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A miniaturized voltammetric pH sensor based on optimized redox polymers

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ABSTRACT

A miniaturized pH sensor based on the voltammetric determination of the potential difference between a pH dependent toluidine blue O modified redox polymer and a pH independent osmium complex modified redox polymer was developed. Gold micro electrodes of various dimensions were modified with the two redox polymers by non-manual deposition procedures. Square wave voltammetry was used to determine the oxidation potentials of the polymer bound redox species allowing the precise determination of the pH value of the electrolyte solution without the need of a stable reference electrode.

1. Introduction

Determination of the pH value is one of the most often performed measurements in modern laboratories. Depending on the application and the precision needed, there are many different pH sensors available today, ranging from pH strips with low precision up to very precise pH meters based on glass electrodes.

Most commercial pH-meters are based on the potentiometric determination of the activity of protons typically using glass membranes or ionophors. However, one major drawback of precise potentiometric measurements is the logarithmic relation with a slope of only 59 mV per pH unit. Evidently, this implies a very accurate determination of a small potential difference which is only possible at the required accuracy using a highly stable reference electrode. Since the potential of a reference electrode is typically determined by the activity of an anion such as Cl^- ions in solution a relatively large electrolyte reservoir is indispensable to ensure constant activity of the counter ion. Thus, despite the development of miniaturized pH sensors e.g. based on ion-selective field-effect transistors were proposed many years ago, miniaturization of potentiometric pH sensors was restricted due to the problems caused by the concomitant miniaturization of the reference electrode.

Alternatively, voltammetric or amperometric methods can be designed to determine the pH value by measuring the redox potential of a suitable pH-dependent redox couple in comparison to a pH-independent redox couple as internal reference [1–3]. In most cases a quinone/hydroquinone redox couple was used [1,2,4] and investigated in detail [5–7]. However, also other pH-dependent redox systems like nitrosophenyl compounds [8], poly[tetra(4-aminophenyl)porphyrin] [9] and poly(4,4′-diaminobiphenyl) [10] were suggested. In most studies the potential of the redox couple and hence the pH value was measured using a conventional Ag/AgCl reference electrode [9,11], hence similarly preventing miniaturization due to the need for the large volume reference electrolyte with constant counter ion activity. To overcome this a few studies proposed to use a second pH-independent redox species, mainly ferrocene and its derivatives [8,12] as internal reference.

Another crucial aspect for the possible miniaturization of voltammetric pH sensors is the stable immobilization of the redox species on the microelectrode surface. Modification of electrode surfaces with monolayers of the two redox species [8,12] or modification of the electrode material itself [13] was shown, however, immobilization of polymers containing bound redox couples was not investigated in deep detail and in most cases the second pH-independent redox couple was not simultaneously immobilized [14,15]. Another strategy is based on the redox activity of oxidized carbon functional groups on the electrode surface itself and the related pH-dependent shift in their formal potential. This approach was successfully applied for monitoring pH value modulations in intact brain tissue [16]. Although the immobilization of the two redox species in a monolayer configuration offers fast response times, the current is proportional with the electrode surface area. Hence, upon miniaturization only very tiny currents are obtained.

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In contrast, polymer-bound redox species allow for electrically connecting a substantially higher number of redox species to the electrode surface thus providing higher current per geometric area.

In order to overcome limitations of miniaturized potentiometric and voltammetric pH sensing strategies, we developed redox polymers with covalently linked Os complexes as pH independent redox species and redox polymers containing a pH-dependent phenothiazine moiety. By electrochemically induced deposition of both polymers on gold microelectrodes followed by electrochemically induced crosslinking of the deposited polymer film voltammetric pH sensors were obtained.

2. Experimental

2.1. Material and methods

Methanol (CH_3OH), ethanol (EtOH), isopropanol ($^{i}PrOH$), 1,4-dioxane, *n*-pentane, *n*-hexane, diethylether (Et_2O), tetrahydrofurane (THF), *tert*-butylmethylether (MTBE), acetone, ethylacetate (EtOAc), toluene, dimethylformamide (DMF), ethylene glycol, dimethylsulfoxide (DMSO), dichloromethane (CH_2Cl_2), HCl (aq., 37% w/w), NaCl, Na_2SO_4, NH_4Cl, NH_4OH (aq., 25% w/w), NaOH, KOH, $NaHCO_3$, Na_2CO_3, K_2CO_3, $KHSO_4$, and citric acid monohydrate were obtained from J.T. Baker (Deventer, Netherland). $K_2HPO_4{}^*3H_2O$ was obtained from Merck (Darmstadt, Germany). Azobisisobutyronitrile (AIBN, 99%), triethylamine (> 99.5%) and 4 M HCl*dioxane were purchased from Fluka (Buchs, Switzerland). 2-Pyridinecarboxyaldehyde, allyl methacrylate (AllMA), *n*-butyl acrylate (BA), glyoxal, pentanol, 2-chloroethylamine hydrochloride, 2,2′-bipyridine, dry dimethylformamide stored under argon over molecular sieve (DMF_{dry}) and di-*tert*-butyl dicarbonate were obtained from Acros (Geel, Belgium). Poly(ethylene glycol) methacrylate (PEGMA), dimethylaminoethyl methacrylate (DMAEMA), styrene (ST), 2 $KHSO_5{}^*KHSO_4{}^*K_2SO_4$, ethylene diamine, oxalylchloride, 2-chloro-5-(hydroxylmethyl)pyridine, pyridiniumtoluene-4-sulfonate, trifluoroacetic acid (TFA), triphenylphosphine (PPh_3), *tetrakis*-triphenylphosphine palladium (0) ($Pd(PPh_3)_4$), Palladium on charcoal (10% w/w, Pd/C), copper iodide (> 99.99%) and NaH (60% w/w in mineral oil) were obtained from Aldrich (Steinheim, Germany). Argon and hydrogen were purchased from Air Liquide (Düsseldorf, Germany). Osmium-bis-*N*,*N*-(2,2′-bipyridyl)-dichloride was synthesized according to [17].

