Microelectrode Combinations of Gold and Polypyrrole Enable Highly Stable Two-electrode Electrochemical Impedance Spectroscopy Measurements under Turbulent Flow Conditions

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Abstract: An electrochemical impedance spectroscopy (EIS) sensor design is proposed based on a standard interdigitated electrode layout in which the smaller working electrode consists of gold (Au) whereas the larger combined counter and reference electrode is coated with a porous layer of polypyrrole (PPy) doped with polystyrene sulfonate (PSS) (PPy:PSS). Each electrode material was first characterized by EIS in a standard 3-electrode setup with subsequent spectra fitting by a modified Randles equivalent circuit. The differences in the spectra obtained by the PPy:PSS coated electrodes can be explained by an increased electroactive surface area due to the porous polymer film. The changes in morphology of the film are discussed with respect to the evolution of the elements of the electric equivalent circuit. When applying the Au/PPy:PSS electrode combination to a standard 2-electrode arrangement, the enlarged highly electroactive surface area of the PPy:PSS coating lowers the interfacial impedance in a way that mainly the gold working electrode contributes to the overall system impedance. Therefore, obtaining reproducible EIS signals depends only on the electrode’s open-circuit potential (OCP) and on additional adsorption events at the gold electrode/electrolyte interface. We present a protocol for microelectrode coating with PPy:PSS, which enables highly stable 2-electrode EIS experiments without the need of a reference electrode. This combination is believed to be very useful if an integration of sensing electrodes inside Micro Total Analysis Systems is aspired.

Keywords: electrical impedance spectroscopy · electrode · gold · interfaces · PPy:PSS

1 Introduction

Electrochemical impedance spectroscopy (EIS) is commonly used as a standard method to characterize electrode/electrolyte interfaces, sensing adsorption processes, formation of surface layers etc. In biosensor systems, EIS is widely used for studies of DNA hybridization interactions [1], immunosensing assays [2], virus detection [3], microbial layer formation [4], specific protein adsorption on self-assembled monolayers (SAMs) [5], or evaluation of lectin binding kinetics on glycopolymer brushes [6]. EIS measurements, especially in microfluidic applications, are typically carried out using an arrangement of two interdigitated electrodes of the same material [6–7]. In comparison to a 3-electrode setup, counter (CE) and reference (RE) electrode are combined to one larger electrode (CE/RE) in the 2-electrode setup, while the working electrode (WE) has a smaller size. The use of micro- or even nano-integrated interdigitated electrodes (IDEs) is preferred since the electric field could be confined close to the surface, which increases the measurement sensitivity and signal-to-noise ratio of molecular recognition compared to planar metal electrodes. It is also well known that the use of interdigitated electrodes of gold (Au) as WE and as CE/RE pair shows low reproducibility of electrical impedance spectra and an increased drift behavior. To tackle this issue e.g. baseline correction is used [8].

To achieve reproducible electrical impedance spectra under turbulent flow conditions, we propose an interdigitated electrode sensor design, in which CE/RE is selectively coated with a conducting conjugated polymer layer based on polypyrrole (PPy) doped with poly(4-styrenesulfonic acid) (PSS), while the WE consists of gold acting as the sensing electrode. In the following, this...
electrode combination is denoted as Au/PPy:PSS. Using a simple potentiostatic deposition protocol, the polymer can precisely be doped with poly(4-styrenesulfonic acid) (PSS) [9]. In general, the deposition process of polymers from monomer-containing electrolytes by applying voltage is termed electropolymerization, where the monomers are oxidized and polymerized in the solution close to the electrode forming polymer molecules [10]. While becoming larger and insoluble, the polymer chains are phase separated from the solution and adsorb onto the electrode surface [11]. It has been shown that the entanglement of two monomers prevents the anionic dopants from being expelled during the polymerization process with the consequence that cations are inserted in the films by direct covalent binding to the conducting polymer backbone [12]. Since the PSS sulfonate groups are irreversibly incorporated in the polymer matrix, the films behave as redox cation exchangers. Film formation can be explained by instantaneous nucleation, followed by three-dimensional growth of the nuclei until they overlap and form a continuous layer [13], although other mechanisms were discussed as well [14]. Depending on film thickness, two different charge transport mechanisms were proposed. For thin films, counter ion diffusion proceeds mainly linearly into the direction transversal to the macroscopic outer surfaces, whereas for thicker films concurrent ionic (diffusion) and electronic transport (migration) inside the porous structure has to be taken into account [15]. Therefore, the electrical impedance spectra of both growth regimes differ from each other. For thin films, they can typically be fitted by a modified Randles equivalent circuit as introduced by Ho et al. [16]. Thicker films can be modeled by a transmission line with ionic and electronic resistances of the polymer [17], respectively. The infinitely enlarged transmission line model can be interpreted as a Warburg impedance, representing homogeneous semi-infinite diffusion processes [18].

