Electrochemical sensing using micro- and nanostructured poly(ferrocenylsilane)s

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**ELECTROCHEMICAL SENSING USING MICRO-
AND NANOSTRUCTURED
POLY(FERROCENYLSILANE)S**

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Gutta cavat lapidem
Ovidius

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Chapter 1

Introduction

1.1 Aim of the project

The work discussed in this thesis is performed as part of the project entitled *Crosslinking polymer microparticles in non-equilibrium with light: Redox-active designer hydrogels for low-cost lab-on-paper diagnostics*. This project belongs to the *NWO ChemThem Out-of-Equilibrium Self-Assembly* programme. This programme focuses on research into systems that are out-of-equilibrium and as a result thereof self-assemble into new structures. In the first paragraph of the project proposal, the main aim was established as: *Developing a new family of low-cost electrochemical and optical biosensors for food or health applications based on enzymatic conversion of e.g. glucose or xanthine followed by (optical) readout using porous microparticles made from a new family of redox responsive polymeric hydrogels based on crosslinked poly(ferrocenylsilane) (PFS). Droplet microfluidics will be used to fabricate monodisperse microparticles. In the end, the newly developed biosensor materials are envisioned to be printed or assembled on paper to enable a low-cost diagnostic device.*

1.2 Poly(ferrocenylsilane)

Poly(ferrocenylsilane)s (PFSs) form a relatively new class of redox-active polymers. PFS has a backbone consisting of alternating silane and ferrocene units (see [Figure 1.1](#)), with every silane unit having two side-groups that can be functionalised to introduce or influence properties such as crosslinkability, solubility, stiffness, glass transition temperature, oxidation potential, and many more. The ferrocene groups in the backbone give the material its redox-responsiveness and make it possible to use it e.g. as an electrochemical sensor.

Figure 1.1 – General structure of Poly(ferrocenylsilane)

Extensive literature reviews on the synthesis, properties and applications of PFS have recently been published by the Manners group^(1, 2). Some noteworthy sensing and particle related applications are briefly discussed here in view of the aim of this work.

1.2.1 Particle formation

Joint work from the MTP group and BIOS describes the fabrication of PFS microparticles using UV-crosslinking to solidify droplets created inside a microfluidic chip⁽³⁾. In this paper, the loading and release of guest molecules is demonstrated. It is this work that served as inspiration for the application and funding of this project.

Other interesting work on PFS particles has been reported by the Manners group. In one approach solid circa 2 μm large PFS particles have been formed by precipitation polymerisation. Using electrostatic interactions, the authors report the self-assembly of heterogeneous particles consisting of a PFS microparticle surrounded by smaller silica particles⁽⁴⁾. In another approach pyrolysis of PFS precursor polymers has been studied to form ceramics such as nanoparticles with e.g. magnetic, conductive and catalytic properties⁽⁵⁾. Pyrolysis has also been used to make porous ceramic microparticles based on a solvent extraction approach as demonstrated by Gou and co-workers⁽⁶⁾.

1.2.2 Sensors

Espada and co-workers⁽⁷⁾ have coated tapered optical fibers with PFS to develop a gas sensor for NH_3 and CO_2 . The measurement principle is based on a change in refractive index. In their paper it is hypothesised that the presence of the transition metal in the polymer backbone might cause an enhanced discriminatory capacity towards certain gasses. Indeed an effect of NH_3 and CO_2 is observed, while no significant response is measured for N_2O . The authors indicate that the work is of a preliminary nature, but we have not found a follow-up article substantiating the

results of this paper.

Electrochemical sensing

PFS has also been used in electrochemical sensing. This includes PFS-based sensors for ascorbic acid^(8–13) and Fe^{3+} ^(12, 13). Although these papers indeed illustrate that PFS can be used as an electrochemical sensor, the used analytes are not very interesting from a sensor perspective. More relevant analytes appearing in PFS-based sensor literature include H_2O_2 ^(9, 12), and glucose⁽¹⁴⁾. What strikes us, is that in most of these papers the results shown are very preliminar, or the experimental details of the sensing are not given in great detail, making it impossible to repeat the reported experiments.

1.3 Biosensing

Biosensors have been particularly successful in healthcare applications. The market share for biosensors is estimated around 13 to 17 billion USD⁽¹⁵⁾. New applications in point-of-care diagnostics and/or theranostics, along with challenges from the ageing society in well-developed countries are key driving forces for continuing development in this field. Especially, the contribution from new nanotechnology, such as carbon nanotubes, and (plasmonic) nanoparticles have resulted in an increasing share of publications on this topic.

However, in a well-cited discussion paper written by Kissinger, it is pointed out that very few electrochemical biosensors have achieved wide-spread commercial success⁽¹⁶⁾. Moreover, Kissinger argues that the impact of a sensor paper can be judged by a set of guidelines formulated as (1) there is a clear demand for the target analyte, (2) the sensor provides a clear benefit over existing sensors for the same analyte, (3) the stability has been tested for long term use and in storage, (4) if it is a sensor intended for biological samples, it has been tested in these biological samples, instead of solely in aqueous buffers, (5) the dynamic range should be properly tested for the intended application in real samples. Although one could debate whether these guidelines of Kissinger are too harsh, it is clear that the published sensing papers using PFS have not been able to live up to these expectations so far.

1.4 Outline

In the [Chapter 2](#), self-assembly and reorganisation of PFS polymer molecules resulting in the formation of porous membranes is discussed. It

is demonstrated that the redox state of these porous membranes can be measured using impedance spectroscopy without the need of an additional reference electrode. Furthermore, we developed an electrochemical cell which can be used for in-situ small angle X-ray scattering measurements.

Using the porous PFS membranes as a robust electrochemical (bio-) sensor proved to be challenging, as described in chapter [Chapter 3](#). The poor conductivity and slow response hinders this material from being applied as routine readout material for enzymatic sensors. Attempts to fabricate an optical sensor by 3D printing photonic crystals are described in [Chapter 4](#). Material properties such as stiffness and melting temperature are essential to print good quality nanostructures.

Microparticle formation using droplet microfluidics was one of the other aims of this project. Out-of-equilibrium assembly of uniform porous microparticles is described in [Chapter 5](#) using droplet microfluidics. At the interface where the disperse and continuous phase meet, liquids are in an out-of-equilibrium state, resulting in the formation of droplets and, after solvent extraction, assembly of particles. Subsequently, the re-organisation of the polymers into porous particles, which was discussed for membranes in [Chapter 2](#) is induced.

Finally, preliminary attempts at making a non-enzymatic glucose sensor are described in [Chapter 6](#) using phenylboronic acid groups on a PMAA brush that swells upon binding with diols such as glucose. It was our intention to measure the swelling of this PMAA hydrogel using impedance spectroscopy, but in the end changes in the spectra due to changes in electrical double layer capacitance of the underlying indium tin oxide electrode turned out to be more dominant. In fact, the indium tin oxide could be used as reference-electrode-free simultaneous pH and conductivity sensor.

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Chapter 2

Synchrotron SAXS and impedance spectroscopy on redox-responsive porous membranes from poly(ferrocenylsilane)

2.1 Abstract

Nanostructured cellular polymeric materials with controlled cell sizes, dispersity, architectures and functional groups provide opportunities in separation technology, smart catalysts, and controlled drug delivery and release. This chapter discusses porous membranes formed in a simple electrostatic complexation process using a NH_3 base treatment from redox responsive poly(ferrocenylsilane) (PFS) based poly(ionic liquid)s and polyacrylic acid (PAA). These porous membranes exhibit reversible switching between more open and more closed structures upon oxidation and reduction. The porous structure and redox behaviour that originate from the PFS matrix are investigated by small-angle x-ray scattering (SAXS) using synchrotron radiation, combined with electrochemical impedance spectroscopy. In order to gain more insight into structure variations during electrochemical treatment, the scattering signal of the porous membrane is detected directly from the films at the electrode surface in-situ, using a custom built SAXS electrochemical cell. All experiments confirm the morphology changing between more “open”

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and more “closed” cells, with approximately 30% variation in equivalent radius or correlation length, depending on the redox state of ferrocene units in the polymer main chain. This property may be exploited in applications such as reference-electrode-free impedance sensing, redox-controlled gating, or molecular separations. Additionally SAXS data indicate the presence of micellar structures at the nanoscale formed by PFS PILs that develop during the base treatment.

2.2 Introduction

Porous nano/micro-structured materials have received much attention owing to their versatile applicability in e.g. separation, controlled release, catalysis, energy storage, adsorption, bio-interfacing and sensing.^(1–5) Porous complexes have been fabricated with success from poly(ionic liquid)s (PILs) owing to their tuneable electrostatic charge and solubility, high physical and chemical stability, and ease of fabrication.^(6–10)

Recently, Yuan et al. reported on the preparation and characterisation of porous materials fabricated from PILs via an electrostatic complexation approach.^(8, 11–13) Their systems have been tested for catalysis, adsorption, sensing and actuating. Pore formation was triggered by base treatment, upon which the ionic cross-linking that stabilises the membrane pores was formed between vinylimidazolium-based cationic PIL and an organic acid. By employing a similar method for the stimulus-responsive organometallic polymer poly(ferrocenylsilane) (PFS), we have developed a redox active, responsive porous membrane and illustrated its potential for applications in gated filtration.⁽¹⁴⁾ PFSs belong to a new class of organometallic polymers, which feature alternating Si atoms and ferrocene units in their main chain.^(15, 16) Substitution on Si (symmetric or asymmetric) allows one to vary the properties of this material. Due to the presence of ferrocenes, PFS exhibits pronounced redox responsive behaviour, which can be stimulated by electrochemical potential,⁽¹⁷⁾ or chemical redox agents.⁽¹⁸⁾

The redox activity and processability of PFS or PFS-based PILs in form of surface-grafted films and layer-by-layer electrostatic assemblies have previously been reported with applications in for example cyclic voltammetric or amperometric electrochemical sensing.^(19–21) The morphology images (i.e. SEM) of the redox responsive porous membranes show that the films have a higher density of openings in the oxidised form and a higher density of closed cells in the reduced state.

Here, we subject our responsive polymer-based porous membranes to combined small angle X-ray scattering (SAXS) and electrochemical measurements, to supplement the information previously obtained using scanning electron microscopy (SEM). We report on complementary data

such as pore characteristics in the bulk, electrical impedance and their dependence on the redox state of the material, and provide a morphological model for the compact PIL-based films prior to forming porous structures by NH_3 treatment.

2.3 Experimental

2.3.1 Materials

Vinylimidazolium-functionalized PFS ($\text{PFS} - \text{VIm}^+$), with the large bis(trifluoromethylsulfonyl)imide (Tf_2N^-) counter anion, was synthesised as described previously.⁽¹⁴⁾ Poly(acrylic acid) (PAA, average $\text{Mn} \approx 1800 \text{ g mol}^{-1}$), dimethylformamide (DMF), ammonium hydroxide solution (28 % NH_3 in H_2O), sodium perchlorate (NaClO_4), iron(III) perchlorate ($\text{Fe}(\text{ClO}_4)_3$), and ascorbic acid were purchased from Sigma-Aldrich. All compounds were chemical grade and used as received.

2.3.2 Synthesis of PFS-based porous membrane

Porous membranes were prepared as described previously⁽¹⁴⁾: $\text{PFS} - \text{VImTf}_2\text{N}$ and PAA were dissolved in DMF at equivalent molar ratio based on monomer units. A range of $\text{PFS} - \text{VImTf}_2\text{N}$ concentrations (20 mg mL^{-1} to 200 mg mL^{-1}) was used, to achieve different thicknesses by varying viscosity. Homogeneous solutions were then cast onto the substrates ($150 \mu\text{m}$ thick glass supports for ex-situ SAXS measurements and gold coated 10 to $20 \mu\text{m}$ thick mica supports for in-situ SAXS and impedance measurements), and dried at 80°C for 2 h, which resulted in dense bulk membranes. To develop the porosity, following the recipe by Yuan et al.^(8, 11–13) the samples were immersed into an aqueous NH_3 solution (0.5 wt.%, pH 11.2, 20°C) until they were completely porous. Chemical oxidation and reduction of the membrane were achieved by using an 0.1 M aqueous solution of $\text{Fe}(\text{ClO}_4)_3$ or ascorbic acid, respectively. Membranes used for ex-situ SAXS measurements were approximately $250 \mu\text{m}$ thick, to have ample material to induce scattering. In-situ samples were approximately $10 \mu\text{m}$ thick, to improve the electrochemical dynamics.

2.3.3 Scanning electron microscopy (SEM)

SEM was carried out using a HR-LEO 1550 FEF SEM instrument at 1 kV. The specimens were first freeze-fractured in liquid nitrogen and then mounted on an aluminum sample holder for SEM analysis.

2.3.4 Small angle X-ray scattering (SAXS)

SAXS measurements were performed on the ID02 high brilliance beam line of the European Synchrotron Radiation Facility (ESRF, Grenoble, France). The X-Ray energy employed was 12.4 keV. Measurements were performed at 3 different sample-detector distances: 30 m, 15 m and 2.5 m, which resulted in a $0.002 \text{ nm}^{-1} < q < 3.05 \text{ nm}^{-1}$ wave vector range ($q = \frac{4\pi}{\lambda} \sin \frac{\theta}{2}$, with λ the incident wavelength of the X-Ray beam and θ the scattering angle). A standard Rayonix detector was used to record the scattered intensity. $I(q)$ intensity curves obtained by azimuthal averaging were corrected for grid distortion, dark current, sample transmission and background scattering. Intensities were normalized to a standard sample (water) to obtain absolute scattering units.^(22, 23)

2.3.5 Electrochemical cell

A commercially available flow cell (DRP-FLWCL, DropSens, Spain) was modified to be suitable for in-situ SAXS measurements. The original flow inlet was closed off with a mica window (diameter 4 mm; thickness 10 to 20 μm) and used as entrance window for the beam. A conical beam exit window was drilled in the base plate of the cell. The original flow outlet was adjusted to hold the reference electrode. New inlet and outlets were drilled into the sides of the cell. Pictures of the modified cell are shown in Figure 2.1. A Ag/AgCl (3 M NaCl, liquid junction, RE-6, BASi, USA) reference electrode was used; 0.1 M NaClO₄ was used as background electrolyte. The cell was filled with electrolyte prior to measurements. The absence of air bubbles in the cell was confirmed both visually and by assuring there was electrical contact between the reference and counter electrodes. During the SAXS and electrochemical measurements there was no liquid flow through the cell. Using a potentiostat (SP200, Bio-Logic SAS, France), offset potentials were applied right before the SAXS measurements until currents dropped below 1 μA . Impedance measurements were performed in a three-electrode configuration, using a bipotentiostat (SP300, Bio-Logic SAS, France) after 5 min equilibration time at the indicated DC offset potentials.

2.3.6 Electrode fabrication

The working electrode was fabricated by sputtering a gold layer on top of mica substrates. The porous layer was prepared on top of this gold layer. Connections from the gold electrode to the potentiostat were made using copper tape with conducting adhesive (1181 Tape, 3M, USA). An insulating layer of 0.06 mm thick Kapton tape with a 4 mm hole was used to separate the working electrode and its connection from the counter electrode, which was formed by a final layer of copper tape with a 6 mm

Figure 2.1 – Pictures of the in-situ electrochemical cell used for the SAXS measurements.

hole. The holes in the two layers were aligned such that they left the polymer layer open to the electrolyte and the X-rays.

2.3.7 Reproducibility

To ensure the reproducibility of our results we measured multiple samples and electrodes, as far as the limited beam-time available permitted. The ex-situ SAXS measurements were performed on oxidised, reduced and nonporous samples of three batches of PFS. All results were comparable; the largest variation between batches was probably caused by thickness variations and incomplete oxidation of the thickest layers. In-situ SAXS measurements were performed on three electrodes, again with comparable results. Impedance measurements were performed on two of these electrodes in the in-situ cell used for the SAXS measurements, as well as on more than 5 electrodes in a different electrochemical cell during preparation for the SAXS measurements. The shapes of the impedance graphs, and their dependence on offset potential was similar for all layers, with only very thick (kinetics too slow) and very thin (virtually direct access of the electrode to the electrolyte through the pores) layers showing deviations from this behaviour. Because the absolute values of all signals depend heavily on the thickness of the layer, direct comparison of two similar samples is difficult. After ensuring with coarse measurements that all batches gave comparable results, we performed detailed and extensive measurements on a single ex-situ and a single in-situ sample in view of the limited beam time. The results of these detailed measurements are discussed in this article.

