take up large amounts of water, show promising applications as soft materials.⁶⁷ Cross-links or grafting points within the materials stabilize these structures, resulting in enhanced resistance against dewetting, detachment, and dissolution in changing environments.⁶⁸ Compared to bulk hydrogels, hydrogel thin films combine a fast response to external stimuli with a high loading capacity for functional compounds, which may be favorable for applications in sensing, actuation and drug delivery.⁶⁹

There are several ways for producing surface-bound hydrogel thin films at surfaces.⁷⁰ For example, a reaction mixture containing the monomer *N*-isopropylacrylamide (NIPAAm), an initiator and a crosslinking agent was spin-coated onto a planar substrate and polymerized *in situ*. A cross-linking copolymerization reaction occurred, which led to the formation of a cross-linked PNIPAAm

thin film.⁷¹ By immobilizing the photoinitiator on the substrate, the monomer and cross-linker could be polymerized under UV-irradiation, forming a film on the substrate with complex geometry,⁷² while the film thickness could be tuned by varying the exposure time. By introducing photoreactive pendant groups (e.g. benzophenone) to the polymer, cross-linking could also be carried out after thin film deposition in the dry state.⁷³ Plasma polymerization⁷⁴ also enables the preparation of highly cross-linked hydrogel thin films without the use of cross-linking agents. Finally, we note that cross-linking of polymer brushes or LbL multilayers can also yield hydrogel thin films.

3.8 Conclusions

Various fabrication techniques of polymer thin films from functional materials, employing a variety of physical and chemical interactions and deposition methods, have grown in fascinating and diverse research directions. The approaches used to prepare polymer thin films on surfaces have been discussed in this introductory chapter. Exciting applications of these polymer thin films have already been realized and, as new functional components and interactions are continuously being explored.

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Chapter 4

Electrografting of Stimuli-Responsive, Redox Active Organometallic Polymers to Gold from Ionic Liquids

Robust, dense, redox active organometallic poly(ferrocenylsilane) (PFS) grafted films were formed within 5 min by cathodic reduction of Au substrates, immersed in a solution of imidazolium-functionalized PFS chains in the ionic liquid 1-ethyl-3-methylimidazolium ethyl sulfate. The electrografted polymer films were employed as an electrochemical sensor, exhibiting high sensitivity, stability, and reproducibility.

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4.1 Introduction

Surface modifications of electrodes have played a pivotal role in the building of novel interfaces for potential applications in ion recognition,¹ anticorrosion coatings,^{2, 3} electronic devices,⁴ sensors,⁵ biofuel cells⁶ and other fields.⁷ Various strategies have been proposed to form mono or multilayered structures aiming at controlling physicochemical properties of interfaces. The modification of electrodes with redox-active materials is an area of intense activity, featuring wide-ranging strategies that include electropolymerization,^{8–10} self-assembly through tethered functional groups,¹¹ thermolysis, click chemistry, and electrografting.¹² Among these approaches, the electrografting method has been proposed as a convenient approach to produce modified and functionalized surfaces, obtain molecularly altered electrodes and other metal surfaces.¹² Here we report on the grafting of redox stimulus responsive poly(ferrocenylsilane) (PFS) films to Au electrode surfaces by employing cathodic electrografting from an ionic liquid solution of the polymer.

Electrografting encompasses electrochemical reactions for the attachment of organic layers to conducting, solid substrates.¹² Various small molecules have been attached with success to electrodes by the electrografting method. For example, the electrochemical reduction of diazonium groups has been investigated in organic, aqueous and ionic liquid media.^{13, 14} Covalent attachment of ferrocenyl derivatives and porphyrin through ethynyl linkages to electrode surfaces and the direct anodic oxidation of the vinyl group through a radical-based electrografting procedure were also investigated.^{15, 16} Ghilane et al. reported the inclusion of ferrocene into the electrode material by cathodic treatment.^{17, 18} It has been shown that under cathodic polarization of noble metals, the metal could be electrochemically modified. Gold is unique among the transition metals in its ability to form isolable non-metallic compounds that contain a monoatomic anion. The auride ion (Au^-) is the first monoatomic metal anion proposed to exist in liquid ammonia.¹⁹ Bard reported on the electrochemical formation of auride ion in liquid NH_3 containing KI as supporting electrolyte.²⁰ The formation of tetramethylammonium auride in which gold carries a negative charge²¹ was also reported. The auride ion displays similar behavior as halogen ions.²² In the above mentioned studies the possibility to prepare grafts electrochemically has been demonstrated.

PFSs are a fascinating class of redox-active materials which are composed of alternating ferrocene and silane units in the main chain and combine a high density of redox centers with excellent processability and redox characteristics.^{23–25} The post functionalization of polymers is an appealing approach to obtain new

materials with tailor-made properties.²⁶ For example, functionalization of PFSs with imidazole side groups yielded novel poly(ionic liquid)s^{27, 28} which offer novel properties.²⁹

In this Chapter, a new, simple and efficient method for the fabrication of redox active polymer grafts from ionic liquid is described, using imidazole-functionalized PFS.

4.2 Results and discussion

4.2.1 Preparation of PFS-MID-Cl ionic liquid solution

The synthetic route employed to obtain methylimidazolium functionalized PFS-MID-Cl is shown in Figure 4.1 a. Interestingly, we found that the PFS-MID-Cl is well soluble (up to 20 mg/mL) in the ionic liquid 1-ethyl-3-methylimidazolium ethyl sulfate (Figure 4.1 b) with the help of heating (Figure 4.1 c left vial). With increasing concentration of PFS-MID-Cl, the color of the solution changed from light yellow to dark orange (Figure 4.2). To the best of our knowledge, this is the first example of a redox-active organometallic poly(ionic liquid) that is soluble in ionic liquids. The ionic liquid here fulfills a dual role as a solvent and electrolyte; it is a nonvolatile liquid, has intrinsic ionic conductivity and a wide potential window suitable for electrochemical applications.^{30–32}

Figure 4.1: (a) Synthesis of PFS-methylimidazole (PFS-MID-Cl), (b) the ionic liquid used in this study, (c) pure ionic liquid 1-ethyl-3-methylimidazolium ethyl sulfate (right vial) and PFS-MID-Cl dissolved in this ionic liquid (5 mg/mL, left vial).

Figure 4.2: PFS-MID-Cl in ionic liquid at different concentrations.

4.2.2 Electrografting of PFS

Cathodic electrografting of the PFS was achieved using the PFS/ionic liquid solution (5 mg/mL). It was shown that the electrochemical reduction of Au in the PFS ionic liquid solution takes place at -2.0 V (Figure 4.3). Following a cyclic voltammetry (CV) scan from -2.55 V to -0.55 V, the surface modification was performed by cathodic polarization at -2.2 V during 200 s. After electrografting, the modified electrodes were removed from the grafting solution and were sonicated in acetonitrile for 5 min, and thoroughly rinsed with acetonitrile and water to remove any weakly adsorbed molecules. Then the electrodes were transferred to 0.1 M NaClO_4 aqueous solution for the electrochemistry studies.

Figure 4.4 b shows the electrochemical response of the decorated Au electrode

Figure 4.3: Cyclic Voltammogram of Au substrates in (a) pure ionic liquid, (b) PFS-MID-Cl / ionic liquid solution (5 mg/mL). Scan rate: 50 mV/s. Pt wires served as reference and counter electrodes.

Figure 4.4: (a) Schematic illustrating the electrografting of PFS-MID-Cl in ionic liquid. (b) Cyclic voltammogram of a PFS modified Au electrode (1) with deposition potential at -2.2 V (2) with deposition potential at -0.8 V in 0.1 M $NaClO_4$, using an Ag/AgCl reference electrode and Pt counter electrode. Scan rate: 50 mV/s. (c) AFM height image of PFS grafts on Au, tapping mode in air; Scan size: $1\ \mu m \times 1\ \mu m$, z-scale: 30 nm.

in 0.1 M $NaClO_4$ aqueous solution. A double-wave voltammogram, displaying the characteristic peaks of PFS, is well visible.³³ The presence of these signals confirms the existence of the organometallic polymer at the Au electrode surface. A differential pulse voltammogram (DPV) also displayed the double waves. The best fit of the area under the DPV curve (Figure 4.5) was obtained with a peak area ratio of $1.1:1:1$, which reflects the influence of intra- and interchain interactions, and the distance of the redox centers to the electrode surface, on oxidation potentials. When the Au electrode was kept at -0.8 V in the presence of PFS in the ionic liquid, the characteristic I–V response of the polymer was not observed (Figure 4.4 b), indicating that at this potential no grafting takes place. The cathodic polarization at -2.2 V which exceeds the Au reduction potential is essential for the electrografting of the polymer onto the Au surface.

The electrochemical stability of the PFS grafts was examined by successive potential cycling in electrolyte solution with a potential sweep rate of 50 mV/s. The current variation in the CVs was negligible, about 3% , after 30 potential cycles (Figure 4.6 a), showing that no PFS chains desorbed from the substrate surface. After a fixed oxidizing potential of 0.6 V (vs. Ag/AgCl) was maintained

Figure 4.5: DPV recorded for the electrografted PFS in 0.1 M NaClO₄ aqueous solution, scan rate 5 mV/s, Ag/AgCl and Pt served as reference and counter electrodes. The line with open circles represents the measured data; the dashed lines are fitted curves. The peak area ratio is 1.1 : 1 : 1.

for 2 h, which is a rather severe test for gauging film stability,^{34, 35} only 15 % material loss occurred (Figure 4.6 b).

The voltammetric performance of the grafts remained unchanged following storage of the modified Au substrate for several weeks under ambient conditions. Integration of the anodic peak current gave an apparent surface coverage of ferrocene sites (Γ) of 1.85×10^{-9} mol cm⁻². Peak currents plotted against the scan rate showed a linear dependence and the ratio of anodic and cathodic current was close to unity (Figure 4.7). These characteristics indicated that the redox process

Figure 4.6: (a) Cyclic voltammogram of PFS-MID-Cl modified Au electrode in 0.1 M NaClO₄, with Ag/AgCl reference electrode and Pt counter electrode. Scan rate: 50 mV/s. (b) Remaining percentage of integrated charge involved in the oxidation process relative to that from time 0 as a function of accumulated holding time at the potential of 0.6 V vs. Ag/AgCl in 0.1 M NaClO₄ aqueous solution.

on these electrodes was controlled by charge-transfer kinetics³⁶ and confirmed that PFS grafts were immobilized on the gold surface.

Figure 4.7: (a) CVs of PFS on Au at various scan rates (10 mV/s — 100 mV/s) in 0.1 M NaClO₄, with Ag/AgCl reference electrode and a Pt counter electrode. (b) Dependence of the anodic and cathodic peak current on the scan rate of the PFS grafts on Au.

XPS was used to accurately analyze the surface chemical composition of the electrografted electrode (Figure 4.8). Following cathodic electrochemical grafting, the gold signal (the doublets at 84.4 and 88.0 eV for Au4f_{7/2} and Au4f_{5/2}) was strongly attenuated. Conversely, the relative intensity of carbon increased considerably. The presence of carbon on the gold surface before the electrochemical

Figure 4.8: XPS survey scans of (a) Au substrate electrografted with PFS-MID-Cl in ionic liquid, (b) Au substrate without any modification.

treatment is due to surface contamination. The marked increase of the carbon signal after electrografting suggests the presence of an organic layer at the gold surface and the observation of substrate signals indicates that the organic layer is thinner than the XPS escape depth (on the order of 10 nm). In addition, after electrografting, new XPS peaks appeared at 152.1 eV, 708.2 eV and 721.3 eV corresponding to Si (2s), Fe(2p_{3/2}) and Fe(2p_{1/2}), which originate from the PFS main chain;³⁷ the peak found at 401.2 eV is assigned to nitrogen N(1s) from the imidazole ring.³⁸ The ratio of Fe/ Si is approximately 1, and N/Fe almost equals to 2, which agrees well with the theoretical composition of the polymer. These results confirm the presence of PFS which is strongly attached to the gold surface after the electrochemical reduction.

The morphology of poly(ionic liquid) grafts was investigated by AFM (Figure 4.9). The zoom-in AFM images shown in Figure 4.4c exhibited a globular surface structure, superimposed on the Au surface with an average roughness of 4.56 nm.

Electrochemistry, XPS and AFM results indicate that the PFS is intercalated in the Au surface after the electrografting. The reaction can be described as follows:

By analogy with a previous study with small molecules,^{17, 18} the electrografting of organometallic polymer followed Equation (4.1), where X⁻ is the anion in the solution. The imidazolium side group of the PFS forms a complex with the

Figure 4.9: AFM images of (a) annealed Au before electrografting, (b) annealed Au after electrografting with PFS. Scan size: 10 μm $\times$ 10 μm .

auride ion generated during the cathodic reduction of Au substrate leading to the formation of new phases with general formula $[\text{PFS-MID}^+\text{Au}^-]$.

The decorated electrode also displayed an XPS peak for $\text{S}(2\text{p}_{3/2})$ at 168.7 eV, which is attributed to sulfate groups;³⁹ while the peak for $\text{Cl}(2\text{p}_{3/2})$ (200.6 eV)³⁹ is almost invisible in the noise. In control experiments, a substrate was exposed to the same electrografting process in pure ionic liquid without PFS. In this case, sulfur (S) was not detected by XPS, indicating the absence of ionic liquid anion (ethyl sulfate) at the electrode surface. These two facts demonstrate that: 1) in our case, X^- in Equation (4.1) represents the anion ethyl sulfate; 2) the anchored PFS chains possess unreacted imidazolium ethyl sulfate side groups.

4.2.3 Electrochemical sensor

We further studied the performance of the Au electrode electrografted with PFS as a potential sensor for the detection of ascorbic acid.

Ascorbic acid, one form of Vitamin C, is well known for its radical-scavenging capacity, present naturally in fruits and vegetables. It is an important preservative and antioxidant agent used in the food and drinks industry, pharmaceutical

Figure 4.10: Cyclic voltammograms of Au electrode grafted with PFS (1) in the absence of the analyte; and in the presence of (2) 0.1 mM, (3) 0.2 mM, (4) 0.3 mM, (5) 0.4 mM, (6) 0.5 mM ascorbic acid in 0.1 M NaClO_4 aqueous solution. Scan rate was 50 mV/s, Ag/AgCl and Pt were used as the reference and counter electrodes. Inset: Amperometric response of the sensor to successive additions of ascorbic acid into stirred aqueous 0.1 M NaClO_4 at room temperature. Each addition represented 2 μM ascorbic acid added. Applied potential = 0.52 V.

formulations, animal feed and cosmetic applications.⁴⁰ It also plays a vital role in biological metabolism. The accurate determination of its concentration is of considerable importance. Thus the development of a simple, rapid and reliable method to detect ascorbic acid has attracted great attention and is desirable for diagnostic and food-safe applications.^{41–44} Hence we chose this molecule to demonstrate the redox sensing ability of the Au electrodes electrografted with PFS. Figure 4.10 illustrates the cyclic voltammograms of the PFS decorated Au substrate in the absence and presence of ascorbic acid, showing the electrocatalytic responses of the electrode. The peak potentials do not change, and the oxidation peak currents increase with the addition of ascorbic acid, indicating that the electrografted PFS on Au effectively catalyzes the oxidation of ascorbic acid. This amperometric response forms the basis for the application of these PFS decorated Au electrodes in electrochemical sensing. The amperometric response (Figure 4.10 inset and Figure 4.11) of the modified electrode to successive additions of ascorbic acid was evaluated by applying a fixed potential of 0.52 V (vs. Ag/AgCl) at room temperature. The current-time curve indicates that the PFS grafts show a rapid response and a high sensitivity.

Figure 4.11: Amperometric response of the sensor to successive additions of ascorbic acid into stirred aqueous 0.1 M NaClO_4 at room temperature. Ag/AgCl and Pt were used as reference and counter electrodes, respectively. Each addition represented (a) 5 μM and (b) 2 μM ascorbic acid. The inset is the calibration curve. Applied potential = 0.52 V ($R=0.998$).

After the first amperometric response test (Figure 4.11a), the PFS-modified electrode was washed thoroughly with distilled water. The second amperometric measurement was conducted on the same electrode to study its sensing reproducibility (Figure 4.11b). The working plot of the oxidation peak current of oxidation current density vs. ascorbic acid concentration is shown in Figure 4.12. A linear relationship between oxidation current and ascorbic acid concentration was obtained up to 25 μM . The sensitivity of the modified electrode remained

unchanged. The limit of detection was estimated to be $0.9 \mu\text{M}$ at a signal-to-noise ratio of 3. Overall, the electrodes electrografted with PFS exhibit a high sensitivity, stable responses, and a low detection limit. The sensing ability compares favorably to other reported ascorbic acid sensors based on ferrocene derivatives.^{45, 46}

Figure 4.12: The calibration curve of the sensor. Measurements were performed in 0.1 M NaClO_4 aqueous solution. Applied potential = 0.52 V.

4.3 Conclusions

In summary, a simple and fast electrografting method to chemically modify Au electrodes with redox-active organometallic polymer, based on the direct cathodic reduction of Au substrates in polymer/ionic liquid solution, is described. This technique allows the generation of a robust redox-active graft on the electrode. Construction and performance tests of an ascorbic acid sensor based on the grafts have been demonstrated. These findings constitute a significant step in the development of a new class of modified electrodes for sensors, fuel cells and energy conversion.

4.4 Experimental part

Materials: Poly(ferrocenyl(3-iodopropyl)methylsilane) (PFS-I) (**1**) (M_n : 3.42×10^5 g/mol, M_w : 6.87×10^5 g/mol, M_w/M_n : 2.0) was prepared according to established procedures.⁴⁷ 1-Methylimidazole (Aldrich, 99%), dimethylsulfoxide

(DMSO, Biosolve), tetrahydrofuran (THF, Biosolve), sodium chloride (Aldrich, 99.5%) and sodium perchlorate (98.0%) were used without further purification. Water was purified with a Millipore desktop system.

