Electrochemically controlled release of molecular guests from redox responsive polymeric multilayers and devices

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Abstract

A novel platform technology for a tunable molecular payload release, employing complex release profiles, from electrode surfaces is reported. Organometallic poly(ferrocenylsilanes) (PFSs), featuring redox responsive ferrocene units in their main chain, are used as a carrier medium to prepare thin films by the layer-by-layer (LbL) method for redox triggered delivery. These films form the active component of the device. The release of guest molecules from PFS multilayer templates was monitored by fluorescence spectroscopy by varying the supporting electrolyte, the ionic strength of electrolyte, redox inactive components, blocking layers and the molar mass of the polymer. Incorporation of Dextran–TRITC and Dextran–Alexa 488 dye molecules into PFS multilayers, at various depths of the film, enabled tuning of the release profiles with different release kinetics for each component. Composite multilayers encompassing dual redox and pH responsive polyelectrolytes show double responsive control over the dye release. Finally a device was build featuring the combination of a microelectrode array (MEA) and PFS multilayers to demonstrate area addressable pulsed release for potential applications.

1. Introduction

“Smart” macromolecules can provide suitable media, triggered by variety of stimuli, for prospective applications in biotechnology and drug-delivery systems\textsuperscript{[1–9]}. Redox potential triggers are explored in this context, as these offer multiple benefits. Redox stimuli can be controlled either by electrical device circuits or by using appropriate redox chemical agents. Redox stimuli allow one to use remote control with high precision and offer nanoscale addressability in contrast to medium control by pH or temperature\textsuperscript{[10–12]}. Redox stimuli can also address specific metabolic processes with various oxidation and reduction potentials and respond to large redox environment differences e.g. between extracellular and intracellular space\textsuperscript{[13,14]}. Despite of this high application potential, there is only a limited number of studies presenting area-sensitive redox-addressable devices\textsuperscript{[10,11]}. The majority of macromolecular redox active systems used for the drug and gene delivery\textsuperscript{[4]}, exploit disulfide bonds which are sensitive to excess of redox active glutathione (GSH) inside the cell\textsuperscript{[15]}

Polyelectrolyte multilayers, obtained by the layer-by-layer (LbL) deposition technique, are well suited for fabrication of composite films. The LbL is a convenient approach to incorporate a variety of functional molecules such as dyes, drugs, proteins, polysaccharides, enzymes, and nucleic acid with preserved activity at a desired position within the multilayer films\textsuperscript{[16–21]}. For example, drug loaded multilayers used as antimicrobial, anti-inflammatory, and anticancer coatings have been demonstrated
These systems provide significant therapeutic benefits, and often rely on the capability of polymeric films to provide spatial and temporal control over the release rate. Redox active LbL composites containing redox sites such as viologen, Prussian Blue, ferrocene (Fc), poly(thiophene), and osmium bipyridyl complex (Os-bpy) have been explored for their potential applications in mechanical actuators, sensors, photochromic devices and surface mediated controlled drug release [24–29]. Polyelectrolyte derivatives of poly(ferrocenylsilanes) (PFSs), containing ferrocene units, are good candidates for the fabrication of redox responsive multilayers by the Lbl technique [30–32]. Iron within the PFS backbone can be reversibly switched between ferrocene and ferrocenium and acts as a transducer of redox signals [33].

There are challenges and opportunities in using redox responsive multilayers as matrices for controlled release of therapeutics from surfaces. Concerning the multilayer itself, one must consider the importance of its chemical and physical properties, such as the internal structure of the film, film hydrophobicity, binding affinity of guest molecules, and assembly/disassembly mechanism. In our previous study, we reported on a systematic investigation of a redox responsive LbL system, constructed with PFS polyelectrolytes, with particular emphasis on disassembly mechanistic studies of the films. We proposed cyclic voltammetry (CV) in a novel approach, as a method for in situ disassembly observations [34,35]. In addition, the cytotoxicity of PFS polymers were reported [36].