2.4 Results and discussion

2.4.1 Membrane fabrication

Porous membranes were fabricated by a two-step treatment on the casted films composed of PFS-VImTf₂N and PAA dissolved in DMF following previously published protocols⁽¹⁴⁾ (Figure 2.2a). SEM images reveal a sub-micron porous structure with pore sizes of 250(70) nm developed in the membrane. The originally clear and dark-red bulk membrane (Figure 2.2b) becomes opaque and yellow (Figure 2.2c) during the formation of the micro-sized pores, as a result of the treatment with aqueous NH₃ solutions. The thickness increase of 85 % corroborates the development of a porous structure during immersion. Deprotonation of PAA and the poor water solubility of PFS-VImTf₂N play an important role in the process of pore formation, as the electrostatic complexation between PFS-VIm⁺ and PAA⁻ finally yields the ionic network, which stabilises

the porous morphology. The weight loss, measured by comparing the dry masses before and after immersion, was 32 %, which indicates a large mass release due to the counter ion exchange replacing the large anion TF_2N^- with deprotonated PAA. The porosity of the formed membrane is calculated to be 63 %, which is in agreement with estimations from cross-sectional SEM images (65 %).⁽²⁴⁾ Oxidation and reduction of the porous membranes were accompanied by a color change from yellow in the neutral state to green in the oxidised state (Figure 2.2c and (d), respectively) as a result of the electrochromic response of PFS.

2.4.2 Ex-situ SAXS

Figure 2.3a summarises the SAXS intensity curves for the bulk and porous membranes. The porous membranes, both in the oxidised (Ox) and reduced (Re) states, exhibit an extended range of power law behaviour. The exponent of -4 is characteristic of scattering from smooth surfaces with sharp boundaries between two phases.⁽²⁴⁾

In both cases a correlation peak can be observed at around $q = 0.009 \text{ nm}^{-1}$, which corresponds to a characteristic distance of 700 nm ($d = 2/q$). The most striking observation is that the bulk polymer prior to forming the porous morphology also exhibits a similar SAXS response. The power law behaviour of $I(q) \equiv q^{-4}$ is restricted to a slightly smaller q -range, but still very well pronounced. This reveals the presence of sharp interfaces even within the bulk polymer. The correlation peak that becomes a “hump” in this case is slightly shifted to higher q value, and corresponds to a characteristic distance of about 400 nm. In the lowest q -region another power law behaviour can be observed with an exponent of -2.5 , characteristic of volume fractals.⁽²⁴⁾

A representation that masks the surface scattering (by plotting $I(q) \cdot q^4$ vs q , Figure 2.3b) allows the determination of the size of the large-scale scattering objects. The oscillations on the Porod plot (Figure 2.3b) can be explained by the presence of (quasi)spherical clusters.⁽²⁵⁾ The radius of these units can be determined from the position of the first maximum ($R_{max} = 2.74/q_{max}$). The average values obtained (198(3) nm, Table 2.1) are similar for all three samples. However, one should keep in mind that the position of the first maxima is highly sensitive to size polydispersity and, as the attenuation of the oscillations shows, this is the case in the present situation.

The pore characteristics of the samples have been explored using the Porod approach.⁽²⁴⁾ This method assumes that the adsorbing surfaces are flat on the length scale of the measurement and have sharp density discontinuities at the interface of the uniform substrate and the outside medium. However, in practice the substrate structure is far from regular over a larger q -range. The Porod analyses were therefore restricted to

Figure 2.2 – PFS-PAA-based porous membrane with redox-responsive morphology variation. Chemical structure of formed porous membrane and electrostatic complexation between PFS and PAA (a). Polymer film deposited on glass before base treatment-induced pore formation (b). Reduced (c) and oxidised (d) porous membrane treated by 0.1 M ascorbic acid and 0.1 M $\text{Fe}(\text{ClO}_4)_3$, respectively. Surface SEM image of or bulk membrane before base treatment (e). SEM images of reduced (f) and oxidised (g) porous membrane with more-closed and more-open porous structure, respectively.

Figure 2.3 – SAXS intensity curves of the bulk (circles), oxidised porous (triangle) and reduced porous (cross) membranes. Intensity curves; the dashed line shows the position of the correlation peak maxima in the porous membranes ($q = 0.009 \text{ nm}^{-1}$) (a). Porod plots of the SAXS responses of the bulk and porous membranes in oxidised and reduced state (b). Porod-Debye plots of the bulk (circles) and oxidised porous (triangles) and reduced porous (crosses) membranes. Solid lines represent linear fits in the Porod region (c).

the range where the intensity varies as Equation 2.1:

$$I(q) = Kq^{-4} + b \quad (2.1)$$

where K is the Porod slope and b is the scattering from atomic disorder in the sample.⁽²⁶⁾ The surface area of the large clusters which yield the previously described low- q behaviour of the spectra is negligible compared to the total internal surface area of the membranes resulting from the porosity. Figure 2.3c shows the Porod-Debye plot (Iq^4 vs q^4) of the scattering response curves, where Equation 2.2 holds:

$$I(q)q^4 = K + bq^4 \quad (2.2)$$

A striking result is that the intercept K was found to be > 0 in all cases, including the bulk membrane, which means that the bulk film prior to forming the porous morphology exhibits a heterogeneous structure. However, the value of K in this case is an order of magnitude smaller in the bulk membrane than in the porous ones, indicating the presence of a much more restricted structural heterogeneity. We postulate that the origin of this structural heterogeneity is related to a phase separation in the bulk films prior to NH_3 treatment. The bulk membrane films were solvent-cast from solutions of highly polar DMF (see membrane fabrication). In the films thus formed, poly-ionic PFS with a compensated counter-anion charge could form phase-separated micelles with non-polar PFS backbones in the center of the micelles, surrounded by protonated PAA material. The size of such micelles should be in the range of the dimensions of the PFS coil size. As discussed in earlier work,⁽¹⁴⁾ the weight average molar mass of the starting poly(ferrocenyl(3-iodopropyl)methylsilane) was in the range of M_w : $3.21 \times 10^5 \text{ g mol}^{-1}$; the coil dimensions of a typical polymer in this molar mass range are around 10 nm. The PFS phase separated domains include Fe and Si, which have a much stronger scattering power than typical organic elements, resulting in the SAXS signal contrast. We therefore explain the observed existence of structural heterogeneities with sizes in the range of 10.6 nm (Table 2.1), by the presence of Fe- and Si-rich phase separated organometallic micelles in the bulk film. This assumption was further strengthened by high resolution SEM images as shown in Figure 2.2c, displaying structural heterogeneities at this length scale. In this image, small (brighter) features can also be seen, which could be due to loose, individual micelles at the specimen surface.

Between the reduced and the oxidised membranes, a factor of two difference in K was found (Table 2.1), suggesting the same relation in the surface-to-volume ratio of the two states. The constant K is related to the surface-to-volume ratio, S/V via Equation 2.3, where S is the interfacial area within a sample of volume V and $(\Delta\rho)^2 = (\rho_{matrix} - \rho_{air})^2$ is the

Table 2.1 – Data extracted from SAXS results

	Bulk	Reduced	Oxidised
R_{max}^I (nm)	203 ± 0.3	197 ± 10	195 ± 2
K^{II}	0.13 ± 0.01	4.07 ± 0.34	2.25 ± 0.20
b^{III}	0.19 ± 0.01	0.19 ± 0.02	0.15 ± 0.01
Q	1.08 ± 0.05	310.9 ± 26.1	221.9 ± 19.4
K/Q	0.12 ± 0.01	0.01 ± 0.002	0.01 ± 0.001
b/K	1.47 ± 0.15	0.05 ± 0.01	0.07 ± 0.01
R_{equiv}^{IV} (nm)	10.6 ± 0.9	97.4 ± 11.5	125.9 ± 15.4

^I R_{max} : radius of large scale units from Porod-plot; ^{II} K : Porod-invariant; ^{III} b : Intercept from Equation 2.2, related to atomic disorder; ^{IV} R_{equiv} : equivalent radius of heterogeneities (micelles, pores), as determined using Porod-analysis

electronic contrast factor between the matrix and air. (The electronic density of air, ρ_{air} , is negligible with respect to ρ_{matrix} .)

$$K = (2\pi(\Delta\rho)^2) \frac{S}{V} \quad (2.3)$$

Another important parameter, the Porod invariant (Q) can also be determined from the Porod-analysis by the numerical integral in Equation 2.4.⁽²⁴⁾

$$Q = \int_0^\infty [I(q) - b]q^2 dq \quad (2.4)$$

Q is directly related to the mean square electron density of the scattering units, and is proportional to the total scattered energy. The mean radius of the pores can be calculated according to Equation 2.5.

$$R_{pore} = \frac{4Q}{\pi K} \quad (2.5)$$

The obtained equivalent R_{pore} values of 100 nm and 130 nm for the reduced and oxidised states, respectively, confirm the visual observation (Table 2.1 and Figure 2.2f and (g)): the porous membrane in the reduced state has a more closed porosity than in the oxidised state.

2.4.3 In-situ SAXS

Typical changes observed in the SAXS response of the porous membranes during in-situ oxidation and reduction in the electrochemical cell developed for this purpose (see and experimental section) are presented in Figure 2.4. To quantify the observed changes, the curves have been fitted by an empirical correlation length model, using the functional form in Equation 2.6:

$$I(Q) = \frac{C}{1 + (Q\xi)^m} + B \quad (2.6)$$

where C and B are Q -independent constants, m is the Porod-exponent, and ξ is the correlation length.⁽²⁷⁾

Figure 2.4 – In-situ SAXS intensity curves after background subtraction and normalisation (a); Effect of switching the potential between 0 and 0.9 V on the correlation length (ξ), multiple cycles (b). Correlation length as a function of gradually changing potential (c).

The values obtained for repetitive reduction-oxidation cycles are presented in Figure 2.4b. This plot clearly shows the reversible and repeatable switching behaviour of the material. The correlation length found in reduced samples is smaller than that of the oxidised ones. This is in agreement with the variation of the pore-sizes observed in ex-situ measurements, hence ξ is characteristic for the polymer and not for the pores. In the oxidised state the structure of the membrane opens

up: the pores get larger, which implies that the polymer gets in a more confined state and its correlation length is decreasing. Reduction results in the opposite phenomena, i.e. closing pores and more relaxed polymer phase. Figure 2.4c shows the ξ -values obtained during stepwise increase and decrease of the applied potential, revealing that the conformation change of the membrane does not follow the gradual potential change. Structural changes are restricted to the same potential range (0.4 V to 0.8 V) in which electrochemical oxidation or reduction of the PFS chains is observed during cyclic voltammetry measurements, see also Figure 2.5.

Figure 2.5 – Cyclic voltammogram of a porous layer on gold on mica (scan rate: 10 mV s^{-1}). The double-wave indicates repulsive interactions between neighbouring ferrocene units along the PFS main chain.^(17, 28) Oxidation starts at 0.4 V, stabilising the potential at or above 0.4 V ensures that the membrane is fully oxidised.

2.4.4 In-situ impedance

Impedance spectra were measured in the cell used for the SAXS measurements, using the same electrode. A small (10 mV) AC disturbance was applied on top of various DC offsets that controlled the redox state. The obtained spectra (Figure 2.6) are unique at each state, showing how the impedance spectrum and the corresponding pore conformation are

changing upon changing DC offsets.

Figure 2.6 – Impedance spectra of a porous layer on gold on mica, at a number of DC offsets. Lines: measured data; markers: fitted data. Dashed vertical line indicates the 100 Hz points, where each redox state has a unique combination of $|Z|$ and phase.

The circuit used in modelling the impedance response is shown in [Figure 2.7a](#). In general, a porous polymer layer shows Warburg-like behaviour.^(29–33) However, in this case the material is redox active. In its fully reduced and fully oxidised states, the polymer has a high resistance, therefore a Warburg-like behaviour is expected below 0.4 V and above 0.8 V. At the intermediate potentials, the resistance of the polymer is much lower, as it can take up or donate electrons during reduction and oxidation, implying a more resistor-like behaviour. To include these different characteristics in one model circuit, we use a constant phase element (CPE) in the representation of the porous polymer layer. The impedance of a CPE is given by [Equation 2.7](#),

$$Z_{CPE} = \frac{1}{Q(i\omega)^a} \quad (2.7)$$

where $0 \leq a \leq 1$ determines the phase of the CPE. For $a = 0$ the element is a resistor with resistance Q^{-1} ; for $a = 1$ it is a capacitor with capacitance Q ; when $a = 0.5$ the element is a Warburg element. The model circuit furthermore contains a resistor representing the bulk electrolyte, a capacitor for the double layer of the membrane and a parasitic capacitance. All elements in the model circuit are:

(a) Circuit representing the model.

(b) Optimised parameters for the constant phase element. In blue: CPE magnitude Q ; in red: CPE exponent.**Figure 2.7** – Modelling the impedance response of the porous polymer layers.

$Q_{pol}(Q, a)$: The constant phase element representing the porous polymer layer;

C_{dl} : The double layer capacitance of the counter electrode. (A three-electrode system was used, which circumvents the double layer of the counter electrode.);

R_{sol} : The resistance of the bulk electrolyte between polymer layer and counter electrode;

C_{par} : The parasitic capacitance of the entire system, including cables etc.

The values of the parasitic capacitance, the double layer capacitance and the resistance of the bulk electrolyte have been fixed at 6 nF; 6 mF and 150 Ω , respectively, as they should be constant for all DC offset potentials. The optimised parameters for the constant phase element and the resulting impedance graphs are shown in Figure 2.7b and Figure 2.6 respectively.

The obtained phase values of the CPE (Figure 2.7b) confirm the Warburg-like behaviour of the polymer layer at the extreme potentials, and the resistor-like behaviour around its oxidation potential. The resulted resistance values (Q^{-1}) around the oxidation potential are much lower than at the extreme potentials. The potential ranges where the resistance changes and where the correlation length of the polymer decreases (Figure 2.4c) were found to be the same (0.4 V to 0.8 V), showing a direct link between the redox state and the structure of the polymer membrane.

The observed findings imply direct advantages in the applicability of our membrane. In general, an electrochemical sensor includes a sensing and a reference electrode. A reference electrode often complicates miniaturisation of a sensor, since miniaturisation of the reference electrode generally impairs its stability. Because in our porous layer system each redox state has a unique impedance spectrum, it can be used as a reference-electrode-free redox sensor. Measuring a spectrum at 100 Hz, for example, allows rough determination of the redox state, as completely reduced, completely oxidised and intermediate states have distinctly different impedances at this frequency (Figure 2.6). Combining the impedance of multiple frequencies will increase the accuracy of such a sensor. As the use of a reference electrode in a sensor comes with many extra requirements and complicating factors, a reference-electrode-free sensor can significantly simplify the sensor design. Furthermore, combining our porous layer with specific biomolecules that can interact directly with the ferrocene units, such as certain oxidase enzymes, would result in an impedance based biosensor.^(34, 35)

2.5 Conclusions

Studying the impact of redox triggers on redox active PFS-based porous membranes by modelling and analysis of SAXS and impedance spectra, we evidenced that these membranes exhibit a transition between more “open” and more “closed” structures in oxidised and reduced states, respectively. Combining in-situ electrochemistry and SAXS enabled us to characterise not only the two extreme, but also the intermediate structures of the membranes. With sub-micron pores (50 nm to 200 nm diameter), high physical and chemical stability, and a tuneable porous structure, the PFS-based responsive membranes show great potential for controlled loading and release, advanced separation and reference-electrode-free impedance sensing. Furthermore, SAXS and SEM analysis of the non-porous precursor layer revealed 10 nm structural heterogeneities in the bulk membranes, indicating formation of micelles that consist of hydrophobic backbone of PFS, surrounded by a charged shell including PAA.