PFS-methylimidazolium chloride (PFS-MID-Cl) (2): 1-Methylimidazole (0.246 g, 3.0 mmol) and DMSO (6 mL) were added to a solution of PFS-I (1) (0.4 g, 1.0 mmol) in THF (12 mL), the mixture was stirred at 60 °C for 24 hrs. After removing the THF, the mixture was transferred into a Spectra/Por 4 dialysis hose (MWCO 12-14000 g/mol) and dialyzed against 0.1 M NaCl (4 × 1 L) and MilliQ water (4 × 1 L). Concentration of the salt-free polyelectrolyte solution by a flow of N₂ produced **PFS-MID-Cl (2)** as orange flakes. ¹H NMR (D₂O): δ 0.19 (SiCH₃, s, 3H); 0.56 (1-CH₂, m, 2H); 1.57 (2-CH₂, m, 2H); 3.5 (N-CH₃, s, 3H); 3.68-4.00 (Cp and 3-CH₂, m, 10H); 7.06 (1H); 7.11 (1H); ¹³C NMR (D₂O): δ -3.41 (SiCH₃); 12.08 (1-CH₂); 24.77 (2-CH₂); 35.77 (N-CH₃); 51.95 (3-CH₂); 70.11-73.59 (Cp), 122.07 (imidazole ring); 123.63 (imidazole ring); 136.02 (imidazole ring).

PFS-MID-Cl / ionic liquid solution: PFS-MID-Cl was dissolved in ionic liquid 1-ethyl-3-methylimidazolium ethyl sulfate at given concentrations by heating the mixture at 80 °C for 3 hours. After cooling to room temperature, a transparent organic liquid was obtained. Prior to electrochemical experiments, the ionic liquid solution was kept under vacuum (10 mbar) at 40 °C overnight.⁴⁸

Electrochemical experiments A conventional three-electrode cell was used. Platinum wire was used as auxiliary electrode. Ag/AgCl was used as reference electrode for the electrochemical measurements in aqueous media. Another Pt wire was used as quasi-reference electrode for the experiments performed in ionic liquid.

Analytical instrumentation:

¹H NMR and ¹³C NMR spectra were recorded using a Bruker Avance III (400 MHz) instrument.

Fourier Transform Infrared (FTIR) spectra were acquired with a Bruker Vertex V70 spectrometer. A background spectrum was obtained by scanning a clean gold substrate.

Atomic Force Microscopy (AFM) measurements were performed using a Multimode AFM (Bruker Nano Surfaces, Santa Barbara, CA, USA) with a NanoScope V controller with tapping mode.

XPS measurements were performed on a Quantera SXM (scanning XPS microprobe) from Physical Electronics, using a monochromatic Al K α X-ray source (1486.6 eV).

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Chapter 5

Surface Attached Poly(ferrocenylsilane): Preparation, Characterization and Applications

Chemically modified electrodes, decorated with covalently immobilized poly(ferrocenylsilane) (PFS) chains, were fabricated by a simple “grafting to” method. The electrochemical properties of the redox active surface-tethered PFS grafts were studied thoroughly in aqueous and organic media. Information on the properties of these films as a function of redox state was obtained using quantitative adherence measurements between the films and AFM tips with in-situ electrochemical AFM. The influence of the AFM probe hydrophilicity on the adhesion force was investigated as well. An ascorbic acid electrochemical sensor based on these surface-anchored PFS chains, exhibiting a high sensitivity and stability, was fabricated. The PFS grafts described are easily derivatized, thus forming a platform for creating highly tailorable redox-active interfaces.

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5.1 Introduction

In this Chapter, we develop a simple “grafting to” approach to tether organometallic polymer – poly(ferrocenylsilane) (PFS)^{1–3} chains on silicon (or gold) substrates, forming redox active ultra thin grafts. The preparation, characterization and application of the grafts in the electrochemical detection of ascorbic acid are reported.

Ascorbic acid, a water-soluble vitamin, is present naturally in fruits and vegetables. It is an important preservative and anti-oxidant used in food industry, pharmaceutical formulations and cosmetic applications. Thus, the development of a simple and rapid method for its detection has attracted great attention.^{4–6}

Electrodes chemically modified with conducting polymers, carbon nanotubes or graphene, ferrocene derivatives and other transition metal complexes play a central role in the detection of many biologically important species.^{7–9} Such chemical modifications are aimed at tailoring electrochemical responses to analytes to improve detection sensitivity, selectivity and device stability.^{10–13} In addition, chemically modified electrodes may be combined with biospecific recognition entities such as redox enzymes for the detection of e.g. glucose.¹⁴ In these cases, redox-active components are often employed to serve as redox mediators, facilitating charge transfer between analytes and electrode.

PFS, composed of alternating ferrocene and silane units in the main chain, are a fascinating class of processable materials with redox characteristics suitable for the electrochemical detection of biological analytes.^{15, 16} Several reports have appeared on the formation of thin PFS films on electrode surfaces using electrostatic layer-by-layer (LbL) deposition^{17–19} of PFS polyions or by solution-casting of PFS homo- or block copolymers.^{20, 21} However, only a few accounts of covalently surface-tethered PFS films, including thiol end-functionalized PFS chains attached to gold by chemisorption, have been reported in the literature.^{16, 22–24}

Here we describe a “grafting to” approach^{14, 25} for the covalent attachment of PFS chains to an electrode surface, employing amine alkylation reactions. The fabrication of such chemically modified electrodes, decorated with stable, ultrathin redox-active films is of interest for electrochemical sensing applications. The electrochemical properties of the surface-immobilized PFS grafts are studied using cyclic voltammetry (CV) and differential pulse voltammetry (DPV), both in aqueous and organic media. Additionally, quantitative adherence measurements between the immobilized PFS grafts and Atomic Force Microscopy (AFM) tips were conducted to gain further information on the behavior of the thin films in the oxidized and reduced states, respectively. The impact of AFM probe hydrophilicity

on the adhesive forces measurements was carefully taken into consideration and controlled. An ascorbic acid electrochemical sensor based on these PFS films (Figure 5.1) exhibited a high sensitivity, stable responses, and a lower detection limit below $5\ \mu\text{M}$ which is comparable to other reported ascorbic acid sensors.²⁶ The grafted-to layers described here are readily derivatized further and can therefore be regarded as a versatile platform for creating tailorable redox-active interfaces.

Figure 5.1: Redox active, surface-tethered PFS grafts were employed as electrochemical sensor for ascorbic acid.

5.2 Results and discussion

5.2.1 Preparation of PFS grafts

Surface grafted PFS layers were realized in two steps: firstly, an amine-terminated monolayer was formed, then poly(ferrocenyl(3-iodopropyl)methylsilane) (PFS-I) (1) was covalently immobilized onto the surface by amination of the iodopropyl side groups of PFS at $50\ ^\circ\text{C}$ (Scheme 5.1). Control experiments were carried out to study the stability of PFS-I (1). A thermogravimetric analysis (TGA) study (Figure 5.2 a) showed that PFS-I (1) remained thermally stable up to $250\ ^\circ\text{C}$.

The integrity of the surface-bound PFS layer was investigated electrochemically as the desorption of physisorbed, non-covalently bound PFS chains is easily monitored in this way. Cyclic voltammograms were recorded before and after soaking a covalently bound PFS-I film and an inert poly(ferrocenyldimethylsilane) (PFDMS) film in THF (Figure 5.2 b). The voltammogram observed for PFS-I (1) grafts showed no change prior to, and following soaking in THF, providing evidence that PFS-I (1) forms a stable layer¹⁶ on amine functionalized gold

Scheme 5.1: Schematic representation of the covalent surface-attachment of PFS chains (a) on a silicon substrate and (b) on a gold substrate.

surfaces. For the physisorbed, non-reactive PFDMS, no appreciable current signal was found.

Figure 5.2: (a) Thermogravimetric analysis of PFS-I. (b) Cyclic voltammogram of (1) PFS-I covalently bound to an amine-terminated SAM on gold; (2) PFMDMS on the gold substrate with the cysteamine SAM; (3) bare gold in 0.1 M NaClO_4 with Pt wires as the reference and counter electrode. All the substrates were soaked in THF overnight before measurements.

Since substrate anchoring occurs through the PFS side groups, the layer is envisaged to be composed of tethered polymer strands of varying length with some fraction of polymer loops. The surface morphology and film height were imaged by tapping mode AFM (Figure 5.3). In the dry state, the PFS films showed a uniform thickness of 9 nm on silicon substrates.²⁷ SEM images also confirmed this film thickness and the uniformity of the PFS layer over larger areas.

Figure 5.3: Height image and surface morphology of the PFS film immobilized on a silicon substrate.

Fourier Transform Infrared (FTIR) spectroscopy was used to verify the presence of PFS chains on the gold surface (Figure 5.4). The spectra for the surface-anchored PFS-I and PFS-I in bulk showed similar absorptions in both the high energy region (3087 cm^{-1} , C–H stretching peaks of ferrocene rings) and the low energy region (1165 cm^{-1} , asymmetric ring in-plane vibration of ferrocene, and 1037 cm^{-1} , the

Figure 5.4: FTIR spectra of PFS-I in bulk (bottom) and PFS-I grafts on a gold substrate (top) in the (a) high energy and (b) low energy region.

out-of-plane C–H vibration of ferrocene).²³

Static contact angles for water were measured to gauge the efficiency of the PFS anchoring step. On gold substrates, the contact angle changed from 60° for the amine-terminated monolayer to 90° after attachment of the PFS chains, confirming the formation of a hydrophobic layer. The surface coverage of ferrocene units was calculated to be $3.8 \times 10^{-9} \text{ mol cm}^{-2}$ from electrochemical measurements, which is a factor of 1.5 to 1.8 higher than previously found for “grafted to” layers formed from thiol end-functionalized PFDMS chains of varying molar mass.^{23, 24} These results indicate that thin, uniform, relatively dense PFS films were introduced by covalent attachment to gold and silicon substrates.

5.2.2 Electrochemical properties of PFS grafts

The electrochemical properties of the PFS films were investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in aqueous solution

Figure 5.5: (a) CVs of PFS grafts immobilized on gold at different scan rates (in 0.1 M aqueous NaClO_4 , Pt as reference and counter electrodes); (b) plots of peak current i_p versus scan rate. (c) ΔE versus scan rate; (d) DPV recorded for these PFS films (scan rate 5 mV/s), the line with open circles represents the measured data; the dashed lines are fitted curves.

and organic solvent. Figure 5.5 shows the results in 0.1 M aqueous NaClO_4 with a potential range between -0.4 V and $+0.4$ V *vs.* the reference electrode. The double-wave voltammogram (Figure 5.5 a) indicates that repulsive interactions exist between the neighboring ferrocene units along the PFS chain.²⁸ Peak currents plotted against scan rate are shown in Figure 5.5 b. Linear dependencies were found for all oxidation and reduction peaks which are characteristic for surface-confined electroactive layers.²⁹

In order to probe the reversibility of the redox process, the separation between the anodic peak potential (E_{pox}) and cathodic peak potential (E_{pred}), ΔE ($\Delta E(1) = E_{\text{pox}1} - E_{\text{pred}1}$, $\Delta E(2) = E_{\text{pox}2} - E_{\text{pred}2}$) was plotted with the scan rate (Figure 5.5 c). A reversible one electron transfer should exhibit a ΔE of 59 mV.²⁹ In aqueous NaClO_4 , the surface-bound PFS layers display electrochemical reversibility up to a scan rate of 30 mV/s for $\Delta E(2)$, and 100 mV/s or higher for $\Delta E(1)$. This is consistent with previous findings.²³

A DPV curve, recorded after performing the CV measurements, is shown in Figure 5.5 d. The best fit of the area under the DPV curve was obtained by considering multiple oxidation events where oxidation potentials are influenced by intra- and interchain interactions and also by the distance of the redox centers to the electrode surface. In this case, an accurate fit was obtained by using a peak area ratio of 1 : 1.2 : 2. When immersed in aqueous NaClO_4 , the hydrophobic PFS chains will be in a collapsed state. Our experimental results show that in the first oxidation step a quarter of the ferrocene units are oxidized, probably predominantly those that are in close proximity to the gold surface. Another quarter of the remaining Fe atoms are oxidized if a higher potential is applied. The electrochemical oxidation is completed if the potential is increased further. This behavior is in accordance with that of end-grafted PFDMS chains on gold.²³

As is well-known, swellability of surface-confined redox-active polymer films in the medium used for cyclic voltammetry greatly facilitates the diffusion of solvated electrolyte ions into the film, which strongly influences electrode processes in the film.³⁰ Electrochemical studies on the immobilized PFS films were also performed in CH_2Cl_2 , a good solvent for PFS, using 0.1 M NBu_4PF_6 as a supporting electrolyte. The shape of the cyclic voltammograms obtained (Figure 5.6 a) is clearly different from those recorded in aqueous electrolyte solution.

Peak currents plotted against scan rate (Figure 5.6 b) showed a linear dependence and the $i_{\text{pox}}/i_{\text{pred}}$ ratio was close to unity. In Figure 5.6 c, both $\Delta E(1)$ and $\Delta E(2)$ showed electrochemical reversibility with scan rates of up to 100 mV/s or higher. These characteristics indicated that the redox process on these electrodes was controlled by charge-transfer kinetics³¹ and confirmed that PFS grafts were immobilized on the gold surface.

Figure 5.6: (a) CVs of PFS grafts immobilized on gold at different scan rates (in 0.1 M NBu_4PF_6 in CH_2Cl_2 , Pt as reference and counter electrodes), (b) plots of peak current i_p versus scan rate. (c) ΔE versus scan rate; (d) DPV recorded for these PFS films (scan rate 5 mV/s), the line with open circles represents the measured data, the dashed lines are fitted curves.

The area under the DPV curve (Figure 5.6 d) was again fitted using multiple oxidation waves, in this case a peak area ratio of 1 : 1 : 1 gave the best fit result.

The swollen grafts in CH_2Cl_2 provide a higher electrolyte permeability and lower diffusion resistivity, leading to readily accessible electroactive centers on the PFS chains and an increased homogeneity within the redox-active film. Compared to Figure 5.5, we can conclude that the oxidation reaction of PFS appears to be particularly sensitive to its solvation in the electrolyte medium.

The electrochemical stability of PFS films was examined by successive potential cycling in both aqueous electrolyte solution and CH_2Cl_2 media from -0.4 to $+0.4$ V versus Pt with a potential sweep rate of 50 mV/s. The peaks in the CVs were unchanged and reproducible after 20 potential cycles (Figure 5.7). Clearly, no PFS chains desorbed from the substrate surface.

Figure 5.7: Cyclic voltammograms of surface-tethered PFS-I on gold, Pt as reference and counter electrode. (a) In 0.1 M aqueous NaClO₄; (b) in CH₂Cl₂ containing 0.1 M NBu₄PF₆.

5.2.3 Redox cycle responsive properties of PFS grafts

Stimuli-responsive polymer interfaces possessing switchable physical and chemical properties in response to changes in the external environment,³² are playing an increasingly important role in a diverse range of applications. AFM is a key technique for surface morphology characterization and surface/interfacial force studies.^{33–35} The AFM probing technique has enabled investigation of interaction forces in various platforms, allowing insight into surface transitions and structural changes triggered by external stimuli, such as temperature,^{36, 37} pH,^{38, 39} solvent,⁴⁰ ionic strength⁴¹ or electrochemical potential.¹⁵ In previous work, our group reported several investigations of redox responsive polymer systems studied by AFM force spectroscopy.^{15, 42–44} An external electrochemical stimulus allows one to induce reversible changes in individual PFS chains as positive charges are introduced upon oxidation.

In this section, we examined the influence of the oxidation state on the properties of the surface-immobilized PFS grafts. The adherence between the PFS grafts and an AFM tip under oxidizing and reducing potential was assessed *in situ* by electrochemical Atomic Force Microscopy (ECAFM)^{44–47} with pull-off force measurements.

Additionally, we paid particular attention to the influence of the AFM tip surface properties on the adhesion force (pull-off force) measurements of PFS grafts in the alternating redox state. AFM measurements of surface forces are strongly affected by the nature of the AFM tip, the sample surface and the environment in which the imaging is done.^{46, 48–50} The effect of surface treatment on the hydrophilicity of silicon nitride AFM probes and correspondingly obtained adhesive force measurements on redox-active PFS grafts are described. Different

treatment procedures drastically affected the observed adhesion forces. The results of adhesive forces between the PFS grafts and the AFM tips are discussed.

AFM tip treatment

Silicon nitride AFM tips were cleaned with organic solvents (acetone and ethanol) or piranha solution before adhesion measurements. The direct characterization of AFM tip modification is practically impossible due to the small size of the AFM tip. For this reason, in general the corresponding AFM probe chip serves as indicator for a successful tip modification. Hence, complementing water contact angle measurements of sessile water droplets on the AFM probe chips are relevant for the evaluation of the hydrophilicity of the AFM tip surface (Figure 5.8). The standard silicon nitride cantilever which was washed with organic solvents revealed a hydrophobic surface, as evidenced by the contact angle measurements on the probe chips (Table 5.1). When cleaned with piranha solution the AFM probe chip became hydrophilic, reducing the contact angle of the drop to 58.5° . Hence by choosing different treatment methods, the hydrophilicity of the AFM tip could be tuned.

Figure 5.8: Water contact angle measurement on (a) organic solvent treated AFM probe, (b) piranha treated AFM probe.