^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker DPX 200, Bruker DPX 250 and a Bruker DRX 400 spectrometer. Deuterated solvents were obtained from Deutero (Kastellaun, Germany). Chemical shifts in ppm (δ) were referenced to the residual solvent signal. All reference signals were set to values according to *Gottlieb et al.* [18]. NMR data were evaluated using the MestReNova v6.2.0 software or MestRec Lite v 4.59 software (MestRec, Santiago de Compostela, Spain). MS spectra were recorded on a Finnigan MAT LCQ Classic.

The solid content of the Os-complex modified polymers were evaluated gravimetrically. For this, samples of redox polymer suspensions were weighed and afterwards dried at 120 °C for 30 min and reweighed.

2.2. Procedures

2.2.1. Immobilization of the Os-complex modified redox polymer on gold disc electrodes

Gold disc electrodes (100 μm or 50 μm diameter) were polished manually on polishing cloth successively with each of the following suspensions for 5 min: diamond 3.0 μm, Al_2O_3 1.0 μm, Al_2O_3 0.3 μm. Polishing cloth and the suspensions were purchased from Leco (Mönchengladbach, Germany). The freshly polished electrodes were cleaned by ultrasonication in deionized water and then dried in air. The deposition of the redox polymers (P029-TB (50 μL, 3.0% w/w in H_2O) and P032-P231 (200 μL, 0.5% w/w in CH_3CN) was performed in a gold coated micro well plate, which served as counter electrode (CE) and pseudo reference electrode (RE). For the electrodeposition the following potential pulse sequences were applied using an Autolab PGSTAT 12 potentiostat (Autolab, Utrecht, The Netherlands) controlled by the Nova 1.7 or Nova 1.9 software:

100 μm gold disc electrode: (-2.5 V/0.1 s/-0.2 V/1.0 s) 100 repetitions

50 μm gold disc electrode: (-2.5 V/0.1 s/0.2 V/1.0 s) 100 repetitions

2.2.2. Electrochemical characterization of the redox polymers

The synthesized redox polymers were characterized in a three electrode setup with a Pt working electrode (WE), a Pt wire as CE and a Ag/AgCl (3 MKCl) RE. KCl (3 M, aq.) was used as electrolyte. The Os-complexes were investigated in solution while the redox polymers were drop-coated on the WE and left to dry in air. The following parameters were used: Cyclic voltammetry (CV): scan rate = 50 mV/s. Differential pulse voltammetry (DPV): modulation time = 0.06 s, interval time = 0.2 s, step potential = 5 mV, modulation amplitude = 15 mV.

2.2.3. Characterization of the redox polymer modified gold electrodes

The modified gold electrodes were characterized using an electrochemical robotic system equipped with a three electrode bundle consisting of the modified gold electrode as WE, a gold micro well plate as CE and a Ag/AgCl 0.1 M KCl as RE. The wells were filled with buffer of different pH which were prepared according to [19]. The electrode bundle was placed in the wells using the micropositioning system of the electrochemical robotic system. After 1 min equilibration time a SWV (-0.4 V - 0.9 V, 25 Hz, 20 mV amplitude, 2.5 mV step width) was recorded. Between the wells the electrode bundle was automatically rinsed in two deionized water containing wells of the microtiter plate. A “handylab pH 12” pH meter equipped with an “inlab micro pH” electrode (SI analytics, Mainz, Germany) was used for comparison measurements.

2.2.4. Synthesis

Synthesis of the redox polymers

The synthesis of the polymer backbones P029 and P032, dimethyldioxyrane, epoxidation of the polymer backbones as well as the synthesis of the final redox polymers P029-TB and P032-P231 were carried out following procedures similar to those described in [20] with the following monomer amounts and solvents:

P029: AllMA (3.16 g, 25.0 mmol, 50.0 mol-%), PEGMA (1.98 g, 3.75 mmol, 7.50 mol-%), BA (0.81 g, 6.28 mmol, 12.5 mol-%) und DMAEMA (2.36 g, 15.0 mmol, 30.0 mol-%); solvent for the polymerization was $^{i}PrOH$ (10 mL) and the polymer was precipitated from Et_2O.

P030: AllMA (1.26 g, 10.0 mmol, 50.0 mol-%), BA (1.00 g, 7.00 mmol, 35.0 mol-%) und ST (0.31 g, 3.00 mmol, 15.0 mol-%)-%); solvent for the polymerization was $^{i}PrOH$ (10 mL) and the polymer was precipitated from pentane. AIBN was recrystallized from toluene to remove impurities.

Synthesis of the osmium complex P231

Synthesis of *tert*-butyl-(2-aminoethyl)-carbamate

Ethylene diamine (4.51 g, 75.0 mmol, 3.00 eq) was dissolved in CH_2Cl_2 (50.0 mL) and cooled to 4 °C. Di-*tert*-butyldicarbonate (5.46 g, 25.0 mmol, 1.00 eq) was dissolved in CH_2Cl_2 (20.0 mL) and added dropwise to the cooled reaction mixture. The reaction mixture was stirred for 3 h at 4 °C, followed by 16 h stirring at room temperature. The white precipitate was removed by filtration,

washed with CH_2Cl_2 (15 mL) and the solvent was removed to dryness. Further drying under high vacuum yielded a colourless oil (11.41 g, 95%), which was used without further purification.