In this contribution, we briefly recapitulate the redox behavior to justify the choice of the supporting electrolyte, ionic strength, redox inactive components, blocking layers and molar mass of polyelectrons for electrochemically triggered release of the molecular guests. We then propose the use of a versatile platform, which can release multiple guest molecules with controlled release profiles. By introducing pH responsive polyelectrolytes, dual responsive multilayers can also be incorporated. A redox electrochemical signal of this platform can be used over release film areas down to the microscale. We illustrate the usefulness of local addressability of potentials by employing microelectrode arrays and demonstrate its applicability for spatially controlled release. The device allows one to control the guest release profiles ranging from sustained to pulsatile release in a pre-programmed manner, or in real time response to physiological changes. The highly controllable and versatile properties of PFS multilayers form a tunable platform for surface mediated delivery applications.

2. Experimental

2.1. Materials

Poly(ethyleneimine) (PEI, $M_w = 2.5 \times 10^4$ g/mol), poly (styrene sulfonate) (PSS, $M_w = 7.0 \times 10^4$ g/mol), poly (acrylic acid) (PAA, $M_w = 4.5 \times 10^5$ g/mol), poly(allylamine hydrochloride) (PAH, $M_w = 5.6 \times 10^4$ g/mol), 3-aminopropyltrimethoxysilane, sodium chloride, sodium nitride, sodium perchlorate, sodium sulphate were obtained from Aldrich and used as received. The fluorescence dye labeled dextran, Alexa Fluor®488 (Dextran–Alexa 488) ($M_w = 1 \times 10^5$ g/mol), was purchased from Invitrogen (Carlsbad, USA) and tetramethylrhodamine isothiocyanate-labeled dextran (Dextran–TRITC, $M_w = 4.4 \times 10^4$ g/mol) was obtained from Aldrich.

2.2. PFS synthesis

Positively (PFS+) and negatively (PFS−) charged poly-ferrocenylsilanes with $M_w = 1.67 \times 10^4$ g/mol, PDI = 1.3; $M_w = 2.5 \times 10^4$ g/mol, PDI = 2.7 were synthesized by ROP of stranded chlorinated cyclophenacene followed by the side group modification as described previously [37].

2.3. Multilayer fabrication

Multilayers were deposited on Indium Tin Oxide (ITO)-glass or ITO-Quartz substrates (Sens, Hengelo, The Netherlands) which were cleaned prior to use by immersing them into a mixture of H2O, H2O2 and NH4OH with a volume ratio of 5:1:1 for 20 min, followed by extensive rinsing with Milli-Q water and drying under nitrogen stream. The cleaned ITO substrates were first immersed in a toluene solution of 3-aminopropyltrimethoxysilane (0.1 mM) to impart positive charges onto the substrates. The modified substrates were alternatively immersed in the polycation and polyanion aqueous solutions (1 mg/mL, 0.5 M NaCl) for 10 min with rinsing, dipping into pure Milli-Q water and drying with a stream of nitrogen gas. Dextran–TRITC and Dextran–Alexa 488, negatively charged model guest macromolecules, were deposited in the Lbl processes by replacing PFS− polyanions at the specific layer. Substrates were submerged into dye labeled Dextran solution (0.1 mg/mL) for 30 min followed by the rinsing and drying step.

2.4. Characterization

UV/Vis spectra were recorded on a Varian Cary 300 Bio instrument in double beam mode using an uncovered quartz slide as reference. Cyclic voltammetry measurements were carried out by an Autolab PGSTAT 302N (Metrohm, Utrecht, The Netherlands) potentiostat in a three-electrode configuration. The ITO substrates with deposited multilayers acted as the working electrode, Pt as the reference electrode, and Pt wire as the counter electrode. 0.1 M electrolyte solutions including (NaClO4, NaCl, NaNO3, Na2SO4) were used. Prior to the measurements, the electrochemical cell was degassed by passing nitrogen through the electrolyte solution for 5 min. A series of cyclic voltammograms were recorded after holding the oxidation potentials at different values for different time intervals. The amount of transferred charge Q was calculated based on the integration of the area under each cyclic voltammogram.