As mentioned earlier, the spring constants of the AFM cantilevers were determined using the thermal tune method.⁵¹ The probe was washed with organic solvent first and the spring constant of the tip was measured to be 0.12 N/m. The same probe was then treated with piranha solution, the spring constant after treatment was 0.11 N/m. As the piranha solution is a strong oxidizing agent, it will remove the organic matter on the tip and also hydroxylate the surface.

Table 5.1: Comparison of contact angles and spring constants of the same AFM probe, first washed with organic solvent, then cleaned with piranha solution.

	Contact angle (°)	Spring constant (N/m)
Washed with organic solvent	107.2 ± 2	0.12
Washed with piranha solution	58.5 ± 2	0.11

Pull-off force measurements

The adherence between the PFS-I grafts and the AFM tip was assessed by electrochemical AFM (Scheme 5.2). Force measurements were performed in 0.1 M NaClO_4 aqueous solution at room temperature, using silicon nitride tips treated as described above. By statistical analysis of over 300 consecutive force curves for each type, a representative set of pull-off force distributions was obtained between neutral and oxidized PFS grafts with the two kinds of AFM tips, the histograms are presented in Figure 5.9.

Scheme 5.2: Schematic representation of the different oxidation states of PFS-I in the ECAFM.

While measuring with the organic solvent treated tip, the average of the distribution of the observed pull-off forces for the grafts in the neutral state is clearly higher than that for the grafts in the oxidized state (Figure 5.9 a and c). Statistical analysis of consecutive force curves shows an average adhesive force of 1.25 nN for the neutral polymer grafts ($E = -0.05 \text{ V}$) and 0.23 nN ($E = +0.60 \text{ V}$) for the oxidized PFSs grafts. The adhesion between the tip and PFS grafts decreased following the conversion of the ferrocenyl groups to the ferrocenium form by

electrochemical oxidization. In great contrast, while measuring with the piranha treated tip, the trend is opposite. The interaction between the oxidized polymer films with a piranha treated tip is stronger than that between the neutral polymer films (Figure 5.9 b and d). The adhesion between the tip and PFS grafts increased upon electrochemical oxidization of the PFS grafts.

Figure 5.9: Histograms of pull-off forces recorded for reduced (a and b) and oxidized (c and d) PFS-I grafts with organic solvent treated AFM tips (a and c) and piranha treated AFM tips (b and d) in 0.1 M aqueous NaClO_4 . The applied electrochemical potential was -0.05 V (a and b) and $+0.6$ V (c and d) vs. Ag wire electrode.

We ascribe the opposite trends in pull-off forces primarily to differences in the hydrophilicity of the AFM tip. We found that the contact angle of the neutral PFS-I grafts was 90° , whereas 78° was measured for oxidized grafts, revealing an increase in hydrophilicity of the PFS-I grafts upon oxidation. Using the organic solvent treated silicon nitride tip which possesses a hydrophobic surface,⁵⁰ a stronger interaction was found with the PFS-I grafts in the neutral state. The lower adhesion force measured between the AFM tip and the oxidized PFS film provides evidence of the increased hydrophilicity of the film upon oxidation. With the

piranha treated silicon nitride tip, the results are opposite. Since the probe became hydrophilic, it revealed stronger adhesion to the oxidized PFS-I grafts and vice versa.

To verify the reversibility of our PFS-I grafts system, the pull-off forces were measured in three consecutive electrochemical cycles. Figure 5.10 shows the pull-off force measurements on the PFS grafts with the tip treated by organic solvent (Figure 5.10 a) and piranha solution (Figure 5.10 b), demonstrating good reversibility of the adhesion response of the PFS-I grafts under electrochemical redox stimulus.

Figure 5.10: Average pull off force measurement of stimulus responsive PFS-I grafts with (a) organic solvent treated tip and (b) piranha solution treated tip in subsequent electrochemical cycles.

5.2.4 Electrochemical sensor for ascorbic acid

The PFS-decorated gold substrates are of interest as the active component in electrochemical sensors. Figure 5.11 shows the electrocatalytic responses of bare gold (Figure 5.11 a) and of a PFS-I layer on gold (Figure 5.11 b) in 0.1 M aqueous NaClO_4 solution, in the presence and absence of 0.6 mM ascorbic acid. The PFS-modified electrode showed a quasi-reversible redox response in the absence of ascorbic acid. The bare gold electrode showed only a redox wave corresponding to ascorbic acid oxidation.⁵² For the gold electrode modified with PFS grafts, however, upon ascorbic acid oxidation, the CV displayed a well-defined peak around +0.34 V (vs. Pt) and greatly enhanced peak currents. This behavior shows that the immobilized PFS films on gold can effectively catalyze the oxidation of ascorbic acid. The defined amperometric response forms the basis for the use of these PFS films in electrochemical sensor applications.

The amperometric response of the modified electrode to successive additions

Figure 5.11: Cyclic voltammogram of (a) bare gold and (b) modified electrode in the absence and presence of 0.6 mM ascorbic acid in 0.1 M NaClO_4 aqueous solution. Scan rate is 50 mV/s, Pt wires were used as the reference and counter electrode.

of ascorbic acid was evaluated by applying a fixed potential of +0.3 V. The current-time curve shown in Figure 5.12 indicates that the PFS films show a rapid response and high sensitivity. The nearly equal current steps observed for each addition reflect a stable and predictable catalytic activity. A linear relationship between oxidation current and ascorbic acid concentration was obtained up to 40 μM . At

Figure 5.12: Amperometric response of the PFS-I grafts to successive additions of ascorbic acid into stirred aqueous 0.1 M NaClO_4 at room temperature, Pt wires were used as reference and counter electrode. The first six additions increased the concentration of ascorbic acid by 5 μM each, later each injection increased the concentration by 10 μM . The inset is the calibration curve. Applied potential = 0.3 V ($R=0.99908$).

high ascorbic acid concentrations a deviation from the straight line was observed. This deviation is likely attributed to the rate of ascorbic acid delivery to the electroactive sites.⁵³ Similar deviations from linearity have been observed in the literature for other amperometric biosensors.^{54, 55} Overall, the ascorbic acid electrochemical sensor based on these PFS films exhibits a high sensitivity, stable responses, and a low detection limit which compares favorably to other reported ascorbic acid sensors based on ferrocene derivatives.^{56–59}

5.3 Conclusions

In conclusion, the present work demonstrates a novel approach to the development of electrochemical sensors based on surface-immobilized PFS chains. Surface-anchored PFS films were formed by an alkylation reaction of an amine monolayer on silicon or gold surfaces with PFS chains featuring iodopropyl side groups. AFM and SEM measurements showed the formation of thin, relatively dense PFS films.

The electrochemical properties of these films were studied both in water and in methylene chloride. In a good solvent which could swell the films, such as CH_2Cl_2 , a more reversible redox behaviour was observed than in water. Due to the covalent anchoring, PFS chains remained on the substrate during repeated redox cycling.

Furthermore, adherence between Si_3N_4 tips and the immobilized PFS films was assessed by ECAFM to gain information on the hydrophobicity / hydrophilicity of the films as a function of redox state. The adhesion force of the PFS-I grafts showed a reversible change upon electrochemical redox stimuli. The different AFM tip treatment procedures drastically affected the observed adhesion forces as reflected in the opposed trends in observed adhesion forces, showing that the influence of the tip hydrophilicity on AFM force measurements is highly significant. These findings are of pivotal importance for AFM based adhesion probing as the AFM tip condition fundamentally governs the experimental results.

The PFS films exhibited a high sensitivity and stable responses to ascorbic acid, which renders these films of interest in electrochemical sensing. The anchored PFS chains possess unreacted iodopropyl side groups which are readily derivatized into a range of functionalities including cationic, anionic, hydrophilic, hydrophobic etc. moieties. These robust redox-active films, possessing tunable characteristics, therefore constitute a highly versatile platform for the chemical modification of electrodes.

5.4 Experimental section

Materials: Poly(ferrocenyl(3-iodopropyl)methylsilane) (PFS-I) (**1**) (M_n : 3.42×10^5 g/mol, M_w : 6.87×10^5 g/mol, M_w/M_n : 2.0)⁶⁰ and poly(ferrocenyldimethylsilane) (PFDMS)⁶¹ were prepared by metal-catalyzed ring-opening polymerization according to established procedures. Cysteamine ($\geq 98.0\%$), (3-aminopropyl)trimethoxysilane(99%), sodium perchlorate ($\geq 98.0\%$), tetrabutylammonium hexafluorophosphate ($\geq 99.0\%$), dichloromethane and ascorbic acid ($\geq 99.0\%$) were obtained from Sigma-Aldrich and used as received. Tetrahydrofuran (THF) was purified by distillation from sodium-benzophenone under argon. All water used in the experiments was Milli-Q grade.

Formation of (3-aminopropyl)trimethoxysilane monolayers: Silicon substrates were first cleaned with piranha solution and then rinsed extensively with water and ethanol. (*Caution! Piranha solution reacts violently with many organic materials and should be handled with great care.*) The dried substrates were placed at the bottom of a desiccator around a vial containing 100 μ L of (3-aminopropyl)trimethoxysilane. The desiccator was then evacuated with a rotary vane pump for 10 min and subsequently closed. Vapor-phase silanization was allowed to proceed overnight. The substrates were then rinsed with toluene and ethanol, dried in a stream of N_2 , and immediately used for PFS immobilization.

Formation of cysteamine SAMs: Gold substrates (100 nm Au on 10 nm Cr on silicon) were cleaned with piranha solution and extensively rinsed with water and ethanol. Cysteamine SAMs were prepared by immersing gold substrates in ethanol solutions, containing 1 wt % cysteamine, for 16 h. The substrates were then rinsed with ethanol, dried in a stream of N_2 , and immediately used for PFS attachment.

Surface attachment of PFS chain: A solution of PFS-I (**1**) in THF (10 mg/mL, 50 μ L) was deposited on the amine-functionalized silicon or gold substrate surfaces. Another 50 μ L of PFS-I solution was added onto the surface after 30 mins. Then the substrates were left to react overnight at 50 °C in a vacuum oven. The PFS-I modified substrates were soaked three times in THF for 30 min to remove physisorbed polymer chains.

Characterization instrumentation:

Fourier Transform Infrared (FTIR): Grazing angle FTIR spectroscopy was employed to establish which groups are present in the tethered films after substrate anchoring. FTIR spectra were obtained using a Bruker Vertex 70V spectrometer. A background spectrum was obtained by scanning a clean gold substrate.

Static Contact Angle (SCA): SCA measurements were performed by the

sessile drop technique using an optical contact angle device equipped with an electronic syringe unit (OCA15, Dataphysics, Germany). The sessile drop was deposited onto the surface of the materials with the syringe, and the drop contour was fitted by the Young–Laplace method. At least three different measurements of each sample were performed.

Thermal stability measurements: The thermal stability of samples was examined on a Perkin Elmer Thermo Gravimetric Analyzer (TGA 7, Waltham, MA, U.S.A.) with a heating rate of 20 °C/min from 50 to 600 °C under a nitrogen atmosphere. All samples were dried under vacuum for 24 h prior to TGA measurements.

Scanning Electron Microscopy (SEM) measurements: SEM images of PFS films were captured with a HR-LEO 1550 FEF SEM instrument.

Electrochemical measurements: Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out with PFS films on gold substrates in aqueous NaClO₄ (0.1 M) or NBu₄PF₆ in CH₂Cl₂ (0.1 M) using an Autolab PGSTAT 10 electrochemical workstation. Cyclic voltammograms were recorded between −0.4 V and +0.4 V at different scan rates, using a Pt reference electrode and a Pt counter electrode. The amperometric sensing of ascorbic acid was performed by using the immobilized PFS films as the working electrode and aqueous NaClO₄ (0.1 M) as the electrolyte solution, the potential was set at 0.3 V for a certain time to stabilize the current. Successively, ascorbic acid aqueous solution was added to the sensing system and mixed under magnetic stirring to form a homogenous solution with a controlled concentration. Simultaneously, the current response was recorded.

Atomic Force Microscopy (AFM) measurements: A Dimension D3100 (Digital Instruments, Veeco-Bruker, Santa Barbara, CA) was operated in tapping mode to obtain the thickness and surface morphology of the PFS graft layers.

In-Situ Electrochemical Atomic Force Microscopy (ECAFM) measurements: AFM force spectroscopy measurements were performed in a combined electrochemistry AFM setup using a PicoForce AFM (Bruker Nano Surfaces, Santa Barbara, CA, USA) with a NanoScope IVa controller and an electrochemical liquid cell in combination with an Autolab PGSTAT10 potentiostat (Ecochemie, Utrecht, The Netherlands). Commercially available V shaped silicon nitride cantilevers (DNP, Bruker AFM probes, Camarillo, CA, USA) were treated with organic solvent (acetone and ethanol) or with piranha solution (H₂SO₄ and H₂O₂ with a volume ratio of 7:3) before the measurements. Cantilever spring constants were determined using the thermal tune method⁵¹ and showed values in the range of ~ 0.1–0.15 N/m. The electrochemical measurements were performed in a three-electrode arrangement in 0.1 M NaClO₄ aqueous solution. The gold substrates

with immobilized PFS grafts were used as the working electrode, while the Ag wire and Pt plate functioned as the reference and counter electrodes which were mounted to the AFM electrochemical liquid cell. Cyclic voltammograms were recorded between -0.05 V and $+0.65$ V vs. Ag wire with a scan rate of 50 mV/s. Prior to the experiments, the electrolyte was degassed by purging the solution with nitrogen gas for 20 min. Each set of pull-off force experiment involved collection of 300 force curves at each redox state (-0.05 V and $+0.60$ V, respectively) at different sample positions.

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Chapter 6

Covalent Layer-by-Layer Assembly of Redox-Active Polymer Multilayers

Poly(ferrocenyl(3-bromopropyl)methylsilane) and poly(ethylene imine) are employed in a layer-by-layer deposition process to form covalently connected, redox-active multilayer thin films by means of an amine alkylation reaction. The stepwise buildup of these multilayers on silicon, ITO, and quartz substrates was monitored by UV-vis absorption spectroscopy, Fourier transform infrared spectroscopy (FTIR), static contact angle measurements, surface plasmon resonance (SPR), atomic force microscopy (AFM), ellipsometry, and cyclic voltammetry, which provide evidence for a linear increase in multilayer thickness with the number of deposited bilayers. Upon oxidation and reduction, these covalently interconnected layers do not disassemble, in contrast to poly(ferrocenylsilane) (PFS) layers featuring similar backbone structures that are held together by electrostatic forces. The PFS/PEI multilayers are effective for the electrochemical sensing of ascorbic acid and hydrogen peroxide and show improved sensing performance at higher bilayer numbers. These covalently linked layers are readily derivatized further and can therefore be regarded as a versatile platform for creating robust, tailorable, redox-active interfaces with applications in sensing and biofuel cells.

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6.1 Introduction

The modification of electrode surfaces with electroactive species has been a topic of major interest in the past decades because of the tremendous application potential of functionalized electrodes in the areas of ion recognition,^{1–3} electrocatalysis,⁴ amperometric biosensors,^{5–7} biofuel cells,^{8–11} and molecular electronics.¹² The surface-anchoring of thiol- or disulfide-containing redox-active molecules onto gold surfaces has resulted in self-assembled monolayers (SAMs) and mixed SAMs.¹³ In addition to small molecules, polymeric redox-active species have been widely employed to create chemically modified electrodes.^{14–16} Typical examples include poly(vinylferrocene) and polymerized $\text{Ru}(\text{vbpy})_3^{2+}$, polymers consisting of poly(allylamine),¹⁷ poly(ethylene imine),⁵ or poly(methacrylate)¹⁸ backbones with pendant redox-active units, and conducting polymers such as poly(pyrrole) and poly(thiophene).¹⁹

Because many applications require the incorporation of additional functions such as biorecognition elements within the redox-active layer, it is desirable to construct the redox-active film in a stepwise manner. A highly versatile approach for the stepwise construction of thin polymer films with controlled composition and thickness is the electrostatic layer-by-layer (LbL) deposition process.^{20, 21} Initially, LbL-related research was focused primarily on the use of commercially available polyelectrolytes for constructing thin films.^{22, 23} Subsequent work demonstrated that many other materials can also be used in the preparation of films. Furthermore, in addition to electrostatics, various driving forces for film assembly were exploited, including hydrogen bonding,^{24–26} DNA hybridization,^{27, 28} sequential chemical reactions,^{29–38} metal coordination,³⁹ and ligand–receptor interactions,⁴⁰ implying that the LbL method is not restricted to charged materials. The use of covalent bonds to assemble LbL films is an emerging area of interest. Covalent connections between deposited chains prevent layer disassembly, which may occur for electrostatically assembled multilayers with changes in pH or ionic strength.

A fascinating class of redox-active materials, poly(ferrocenylsilanes) (PFSs),^{41–45} composed of alternating ferrocene and silane units in their main chain combine a high density of redox centers with excellent processability and possess redox characteristics^{46–48} suitable for the electrochemical detection of biological analytes. Several reports have appeared on the formation of thin PFS films on electrodes and other surfaces using the layer-by-layer deposition of PFS polyions.^{49–52}

Multilayered organometallic thin films, held together only by electrostatic interactions, were used for the redox-controlled release of molecular payloads

stored within the films.⁵³ PFS films can also be anchored on solid substrates through covalent bonds. In Chapter 5, we reported the fabrication of a covalently immobilized poly(ferrocenylsilane) film on a gold electrode using reactive side groups of the PFS chains for surface anchoring.^{54–57} The stable, ultrathin redox-active films resulting from this “grafting-to” approach⁵⁸ were successfully used in the electrochemical sensing of ascorbic acid. These layers are readily derivatized further and can therefore be regarded as a versatile platform for creating robust, tailorable redox-active interfaces.