^{1}H NMR (200 MHz, $CDCl_3$, 300 K): δ (ppm) = 4.93 (br, 1H); 3.13 (m, 2H); 2.77 (t, J = 5.8 Hz, 2H); 1.42 (s, 9H). The spectroscopic data was in accordance with the literature [21].

Synthesis of 6-chloropyridine-3-aldehyde

According to a procedure of *Lee et al.* [22] oxalylchloride (3.81 g, 30.0 mmol, 3.0 eq) was dissolved in CH_2Cl_2 (35 mL) at -78 °C (dry ice/acetone). Then DMSO (46.9 g, 0.60 mol, 60.0 eq.) was added dropwise under stirring and the reaction mixture was stirred for additional 30 min at -78 °C. Then 2-chloro-5-(hydroxymethyl)pyridine (1.44 g, 10.0 mmol, 1.00 eq) was dissolved in CH_2Cl_2 (10 mL) and added dropwise with a syringe to the cold reaction mixture. Afterwards, the reaction mixture was stirred for 40 min at -78 °C and NEt_3 (91.9 g, 0.90 mol, 90.0 eq) was added to the reaction mixture at a rate of 2.0 mL/min. The reaction mixture was stirred for 1 h at -78 °C, followed by another 1.5 h stirring at r.t. The reaction mixture was then diluted with Et_2O (60 mL) and the organic phase was washed successively with $NaHCO_3$ (sat., aq., 2 × 30 mL), $KHSO_4$ (1 M, aq., 60 mL) and $NaHCO_3$ (sat., aq., 30 mL). After phase separation the organic phase was dried over Na_2SO_4, the solid removed by filtration and the solvent was removed in vacuum. Further cleaning with liquid chromatography (LC) (SiO_2, n-pentane/EtOAc/NEt_3 = 200/100/6) gave the desired product as yellowish solid (1.35 g, 96%).

R_f (n-hexane:EtOAc = 2:1) = 0.59.

^{1}H NMR (200 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.09 (s, 1H), 8.86 (d, J = 2.3 Hz, 1H), 8.13 (dd, J = 8.3, 2.3 Hz, 1H), 7.50 (d, J = 8.3 Hz, 1H). The spectroscopic data was in accordance with the literature [22].

Synthesis of 2-chloro-5-(1,3-dioxolan-2-yl)pyridine (2)

6-Chloropyridine-3-aldehyde (1.35 g, 9.60 mmol, 1.00 eq), pyridiniumtoluene-4-sulfonate (0.48 g, 1.92 mmol, 0.20 eq) and ethylene glycole (1.79 g, 28.8 mmol, 3.00 eq) were successively dissolved in toluene (100 mL) and refluxed for 2 h using a Dean-Stark apparatus for removal of the water. After cooling to room temperature the solvent was removed at 70 °C under high vacuum to dryness. The residue was dissolved in Et_2O (30 mL) and the organic phase was washed with $NaHCO_3$ (sat., aq., 10 mL). After phase separation the aqueous phase was extracted with Et_2O (2 × 30 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuum. Further cleaning by LC (SiO_2, n-pentane/EtOAc/NEt_3 = 200/100/3) gave the desired product (1.39 g, 78%) as pale yellow solid.

R_f (n-pentane:EtOAc:NEt_3 = 200:100:3) = 0.47.

^{1}H NMR (400 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.47 (d, J = 2.4 Hz, 1H), 7.74 (dd, J = 8.3 Hz, J = 2.4 Hz, 1H), 7.32 (d, J = 8.3 Hz, 1H), 5.83 (s, 1H), 4.29–3.84 (m, 4H).

^{13}C NMR (100 MHz, $CDCl_3$, 300 K): δ = 152.1, 148.3, 137.2, 132.9, 124.2, 101.4, 65.6.

FAB-MS: calculated for $C_8H_8ClNO_2H$ ($[M+H]^+$): 186.0 found: 186.0

Synthesis of 2-(tributylstannyl)pyridine (1)

Following a modified procedure of *Bianchini et al.* [22] 2-bromopyridine (4.86 mL, 50.0 mmol, 1.00 eq) was dissolved under argon in Et_2O_{dry} (50 mL), added dropwise with ca. 1 mL/min to a solution of ^{n}BuLi (1.6 M in n-hexane, 31.3 mL, 50.0 mmol, 1.00 eq) in Et_2O_{dry} (25 mL) at–78 °C and the resulting reaction mixture was stirred for 1 h at -78 °C. Then tributyltinchloride (12.00 mL, 17.90 g, 55.00 mmol, 1.10 eq) dissolved in THF_{dry} (10 mL) was added dropwise at -78 °C and the reaction mixture was stirred 1 h at -78 °C followed by another 2 h stirring at room temperature. The reaction was stopped by the addition of NH_4Cl (aq., sat., 30 mL). Subsequently the reaction mixture was washed with additional NH_4Cl (aq., sat., 1 × 50 mL) and the aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic phases were successively washed with H_2O (30 mL) and brine (30 mL), dried over Na_2SO_4 and the solvent was removed in vacuum. Further drying under high vacuum gave the product (16.84 g, 92%) as orange oil, which was used without further purification.

R_f (MTBE:MeOH = 6:4) = 0.86

^{1}H-NMR (200 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.69 (m, 1H); 7.46 - 7.32 (m, 2H); 7.08 - 7.00 (m, 1H); 1.60 - 1.48 (m, 6H); 1.30 (m, 6H); 1.13 - 1.06 (m, 6H); 0.87 - 0.81 (m, 9H). The spectroscopic data was in accordance with the literature [23].