2.5. MEA experiment

Microelectrode array (MEA) biochips (Ayanda Biosystems, Switzerland) were used to stimulate the redox
responsive PFS multilayers on localized ITO microelectrodes which are connected to MEA60 signal amplification system from Multi Channel Systems MCS (Reutlingen, Germany). The multiplexing unit was connected to a potentiostat, PGSTAT 10 (Ecochemie, The Netherlands). (PFS\(^-\)/PFS\(^+\))\(_5\) bilayers with Dextran–Alexa 488 dye at the 3rd layer were prepared with LbL techniques. The Dextran–Alexa 488 was encapsulated in the PFS multilayers by replacing 3rd layer of PFS\(^-\) polyanions. Desorption of multilayers and guest molecules were performed by applying PFS oxidation potential (1 V vs. Pt wire) to ITO electrodes with a 0.1 M NaCl electrolyte solution. The fluorescence images were taken with a Zeiss LSM 510 (63 × oil immersion objective) confocal scanning system.

3. Results and discussion

Synthesis of PFS polyelectrolytes and their LbL assembly was reported previously by our group [30–32,38]. Various film configurations of PFS polyelectrolytes with two different dye molecules were investigated (Schemes 1 and 2). Dextran Alexa 488 was used as a basic molecule for release observations and Dextran–TRITC was employed as second release guest.

3.1. The effects of electrolyte counter ion and ionic strength

The disassembly of the redox responsive PFS LbL was electrochemically controlled by prolonged exposure to a low oxidation potential. Oxidation of the PFS backbone introduces charge imbalance and electrostatic repulsion force between the charged polyelectrolyte domains and together with osmotic pressure contribute to multilayer disassembly as we discussed in previous papers [34,35]. The charge transport in the electrochemical oxidation process of the PFS multilayer is rather complex with several steps occurring, such as electron hopping between redox centers, counter ion and water molecule movement during oxidation and polymer diffusion. Ion diffusion causes an increase in osmotic pressure. Ion pairs also form between oxidized ferrocenium ions and migrated anions. Thus, the supporting electrolyte counter anion species and ionic strength are expected to influence the guest molecule release.

Multilayers (PFS\(^-\)/PFS\(^+\))\(_5\) were fabricated using dye labeled molecules as model payload (Scheme 1). The PFS polyanions of the 3rd layer were replaced with the anionic
fluorescent dye labeled Dextran–Alexa 488, during the sequential LbL deposition process to encapsulate these model payloads. The area under the I–V curves obtained by voltammetry is proportional to the number of redox units that were probed by CV, which can be quantitatively determined by integration [34]. The voltammetric signals can be used to determine the loss of electroactive species deposited on the electrode. The fluorescence emission of the dye was monitored by fluorescence spectroscopy in the electrolyte medium as the guest molecules were released into solution driven by PFS film disassembly. An oxidation potential of 0.6 V (vs. Pt) was used for the release experiments. Such a potential corresponds to complete oxidation of PFS.

Typical PFS multilayer disassembly voltammograms for five bilayers with encapsulated Dextran–Alexa 488 are shown in Fig. 1a. The decrease of the area under the I–V curves indicates removal of PFS from the electrode surface. Dye release was performed in four different solutions of supporting electrolytes (NaClO₄, Na₂SO₄, NaNO₃, NaCl) with the same 0.1 M ionic strength (Fig. 1b). All multilayers were exposed to the oxidation potential (0.6 V vs. Pt) for a prolonged time. The dye release rate is highly dependent on the type of the counterion used and follows the sequence ClO₄⁻ < NO₃⁻ < Cl⁻ < SO₂₄⁻. The sequence is identical with results observed previously for the PFS multilayer disassembly rate, and follows hydration differences of the counter ions described by the Hofmeister series [35]. Chaotropic ions (ClO₄⁻) show stronger ion pairing effects with ferrocenium centers in the polymer main chain than kosmotropic ions (NO₃⁻, Cl⁻, SO₂₄⁻). Strong ion pairing provides more stability, thus slowing down disassembly. The amount of water in the hydration shell of the anion increases from ClO₄⁻ to SO₂₄⁻, enhancing deceleration problems with migration of ions inside multilayers.

As a result, upon oxidation, the effective size of charged polymer domains is larger for highly solvated anions (e.g. SO₂₄⁻) making the multilayer disassembly faster.

The dye release was monitored for two different ionic strength NaClO₄ solutions and was visibly faster for the low ionic strength solution (Fig. 1c). Although this explanation is in line with general “salting out” effects observed in polyelectrolytes, due to many possible contributions here (diffusivity, osmotic effects, entropy variation), we do not speculate further about the related mechanism.