Figure 6.1: Covalent LbL deposition of poly(ferrocenyl(3-bromopropyl)methylsilane) (PFS-Br) and poly(ethylene imine) (PEI). The multilayered films were employed as electrochemical sensor for hydrogen peroxide.

Here we extend this “grafting-to” method to a covalent LbL deposition process for the sequential buildup of covalently interconnected PFS layers on ITO and Si substrates, using an amine alkylation reaction between PFS bromopropyl side groups and poly(ethylene imine) (Figure 6.1). In contrast to the widely explored electrostatic LbL deposition process, LbL processes where covalent bonds are formed between layers are relatively rare.^{20, 21} Very few examples exist of covalently constructed LbL films involving a polymer with pendant redox units,⁵⁹ and to our knowledge, there are no accounts of LbL films based on covalently linked redox-active polymers with skeletal redox units. We monitored multilayer growth by UV–vis spectroscopy, static contact angle (SCA) measurements, surface plasmon resonance (SPR) spectroscopy, atomic force microscopy (AFM), and cyclic voltammetry (CV). The performance of these stable, ultrathin redox-active films in the electrochemical sensing of hydrogen peroxide⁶⁰ as a function of the number of bilayers is discussed.

Figure 6.2: Photographs of solutions of the multilayer building blocks PFS-Br (**1**) and PEI in the layer deposition medium, and the precipitate formed when mixing these components, indicating that intermolecular alkylation occurs.

6.2 Results and discussion

6.2.1 Covalent LbL assembly of multilayers

The redox-active component employed in the LbL construction of the redox-active multilayers, poly(ferrocenyl(3-bromopropyl)methylferrocene) (PFS-Br) (**1**), was synthesized according to a published procedure.⁶¹ High-molar-mass, branched poly(ethylene imine) (PEI) was used to interconnect the PFS chains via amine alkylation reactions. To verify sufficient reactivity, PFS-Br (**1**) and PEI were mixed in the deposition medium, 4:1 v/v THF/DMSO. A precipitate formed within 10 min (Figure 6.2). Multilayers of PFS-Br (**1**) and PEI were fabricated on various

Scheme 6.1: Schematic representation of PFS/PEI multilayer fabrication on amine-functionalized substrates.

substrates to determine if well-defined film growth occurred.

An alternate adsorption of PFS **1** and PEI was conducted as depicted in Scheme 6.1. First, silicon or ITO substrates were treated with (3-aminopropyl)-trimethoxysilane to create an amine-terminated surface for the attachment of PFS-Br (**1**). The substrate was subsequently immersed in a 2 mg/mL solution of **1** in THF/DMSO, rinsed, and immersed in a PEI solution of the same concentration.

6.2.2 Characterization of the multilayers

The sequential buildup of PFS/PEI multilayers on quartz slides was monitored by UV-vis spectroscopy. After each deposited bilayer, a spectrum was recorded. Figure 6.3 shows the increase in the absorbance at $\lambda = 216$ nm associated with the intense ligand-to-metal charge-transfer transition (LMCT)⁶² characteristic of PFS as a function of the number of bilayers. A linear increase in this absorbance with the number of PFS/PEI bilayers was observed, which is indicative of a well-defined deposition process. Interestingly, the linear increase in absorbance is already observed in the first few bilayers, and the fitted line passes through the origin. Thus, this deposition process does not show a transient for the first few layers, as commonly observed for electrostatic LbL depositions where the substrate influences the number of adsorbed chains.⁶³

FTIR was used to obtain further information on bonds formed within the PFS/PEI multilayer. Figure 6.4 shows the FTIR spectra of PEI, PFS-Br **1**, and (PFS/PEI)₈-PFS. In the spectrum of PEI, the band at 1578 cm^{-1} is associated with the N-H bending vibration of primary amines.^{64–66} The absorption at 1120 cm^{-1} belongs to the C-N stretching of secondary amine groups.^{64–66} In the spectrum

Figure 6.3: (a) UV-vis absorption spectra of sequentially adsorbed layers of PFS-Br **1** and PEI deposited from 4:1 v/v THF/DMSO on quartz. (b) Absorption at $\lambda = 216$ nm as a function of the number of bilayers.

Figure 6.4: FTIR spectra of (1) PEI, (2) (PFS/PEI)₈-PFS, and (3) PFS-Br **1** on Si substrates at (a) high energy region and (b) low energy region.

of the (PFS/PEI)₈-PFS multilayer, the band at 1578 cm^{-1} became weaker and a strong absorption appeared at 1110 cm^{-1} , indicating that primary amine groups changed to secondary amine groups.

Table 6.1: Static contact angles measured during PFS/PEI multilayer buildup.

	PFS-Br 1 (°)	PEI (°)
bilayer 1	67 ± 2	30 ± 2
bilayer 2	65 ± 2	42 ± 2
bilayer 3	60 ± 2	45 ± 2
bilayer 4	63 ± 2	45 ± 2

The layer-by-layer buildup on silicon substrates was monitored by static contact angle measurements. It is known that the outermost layer of LbL-deposited films governs the wettability of multilayer assemblies.⁶⁷ Because hydrophobic (PFS-Br) and hydrophilic (PEI) building blocks are used, successful multilayer growth should result in alternating contact angle values. As layers were added, contact angles alternated between $63 \pm 2^\circ$ for PFS outer layers and $45 \pm 2^\circ$ for PEI outer layers (Table 6.1), providing clear evidence for the stepwise LbL assembly of the PFS/PEI multilayer films. The obtained contact angles for PEI outer layers matched the literature values for PEI-based multilayers.^{68, 69}

The covalent layer-by-layer assembly process was also gauged by surface plasmon spectroscopy (SPR). Figure 6.5 shows the various reflectivity curves obtained during the LbL deposition of PFS-Br **1** and PEI. SPR responses originate from refractive index changes, and the shifts in the minimum of the angular θ scans of reflected intensity signify the sequential assembly of the different building

Figure 6.5: (a) Reflected intensity as a function of the angle-of-incidence scan (θ) plot showing the assembly of the multilayered structure on the Au substrate. (b) Expanded view of the SPR spectra in the range of the reflectivity minimum. The various reflectivity curves correspond to (1) the amine-functionalized Au surface, (2) (PFS)₁, (3) (PFS/PEI)₁, (4) (PFS/PEI)₁-PFS, (5) (PFS/PEI)₂, (6) (PFS/PEI)₂-PFS, (7) (PFS/PEI)₃, (8) (PFS/PEI)₃-PFS, and (9) (PFS/PEI)₄.

blocks into the layered architecture. A Fresnel fit to the resonance curve of bare and coated Au surfaces gave optical thicknesses of the multilayer films. On the basis of known refractive indices of the film components ($\text{RI}_{\text{PFS}} = 1.57$,⁷⁰ $\text{RI}_{\text{PEI}} = 1.50$ ⁷¹) a bilayer thickness of 1.4 nm was found for the multilayer films.

Figure 6.6: Tapping-mode AFM images of (a) an amine-functional Si substrate after attaching PFS-Br 1 (i.e., (PFS)₁), (b) (PFS/PEI)₁, (c) (PFS/PEI)₄-PFS, and (d) (PFS/PEI)₈-PFS, scanned over a $0.5 \times 0.5 \mu\text{m}^2$ area. (e) AFM image and cross-sectional height profile of a (PFS/PEI)₈-PFS film assembled on a Si substrate. The rms roughness values for these layers are (a) 0.6, (b) 0.7, (c) 1.4, and (d) 1.9 nm.

Figure 6.6 shows the surface morphology of PFS/PEI films as a function of the number of bilayers, measured by tapping mode AFM, and the film height of a (PFS/PEI)₈-PFS multilayer on silicon substrates. The rms roughness of the multilayer over a scanned area of $0.5 \times 0.5 \mu\text{m}^2$ increased with the number of bilayers. For a single layer of PFS-Br **1** attached to an amine functional Si substrate, (PFS)₁, the value of the rms roughness was 0.6 nm, 0.7 nm for (PFS/PEI)₁, 1.4 nm for (PFS/PEI)₄-PFS, and 1.9 nm for (PFS/PEI)₈-PFS.

A cross-sectional height profile of a (PFS/PEI)₈-PFS film on Si had a thickness of 9.13 nm in the dry state. Ellipsometry measurements (Figure 6.7) and the SPR results (1.4 nm/bilayer) confirmed this film thickness.

Figure 6.7: (a) Ellipsometry spectra as a function of wavelength for PFS/PEI bilayers for three angles. (b) Film thickness evolution with increasing numbers of bilayers.

The electrochemical characteristics of the multilayers were studied by cyclic voltammetry (CV). Figure 6.8a shows the cyclic voltammograms corresponding to (PFS/PEI)_n-PFS thin films with different numbers of bilayers ($n = 1, 3, 5$, and 7). The double-wave voltammograms result from intermetallic coupling between the ferrocene units in the PFS chain. Because of intermetallic coupling, the oxidation potential of a ferrocene unit is increased when a neighboring ferrocene is already in the oxidized state. Therefore, ferrocene units in alternating positions along the PFS chain are oxidized first at low potential, followed by the oxidation of the remaining ferrocene units at higher potential, resulting in the double-wave voltammogram typical of PFS chains.⁴⁸ The charge passed during oxidation and reduction increased linearly with the number of bilayers in the LbL assembly, showing that the number of ferrocene units on the surface grew linearly (Figure 6.8 b). By integration of the area under the redox peaks, the surface coverage of ferrocene sites for (PFS/PEI)₇-PFS was calculated to be $2.29 \times 10^{-9} \text{ mol cm}^{-2}$.⁷²

To characterize the electroactive behavior of the redox-active assembly further, we studied the scan rate (v) dependence of the peak current (I_p), which provides

Figure 6.8: (a) Cyclic voltammograms for PFS/PEI multilayers on ITO substrates in a 0.1 M NaClO₄ aqueous solution, with a Ag/AgCl reference electrode and a Pt counter electrode. The scan rate is 50 mV/s. (b) Charge passed during oxidation/reduction for varying numbers of bilayers. $R_{ox} = 0.993$ and $R_{re} = 0.988$.

powerful insight into the electrode processes that govern the redox characteristics of the film. The linear dependence of I_p on v , found for all oxidation and reduction peaks (Figure 6.9), is typical for surface-confined electroactive species.^{72, 73}

We investigated the stability of the multilayers in the reduced and oxidized states. In the reduced state, when held at a fixed potential of -0.1 V, the films were stable. Also, no material loss was observed after 30 CV cycles at a scan rate of 50 mV/s. After a fixed oxidizing potential of 0.6 V (vs. Ag/AgCl) was maintained for 3 h, which is a rather severe test for gauging film stability, only

Figure 6.9: (a) Cyclic voltammograms of a (PFS/PEI)₈-PFS multilayer on ITO at various scan rates in 0.1 M NaClO₄ aqueous solution, with a Ag/AgCl reference electrode, and a Pt counter electrode. The scan rate was varied from 10 to 100 mV/s, and the potential range applied was -0.1 to 0.9 V vs. Ag/AgCl. (b) Dependence of the peak currents on the scan rate for a (PFS/PEI)₈-PFS multilayer on ITO.

Figure 6.10: Remaining percentage of integrated charge Q involved in the oxidation process in time as a function of accumulated holding time at a potential of 0.6 V (vs. Ag/AgCl), demonstrating the stability of a (PFS/PEI)₈-PFS multilayer under an applied potential.

10% material loss occurred (Figure 6.10). In contrast, a five-bilayer film composed of PFS polyanions and polycations, held together by electrostatic forces, showed much higher material losses when kept at aging potentials as low as 0.1 V.⁵³

Figure 6.11: Cyclic voltammograms of (PFS/PEI)₈-PFS (a) in the absence of and in the presence of (b) 0.1 mM and (c) 0.3 mM ascorbic acid at a scan rate of 50 mV/s in 0.1 M NaClO₄ aqueous solution. Ag/AgCl and Pt wire served as the reference and counter electrode, respectively.

6.2.3 Electrochemical sensing applications

Poly(ferrocenylsilanes) are promising redox mediators with application potential in electrocatalysis and in electrochemical sensing. In Chapter 4 and 5, we reported an ascorbic acid sensor based on an electrografted, and on a single poly(ferrocenylsilane) layer bound covalently to a gold electrode.⁵⁴ Extending this approach, we were interested in gauging the performance of the current LbL assembled films as ascorbic acid and hydrogen peroxide sensors, in particular, as a function of the number of bilayers. The amperometric response of (PFS/PEI)₈-PFS multilayer to ascorbic acid under an applied potential of 0.6 V (vs. Ag/AgCl) was measured (Figures 6.11 and 6.12). The multilayer films showed a good sensing ability towards ascorbic acid.

Figure 6.12: (a) Amperometric response of (PFS/PEI)₈-PFS to ascorbic acid under an applied constant potential of 0.6 V (vs. Ag/AgCl), each step represents 5 μM ascorbic acid. (b) Plot of chronoamperometric current vs. ascorbic acid concentration for a (PFS/PEI)₈-PFS multilayer.

To demonstrate that the performance of electrochemical sensors based on PFS redox mediators can be enhanced by tuning the number of bilayers, we examined multilayers consisting of PFS-Br **1** and PEI with five and nine bilayers as mediators in the electrochemical sensing of hydrogen peroxide (H₂O₂). The detection of hydrogen peroxide is of importance in pharmaceutical, clinical, environmental, textile, and food manufacturing applications.⁶⁰ It is known that at unmodified electrodes, slow electrode kinetics and high overpotential hinder the oxidation and reduction of H₂O₂ in analytical applications.⁶⁰ Figure 6.13 illustrates the cyclic voltammograms of (PFS/PEI)₈-PFS in the absence (a) and presence (b–e) of H₂O₂. The peak potentials do not change, but the anodic and cathodic peak currents increase with the addition of H₂O₂, indicating that PFS mediates H₂O₂ redox processes effectively.

Figure 6.13: Cyclic voltammograms of (PFS/PEI)₈-PFS (a) in the absence and presence of H₂O₂ (b, 0.5; c, 1; d, 5; and e, 10 mM) at a scan rate of 50 mV/s in a 0.1 M NaClO₄ aqueous solution. Ag/AgCl and a Pt wire served as the reference and counter electrodes, respectively.

Figure 6.14 presents the sensor response of (PFS/PEI)₄-PFS and (PFS/PEI)₈-PFS multilayers to consecutive additions of H₂O₂, where its concentration increases with 25 μ M increments. A very significant difference in the current densities between these multilayers is observed. Clearly, the number of accessible ferrocene moieties per unit area at the electrode directly affects the performance of the sensor. A plateau is visible in the curve of (PFS/PEI)₄-PFS. At the plateau, the

Figure 6.14: Amperometric responses of (PFS/PEI)₄-PFS and (PFS/PEI)₈-PFS to H₂O₂ at -0.1 V (vs. Ag/AgCl) constant potential, where each step represents 25 μ M H₂O₂.

Figure 6.15: (a) Amperometric response of (PFS/PEI)₈-PFS to H₂O₂ at -0.1 V (vs. Ag/AgCl) constant potential. Each step represents $5 \mu\text{M}$ H₂O₂, (b) Plot of chronoamperometric current vs H₂O₂ concentration.

(PFS/PEI)₄-PFS redox mediator is saturated by the high concentration of H₂O₂. We expect a plateau also for (PFS/PEI)₈-PFS; however, this may occur at higher concentrations of H₂O₂ because more redox centers are present at the electrode in the case of (PFS/PEI)₈-PFS.

The amperometric response of the most sensitive multilayer, (PFS/PEI)₈-PFS, to a smaller H₂O₂ concentration increment of $5 \mu\text{M}$ was subsequently investigated and is displayed in Figure 6.15a. The rapid increase in the recorded current proves that these multilayer films can detect H₂O₂ at low concentration levels. Also, these sensor layers display a fast response to H₂O₂ addition.⁷⁴ The vertical sections of the amperometric response plot (Figures 6.14 and 6.15a) indicate response times of less than 3 s. The analytical curve ($R = 0.997$) obtained from the chronoamperogram of (PFS/PEI)₈-PFS is displayed in Figure 6.15b. The current was found to increase linearly with H₂O₂ concentration up to $30 \mu\text{M}$. At higher concentrations, a moderate deviation from linearity was observed. This deviation may be attributed to changes in the rate of H₂O₂ delivery to the electroactive sites. Similar deviations from linearity have been observed in the literature for other amperometric biosensors.^{74–76} The limit of detection was estimated to be $3 \mu\text{M}$ at a signal-to-noise ratio of 3. It is noteworthy that the low applied working potentials (-0.1 V) used with these redox-active PFS layers are an important characteristic of interest for oxidase-based biosensors, which display poor selectivities at high applied working potentials when interfering substances are present.⁷⁷ For ascorbic acid (Figure 6.12), a detection limit of $1.6 \mu\text{M}$ was estimated. These detection limits match the performance of sensors reported in the literature. In the electrochemical sensing of hydrogen peroxide, sensors based on noble metal nanoparticles,⁷⁸ heme

proteins and nanomaterials,⁶⁰ or porous carbon-modified electrodes⁷⁹ achieve limit of detection (LOD) values ranging from 10 to 0.1 μM , with exceptions where a LOD of 0.028 μM was demonstrated.⁸⁰ Similar to electrochemical hydrogen peroxide sensing, most of the electrochemical sensors for ascorbic acid display detection limits of between 10 and 0.1 μM .^{14–16, 81–86}

6.3 Conclusions

This study describes the first example of a redox-active multilayer film, fabricated from a main-chain redox-active polymer (PFS-Br) and a redox-inert polymer (PEI) interconnected by covalent bonds. The multilayer films, formed on silicon, ITO, quartz, and Au substrates by LbL deposition, were characterized by UV-vis spectroscopy, FTIR, static contact angle measurements, SPR, atomic force microscopy, ellipsometry, and cyclic voltammetry, which showed that multilayer growth occurred in a well-defined manner, with the multilayer thickness increasing linearly with the number of deposited bilayers. PFS/PEI multilayers were successfully used in the electrochemical sensing of ascorbic acid and hydrogen peroxide. The efficiency of the multilayer films in these electrocatalytic processes was enhanced by increasing the number of bilayers. These layers are readily derivatized further and can therefore be regarded as a versatile platform for creating robust, tailorable redox-active interfaces.