Thanks to the superior cation-exchange behavior and the large electrochemically active surface area, PPy:PSS films are today used in polymer batteries [19], as charge-controllable membranes for water purification [20], as a CE for solar cells [21], as neural recording electrodes [22] or as pseudo-reference electrodes [9,12a,23]. In EIS, gold is preferred as electrode material for sensing, especially for biochemical applications, because of its chemical inertness and the ease of binding SAMs as intermediate layers for subsequent immobilization of biomolecules [24]. PPy is also used as a pH-responsive film on platinum IDEs, where a thin polymer film is formed between adjacent digits to monitor penicillin induced acidifying of the PPy membrane by amperometric sensing [25]. Doped overoxidized PPy:PSS coated gold IDEs, where all digits are coated, are used in an amperometric setup for detection of dopamine released from cells [26].

The objective of this work is the development of miniaturized EIS sensors working in a 2-electrode configuration, which enable stable and repeatable EIS measurements for use in biosensing applications. We propose an EIS sensor based on a standard interdigitated electrode arrangement in which the WE consists of gold while the other, combined CE/RE, is coated with PPy:PSS. To our knowledge, the use of an IDE based EIS sensor with gold WE and PPy:PSS CE/RE has not been reported up to date.

The hypothesis is that due to the PPy:PSS coating the electroactive surface area increases and thus the interfacial impedance decreases in a way that almost only the gold WE contributes to the overall impedance of the system and therefore significantly improves the repeatability of the EIS signals, especially under turbulent flow conditions. It will be shown that this reproducibility depends only on the open circuit potential (OCP) behavior and additional molecular adsorption events at the gold electrode/electrolyte interface.

The paper is structured as following: After explaining the sensor chip fabrication process, the electrode regeneration process is investigated. Here, the cleanliness of the gold CE/RE is scrutinized by cyclic voltammetry (CV). Then, the two electrode materials are characterized individually by EIS in a standard 3-electrode setup. Detailed investigations are performed on the PPy:PSS layers by data fitting to a meaningful equivalent circuit modeling the electrode/electrolyte interface and addressing changes on the circuit elements to physical film formation processes. Additionally, the OCP is recorded for both electrode materials, respectively. Finally, the Au and PPy:PSS electrodes are combined in a 2-electrode configuration and the stability of the electrode signals is characterized.

2 Materials and Methods

2.1 Materials

For photolithography processes, all photoresists were purchased from MicroChemicals GmbH (Ulm, Germany).

For the electrochemical deposition of PPy:PSS, pyrrole (reagent grade, 98 %, Sigma-Aldrich Chemie GmbH, Munich, Germany) and poly(4-styrenesulfonic acid) solution (Sigma-Aldrich Chemie GmbH, Munich, Germany) were diluted with deionized water to a concentration of 200 mM each. Electropolymerization was carried out by mixing both solutions in a ratio of 1:1.

Electrode regeneration was performed using a solution of deionized water, ammonium hydroxide (NH₄OH, 25 %, Th. Geyer GmbH & Co. KG, Renningen, Germany) and hydrogen peroxide (H₂O₂, 30 %, MicroChemicals GmbH, Ulm, Germany) in the volume ratio of 7:2:1.

For electrode characterization, a phosphate buffer (PB) containing sodium chloride and phosphate buffered saline (PBS) were used. PB consisted of 50 mM NaH₂PO₄ and 150 mM NaCl while PBS consisted of 137 mM NaCl, 2.7 mM KCl and 10 mM phosphate buffer solution. Both electrolytes yielded a pH-value of 7.4.
As a redox system for CV measurements, the redox couple \([\text{Fe(CN)}_3]^{3-} / 4\) (HCF, Sigma-Aldrich Chemie GmbH, Munich, Germany) was added to PB.