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Chapter 3

Electrochemical sensing using PFS-vinylimidazolium

3.1 Introduction

Poly(ferrocenylsilane)s (PFSs) form a class of redox-active polymers. PFS has a backbone consisting of alternating silane and ferrocene units, with every silane unit having two side-groups that can be functionalised to introduce or influence properties such as crosslinkability, solubility, stiffness, glass transition temperature, standard potential, and many more. The ferrocene groups in the backbone give the material its redox-responsiveness and can be electrochemically addressed or probed. One of the commonly mentioned applications for these materials are electrochemical sensors. Indeed other authors have reported PFS-based sensors for ascorbic acid⁽¹⁻⁶⁾, H_2O_2 ^(2, 5), Fe^{3+} ^(5, 6), glucose⁽⁷⁾ and other analytes^(5, 6, 8). What strikes us, is that in most of these papers the results shown are very preliminar, or the experimental details of the sensing are not given in detail. We therefore decided to attempt to construct our own PFS-based sensors, using the porous PFS membranes discussed in [Chapter 2](#). In this research PFS-vinylimidazolium (PFS-VIm) was used, containing cationic vinylimidazolium sidegroups, see [Figure 3.1](#). The PFS was mixed with poly(acrylic acid) (PAA) and exposed to ammonia, to prepare a porous membrane⁽⁹⁾ (see also [Chapter 2](#)). An optical pH sensor, based on a similar porous layer was previously reported⁽¹⁰⁾. Because, in contrast to the material used in that report, PFS contains redox active sites, we expect to be able to use the porous layer as an electrochemical sensor.

Figure 3.1 – PFS-Vinylimidazolium (PFS-VIm⁺)

3.1.1 Theory: Transport and kinetics

Two frequently used methods in electrochemical sensing, which we also apply in this chapter are cyclic voltammetry and amperometry. In cyclic voltammetry the potential is scanned between two values with a linear change in potential (called the scan rate). The current measured at the electrode surface can be related to the concentration of redox active species using Randles-Sevcik theory. The Randles-Sevcik equation (Equation 3.1) gives the relation between the concentration of the oxidising species and peak height. In this equation I_p is the peak current; F the Faraday constant; R the gas constant; T the absolute temperature; n the number of electrons in the reaction; A the surface area of the electrode, D_O the diffusion coefficient of the oxidised species; C_O^* the equilibrium concentration of the oxidised species in solution and v the scan rate.

$$I_p = 0.4463 \left(\frac{F^3}{RT} \right)^{1/2} n^{3/2} A D_O^{1/2} C_O^* v^{1/2} \quad (3.1)$$

In amperometry, the current response of an electrode upon application of a constant potential step is recorded. For a planar electrode and in the absence of convection and migration, this response is described by the Cottrell equation (Equation 3.2)⁽ⁱⁱ⁾. In this equation, $I(t)$ is the current response over time; C_O^* the concentration of the oxidised species in the bulk solution (the boundary condition used in the Cottrell derivation) and $t = 0$ the instant at which the potential step is applied.

$$I(t) = \frac{nFA D_O^{1/2} C_O^*}{\pi^{1/2} t^{1/2}} \quad (3.2)$$

To be able to explain the observed results in later sections, we need

to take a close look at the electrode/porous PFS/solution interface. A schematic overview is shown in Figure 3.2a, indicating an electrode (grey) with a porous PFS layer on top (orange), where the analyte is indicated in blue. For the sake of simplicity we assume that migration and convection do not play a role in this system, which is a reasonable assumption since we do not stir the solution, and we work with analytes with a high concentration (0.1M) of supporting electrolyte. Upon application of an oxidising or reducing potential at the electrode, a corresponding current response is measured. The key question is what happens with the measured current upon addition to the analyte of a reducing or oxidising agent such as hydrogen peroxide or ascorbic acid. An important factor in formulating a hypothesis is the role of the PFS in the overall electrochemical reaction. The general consensus is that the transfer of charge within an electroactive polymer (such as PFS) is either due to electron hopping between redox sites, or to the internal mobility of the redox sites. Simultaneously, the transfer of charge may be limited by the availability of counter ions, required to maintain electroneutrality. In practice it depends on the fluidity of the polymer matrix, or the facility of intersite electron transfer^(12, 13) (and refs 1-22 within⁽¹²⁾). We believe that the limit caused by the availability of counterions is less likely to occur since we work at high supporting electrolyte concentrations. We consider three extreme cases:

Case 1: PFS is inert If the PFS is merely a porous membrane on top of the electrode, it will act as a diffusion barrier for the oxidising or reducing agent to reach the electrode surface. This situation is depicted by species C and D in Figure 3.2a. Since the diffusion constant inside the membrane is most likely lower (slower) than in the solution, the concentration profile will look like Figure 3.2b. Of course this is a simplified situation, where it is assumed that the reaction is limited by the mass transport in the PFS layer, and not by other steps such as the electron transfer at the electrode surface. Furthermore, Figure 3.2b illustrates a steady state situation.

Case 2: PFS is highly reactive, and a good conductor If the ferrocene groups present in PFS are easy to oxidise or reduce, it is likely that the oxidising or reducing agent in the solution reacts at the electrolyte/PFS interface. If we furthermore assume that PFS is a good current conductor, meaning easy intersite electron transfer, PFS is in turn oxidised or reduced by the electrode. PFS can then act as mediator between the oxidising or reducing agents in solution and the electrode. Since in this case the PFS is considered a good conductor, the reaction-rate-limiting step becomes the mass transport of oxidising or reducing agent from the bulk of the solution towards the PFS interface. The resulting current response upon applying a constant

potential will follow standard theory described by Cottrell⁽¹¹⁾, i.e. the concentration of redox active species at the PFS/solution interface is zero, and a concentration profile will develop following the scheme shown in Figure 3.2c.

Case 3: PFS is highly reactive, but poorly conducting If the ferrocene groups present in PFS are easy to oxidise or reduce, yet the PFS is considered a poor conductor, the response becomes more complicated. In this case, the oxidised or reduced ferrocene groups far away from the electrode can not directly react with the electrode. Only if the reducing or oxidising agent diffuses into the membrane and reacts with the ferrocene groups close to the electrode surface, a change in current will be measured. Alternatively, the ferrocene groups ‘pass’ their charge to neighbouring groups due to internal mobility of the ferrocene, until that charge is measured at the electrode surface. This passing of charge by internal mobility is considered to be another slow ‘diffusive’ process.

Mixed response From measurements it is known that PFS is an insulator⁽¹⁴⁾. It is therefore most likely that we will observe a response that is a mix of case 1 and 3. Which of the two mechanisms is dominant depends on many factors, such as, amongst others, the diffusion constants of the electrochemically active species and the thickness and fluidity of the PFS layer. Complicating matters even further is the closing of the pores of the PFS membrane upon reduction, as shown in Chapter 2, which effectively lowers the opportunity of (counter)ions to diffuse into the layer.

3.2 Experimental Details

3.2.1 Materials

PFS-VIm was synthesised as described previously, see Chapter 2 and^(9, 15). All other chemicals—poly(acrylic acid) (PAA), ascorbic acid, hydrogen peroxide, $\text{Fe}(\text{ClO}_4)_3$, NaClO_4 , dimethylformamide (DMF), ammonium hydroxide solution (28% NH_3 in H_2O), sodium acetate trihydrate, acetic acid, potassium chloride (KCl), D-glucose and glucose oxidase from *Aspergillus niger*, Type X-S, lyophilized powder, 100,000-250,000 units/g solid without added oxygen—were ordered from Sigma-Aldrich (chemical grade) and used as received.

(a) Porous electrode

3.2.2 Porous layer formation

Porous layers were prepared on top of gold electrodes as described previously⁽⁹⁾. PFS and PAA were dissolved in DMF in a 1:1 ratio (based on monomer units).

3.2.3 Electrochemistry measurements

Measurements were performed in the electrochemical cell shown in Figure 3.3a, except experiment CVGD, which was performed in a glass beaker. Figure 3.3b shows the two types of chips that were used for measurements with porous layers. In both cases the electrode was gold; either on a silicon chip or on glass. Figure 3.3c shows the counter electrode used in all measurements. Electrolyte concentration was 0.1 M in all cases. When a buffer was added, it was 50 mM acetic acid buffer pH 5.1. Table 3.1 gives further experimental details. All CVs were measured clockwise. Exact descriptions of the experimental procedures can be found in Appendix A.

Figure 3.3 – Cell in which electrochemical measurements were performed. Examples of silicon (left) and glass chips (right) as used in the experiments show in (c). Only the circular top electrode of this chip was used.

Table 3.1 – Experimental details of performed experiments

Code	Technique	Working electrode	Reference electrode	Scan rate CV
CVAA	CV	PFS/PAA porous layer on gold	Hg/Hg ₂ SO ₄ (sat K ₂ SO ₄)	10 mV s ⁻¹
IPFE	EIS	PFS/PAA porous layer on gold	SPEIS: Ag/AgCl (3 M NaCl); none	
IPAA	EIS	PFS/PAA porous layer on gold	None	
CVH1	CV	PFS/PAA porous layer on gold	Hg/Hg ₂ SO ₄ (sat K ₂ SO ₄)	10 mV s ⁻¹
CVH2	CV	PFS/PAA porous layer on gold	Hg/Hg ₂ SO ₄ (sat K ₂ SO ₄)	10 mV s ⁻¹
CAHP	CA&CV	PFS/PAA porous layer on gold	Hg/Hg ₂ SO ₄ (sat K ₂ SO ₄)	5mV/s
CVGD	CV	Screen printed carbon electrode ^a	Ag/AgCl (3 M)	50 mV s ⁻¹
CVGP	CV	PFS/PAA porous layer on gold containing enzyme	Ag/AgCl (sat KCl) ^b	10 mV s ⁻¹
CAGP	CA	PFS/PAA porous layer on gold	Ag/AgCl (sat KCl) ^b	
<i>a: DRP 110, Dropsens, Spain; b: REF200, Radiometer Analytical, France.</i>				
Code	Electrolyte	Analyte	Analyte concentration over time	Chip Material
CVAA	NaClO ₄	Ascorbic acid	Both increased and decreased	Glass
IPFE	NaClO ₄	Fe(III)	None	Silicon
IPAA	NaClO ₄	Ascorbic acid	0.5 M	Silicon
CVH1	NaClO ₄	H ₂ O ₂	Increased	Silicon
CVH2	NaClO ₄	H ₂ O ₂	Both increased and decreased	Silicon
CAHP	NaClO ₄	H ₂ O ₂	Randomized order	Silicon
CVGD	KCl with buffer and 160 µg mL ⁻¹ GOx	Glucose	Increased	
CVGP	NaClO ₄ with buffer	Glucose	Increased	Glass
CAGP	NaClO ₄ with buffer and 65 µg mL ⁻¹ GOx	Glucose	Increased	Glass

3.3 Results and Discussion

3.3.1 Fe(III) and ascorbic acid sensing experiments

Cyclic Voltammetry (CVAA)

In a first experiment, cyclic voltammograms were measured of the porous membrane and between measurements the ascorbic acid concentration in the solution was changed (sometimes increased and sometimes decreased), see [Figure 3.4a](#). Ascorbic acid reduces the ferrocene groups in the PFS layer, resulting in decreased reduction at the electrode. Correspondingly, oxidation at the electrode increases. Higher ascorbic acid concentrations therefore lead to decreased reduction and increased oxidation peaks in the CV. The Randles-Sevcik equation ([Equation 3.1](#)), states that the peak current of a CV in a diffusion-controlled measurement is linearly dependent on the concentration of the diffusing species. In our case this diffusing species can be either the ascorbic acid in solution, or the charge through the porous layer. Since the amount of charge in the layer depends on the ascorbic acid concentration in solution, however, we can use [Equation 3.1](#) with C_O the concentration of ascorbic acid. The peak currents of the second oxidation peak are therefore plotted versus the corresponding ascorbic acid concentrations, see [Figure 3.4b](#). This plot indeed shows a linear correlation between concentration and peak current up to 25 mM, after which the current seems to have reached a maximum. Possibly the entire layer is reduced by the ascorbic acid at this (and higher) concentration(s), or the rate limiting step of the process changes at this point. The linear fit of the data up to 25 mM is also given in [Figure 3.4b](#).

Impedance spectroscopy (IPFE & IPAA)

As discussed in [Chapter 2](#), a porous PFS layer displays a unique impedance spectrum for every oxidation state, as a result of changes in the pore structure upon increased charge in the material. We tried to use this phenomenon for the construction of a reference-electrode-free sensor, based on impedance spectroscopy as a readout method. We therefore exposed the porous layers to 10 mM $\text{Fe}(\text{ClO}_4)_3$ for 45 minutes prior to measuring a two-electrode impedance spectrum. [Figure 3.5](#) shows the Staircase Potentio-Electrochemical Impedance Spectroscopy (SPEIS) result measured prior to, and the two-electrode impedance spectrum obtained after exposure to Fe^{3+} . The Fe^{3+} oxidises the membrane, thereby changing its potential. The black line in [Figure 3.5](#) shows the two-electrode impedance spectrum measured after the Fe^{3+} treatment. This spectrum closely resembles the spectra obtained at a DC offset of around 0.55 V. The standard oxidation potential of $\text{Fe}^{3+}/\text{Fe}^{2+}$ with respect to the used

(a) CVs varying with ascorbic acid concentration.

(b) Peak currents vs ascorbic acid concentration. Inset: zoom of the lower concentrations. Fitted line: $y = 0.0012x + 0.014$; $R^2 = 0.98$.

Figure 3.4 – CVs and fits of ascorbic acid additions. Experiment code: CVAA.

reference electrode (Ag/AgCl (3 M NaCl), potential 0.2 V vs NHE)⁽¹⁶⁾ is 0.57 V. It is expected that the Fe^{3+} treatment oxidises the membrane to this potential, since there was enough Fe^{3+} in the solution to oxidise the entire membrane. The close match between the apparent potential of the membrane and the standard potential of $\text{Fe}^{3+}/\text{Fe}^{2+}$ shows that this is indeed the case.

Figure 3.5 – Impedance of porous layer in pure electrolyte at various offset potentials, and after immersion in $\text{Fe}(\text{ClO}_4)_3$. Experiment code: IPFE

In a follow-up experiment, two-electrode impedance measurements were performed on the porous layer at increasing times after addition of ascorbic acid (resulting concentration 0.5 mM), see Figure 3.6. We see the impedance plot slowly changing towards more reduced spectra (compare with Figure 3.5). The curves measured at 85 and 101 minutes after addition of the ascorbic acid correspond to the 0.1 V to 0.15 V curves in Figure 3.5, which matches the oxidation potential of ascorbic acid⁽¹⁷⁾. Surprisingly, the final measurement shows a more reduced spectrum again, comparable to the 0.25 V DC offset curve of the SPEIS measurement.

The above results show that it is in principle possible to construct a reference-electrode-free ‘oxidation potential’ sensor using the porous layer. However, the results in Figure 3.6 show that the layer takes over half an hour to respond to a change of 0.5 mM in the solution, which is impractically slow for a sensor. For lower concentrations this will probably take even longer and this, combined with the fact that the signal begins to return to the original state after 2 hours will make it practically

Figure 3.6 – Impedance of porous layer at increasing intervals after addition of ascorbic acid. Note that the artefacts at 200 kHz are resulting from issues with the stability of the potentiostat or reference electrode, not from the working electrode or electrolyte. Experiment code: IPAA

impossible to determine the best time between addition of a sample and measurement. Alternatively, since the speed at which the impedance spectrum changes is probably related to the concentration of the analyte, using this as extra readout may increase the functionality of the sensor. Where measuring the equilibrium spectrum only tells one the oxidation potential of the species in the solution, measuring the rate at which the system reaches this equilibrium may allow for determination of the concentration of the species. In all cases the amount of oxidising or reducing agent in the solution has to be enough to oxidise or reduce the membrane to an equilibrium state, or the measured spectrum will not correspond to the oxidation potential of the species, but to a potential between the initial potential of the membrane and the oxidation potential of the species. Thinner porous layers will require less analyte and have faster response times, but they no longer show distinct differences between the impedance spectra of the oxidised and reduced states. Possibly the kinetics of the layer can be improved by incorporating a conducting polymer in the membrane.

3.3.2 Hydrogen peroxide sensing experiments

Well-known oxidising and reducing agents such as Fe^{3+} and ascorbic acid are relatively simple analytes, so the next step was to test a more complicated analyte: hydrogen peroxide (H_2O_2). Hydrogen peroxide is more complicated, since it can act as both oxidising (Equation 3.3) and reducing agent (Equation 3.4 to Equation 3.6).⁽¹⁶⁾ Hydrogen peroxide is a highly relevant analyte, since it is for instance a marker in lung diseases⁽¹⁸⁻²⁰⁾, as well as a product of many peroxidase enzymes used for sensing purposes. We investigate the applicability of the porous membrane both as a voltammetric and an amperometric hydrogen peroxide sensor.