3.2. Redox active–redox inactive composite multilayer templates

To investigate the influence of type of PFS polyelectrolyte on the dye release process, composite multilayer films with either redox active polycations combined with non-responsive polyanions (PFS⁺/PAH⁻)₅, or redox active polycations with non-responsive polyanions (PSS⁺/PFS⁻)₅, were fabricated. The schematic of the composite multilayers with dyes encapsulated is shown in Scheme 2b. The anionic dye macromolecules were encapsulated at the 3rd bilayer from the top. The corresponding dye release profiles are shown in Fig. 2a. The dye release from the (PFS⁺/PAH⁻)₅ multilayer films were faster than the release from the (PSS⁺/PFS⁻)₅. This follows observations as described in our previous study [34], where composite multilayers build from PFS⁻ were disassembling faster than those constructed from PFS⁺ and corresponding counterpolymers. We associate this phenomenon with interactions at the interfaces between the oppositely charged polyelectrolytes layers. The interfacial ion pairs are easier to brake in the negative PFS⁻, due to intramolecular compensation of PFS⁻ and formation of zwitterionic polymer upon oxidation. This contributes to faster multilayer disassembly and dye release.

Release profiles can be further tuned by using redox inactive blocking layers. To demonstrate this effect,
electroactive (PFS\(^{-}/\)PFS\(^{+}\))\(_5\) bilayers were fabricated on top of the non-redox active spacer (PAA\(^{-}/\)PAH\(^{+}\))\(_5\). In this configuration inactive film with thickness of five bilayers was placed between redox active PFS multilayer and the electrode (Scheme 2c). As shown in Fig. 2b, the dye release is much slower for the samples fabricated with blocking layers. Films with thick blocking layers (>9 nm, five bilayers) release dyes slowly by diffusion, similarly to reference films immersed in the supporting electrolyte without electrochemical stimulus. Films with non-blocking layer (PAA\(^{-}/\)PAH\(^{+}\))\(_5\) (PFS\(^{-}/\)PFS\(^{+}\))\(_5\) with electrochemical stimulus; (\(\triangle\)) without blocking layer (PFS\(^{-}/\)PFS\(^{+}\))\(_5\) with electrochemical stimulus.

Fig. 2. (a) Release profile of dye molecules in different composite multilayers. (\(\Box\)) (PSS\(^{-}/\)PFS\(^{+}\))\(_5\) bilayer film (Dextran–Alexa 488 was encapsulated in the 3rd layer from the top, (\(\bigcirc\)) (PFS\(^{-}/\)PAH\(^{+}\))\(_5\) bilayer film (Dextran–Alexa 488 was encapsulated in the 3rd layer from the top) for 0.6 V (vs. Pt) oxidation potential; (b) release profile of dye molecules in NaClO\(_4\) electrolyte a (PFS\(^{-}/\)PFS\(^{+}\))\(_5\) bilayer film (Dextran–Alexa 488 was encapsulated in the 3rd layer from the top) for 0.6 V (vs. Pt) oxidation potential; (\(\bigcirc\)) with blocking layer (PAA\(^{-}/\)PAH\(^{+}\))\(_5\) (PFS\(^{-}/\)PFS\(^{+}\))\(_5\) without electrochemical stimulus; (\(\bigcirc\)) with blocking layer (PAA\(^{-}/\)PAH\(^{+}\))\(_5\) (PFS\(^{-}/\)PFS\(^{+}\))\(_5\) with electrochemical stimulus; (\(\triangle\)) without blocking layer (PFS\(^{-}/\)PFS\(^{+}\))\(_5\) with electrochemical stimulus.

3.3. Dual responsive multilayer

A composite multilayer such as (PFS\(^{-}/\)PAH\(^{+}\))\(_5\) enables the development of materials exhibiting both redox and pH dependences, respectively. PAH has pH-sensitive properties due to the protonation–deprotonation equilibrium of the amino groups and displays reversible conformational transitions in response to the net charge. In this case, the encapsulated guest molecules could be precisely manipu-
the release dynamics strongly depends on the guest deposition depth, allowing for the construction of a controlled dosing film. For the Dextran–TRITC, embedded in a shallow layer, the dye molecule was released faster. In contrast, for deeper embedded molecules of Dextran–Alexa 488 were released slower. Exploiting this design multiple components can be loaded into PFS multilayer films and tune the release kinetics of these cargos separately via the architecture of the film.