### 2.2 Sensor Fabrication

Interdigitated electrodes were fabricated on 4” borosilicate glass wafers (Siegert Wafer GmbH, Aachen, Germany). For microelectrode definition, the photoresist AZ nLOF 2070 was spin-coated and patterned by standard UV-photolithography. Subsequently, 30 nm titanium (Ti) as an adhesion layer, 220 nm gold (Au) as the electrode material and 50 nm Ti as a protective layer were sputtered in a Nordiko NS 2550 sputtering system followed by a lift-off process. For insulation, a thin layer of Parylene C (PDS 2010 E LabCoter®, 1, Specialty Coating Systems, Indianapolis, IN, USA) was deposited. The layer was circularly patterned allowing the electrolyte to access the sensor area by a second photolithography step followed by a reactive ion etching in oxygen. Finally, the protective Ti layer on top of the gold electrodes was removed by wet etching with ammonium hydroxide-hydrogen peroxide solution just before the measurements to obtain a clean surface.

To modify the surface of the electrode, PPy : PSS was deposited by means of electropolymerization on the CE/RE using a Potentiostat/ Galvanostat Model 283 (EG&G Instruments, Princeton Applied Research, Berwyn, PA, USA) as described by El Hasni et al. [27]. The footprint of the bare gold WE (46402 μm²) was smaller than the footprint of the CE/RE (62775 μm²). A 200 μL droplet containing 100 mM PPy and 100 mM PSS was pipetted onto the electrode area and a potential of 1 V was applied to the CE/RE, while the WE was connected to the ground. Coatings with termination charges of 20 μC up to 270 μC were deposited. After deposition, the droplet was removed by rinsing with deionized water and isopropanol and followed by drying of the chip under a nitrogen stream.

To demonstrate their robustness, some chips were regenerated several times. A 60 minute cleaning in ammonium hydroxide-hydrogen peroxide solution does not only remove any deposited biomolecules from gold surfaces but also the PPy : PSS film formed by electropolymerization [28]. It has recently been shown by EIS that a similar treatment reveals a clean gold surface [29].

### 2.4 EIS Measurements

Au as well as PPy : PSS coated electrodes were initially characterized in a 3-electrode configuration with a platinum wire CE and an Ag/AgCl RE. The platinum wire had a significantly larger surface area than the WE to deliver sufficiently high current. A Novocontrol Technologies Alpha-A High Performance Frequency Analyzer (Novocontrol Technologies GmbH & Co. KG, Montabaur, Germany) was used as impedance analyzer. Spectra were obtained in the frequency range between 1 Hz and 10 MHz with an applied voltage amplitude of 10 mV. In this frequency regime, 800 points were obtained to ensure sufficient data points for equivalent circuit fitting.

For the Au/Au and Au/PPy : PSS electrode combinations, EIS was carried out in a 2-electrode setup. Here, a Hewlett Packard 4294 A Precision Impedance Analyzer (Palo Alto, CA, USA) was used. A sensor chip was clamped in a custom-made fixture and electrically contacted by pogo pins (Mill-Max Manufacturing Corp., Oyster Bay, NY, USA). The sensor was connected to the analyzer via coaxial cables to enable 4-point probe measurements. Impedance values were recorded at 50 frequencies ranging from 100 Hz to 3 MHz, uniformly distributed at the logarithmic axis. A full spectrum was taken within approximately 15 s. A voltage amplitude of 10 mV was applied. For monitoring the time-dependent change of the double-layer capacitance, the imaginary part of the impedance at 664 Hz was selected as sensor signal.

All EIS experiments were carried out at OCP in PB or PBS, respectively.

### 2.5 OCP Measurements

The OCP was recorded over time for Au as well as for PPy : PSS coated electrodes in PB using a Potentiostat/ Galvanostat Model 283 (EG&G Instruments, Princeton Applied Research, Berwyn, PA, USA). Here, a 3-electrode setup with an Ag/AgCl electrode pellet (Type EP2, World Precision Instruments, Inc., Sarasota, FL, USA) as CE and an Ag/AgCl double junction RE (World Precision Instruments, Inc., Sarasota, FL, USA) were utilized. PPy : PSS and Au were connected to the WE terminal of the potentiostat, respectively. In contrast to standard configurations where platinum is used as CE, the Ag/AgCl pellet provides a more stable electrode potential in combination with a large area to deliver enough current to the system.

