Establishment of a Matrix for Biorecognition Reactions Based on Nanoscaled Films of Responsive Polymeric Compounds

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ABSTRACT

Polymer based surfaces attract an increasing interest as useful elements of analytical devices. A special class of macromolecular compounds, the "smart" polymers, undergo phase transitions in response to environmental triggers like temperature or pH changes.

We have immobilised a poly(OEGMA) type of polymer on gold electrode as a thin film and studied the temperature induced phase transition by electrochemical means.

Keywords: electrochemistry, cyclic voltammetry, electrochemical impedance spectroscopy, surface plasmon resonance, quartz crystal microbalance

1 INTRODUCTION

Thin organic polymer films on solid surfaces find increasing applications in diverse scientific and technical fields. A very useful class of polymers for surface modifications is the group of so called stimuli-responsive polymers (also called "smart" polymers) [1]. These are macromolecular chemical compounds which undergo very sharp phase transitions in response to certain environmental changes. Triggers of physical, biological, chemical nature thus enable a simple way for rapid variations of surface properties. Properties, which can be varied "on demand" are among others film thickness, hydrophilicity, isolating properties [2].

Different methods have already been used in investigating switching phenomena of thermoresponsive polymers on gold surfaces. The choice of a suitable technique depends on the property of interest which changes during the phase transition process to be observed. Contact angle measurments allow monitoring of hydrophobicity changes [3], whereas ellipsometry is a suitable technique for film thickness tests [4]. In this study, it is shown how the structural reorganization process in the polymer film can be monitored by electrochemical means. For this purpose, an oligo(ethylene glycol) methacrylate based thermoresponsive polymer was synthesized by radical polymerization (figure 1).

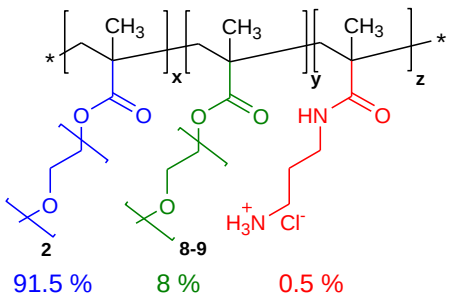

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Figure 1: Structural formula of the investigated thermally switchable copolymer

This polymer is a copolymer of 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA), oligo(ethylene glycol) methacrylate (OEGMA) and N-(3-aminopropyl) methacrylate hydrochloride (APMA). It is water soluble at room temperature and shows a cloud point at about 38 °C. The ammonium group containing monomer is added in order to allow covalent immobilization of the synthesized polymer onto the surface of gold wire electrodes but also to allow coupling of further molecules on the polymer.

Cyclic voltammetric and impedimetric measurements with modified electrodes were realized in potassium ferro-/ferricyanide solutions at room temperature and under temperature variation in the range 25 – 45 °C. Room temperature measurements demonstrate the successful immobilization of the polymer on the gold surface. These experiments are supported by quartz crystal microbalance measurements. A temperature-dependent electrochemical

study allows observation of the reorganization process of the polymer on the gold surface.

Further experiments are related to the idea of using this polymer interface as carrier for biorecognition elements and utilizing it for the analysis of biochemical processes. The switchable system is combined with biological components (short peptide and corresponding antibody) and the effect on the responsive behavior by temperature-dependent measurements is tested.

2 EXPERIMENTAL

2.1 Materials

2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA), oligo(ethylene glycol) methacrylate (OEGMA, M_n = 475 g/mol), ferri- and ferrocyanide, 3 mercaptopropionic acid (MPA), N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) were provided by Sigma-Aldrich (Taufkirchen, Germany); N-(3-aminopropyl)methacrylamide hydrochloride (APMA) by Polyscience; azobisisobutyronitrile (AIBN) by Acros; ethanol (undenaturated) by Roth (Karlsruhe, Germany). Gold wire electrodes with a diameter of 0.5 mm were provided by Goodfellow (Bad Nauheim, Germany). All aqueous solutions were prepared in deionised water.