Cyclic Voltammetry (CVH1 & CVH2)

In a first experiment, CVs were measured between additions of H_2O_2 to the electrolyte, see Figure 3.7a. An increase in the amount of hydrogen peroxide resulted in increase of both the oxidation and reduction peaks, see Figure 3.7b. This confirms that hydrogen peroxide both oxidises and

Figure 3.7 – CVs of porous membrane for increasing H_2O_2 concentrations. The arrows in (a) indicate peaks used for further analysis. Oxidation and peak currents vary with H_2O_2 concentration (b) and with time (c). Experiment code: CVH1

reduces the porous membrane. However, because the concentration was only increased over time, it is not conclusive whether or not the observed changes are purely an effect of concentration. It is possible that the porous membrane is slow to respond to the presence of hydrogen peroxide and that the effect we see is in fact mostly a result of increasing time after the first exposure to hydrogen peroxide. Figure 3.7c supports this hypothesis. Especially regarding the slow response observed in earlier measurements (Paragraph 3.3.1), some time is probably needed for the layer to reach an equilibrium state after every addition of hydrogen peroxide. If we added the new aliquot of hydrogen peroxide before the membrane has reached this equilibrium state, we are convoluting both the time and concentration effects, which most likely has been the case here. This can also explain why the peak current vs time plot does not show a linear trend, as would be expected from the Randles-Sevcik equation (Equation 3.1).

Figure 3.8 – CVs of porous membrane for various H_2O_2 concentrations. Oxidation peak current vs concentration (b) and vs time after first measurement (c). Experiment code: CVH2

To separate the effects of concentration and of time, the experiment was repeated with not just additions of hydrogen peroxide, but also additions of electrolyte, diluting the hydrogen peroxide concentration. The

results hereof are shown in Figure 3.8. Comparing figures Figure 3.8b and Figure 3.8c, it is clear that in this experiment the observed effect is primarily a result of time and not of hydrogen peroxide concentration. The observed effect is opposite to that observed in Figure 3.7, however: the peaks decrease over time. This is probably a result of deterioration of the porous layer, an effect which was observed before, especially in the presence of hydrogen peroxide.

Chronoamperometry (CAHP)

A second experiment with a randomised order of concentrations was performed: a dilution series of hydrogen peroxide solutions was prepared and the order in which they were measured was based on a random number generator. The read-out method used was chronoamperometry. The Cottrell equation (Equation 3.2), describes the time dependence of the current signal for diffusion-limited chronoamperometry measurements. In this experiment the rate-limiting step can be the diffusion of the hydrogen peroxide towards the porous layer (as argued in case 2), or the diffusion of charge through the porous layer (as argued in case 3). However, since the concentration of charge on the top surface of the layer depends on the hydrogen peroxide concentration, in both cases it will be the concentration of hydrogen peroxide in solution which effectively determines the current response.

It follows from the Cottrell equation, that the measured current plotted vs $t^{-1/2}$ results in a straight line with a slope dependent on C_{O}^{*} : the hydrogen peroxide concentration. We therefore represented the measured data in this way, see Figure 3.9a. We then used least-squares fitting to determine the slope of the measured data in the time span between 10 and 150 seconds, the linear part of the plots. The values for the slope we thus found are plotted in Figure 3.9b and c versus hydrogen peroxide concentration and time after the first measurement, respectively. These plots clearly show an increasing trend for the slope vs concentration, as dictated by the Cottrell equation, whereas there is no trend of slope vs time visible.

Measuring a CV on this membrane in fresh electrolyte the next day revealed that the porous layer was seriously damaged by the hydrogen peroxide treatment, see Figure 3.9d. All in all, since the response of these layers to hydrogen peroxide is unpredictable at best, it does not seem to be a good material for the construction of a hydrogen peroxide sensor.

Figure 3.9 – CAs of solutions with various H_2O_2 concentrations (a). Slope of the fit through these curves as a function of concentration (b). Same slope as function of elapsed time after first measurement (c). CVs measured prior to, and day after hydrogen peroxide exposure (d). Both CVs measured in H_2O_2 -free electrolyte. Experiment code: CAHP

3.3.3 Enzymatic glucose sensing experiments

Enzymatic glucose sensors have undergone much development since their first occurrence. Glucose sensors usually use the enzyme glucose oxidase (GOx) as recognition element because it is a relatively robust enzyme⁽²¹⁾. Glucose oxidase, carrying the co-factor flavin adenin dinucleotide (FAD), oxidises glucose to gluconolactone (Equation 3.7). FAD is reduced to FADH₂ in this reaction. The first generation of sensors required the presence of oxygen to ‘reset’ the enzyme in order to function optimally (Equation 3.8). The amount of hydrogen peroxide produced in this reaction was measured to obtain the glucose concentration in the sample. In second-generation glucose sensors, a mediator was added to shuttle between the electrode and the enzyme, see Equation 3.9 and Equation 3.10. Apart from no longer needing oxygen to be present, this had the advantage that the potential of the electrode could now be tuned to the mediator, instead of to hydrogen peroxide. This allowed for more selective detection. Third generation glucose sensors co-immobilise the mediator and the enzyme on the electrode surface. This not only prevents the mediators from diffusing away from the electrode, but can also speed-up the reactions, resulting in high current densities and fast sensor responses.⁽²²⁾

Ferrocene is a commonly used mediator in enzymatic glucose sensing experiments, as it is able to reach the active site of the glucose oxidase enzyme and thereby reactivate the enzyme. Since PFS contains ferrocene groups in the backbone, we tested the applicability of PFS as a mediator in glucose sensing. We tried to make a second generation glucose sensor using PFS, having the PFS in solution, as well as a third generation glucose sensor, based on the PFS porous membranes that were described in Chapter 2.

Cyclic Voltammetry (CVGD & CVGP)

Dissolved PFS To give the ferrocene groups the freedom to interact with the enzyme, in a first experiment we used dissolved PFS-VIm⁺ instead of the porous layer on an electrode. We measured CVs of the solution and increased the glucose concentration in the solution between measurements. We see the CV change as expected: as the PFS is reduced in the solution, we see more oxidation and less reduction occurring at

Figure 3.10 – CVs measured with increasing glucose concentration in solution, bubbled with nitrogen (a). Peak currents of CV vs glucose concentration (b). Michaelis-Menten plot: glucose concentration divided by peak current vs glucose concentration (c). Peak currents of CVs vs measurement time (d). Experiment code: CVGD

the electrode, see Figure 3.10a. Enzyme kinetics are described by the Michaelis-Menten equation (Equation 3.11), where v is the reaction rate; v_{max} the maximum reaction rate, $[S]$ the substrate concentration and K_m the enzyme-specific Michaelis constant.

$$v = \frac{v_{max}[S]}{K_m + [S]} \quad (3.11)$$

The CV plots show a plateau at higher glucose concentrations, in stead of a $t^{-1/2}$ ($\propto E_{we}^{-1/2}$) decrease as is the case for normal diffusion-limited CV plots. The appearance of a plateau is a result of the constant regeneration of the reduced species by the enzyme. The height of the plateau, I_{peak} , is therefore related to the reaction rate of the enzyme v . Rewriting Equation 3.11 to Equation 3.12 shows that a plot of concentration divided by peak current versus the concentration should result in a straight line.

$$\frac{[S]}{v} = \frac{K_m}{v_{max}} + \frac{[S]}{v_{max}} \quad (3.12)$$

This plot, including the least squares fit of the data, is shown in Figure 3.10c. However, as in experiment CVH1, the concentration of the analyte was only increased over time, making it impossible to exclude time effects, see also Figure 3.10c.

Porous layer The measurements discussed above were repeated on a porous PFS layer which contained GOx covalently linked to the layer. The result hereof is shown in Figure 3.11a. Surprisingly, this time we measure a decrease in the oxidation peak height with increasing glucose concentration and an increase in reduction peak height, see Figure 3.11b. The effect of time can again not be excluded, see Figure 3.11c. Possibly the covalent link between enzyme and polymer prevents the ferrocene groups in the backbone from reacting with the enzyme. In that case, the enzyme therefore probably reacts with oxygen in stead of PFS, resulting in hydrogen peroxide production. This in turn may explain the observed changes. Although this is only a single experiment, the used method of linking enzyme and porous layer does not seem optimal. Possibly catching the enzymes in the pores of the layer or some other method of restricting the enzyme inside the layer which still leaves it some freedom to move will give better results.

Chronoamperometry (CAGP)

A third glucose sensing experiment was performed with the porous layer on the electrode and the enzyme in solution. Chronoamperograms were measured of solutions with increasing glucose concentration, see Figure 3.12a. As before, we plotted the data vs $t^{-1/2}$, to be able to fit a

Figure 3.11 – CVs of increasing glucose concentration (a) and oxidation and reduction peak currents vs concentration (b) and vs time (c). Experiment code: CVGP

straight line according to the Cottrell equation. We used the datapoints obtained between 25 and 100 seconds for this fit (the grey rectangle in Figure 3.12a). The results of this fit are shown in Figure 3.12b (slope and intercept plotted versus glucose concentration) and Figure 3.12c (slope and intercept plotted versus time). Both the slope and the intercept value increase with increasing glucose concentration and over time. To keep the GOx concentration in the system constant, this was added to the glucose solution prior to additions to the cell. As a result hereof, the glucose oxidation may have started in this solution already, with the enzyme being reactivated by oxygen. This would mean that over time, not just the glucose concentration in the cell was increased, but that every addition of glucose also contained an increasing concentration of hydrogen peroxide. This can have played a large role in the observed effects. Furthermore, according to Cottrell's law the intercepts should equal zero, but this is clearly not the case here, moreover, a clear trend is visible for the intercepts (whether it is an effect of time or concentration).

Three control experiments were performed for this experiment, each time with a single condition different from the first experiment. During the first of these control experiments, the solution was deoxygenated with argon before every measurement. The results hereof are shown in

Figure 3.12 – CA curves of increasing glucose concentrations. The grey rectangle indicates the 25 s to 100 s interval (a); slope and cut-off of Cottrell fit of 25 s to 100 s of CA vs glucose concentration (b) and time (c). Experiment code: CAGP

Figure 3.13. The observed slopes are much larger in this measurement, possibly because the GOx is now ‘forced’ to react with PFS in stead of with dissolved oxygen. The fitted intercepts, although nonzero, do not show any trend in this experiment as was expected based on Cottrell’s law.

The other two control experiments were repeats of the first experiment, with the exceptions that either no GOx (**Figure 3.14**) or no glucose was added during the entire experiment (**Figure 3.15**). For these measurements the Cottrell fit was taken between 1 and 4 seconds, as the noise in the later parts of the measurements was too large to allow for a good fitting. In both cases, there is no trend visible in the slope or intercept and the intercepts are almost zero. This means that glucose does not react directly with the electrode, nor does the solution contain anything for GOx to react with, resulting in a stable state for the membrane. The only disruption of this state occurs when a measurement is performed, after which a new stable state is reached. For measurements that were performed relatively long after the previous one, the observed (absolute values of the) slope and intercept are much larger than for the other measurements, probably because the membrane slowly returns to its initial state when left unperturbed.

The results of the four experiments are summarised in **Table 3.2**. A trend for the intercept v.s. concentration of glucose is only observed for the first experiment in which GOx can react both with glucose and with oxygen. The source of the trend is unknown, but it seems likely that oxygen and/or hydrogen peroxide play a role. The experiment where argon was used to exclude oxygen has the best results: no dependence of the intercepts, and the largest dependence of the slope on glucose concentration. This result is promising for the use of the porous membrane as a mediator in glucose sensing. However, we have to note that we did not extensively check the time dependence of the signal. The two experiments in which, respectively, no glucose and GOx were added show a very stable signal for the membrane, however, so we don’t expect significant drift to occur. Finally, we would have expected a Michaelis-Menten-shaped curve for the slope-vs-concentration plot, which is not what we obtained. A possible explanation for this observation could be a too slow response of the layer, thus violating the boundary conditions for Michaelis-Menten kinetics where the enzyme reaction is the rate limiting step.

Table 3.2 – Summary of the CAGP conditions and results.

Figure results	Present chemicals	trend of slope	trend of intercept
Figure 3.12	GOx, O ₂ , glucose	$\pm 2 \text{ mA} \sqrt{\text{s}}/\text{M}$	linear, $\pm 0.5 \text{ mA M}^{-1}$
Figure 3.13	GOx, glucose	$\pm 7 \text{ mA} \sqrt{\text{s}}/\text{M}$	random
Figure 3.14	O ₂ , glucose	no trend	± 0
Figure 3.15	GOx, O ₂	no trend	± 0

Figure 3.13 – CA curves of increasing glucose concentrations measured after deoxygenating with argon. The grey rectangle indicates the 25–100 s interval (a); slope and cut-off of Cottrell fit of 25–100 s of CA vs glucose concentration (b) and time (c). Experiment code: CAGP

Figure 3.14 – CA curves of increasing glucose concentrations in the absence of glucose oxidase. The grey rectangle indicates the 1 s to 4 s interval. Please note the difference in scale compared to Figure 3.12 and Figure 3.13 (μA vs mA) (a); slope and cut-off of Cottrell fit of 25 s to 100 s of CA vs glucose concentration (b), zoom-in of the same (c), vs time (d) and zoom-in (e). Experiment code: CAGP

Figure 3.15 – CA curves measured after additions of electrolyte and glucose oxidase solution containing no glucose. The grey rectangle indicates the 1 s to 4 s interval. Please note the difference in scale compared to Figure 3.12 and Figure 3.13 (μA vs mA) (a); slope and cut-off of Cottrell fit of 1 s to 4 s of CA vs “glucose concentration” (what the concentration had been if a solution containing glucose as in the other experiments had been used) (b), zoom-in of the same (c); vs time (d) and zoom-in (e). Experiment code: CAGP

3.4 Conclusion

The porous PFS/PAA layer was used in a number of electrochemical sensing experiments, with varying success. Some of the experiments give promising results, but in most cases the slow response of the layer makes it hard to distinguish time effects from concentration variations. Furthermore, there is a problem with repeatability, as similar experiments have resulted in opposite effects, for example the glucose sensing using CV, which in one case resulted in increasing currents with increasing glucose concentration, where in a second case the currents decreased with increasing concentration. Finally, the porous layers often degraded over the course of the measurements. The porous layer therefore does not seem to be a robust enough system to base electrochemical sensors on, at this point in time. Improvements in response time could probably be achieved by making thinner layers, but this is likely to result in even faster degradation and worse selectivity because the underlying electrode will then also be more directly accessible through the pores.

3.5 References

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Chapter 4

Comparison of three types of redox active polymer for two-photon stereolithography

4.1 Abstract

3D-printing and stereolithography of functional materials for nanofabrication has recently spiked a lot of interest, as have responsive materials. We have investigated the applicability of the redox-responsive polymer poly(ferrocenylsilane) (PFS) for stereolithography purposes. Three types of PFS were synthesized, each functionalized with specific properties to make them interesting for use in nanofabrication. These properties include various stiffnesses, crosslink densities and hydrophobicities. One of the three types of PFS is polycationic, therefore resulting in a hydrogel structure. Structures, fabricated from these materials, are shown and the most often encountered challenges in using new materials such as these for two-photon stereolithography discussed.

4.2 Introduction

Two photon polymerization (TPP) is a lithography technique, in which a laser beam is focussed into the resist. The wavelength of the laser beam is double the excitation wavelength of the initiator in the resist. As a result

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hereof, only spots which are illuminated with such a high intensity that two photons excite one initiator molecule almost simultaneously, are cross-linked. Because the chance of this happening is quadratically dependent on the light intensity, the area that is effectively illuminated is much smaller than the actually illuminated spot, as is shown in Figure 4.1⁽¹⁾. As a result hereof, the feature sizes of the fabricated structures can be much smaller than with single photon lithography.

Figure 4.1 – Single-photon (left) and two-photon (right) excitation of a rhodamine B solution. The two-photon excitation is so localised, it is hard to see. Image by LaFratta et al.⁽¹⁾ reproduced with permission.