### 3 Results and Discussion

#### 3.1 Sensor Fabrication

A circularly patterned interdigitated Au-electrode is shown in Figure 1a). We utilized a circular-shaped interdigitated electrode design, where the smaller sized electrode defines the WE, whereas the slightly larger one
This demonstrates the high reproducibility of our method during electropolymerization and are in a close range. Coated electrodes and a bare gold electrode were conducted using a Tencor P-10 surface profiler (KLA-Tencor, Milpitas, CA, USA). The overlaid scans are depicted in Figure 1 in the supporting information for three deposition processes with different charge termination criteria as shown in Figure S-2c). Thin layers at charges below 120 μC exhibit optical interferences indicating a thin layer, which matches the results obtained by profilometry. For a termination charge value of 170 μC, a brownish homogenous layer was formed. Since the growth was not only in the normal direction of the surface, an increasing number of dark brownish islands appeared for a termination charge value of 220 μC. Layers deposited with Q=270 μC tended to delaminate most likely due to too large mechanical stress during growth. This effect was in clear correlation with the decreased growth rate. Scanning electron microscopy (SEM) pictures shown in Figure 1c) reveal that the bare gold electrode shows small grains. For gold layer deposition, a standard DC magnetron sputtering process was used. Typically, sputtered gold films show a grainy structure compared to thermally evaporated films [32]. The preparation of PPy:PSS layer on gold electrodes can be easily achieved in a potentiostatic electropolymerization process [27]. An SEM picture of the deposited PPy:PSS (170 μC) layer is shown in Figure 1d). Polymer layers grown by electrodeposition typically exhibit an enhanced surface area compared to the bare gold layers [33].

### 3.2 Electrode Regeneration

It is worthwhile to note that stability and reliability of the electrode/electrolyte interface are crucial for every EIS experiment. The purity of the surface affects the drift of the impedance signal significantly. As already investigated in detail in [28–29,34], we favored cleaning in ammonium hydroxide-hydrogen peroxide solution which removes biomolecules as well as the PPy:PSS coating. A clean surface is a precondition to ensure repeatable and reproducible EIS sensor signals. To ensure quality of the electrode after regeneration, the CE/RE has been cleaned according the procedure described above. Then, a layer of PPy:PSS (Q=170 μC) was potentiostatically deposited. Afterwards, the electrode was regenerated leading to removal of the PPy:PSS layer. This procedure of regeneration and deposition was carried out for three times. Finally, a last regeneration step was conducted. After each regeneration step, CV measurements were performed in PB in scan range between -0.9 V and 0.9 V vs. RE at a rate of 50 mV/s. For each measurement, ten cycles were collected. The CV’s of the last cycle for each measurement are shown in Figure 2.

The CV curves have a similar shape evidencing a reproducible clean surface condition after multiple electrode regeneration in the ammonium hydroxide-hydrogen peroxide solution.
For the CV measurements, PB without redox couples was used corresponding to the electrolyte in the EIS measurements. To gather more details about surface’s purity, a CVs was conducted in PB including HCF after 5-fold PPy : PSS deposition and cleaning cycles. The curves of $n=10$ CV cycles are shown in Figure S-3 in the supporting information. The peak position observed during $n=10$ cycles did not change. This indicates a reproducible cleaning process in ammoniumhydroxide-hydrogen peroxide solution and a clean surface. It is worthwhile to note that the use of HCF as redox couples hampers the stability of the gold electrodes [29], so the experiments using HCF as an additive were only done once on each sensor chip.

### 3.3 Electrode Characterization

At first, both electrode materials, Au and PPy : PSS, were characterized independently by EIS. Impedance spectra of the bare gold CE/RE as well as spectra of differently deposited PPy : PSS layers on CE/RE were taken with the 3-electrode setup as described above. Figure 3 depicts the magnitude of the electrical impedance over frequency.

The electrodes exhibit a typical capacitive behavior at low frequencies and a resistive behavior at higher frequencies. After electrodeposition of PPy : PSS on the CE/RE, the resistive plateau observed in Figure 3 broadens to a frequency range between 10 kHz and 2 MHz combined with a significant decrease of the interfacial impedance at low frequencies. This effect has also been observed by Cui et al. [30c] and Sun et al. [35]. Compared to Cui et al., the cutoff frequency, deposition charge, film thickness and the absolute impedance values differ because they used a different deposition setup with different electrode area, shape and arrangement. In addition, different polymerization voltage and buffer solutions for the EIS measurements were used. For our setup it is argued that the adjacent fingers of the interdigitated electrode design lead to more E-field contributions in tangential direction of the electrodes and forces more spherical diffusion instead of planar diffusion behavior. Vorotyntsev et al. pointed out that the initial growth up to about 200 nm in thickness takes dominantly place on the upper part of the electrode surface [31]. Further polymerization combines this vertical growth with film extension along the insulator surface. It can be concluded that this lateral growth can be correlated to closing of the pores even at thin layers, depending on the deposition conditions. At frequencies beyond 1 MHz, parasitic capacitive contributions from the measurement setup have an increasing impact.

