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Multilayered sulfonated polyaniline network with entrapped pyrroloquinoline quinone-dependent glucose dehydrogenase: tunable direct bioelectrocatalysis

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A feasible approach to construct multilayer films of sulfonated polyanilines - PMSA1 and PABMSA1 - containing different ratios of aniline, 2-methoxyaniline-5-sulfonic acid (MAS) and 3-aminobenzoic acid (AB), with the entrapped redox enzyme pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH) on Au and ITO electrode surfaces is described. The formation of layers has been followed and confirmed by electrochemical impedance spectroscopy (EIS), which demonstrates that the multilayer assembly can be achieved in a progressive and uniform manner. The gold and ITO electrodes subsequently modified with (PMSA1;PQQ-GDH) and PABMSA1 films are studied by cyclic voltammetry (CV) and UV-Vis spectroscopy and show significant direct bioelectrocatalytical response to the oxidation of the substrate glucose without any additional mediator. This response correlates linearly with the number of deposited layers. Furthermore, the constructed polymer/enzyme multilayer system exhibits a rather good long-term stability, since the catalytic current response is maintained for more than 60% of the initial value even after two weeks of storage. This verifies that a productive interaction of the enzyme embedded into the film of substituted polyaniline can be used as a basis for the construction of bioelectronic units, which are useful as indicators for processes liberating glucose and allowing optical and electrochemical transduction.

Introduction

Entrapment of enzymes in conducting polymers or polymeric nanocompositions has become a popular method for fabricating biosensors.[1-3] The electroactive polymer films have to be chosen in a way that not only a loss of the bioactivity of entrapped proteins is prevented, but also the contact to the electrode surface is ensured. Besides numerous redox polymers which are characterized by the presence of spatially separated active sites, conjugated conducting polymers are particularly attractive for this purpose. Even when the concept of contacting enzyme molecules with conjugated polymers chains is not new, only a limited number of functional systems have been demonstrated yet.[4-6] Also the mechanisms behind are often not simple, since enzymatic reaction products might be involved into the overall electron transfer. One of the rational ways to obtain such functional enzyme organization is to immobilize the proteins into well-defined thin layers.[7-10] In order to form such a network, in which enzyme molecules not only retain their activities, but are also electroactive, one needs to choose building blocks that are capable of exchanging electrons with the enzyme and do not cause enzyme denaturation. Conjugated polymers such as polypyrroles and polyanilines have been already proven to represent suitable materials for this purpose.[4, 6, 11-15]

Particularly polyaniline and its substituted forms have found to be useful for the construction of biosensor interfaces not only because of their unique electrical behavior, good environmental stability in doped and neutral states and ease of synthesis,[16-19] but also due to their action as electron carriers in redox and enzymatic reactions.[20-23] Furthermore, they can form dense and

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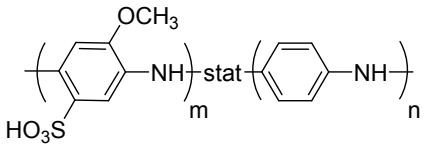
ordered films and/or possess a defined charge being able to work as anti-interference layers in enzymatic sensors.[2, 16, 21, 24] Especially self-doped polyanilines have received great interest in recent years, since they exhibit higher conductivities compared to unsubstituted polyanilines together with several other advantages such as improved solubility and redox activity over a wide pH range. The self-doping can be done, for example, by sulfonation of polyaniline rings or copolymerization of aniline with sulfonated aniline derivatives.[18, 23, 25-27]

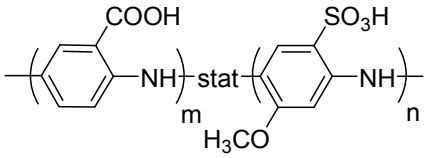
Interactions of polyaniline derivatives with selected bioentities, such as amino acids, antibodies, and oligonucleotides have already been reviewed.[28-31] Also for the biosensing of DNA hybridizations differently structured nano-networks of sulfonated polyanilines have been used.[32,33] Moreover, these conducting polymers have been reported in terms of interactions with redox proteins such as cytochrome c[26, 34] and hemoglobin.[15] Up to now, formation of novel multilayer architectures based on differently substituted polyaniline and cytochrome c,[35] choline oxidase,[36] xanthine oxidase,[37, 38] oxalate oxidase,[39] bilirubin oxidase,[37, 40] glucose oxidase,[41] and sulfite oxidase[42] has already been established.