Synthesis of 5-(1,3-dioxolan-2-yl)-2,2′-bipyridine

2-Chloro-5-(1,3-dioxolan-2-yl)pyridine (745 mg, 4.00 mmol, 1.00 eq) (solution 1) and 2-(tributylstannyl)-pyridine (1.74 g, 4.80 mmol, 1.2 eq) (solution 2) were dried in two separate Schlenk flasks under high vacuum for 10 min at room temperature. Both compounds were dissolved under argon in DMF_{dry} (each in 3.0 mL) and both solutions were degassed by cycling between high vacuum/argon three times. Then PPh_3 (1.30 g, 5.00 mmol, 1.25 eq) and $Pd(PPh_3)_4$ (462 mg, 0.40 mmol, 0.10 eq) were added to solution 1(→(**I**)), while CuI (152 mg, 0.80 mmol, 0.20 eq) was added to solution 2 (→(**II**)). Both solutions were stirred for 20 min at room temperature. Then (**II**) was added to (**I**) using a syringe and the reaction mixture was stirred for 72 h at 125 °C. After cooling to room temperature THF (50 mL) and NH_4OH (30% w/w, aq., 30.0 mL) were successively added to the reaction mixture and the solution was stirred vigorously for 30 min. Then the solution was diluted with additional THF (100 mL) and NH_4OH (30% w/w, aq., 25.0 mL) and stirred again vigorously for 16 h at room temperature. After phase separation the aqueous phase was extracted with THF (3 × 25 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuum. Further cleaning with LC (SiO_2, *n*-pentane/EtOAc/NEt_3 = 200/100/3) yielded the desired product (0.90 g, quant.) as pale yellow solid.

R_f (*n*-pentane:EtOAc:NEt_3 = 200:100:3) = 0.47.

^{1}H-NMR (400 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.75 (d, J = 2.2 Hz, 1H), 8.66 (m, 1H), 8.41–8.38 (m, 2H), 7.89 (dd, J = 8.2 Hz/2,2 Hz, 1H), 7.78 (m, 1H), 7.28 (m, 1H) 5.90 (s, 1H), 4.12–4.03 (m, 4H).

^{13}C-NMR (100 MHz, $CDCl_3$, 300 K): δ = 157.0, 156.0, 149.3, 147.9, 137.0, 135.2, 133.7, 123.9, 121.4, 120.8, 102.1, 65.5.

FAB-MS: calculated for $C_{13}H_{12}N_2O_2H$ ($[M+H]^+$): 229.1 found: 229.1

Synthesis of 2,2′-bipyridin-5-carbaldehyde (3)

5-(1,3-Dioxolan-2-yl)-2,2′-bipyridin (890 mg, 3.9 mmol, 1.0 eq) was dissolved in CH_2Cl_2 (15 mL). Then TFA (50% w/w, aq., 5.0 mL) was added and the reaction mixture was stirred vigorously for 16 h at room temperature. The reaction was stopped by addition of Na_2CO_3 (sat., aq., 10 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with a solution of NaCl/NaOH (sat., aq., 5 mL/6 M, aq., 1 mL), dried over Na_2SO_4 and the solvent removed in vacuum. Additional drying under high vacuum gave the desired product **3** (702 mg, quant.) as yellow solid which was used without further purification.

^{1}H-NMR (400 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.16 (s, 1H), 9.12 (d, J = 2.1 Hz, 1H), 8.72 (m, 1H), 8.61 (d, J = 8.2 Hz, 1H), 8.50 (m, 1H), 8.27 (dd, J = 8.2 Hz/2.1 Hz, 1H), 7.85 (m, 1H), 7.37 (m, 1H).

^{13}C-NMR (100 MHz, $CDCl_3$, 300 K): δ = 190.7, 160.8, 154.9, 151.8, 149.6, 137.2, 137.0, 131.3, 124.9, 122.4, 121.4.

ESI-MS: calculated for $C_{11}H_9N_2OH$ ($[M+H]^+$): 185.07 found: 185.11

Synthesis of 2,2′-bipyridin-5-methylamino(ethyl-2-(*tert*-butylcarbamate)

2,2′-Bipyridine-5-carbaldehyd (**3**) (350 mg, 1.9 mmol, 1.0 eq) and *tert*-butyl-(2-aminoethyl)-carbamate (320 mg, 2.00 mmol, 1.05 eq) were dissolved in EtOH (25 mL) and degassed with 3 cycles high vacuum/argon. Then Pd/C (10% w/w, 50 mg) was added and the reaction flask was sealed with a septum. To exchange the argon

atmosphere against a hydrogen atmosphere a small balloon was filled with hydrogen. Then the flask was evacuated until the solvent started to boil and the flask was filled with hydrogen from the balloon through the septum. The reaction mixture was stirred under hydrogen atmosphere for 16 h at room temperature. Then the catalyst was removed by filtration (celite) and the filter cake was washed with additional EtOH (25 mL). Removal of the solvent in vacuum and cleaning via LC (SiO_2, DCM/MeOH/NEt_3 = 100/10/1) yielded the desired product (554 mg, 89%) as pale yellow solid.

R_f (DCM/MeOH/NEt_3 = 100/10/1) = 0.13

^{1}H-NMR (400 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.68 - 8.60 (m, 2H), 8.38–8.32 (m, 2H), 7.82–7.75 (m, 2H), 7.31 (m, 1H), 4.94 (br, 1H), 3.86 (s, 2H,), 3.72 (m, 2H), 2.77 (t, J = 5.8 Hz, 2H), 1.43 (s, 9H).

^{13}C-NMR (100 MHz, $CDCl_3$, 300 K): δ = 161.9, 156.3, 155.4, 149.3, 149.2, 137.0, 136.6, 135.4, 123.8, 121.1, 120.9, 79.4, 50.8, 48.8, 40.3, 28.5.