For more detailed investigation of the change in electrical impedance of the electrode/electrolyte interface, the obtained spectrum of the bare gold CE/RE before electrodeposition and the PPy : PSS coated CE/RE at different deposition charges as indicated in the legend. EIS measurements were carried out in PB in a standard 3-electrode setup using a platinum wire as CE and an Ag/AgCl pellet as RE.
The impedance of the CPE is given by $Z = 1/[Q \cdot (j\omega)^n]$ according to [39c, 40].

Figure 5 shows the fitted curve and the measured spectrum in comparison. For the bare gold CE/RE, $R_{SOL}$ is determined to be 562.18 \(\Omega\) (±2.27%). The elements modeling the electric double-layer are calculated to $Q_{CPE_{DL}} = 21.1 \text{nF} \cdot \text{s}^{-1/2}$ (±2.28%) and $n(CPE_{DL}) = 0.92$ (±0.25%), respectively. The charge-transfer resistance $R_{CT}$ is determined to be $5.7 \cdot 10^{20} \Omega$. The comparatively high value of $R_{CT}$ is because no redox couples were present in the electrolyte and thus mainly non-faradaic processes occurred at the electrode. The gold electrode exhibited a rough surface, which can be deduced from the SEM picture in Figure 1c) and is consistently represented in the data by an exponent $n(CPE_{DL})$ smaller than 1 [39b]. For sputter-deposited gold thin films, the roughness was investigated in more detail before [32, 41].

Although the fitted data matches the spectrum in a wide frequency range, a slight mismatch in the high frequency regime can be observed (see Figure 5). In addition, the limited electrical resistance of the PPy:PSS layer needs to be addressed. Thus, the electric equivalent circuit in Figure 4a) is slightly modified and expanded as shown in Figure 4b).

For a more accurate fit, the circuit is expanded by a capacitor modeling parasitic effects $C_{PAR}$ and a resistor $R_{LEAD}$ modeling the Ohmic losses between the terminal of the impedance spectrometer and the electrode/electrolyte interface. This includes the electrical resistance of the deposited PPy:PSS layer on CE/RE (different transferred charge during electropolymerization) and the resistance of the coaxial cables, pogo pins to connect the chip, as well as gold leads on the chip and the electrical resistance of the deposited PPy:PSS layer on top of the gold electrode.

The fitted values of the circuit elements including the relative errors for different PPy:PSS layers on CE/RE (different transferred charge during electropolymerization) are summarized in Table S-1 in the supporting information and depicted in Figure 6. Values for bare gold CE/RE are indicated by “0 \(\mu\text{C}\\) deposited charge. The fitted curve is added to Figure 5 to demonstrate the improvement of the fit in the high frequency regime due to taking parasitic effects into account. This improvement is clearly visible in the inset of Figure 5. In addition, values for the bare gold WE are listed in Table S-1 (gray column) and a comparison between gold WE and gold CE/RE is depicted in Figure S-4 of the supporting information. As expected, the impedance magnitude value (at 1 Hz) of the WE is higher than the magnitude of the CE/RE and it is scaling with the ratio of the respective electrode areas.
The change in electric properties due to the PPy:PSS coating deposited with different charge termination criteria did not only increase the electrochemical stability of the combined CE/RE, but also provided information about the growth mechanism and the layer structure of the polymer film. For a complete discussion, it is noteworthy that the parasitic capacitance $C_{PAR}$ is comparably small and thus contributes only at higher frequencies (compare inset Figure 5). Since $C_{PAR}$ is constant and due to the experimental setup, we will not focus on the discussion of this circuit component.

It can clearly be seen, that a bare gold electrode exhibits by 2 orders of magnitude a significantly higher interface impedance in the low frequency regime than the same electrode coated with PPy:PSS (Figure 3). The low and medium frequency regime is dominated by the electrical double-layer. For electrolytes containing active redox species, the charge-transfer would dominate at low frequencies [18a]. Due to the lack of active redox couples in our system, only non-faradaic processes occur. This can be derived from the fact that in our data the electrode/electrolyte interface can be modeled using the equivalent circuit shown in the red dashed box of Figure 4b). After the deposition of PPy:PSS, the double layer capacitance $Q(CPE_{DL})$ increases by more than two orders of magnitude (compare Figure 6a) and Table S-1). This can most likely be explained by a pore formation in the polymer film, which increases the electroactive area and thus the volumetric capacitance, as already discussed by Proctor et al. [43]. From our SEM images in Figure 1c) and 1d), one can see that the porosity of the polymer electrode increases compared to the gold electrode. This result matches with the values extracted from the equivalent circuit taking into account that the exponent $n(CPE_{DL})$ is typically a measure for the roughness of the electrode, where $n=1$ represents ideally flat surfaces while lower values represent increasing surface roughness [39]. The increased roughness of the PPy:PSS layer compared to bare gold was already investigated by atomic force microscopy [44].