In our recent report we have demonstrated the ability of copolymers containing different ratios of 2-methoxyaniline-5-sulfonic acid (MAS), 3-aminobenzoic acid (AB) and aniline units to react directly with the redox enzyme pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH).[23] Since this enzyme is insensitive to the oxygen level in the sample and exhibits high catalytic activity at physiological pH, it represents an interesting bioentity for the engineering of biocatalytic glucose electrodes.[43-45] However, until now no report on a multilayered, mediatorless glucose biosensor constructed with this homodimeric enzyme is known, even though the direct bioelectrocatalysis at different surfaces has been demonstrated.[23, 44, 46-50] Therefore, based on the achievement of a direct electron exchange with sulfonated polyanilines, we have chosen PQQ-GDH for the construction of novel multilayered architectures.

Exploiting the advantageous features of substituted polyaniline copolymers[23] in their interaction with PQQ-GDH, we investigate herein thickness-tunable multilayered polymer films with embedded PQQ-GDH. For this purpose the immobilization conditions are varied by changing the sequence of polymer-enzyme/polymer layers and the polymer/enzyme ratios. On the basis of electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and UV-Vis spectroscopy studies we demonstrate the defined formation of multilayers with active enzyme.



Scheme 1. Chemical structure of poly(2-methoxyaniline-5-sulfonic acid)-co-aniline (PMSA1: m/n =4.5/1)



Scheme 2. Chemical structure of poly(3-aminobenzoic acid-co-2-methoxyaniline-5-sulfonic acid) (PABMSA1: m/n=1/1.8)

Experimental Section

Chemicals

MES buffer was purchased from Sigma-Aldrich (Taufkirchen, Germany), dehydrated calcium chloride and anhydrous D-(+)-glucose were obtained from Fluka Analytics (Taufkirchen, Germany). They were used without further purification. sGDH (Acinetobacter calcoaceticus) was kindly gifted to us by Roche Diagnostics GmbH. The enzyme was recombinantly expressed in Escherichia coli. PQQ was purchased from Wako Pure Chemical Industries. 18 MΩ Millipore water (Eschborn, Germany) was used for all types of measurements.

Sulfonated polyaniline copolymers

Poly(2-methoxyaniline-5-sulfonic acid)-co-aniline polymer (PMSA1, Scheme 1) and poly(3-aminobenzoic acid-co-2-methoxyaniline-5-sulfonic acid) (PABMSA1, Scheme 2) were synthesized as reported before.[23, 26]

Preparation of the enzyme solution

sGDH was dissolved in 5 mM MES buffer in the presence of 1 mM $CaCl_2$ and the pH adjusted to 5. apoGDH was reconstituted by a PQQ/GDH ratio of 1 according to Olsthoorn.[51] For this purpose sGDH and PQQ were incubated together for 1 h at room temperature in the dark. Aliquots were stored at -20 °C. Prior to each measurement, the specific activity of the reconstituted enzyme was determined to be 2200 ± 30 U mg^{-1} by using 2,6-dichlorophenol indophenol (DCIP) as electron acceptor.

Construction of the single/multilayer films

Gold wire electrodes with a diameter of 0.5 mm were obtained from Goodfellow (Bad Nauheim, Germany). First, they were boiled in 2.5 M solution of potassium hydroxide, then washed with distilled water and placed in the concentrated sulfuric acid overnight. Prior to each usage the wires must be washed with water again, then they are to be placed in concentrated HNO_3 for 15 minutes. Finally they are to be carefully washed with water.

For the preparation of single layer films, the previously cleaned gold wires or rectangular Indium Tin Oxide (ITO) coated glass slide with surface resistivity 15-25 Ω/sq (obtained from Sigma-Aldrich, Taufkirchen, Germany) were initially incubated in buffer solutions of PMSA1;PQQ-GDH mixture