In TPP, the photoresist layer can usually be moved around in three dimensions over a fixed focus position of the laser beam, giving almost complete freedom in the paths that are illuminated and therefore in the resulting fabricated structures. TPP, and stereolithography in general, have been extensively investigated in recent years, resulting in great improvements in both methodology and materials. This has led to faster fabrication, smaller minimum feature sizes (lateral resolution around 100 nm, addition of quenchers gives resolutions down to 20 nm, axial resolution around 500 nm) and more diversity in the types of materials that can be used.^(2–6) Simultaneously, use of TPP for microfluidics⁽⁷⁾ and use of responsive materials in microfabrication have taken flight. Recent papers have discussed embedding of microfabricated structures in responsive materials⁽⁸⁾ and functionalization of microfabricated structures, leading to devices responsive to changes in for example pH⁽⁹⁾, magnetic fields^(10–14), and light intensities⁽¹⁵⁾. Direct fabrication of responsive nanostructures is not as common, however. Marino et al. published TPP using piezoelectric materials⁽¹⁶⁾ and Wickberg et al. used TPP to directly fabricate temperature sensors⁽¹⁷⁾, but to the best of our knowledge, no redox active resists have been developed for two photon polymerization yet. Use of redox-active resists enables the direct fabrication of redox responsive nanostructures. Examples of possible applications of such redox-active nanostructures could be nano-actuators and sensors, with the readout of the sensors being either photonic or electrochemical. We

here report our use of the redox active polymer poly(ferrocenylsilane) (PFS) in TPP. PFS has a backbone consisting of alternating silane and ferrocene units. Desired properties, such as the crosslink functionality needed for photolithography purposes, can be introduced by functionalization of one or both of the two remaining side groups of the silicon atom. Even more freedom in tailoring the material to one's wishes can be obtained by using copolymerization of multiple types of functionalized ferrocenylsilane. The wide range of possibilities that PFS offers, combined with its responsiveness and the unique possibilities of TPP, makes TPP using PFS a very interesting pursuit. PFS was previously patterned into 2D micro-structures by directed self-assembly and self-assembled nanostructures of PFS have been used as a mask in lithography techniques^(18, 19), for instance for the fabrication of cobalt magnetic dot arrays⁽²⁰⁾. Using TPP will enable fabrication of 3D PFS nanostructures. We here report a comparison of three types of PFS and their applicability as a resist in two-photon lithography. Furthermore, we discuss a number of challenges one is likely to encounter when using non-standard resists for TPP.

4.3 Methods

Synthesis: Two precursors — poly(ferrocenyl(3-chloropropyl)methylsilane) and poly(ferrocenyl(3-chloropropyl)methylsilane)-co-poly(ferrocenyl-methylphenylsilane) — were readily accessible by transition-metal-catalyzed ring-opening polymerization of the corresponding (3-chloropropyl)-methylsilyl[1]ferrocenophane and methylphenylsilyl[1]ferrocenophane^(21, 22). By means of halogen exchange, the products were converted quantitatively into iodopropyl analogues, which are particularly suitable for further functionalization by nucleophilic substitution. Methacrylate functionalized PFSs (**PFS1** and **PFS2**) were obtained by allowing poly(ferrocenyl(3-iodopropyl)methylsilane) and poly(ferrocenyl(3-iodopropyl)methylsilane)-co-poly(ferrocenyl-methylphenylsilane) to react with sodium methacrylate and 15-crown-5 in THF/DMSO (2:1 v/v) at room temperature for 7 days under a nitrogen atmosphere. The resulting mixture was precipitated into cold methanol and the resulting solid was collected and washed thoroughly with water and methanol. Methacrylamide functionalized PFS (**PFS3**) was obtained by allowing poly(ferrocenyl(3-iodopropyl)methylsilane) to react with *N*-[3-(dimethylamino)propyl]-methacrylamide in THF/DMSO (2:1 v/v) at room temperature for 24 h. The iodide counterions were exchanged with chloride counterions by dialysis against 0.1 M NaCl and MilliQ water in Spectra/Por 4 dialysis hose (MWCO 12-14,000 g/mol). Concentration of the salt-free polyelectrolyte solution by a flow of nitrogen produced **PFS3** as orange flakes.

Structures were fabricated using two photon lithography in the Photonic Professional (Nanoscribe GmbH, Germany). Droplets of dissolved PFS and initiator were placed on a glass cover slide (30 mm diameter; 170 μm thickness, ThermoScientific, Germany) and allowed to dry on air. A 100x immersion objective was used. Laser power was optimised during the experiment by writing of test lines at increasing laser power and visually checking at what power the lines were seen to appear but not melt or burn, as described above. The used initiators, solvents and concentrations of polymer and initiator are given in Table 4.1.

Table 4.1 – Sample preparation parameters.

	PFS1	PFS2	PFS3
Solvent	Toluene	Toluene	Water
Developer	Chlorobenzene	Chlorobenzene	Water
[PFS] before drying	80 mg mL ⁻¹	80 mg mL ⁻¹	80 mg mL ⁻¹
Initiator (I)	Irgacure 651	Irgacure 651	Irgacure 2959
[I] before drying	15 mg mL ⁻¹	15 mg mL ⁻¹	8 mg mL ⁻¹

4.4 Results and Discussion

The three types of PFS used in this research are given in Figure 4.2. PFS-methacrylate (**PFS1**) contains methacrylate sidegroups that supply the crosslinking functionality. **PFS2** is a copolymer of **PFS1** and a PFS containing phenyl sidegroups. These phenyl groups increase the glass transition temperature of the material, but dilute the crosslinkable groups: from the original ratio of one crosslinkable group on every ferrocenylsilane unit, to 1:4. The final PFS used in this research, **PFS3**, is a water-soluble, polycationic PFS, with dimethylaminopropyl methacrylamide (DMAPMA+) sidegroups. As a result of the cationic nature of this PFS, structures made from it are hydrogels.

We fabricated two types of structures using these materials: single lines (nanowires) and woodpiles (stacks of 4 repeating layers of parallel bars, with alternating layers having the bars perpendicular to each other). When properly defined and fabricated, woodpiles can act as photonic crystals. The line structures fabricated from the three types of PFS, can be seen in Figure 4.3. Figure 4.3a-d shows the lines fabricated from **PFS1**. The lines in Figure 4.3a image appear to be nicely thin wires, but seen from the top using incident light (Figure 4.3b), it can be seen that they are in fact thin slabs that are sagging. The lines in Figure 4.3c,d appear to be much flatter, but these contain squiggles which we cannot explain satisfactorily. A possible explanation for these squiggles includes swelling

Figure 4.2 – The three types of poly(ferrocenylsilane) used in this research.

of the material upon crosslinking, where the material is only attached to the surface at regularly spaced points. The material connecting these points bulges outwards, resulting in the squiggles. The logical source of this spacing would be the point distance of the apparatus, which is the distance between points where the laser rests for a short while during the writing process. However, in this experiment the point distance was set to 50 nm, which does not match the length of the squiggles. The glass transition temperature (T_g) of **PFS1** (before crosslinking) was measured to be 17 °C. Since this is rather low, compared to the temperature in the apparatus, the fabricated structures may melt during the writing process.

We therefore introduced the phenyl groups in **PFS2**, to increase both the stiffness and T_g of the material. The T_g of **PFS2** (before crosslinking) was indeed higher: 70 °C. Lines fabricated from **PFS2** are shown in [Figure 4.3e-f](#). As can be seen, these lines are not straight either, they squiggle as well. Furthermore, the lines are attached to the glass slide on which they are fabricated on one end, but not over their entire length. This is a typical example of one of the main problems we encountered with this technique: it is very hard to align and focus the sample and microscope such that the structures are fabricated right on top of the substrate. When the focus of the interface is set is too high, structures are released from the substrate when they are developed; when the interface was mistakenly put too low, the lower parts of the structures are not fabricated because they would have to be located inside or below the substrate. The Nanoscribe apparatus has an autofocus function, which looks for a contrast in index of refraction between the glass and the resist. In the case of our resists, however, this contrast was very low, which resulted in the autofocus function finding the bottom of the glass

Figure 4.3 – Pictures of H-shaped line structures. a-d, fabricated using **PFS1**. a,b: transmitted light (TL), c,d: incident light (IL). Pictures a and b are taken at the same location as c and d, respectively. a,c: dwell time 200 ms, laser power 48 mW; b,d: dwell time 100 ms; laser power 8 mW. e,f: fabricated using **PFS2**; g: fabricated using **PFS3**, with laser power increasing from left to right (18 mW, 21 mW, 24 mW, 27 mW and 30 mW.)

substrate or the top of the resist layer, instead of the correct interface. Focussing therefore had to be done manually and this made it impossible to correct for the slight tilt the sample might have with respect to the sample holder and the apparatus.

In [Figure 4.3g](#), lines fabricated from **PFS3** are shown. The squiggling of the lines is less pronounced in this case, but again parts of the lines have released from the substrate and some squiggling is still visible. The outer right line was fabricated using the highest laser power for this experiment (30 mW), which, for all three resists, often results in ill-defined, swollen or even burnt-looking, structures. The T_g of **PFS3** is 30 °C, which means that the swollen looking structures may be a result of melting during fabrication.

Woodpiles, fabricated from each of the three types of PFS are shown in [Figure 4.4](#). SEM images of woodpiles from **PFS1** and **PFS2** can also be found there. The same phenomena which were observed in the fabricated lines can be recognised in these structures: **PFS1** ([Figure 4.4a,b](#)) appears to have the best-defined lines; the bars in the woodpiles from **PFS2** ([Figure 4.4c,d](#)) are sagging and squiggling. The woodpiles made from **PFS3** ([Figure 4.4e](#)) even appear swollen. As the structures were dried completely at the time of imaging, these deformations can not be the result of an uptake of solvent, but are probably due to flexibility of the materials or too high laser intensity during fabrication, as in the case of the 30 mW laser power line in [Figure 4.3g](#).

Figure 4.4 – Pictures of woodpiles, fabricated in **PFS1** (a,b), **PFS2** (c,d) and **PFS3** (e). a,c,e: light microscopy pictures; b,d: electron microscopy images. Structures in b are incomplete, only one or two layers of bars have been formed on the substrate.

4.5 Summary and Outlook

We were able to fabricate nanostructures using our new, home-made redox-active resists, but we ran in to a number of challenges when using these new resists in TPP. The challenges we encountered, and suggestions for future improvement are listed below:

Focussing As a result of a low contrast in the index of refraction of the used substrates and the uncured resist, it was hard to find the exact interface of the two. Finding this interface is crucial, in order to be able to fabricate structures that will adhere to the substrate yet do not miss the bottom parts. Because the contrast was so low, the autofocussing function of the apparatus could not find the right interface; it most often found the interface of the immersion oil and the glass substrate, as the contrast here was larger. In order to find the correct interface, we focussed on a number of locations outside the uncured resist where the glass substrate was exposed to air, leading to a detectable contrast. We then interpolated the thus found coordinates, to estimate the position of the interface in the center of the resist. Alternative solutions could be the use of a substrate with an index of refraction that is distinguishable from that of the resist, or the placement of focussing markers (e.g. sputtered lines of metal) on the substrate. In any case: when the substrate is not completely level inside the sample holder, writing larger structures with a single focus setting can still result in parts of the structure

being written inside or above the substrate.

Stiffness of polymer As always, to be able to fabricate a structure, the used material has to have the correct mechanical properties. The crosslink density in the fabricated material has to be large enough, such that the structures can support their own weight and do not sag, as was the case with our materials.

Melting temperature Similar arguments hold for the melting temperature of the materials. When a focussed laser beam is aimed into the sample, the resist can be heated locally, leading to deformation of the structures during fabrication. In some of our samples the polymer even appeared to have been burnt by the laser.

Reproducibility Using identical settings for parameters such as *laser-power* and *powerscaling*, often very different results were obtained. In some cases nice structures were obtained using settings which in a later experiment resulted in burned or molten structures. One possible explanation for this may be local variations in the initiator and polymer concentrations, as a result of drying of the resist before illumination, where drying patterns and possible even some kind of phase separation occur. Another explanation could be scattering of the laser in the sample reducing the actual intensity. To determine the correct settings for each individual experiment, we started each experiment by writing a number of test lines with increasing laser power. When polymerisation occurs, this can be seen using the microscope in the apparatus and when the substrate burns or melts this can also be observed. This allows one to determine a laser power at which structures are fabricated, but not deformed. At the same time, when lines are seen to appear, one can be sure that the focus position is not too low, i.e. in the substrate. It does not prevent focussing above the interface, however.

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Chapter 5

A facile microfluidic approach to porous microspheres from poly(ferrocenylsilane)s

5.1 Introduction

Porous polymeric microspheres represent a highly attractive class of materials today, which is of great interest in the diverse field of sciences. Exhibiting unique characteristics such as a high permeability, a large surface area and a low density, porous particles have successfully been used for a variety of applications during the last decade^(1, 2). These applications include, for example, ion-exchange and catalysis⁽³⁾, chromatography⁽⁴⁾ as well as drug delivery⁽⁵⁾. Since there is still an increasing interest in research on porous particles, they show a great potential for fulfilling future needs of health care, energy storage and catalysis, in particular by using stimuli-responsive materials^(1, 6). Therefore, the fabrication of porous particles is a promising field of research with the aim to develop processes that enable the ultimate control on particle size and shape, morphology as well as polydispersity⁽¹⁾.

Due to the great importance of polymers in materials science and industry, there is a wide range of different top-down processes for the fabrication of polymeric particles, whereas most of them are subclasses of the heterogeneous polymerisation⁽⁷⁾. By the incorporation of porogens in general, these conventional bulk methods can be slightly modified to alter the particle's structure from solid to porous⁽⁸⁾. Suspension polymerisation, which is probably the simplest of these conventional methods, has shown to be compatible to the use of most types of porogens, for example nonsolvents (regarding the polymer),⁽⁹⁾ linear polymers (in combination with solvents)⁽¹⁰⁾, water⁽¹¹⁾ or even solids⁽¹²⁾.

Since many bulk processes for porous polymeric particle fabrication suffer from low controllability regarding particle size and shape, new bottom-up methods arose⁽¹⁾. With the use of porous membranes^(13, 14) and microchannels⁽¹⁵⁾ for the formation of single monodisperse droplets, heterogeneous polymerisation methods were highly improved because rigorous stirring was no longer necessary for dispersion. Later, the development of poly(dimethylsiloxane) (PDMS) for prototyping of microchips by Whitesides and co-workers in 1998⁽¹⁶⁾ paved the way for microfluidics, which is the youngest but maybe also the most promising field of porous particle fabrication today. Within the past ten years, various novel microfluidic devices were introduced, including microcapillary devices⁽¹⁷⁾ and ‘off-the-shelf’ devices,^(18, 19) which both proved to be suitable for the preparation of emulsion droplets and thus for porous particle fabrication.

In contrast to bulk processes, these devices enable discrete droplet formation by introducing a dispersed phase of dissolved monomer species into a continuous phase. Afterwards, polymerisation can be induced via UV irradiation to obtain solid polymer particles⁽²⁰⁾. As a study by Kumacheva and co-workers in 2008⁽²¹⁾ exemplifies, the utilization of porogens in microfluidic devices is generally comparable to bulk methods, since the pore-forming agents are readily added to the dispersed phase of dissolved monomer species. Furthermore, the use of gaseous⁽²²⁾ and even reactive⁽²³⁾ pore-forming agents was reported, which demonstrates the diversity of the porogen-based approach. In contrast, Watanabe and co-workers recently reported two porogen-free methods to fabricate porous polymer particles via solvent diffusion⁽²⁴⁾ and solvent extraction^(25, 26) within a microfluidic device. The formation of pores was controlled by using an amphiphilic diblock copolymer with a tunable solvent compatibility⁽²⁴⁾ and via complex phase inversion processes within a ternary system of polymer, solvent and nonsolvent,^(25, 26) respectively.

As already mentioned above, developments in the field of porous polymeric particles can strongly benefit from the use of stimuli-responsive materials.^(6, 27) In the 1990s, poly(ferrocenyl-silane)s (PFS) emerged as a promising new class of redox-responsive metallopolymers, since they combine a good accessibility and a broad range of unique characteristics.^(28, 29) Vancso and co-workers⁽³⁰⁾ recently used a PFS-based cationic polyelectrolyte for the porogen-free preparation of redox-responsive membranes with controlled pore sizes, whereby pore formation was induced via an ammonia treatment. In principle, this membrane-based concept can also be easily applied for the fabrication of porous particles.