Figure 6c) shows the evolution of the electrolyte resistance $R_{SOL}$ and $R_{LEAD}$. While $R_{SOL}$ was almost constant as expected (same electrolyte), the lead resistance increased slightly with increasing charge transferred during electropolymerization of the PPy:PSS layer for $Q \leq 170 \mu C$. For $Q=220 \mu C$, a severe increase can be observed. To understand these changes, the origin of $R_{LEAD}$ needs to be discussed first. In a 3-electrode setup, which we used for the characterization of the bare CE/RE and the PPy:PSS coated CE/RE, the Ohmic resistance between the working electrode terminal of the impedance analyzer and the electrode/electrolyte interface can’t be neglected completely. Coaxial cables, pogo pins to connect the chip as well as gold leads on the chip contribute to the measured impedance. It is obvious that the PPy:PSS layer does not only form a volumetric capacitance, but increases the Ohmic resistance due to a prolonged current path. For low deposition charges, the growth mechanism is mainly in normal direction to the surface as discussed before [31]. The layer thickness increases as depicted in Figure S-2a) and S-2b) and thus, the Ohmic resistance increases. It was earlier proposed that at higher deposition charges pores close at the bottom and deep in the bulk of the film, while on its top new pores form. This mechanism was described by Cui et al. [22a], but has so far not been discussed in EIS with respect to a precise equivalent circuit modeling. The proposed mechanism could also explain the data shown in Figure S-5 of the supplementary material. There, we plotted the impedance magnitude versus different charge termination for three different frequencies. The build-up of a large volumetric capacitance is a low frequency effect (dashed blue line), while the increase of Ohmic losses plays a role at higher frequencies due to neglectable influence of the double-layer capacitance $CPE_{DL}$ (dashed black line).

While the characterization of each electrode material was carried out by EIS in a 3-electrode setup, the combination of both materials with a bare gold electrode as smaller WE and a PPy:PSS electrode as larger CE/RE was investigated in a 2-electrode setup. For the following experiments, a PPy:PSS layer deposited with charge termination at 170 $\mu C$ has been chosen. This is a good tradeoff between low interfacial impedance compared to gold and a high porosity as indicated by the low exponent $n$ of $CPE_{DL}$. In preliminary experiments, we observed
cracks in thinner layers after their exposure to liquids for 1 h (data not shown). Thicker layers exhibited a high resistance $R_{\text{LEAD}}$ and tend to delaminate. To investigate the performance of the two electrode combinations, transient EIS measurements were performed in the 2-electrode setup using electrode combination 1 (WE: gold; CE/RE: gold) and combination 2 (WE: gold; CE/RE: PPy: PSS), respectively. After an electrode regeneration step, a 10 μL droplet of PB was incubated onto electrode combination 1 at $t=0$ s for 30 minutes. Afterwards, the chip was rinsed with 5 mL deionized water provided by a syringe and dried under a stream of nitrogen. Such a manual procedure of media exchange is in general disturbing the electrochemical equilibrium at the interface and thus, hampering the stability of the entire system. After drying, a fresh 10 μL droplet of PB was incubated again for 30 minutes. During that whole first cycle, EIS measurements were continuously taken. After the first cycle, the chip was regenerated and the CE/RE electrode was modified by electropolymerization of PPy: PSS as described. Then, a second cycle with electrode combination 2 was done. For verification of the repeatability of the measurement, the chip was regenerated and the two cycles were repeated four additional times. It is noteworthy that the same chip was used for all transient EIS measurements.

For monitoring the change of the double-layer capacitance over time, the imaginary part of the impedance at 664 Hz was used. The phase progression curves in Figure S-6b) and S-7b) for both electrode combinations reveal that at this frequency the capacitive effects dominate the impedance, because the phase angle approaches $90^\circ$. Our choice to monitor the double-layer changes at this frequency is due future application of the microelectrodes as biosensors. Adsorbed biomolecules, which bind onto or near the WE, will enlarge the average distance between counter ions and electrodes leading to a change of the electric double-layer capacitance.