(1.5 mg ml^{-1} PMSA1, 5 µM PQQ-GDH; 20mM MES + 5mM $CaCl_2$, pH 6) in darkness for 2 hours. Afterwards, the Au/(PMSA1;PQQ-GDH) electrode was dipped into the same buffer to wash away the unbound material. In order to deposit an intermittent polymer layer, this electrode was subsequently immersed in a PABMSA1 solution without enzyme (0.5 mg ml^{-1} PABMSA1, 20mM MES + 5mM $CaCl_2$, pH 6) for 30 minutes. After being washed with the same buffer, the derived Au/(PMSA1;PQQ-GDH)-PABMSA1 electrode was placed in the PMSA1;PQQ-GDH mixture solution again for 30 minutes. Repetition of the above procedure led to alternating PABMSA1/PMSA1;PQQ-GDH multilayer films with the desired number of bilayers (PABMSA1/PMSA1;PQQ-GDH)$_n$. For the preparation of the above multilayer assembly on paper strips filter paper (thickness 0.2mm; obtained from Macherey-Nagel GmbH, Düren, Germany) was used.

Instruments

Electrochemical measurements were performed in a homemade 1 ml cell using an Ag/AgCl/1 M KCl reference (Biometra, Germany) and a platinum wire counter electrode. Cyclic voltammetry experiments were carried out with an µAutolab Type II device (Metrohm, the Netherlands). The scan rate was set to be 5 mV s^{-1}. The potential range was chosen as between -0.4 and +0.4 V vs. Ag/AgCl. Data analysis was performed using GPES software (General Purpose for Electrochemical System, Eco Chemie, Utrecht, the Netherlands). Electrochemical impedance spectroscopy (EIS) measurements were performed with a model 660B electrochemical workstation from CHI Instruments (Austin, TX, USA). All EIS measurements were carried out in the presence of 5 mM $K_3[Fe(CN)_6]$/5 mM $K_4[Fe(CN)_6]$ mixture as a redox probe in the frequency range between 0.1 and 100000 Hz at the open circuit potential of +0.18 V. The amplitude of the alternating voltage was 10 mV.

Results and discussion

Fabrication and characterization of the Au/(PMSA1;PQQ-GDH) electrode

PMSA1 (Scheme 1) has been shown to react directly with PQQ-GDH,[23] which is used as a starting point for the study reported here. Contrary to our previous studies,[23-26] here the enzyme is not coupled to a prepared polymer film, but we have tried to embed the protein within the polymer. In order to generate a suitable interface for the bioelectrocatalytic conversion of glucose thin films with incorporated enzyme are prepared on gold wire and ITO electrodes. As a confirmation of the film formation, direct cyclic voltammetry of the polymer/enzyme-coated electrodes has been performed. Figure 1A (curve 1) illustrates the cyclic voltammetric behavior of Au/(PMSA1;PQQ-GDH) electrode. A clear redox couple at E_f = 0.18 ± 0.01 V vs. Ag/AgCl might be attributed to the polymer conversion from emeraldine to pernigraniline state.

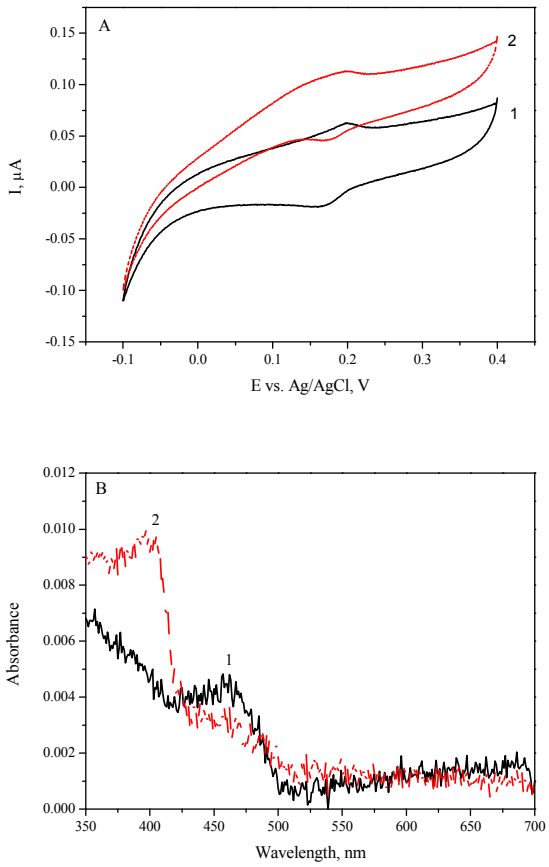
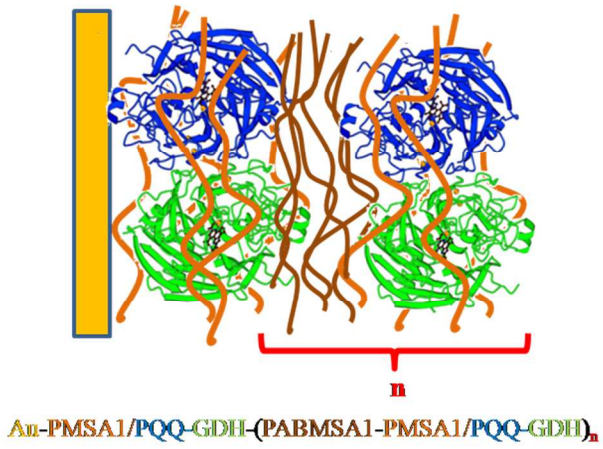