Based on the concept for porous membranes, this chapter presents a novel porogen-free approach for the microfluidic fabrication of monodisperse porous polymer particles from a PFS-based polymer, using a simple capillary-tubing device. In a controlled two-stage process, microspheres are generated from a dispersed polymer solution via ex-situ

solvent diffusion, which are later infused with ammonia to induce pore formation. Obviously, this method is very simple, ranges at low costs and does not require advanced microfluidic skills, but enables reproducible porous particle fabrication from a unique redox-responsive material.

5.2 Experimental

The synthesis of PFS–VImTf₂N is described elsewhere in literature⁽³⁰⁾. For droplet microfluidic experiments, a solution of the polyelectrolyte pair PFS–VImTf₂N/PAA (100 mg mL⁻¹ and 20 mg mL⁻¹, respectively) in N,N Dimethylformamide (Sigma-Aldrich) was used as the dispersed phase, whereas the continuous phase was PDMS oil (Sigma-Aldrich, viscosity of 1 cSt), containing 5 wt.% of Dow corning 749 fluid (Dow Corning) as surfactant.

Glass Syringes (Hamilton Gastight) and a syringe pump (Cetoni, neMESYS Low Pressure Syringe Pump) were used to introduce both phases into the co-flow microfluidic device, which was assembled by only using commercially available components, as described by Hadziioannou and co-workers⁽¹⁸⁾. Within the rectilinear passage of a T junction, a capillary for the dispersed phase was axisymmetrically fitted into a plastic tubing, which acts as outlet tubing (T junction: IDEX H&S, P-712 PEEK Low Pressure Tee Assembly; Capillary: Molex, Flexible Fused Silica Capillary Tubing, 360 µm OD x 100 µm ID; Tubing: IDEX H&S, 1516L Tefzel ETFE Tubing, 1/16" OD x 0.020" ID). The continuous phase was separately introduced into the rectangular inlet of the T-junction via another plastic tubing (IDEX H&S, see above). A schematic of this capillary-tubing device is shown in Figure 5.1.

Flow rates of the continuous and the dispersed phase were 100 µL min⁻¹ and 5 µL min⁻¹, respectively. The droplet formation at the tip of the capillary was observed with a high-speed camera (Point Grey Research Inc., Grasshopper3). The droplets that leave the outlet tubing were collected in a continuously shaken petri dish containing PDMS oil and 5 wt.% of Dow corning 749 fluid (Dow Corning). Afterwards, they were transferred to a large petri dish to further dry in PDMS oil at 50 °C for 12 h, washed with n hexane (Sigma-Aldrich) and infused with a 0.5 wt.% aqueous NH₃ solution for 2 h (Sigma-Aldrich) to induce pore formation. SEM images of the porous particles were taken using a Quanta 450 SEM (FEI).

Figure 5.1 – Schematic illustration of the used microcapillary device. As an ‘off-the-shelf’ device⁽¹⁹⁾, it can be readily assembled from commercially available low-cost components. Emulsion droplets from the dispersed polymer phase are formed at the tip of a capillary, that is centered on the inside of a tubing. Thus, particles are carried out by the continuous phase and collected in a petri dish filled with PDMS oil.

5.3 Results and Discussion

Droplets are being generated using the simple T-junction configuration as previously described by Hadziioannou and co-workers⁽¹⁸⁾. The dispersed phase, which contains the polyelectrolyte pair PFS – VImTf₂N/PAA⁽³⁰⁾ in N,N Dimethylformamide (DMF), is introduced via the central capillary. PDMS oil is used as continuous phase, which flows around the capillary through the plastic tubing. Emulsion droplets from the dispersed phase form at the tip of the capillary and are subsequently carried away through the outlet tubing, where solvent diffusion starts. As shown by a series of pictures in Figure 5.2, emulsion droplets with a diameter of approximately 450 μm are formed via co-flow of the continuous phase and dispersed phase with flow rates of 100 $\mu\text{L min}^{-1}$ and 5 $\mu\text{L min}^{-1}$, respectively. The frequency of droplet formation is around 90-100 droplets per minute. During this process, the dispersed phase forms a continuously growing semi-sphere at the tip of the capillary, which is eventually sheared off by the continuous phase. Interestingly, a low tuneability of droplet size was observed, which we would not have expected based on earlier reports found in literature.^(24, 25, 31, 32) The dispersed phase obviously adheres to the flat side of the capillary’s tip, which can be explained by adsorption of the relatively polar solvent DMF at the capillary’s hydrophilic glass surface. Additionally, the rectangular shape of the tip causes a disadvantageous hydrodynamic effect, since the droplets are in a “hydrodynamic slipstream” that prevent a faster droplet break-off (Figure 5.3). Due to this effect, changes in the flow rates mainly

influence frequency of droplet formation. However, the decoupling of flow rates and droplet size ensures robustness of the process and a high monodispersity of the fabricated particles. Additionally, the droplet size can be controlled by changing the outer diameter of the used capillary which is readily accessible.

Figure 5.2 – Schematic illustration of the herein reported porous particle fabrication via a two-stage approach. In the first stage, droplets of PFS – VImTf₂N/PAA dissolved in DMF are formed within a co-flow microfluidic device. The droplets are collected in PDMS oil containing 5 wt.% surfactant and dried ex-situ at 50 °C for proper solvent diffusion. In the second stage, the dried droplets are washed with n-hexane to remove oil residues and treated with a 0.5 wt.% aqueous NH₃ solution to induce pore formation.

The freshly formed droplets are continuously carried away by the PDMS oil, whereas a new semi-sphere is left at the tip of the capillary. To avoid droplet coalescence in the tubing or the collection container which was observed in early experiments, a siloxane-based surfactant was added to the continuous phase. Further observations revealed that initial droplets are deformed a short distance from the tip to become platelet-shaped, being compressed alongside the tubing and slightly touching the walls (Figure 5.3). This deformation is supposed to be driven by hydrodynamic pressure of the continuous phase and to be further intensified because droplets fill around 80 % of the channel's cross section right after

shear-off. However, this effect seems to not have any influences on the later shape of particles, which are apparently spherical (Figure 5.4B).

Figure 5.3 – Images of the droplet formation from the polymer phase in PDMS oil at flow rates of $100 \mu\text{L min}^{-1}$ and $5 \mu\text{L min}^{-1}$ for the continuous phase and dispersed phase, respectively. The green bar visualizes the inner diameter ($100 \mu\text{m}$) of the capillary, which is apparently much smaller than the generated droplets. The red arrows emphasise the deformation of the droplets a short distance from the tip, as a result of which they touch the tubing.

Since DMF has a low solubility in PDMS oil⁽³²⁾, the solvent slowly diffuses out of the droplets that are carried through the outlet tubing of the device. With a tubing length of 1.5 m, the dwell time of the droplets is around 150 s, before they enter a petri dish filled with fresh PDMS oil. However, droplets are not solid but soft and gel-like when they leave the tubing, apparently still holding residual solvent, which is a known problem in literature⁽²⁵⁾. Additionally, solvent diffusion is very slow, which may relate to the small PDMS volume around each droplet. Thus, saturation of the continuous phase with DMF counteracts further solvent diffusion from the droplet. Further investigations are necessary to fully understand the herein reported problem.

To solve this, an ex-situ approach is used to complete the solidification process. The droplets are widely spread in a thin PDMS oil layer inside a petri dish and then dried at 50°C for several hours. During this step physical separation of particles is of utmost importance to prevent particle coalescence even after solidification, since the glass transition temperature of the polymer is 30°C . At last, solid particles with a bright orange color and an approximate size of $250 \mu\text{m}$ are obtained after this procedure with just few merged particles, as depicted in Figure 5.4A. Droplet shrinkage, which could not be observed within the microfluidic device, apparently took place during the extensive drying in the oven and amounts to about 50 %. The SEM images in Figure 5.4B/C reveal a

smooth surface and a regular spherical shape but also some irregularities on the backside of the shown particle. This demonstrates that herein, porosity does not originate from solvent diffusion.

Figure 5.4 – Photographs and SEM images of the fabricated PFS–VImTf₂N/PAA particles. The upper row (A–C) shows solid particles, which are obtained from the ex-situ solvent diffusion (stage 1), as described in Figure 5.2. A) Photo of dried particles in a petri dish. B) SEM image of a dried particle with a size of 250 μm. C) SEM image of the surface of a dried particle, revealing the absence of pores. The bottom row (D–F) shows porous particles, which are finally obtained after ammonia treatment (stage 2), as described in Figure 5.2. D) Photo of porous particles in a petri dish. E) SEM image of a porous particle with a size of 280 μm. F) SEM image of the surface of a porous particle with pores of approximately 400 nm to 600 nm.

Before the particles are dispersed in an 0.5 wt.% aqueous solution of ammonia, a proper washing procedure using n-hexane is necessary to remove residues of PDMS oil from the particles. The process of washing as well as pore formation using ammonia is schematically illustrated in Figure 5.2. During ammonia treatment, the color of the particles changes from orange to pale yellow (Figure 5.4D), indicating a metamorphosis of particle morphology. The mechanism behind these structural rearrangements, however, is not fully understood yet. It was shown that most of the COOH groups of PAA are deprotonated at pH>10, which is supposed to be important for pore formation. Additionally, low solubility of the PFS-based cationic poly(ionic liquid) may play an important role in this process⁽³⁰⁾. The successful pore formation is shown by SEM images of a

porous microsphere and its surface in Figure 5.4E/F. Pores with different diameters are dispersed on the particles surface, which gives the particle an overall rough surface texture. Additionally, the size of the particle increased to approximately 280 μm due to the structural rearrangements. The size of the pores basically ranges from 400 nm to 600 nm, as depicted in Figure 5.4F, but much larger pores are also located on the surface (Fig 2E). Apart from that, the pore shape is observed to be irregular, with most pores having a circular shape.

5.4 Conclusion

A simple two-stage, porogen-free approach for the fabrication of porous polymeric microspheres from poly(ferrocenylsilane)s was demonstrated, using an ‘off-the-shelf’ microfluidic device. Emulsion droplet formation at the tip of a co-flow-fitted capillary led to monodisperse emulsion droplets, which solidified during ex-situ solvent diffusion. Pore formation was then induced via an ammonia treatment, yielding porous particles of approximately 280 μm with an average pore size around 500 nm.

In the near future, further experiments are planned to investigate the extensive redox-responsive behaviour that porous PFS – VImTf₂N/PAA is known to have. This feature has already been demonstrated in the past using porous membranes⁽³⁰⁾. The pores underwent a reversible change in pore size upon treatment with redox agents, which was used to control the permeability of membranes. Additionally, the ability to grow silver nanoparticles on the polymer’s surface using an aqueous solution of AgNO₃ was proven. Therefore, experiments will be carried out by our group to test if the herein produced porous particles bear similar characteristics. This could enable great opportunities for controlled release of molecules as well as for drug delivery.^(1, 2)

5.5 References

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Chapter 6

Reference-electrode free pH and conductivity sensor

6.1 Introduction

In this chapter we set out to measure glucose using a polymer brush functionalised with boronic acid (BA) groups on indium tin oxide (ITO) electrodes by impedance spectroscopy. Traditionally, (first to third generation) glucose sensors work based on the conversion of glucose using enzymatic means (usually using glucose oxidase)⁽¹⁾. An interesting alternative non-enzymatic method is to use a hydrogel containing phenylboronic acids (PBA). These PBA groups are Lewis acid sites, forming favourable configurations for bidentate condensation with diols such as glucose. As a result the hydrogels change properties, caused by swelling forces. An extensive review on this work is written by Siegel et al.⁽²⁾. The swelling of the hydrogels is typically measured using optical means^(3, 4), electrochemically^(5, 6), or by mechanical sensors⁽⁷⁾, including a silicon based wireless capacitive sensor⁽⁸⁾. Moreover, Ma and co-workers modified the surface of a gold electrode with PBA and measured the concentration of four different saccharides using impedance spectroscopy⁽⁹⁾.

The mechanism of glucose sensing using PBA is highly pH sensitive. However, the ITO is also known to be sensitive to changes in pH by itself, as shown by research where ITO is used as extended gate ion sensitive field effect transistor (ISFET) from both Yin et al.⁽¹⁰⁾ and Lin et al.⁽¹¹⁾. Unfortunately, in neither publication do the authors explain in depth what the physical principle behind the pH sensitivity is. From more general ISFET theory it is known that OH-groups at the surface can protonate or deprotonate depending on the pH, causing a change in surface charge at the gate⁽¹²⁾. A similar mechanism is at play for the changes in in-plane conductivity of thin (15 nm to 30 nm) layers of ITO

for changes in pH recorded using 4-point measurements⁽¹³⁾.

In this chapter, we look in more detail at the effects of pH on the stability of impedance measurements when using ITO electrodes. We find small, but clearly noticeable changes in what is typically called the electrical double layer (EDL) capacitance of ITO when the pH is varied. We have ruled out the change in electrolyte conductivity caused by these pH titrations. We contribute this effect to the change in pseudo-capacitance from protonation and deprotonation of OH-groups at the ITO surface, which can be explained by the site-binding model⁽¹²⁾. Modification of the electrode surface with a PMAA brush shows similar but smaller changes in the EDL than the bare ITO. After functionalisation of the PMAA brush with boronic acid groups, we have tested its sensitivity for glucose, but we did not find any effect.

6.2 Impedance of the ITO/electrolyte interface

To explain possible differences in the double layer capacitance of ITO with changing pH, we have looked into models for the ITO/electrolyte interface that include the protonation/deprotonation of OH-groups at the surface. A model that described these phenomena in a quantitative manner for silicon dioxide surfaces is published by Bousse and Bergveld⁽¹²⁾. Standard electrical double layer theory describes the electrode/electrolyte interface by a Stern layer, including both the inner Helmholtz plane (IHP), and the outer Helmholtz plane (OHP), and the diffuse layer (see Figure 6.1a). Solvated ions can only approach the electrode surface up to the OHP, while adsorbed ions are located at the IHP.

In the model of Bousse and Bergveld, the impedance of the total ITO/electrolyte interface consists of a diffuse double layer capacitance (C_{dl}) based on Gouy-Chapman-Stern theory, parallel to a capacity from absorbed charge (C_a) and a Warburg impedance (Z_w) corresponding to the variations of surface pH induced by the AC signal from the EIS measurement (see Figure 6.1b). The equations for these equivalent circuit elements as described in⁽¹²⁾ are:

$$C_{dl}^{-1} = C_{Stern}^{-1} + \left(\frac{q\sqrt{2\epsilon_0\epsilon_r kTC}}{kT \cdot \cosh(q\psi_d/(2kT))} \right)^{-1} \quad (6.1)$$

$$C_a^{-1} = \frac{kT}{\delta N_s q^2} \left(1 + \left(\frac{\sigma_0}{\delta N_s q} \right)^2 \right)^{-1/2} \quad (6.2)$$

$$Z_w = \frac{kT}{q^2 \sqrt{2\omega} [\text{H}^+] \sqrt{D_{\text{H}^+}} + \text{OH}^- \sqrt{D_{\text{OH}^-}}} \frac{1-j}{\sqrt{2\omega}} \quad (6.3)$$

Figure 6.1 – Standard model of the electrical double layer, including the Stern layer with inner and outer Helmholtz plane, and the diffuse layer. Image modified from⁽¹⁴⁾ (a). The equivalent circuit model used in this paper is shown in (b), where R_1 is the electrolyte resistivity, R_2 the interfacial resistance, C_{dl} the diffuse layer capacitance, C_a the capacitance due to absorbed charge, and Z_w a Warburg impedance due to variations of surface charge pH. (c) shows the simplified equivalent circuit used to analyse the data in this chapter.

C_{Stern} is the compact layer capacitance (assumed to be 0.2 F m^{-2}), ϵ_0 and ϵ_r the permittivity of vacuum and water, k is Boltzmann's constant, T temperature, C the concentration of the electrolyte, q the elementary charge, and ψ_d the potential at the OHP. δ characterizes the reactivity of the surface, whereas N_s is the number of sites per unit area. D_{H^+} and D_{OH^-} are the diffusion constants of protons and OH^- ions. Please note that C , $[H^+]$ and $[OH^-]$ are given in units of molecules (and not moles) per volume.