In the low frequency range, the double-layer capacitance modeled by a CPE dominates the overall impedance of the electrode/electrolyte interface. From the obtained circuit elements by means of equivalent circuit fitting, the CPE of the gold WE is $Q(CPE_{\text{DL}}) = 15.6 \, \text{nF s}^{(n-1)}$ and $n(CPE_{\text{DL}}) = 0.94$ leading to an impedance at 664 Hz of $Z_{\text{WE}} = 25.337 \, \text{k}\Omega$. For the PPy: PSS (170 μC) coated electrode, $Q(CPE_{\text{DL}}) = 3.22 \, \mu\text{F s}^{(n-1)}$ and $n(CPE_{\text{DL}}) = 0.65$ are obtained (compare table S-1 in the supporting information) leading to an impedance of $Z_{\text{CE}} = 1.377 \, \text{k}\Omega$. In a 2-electrode setup, these two interface impedances are connected in series. The solution resistance $R_{\text{SOL}}$ between the two electrode/electrolyte junctions is comparably small. Here, almost only the electrode bearing the higher impedance and thus the electrode/electrolyte interface at the gold WE contributes to the overall impedance of the 2-electrode system. This is due to the increased electroactive surface area of the CE/RE and the resulting high volumetric capacitance of the PPy: PSS electrode.

Figure 7 shows the transient of the imaginary part of the impedances normalized to the initial values when PB was manually applied at $t=0$ s. The numbers in the insert refer to the number of the cycle.

With regard to the Au/Au electrode combination 1, the time-depending progressions of the imaginary part show significant deviations after each electrode regeneration without any reproducibility (see Figure 7a), whereas for electrode combination 2 reproducible electrical impedance spectra were obtained (see Figure 7b) even after a 10-fold regeneration of the chip.

Corresponding impedance measurements, carried out at the beginning of the cycles, are depicted as Bode plots in the supplementary information for electrode combination 1 (Figure S-6) and 2 (Figure S-7), respectively. The AC magnitude and phase using electrode combination 1 differ in every cycle even though the same sensor chip was used. This irreproducibility can be eliminated by using a PPy: PSS film as CE/RE as in electrode combination 2. The magnitude and phase progression presented in

![Figure 7](image-url)
Figure S-7 only show slight variations even after 10-fold usage of the same chip.

The same experiments were repeated for PBS electrolyte. The results are presented in the supporting information (Figure S-8, S-9 and S-10) and show the same trend. The use of electrode combination 1 (Au/Au) tends to exhibit uncontrollable drift, while the use of PPy:PSS as CE/RE electrode material (electrode configuration 2) improves the repeatability. The drift of the system in PBS was slightly higher, most likely because the additional, relatively large potassium ions adsorb at the gold surface compared to PB, where only smaller ions are present.

In our studies, we generally focus on these two buffer systems because we aim for a direct detection of biomarkers from physiological solutions without the need of adding a redox system. We already proposed such a sensor concept as a biosensor to monitor the binding of streptavidin to a thiolated SAM, which was immobilized to the gold WE, followed by binding of a biotinylated horseradish peroxidase [44]. The benefit of PPy:PSS as the CE/RE material is that unspecific binding was suppressed. In this case, only changes at the WE are monitored and sensitivity and stability of the bioassays are improved [44]. In future, we aim to embed the proposed sensor type into a digital microfluidic platform. In such systems, very small amounts of liquids in terms of discrete droplets are manipulated between hydrophobic surfaces by means of electrostatic forces [45]. The sensor surface is wetted with the analyte and the analyte is replaced after incubation by so-called “passive dispensing” [46]. This exchange disturbs the electrochemical equilibrium. In comparison, many continuous flow microfluidic systems with integrated EIS sensors were described [6–7, 47]. Under continuous flow conditions, the electrode potentials can stabilize and are not disturbed by a turbulent media exchange and by drying the sensor spot as in our case.

To investigate the electrode combinations in detail, we performed transient OCP measurements with both electrode materials separately using PB electrolyte in a 3-electrode setup. Here, we carried out the experiments in a beaker to meet the spatial requirements for the bulky external counter- and reference electrodes.

The obtained OCPs for a gold electrode and PPy:PSS coated electrode are shown in Figure 8. For the gold electrode, the OCP exhibits a severe drift behavior with overlaid fluctuations, and in addition, we observed a significant peak after media exchange. In comparison, the OCP for the PPy:PSS electrode drifts only slightly except the short duration of electrolyte exchange, which was done in the same way as for the transient EIS measurements. The OCP of the PPy:PSS coated electrode was not affected although the electrochemical equilibrium was heavily disturbed by the electrolyte exchange process.