Figure 1. A) CV for the Au/(PMSA1;PQQ-GDH) electrode without (1) and with (2) glucose (scan rate = 5mV/s); B) UV-Vis spectra on ITO electrode covered with (PMSA1;PQQ-GDH) without (1) and with (2) glucose. Measurements are taken in 20 mM MES+5mM $CaCl_2$ buffer pH 6, [glucose] = 5mM

Interestingly, the presence of the enzyme in the film cannot be seen in cyclic voltammograms, since no additional redox peaks appear. However, the appearance of a defined catalytic current upon addition of the substrate glucose gives proof of the presence of active enzyme in the film (Figure 1A-curve 2). The current starts from a potential of about E = -0.025 V vs. Ag/AgCl and reaches at 0.35 V a change of ΔI = 57 ± 6 nA, n = 3. The bioelectrocatalysis demonstrates a direct electron transfer between the redox center of the entrapped enzyme and the electrode, indicating that the polymer environment does not inhibit the catalytic activity of PQQ-GDH, but allows an efficient electron withdrawal from the reduced enzyme. However, investigation in varying the polymer-enzyme ratios shows that there is only a limited concentration range, in which bioelectrocatalysis occurs. At [4 mg ml^{-1} PMSA1/5 µM PQQ-GDH] concentration ratio precipitation of polymer occurs. At polymer concentrations 1.5mg/ml^{-1} < PMSA1 < 4mg ml^{-1} rather smaller catalytic currents can be detected. Thus, all further measurements have been performed with this medium concentration ratio of [1.5 mg ml^{-1} PMSA1/5 µM PQQ-GDH] (see Experimental section).



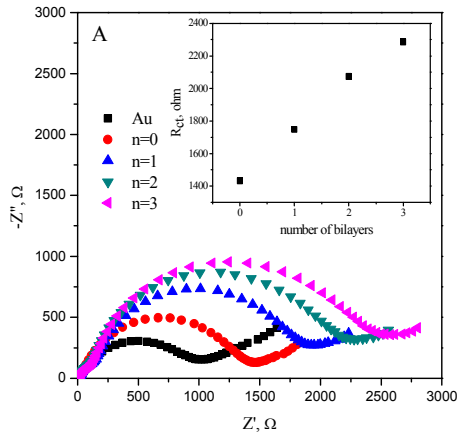
Scheme 3. Schematic representation of the multilayered enzyme network.

To prove, whether it is possible to verify the biochemical PMSA1 polymer reduction spectrophotometrically ITO/PMSA1;PQQ-GDH electrodes have been prepared in the same way as on gold. Figure 1B demonstrates the spectral changes of the mixed polymer-enzyme film on ITO in the absence (curve 1) and in the presence (curve 2) of glucose. According to the known redox state-dependent spectral characteristics of PMSA1[23, 52] it can be seen, that the reduction of the polymer upon addition of glucose occurs with the appearance of the characteristic band at 408 nm. It should be pointed out, that because of the small thickness of the immobilized polymer-enzyme film the absorbance is rather low (Figure 1B), but nevertheless, the direct electron transfer between the entrapped PQQ-GDH and the surrounding polymer can be followed.