To be able to calculate the total impedance of the ITO/electrolyte interface as a function of pH, we need to know σ_0 , the charge on the ITO surface due to protonation and deprotonation of H^+ and OH^- ions, as described by the site-binding model. This surface charge can be calculated from the point of zero charge (PZC), and the two dissociation constants (pKa, pKb) of the two possible acid-base reactions taking place at the ITO surface, following the theory described by van Hal, Eijkel and Bergveld⁽¹⁵⁾. Moreover, these parameters can be used to calculate the intrinsic buffer capacity β of the surface, as described in detail in the paper of van Hal et al.⁽¹⁵⁾. Since we could not find this information for ITO in sufficient detail in literature, we only show qualitative results assuming that σ_0 is equal to the charge in the diffuse layer, which can be calculated by the Gouy-Chapman-Stern theory⁽¹²⁾:

$$\beta = \frac{\delta N_s q^2}{kTC_{dl}} \quad (6.4)$$

In Figure 6.2, Bode plots are shown for variations in pH between 3 and 8. An effective surface area of $6.4 \times 10^{-4} \text{ m}^2$ is assumed, while the electrolyte resistance is 532Ω , and the interfacial resistance $250 \text{ k}\Omega$, similar to the experimental conditions shown in the next sections. We use a value of 0.3 for the parameter β to get a value for the product of δ and N_s . It is clear that variations in pH only affect the shape of the curves around 10 Hz, where the electrical double layer capacitance is dominant. Since we are using the same assumptions for σ_0 as Bousse and Bergveld⁽¹²⁾, we observe a non-linear change of the EDL capacitance with pH. A more refined model taking into account the PZC, pKa, and pKb of ITO following the model of van Hal et al.⁽¹⁵⁾ would most likely make the simulated results more accurate.

6.3 Experimental Details

6.3.1 Chemicals

Phosphate buffered saline (PBS) tablets were ordered from Sigma-Aldrich, dissolving 1 tablet in 200 mL DI gives 0.01 M phosphate buffer; 0.0027 M

Figure 6.2 – Simulations of the Bode plots at pH values between 3 and 8 for the ITO setup as used in the experiments.

KCl; 0.137 M NaCl; pH 7.4 at 25 °C. PBS buffers at various pH values were prepared by adjusting the pH of this standard PBS buffer (original pH 7.4) with Na_2HPO_4 or phosphoric acid. Na_2HPO_4 and phosphoric acid (≤ 85 wt% solution in water) were ordered from Sigma-Aldrich and used as received.

6.3.2 Impedance sensing

Impedance measurements were performed using a bipotentiostat (SP300, Bio-Logic SAS, France) in a two-electrode configuration. The electrochemical cell used is shown in Figure 6.3a; the electrodes are shown in Figure 6.3b. ITO-covered glass slides were ordered from Sigma Aldrich (Surface resistivity 70 to 100 Ω/sq), cleaned using RCA-1 clean, rinsed extensively with water and ethanol and cut to 1 cm $\times$ 2 cm. Additionally, three parallel shallow cuts were made in the chips, dividing the ITO into four separate lanes, thus giving four electrodes on one chip. To prevent electrolyte leaking from the cell through these cuts, drops of UV-curable glue (NOA 81, Norland, USA) were positioned on the cuts and the glue was allowed to fill the cuts due to capillary interactions. When the glue in the cuts had entered the sensing area and therefore water-tightness was ensured, the glue was cured using an UV light (in-house built, 6 s pulses; 350 mW cm^{-2} , 365 nm). During measurements, the first and third ITO lanes were connected and used together as counter (and pseudo-reference) electrode; the second ITO lane was used as working electrode. Impedance spectra were measured at the open circuit poten-

tial with an AC disturbance of 10 mV, see Figure 6.4a to c for a typical example of such a spectrum. From the full spectrum, the frequencies at which the phase was maximal ($\pm 0^\circ$, 300 kHz) and minimal ($\pm -80^\circ$, 10 Hz) were obtained. The impedance at these frequencies was recorded over time while the pH or concentration of buffer in the cell was varied. pH sensing experiments were performed in PBS (0.01 M phosphate buffer). The starting concentration for the conductivity sensing experiments was the same PBS buffer at pH 7.3. During the concentration variations the pH of this (diluted) buffer varied between 7.3 and 7.5. A commercial pH meter (SevenMulti, Mettler Toledo, the Netherlands) was used as a reference to determine the pH of the solution in the cell. Because the volume of the solution in the cell was too low to use the pH meter directly in the cell, identical manipulations as performed on the solution in the cell were performed on a larger volume of the same solution in a separate vial and the pH of this reference solution was monitored.

Figure 6.3 – Electrochemical cell used for impedance measurements (a). Electrodes as used in measurements (b). The dashed circle indicates the area that is exposed to electrolyte.

6.4 Results and Discussion

6.4.1 bare ITO electrode

Figure 6.5a shows the measured impedance value at 10 Hz (in blue) during changes over time in the pH of the solution (red curve). The large spikes are a result of mixing using a pipette right after the addition of

(a) Impedance spectrum, Bode plot

(b) Nyquist plot

(c) Detail of (b)

(d) Bode plot of fitted spectrum

(e) Nyquist plot of fitted spectrum

Figure 6.4 – Typical impedance spectrum of an indium-tin-oxide electrode (a). Green and blue dashed lines indicate minimum and zero phase frequencies, respectively. Nyquist plot of the same data (b). Zoom of the origin region of the Nyquist plot (c). Bode plot of the impedance response of the equivalent circuit used for fitting (see Figure 6.1c) (d). Nyquist plot of the impedance response of the equivalent circuit used for fitting with points of minimum and maximum phase angles indicated (e).

250 μL of new solution to the electrolyte already in the cell. As can be seen from the red line, the pH was both reduced and increased during the same measurement to exclude drift effects over time. The average impedance during each stable pH interval (excepting the first and final 10 measurement points per pH to avoid averaging in the spikes) for both frequencies, is plotted in Figure 6.5c. This plot shows a strong dependence of the 10 Hz impedance (blue) on the pH, whereas the 300 kHz impedance (red) does not depend on pH. The measured 300 kHz impedance (red) value during varying of the buffer concentration (green) is shown in Figure 6.5b. The spikes are again a result of the mixing by pipette, but as can be seen, the value of the impedance at this frequency is less sensitive to this. Only when air is injected directly on top of the measurement section a large spike is observed. Averaging of the plateau values of the impedance and plotting them versus the dilution factor for both frequencies results in Figure 6.5d. This plot shows the strong dependence of the 300 kHz impedance on buffer concentration, whereas the 10 Hz impedance remains virtually constant. A small slope can be observed, but this is probably due to a small change in pH value during the experiment which coincides with the dilution factor: from pH 7.3 at no dilution to pH 7.5 at dilution factor 7. Together, Figure 6.5c and d confirm that the bare ITO electrode can be used simultaneously as a reference-free pH and conductivity sensor.

We have determined the values of the electrolyte resistance (R_1), the interfacial resistance (R_2) and the combined double layer capacitance C_2 (which is an approximation of the $C_{dl} // C_a + Z_w$ branch in Figure 6.1b). We use this approximation because we measured the impedance at only two frequencies, resulting in 4 data points. The circuit shown in Figure 6.1b has 6 parameters and is therefore too complicated for a fit with our data points. The circuit used for fitting is shown in Figure 6.1c. The general shape of an impedance spectrum of this circuit is shown in Figure 6.4d (Bode plot) and Figure 6.4e (Nyquist plot). In these figures, we see that the $|Z|$ value we measure at 300 kHz equals R_1 . The minimum angle of the impedance lies on the semicircle of the Nyquist impedance plot where the tangent through the origin touches (see Figure 6.4e). This means that we can calculate R_2 using Equation 6.5, in which $|Z_{10\text{ Hz}}|$ and $\phi_{10\text{ Hz}}$ are the amplitude and the phase of the impedance measured at 10 Hz, respectively.

$$R_2 = 2|Z_{10\text{ Hz}}| \tan(-\phi_{10\text{ Hz}}) \quad (6.5)$$

The frequency-dependent impedance of the equivalent circuit shown in Figure 6.1 is given in Equation 6.6, with f the frequency in Hz. Using this formula, the data measured at 10 Hz and the obtained values for R_1 and R_2 , we calculate C_2 .

Figure 6.5 – Impedance of the bare ITO electrode at 10 Hz (blue) and pH (green) vs time (a). Impedance of the bare ITO electrode at 300 kHz (red) and 1/relative concentration (= dilution factor, green) vs time (b). Mean $|Z|$ values per applied pH value (blue: 10 Hz; red: 300 kHz) (c). Mean $|Z|$ values per applied dilution factor (blue: 10 Hz; red: 300 kHz) (d).

$$Z(f) = R_1 + \frac{R_2}{1 + i2\pi f R_2 C_2} \quad (6.6)$$

The values thus obtained for C_2 , R_2 and R_1 are plotted versus pH in Figure 6.6. A clear dependence of R_2 and C_2 on pH can be seen. The value of R_1 is independent of pH: the decrease at higher pH values is a result of the higher conductivity at these pH values due to the addition of Na_2HPO_4 to increase the pH. As predicted by the theory from Section 6.2, the value of C_2 depends on pH and seems to have an almost linear response for pH values between 3 and 8.

Figure 6.6 – Derived model parameter values vs pH.

6.4.2 pH-sensitive and glucose-sensitive brush functionalised ITO electrode

The same measurements were performed on an electrode from which a poly(methacrylic acid) (PMAA) brush was grafted and on the same brush after functionalisation with boronic acid groups. The procedure for the ITO surface modification using reversible addition-fragmentation chain transfer (RAFT) polymerization and subsequent modification of the PMAA brush with boronic acid groups can be found in Appendix B. Both brushes showed pH sensitivity, but a mixed response was obtained. This

led us to the conclusion that a simple bare ITO electrode performs better as a pH sensor than either of these modified electrodes. The boronic acid brush was expected to have glucose sensitivity, but this could not be observed in the measurement results. See [Appendix B](#) for details and measurement data on the PMAA and boronic acid brushes.

6.5 Conclusion

We demonstrated the use of a simple indium-tin-oxide electrode as reference-electrode-free combined pH and conductivity sensor. The simplicity and robustness of this system holds much promise for application in microdevices and long-term sensing applications. The observed change in double layer capacitance can be explained by theory from earlier work on ISFETS. However, more work on the theoretical model is required to match theory with practical observations on a quantitative level. Moreover, functionalisation of the electrode with a pH sensitive brush did not improve its performance as pH sensor, and no sensitivity towards glucose was observed for an electrode functionalised with a boronic-acid-containing brush.

6.6 References

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Appendix A

Experimental details sensing experiments Chapter 3

A.1 Fe(III) and ascorbic acid sensing experiments

CVAA The porous membrane on an electrode was put in the electrochemical cell, with 5 mL NaClO_4 (0.1 M). Aliquots of 10 mM ascorbic acid solution were added and everything was mixed with a pipette. After 10 additions, the entire solution was replaced by 5 mL of 0.05 M ascorbic acid in 0.1 M NaClO_4 and subsequently diluted two times with 0.1 M NaClO_4 twice. A third short series was then started, beginning with 5 mL of 0.01 M ascorbic acid in 0.1 M NaClO_4 and diluted three times with 0.1 M NaClO_4 . Finally the cell was filled with only electrolyte (0.1 M NaClO_4). For each of these concentrations, a CV was measured, with scan rate 10 mV s^{-1} using a $\text{Hg}/\text{Hg}_2\text{SO}_4$ (sat K_2SO_4) reference electrode.

IPFE The PFS porous membrane was soaked in 10 mM FeCl_3 solution for 45 min prior to the impedance measurements. The measurement was performed in a 0.1 M NaClO_3 solution.

IPAA The porous layer was prepared on a platinum electrode on a glass chip. A SPEIS measurement was performed in 0.1 M NaClO_3 electrolyte, with a Ag/AgCl (3 M NaCl) reference electrode was used and a platinum spiral counter electrode. Ascorbic acid was added to a concentration of 0.5 M and more impedance spectra were measured, after 0, 3, 23, 80, 96 and 122 minutes.

A.2 H₂O₂

CAHP Chronoamperograms were measured of a number of solutions with different concentrations of H₂O₂, in a randomized order, see Table A.1. The solutions were prepared following the dilution scheme shown in Figure A.1. For all measurements, the background electrolyte was 0.1 M NaClO₄. The porous layer was applied on a gold layer on silicon; a platinum spiral was used as counter electrode and a Hg/HgSO₄ (saturated K₂SO₄) reference electrode was used. CAs were measured at 0.3 V vs this reference electrode, with a I-scale setting of 10 μ A. The following day a CV was measured in fresh electrolyte (scan rate 5 mV s⁻¹).

Figure A.1 – Dilution scheme used in the preparation of the sample solutions for experiment CAHP.

CVH1 CV measurements were performed on a porous membrane on gold on silicon with a scan rate of 10 mV s⁻¹. After every CV, a certain amount of H₂O₂ (10 M) was added to the solution already present in the cell and mixed using a pipette. The concentrations of the solutions measured, are given in Table A.2. A Hg/HgSO₄ (saturated K₂SO₄) reference electrode was used and a platinum spiral counter electrode

CVH2 The previous experiment was repeated, but this time additions of H₂O₂ were alternated with dilutions by adding background electrolyte, to assess whether the observed effects are a result of time or concentration. The thus created concentration series is given in Table A.3.

Table A.1 – Codes, measurement number (randomized) and $[\text{H}_2\text{O}_2]$ for the sample solutions of experiment CAHP

Solution code	Measurement no.	$[\text{H}_2\text{O}_2]$ (mM)
A	4	50
B	5	25
C	9	12.5
D	10	6.25
E	8	10
F	2	5
G	6	5
H	3	2.5
I	7	2.5
J	1	2.5

A.3 Glucose

CVGD A Dropsens screen printed carbon electrode was used as working electrode in a glass beaker. The starting solution was 25 mL 0.1 M KCl containing 50 mM acetic acid buffer pH 5.1. 400 μL of 10 mg mL^{-1} PFS-VImCl (aq) was added, so the enzyme concentration in the cell was 160 $\mu\text{g mL}^{-1}$. Glucose oxidase solution (400 μL 10 mg mL^{-1} GOx in 50 mM acetate buffer pH 5.1) was added, resulting in 80 $\mu\text{g mL}^{-1}$. Increments of 0.1 M D-glucose solution were added and after each addition, the solution was bubbled with nitrogen to achieve mixing and possible deoxygenation. For every concentration, a CV (50 mV s^{-1}) was measured. The concentrations of the measured solutions are given in Table A.4.

CVGP A PFS porous layer was prepared with glucose oxidase in the layer. CVs of these layers were measured for a number of glucose concentrations at a scan rate of 10 mV s^{-1} . The background electrolyte was 0.1 M NaClO_4 . A redrod Ag/AgCl (saturated KCl) reference electrode (Radiometer Analytical, France) was used and a platinum counter electrode. The measured concentrations were: 0 mM, 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM, 2.0 mM, 3.9 mM and 4.8 mM.

CAGP CAs were measured of solutions with increasing glucose content. The background measurement was performed in the electrolyte: 0.1 M NaClO_4 and 50 mM acetic acid buffer pH 5.1. To this

Table A.2 – Concentrations H_2O_2 in the solutions of experiment CVH1.

CV number	$[\text{H}_2\text{O}_2]$ (mM)
0	0
1	1
2	2
3	4
4	6
5	10
6	15
7	20
8	25
9	30
10	40
11	60

was added $65 \mu\text{g mL}^{-1}$ glucose oxidase. A glucose/GOx mixture was prepared, (0.06 M glucose; 0.4 mg mL^{-1} GOx), which was incrementally added to the cell. After each addition, a pipette was used to mix the contents of the cell. The layer was fabricated on top of a gold electrode; a Ag/AgCl (saturated KCl) reference electrode was used, a platinum spiral counter electrode and CA measurements were performed at 0.8 V . The experiment was performed three more times as controls: in the first case bubbling with argon was used to provide mixing and remove oxygen from the solution; in the second case no glucose, but electrolyte containing only GOx was added; in the third case no GOx was added.