FAB-MS: calculated for $C_{18}H_{24}N_4O_2H$ ($[M+H]^+$): 329.2 found: 329.1

Synthesis of 2,2′-bipyridine-5-methylamino-(ethyl-2-amine) (4)

2,2′-Bipyridine-5-methylamino(ethyl-2-(*tert*-butylcarbamat) (328 mg, 1.0 mmol, 1.0 eq) was dissolved in CH_2Cl_2 (20 mL) and cooled to 4 °C. Then HCl (4 M in dioxane, 20 mL) was added dropwise and the reaction solution was stirred for 30 min at 4 °C followed by 1 h stirring at room temperature. The solvent was removed under high vacuum and the residue was dissolved in CH_2Cl_2 (20 mL)/NaOH (1 M, aq., 5 mL). After phase separation the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuum. Further drying under high vacuum gave product **4** (140 mg, 61%), which was used without further purification.

^{1}H-NMR (200 MHz, CD_3CN, 300 K): δ (ppm) = 8.71–8.54 (m, 2H), 8.45–8.28 (m, 2H), 7.97–7.76 (m, 2H), 7.46–7.25 (m, 1H), 3.81 (s, 2H), 2.76–2.53 (m, 4H).

ESI-MS: calculated for $C_{13}H_{16}N_4H$ ($[M+H]^+$): 229.30 found: 229.27

Synthesis of [Os-(2,2′-bipyridine-5-methylamino-(ethyl-2-amin))-*bis*-(2,2′-bipyridine)]-*bis*-(hexafluorophosphate) (P231)

2,2′-Bipyridin-5-methylamino-(ethyl-2-amine) (**4**) (25 mg, 0.11 mmol, 1.10 eq) and $Os(bpy)_2Cl_2$ (57 mg, 0.10 mmol, 1.0 eq) were dissolved successively in ethylene glycol (2.0 mL) under argon atmosphere and the solution was degassed by 3 cycles between high vacuum/argon. The reaction mixture was stirred 10 days at 140 °C. After cooling to room temperature the reaction mixture was added dropwise to a NH_4PF_6 solution (0.86 g in 20 mL H_2O) and the suspension was stored at 4 °C for 16 h. The precipitate was filtered, washed successively with cold H_2O (3.0 mL) and Et_2O (10 mL) and dried under high vacuum to yield the desired Os complex **P231** as dark green solid (76 mg, 75%).

^{1}H-NMR (400 MHz, CD_3CN, 300 K): δ (ppm) = 9.13 - 9.11 (m, 1H); 8.56 - 8.50 (m, 2H); 7.98 - 7.96 (m, 2H); 7.90 - 7.86 (m, 2H); 7.84 - 7.73 (m, 4H); 7.70 - 7.62 (m, 2H); 7.59 - 7.52 (m, 2H); 7.46 - 7.42 (m, 2H); 7.33 - 7.18 (m, 4H); 7.15 - 7.10 (m, 1H); 6.38 (d, 1H, J = 1,2 Hz); 6.31 (d, 1H, J = 1.2 Hz); 4.70 (t, 2H, J = 5.7 Hz); 3.65 - 3.59 (m, 2H)

ESI-MS: calculated for $C_{33}H_{32}N_8Os$ ($[M-(PF_6)]^{2+}$): 366.12 found: 365.99