Au/(PMSA1;PQQ-GDH)/(PABMSA1-PMSA1;PQQ-GDH)n multilayers

The idea to construct multilayers with controlled building blocks based on a Au/(PMSA1;PQQ-GDH) electrode originates from advantages of enzyme multilayer systems of tunable sensitivity in general, together with the possibility to make use of direct interactions of the biocatalyst with the polymer used for layer construction in particular. For this purpose, PMSA1 and PABMSA1 (Scheme 2) have been chosen for the formation of a layered structure. Although PMSA1 provides a suitable environment for the immobilization of active PQQ-GDH, it does not allow the stable adsorption of several layers of (PMSA1;GDH) on top of each other. Therefore, intermittent polymer layers without enzyme are intended to be deposited between polymer/enzyme layers. It is found that PABMSA1 is suitable for this purpose, allowing further (PMSA1;GDH) deposition.

Scheme 3 shows the construction pathway of the Au/(PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)$_n$ multilayer films. The key to this process is the attachment of the additional negatively charged PABMSA1 polymer layer to the Ca^{2+}-containing PMSA1 conformer via electrostatic



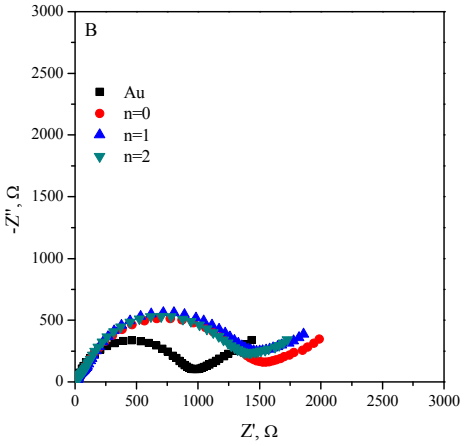


Figure 2. Impedance spectra for the A) Au/(PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)$_n$ electrodes with different numbers of layers (n=0 to 3). Inset: the relationship of R_{ct} vs. the number of bilayers; B) Au/(PMSA1;PQQ-GDH)$_n$ electrodes (n=0 to 3). Measurements are taken in in 5 mM $K_3[Fe(CN)_6]$/5 mM $K_4[Fe(CN)_6]$ solutions of 20 mM MES+5mM $CaCl_2$ buffer, pH 6.

binding. Since the coordination of Ca^{2+} to sulfonate ring substituents in emeraldine salt-polyanilines is known[52, 53] and expected to stabilize their more planar conformers, the PMSA1 is applied here in a Ca^{2+}-containing buffer. The Ca^{2+} ions can compensate the negative charge at the SO_3^- groups on the surface,[52, 54] allowing the electrostatic attachment of PABMSA1 on the surface.

Since EIS is an effective method that is often used for probing the changes in surface-modified electrodes, we apply it for characterization of the multilayer assembly. An impedance spectrum mainly contains three distinguished regions corresponding to mass transfer limitation (W), charge transfer process (R_{ct}) and current transport in solution (R_{sol}). The charge transfer resistance R_{ct} can be obtained by analysing the diameter of the semicircle in the impedance spectrum. Therefore, we can determine the R_{ct} to characterize the

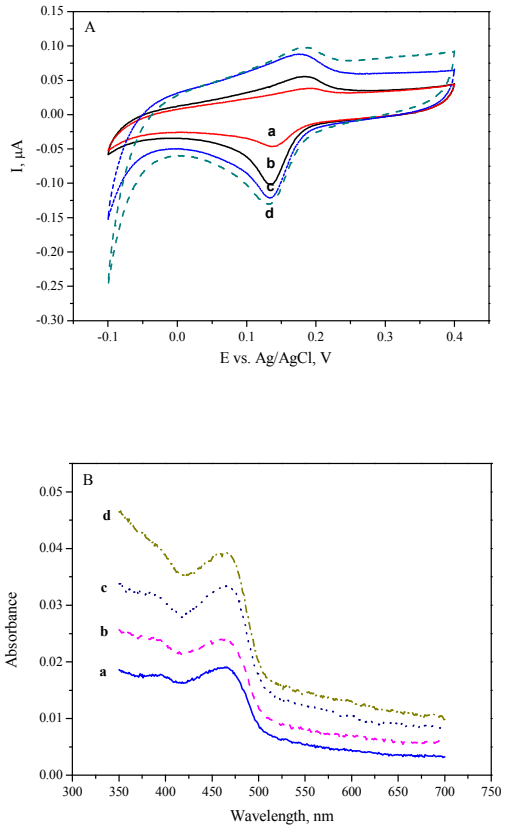


Figure 3. A) Cyclic voltammograms and B) UV-Vis spectra of Au(or ITO)/(PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)n multilayer electrodes in the order of increasing number of bilayers (a-n=0, b-n=1, c-n=2 and d-n=3). Inset in A): the relationship of reak current vs. the number of bilayers. Scan rate – 5 mV/s. Measurements are taken in 20 mM MES+5mM CaCl2 buffer pH 6.