3.5. Guest molecule release from a microelectrode array (MEA)

The field of controlled release initially focused on achieving a sustained release of drugs over an extended period of time with minimal influence of stimulus such as pH, light, and temperature. However, sometimes sustained release is not the optimal method of drug delivery. Instead, delivery of pulses of drug at variable time intervals can also be preferred and is commonly referred to as pulsatile release [41]. This delivery method works better in certain cases because it closely mimics the way in which the human body naturally produces some regulating substances at different time intervals. Microelectrode arrays (MEAs), consisting of a set of separately addressed surface electrodes, provide a suitable platform when complicated release patterns and good spatial resolution are required. After integration with polymeric thin films, MEA has the ability to store and release chemicals on demand. This technique offers advantages such as multiple chemicals release, complex release patterns or local delivery, and is particularly useful if the drug has adverse side effects or is administered in high doses [41–44].

To fabricate a device which features localized redox responsive surfaces and to investigate controlled release of guest molecules from individually addressed microcompartments, (PFS /PFS)5 multilayers with encapsulated Dextran–Alexa 488 dye molecules were deposited on a commercial MEA. An electrical potential (1 V vs. Pt reference) was applied on the modified electrode for different oxidation times (Electrode 1: 0 s; Electrode 2: 1 s; Electrode 3: 1 min; Electrode 4: 5 min) in 0.1 M NaCl electrolyte solution; (b) Fluorescence intensities on microelectrode with or without electrochemical stimulus in NaCl electrolyte solution.

![Fig. 4.](image-url)  
(a) Fluorescence intensity of two dyes released which were embedded in a PFS10 multilayer upon different multilayer oxidation time. (Dextran–TRITC was absorbed at the 3rd layer from the top and Dextran–Alexa 488 was absorbed at 8th layer from the top); (b) release profile of two dyes molecules of a 10 bilayer film for 0.6 V oxidation potential, fluorescence was normalized for each component; (c) example of data fitting for release at 45 min, double asymmetric Gaussian function was used for each component [40].

![Fig. 5.](image-url)  
(a) Confocal fluorescence images of surface modified ITO microelectrode (d = 40 μm). PFS5 bilayers with embedded Dextran–Alexa 488 dye molecules were deposited on ITO microelectrode. An electrical potential (1 V vs. Pt reference) was applied on the modified electrode for different oxidation times (Electrode 1: 0 s; Electrode 2: 1 s; Electrode 3: 1 min; Electrode 4: 5 min) in 0.1 M NaCl electrolyte solution; (b) Fluorescence intensities on microelectrode with or without electrochemical stimulus in NaCl electrolyte solution.
the fluorescence image of a composite multilayer, which was not electrochemically stimulated, on the Au electrode of the MEA. When an electrical potential of 1 V (vs. Pt wire) was applied onto the circular Au microelectrode for 1 s, (electrode 2), 1 min (electrode 3) and 5 min (electrode 4) in 0.1 M NaCl solution, a decay of fluorescence intensity on the electrode areas was clearly observed. The average fluorescence intensity decrease to 95% of original value on electrode 2, decreased to 75% of original values on electrode 3 and decreased to 50% of original values on electrode 4 (shown in Fig. 5b). This indicates a significant desorption of fluorescence dye molecules from the Au electrode after the stimulus varying from spot to spot. The fluorescence intensity on the surrounding area and electrode 1 did not show obvious changes, and it is evident that in this localized process, only the material deposited on the activated electrode area was released. Ongoing studies are currently in progress to further study multiple drug release patterns with a new data acquisition interface and a stimulus generator.

4. Conclusions

This study provides proof of principle for the use PFS multilayer platform to release single or multiple molecular payloads upon prolonged electrochemical exposure to a small potential. Kinetics and profile of payload release can be controlled by the type and ionic strength of the supporting electrolyte solution. Since a wide variety of bioactive components can be readily incorporated into polyelectrolyte multilayer films, this technique in combination with microelectrodes, would be a new tool for applications that require precisely controlled local delivery of molecular payloads, e.g. drugs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2013.01.029.

References