3. Results and Discussion

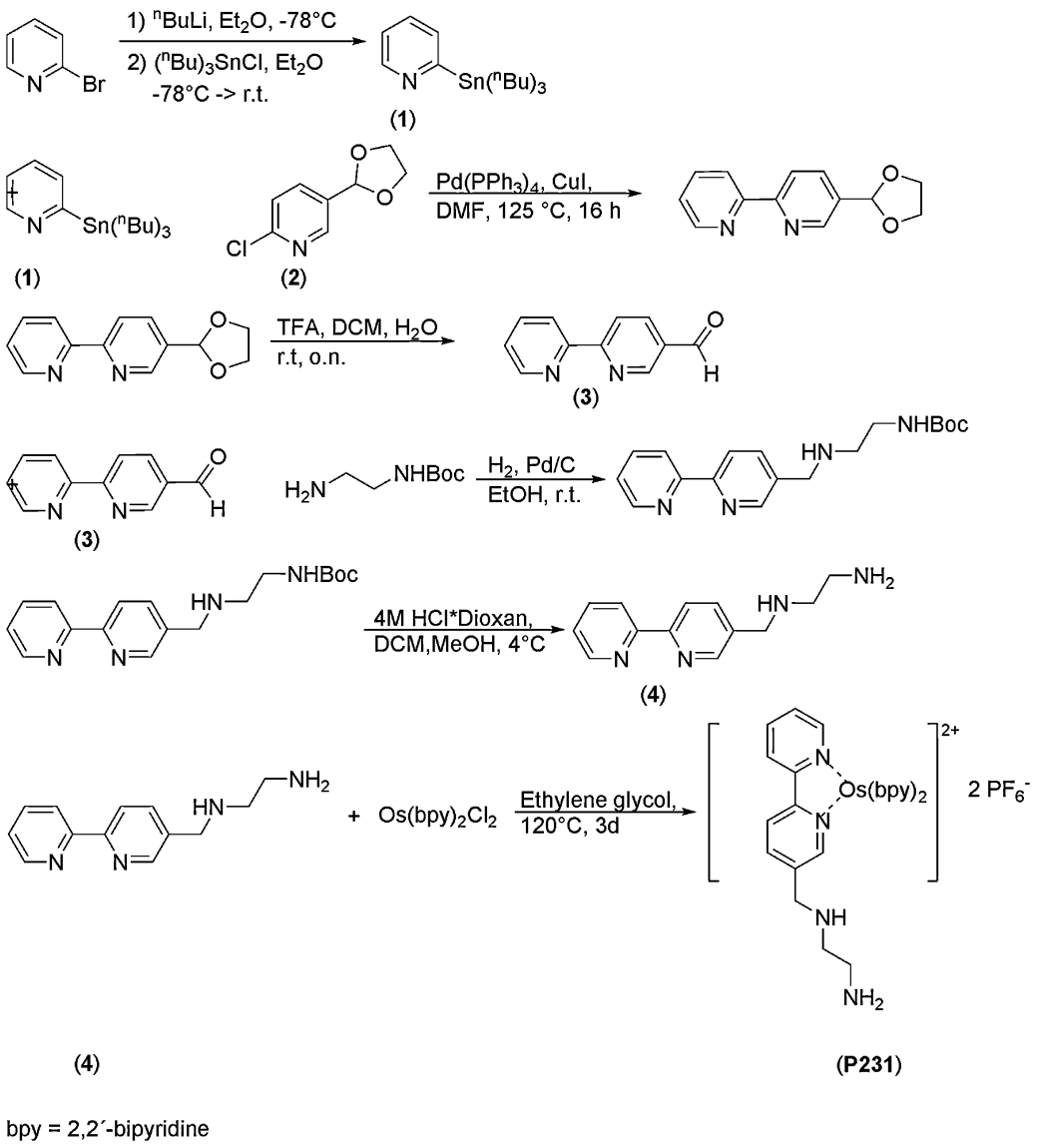
The anticipated concept for voltammetric pH value determination involves the binding of two quasi-reversible redox species on an electrode surface. Due to the surface confinement and the limited amount of redox compounds a fast voltammetric scan using e.g. square-wave voltammetry is addressing quantitatively all surface-bound redox species leading to a maximal current signal. Most importantly, two redox waves will be obtained; one for the redox conversion of the pH-sensitive redox species and one for the redox conversion of the pH-insensitive redox species which serves as internal potential reference. In earlier work by Xiong et al. ferrocene derivatives were used as internal pH-insensitive standard [8]. However, due to the rather low formal potential of the used ferrocene derivatives the potential difference between the redox conversion of the pH-dependent and pH-independent signal was often not sufficient to avoid overlapping of the two signals. Therefore we were aiming on the design of a pH-independent and stable redox species with a comparatively high redox potential. The formal potentials of the Os^{2+}/Os^{3+} reaction of octahedrally coordinated Os complexes are independent of the pH value in solution and their formal potential can be adapted to an envisaged application by manipulating the electron density of the bidentate bipyridine ligands. Based on the experience gained by the synthesis of Os complex modified redox polymers we designed a synthesis scheme of the high-potential pH-independent Os complex **P231**. The targeted formal potential was 700 mV vs Ag/AgCl/3 M KCl which should be achieved with a coordination by three 2,2′-bipyridyl ligands (Scheme 1).

It was known that basic nitrogen sites as part of the ligand sphere, like in the case of imidazole derivatives, lead to a significant anodic shift of the formal potential with decreasing pH value. To avoid any pH dependence caused by deprotonation or protonation of groups at the used bidentate ligands with an impact on the electron density of the ligand and consequently on the formal potential of the Os complex, we used 2,2′-bipyridine as ligands. However, 2,2′-bipyridine does not provide any binding site for the linkage of the Os complex to a preformed methacrylate based electrodeposition polymer backbone. Therefore it was necessary to modify one of the three 2,2′-bipyridine groups with a linker with a terminal primary amine group to allow covalent binding to epoxide functions in the side chains of the polymer backbone. The synthesis of the bipyridine ligand with the amino terminated anchor group was achieved by Stille cross coupling of pyridines **1** and **2** to yield the bipyridine aldehyde **3** after deprotection of the acetal. The bipyridine aldehyde **3** was then functionalized with the primary amine group by reductive amination to give the bipyridine **4**. Finally the Os complex **P231** was obtained by ligand exchange reaction of bipyridine **4** with Os (II)-bis-(2,2′-bipyridine) dichloride exchanging the two labile chloro ligands against the modified 2,2′-bipyridine chelate ligand (Scheme 1). The synthesis is described in detail in the experimental section including the characterization of all products.

Electrochemical characterization of the synthesized Os complex **P231** showed a single quasi reversible redox species with a formal potential of 640 mV vs. Ag/AgCl/3 M KCl (Fig. 1) which is in good agreement with the calculated redox potential for a tris-bipyridine coordinated Os complex [24]. The successful formation of the target Os complex was furthermore confirmed by NMR and MS.

For immobilization of the synthesized Os complex **P231** it was bound to the hydrophobic polymer backbone **P032** via epoxide ring opening reaction to yield the pH-independent redox polymer **P032-P231** (Fig. 2, right). As pH-dependent redox couple the toluidine blue O modified redox polymer **P029-TB** was chosen (Fig. 2, left). This polymer can be deposited upon an electrochemically induced pH modulation causing a deprotonation of the previously protonated dimethylammonium group. During the precipitation on the electrode surface it serves simultaneously as immobilization matrix for the polymer **P032-P231**.

The redox polymer **P032-P231** was coprecipitated with **P029-TB** and the whole polymer film was further stabilized by electrochemically induced crosslinking following a recently proposed protocol [25]. The deposition of the mixed redox polymer



Scheme 1. Synthesis of the pH-independent Os complex **P231**.

films was achieved by applying potential pulses to the gold micro electrodes [(-2.2 V; 0.1 s/-0.2 V; 1.0 s) 100 repetitions)]. The modified gold micro electrodes were subsequently characterized in an electrochemical robotic system by measuring a series of square-wave voltammograms (SWV) in phosphate buffer (0.1 M) adjusted to different pH values. The peak potentials for both redox species were determined from the SWV and the average of 5 measurements were used to precisely determine the potential difference between both electrode confined redox species in dependence from the pH value. Additionally the pH-dependence of the two redox couples was determined from the slope of a linear fitting.