2.2 Preparation of the copolymer

MEO_2MA (91 eq.), OEGMA (8 eq.), APMA (0.5 eq.) were dissolved in ethanol (85 wt.-%). To this mixture AIBN (0.5 eq.) was added and the solution was purged through a rubber septum with argon for 30 minutes. The polymerization was conducted at 60°C for 24 h. The mixture was diluted with deionised water and purified by dialysis against deionised water (Roth, ZelluTrans membrane, molecular weight cut off: 4000-6000). The polymer was isolated by freeze drying to yield 90 % of colourless glue ($M_{w,GPC} = 4.4 \cdot 10^5$ g/mol, $PDI_{GPC} = 6.3$). The copolymer composition was verified by ^{1}H-NMR spectroscopy, the presence of the amine groups in the polymer was verified qualitatively by a ninhydrine test.

SEC was run at 50°C in DMF (flow rate 1mL/min) using a Spectra Physics Instruments apparatus equipped with a UV-detector SEC-3010 and a refractive index detector SEC 3010 from WGE Dr. Bures [Columns: Guard (7.5 x 75 mm), PolarGel-M (7.5 x 300 mm)], calibration with linear polystyrene standards (PSS, Germany).

^{1}H-NMR spectra were recorded in $CDCl_3$ with a Bruker Avance 300 spectrometer.

2.3 Cyclic voltammetric and electrochemical impedance spectroscopy measurements

Electrochemical measurements were performed with a model 660B electrochemical workstation from CHI Instruments (Austin, TX, USA).

For the electrochemical studies, a custom-made 1 mL cell, an Ag/AgCl/ 1 mol/L KCl reference electrode (Biometra, Göttingen, Germany), and a platinum counter electrode was used. Gold electrodes were cleaned following an established protocol [2]. The immersion depth of the electrodes during the measurements was 4 mm.

2.4 Preparation of modified electrodes

The switchable polymer surface on gold electrodes was prepared in three steps (figure 2).

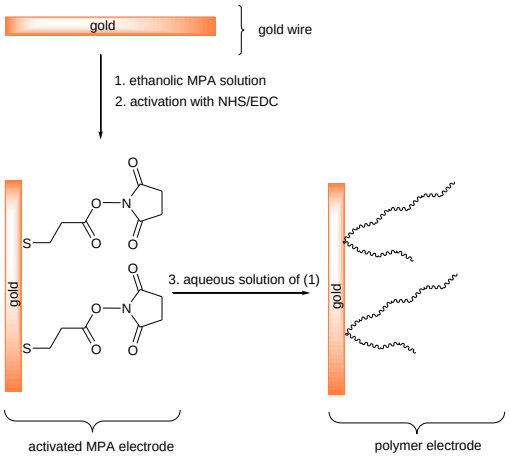

Figure 2: Preparation scheme for the immobilization of the thermoresponsive polymer 1 on gold surface

Preparation of MPA electrodes: Electrode modification with MPA was conducted by immersion of cleaned gold electrode in a 10 mmol/L MPA solution in ethanol for 2 hours. The MPA modified electrode was rinsed with water before measurement.

Preparation of polymer modified electrodes: The polymer modification was performed on a MPA modified gold electrode. The MPA electrodes were incubated for 15 minutes in an aqueous EDC/NHS mixture (200 mmol/L / 50 mmol/L) and rinsed with water. Then the electrode was dipped into an aqueous polymer solution (7 mg/mL) for 2 hours.

Preparation of bioconjugate modified electrodes: After the polymer coupling the electrode was incubated for 2 hours in a FLAG peptide (0.1 mg/mL) mixture with EDC/NHS (200 mmol/L/50 mmol/L) in HEPES buffer (10 mmol/L, pH 7). Afterwards the antibody was bound from 5 µg/mL solution in HEPES buffer (10 mmol/L, pH 7) for 2 hours (scheme 2).