Table A.3 – Concentrations H_2O_2 in the solutions of experiment CVH2.

CV number	$[\text{H}_2\text{O}_2]$ (mM)
0	0
1	50
2	25
3	50
4	25
5	12.5
6	6.25
7	3.13
8	1.55
9	6.55
10	16.55
11	26.55
12	36.55
13	46.55
14	0

Table A.4 – Glucose concentrations corresponding to the CV numbers measured during CVGD.

CV/CA number	[glucose] (mM)
1	0
2	2.3
3	3.8
4	4.7
5	5.5
6	6.4
7	7.2
8	8.9
9	10.5
10	12
11	16.3
12	19
13	21.5
14	28.1

Appendix B

Impedance sensing using polymer-brush functionalised electrodes

B.1 Introduction

The pH sensing experiments discussed in [Chapter 6](#) were repeated on ITO electrodes functionalised with poly(methylmethacrylate) (PMAA) brushes. Furthermore, pH and glucose sensing experiments were performed on these electrodes after functionalisation with boronic acid (BA).

B.2 Experimental Details

B.2.1 Chemicals

(3-Aminopropyl) trimethoxysilane, 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid *N*-succinimidyl ester, 2-cyano-2-propyl benzodithioate, methacrylic acid, neutral alumina, MgSO_4 , and AIBN solution (0.2 M in toluene) were obtained from Aldrich and used without further purification. Toluene, dimethylformamide (DMF), ethanol and dichloromethane were obtained from Biosolve. Milli-Q water (Millipore) was used in all experiments.

B.2.2 Brush fabrication

Synthesis of the surface RAFT agent

4-Cyano-4-(phenylcarbono-thioylthio)pentanoic acid *N*-succinimidyl ester (300 mg, 0.80 mmol) and (3-aminopropyl) trimethoxysilane (139 μL , 0.80 mmol) were dissolved in anhydrous dichloromethane (50 mL). The

mixture was stirred for 12 hours at room temperature. Subsequently, the solution was washed with brine and the organic phase was dried over MgSO_4 . The solvent was removed under reduced pressure to leave a pink oil, which was used without further purification (0.42 g, 95 %).

Immobilisation of the surface RAFT agent

Indium tin oxide (ITO) substrates were first cleaned with RCA-1 clean and then rinsed extensively with water and ethanol. Cleaned ITO substrates were immersed in a 1 mM dry toluene solution of the surface RAFT agent. The substrates were kept in solution in the dark for 12 h at room temperature. After that, the ITO substrates were rinsed with dichloromethane three times, dried under a flow of nitrogen and stored in the dark for the surface-initiated RAFT polymerisation.

Surface-Initiated RAFT Polymerisation

Methacrylic acid was purified just before use by passing it over a short column of activated neutral alumina (Al_2O_3). Anhydrous DMF (10 mL), methacrylic acid (3.0 mL, 3.54×10^{-2} mol), sacrificial RAFT agent, 2-cyano-2-propyl benzodithioate (4.5 mg, 2.0×10^{-5} mol) and RAFT agent-functionalised ITO substrates were added to a 50 mL flask. AIBN solution (0.2 M in toluene) (0.075 mL, 1.5×10^{-5} mol) was added. The solution was degassed by purging with argon for 45 min, and then placed in a preheated oil bath of 70 °C. Polymerisation was allowed to proceed overnight (or 1 day). After polymerisation, the substrate was removed from the vial, washed with DMF and deionised water and immersed in Milli-Q water to remove any physisorbed polymer. Finally, the substrate was rinsed with ethanol and dried under a flow of nitrogen.

Post-polymerisation modification on PMAA brush:

First, the carboxylic acid groups of the poly(methacrylic acid) (PMAA) brush modified ITO substrates were activated by placing the samples in a solution of EDC (0.1 M) and NHS (0.2 M) in deionised water for 4 h followed by rinsing with ethanol. To introduce 3-aminophenylboronic acid (APBA), the NHS-activated PMAA brushes were immersed overnight in a 10.0 mg mL⁻¹ solution of APBA in 10 mM phosphate buffer (pH 7.2). After that, the substrates were rinsed with water and finally dried under a flow of nitrogen.

B.2.3 Impedance sensing

Impedance measurements were performed as described in Section 6.3.2. Glueing of the brush-functionalised electrodes was performed after

growth of the PMAA brushes, but before functionalisation with boronic acid. Glucose sensing experiments were performed for various glucose concentrations in PBS (0.01 M phosphate buffer) at pH 7.3 and at pH 8.9. pH sensing experiments were performed in PBS (0.01 M phosphate buffer).

B.3 Results and Discussion

B.3.1 PMAA-brush functionalised electrode

A PMAA brush is pH responsive: at low pH (pH below pK_a of the brush) the brush is in a collapsed state; at high pH (above the pK_a of the brush), it exists in a swollen state⁽¹⁾. Previous studies have investigated the effect of similar brushes on the electrochemical behaviour of the electrode⁽²⁾. Addition of the PMAA brushes did not improve our pH sensor however, but resulted in a mixed response: below pH 5 the slope of the $|Z|$ vs pH curve (the sensitivity of our sensor) is slightly larger (about 25 %) than for the bare ITO electrode, but above pH 5 the sensitivity decreases by a factor two, see Figure B.1. The pK_a of the surface of the PMAA brush is about 4.6⁽¹⁾; possibly above this pH the negative charge on the surface of the brush partially shields the underlying electrode from further increases in pH.

B.3.2 Boronic-acid brush functionalised electrode

In a second modification step the PMAA brush was functionalised with boronic acid (BA) groups. BA-containing materials are known to be responsive to glucose (and other sugars)⁽³⁾. The impedance of the BA-brush showed no response to any changes in glucose concentration, however (see Figure B.2). The pH response of the BA-brush was comparable to that of the PMAA-brush, see Figure B.3.

Figure B.1 – Response of the impedance of the PMAA-brush functionalised ITO electrode to changes in pH.

Figure B.2 – Response of the boronic acid functionalised brush to changes in glucose concentration at two pH levels.

Figure B.3 – Response of the boronic acid functionalised brush to changes in pH.

Summary

In this thesis, we look for ways to use the polymer poly(ferrocenylsilane) in sensor applications. Poly(ferrocenylsilane)s (PFS) contain ferrocene groups, resulting in redox activity. In the polymer's backbone, the ferrocene groups are alternated with silicon atoms. The two remaining side groups of the silicon atom can be freely varied and used to introduce additional functionalities in the polymer.

A poly-cationic polymer is obtained by introducing a vinylimidazole group, resulting in PFS-Vim⁺. Drying a mix of PFS-Vim with polyacrylic acid (PAA) results in a partially phase-separated layer. We have visualised this using electron microscopy (SEM) and X-ray scattering (SAXS). When the dried layer is exposed to ammonia, a porous membrane is formed. The size of the pores in the membrane can be influenced by oxidation and reduction of the PFS. We investigated fully oxidised and fully reduced membranes using electron microscopy (SEM) and X-rays (SAXS). Furthermore, we developed an electrochemical cell which allows us to carry out in-situ SAXS measurements: we can perform SAXS measurements while electrochemically changing the oxidation state of the layer. Using this cell, we cannot only observe the two extreme membrane states, but also the transition between them. Finally, we found that each redox state of the membrane has a unique impedance spectrum.

Subsequently, we attempted to use the porous PFS membrane to fabricate a sensor. The applicability of the porous membrane was tested for ascorbic acid, Fe³⁺, hydrogen peroxide and enzymatic sensors. We obtained some encouraging results, but the stability of the membrane, the slow response and the limited conductivity complicate matters. There is a lot left to be desired in the reproducibility and reliability of sensors based on a porous PFS layer.

Alternatively, in an attempt to construct a photonic sensor, we tested three types of PFS for use in two-photon-lithography. All three variants were suitable, but a number of obstacles had to be overwon. These obstacles—which will generally be encountered when developing a new resist for two-photon-lithography—and possible solutions were addressed in detail.

Additionally, we discovered that the technology used for fabricating porous membranes can also be used to make porous microparticles. The first step herein is the fabrication of PFS/PAA micro particles. We managed to do this by means of a simple microfluidic device: a T-junction that forms droplets of polymer solution in PDMS oil. Because the polymer solvent mixes with the PDMS oil, it slowly vacates the droplets, leaving behind a PFS/PAA particle. After a drying step they can be exposed to ammonia and porous PFS/PAA particles are obtained.

Finally, a reference-electrode-free pH and conductivity sensor based on indium-tin-oxide (ITO) electrodes was developed. During an attempt to make a pH and glucose sensor using polymer brushes on ITO, the response of ITO turned out to be dominant over the response of the polymer brushes. Moreover, the impedance was found to be a very good indicator of the pH and the conductivity of a solution. The impedance value at a low frequency (10 Hz) mainly depends on the double-layer capacity of the electrode, resulting in a pH sensor, while the value at a high frequency (300 kHz) depends on the resistance of the electrolyte and thus gives a measure of the solution's conductivity.

Samenvatting

Elektrochemisch meten met micro- en nanogestructureerde poly(ferrocenylsilaan) In dit proefschrift wordt gezocht naar manieren om de polymeer poly(ferrocenylsilaan) (PFS) te gebruiken voor het maken van sensoren. Poly(ferrocenylsilaan) is een redox-actief polymeer door de aanwezigheid van een ferrocen-groep in de keten. Deze groepen worden in de backbone van de polymeer afgewisseld met siliciumatomen. De twee overgebleven zijgroepen van het siliciumatoom kunnen vrij gevarieerd worden om extra functionaliteiten in de polymeer te introduceren.

Door het toevoegen van een vinylimidazolgroep aan de polymeer ontstaat een poly-kationisch polymeer. Wanneer deze polymeer gemengd wordt met polyacrylzuur (PAA) ontstaat een laag waarin fasescheiding optreedt. We hebben dit zichtbaar gemaakt met behulp van SEM en SAXS. Als deze laag vervolgens wordt blootgesteld aan ammonia, ontstaan er poriën in de laag. Door middel van oxidatie en reductie kan de grootte van deze poriën veranderd worden. Dit hebben we eveneens bestudeerd met elektronenmicroscopie (SEM) en röntgenstraling (SAXS). We hebben hiervoor een elektrochemische cel ontwikkeld, die ons in staat stelt om SAXS-metingen uit te voeren terwijl we de toestand van de laag veranderen. Hierdoor kunnen we niet alleen de extreme toestanden, maar ook de overgang tussen de twee extremen waarnemen met SAXS. Daarnaast bleek elke redox-toestand van het membraan een uniek impedantie-spectrum te hebben.

Vervolgens hebben we gepoogd het poreuze PFS membraan te gebruiken voor het maken van een sensor. Het poreuze membraan is getest voor zijn toepasbaarheid in ascorbinezuur-, Fe^{3+} -, waterstofperoxide- en enzymatische glucosesensoren. Er zijn hierbij bemoedigende resultaten behaald, maar de stabiliteit van het membraan, evenals de lage reactiesnelheid en de slechte geleidbaarheid van het materiaal zorgen voor de nodige problemen. Hierdoor laten de reproduceerbaarheid en betrouwbaarheid van de op de poreuze PFS-laag gebaseerde sensoren te wensen over.

Drie andere typen PFS zijn getest voor hun toepasbaarheid in twee-

foton-lithografie. Alledrie deze typen PFS bleken hiervoor geschikt te zijn, maar er moest wel een aantal obstakels overwonnen worden. Deze obstakels—die men in het algemeen zal tegenkomen bij het ontwikkelen van een nieuwe resist voor twee-foton-lithografie—en de mogelijke oplossingen hebben we uitgebreid besproken.

De techniek die werd gebruikt voor het maken van poreuze membranen is ook gebruikt voor het maken van poreuze micro-deeltjes. Hiervoor moesten er eerst micro-deeltjes van PFS/PAA gemaakt worden. Dit is gedaan door middel van een simpel microfluidisch device: een T-junctie waarin druppels van de polymeeroplossing gemaakt worden in PDMS-olie. Omdat het oplosmiddel van de polymeren mengt met de gebruikte olie, verlaat het langzaam de druppels, waarna een PFS/PAA deeltje overblijft. Na een droogstap en blootstelling aan ammoniak resulteert dit in poreuze PFS/PAA deeltjes.

Tot slot is een referentie-elektrode-loze pH- en geleidbaarheidssensor op basis van indium-tin-oxide (ITO) elektrodes ontwikkeld. In een poging een pH- en glucosesensor te maken met behulp van polymeerborstels op ITO, bleek de respons van ITO dominant over die van de polymeerborstels. Daarbij bleek impedantie een zeer goede uitleesmethode te zijn om onafhankelijk van elkaar de pH en de geleidbaarheid van een oplossing te meten. Door bij een lage frequentie (10 Hz) te meten wordt vooral de pH-afhankelijke dubbellaagcapaciteit van de elektrode bemonsterd, resulterend in een pH-sensor. Impedantiemetingen bij een hoge frequentie (300 kHz) bemonsteren juist de weerstand van de elektrolyet en zijn dus een maat voor de geleidbaarheid van de oplossing.

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Contributions

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Chapter 2

This chapter is the result of collaboration with Kaihuan Zhang, Dr. Mark Hempenius and Prof. Julius Vancso of the MTP group of the University of Twente and with Orsolya Czakkel from the Institut Max von Laue - Paul Langevin (ILL) in Grenoble, France. Polymer synthesis and porous layer formation were performed by Kaihuan Zhang. The electrochemical cell was modified by Hans L. de Boer from the BIOS-lab on a chip group. SAXS data were analysed by Orsolya Czakkel. Electrochemical measurements and subsequent data analysis were performed by Laura Folkertsma.

Chapter 3

This chapter is the result of collaboration with Kaihuan Zhang, Dr. Mark Hempenius and Prof. Julius Vancso of the MTP group of the University of Twente. Polymer synthesis and porous layer formation were performed by Kaihuan Zhang. Electrochemical measurements and data

analysis were performed by Laura Folkertsma.

Chapter 4

This chapter is the result of collaboration with Kaihuan Zhang, Dr. Mark Hempenius and Prof. Julius Vancso of the MTP group of the University of Twente and with Huib Vossen from the MESA+ Nanolab. The different polymers were designed by and synthesised in collaboration with Dr. Mark Hempenius and Kaihuan Zhang. Hans L. de Boer made the sample holder used for developing the samples. Huib Vossen manages the Nanoscribe machine. All nanoscribe experiments and subsequent analyses were performed by Laura Folkertsma

Chapter 5

This chapter is the result of collaboration with Kaihuan Zhang, Dr. Mark Hempenius and Prof. Julius Vancso of the MTP group of the University of Twente. Polymer synthesis was performed by Kaihuan Zhang. Microfluidic particle generation experiments were performed by Jonas Will under guidance of Laura Folkertsma. Further treatment of these particles was performed by Kaihuan Zhang, together with Jonas Will.

Chapter 6

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Publications

Journal papers

Laura Folkertsma, Kaihuan Zhang (co-first author), Orsolya Czakkell (co-first author), Hans L. de Boer, Mark A. Hempenius, Albert van den Berg, Mathieu Odijk and G. Julius Vancso. Synchrotron SAXS and impedance spectroscopy unveil nanostructure variations in redox-responsive porous membranes from poly(ferrocenylsilane) poly(ionic liquid)s. *Macromolecules*, (2017), Volume 50, Issue 1, Pages 296–302.

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About the author

Laura Hendriks was born in Boxmeer, the Netherlands, in 1988. After primary school in Sint Anthonis, she went to the Elzendaalcollege in Boxmeer. There she completed grammar school, combining the “Nature and Health” (Biology), “Nature and Technology” (Math, Physics and Chemistry) and “gymnasium” (Latin and Greek) tracks.

In 2006 she started the Bachelor’s programme Chemistry at the Radboud University in Nijmegen. She received her BSc degree in 2009 cum laude. In 2012 she also completed the Chemistry research Master cum laude in Nijmegen. As a part of her Master she went to Boston, USA to spend 6 months performing research in the Aizenberg Biomimetics and Biomineralization lab of Harvard University.

In June 2012 she started her PhD research in the BIOS-lab on a chip group of Albert van den Berg at the Twente University in Enschede (the Netherlands). Her main scientific interests are crystal growth, NMR, microfluidics and electrochemistry.

Laura is currently living in Enschede with her husband and their two children (2015, 2017).