The results obtained for a 100 μm gold electrode modified with **P029-TB** and **P032-P231** are shown in Fig. 3. The SWV showed clearly two redox peaks corresponding to the two

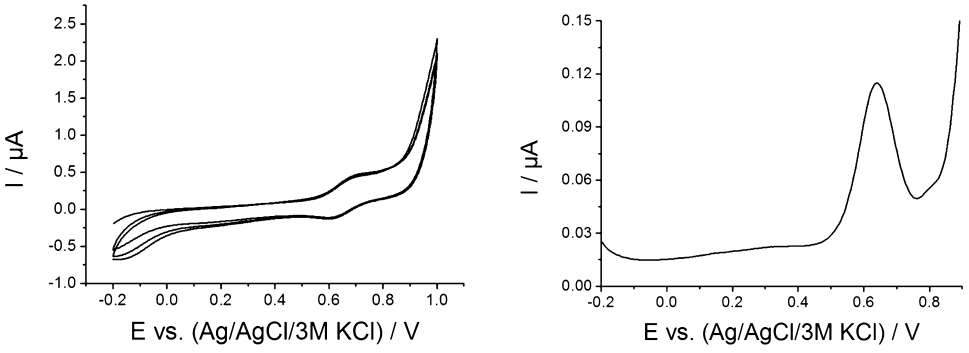


Fig. 1. CV (left; 50 mV/s) and DPV (right; 15 mV ModAmp; 0.06 s Modtime; 0.2 s interval time) of the Os complex **P231** in 3 M KCl (WE = Pt 1 mm).

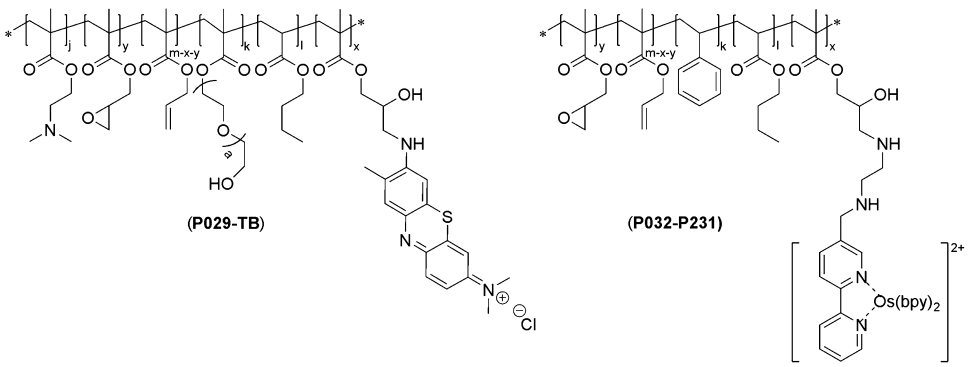


Fig. 2. Proposed structural formulas of the redox polymers **P029-TB** und **P032-P231**.

polymer bound redox species, which were both shifted slightly to more anodic potentials as compared to the unbound, free diffusing redox species. Since the free diffusing species were characterized in 3 M KCl, the slower counter ion diffusion inside the crosslinked polymer films is seen as the reason for this potential shift.

As expected, the linear fit for the pH-independent redox polymer **P032-P231** clearly showed the stable formal potential of the polymer bound Os complex (Fig. 3B, red), while the pH-dependent redox polymer **P029-TB** showed a pronounced shift of the peak potential with the pH value. The linear fit for **P029-TB** was divided into two regions. While a shift of -43 mV/pH was observed for acidic pH values only a slope of -26 mV/pH was observed for neutral to alkaline pH values. The change in the pH dependence indicates a change in the electron transfer mechanism from a $2e^-/2H^+$ mechanism (pH = to pH = 6) to a $2e^-/1H^+$ mechanism at higher pH values. The overall deviation of the peak potentials between five subsequent measurements was not significant for both redox polymers.

The modification procedures used for the 100 μm gold electrode were then applied for the modification of two different 50 μm gold electrodes and the modified electrodes were characterized by SWV as before. As expected again two redox peaks were observed in the SWV, again with 100 mV anodic shift as compared to the free diffusing redox species. The calibrations plots for the two electrodes are shown in Fig. 4.

The formal potential of the Os complex modified redox polymer **P032-P231** was stable over the entire investigated pH value range at around 800 mV for both electrodes. In contrast to this a shift of -58 mV/pH and -57 mV/pH was observed in the acidic pH range (pH = 3 to pH = 6) for the pH dependent redox polymer **P029-TB**, which almost matches the -59 mV/pH expected for a $2e^-/2H^+$ mechanism according to the Nernst equation. Furthermore a shift of -23 mV/pH and -30 mV/pH was observed for **P029-TB** in neutral to alkaline pH range (pH = 6 to pH = 7.5), which also corresponds nicely to the -29.5 mV expected for a $2e^-/1H^+$ transition. The deviation between five measurements as well as between the two electrodes was insignificant demonstrating the excellent reproducibility due to a controlled electrochemically induced polymer deposition and crosslinking procedure.