6.4 Experimental section

Materials: Poly(ferrocenyl(3-bromopropyl)methylsilane) (PFS-Br) (**1**) ($M_n = 1.61 \times 10^5$ g/mol, $M_w = 3.93 \times 10^5$ g/mol, $M_w/M_n = 2.4$) was synthesized according to established procedures.⁶¹ (3-Aminopropyl)-trimethoxysilane (97%), poly(ethylene imine) (branched, average $M_n \approx 1.00 \times 10^4$ g/mol by GPC, average $M_w \approx 2.50 \times 10^4$ g/mol by LS according to the supplier, $\leq 1\%$ water), cysteamine ($\geq 98.0\%$), and sodium perchlorate ($\geq 98.0\%$) were obtained from Sigma-Aldrich and used as received. Tetrahydrofuran (THF) was purified by distillation from sodium benzophenone under argon. All water used in the experiments was Milli-Q grade. Hydrogen peroxide (for analysis, 35 wt% solution in water) was obtained from Acros Organics.

Formation of (3-aminopropyl)trimethoxysilane monolayers: Indium tin oxide (ITO, for CV measurements), silicon (for AFM and ellipsometry measurements), and quartz (for UV-vis spectroscopy) substrates were first cleaned with piranha solution (base or acid) and then rinsed extensively with water and ethanol.

(Caution! Piranha solution reacts violently with many organic materials and should be handled with great care.) The dried substrates were placed at the bottom of a desiccator around a vial containing 100 μL of (3-aminopropyl)trimethoxysilane. The desiccator was then evacuated with a rotary vane pump for 10 min and subsequently closed. Vapor-phase silanization was allowed to proceed overnight. The substrates were then rinsed with toluene and ethanol, dried in a stream of N_2 , and immediately used for LbL assembly.

Formation of cysteamine SAMs: Gold substrates (for SPR measurements) were cleaned with piranha solution and extensively rinsed with water, ethanol, and dichloromethane. Cysteamine SAMs were prepared by immersing gold substrates in ethanol solutions, containing 1 wt % cysteamine, for 16 h. The substrates were then rinsed with ethanol, dried in a stream of N_2 , and immediately used for LbL assembly.

Multilayer fabrication: The amine-modified substrates were alternately immersed in solutions of PFS-Br 1 (in 4:1 v/v THF/DMSO, 2 mg/mL) and PEI (in 4:1 v/v THF/DMSO, 2 mg/mL) for 30 min and after each deposition step rinsed with THF (PFS-Br 1 outer layer) or Milli-Q water (PEI outer layer), immersed in pure THF or Milli-Q water for 2 min, rinsed with ethanol, and dried in a stream of nitrogen.

Characterization instrumentation:

UV-vis spectroscopy: UV-vis absorption spectra were recorded using a Perkin-Elmer Lambda 850 UV-vis spectrophotometer.

Fourier Transform Infrared (FTIR) measurements: FTIR spectra were obtained using a Bio-Rad FTS 575C spectrometer. A background spectrum was obtained by scanning a clean silicon substrate.

Static Contact Angle (SCA) measurements: SCA measurements were performed by the sessile drop technique using an optical contact angle device equipped with an electronic syringe unit (OCA15, Dataphysics, Germany). The sessile drop was deposited onto the surface of the materials with the syringe, and the drop contour was fitted by the Young-Laplace method. At least three different measurements of each sample were performed.

Ellipsometry measurements: An M-2000X variable-angle spectroscopic ellipsometer (J.A. Woollam Co., Lincoln, NE, USA) system was used. Measurements were performed at wavelengths ranging from 210 to 1000 nm (1.25–5.85 eV) at three angles (65, 70, and 75°). The spot size of the probing light had a diameter of 2 mm. Data fitting was performed with a commercial software package (Complete EASE v.4.41) supplied with the M-2000X system. The thickness of the multilayer films deposited on silicon substrates was determined, assuming simple Cauchy

dispersions.

Surface Plasmon Resonance (SPR) measurements: SPR spectra were collected by using a Multiskop instrument (Optrel, Germany) equipped with a 632.8 nm He–Ne laser. An SPR chip (gold thickness of 50 nm, XanTec Bioanalytics, Düsseldorf, Germany) and a 90° LaSFN9 prism (Hellma Optik, Jena, Germany) with a refractive index of 1.845 (at $\lambda = 632.8$ nm) were assembled in the Kretschmann configuration. The SPR chip was optically matched to the base of the prism using a refractive index matching oil (Series B, Cargille Laboratories, Cedar Grove, NJ, USA). The intensity of the reflected light as a function of the angle of incidence was recorded. The reflected intensity showed a sharp minimum at a resonance angle that depends on the precise architecture of the interface. The measured angular reflectivity curves were fitted using the Optrel GbR surface plasmon data evaluation and simulation program (v. 2.1). Layer thicknesses were determined by nonlinear least-squares fitting to a layer model using Fresnel theory and refractive index values of 1.57 for PFS⁷⁰ and 1.50 for PEI.⁷¹

Table 6.2: The plasmon coupling angle of PFS/PEI multilayer reading from Figure 6.5 and the fitting results of optical thickness of the multilayer films.

Sample	The plasmon coupling angle (°)	Added layer	Thickness of added layer (nm)
The amine- Au	43.48	NH ₂	2.9
PFS ₁	43.77	PFS 1	1.5
(PFS/PEI) ₁	44.01	PEI 1	1.4
(PFS/PEI) ₁ -PFS	44.11	PFS 2	0.3
(PFS/PEI) ₂	44.335	PEI 2	1.1
(PFS/PEI) ₂ -PFS	44.403	PFS 3	0.4
(PFS/PEI) ₃	44.615	PEI 3	1

Atomic Force Microscopy (AFM) measurements: AFM measurements were performed with a Dimension D3100 AFM equipped with a NanoScope IVa controller (Bruker, Santa Barbara, CA, USA) in tapping mode using commercially available silicon cantilevers (PointProbe Plus silicon probes, PPP-NCH, Nanosensors, Neuchâtel, Switzerland) to measure the thickness and surface morphology of the grafted layers.

Electrochemical measurements: Electrochemical measurements were carried out with multilayers on ITO substrates in aqueous NaClO₄ solution (0.1 M) using an Autolab PGSTAT 10 electrochemical workstation. Cyclic voltammograms were recorded between –0.1 and +0.9 V at various scan rates using a Ag/AgCl reference electrode and a Pt counter electrode.

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Poly(ferrocenylsilane) as Redox Mediator in the Enzymatic Sensing of Glucose: Can Enzymatic Sensing Efficiency be Improved by Increasing Enzyme Coverage?

An electrochemical biosensor for the enzymatic sensing of glucose was constructed from cationic poly(ferrocenylsilane) (PFS) and glucose oxidase (GOx), employing a layer-by-layer assembly technique, followed by cross-linking of the layers with PEG dimethacrylate. In the resulting multilayer structures, the PFS chains served as redox mediator and GOx as biorecognition element. The fabrication and performance of the sensor are described and discussed.

7.1 Introduction

The immobilization and electrical “wiring” of enzymes on electrodes have raised interest due to potential applications in sensors,^{1, 2} fuel cells,^{3–5} and biocomputing logic systems.^{6, 7} Applications where surface-bound, immobilized enzymes are involved rely on retaining enzyme activity. This depends on solution parameters and electrode design.⁸ For a high sensor performance, the enzyme must be in good contact with the electrode or mediator without blocking the active site of the enzyme. In addition, the enzyme’s geometry should not be changed significantly upon immobilization.⁹ Methods to immobilize the enzyme include physical entrapment, covalent binding, adsorption or crosslinking.

In sensing applications, redox mediators which facilitate electron transfer between enzyme and electrode, are often used (Chapter 2).¹⁰ Many attempts have been made in the last decades to improve the response and sensitivity of biosensors, not only by tailoring the redox mediator, but also by improving contact or even entrapment of the enzyme by the mediator. Also, the electrode surface coverage by the enzyme influences sensor performance. The early examples of redox mediators were low molecular weight redox couples that could diffuse in and out of the enzyme’s cavity.^{11, 12} Some examples of these small molecules are ferrocene derivatives, ferricyanide, quinones or metal containing complexes.⁹ The next generation of mediators were redox active polycationic polymers.^{13, 14} The redox active polymer penetrates and binds the enzymes, forming a three-dimensional network. For a chemically modified electrode, the use of electroactive polymers has several advantages over the use of monomolecular layers. The response of polymer-based electrodes is larger and therefore easier to detect due to the higher number of redox active sites compared to a monolayer.¹⁵ Electroactive polymers show enhanced performance in biological applications. Adding redox enzymes to the polymeric film reduces the distance between the enzyme and electrode resulting in an efficient charge transfer. Furthermore, the polymeric film can protect the electrode from non-specific protein adsorption.

Organometallic polymers are showing a growing importance in the last few years as these materials combine useful optical, electrical and magnetic properties with the processability of polymers.^{16–18} Organometallic materials are characterized by the presence of transition metals, these transition metals can be a part of the main chain or be present in the side groups. In this Thesis we focus on the organometallic polymer poly(ferrocenylsilane)s (PFS).¹⁹ PFS consists of alternating ferrocene and silane units in the main chain of the polymer. These ferrocene units can be reversibly oxidized and reduced and therefore make PFS a redox responsive polymer.^{20–23}

The electrochemical biosensor for the detection of glucose that we aim to construct requires immobilization of the biorecognition element, the enzyme, and the redox mediator PFS on an electrode surface. From the range of methods discussed in Chapter 3 for fabricating thin films on solid substrates, layer-by-layer deposition was chosen. Electrode modification by redox-active multilayer films, assembled in a stepwise manner (layer-by-layer, LbL), is a highly promising method since multilayer thickness and composition can be controlled accurately.²⁴ The number of redox active centers can be tuned with the number of deposited redox active layers, and therefore the anticipated electrical response can be enhanced compared to that of a monolayer. Since the advent of this approach, polyions have been combined with oppositely charged (not necessarily polymeric) species, including enzymes, as has been reported.^{2, 25–28}

Figure 7.1: Cross-linking of a PFS and GOx layer-by-layer assembled film.

In this Chapter we describe the fabrication and performance of a glucose sensor based on the redox active polymer PFS and the enzyme glucose oxidase (GOx). Crosslinkable cationic PFS chains will be used to construct a LbL assembled film consisting of PFS polycations and the negatively charged GOx, making use of electrostatic interactions. The aim of this work was to investigate whether PFS could act as a redox mediator in a biochemical sensor. PFSs have redox potentials in a range that is suitable for redox mediation. In addition, owing to the high density of redox centers in the PFS chain, we anticipate that electron transport between enzyme and electrode may be mediated efficiently by PFSs. We note that although electrostatic LbL films showed good stability, they can slowly disassemble over time due to lack of covalent bonds between layers, or upon exposure to salt solutions of high ionic strength.²⁹ Keeping this in mind, we designed an LbL system featuring covalently linked layers (Figure 7.1). When there is a sufficient

amount of layers formed (10 layers, i.e. 5 bilayers), the films will be crosslinked to enhance their stability.

7.2 Results and discussion

7.2.1 Synthesis and characterization of PFS⁺-methacrylate and cross-linker

In order to form a film composed of PFS and GOx on an electrode surface suitable for glucose sensing, cationic PFS bearing methacrylate side groups (PFS⁺-methacrylate) and PEG-dimethacrylate (PEG-DMA), a cross-linker, were synthesized. A low molar mass PEG-dimethacrylate (PEG-DMA, $M_n = 500$ g/mol) was synthesized to allow the formation of covalent bonds between the PEG and the PFS chains in the multilayer. The resulting network is expected to physically entrap the GOx enzyme.

The synthesis of PFS⁺-methacrylate is outlined in Scheme 7.1. The iodo groups of PFS-I are easily replaced by a variety of nucleophiles under mild conditions.^{30, 31} For example, Sui et al. reported that sodium acrylate, a weak nucleophile, could be attached quantitatively to PFS in the presence of the crown

Scheme 7.1: Synthesis of cationic PFS⁺-methacrylate.

ether 15-crown-5.³² Similarly, it is possible to partially convert the iodopropyl groups of PFS-I into methacrylate groups to make the polymer suitable for crosslinking. Together with the PEG-DMA spacer a polymeric network can be formed. To obtain a 3D network only a few cross-links are necessary, therefore 10 % of the iodopropyl groups were converted into methacrylate groups. The converted amount was determined from ^1H -NMR spectroscopy. The remaining iodopropyl groups of PFS were converted into the cationic (trimethylamino)propyl groups to make the polymer water soluble and suitable for LbL assembly with the negatively charged GOx enzyme (at physiological pH), followed by ion exchange to obtain chloride counter ions.

The PFSs obtained were characterized by ^1H NMR, ^{13}C NMR, gel permeation chromatography and FTIR. Figure 7.2 shows that the double bond of the methacrylate group is still present in PFS⁺-methacrylate, indicating that no crosslinking of the polymer occurred during its synthesis.

Figure 7.2: FTIR spectrum of PFS⁺-methacrylate at (a) high energy region and (b) low energy region.

7.2.2 Formation of PFS/GOx multilayers

Through electrostatic layer-by-layer assembly, cationic PFS⁺-methacrylate and GOx were deposited on various substrates alternatively. ITO substrates were functionalized with (3-mercaptopropyl)trimethoxysilane to obtain a thiol endcapped monolayer (Scheme 7.2). Silanization was performed in solution which led to an increase in contact angle from $28 \pm 2^\circ$ to $77 \pm 4^\circ$. Silicon substrates were functionalized in the same way, resulting in a contact angle of $69 \pm 2^\circ$ and the functionalization of quartz substrates yielded a contact angle of $73 \pm 2^\circ$.

The first layer was covalently attached through the PFS methacrylate side

Scheme 7.2: Covalent surface-attachment of PFS⁺-methacrylate, constituting the first layer of the PFS/GOx multilayer film.

groups onto the thiol-end functionalized substrate in the presence of *n*-hexylamine. Acrylate moieties are known to react rapidly with thiols by thiol-Michael addition reaction, but also methacrylate groups show sufficient reactivity towards thiols in this addition reaction.³³ In this way, the first layer, which possesses cationic side groups, was firmly anchored on the substrate, forming the basis for the growth of the rest of the layers. The multilayers were built by electrostatic LbL assembly with negatively charged GOx and cationic PFS⁺-methacrylate in buffer solution.

The sequential buildup of PFS⁺-methacrylate and GOx multilayers on quartz slides was monitored by UV-vis spectroscopy. The samples were not cross-linked during the multilayer fabrication process. After each deposited layer, a spectrum was recorded. Analyzing the characteristic absorbance of PFS at $\lambda = 216$ nm which is associated with the intense ligand-to-metal charge transfer transition (LMCT),³⁴ a linear increase in absorbance with the number of layers was observed (Figure 7.3 a). This linear increase indicated a well-defined growth, i.e. the same amount of material was deposited on the electrode in each deposition step.

The LbL deposition of PFS⁺-methacrylate and GOx was also followed by ellipsometry (Figure 7.3 b). The film thickness was determined based on two separate multilayer samples. The thickness of the oxide layer and monolayer were measured using the dielectric function of SiO₂ on a Si substrate. Bilayers were

Figure 7.3: (a) UV-vis absorption as a function of the number of bilayers and (b) film thickness as a function of the number of bilayers, measured by ellipsometry on two separate sample species for PFS⁺-methacrylate and GOx multilayers.

grown on the substrates, and the bilayer thickness was determined using a Cauchy model with $A=1.55$ (offset) and $B=0.005$ (dispersion). Samples with only one or two bilayers showed good fitting results using the Cauchy model, however after three bilayers the Cauchy fits became less accurate. The surface of the samples became spotty, indicating some loss of uniformity. For these samples, the best fit to ellipsometry data was obtained when thickness non-uniformity³⁵ of the samples was taken into account. This result can be explained by the fact that the multilayers are formed from linear polymer chains and spherical enzyme objects which possess a diameter of about 10 nm.

In order to characterize the surface morphology and roughness, AFM height images were obtained in tapping mode for various numbers of bilayers. All samples containing both enzyme and PFS were immersed in a solution of PEG-DMA and Irgacure 2959 overnight, and crosslinked with UV-light of $\lambda = 365$ nm during 60 seconds before measuring AFM. Height AFM images with a scan size of $1 \mu\text{m} \times$

Table 7.1: Surface roughness in nm as a function of the number of bilayers for PFS⁺-methacrylate and GOx multilayers, determined by AFM in tapping mode. Scan size was $1 \mu\text{m} \times 1 \mu\text{m}$.

Layer	Surface roughness (nm)
Monolayer	0.105
PFS	0.448
Bilayer 1	1.77
Bilayer 3	2.69
Bilayer 5	6.15

Figure 7.4: AFM measurements on PFS/GOx multilayers on silicon substrates. (a)-(e) Height images for increasing numbers of bilayers, (f) three-dimensional height plot for five bilayers. Scan size: $1\ \mu\text{m} \times 1\ \mu\text{m}$.