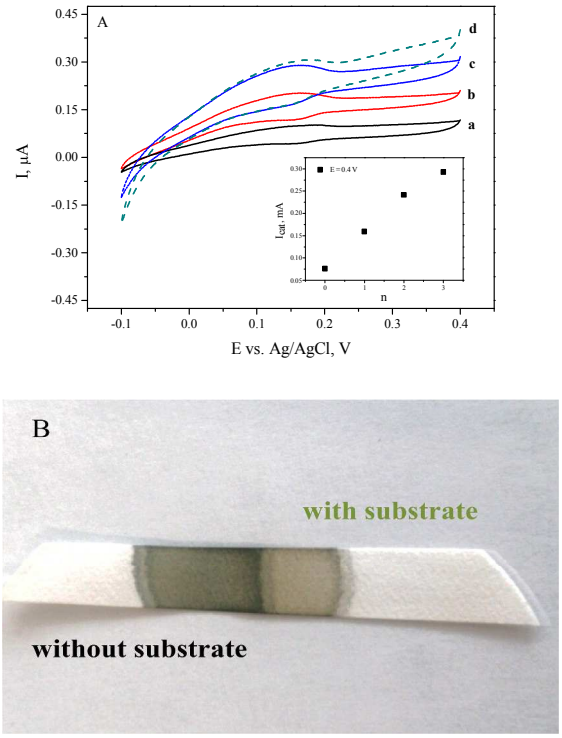


Figure 4. A) Catalytic response of Au/(PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)$_n$ multilayer electrodes in the presence of 5 mM glucose (a-n=0, b-n=1, c-n=2, d-n=3). Inset: The anodic currents vs. number of layers at E = +0.4 V vs. Ag/AgCl. B) Colour change of polymer/enzyme deposit on paper strip with and without substrate. Scan rate = 5 mV/s. Measurements are taken in 20 mM MES+5mM $CaCl_2$ buffer pH 6.

modification steps of the electrodes. Figure 2A shows the EIS presented as Nyquist plot of electrodes modified with different numbers of (PABMSA1/PMSA1;PQQ-GDH)$_n$ bilayers. Significant differences after each layer deposition have been observed, resulting from the raising thickness of the polymer/enzyme film which increases the barrier for interfacial charge transfer. The charge transfer resistance (R_{ct}) is linearly proportional to the number of attached bilayers, as shown in the inset of Figure 2A.

In order to demonstrate the ability of the PABMSA1 polymer to enhance the formation of the desired multilayered assembly, EIS measurements have been performed on electrodes, which have been repeatedly incubated in a PMSA1;GDH solution without intermittent PABMSA1 depositions. No change in impedance spectra with increasing number of incubation steps can be detected, confirming the importance of the additional polymer layer for the achievement of the whole assembly in a uniform manner (Figure 2B). In addition, no change in impedance spectra is detectable, when both incubation steps and measurements are performed in buffer solutions containing no Ca^{2+} ions, confirming the formation of multilayers via electrostatic interactions discussed before. Also cyclic voltammetry and UV-Vis spectroscopy have been carried out to characterize the formation of the multilayered structure. Figure 3A summarizes CVs of Au/(PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)$_n$ electrodes measured in the absence of the substrate. A redox couple with a formal potential of E = +0.18 V vs. Ag/AgCl attributed to sulfonated polyaniline can be observed. The current intensities of this redox couple are increasing with increasing numbers of deposited polymer/enzyme layers, which confirms the formation of the layered assembly. Figure 3B summarizes UV-Vis spectra of the (PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)$_n$ system (n=0-3) on ITO electrodes, demonstrating an increase in absorbance at 469 nm of half-oxidized PMSA1 polymer[23, 26, 52] after deposition of multiple polymer/enzyme/polymer layers. Obviously, the concentration of PABMSA1 in the film is rather small compared to PMSA1 and cannot be detected in the spectra (Figure 3B).