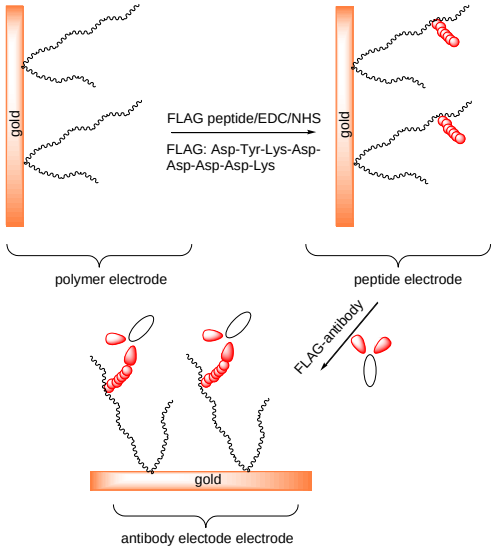

Figure 3: Preparation scheme for the synthesis of the bioconjugate on the switchable polymer interface

2.5 Quarz crystal microbalance measurements

QCMD experiments were performed with a 5-MHz, 14-mm diameter, QSX 301 standard gold quartz crystal chip (QCMD chip) by using a QCMD Q-Sense E4 system .

Prior to use, the gold surface of the QCMD chip was cleaned with piranha for 10 min. After flushing with double-distilled water and once with pure ethanol, the QCMD chip was incubated in a batch cell with 10 mM MPA in pure ethanol for 2 h. This ensures the formation of a carboxy-terminated thiol layer. After three washing steps, once with ethanol and twice with double-distilled water, the chip was mounted in the QCP 01 chamber and flushed with deionized water until equilibration with a constant flow rate of 50 µl/min. After a stable baseline is achieved, the chip was ready to use for polymer coupling. To perform the binding of the polymer, the sensor chip was flushed with a constant flowrate of 50 µL/min with aqueous EDC/NHS mixture (200 mmol/L/50 mmol/L) for 15 minutes. After the activation step, aqueous polymer solution (7 mg/mL) was flushed with a constant flow rate of 10 µL/min for 1.5 hours. Following steps were saturation with aqueous ethanolamine solution (1 mol/L) for 15 minutes (50 µL/min), peptide binding from 0.1 mg/mL solution in HEPES buffer (10 mmol/L, pH 7) mixed with EDC/NHS (200 mmol/L/50 mmol/L) for 20 minutes (50 µL/min) and finally the antibody binding from 5 µg/mL solution in HEPES buffer (10 mmol/L, pH 7) for 30 minutes (10 µL/min). The washing steps in between were performed with deionized water with a constant flow rate at 50 µL/min.

3 RESULTS AND DISCUSSION

3.1 Measurements at room temperature

The results of the electrochemical investigations at room temperature prove successful immobilization of the polymer on the surface of gold. In the cyclic voltammogram (figure 4 a) the peak current decreases and the peak separation increases after the polymer coupling, hinting at an impeded access of the complex ions of the redox couple to the electrode. The hindered electron due to the polymer coupling also leads to an increased interfacial impedance (figure 4 b).

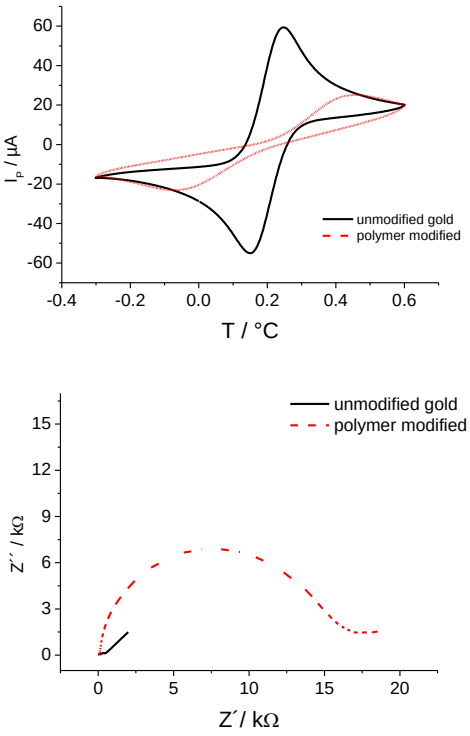

Figure 4: a) Cyclic voltammogram; b) Nyquist impedance plot

The QCM measurements at room temperature verify the polymer immobilisation but also show the successful synthesis of the bioconjugation complex (figure 5).