$1\ \mu\text{m}$ (Figure 7.4) show an increasing roughness of the surface with increasing numbers of bilayers. Figure 7.4 f displays a three-dimensional height plot of a sample on a silicon substrate with five bilayers of PFS⁺-methacrylate and GOx. From this plot it can be clearly seen that there is substantial height variation within the sample, which corresponds to the observations from ellipsometry. The roughness of the surface was characterized by the RMS value of the height data, shown in Table 7.1.

Figure 7.5: AFM tapping mode height image of five bilayers of PFS⁺-methacrylate and GOx.

In order to determine film thickness also by AFM, films were scratched to the depth of the substrate and in the scratched areas multilayers were removed. Thus the resulting step height allowed us to obtain thickness values from AFM height images. Figure 7.5 shows a typical scratch image of a cross-linked sample containing five bilayers. From this image, the total height of the five bilayers was determined to be 48.5 nm. Compared to the height data from ellipsometry, this value is much lower. From Figure 7.4 f, it can be seen that the variation in height can be up to 30 nm within $1\ \mu\text{m} \times 1\ \mu\text{m}$ and the thickness obtained from ellipsometry varies between 70-100 % of the measured value. Due to these variations and by changing the location of the scratch, different film thickness values were obtained. Furthermore, we observed that the layers settled over time to a more compact arrangement, resulting in a lower thickness.

For this part we conclude that film fabrication of PFS/GOx multilayers by the LbL method was successfully achieved. However, surface smoothness and uniformity were decreasing as the number of bilayers increased, which is caused by the spherical enzyme objects embedded in the multilayers.

7.2.3 Sensor performance

The cross-linked multilayers were used as sensors for glucose. Figure 7.6 shows the amperometric response for one bilayer of PFS⁺-methacrylate and GOx upon injection of 1 mM glucose every 200 seconds. It can be concluded that one bilayer is not capable of detecting glucose. Likely, the amount of GOx in the bilayer was too

Figure 7.6: Amperometric response for one PFS/GOx bilayer upon injection of glucose, 1 mM was injected every 200 seconds. The arrows indicate the first injection of glucose. In 0.1 M sodium acetate buffer, pH 5.5, $E=0.44\ \text{V}$ vs. Ag/AgCl.

Figure 7.7: (a) Amperometric response upon injection of glucose for five PFS/GOx bilayers. The arrows indicate the total concentration of glucose injected at that point. (b) working plot of this sensor. In 0.1 M sodium acetate buffer, pH 5.5, $E=0.44$ V vs. Ag/AgCl.

low to generate a measurable current. Figure 7.7 presents the sensor response of a cross-linked multilayer (5 bilayers) of PFS⁺-methacrylate and GOx to consecutive additions of glucose by measuring the current response at a potential of 440 mV vs. Ag/AgCl with time. Injections of glucose resulted in a clear response for concentrations of up to 5 mM. If the total concentration of glucose was increased to 10 mM, there was only a small response, and with higher concentrations there was no response at all. The current increased linearly with the glucose concentration up to 5 mM. When the glucose concentration was increased further, the amperometric response levelled off. We ascribe this behavior to saturation of

Figure 7.8: Amperometric response for five PFS/GOx bilayers after being stored for one week. 1 mM glucose was injected every 400 seconds. The arrows indicate the first injection of glucose. In 0.1 M sodium acetate buffer, pH 5.5, $E=0.44$ V vs. Ag/AgCl.

the enzyme. Comparing the sensor performance of the one-bilayer and five-bilayer systems, we can conclude that the amount of GOx in the multilayer influences the sensing efficiency of these sensors.

After one week storage (4 °C, in 100 mM sodium acetate buffer of pH 5.5), the response to glucose was measured for the second time by measuring the amperometric response at a potential of 440 mV vs Ag/ AgCl. Figure 7.8 shows the amperometric response upon injection of 1 mM glucose every 400 seconds. The response flattened after 4-5 injections of glucose. The magnitude of the response had decreased substantially, compared with the initial measurement (Figure 7.7).

7.3 Conclusions

The objective of the work described in this Chapter was to develop a biosensor which is able to sense glucose by using poly(ferrocenylsilane) as a redox mediator, and to enhance the sensing capacity of this sensor by increasing the surface coverage by GOx, using a multilayer immobilization approach. The synthesis of cationic water soluble PFS polymers, the layer-by-layer assembly, cross-linking of the polymers and characterization of the sensing properties were achieved.

Layer-by-layer assembly of cationic PFS⁺-methacrylate and anionic glucose oxidase was monitored by UV-vis absorption spectroscopy and ellipsometry. For up to five bilayers, there was an increase in film thickness for every deposition step. This study demonstrates that PFS can be used as a redox mediator between a biorecognition element and electrodes in electrochemical biosensors. Five bilayers of PFS⁺-methacrylate and GOx resulted in a clear amperometric response for β -D-glucose at concentrations of up to 5 mM. Unfortunately, redox sensing signals became saturated with increasing analyte concentration. Upon storage, sensor performance decreased, which may indicate that enzyme moieties slowly leached from the multilayer. Possibly, the employed physical entrapment of the enzyme species within the multilayers was not sufficient. Additionally, it has been reported that molecules with protonated amines close to ferrocene units are known to form complexes with anions irreversibly upon oxidation in some buffer solution.^{36, 37} This would significantly limit the mediating performance of PFS in buffer solutions.

7.4 Experimental section

Material: *n*-Butyllithium (2.5 M in hexanes), chloroplatinic acid hexahydrate, 15-crown-5 (98%), dicyclohexano-18-crown-6 (98%), *N,N*-dimethylethylamine

(99%), diphosphorus pentoxide, ferrocene (98%), β -D-glucose (98%), glucose oxidase from *Aspergillus Niger*, hexylamine (99%), iodoethane (99%), (3-iodopropyl)trimethoxysilane ($\geq 95\%$), (3-mercaptopropyl)trimethoxysilane (95%), methacrylic anhydride (94%), poly(ethylene glycol) methacrylate $M_n = 500$ g/mol, sodium 2-mercaptoethanesulfonate ($\geq 98\%$), sodium methacrylate (98%), sodium perchlorate ($\geq 98\%$), sodium tert-butoxide (97%), *N,N,N,N*-tetramethylethylenediamine (99%), triethylamine ($\geq 99\%$), and trimethylamine solution (4.2 M in ethanol) were obtained from Sigma-Aldrich and used as received. Dichloro(3-chloropropyl)methylsilane was obtained from ACBR chemicals. Potassium iodide (99%) was obtained from Fluka. Acetic Acid (glacial) and sodium acetate were obtained from Merck. Tetrahydrofuran (THF) was purified by distillation from sodium benzophenone under argon. The other solvents were obtained from Biosolve and used as received. All water used in the experiments was of Milli-Q grade.

Synthesis of PEG-dimethacrylate (PEG-DMA) spacer: A mixture of PEG-methacrylate (8.22 g, 0.016 mol), methacrylic anhydride (6.16 g, 0.040 mol), triethyl amine (4.05 g, 0.040 mol) and dichloromethane (30 mL) was stirred for 7 days at room temperature under argon atmosphere. Precipitation of the product in ice cold *n*-hexane resulted in a slightly yellow oil.

Synthesis of PFS⁺-methacrylate: Poly(ferrocenyl(3-iodopropyl)methylsilane) (PFS-I) ($M_w: 5.47 \times 10^5$ g/mol, $M_n: 2.94 \times 10^5$ g/mol, $M_w/M_n=1.86$) was prepared according to established procedures.³¹ To a solution of PFS-I (0.2 g, 0.5 mmol) in THF (6 mL), DMSO (3 mL), sodium methacrylate (5.4 mg, 0.05 mmol) and 15-crown-5 (20 μ L, 0.1 mmol) were added and the reaction mixture was stirred at room temperature for 7 days. The resulting mixture was precipitated into cold methanol and the resulting solid was collected and washed thoroughly. PFS-methacrylate were obtained and ¹H NMR spectroscopy indicates that 10 % of the iodopropyl groups were converted into methacrylate groups. ¹H NMR (CDCl₃, ppm): δ 0.47 (SiCH₃, s); 1.00 (1-CH₂, m); 1.88 (2-CH₂, m); 1.95 (CH₃-C=CH₂, s); 3.22 (I-CH₂, m); 3.68-4.23 (Cp rings, m); 4.15 (-O-CH₂, t); 5.55(=CH) and 6.12 (=CH).

Trimethylamine was added to the mixture of PFS-methacrylate (10 % substitution) in DMSO and THF. The mixture became cloudy after one day. The THF was evaporated and fresh DMSO and trimethylamine were added, and stirring was continued for one week. The mixture was transferred to a Spectra/Por 4 dialysis hose (MWCO 12-14000 g/mol) and dialyzed against 0.1 M NaCl (3 $\times$ 1L) and MilliQ water (3 $\times$ 1L). Concentration of the salt-free polyelectrolyte solution by a gentle flow of nitrogen produced PFS⁺-methacrylate as orange flakes. ¹H NMR (DMSO-d₆, ppm): δ 0.54 (SiCH₃, s); 0.87 (1-CH₂, m); 1.75 (2-CH₂, m); 1.88

(CH₃-C=CH₂, s); 3.11 (N-CH₃, s); 3.38 (3-CH₂); 4.00-4.20 (Cp rings, m); 4.15 (-O-CH₂, t); 5.62 (=CH) and 6.07 (=CH); ¹³C NMR (DMSO): δ 3.41 (SiCH₃); 11.48 (1-CH₂); 18.05 (2-CH₂); 23.15 (CH₃-C=CH₂, s); 52.01 (N-CH₃); 66.76 (3-CH₂); 67.70 (-O-CH₂); 69.89-73.59 (Cp); 125.53 (=CH₂); 136.12 (-C=CH₂) and 166.50 (C=O).

Formation of (3-mercaptopropyl)trimethoxysilane SAMs: Indium tin oxide (ITO, for electrochemical measurements), silicon (for AFM and ellipsometry measurements), and quartz (for UV-vis spectroscopy) substrates were first cleaned with Piranha solution (base or acid), then rinsed extensively with water and ethanol. (**Caution!** Piranha solution reacts violently with many organic materials and should be handled with great care.) Toluene was purged for 30 minutes with argon, after that (3-mercaptopropyl)trimethoxysilane (MPTMS) was added to obtain a 4 % (vol) solution. Cleaned and dried substrates were immersed in the MPTMS solution overnight. The substrates were washed with toluene and dried under N₂.

Multilayer fabrication: Multilayers with PFS⁺-methacrylate and GOx from *Aspergillus Niger* were deposited onto various MPTMS functionalized substrates. The first layer (PFS) was covalently attached through the PFS⁺-methacrylate side groups onto the thiol-modified substrate in the presence of hexylamine. The modified substrates were immersed into a solution of PFS⁺-methacrylate (2 mg/mL, 0.5 M NaCl, 300 μ L hexylamine) for 6 hours, followed by rinsing with Milli-Q water, dipping for 2 minutes into pure Milli-Q water, rinsing with Milli-Q water and drying under N₂. The subsequent layers were fabricated by alternating immersion in GOx (1.5 mg/mL in 100 mM sodium acetate buffer pH 5.5) solutions and PFS⁺-methacrylate solutions (2 mg/mL in Milli-Q water, 0.5 M NaCl) for 15 minutes, with rinsing with Milli-Q water, dipping for 2 minutes into pure Milli-Q water, rinsing with Milli-Q water and drying under N₂ between each deposition step. After depositing the desired number of bilayers, the multilayers were immersed in a solution of PEG-DMA and Irgacure 2959 overnight, and crosslinked with UV-light of λ = 365 nm during 60 seconds.

Characterization instrumentation:

¹H NMR and ¹³C NMR spectra: NMR spectra were obtained on a Bruker 400 MHz NMR spectrometer at room temperature in approximately 10 % w/v solutions in deuterated solvents. All chemical shifts were reported in ppm and referenced to the residual solvent resonances. The chemical shift of the CDCl₃ solvent peak at δ = 7.26 and 77.0 ppm, respectively, was used as a reference.

Gel permeation chromatography (GPC) measurements: GPC was carried out in THF (flow rate 2.0 mL/min) at 25 °C using microstyragel columns (Waters) and a dual detection system consisting of a differential refractometer (Waters 410)

and a differential viscometer (Viscotek H502). Molar masses were determined relative to narrow polystyrene standards.

UV-vis spectroscopy: UV-vis absorption spectra were recorded using a Perkin Elmer Lambda 850 UV-vis spectrophotometer.

Fourier Transform Infrared (FTIR) measurements: FTIR spectra were obtained using a Bio-Rad FTS 575C spectrometer. A background spectrum was obtained by scanning a clean silicon substrate.

Static Contact Angle (SCA) measurements: SCA measurements were performed by the sessile drop technique using an optical contact angle device equipped with an electronic syringe unit (OCA15, Dataphysics, Germany). The sessile drop was deposited onto the surface of the materials with the syringe, and the drop contour was fitted by the Young-Laplace method. At least three different measurements of each sample were performed.

UV irradiations: UV irradiations were performed with a high power UV-LED (P8D236, Seoul Optodevice Co., South Korea) with a narrow emission spectrum (365 ± 5 nm) mounted onto a printed circuit board (PCB) in series with a 4.7Ω power resistor and operated with a laboratory power supply (6.0 V, 500 mA).

Ellipsometry measurements: A M-2000X variable angle spectroscopic ellipsometer (J.A. Woollam Co., Lincoln, NE, USA) system was used. Measurements were performed at wavelengths ranging from 210 nm to 1000 nm (1.25-5.85 eV) at three angles (65° , 70° and 75°). The spot size of the probing light had a diameter of 2 mm. Data fitting was performed with a commercial software package (Complete EASE v.4.41) supplied with the M-2000X system. The thickness of the multilayer films deposited on silicon substrates was determined, assuming simple Cauchy dispersions.

Atomic Force Microscopy (AFM) measurements: AFM measurements were performed on silicon functionalized substrates with a NanoScope IVa scanning probe microscope (Bruker, Santa Barbara, CA, USA) in tapping mode operation using commercially available silicon cantilevers (PointProbe Plus silicon probes, PPP-NCH, Nanosensors, Neuchâtel, Switzerland) to measure the thickness and surface morphology of the graft layers.

Electrochemical measurements: Electrochemical measurements were carried out with multilayers on ITO substrates in aqueous NaClO_4 (0.1 M) or buffer solution using an Autolab PGSTAT 10 electrochemical workstation. Cyclic voltammograms were recorded between -0.1 V and $+0.9$ V at various scan rates, using a Ag/AgCl reference electrode and a Pt counter electrode.

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Metal Nanoparticle Foundry with Redox Responsive Hydrogels

A redox-active, organometallic hydrogel was successfully formed from the reaction of poly(ferrocenyl(3-bromopropyl)methylsilane) with a multifunctional amine crosslinker, *N,N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA). When the PFS hydrogel was charged with noble metal salts, corresponding noble metal nanoparticles were formed. Interestingly, the nanoparticles were well dispersed in the hydrogel matrix, and little or no aggregation was observed. The noble metal nanoparticles, which included Au, Ag, Pd, Pt, Ir and Rh, were characterized by TEM, EDX, XPS. The concept of using a redox-active hydrogel for fabricating noble metal nanoparticles, where the PFS network chains provide reducing capability and confinement, and where no external reducing agents or ligands were required, was demonstrated to be effective.

8.1 Introduction

Noble metal materials with nanometer-scale dimensions and a particular shape have attracted great interest for their special properties,^{1–3} such as large surface area, surface plasmon characteristics,⁴ and unique optical and electronic properties. These materials have potential applications in the areas of catalysis,⁵ optoelectronics,⁶ sensors,⁷ biomedical materials and in the energy field.⁸ In order to take full advantage of the unique physical and chemical attributes, it is necessary to explore synthetic methods for controlling the shape, size and composition of the noble metal materials.^{9, 10}

Several methods have been utilized to fabricate metal nanomaterials with tunable shape, size and dimensions.^{9, 11} However, many of the approaches employ reductants, surfactants, polymers or stabilizing agents to bring about reduction and prevent nanoparticle aggregation.^{12–15} The added reducing or protecting agents may adsorb on the nanoparticle surface, which may affect the catalytic activity of the nanomaterials.

The interest in synthetic methods for preparing metal nanoparticles continues to increase. Dai et al. described the spontaneous formation of metal nanoparticles on the sidewalls of single-walled carbon nanotubes (SWNTs) by the direct redox reactions between the metal ion precursors and nanotubes for Au and Pt.¹⁶ Substrate-Enhanced Electroless Deposition (SEED) methods were introduced later for electroless deposition of a large variety of metal nanoparticles including Cu and Ag nanoparticles, on both SWNTs and multiwalled carbon nanotubes (MWNTs) in the absence of any additional reducing agent.¹⁷

Reduced graphene oxide (rGO) nanosheets were used for fabricating and anchoring noble metal (Au, Ag, Pd) nanoparticles^{18–20} and clusters with particle sizes below 2 nm.²¹ The deposition of metallic noble metal nanoparticles on the partially reduced graphene oxide mat was realized by a simple redox reaction between the noble metal precursors and GO in aqueous solution, without using any additional reductants, surfactants or protecting ligands.

Here we propose a new, simple and straightforward method for the in-situ preparation of metal nanoparticles from their corresponding salts, using redox-active polymer hydrogels.

Poly(ferrocenylsilanes) (PFSs), which consist of alternating ferrocene and silane units in the main chain, are a fascinating class of processable materials which can be reversibly oxidized and reduced by chemical and electrochemical means.^{22–24} This organometallic polymer has received considerable attention due to its interesting optical, electronic and redox properties. By post-functionalization of PFS chains, typically involving variation of substituents at the silicon atoms

of PFS, a range of organometallic polymer materials has become available. These include organic solvent soluble PFSs, water soluble PFSs, and polymers of these types possessing crosslinkable side groups. Our group has reported the synthesis of several PFS-based gels using different crosslinking methods^{24–26} and the in-situ preparation of PFS-PNIPAM-Ag composites.²⁷

Figure 8.1: Schematic illustration of (a) metal nanoparticle foundry and (b) one step synthesis of PFS hydrogel.