The OCP measurements at the gold as well as at the PPy:PSS coated electrode perfectly match the transient EIS results. The irreproducibility and drift of the 2-electrode system consisting of two gold electrodes (Au/Au electrode combination 1, Figure 7a) are high, whereas the Au/PPy:PSS electrode combination 2 show reproducible results as presented in Figure 7b.

In a standard 3-electrode setup, a stable (pseudo-) RE with a constant OCP in interaction with a CE of low interface impedance to deliver sufficient compensation current is used. In comparison in the 2-electrode setup proposed in this work, these two features are combined in one electrode, the CE/RE, consisting of PPy:PSS which shows a nearly constant OCP behavior (Figure 8) and low interface impedance (Figure 3) compared to the gold electrode due to its high electroactive surface area. Therefore, the peaks and drift observed in Figure 7b) can be correlated to the settling of the OCP equilibrium at the Au electrode surface at the beginning of each cycle. By carrying out similar experiments, Lazar et al. pointed out that a severe drift in the EIS signal occurred within the first 30 minutes [29]. Drifts were always observed, independent on the particular electrode cleaning procedure. For electrode cleaning with NH$_2$OH:H$_2$O$_2$:H$_2$O, also used in this work, the lowest charge-transfer resistance was observed which was taken as an indicator for a clean surface. The increase of the normalized imaginary part of the impedance (Figure 7b) can be attributed to the adsorption of molecules at the electrode surface and the build-up of a capacitive layer hindering the electron transfer through the interface at the gold electrode.

Furthermore, in most of the EIS experiments described in the scientific literature, CV curves are shown only after establishing a constant OCP or after subtracting the drift, while experimental conditions are not changed. Therefore, in particular in microfluidic applications, constant flow rates above the sensor surfaces are desirable. In contrast, the experiments in this work were carried out under non-reproducible flow conditions at the electrode/electrolyte interface by pipetting tiny droplets onto the sensor electrode area, washing the sensor surface.
with deionized water and drying the sensor spot, which disturbs the electrochemical equilibrium significantly. This treatment hampers reproducible drift behavior, as shown in Figure 7 and Figure 8. In contrast to Figure 7b, the drift depicted in Figure 7a is highly irreproducible in the 2-electrode setup when gold is used as WE and CE/RE. Here, the OCP stabilizes at both electrodes independently. The individual drifts at both electrodes superimpose in the measurements. This is not the case when an OCP stable PPy : PSS electrode is used as CE/RE.

4 Conclusion

We propose a circular EIS sensor design based on a standard IDE layout with smaller gold WE, whereas the larger CE/RE is coated with PPy : PSS by a precise, charge-limited, potentiostatic electropolymerization protocol. The PPy : PSS coating possesses a highly electroactive surface area, which lowers the interfacial impedance in a way that almost only the gold WE contributes to the overall impedance of the system, leading to reproducible EIS values even after electrode regeneration. We explained the electrode characteristics observed in EIS measurements by modified electric equivalent circuits. The combined PPy : PSS CE/RE provides two key features: firstly, a low interface impedance compared to the WE, which ensures that enough current can be delivered into the system. This is a standard requirement for a good CE. Secondly, PPy : PSS provides a stable reference potential, which meets the requirements for a pseudo-reference electrode. In addition, the stable OCP values at the PPy : PSS layer ensure that the drift behavior of the 2-electrode configuration only depends on the electric double-layer capacitance of the gold WE.

This compact, circular electrode design will be of particular importance in a digital microfluidic setup, where electrodes are quickly wetted and the media is replaced by passive dispensing in a repeated manner. We anticipate that the utilization of PPy : PSS as the CE/RE material in our interdigitated sensor designs will increase the sensitivity for future biosensor applications.

Acknowledgements

The authors express their sincere thanks to Jochen Heiss, Dorothee Breuer and Ewa-Janina Sekula for their assistance in sensor fabrication, Nora Menzel and Anabel Mernitz for carrying out some experiments, Lea Hansmann for valuable preliminary work during her bachelor thesis and Dagmar Leisten from the Institute of Materials in Electrical Engineering 2 at RWTH Aachen University for taking the SEM images and Dr. Ilia Valov for carefully reading the manuscript. Open access funding enabled and organized by Projekt DEAL.

Data Availability Statement

All relevant data generated or analyzed during this study are included in this published article and its supplementary information files.

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