Bioelectrocatalytic oxidation of glucose at Au/(PMSA1;PQQ-GDH)-(PABMSA1/PMSA1;PQQ-GDH)n electrodes

To evaluate the usefulness of this strategy, multilayer assemblies have been prepared on Au and their bioelectrocatalytic characteristics have been investigated.

Figure 4A represents cyclic voltammetric responses obtained from electrodes modified with different numbers of (PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)$_n$ layers in the presence of 5 mM glucose. With a rising number of layers the voltammograms change clearly, with an increase in the oxidation current (Figure 4A), starting from the potential at about E = -0.025 V vs. Ag/AgCl. The anodic response exhibits a nearly linear dependence with the number of (PABMSA1/PMSA1;PQQ-GDH)$_n$ bilayers (inset in Figure 4A) due to the increased amount of the enzyme embedded into the layers. The bioelectrocatalysis demonstrates that an efficient electron transfer between the redox centre of the enzyme and the electrode through different polymer/enzyme layers has been achieved. Thus, one can conclude that each bilayer contains nearly the same amount of active enzyme, and (PMSA1;PQQ-GDH)/(PABMSA1-PMSA1;PQQ-GDH)$_n$ films are formed in a rather ordered manner, which offers the possibility of conveniently controlling the active enzyme loading via adjusting the number of (PABMSA1/PMSA1;PQQ-GDH)$_n$ bilayers attached.

In order to visualize the change in redox state of the polymer after enzymatic reaction, such a layered deposition of PQQ-GDH has been also performed on a paper material. Figure 4B demonstrates the change in color of a polymer-enzyme system immobilized on a paper strip in the presence and in the absence of the substrate. Immediately after dropping the solution containing glucose on the strip containing entrapped enzyme, the greenish color characteristic for highly conducting polyanilines in emeraldine salt redox state starts to disappear. This shows that an easy read out by the naked eye is feasible, exploiting the direct electron transfer between the enzyme and the polymer film.

Analytical performance of the Au/(PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)$_n$ electrodes and mechanistic discussion

Figure 5A shows the calibration current density curves obtained from the electrodes with different numbers of (PABMSA1/PMSA1;PQQ-GDH)$_n$ bilayers (n=0-3). The oxidation current has been measured as a function of glucose concentrations at E = +0.4 V vs. Ag/AgCl. It has been found that the dynamic range is between 0.025 and 3mM of glucose with a tendency to increase with raising layer number. It can also be seen that anodic signals of the multilayer network enhance with the increasing number of deposited bilayers for the same glucose concentration. The results clearly suggest that the construction of these electrodes can be realized through precisely controlling the number of attached (PABMSA1/PMSA1;PQQ-GDH)$_n$ bilayers.

Since the long-term stability is an important parameter for the evaluation of the performance of a sensing system, we have traced the stability of our Au/(PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)$_n$ multilayer films by testing their activity in glucose solution, after the electrodes were kept at 4 °C in 20mM MES+5mM $CaCl_2$, pH 6 when not