Given that PFSs have an oxidation potential of around +0.4 V, it is possible to reduce metal salts possessing significantly higher oxidation potentials to metal nanoparticles where the PFS chains act as reducing agent. In addition, the network structure of the crosslinked PFS chains provides a confined environment for metal salt reduction which may influence size and polydispersity of the nanoparticles produced. In this Chapter, we describe a metal nanoparticle foundry based on a redox-active PFS hydrogel and demonstrate that the reduction capability of PFS hydrogels is universal, allowing one to directly reduce metal salts to the corresponding metallic particles (Au, Ag, Pd, Pt, Rh, Ir) at room temperature.

8.2 Results and discussion

8.2.1 Synthesis of poly(ferrocenylsilane) hydrogel

A bulk cationic poly(ferrocenylsilane) hydrogel was formed in one step by cross-linking poly(ferrocenyl(3-bromopropyl)methylsilane) chains with *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA) at room temperature (Figure 8.1). A bulk gel was obtained within 3 hours. Upon swelling in deionized water, the PFS network directly became an amber transparent elastic hydrogel (Figure 8.2). In previous work, our group reported the formation of a cationic PFS hydrogel from poly(ferrocenyl(3-iodopropyl)methylsilane) in two steps.²⁸ Considering the physical properties of the halide ions, a trend in hydrophilicity could be found as follows:²⁹ $\text{Cl}^- > \text{Br}^- > \text{I}^-$. PFSs bearing bromopropyl side groups have sufficient reactivity and the materials are more hydrophilic than the iodopropyl-substituted PFSs after quaternization with tertiary amines. Therefore, it was not necessary to exchange the bromide into chloride counter ions after the amination reaction to obtain water-swallowable networks.

Figure 8.2: Photographs of the gel formation (reaction in Figure 8.1). (a) Mixture of PFS-Br and PMDETA in THF/DMSO; (b) gel formed within 3 h at room temperature, after washing with water.

Fourier Transform Infrared (FTIR) spectra shown in Figure 8.3 indicate that the PMDETA was incorporated in the network. Typical absorptions of PFS were observed at 3087 cm^{-1} ($\nu_{\text{C-H}}$, Fc), 2958 cm^{-1} ($\nu_{\text{C-H}}$, methyl groups), 1248 cm^{-1} (symmetric deformation of Si-CH_3), 1182 and 1163 cm^{-1} (asymmetric ring in-plane vibration for Fc), 1037 cm^{-1} (out-of-plane C-H vibration for Fc), 896 cm^{-1} (rocking vibration of methyl groups), 864 cm^{-1} (Si-C stretching vibration), 825 cm^{-1} (in-plane C-H stretching for Fc), as well as 770 cm^{-1} (out-of-plane C-H deformation for Fc). In terminal alkyl halides, the C-H wag of the $-\text{CH}_2\text{Br}$ group is seen at 1230 cm^{-1} .³⁰ This peak disappeared in the FTIR spectra of the hydrogel (Figure 8.3 b-2), indicating quaternization of the bromopropyl groups.

Figure 8.3: FT-IR spectra of 1) PFS-Br, 2) PFS-Br/PMDETA hydrogel at (a) high energy region and (b) low energy region.

Thermogravimetric analysis was used to study the thermal stability of the network. In Figure 8.8, thermal decomposition of the PFS network occurred by a two-step mechanism, displaying a maximum degradation at 510 °C with a residual mass of 31%, due to the presence of the non-volatile elements iron and silicon in the PFS main chain.

Water-uptake measurements were performed and a swelling ratio of 15.5 was obtained, indicating that a rather densely cross-linked network was formed. This figure is lower than that of the cationic PFS hydrogel possessing Cl^- as the counter ion (containing the same molar quantity of the cross-linker), which may be caused by a somewhat lower hydrophilicity of the $-\text{NMe}_3\text{Br}$ groups compared to the $-\text{NMe}_3\text{Cl}$ groups.

8.2.2 Metal nanoparticle foundry

The redox activity of the PFS chain could be used to prepare metal nanoparticles (NPs) in a facile in-situ manner without using external reducing agents or organic solvents. The synthesis of NPs was carried out by immersion of a piece of hydrogel in a solution of metal ions. For ions with a sufficiently high oxidation potential, the reaction occurred spontaneously as outlined in Figure 8.1. Table 8.1 shows the standard potential of several half reactions of metal salts. If the standard electrode potential is higher than the oxidation potential of ferrocene units in PFS (+0.4 V vs. SHE), then the ferrocene units can transfer an electron to the metal ions, reducing the metal ions eventually to $\text{M}(0)$ within the hydrogel, while the ferrocene units from the hydrogel are oxidized. PFS oxidation is accompanied by a color change from amber to dark green. After the redox reaction, the hydrogel

Table 8.1: Standard electrode potentials of common half-reactions in aqueous solution, measured relative to the standard hydrogen electrode at 25°C with all species at unit activity.³¹

Standard electrode potentials of common half-reactions in aqueous solution	E° / V vs <i>SHE</i>
$\text{Ag}^{+} + \text{e}^{-} \rightleftharpoons \text{Ag}$	+ 0.8
$\text{AuCl}_4^{-} + 3\text{e}^{-} \rightleftharpoons \text{Au} + 4\text{Cl}^{-}$	+ 1.00
$\text{Cu}^{2+} + 2\text{e}^{-} \rightleftharpoons \text{Cu}$	+ 0.34
$\text{PdCl}_4^{2-} + 2\text{e}^{-} \rightleftharpoons \text{Pd} + 4\text{Cl}^{-}$	+ 0.64
$\text{PtCl}_4^{2-} + 2\text{e}^{-} \rightleftharpoons \text{Pt} + 4\text{Cl}^{-}$	+ 0.76
$\text{Ir}^{3+} + 3\text{e}^{-} \rightleftharpoons \text{Ir}$	+ 1.156
$\text{Rh}^{3+} + 3\text{e}^{-} \rightleftharpoons \text{Rh}$	+ 0.76
$[\text{Ferricinium}]^{+} + \text{e}^{-} \rightleftharpoons \text{Ferrocene}$	+ 0.4

was washed with Milli-Q water to remove unreacted salts and freeze-dried for Transmission Electron Microscopy (TEM) analysis.

Figure 8.4 shows TEM images of the metal nanoparticles obtained using the PFS hydrogel. For all the samples, it can be seen that noble metal nanoparticles were formed within the hydrogel.

Figure 8.4 a reveals Au particles of uniform size, homogeneously dispersed in the hydrogel. The Au particles had an average diameter of 7 nm. Silver salts tended to form larger particles with a diameter of 20–40 nm (Figure 8.4 b), which corresponds with results previously reported by our group.²⁷ Platinum nanoparticles were successfully synthesized by treating the hydrogel with K_2PtCl_4 . As is displayed in Figure 8.4 c, Pt nanoparticles with a diameter of around 2 nm and a remarkably narrow size distribution were well dispersed in the gel. Pd nanoparticles could also be produced using the organometallic hydrogel. In the TEM image (Figure 8.4 d), very small particles with an average diameter of 2.4 nm were formed, including some aggregates. For Rh and Ir, images demonstrate the formation of uniform nanoparticles which were not aggregated. The dimensions were evaluated, resulting in a diameter of 2.3 nm for Rh and 1.6 nm for Ir. Energy Dispersive X-ray (EDX) measurements were carried out on the nanoparticle-loaded PFS gels to further characterize the produced metal particles (Figure 8.5). In all cases, the expected signals of the metals were observed in the spectra.

Figure 8.4: TEM images of the noble metal ((a) Au, (b) Ag, (c) Pt, (d) Pd, (e) Rh, (f) Ir) nanoparticles formed in situ within the PFS hydrogel. Scale bar: 20 nm.

Figure 8.5: EDX spectra of the PFS hydrogels loaded with metal nanoparticles. (a) Au, (b) Ag, (c) Pt, (d) Pd, (e) Rh, (f) Ir.

High resolution TEM images of noble metal nanoparticles (Pd, Pt, Rh, Ir) are shown in Figure 8.6. For Pd, the HR-TEM image indicates that these nanoparticles are single-crystal with many (111) facets with a spacing of lattice fringes of 0.219 nm. For Pt, the measured interplanar spacing for the lattice fringes is 0.227 nm, which corresponds to the (111) lattice plane of face-centered cubic (fcc) Pt. The HR-TEM images shown in Figure 8.6 c and d prove that the Rh and Ir nanoparticles are crystallites with visible lattice fringes. Rh nanoparticles displayed a spacing of 0.219 nm, corresponding with metallic Rh(111).³² For Iridium, an interplanar spacing of 0.183 nm was found.

Figure 8.6: HR-TEM images of (a) Pd, (b) Pt, (c) Rh and (d) Ir noble metal nanoparticles.

In addition to verifying the nature of the metal nanoparticles by EDX, we established the valence state of the Au nanoparticles by X-ray photoelectron spectroscopy (XPS). As shown in Figure 8.7 b, the XPS signature of the Au 4f doublet ($4f_{7/2}$ and $4f_{5/2}$) is deconvoluted into two pairs of peaks, corresponding to the reduced Au(0) and the Au(III) ions, respectively. The ratio of the splitted peaks is: 21% of Au $4f_{7/2}$ at 86.3 eV and 79% at 84.69 eV. The XPS measurements prove that metallic Au particles were formed, but also indicate the presence of Au(III) ions. As the XPS data were acquired using a beam size of 200 μm , it was not possible to determine by these measurements whether the Au(III) ions were part of the nanoparticles, or were left as unreacted salt within the hydrogel.

Interestingly, however, there was a 0.69 eV red shift of the XPS peak of Au(0) (84.69 eV) compared with metallic Au (84.0 eV). This shift in the binding energy is typical for very small metal nanoparticles on a variety of support materials and is attributed to reduced core-hole screening in metal clusters.^{33, 34} This result indicates that the electronic properties of the Au nanoparticles formed within the hydrogel are significantly different from bulk materials and provides evidence that the nanoparticles are Au(0).

XPS also proved the presence of other expected groups in the Au-loaded PFS

Figure 8.7: XPS spectra of the (a) C 1s region and (b) Au 4f region of Au/hydrogel. The line with open circles represents the measured data, the dashed lines are fitted curves and the solid line is the sum of the fitted curves.

hydrogel. The high-resolution C 1s XPS spectrum of the Au/hydrogel sample could be fitted into the following peaks, corresponding to C atoms from different functional groups: 284.1 eV, 284.8 eV ($-C$ and $C=C$), 286.0 eV (C in $C-N$ bonds), 286.8 eV (C in $C-Br$ or $C-O$), 288.1 eV (carbonyl C). The peak at 284.1 eV is attributed to the $C-N-Au$ bond.³⁵

Thermogravimetric analysis (TGA) of hydrogel/metal nanoparticle hybrids was performed to gather information on degradation pathways and on residual mass, which typically is associated with the presence of inorganic elements. TGA was conducted in a N_2 atmosphere, the curves are shown in Figure 8.8. For the bare hydrogel, a residual mass of 31% was found. The residual mass found for

Figure 8.8: TGA curves of hydrogel and hydrogel/metal nanoparticle hybrids.

metal-loaded hydrogels was 36.0%, 37.3%, 46.0%, 47.67%, 51.3% and 55.1% in case of Pd, Au, Rh, Ir, Ag and Pt, respectively. As the residual mass of the gel increased when exposed to the various metal salts, these results support the successful formation of metal particles within the hydrogel.

We were interested to see whether the presence of metal nanoparticles in the PFS hydrogel would influence the electrochemical behavior of the constituent PFS chains. Cyclic voltammograms of the PFS-Br/PMDETA hydrogel and Au/hydrogel hybrid are shown in Figure 8.9. For the hydrogel itself, the CV displayed a single wave at a scan speed of 50 mV/s in NaClO₄. With the incorporation of Au nanoparticles, the CV of the hybrid gel showed a strong increase in the oxidation and reduction current and, in addition, displayed the double oxidation and reduction waves characteristic of PFSs. The incorporation of the Au nanoparticles likely increased the conductivity of the gel and facilitated electron transfer within the gel.

Figure 8.9: Cyclic voltammogram of PFS-Br/PMDETA hydrogel (red) and hydrogel+Au (black) at $v = 50$ mV/s in 0.1 M NaClO₄ aqueous solution. Ag/AgCl was used as reference electrode and Pt as counter electrode.

8.3 Conclusions

A redox-active, organometallic hydrogel was successfully formed from the reaction of poly(ferrocenyl(3-bromopropyl)methylsilane) with a multifunctional amine crosslinker, *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA). When the PFS hydrogel was charged with noble metal salts, corresponding noble metal nanoparticles were formed, typically possessing diameters of around several nm and relatively narrow size distributions. Interestingly, the nanoparticles were

well dispersed in the hydrogel matrix, and little or no aggregation was observed. The noble metal nanoparticles, which included Au, Ag, Pd, Pt, Ir and Rh, were characterized by TEM, EDX, XPS. The concept of using a redox-active hydrogel for fabricating noble metal nanoparticles, where the PFS network chains provide reducing capability and confinement, and where no external reducing agents or ligands were required, was demonstrated to be effective.

8.4 Experimental section

Materials:

Poly(ferrocenyl(3-bromopropyl)methylsilane) (**1**) ($M_n = 1.61 \times 10^5$ g/mol, $M_w = 3.93 \times 10^5$ g/mol, $M_w/M_n = 2.4$) was synthesized according to established procedures.³⁶ ^1H NMR (CDCl_3): δ 0.47 (SiCH_3 , s, 3H); 1.01 (1-CH_2 , m, 2H); 1.91 (2-CH_2 , m, 2H), 3.41 (Br-CH_2 , m, 2H); 3.96-4.23 (Cp, m, 8H); ^{13}C NMR (CDCl_3): δ -3.22 (SiCH_3); 15.41 (1-CH_2); 28.01 (2-CH_2); 37.39 (Br-CH_2); 69.97-73.59 (Cp).

N,N,N',N'',N'''-pentamethyldiethylenetriamine (PMDETA), sodium perchlorate (> 98.0 %), gold(III) chloride trihydrate ($\geq 99.9\%$), silver nitrate ($\geq 99.0\%$) and concentrated hydrochloric acid (37%), were obtained from Sigma-Aldrich and used as received. Tetrahydrofuran (THF) was purified by distillation from sodium benzophenone under argon. Potassium tetrachloropalladate(II), potassium tetrachloroplatinate(II), rhodium(III) chloride hydrate and iridium(III) chloride hydrate were purchased from Strem Chemicals, Inc. (Newburyport, MA, USA). All water used in the experiments was Milli-Q grade.

Preparation of PFS hydrogel: PFS-Br (60 mg) was dissolved in THF (400 μL) and DMSO (200 μL) and then PMDETA (12 μL) was added to the solution. The time to form a gel was determined using the vial tilting method.²⁶ In order to completely remove THF, the gel was placed in Milli-Q water which was replaced several times.

Fabrication of metal nanoparticles: The metal salts were dissolved in deionized water to produce a corresponding solution with a concentration of 5 mM. Then, the solution of the metal salt was added into the swollen PFS hydrogel. The reaction was left to proceed in the dark at room temperature. The color of the hydrogel turned from amber to dark green. The composite hydrogel was washed with Milli-Q water to remove unreacted metal salts.

Analytical instrumentation:

^1H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance III (400 MHz) instrument at 400.1 and 100.6 MHz, respectively.

Gel permeation chromatography (GPC) measurements were carried out in THF (flow rate 2.0 mL/min) at 25 °C, using microstyragel columns (bead size 10 μm) with pore sizes of 10^6 , 10^5 , 10^4 , and 10^3 Å (Waters) and a dual detection system consisting of a differential refractometer (Waters model 410) and a differential viscometer (Viscotek model H502). Molar masses were determined relative to narrow polystyrene standards.

Swelling measurements were carried out by immersing dry gel samples in water. The hydrogels were allowed to equilibrate for 24 h until a constant weight was reached. The surface water was carefully wiped off before weighing. The swelling ratio (by weight), S_W , was calculated as follows: $S_W = (W_h - W_d)/W_d$, where W_h and W_d are the hydrated and dry sample weights, respectively.

Fourier Transform Infrared (FTIR) spectra were obtained with a Bruker Alpha spectrometer.

Thermal stability of samples was examined on a Perkin Elmer Thermo Gravimetric Analyzer (TGA 7, Waltham, MA, USA) with a heating rate of 20 °C/min from 50 to 650 °C under a nitrogen atmosphere.

XPS measurements were carried out on a Quantera SXM (scanning XPS microprobe) from Physical electronics, using a monochromatic Al K α X-ray source (1486.6 eV).

Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray spectroscopy (EDX) analyses were performed with a FEI (Philips) CM300ST equipped with a GATAN Tridiem energy filter (2k $\times$ 2k CCD camera), extra GATAN Ultrascan 1000 CCD camera (2k $\times$ 2k), and a Noran System Six EDX analyzer with a Nanotrace EDX detector. The samples were prepared by sonication of small gel fragments in ethanol and placing one droplet of the dilute dispersion on a 200 mesh carbon coated copper grid.

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Chapter 9

Thin Film Hydrogels from Redox Responsive Poly(ferrocenylsilanes): an Outlook

As an outlook, we sketch the realization of a poly(ferrocenylsilane) hydrogel thin film based on the crosslinking chemistry described in Chapter 8.