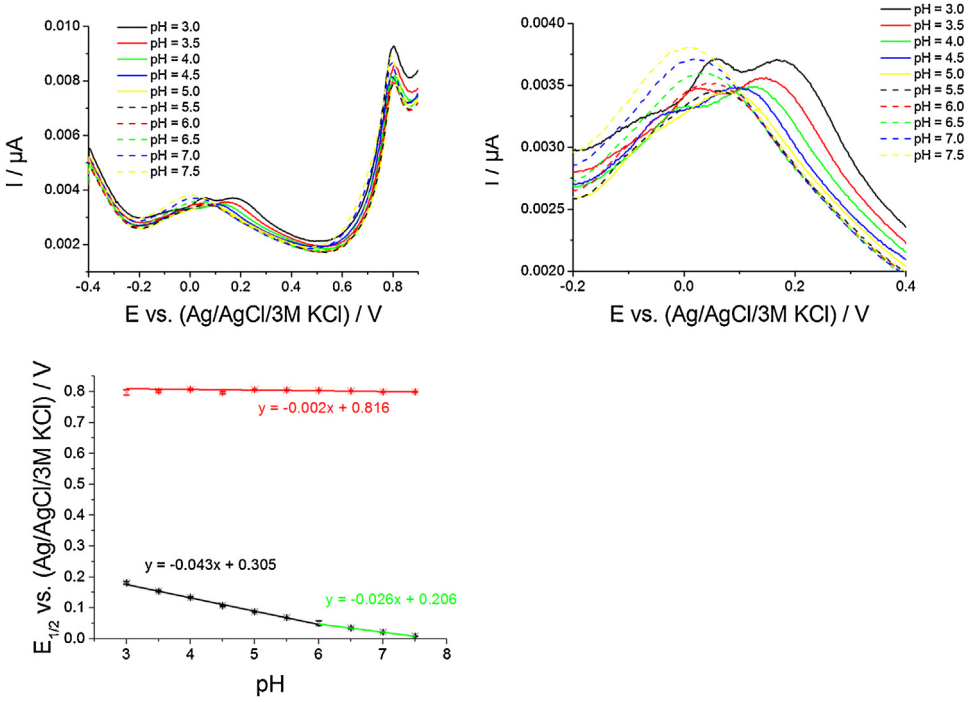


Fig. 3. SWV of a gold disc electrode (100 μm) modified with the redox polymers **P032-P231** and **P029-TB** measured in PCB (0.1 M) with varying pH-values (left), a magnification of the range -0.2 V - 0.4 V (middle) and the calibration graphs calculated from the peak potential measured by SWV (n = 5; **P032-P231** (red); **P029-TB** (black and green)) (right).

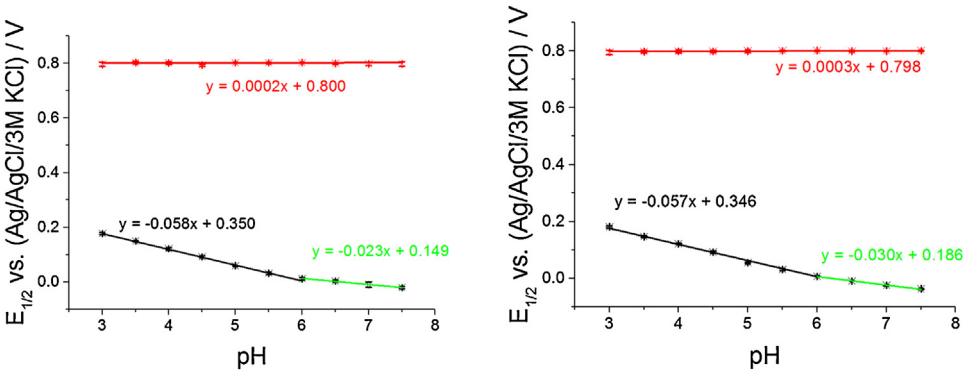


Fig. 4. Calibration plots (n = 5; **P032-P231** (red); **P029-TB** (black and green)) of two different 50 μm gold disc electrodes modified with **P032-P231**/**P029-TB**/CL**32**.

Table 1
Comparison of the pH value measured with a commercial pH meter and the pH value obtained from voltammetric pH determination using SWV and a redox polymer modified gold electrodes.

target pH	pH (glass electrode)	pH (redox polymer electrode)
3.0	2.9	3.0
5.0	4.9	5.0
7.0	7.1	7.0

As an example, for gold electrode 1 (Fig. 4, left) the pH-dependence of the peak potential difference can be calculated from equation 1 for $\Delta E > 0.60\,V$ to $\Delta E < 0.80\,V$ and from equation 2 for $\Delta E > 0.80\,V$ to $\Delta E < 0.85\,V$.

$$pH = \frac{\Delta E - 0.45\Delta}{0.058V} \quad (1)$$

$$pH = \frac{\Delta E - 0.65V}{0.023V} \quad (2)$$

To further evaluate the feasibility of the developed reference-electrode free voltammetric pH microsensors three buffer solutions with different pH values were prepared according to [19] and adjusted to the target pH value by measuring the pH value with a pH meter based on a glass electrode. Directly after the preparation of the standard buffer solutions, their pH values were determined with the redox polymer modified gold electrode 1 by SWV and the pH value was calculated following Eq.1 or Eq.2, depending on the obtained peak potential difference. The results are shown in Table 1.

The pH measured with the redox polymer modified gold electrode showed only minor deviations as compared to the pH value measured with the glass electrode. These minor differences are attributed to minor pH changes due to contact to air or minor changes of the solutions temperature.

4. Conclusion

A voltammetric micro-pH sensor was designed using a pH-independent redox reaction of a specifically designed Os complex modified redox polymer **P032-P231** codeposited with a pH-dependent redox reaction of a toluidine blue O modified redox polymer **P029-TB.** It could be demonstrated that voltammetric determination of the pH value is possible using SWV of polymer modified micro gold electrodes avoiding any large reference electrode as required in the case of potentiometric pH value determination. The micro pH sensors showed excellent reproducibility and good operational and storage stability. In addition to the advantage of miniaturization, the redox polymer modified gold electrodes should offer the possibility to measure proton activity also in dry in organic solvents, which cannot be achieved with pH meters based on glass electrodes.

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