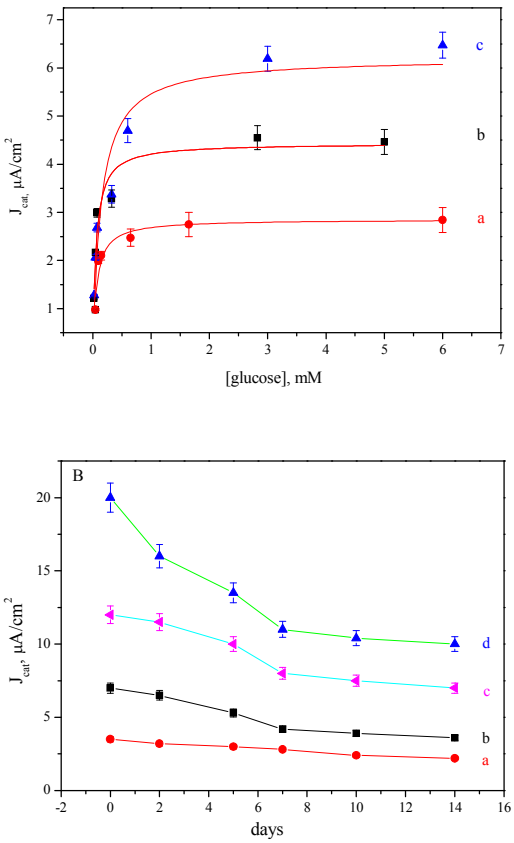


Figure 5. A) Change in catalytic current density for the Au/(PMSA1;PQQ-GDH)/(PABMSA1/PMSA1;PQQ-GDH)$_n$ (a-n=1, b-n=2, c-n=3) as a function of glucose concentrations. Bioelectrocatalytic signal values were registered from the respective cyclic voltammograms at E = +0.4 V; surface area used for the calculation-0.065 cm^2 B) Stability of the multilayers: (a-n=0, b-n=1, c-n=2, d-n=3).

in use. Figure 5B shows that the catalytic current response maintained over 60% of the initial value after 12 days. The decrease seems to be mainly caused by partial disassembly of the layered structure since the enzyme immobilization in one layer (PMSA1;PQQ-GDH) is stable (>90%) over the whole period of investigation (see Figure 5A). This is not usual for PQQ-GDH, which often shows stronger decrease in activity due to disassembly of PQQ from the enzyme.[55-57] These results demonstrate that the entrapment of the PQQ-GDH into the sulfonated polyaniline films leads to an improved long-term stability, making this type of architecture promising to be considered as a candidate for the construction of glucose biosensors.

The measurements strongly support the idea of a direct interaction of the enzyme with the polymer, since no free PQQ has been added during storage and bioelectrocatalysis occurs after 10 days in permanent electrolyte contact. It should also be taken into account that we use PQQ-GDH, which has been reconstituted at a molar ratio of 1 (protein:PQQ),[51] so that no or negligible excess of free PQQ exists and specific activity is rather high (~2200 U/mg). This fact, together with unusual stability of the enzyme electrode makes a hypothesis on a

potential mediation of electron transfer by some denaturated enzyme and liberated PQQ rather unlikely.

Based on these, but also on previously obtained results with polypyrrole-entrapped quinohemo-protein alcohol dehydrogenase QH-ADH,[4, 5] which demonstrated a direct electron transfer via the π-system of the polypyrrole network, it seems to be feasible to build reagentless biosensors when a close contact of polymer and enzyme can be provided. For dehydrogenases this represents a more defined situation, since no interference from reduced species of oxygen can occur. According to our previous report,[23] the emeraldine salt PMSA1 polymer and the enzyme PQQ-GDH act as reaction partners, since the polymer reduction occurs upon addition of glucose to the polymer-enzyme mixture in solution, resulting in significant color change of the polymer without applying any potential.

The same effect we demonstrate in this work by immobilizing both reaction partners on a paper strip without addition of free PQQ. When immobilized on electrodes, the potential can be additionally used to drive the reaction in the desired direction, although some enzyme molecules are located far away from the electrode surface.

Conclusion

In this work, we utilize differently sulfonated polyaniline copolymers as a matrix for the entrapment of the PQQ-GDH by fabricating a multilayered enzyme network via layered polymer deposition. Sulfonated polyanilines with their good biocompatibility and ability to react directly with PQQ-GDH can serve as a matrix without inhibiting the catalytic activity of the entrapped enzyme. The resulting multilayer system shows an enhanced bioelectrocatalytic response with increasing number of deposited building blocks, which indicates that the sensitivity is tunable by controlling the film thickness. Bioelectrocatalysis starts at a potential of the enzyme redox unit, i.e. no large overpotential is needed to drive the reaction. Particularly the mild fabrication process and active role of the polymer films in closing the enzymatic cycle lead to a system with high stability, since good bioelectrocatalysis can be detected even after more than 12 days of storage. Therefore, the proposed construction method is interesting for the development of autonomous sensing units, indicator system for immune reactions based on label detection and miniaturized biosensors.

Acknowledgements

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Graphical Abstract

"Multilayered sulfonated polyaniline network with entrapped pyrroloquinoline quinone-dependent glucose dehydrogenase: tunable direct bioelectrocatalysis"

Differently sulfonated polyaniline copolymers have been utilized as a matrix for the entrapment of the PQQ-GDH by fabricating a multilayered enzyme network via layered polymer deposition, resulting in a direct bioelectrocatalytic response with increasing number of deposited building blocks together with the color change upon addition of the substrate.