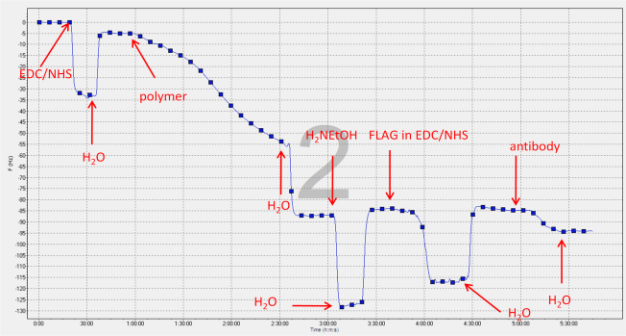

Figure 5: Quartz crystal microbalance measurement

3.2 Measurements under temperature variation

Significant changes in the voltammetric peak current values by varying the temperature in the range 25 – 45 °C clearly demonstrate the thermally induced phase transition (Figure 6) at about 38 °C. In contrast to this, no discontinuieties are observed in the slopes of the temperature dependencies for unmodified electrode. The experiments indicate a better access for the redox couple after the change in polymer conformation.

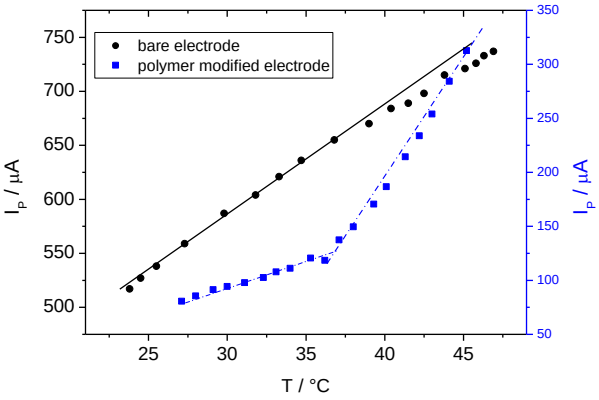

Figure 6: Peak current values as function of temperature for the non-modified gold electrode and for the polymer modified gold electrode

This is also reflected in the change of the temperature dependence of the peak separation. It has to be emphasized here that the polymer film is rather thin (10 - 20 nm) and thus conformational changes in the film may result in the formation of access channels for the diffusion of the redox ions. The study demonstrates that electrochemistry is a sensitive tool to monitor polymeric phase transitions on surfaces. The change in polymeric structure at about 38 °C can also be verified by temperature-dependent SPR measurements. Also here a change in the slope of temperature dependent resonance angle is found.

Further, the influence of coupling reactions on the responsiveness of the interface is investigated (figure 7). It is found that after the peptide binding and the antibody docking the interface is still responsive, but the transition temperature region for the antibody modified electrode is slightly broadened compared to FLAG peptide electrode.

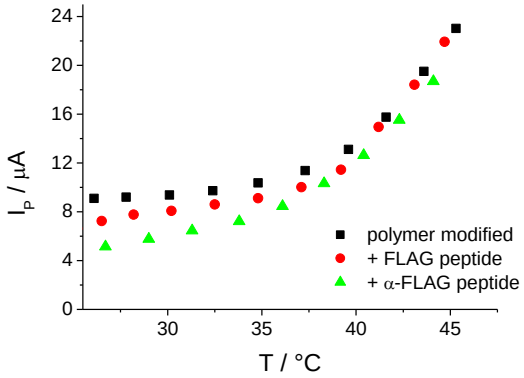

Figure 7: Peak current values as function of temperature for the polymer, peptide and antibody modified gold electrode

The observed effects are rather small, potentially because very little FLAF is bound. Further research is necessary focusing on the polymer composition and the amount of bound biomolecules.

4 CONCLUSION

A poly(OEGMA) type copolymer was prepared and covalently bound onto an electrode surface. The temperature study shows that the reorganization process of the polymer can be observed by electrochemical means.

The effect of a biomolecule binding on the thermoresponsiveness was tested, opening up new opportunities for bioanalytical investigations.

5 ACKNOWLEDGMENT

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