9.1 Introduction

Hydrogels, an appealing category of soft materials, are cross-linked polymeric networks which absorb and retain large amounts of water. By choosing stimulus responsive chains to form their matrix, responsive hydrogels that are sensitive to external stimuli, such as temperature, light, pH, ionic strength, or bioactive species can be obtained.^{1–7} 3-D hydrogel networks are excellent candidates as scaffolds for incorporating various nanoparticles^{8–10} or as “reactors” for the in-situ generation of nanoparticles (Chapter 8). The resulting hybrid materials often possess enhanced mechanical characteristics, or unique optical, magnetic, electric, catalytic, or biological properties.^{11–14} Hydrogel thin films have raised recent interest as crosslinked architectures for creating responsive surfaces and interfaces.¹⁵ Compared with polymer brushes, a covalently crosslinked 3-D polymer network is more robust at interfaces. Compared with bulk gels, hydrogel thin films often possess fast response times¹⁶ to stimuli, which is an advantage that can be exploited in miniaturized devices.¹⁵ Hydrogel thin films can also be transferred from the surface of one material to the surface of another material, or be used as a free-standing film.

The redox responsive organometallic polymer poly(ferrocenylsilane) (PFS) has received considerable attention due to its fascinating redox and optical properties.¹⁷ Our group reported the synthesis and characterization of several PFS-based hydrogels.^{11, 18, 19} To the best of our knowledge, there are no reports on

Figure 9.1: Fabrication of hydrogel thin films from poly(ferrocenylsilanes).

PFS hydrogel thin films in the literature, and the addressability of the redox-active PFS chains will benefit from incorporation in hydrogel thin films. Decreasing (one of the) PFS hydrogel dimensions to several micrometers may lead to novel, responsive structures.

Among the existing PFS hydrogels, we focus on the materials described in Chapter 8 (PFS-Br/PMDETA hydrogel, Figure 9.1) for the following reasons: the gel formed under mild conditions and reaction times for gel formation were long enough to allow the fabrication of thin films.

9.2 Hydrogel thin film formation

The fabrication of the hydrogel film was straightforward. A mixture of PFS-Br/PMDETA was left for 40 min to pre-crosslink, then the mixture was spin-coated on a substrate, and left to react at room temperature for another 3 h (Figures 9.1 and 9.2 a). Interestingly, the moderate reactivity of PFS-Br towards PMDETA allowed the fabrication of homogeneous thin films by spin-coating. Control experiments were conducted to show that the pre-crosslink procedure was important for the thin film formation. Spin-coating fresh solutions of PFS-Br and PMDETA in THF/DMSO gave films which were inhomogeneous, fragile, and could easily be washed away. SEM image show that a film thickness of about $1.37\ \mu\text{m}$ was reached when films were spin-coated at 2000 rpm (Figure 9.2 b). The film thickness could be tuned by adjusting the spin-coating speed. Hydrogel thin films with thicknesses of $2.15\ \mu\text{m}$ and $728\ \text{nm}$ were obtained at 1000 rpm and 3000 rpm, respectively. The thin film can also be peeled off from the substrates, yielding a free-standing PFS hydrogel film. Figure 9.3 shows the free-standing hydrogel film in water.

Figure 9.2: (a) Image of the PFS-Br hydrogel thin film; (b) SEM image of the hydrogel thin film on silicon (cross-section), spin coated at 2000 rpm for 75 s.

9.3 Redox responsive properties of the thin film

The PFS-Br/PMDETA hydrogel thin film could be reversibly oxidized and reduced both chemically and electrochemically. Figure 9.3 illustrates the hydrogel thin film in its neutral and oxidized states. A rapid color change from orange to dark blue occurred when the hydrogel was immersed in 0.1 mM aqueous FeCl_3 solution.

Figure 9.3: Free-standing PFS film in the neutral (a) and oxidized (b) state.

A hydrogel thin film was also fabricated on ITO which was previously functionalized with amine groups in order to covalently bind the film to the electrode surface. A cyclic voltammogram of the PFS-Br/PMDETA hydrogel thin film is shown in Figure 9.4. The characteristic double waves typical of PFS chains appeared when the scan speed was lower than 5 mV/s. Upon oxidation, the color of the film changed from light yellow to blue/green.

Figure 9.4: Cyclic voltammogram of a PFS-Br/PMDETA hydrogel thin film on ITO at $v=5\text{mV/s}$ in 0.1 M NaClO_4 aqueous solution, using Ag/AgCl as reference electrode and Pt as counter electrode.

9.4 Outlook

As discussed in Chapter 8, the redox activity of the PFS chains could be employed to prepare metal nanoparticles in a facile in-situ manner without using external reducing agents or organic solvents. Thin hydrogel films made from the same material could provide a platform for monitoring the metal particle growth and may have promising applications in catalysis and sensing. Corresponding research will be carried out in the future.

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Summary

The aim of the work described in this Thesis was to develop novel redox responsive organometallic poly(ferrocenylsilane)s (PFSs) for the decoration of electrode surfaces and for the fabrication of redox-active hydrogels. The films and gels produced were explored as redox active platforms for electrochemical sensing and to obtain metal nanoparticles in redox reactions (“metal nanoparticle foundry”).

PFSs are a fascinating class of processable materials with redox characteristics suitable for the modification of surfaces and electrodes and have significant potential in the electrochemical detection of biological analytes. We fabricated several kinds of PFS grafts on electrodes through different approaches (electrografting, simple grafting to, covalent layer-by-layer assemblies, hydrogel thin films) and investigated the sensing abilities of these redox-active interfaces.

PFSs can be used for forming responsive 3D structures such as hydrogels by introducing crosslinkable moieties to the PFS main chain. The intrinsic redox-active properties of PFSs enable corresponding hydrogels to be used as noble metal nanoparticle foundry for the in-situ fabrication of a variety of metal nanoparticles.

In Chapter 2, a broad overview on the role of organometallic polymers as active components in electrochemical sensors is given.

In Chapter 3, the approaches for fabricating polymer thin films on solid substrates are discussed.

In Chapter 4, a simple and fast electrografting method for direct immobilization of PFS chains to Au surfaces from ionic liquids is introduced. Robust, dense, redox active organometallic PFS grafted films were formed within 5 min by direct cathodic reduction of Au substrates. The electrografted polymer films were employed as an electrochemical sensor, exhibiting high sensitivity, stability and reproducibility.

In Chapter 5, a “grafting to” approach was utilized for chemically tethering

PFS chains to silicon or gold substrates, employing an amine alkylation reaction. PFS grafts with a thickness of 9 nm were obtained. The electrochemical properties of the grafts were studied thoroughly. Additionally, the adherence between silicon nitride AFM tips and PFS grafts was investigated in situ by pull-off force measurements using electrochemical AFM. The pull-off force was found to depend on the oxidation state of the grafts. Hydrophobic AFM tips showed a stronger adherence with PFS grafts in the unoxidized, hydrophobic state, and displayed lower pull-off forces from oxidized, more hydrophilic PFS graft layers. The PFS grafts served as an electrochemical sensor for ascorbic acid, exhibiting high sensitivity and stability.

In Chapter 6, the first example of a redox-active multilayer film, fabricated from a main-chain redox-active polymer (PFS) and a redox-inert polymer (PEI), interconnected by covalent bonds, is described. The multilayer films, formed on silicon, ITO, quartz and Au substrates, were fabricated by covalent layer-by-layer assembly showing a well-defined growth process. Owing to the formation of covalent bonds between the layers, these covalently interconnected layers do not disassemble upon oxidation and reduction, in contrast to PFS layers featuring similar backbones held together by electrostatic forces. PFS/PEI multilayers were successfully used in the electrochemical sensing of ascorbic acid and hydrogen peroxide and show improved sensing performance at higher bilayer numbers.

Table S.1: Sensor characteristics of PFS films immobilized on electrode surfaces in the detection of ascorbic acid.

PFS deposition method	Film thickness (nm)	Surface coverage (mol cm^{-2})	Sensitivity ($\mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$)	Detection limit (μM)
Grafting to	9	3.8×10^{-9}	0.12	3.6
Layer-by-layer (PFS/PEI) ₈ -PFS	~ 10	2.52×10^{-9}	0.13	1.6
Electrografting	<10	1.89×10^{-9}	0.54	0.9

Table S.1 lists the sensor characteristics of PFS decorated electrodes fabricated through the various strategies discussed in this Thesis. The “grafting to” method (described in Chapter 5) provides the electrodes with the highest surface coverage by ferrocene units. PFS films generated by the covalent LbL deposition approach (Chapter 6) have a lower surface coverage of ferrocene units compared to the “grafting to” grafts (Chapter 5) with same thickness, resulting from the PEI layers incorporated in the film. By increasing the number of layers, the surface coverage of the LbL films increases further. The electrografted PFS grafts (Chapter 4) have

the lowest surface coverage, which is determined by the grafting mechanism. The sensing ability and detection limit of ascorbic acid with the three types of PFS decorated electrodes are given. The PFS films generated by the electrografting method have the highest sensitivity and lowest detection limit, likely caused by the direct contact between the PFS chains and the electrode surface. When comparing the “grafting to” films with the LbL films, it seems that the detection limit is lowered by the increased hydrophilicity of the LbL film.

In Chapter 7, a biosensor based on cross-linked PFS and glucose oxidase (GOx) multilayers was demonstrated, in which PFS acted as redox mediator. The synthesis of cationic PFS bearing crosslinkable side groups, layer-by-layer assembly of PFS and GOx, crosslinking of the layer constituents and sensor characteristics are presented. However, reproducibility of the sensor still needs to be improved.

In Chapter 8, the formation of a redox active PFS hydrogel in a single step is introduced. The hydrogel served as a noble metal nanoparticle foundry. Given that the PFS has an electrode redox potential of around +0.4 V, it is possible to reduce certain metal salts into metal nanoparticles through spontaneous reduction without the aid of other reducing agents. Au, Ag, Pt, Pd, Ir, Rh nanoparticles were fabricated by the PFS NP foundry by this clean and facile method.

In Chapter 9, the fabrication of a stimuli-responsive hydrogel thin film based on materials discussed in Chapter 8 was reported. The thin gel film layer could be detached from the surface and used as a free-standing film. Compared to bulk gels, hydrogel thin films have a fast response to external stimuli and may have advantages for applications in sensing and catalysis.

Samenvatting

Het doel van het werk beschreven in dit Proefschrift was om nieuwe redox-actieve organometaalpolymeren, poly(ferrocenylsilanen) (PFSs), te ontwikkelen voor het functionaliseren van elektrode oppervlakken en voor het maken van redox-actieve hydrogels. De geproduceerde dunne films en gels werden onderzocht als redox-actieve componenten voor gebruik in elektrochemische analyse en detectie en voor het fabriceren van metaal nanodeeltjes in redoxreacties (metaal nanodeeltjes “fabrie”).

PFSs vormen een fascinerende klasse van redox-actieve, verwerkbare materialen met eigenschappen die geschikt zijn voor het chemisch modificeren van oppervlakken en elektroden, en die geschikt zijn voor de elektrochemische detectie van biologische moleculen. Verschillende typen PFS ketens werden aan oppervlakken gehecht door middel van procedures als “electrografting”, “grafting-to”, covalente “layer-by-layer” groei, of als dunne hydrogel films, en de sensor eigenschappen van deze redox-actieve lagen werden onderzocht.

PFSs kunnen worden gebruikt voor de vorming van op stimuli reagerende 3D structuren zoals hydrogels, door de PFS hoofdketen te voorzien van crosslinkbare zijgroepen. De intrinsiek aanwezige redoxactiviteit van PFSs maakt het mogelijk om de hydrogels te gebruiken als “fabriek” voor de in-situ synthese van een reeks van metaal nanodeeltjes.

In Hoofdstuk 2 wordt een overzicht gegeven van de rol van organometaalpolymeren als actieve component in elektrochemische sensoren.

In Hoofdstuk 3 worden de methoden om dunne polymeerfilms op oppervlakken aan te brengen bediscussieerd.

In Hoofdstuk 4 wordt een eenvoudige en snelle “electrografting” methode voor de directe hechting van PFS ketens aan goudoppervlakken vanuit ionische vloeistoffen geïntroduceerd. Robuuste, dichte, redox-actieve PFS organometaal-

films werden binnen 5 minuten gevormd door de directe kathodische reductie van goudsubstraten. De “electrografted” polymeerfilms werden gebruikt als elektrochemische sensor en vertoonden daarin een hoge gevoeligheid, stabiliteit en reproduceerbaarheid.

In Hoofdstuk 5 werd een “grafting-to” methode gebruikt om PFS ketens chemisch te hechten aan silicium of goudoppervlakken, gebruikmakend van een amine alkylerings reactie. PFS films met een dikte van 9 nm werden verkregen. De elektrochemische eigenschappen van de films werden in detail bestudeerd. Bovendien werd de adhesie tussen siliciumnitride AFM tips en PFS films bestudeerd door middel van in-situ “pull-off force” metingen met elektrochemische AFM. De “pull-off force” bleek afhankelijk te zijn van de oxidatietoestand van de films. Hydrofobe AFM tips gaven een sterkere adhesie met PFS films in de ongeoxideerde, hydrofobe toestand en vertoonden lagere “pull-off forces” vanaf geoxideerde, meer hydrofiele PFS films. De PFS films dienden als elektrochemische sensor voor ascorbinezuur. De sensor bezat een hoge gevoeligheid en stabiliteit.

In Hoofdstuk 6 wordt het eerste voorbeeld van een redox-actieve multilaag film, gefabriceerd uit een hoofdketen redox-actief polymeer (PFS) en een redox-inert polymeer (PEI), verbonden door covalente bindingen, beschreven. De multilaag films, gevormd op silicium, ITO, quartz en goudoppervlakken, werden opgebouwd door covalente laag-op-laag hechting. Dit groeiproces bleek goed gedefinieerd te verlopen. Vanwege de vorming van covalente bindingen tussen de lagen vallen deze niet uiteen tijdens hun oxidatie of reductie, in tegenstelling tot PFS lagen die door elektrostatische interacties bij elkaar worden gehouden. PFS/PEI multilagen werden succesvol gebruikt voor de elektrochemische detectie van ascorbinezuur en waterstof peroxide, en vertoonden betere sensorprestaties bij grotere aantallen bilagen.

Table S.1: Sensor eigenschappen van PFS films geïmmobiliseerd op elektrode oppervlakken voor detectie van ascorbinezuur.

PFS film fabricage method	Filmdikte (nm)	Oppervlakte bedekking (mol cm^{-2})	Gevoeligheid ($\mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$)	Detectielimiet (μM)
Grafting to	9	3.8×10^{-9}	0.12	3.6
Layer-by-layer (PFS/PEI) ₈ -PFS	~ 10	2.52×10^{-9}	0.13	1.6
Electrografting	<10	1.89×10^{-9}	0.54	0.9

Tabel S.1 geeft een overzicht van de sensor eigenschappen van met PFS films

gefunctionaliseerde elektroden, gefabriceerd door middel van de strategieën beschreven in dit Proefschrift. De “grafting-to” methode (beschreven in Hoofdstuk 5) leidt tot de hoogste bedekkingsgraad van elektroden door ferroceen eenheden. PFS films gevormd door covalente “layer-by-layer” stapeling (Hoofdstuk 6) hebben een lagere bedekkingsgraad vergeleken met “grafting to” films (Hoofdstuk 5) van dezelfde dikte, door de aanwezigheid van PEI lagen in de films. Door het aantal lagen te verhogen neemt de bedekkingsgraad van de “layer-by-layer” films toe. De “electrografted” PFS films (Hoofdstuk 4) hebben de laagste bedekkingsgraad, wat een gevolg is van het hechtingsmechanisme van de polymeerketens aan het oppervlak. De sensorprestaties en ascorbinezuur detectielimiet van de drie met PFS gefunctionaliseerde elektroden zijn weergegeven in Tabel S.1. De PFS films gemaakt via “electrografting” hebben de hoogste gevoeligheid en de laagste detectielimiet, waarschijnlijk veroorzaakt door het directe contact tussen de PFS ketens en het elektrodeoppervlak. Vergeleken met de “grafting-to” films lijkt het dat de “layer-by-layer” films een lagere detectielimiet te danken hebben aan hun hogere hydrofiliciteit.

In Hoofdstuk 7 werd een biosensor gebaseerd op gecrosslinked PFS en glucose oxidase (GOx) multilagen gedemonstreerd waarin PFS diende als redox mediator. De synthese van kationisch PFS met crosslinkbare zijgroepen, laag-op-laag constructie van PFS en GOx multilagen, crosslinken van de componenten in de laag en sensor karakteristieken worden gepresenteerd. De reproduceerbaarheid van de sensor moet echter nog worden verbeterd.

In Hoofdstuk 8 wordt de vorming van een redox-actieve hydrogel in een enkele stap geïntroduceerd. De hydrogel werd gebruikt als edelmetaal nanodeeltjesfabriek. Aangezien PFS een elektrode redox potentiaal van ongeveer +0.4 V heeft is het mogelijk om bepaalde metaalzouten te reduceren tot metaal nanodeeltjes door spontane reductie, zonder hulp van externe reducerende chemicaliën. Au, Ag, Pt, Pd, Ir en Rh nanodeeltjes werden gevormd met behulp van de PFS nanodeeltjes “fabriek” middels deze schone en eenvoudige methode.

In Hoofdstuk 9 wordt de fabricage van een op stimuli reagerende dunne hydrogel film, gebaseerd op materialen uit Hoofdstuk 8, beschreven. De dunne gel film kon worden losgehaald van het oppervlak en worden gebruikt als “free-standing” film. Vergeleken met bulk gels vertonen dunne hydrogel films een snelle reactie op externe stimuli, wat voordelen kan hebben voor toepassingen in detectie en katalyse.

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Xueling Feng

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Enschede, the Netherlands

2015

Publications

Journal articles

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