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

David Sarauli,^{*a} Chenggang Xu,^b Birgit Dietzel,^c Burkhard Schulz^d and Fred Lisdat^{*a}

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 which show a significant direct bioelectrocatalytic 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 in 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.

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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 such a way that not only a loss of the bioactivity of entrapped proteins is prevented, but also contact with 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 though the concept of contacting enzyme molecules with conjugated polymer 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 in the overall electron transfer. One of the rational ways to achieve such

functional enzyme organization is to immobilize the proteins in 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 already been proven to represent suitable materials for this purpose.^{4,6,11–15}

Particularly polyaniline and its substituted forms have been 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 ordered films and/or possess a defined charge, thus being able to work as anti-interference layers in enzymatic sensors.^{2,16,21,24} Especially self-doped polyanilines have received great attention 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. Self-doping can be performed, for example, by sulfonation of polyaniline rings or copolymerization of aniline with sulfonated aniline derivatives.^{18,23,25–27}

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Interactions of polyaniline derivatives with selected bio-entities, 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 Formation of novel multilayer architectures based on differently substituted polyanilines and cytochrome *c*,³⁵ choline oxidase,³⁶ xanthine oxidase,^{37,38} oxalate oxidase,³⁹ bilirubin oxidase,^{37,40} glucose oxidase,⁴¹ and sulfite oxidase⁴² 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 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 copolymers²³ 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 the active enzyme.

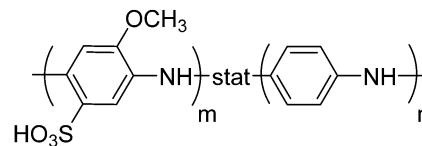
Experimental section

Chemicals

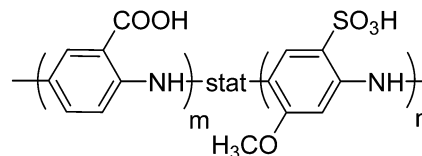
MES buffer was purchased from Sigma-Aldrich (Taufkirchen, Germany), dehydrated calcium chloride and anhydrous D-(+)-glucose were obtained from Fluka Analytics (Taufkirchen, Germany). They were used without further purification. sGDH (*Acinetobacter calcoaceticus*) was kindly gifted to us by Roche Diagnostics 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}



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: $m/n = 1/1.8$).

Preparation of the enzyme solution

sGDH was dissolved in 5 mM MES buffer in the presence of 1 mM CaCl_2 and the pH was 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 $-20\text{ }^\circ\text{C}$. Prior to each measurement, the specific activity of the reconstituted enzyme was determined to be $2200 \pm 30\text{ U mg}^{-1}$ by using 2,6-dichlorophenol indophenol (DCIP) as an electron acceptor.

Construction of 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 concentrated sulfuric acid overnight. Prior to each usage the wires must be washed with water again and then they should be placed in concentrated HNO_3 for 15 minutes. Finally they should 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 slides with surface resistivity $15\text{--}25\text{ }\Omega\text{ sq}^{-1}$ (obtained from Sigma-Aldrich, Taufkirchen, Germany) were initially incubated in buffer solutions of the PMSA1-PQQ-GDH mixture (1.5 mg ml^{-1} PMSA1, $5\text{ }\mu\text{M}$ PQQ-GDH; 20 mM MES + 5 mM 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, 20 mM MES + 5 mM 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 0.2 mm; obtained from Macherey-Nagel GmbH, Düren, Germany) was used.

Instruments

Electrochemical measurements were performed in a home-made 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 a μ Autolab Type II device (Metrohm, the Netherlands). The scan rate was set to 5 mV s^{-1} . The potential range was chosen as between -0.4 and $+0.4 \text{ V vs. Ag/AgCl}$. 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 a $5 \text{ mM K}_3[\text{Fe}(\text{CN})_6]$ – $5 \text{ mM K}_4[\text{Fe}(\text{CN})_6]$ mixture as a redox probe in the frequency range between 0.1 and 100 000 Hz at an open circuit potential of $+0.18 \text{ V}$. 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 the 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. Fig. 1A (curve 1) illustrates the cyclic voltammetric behavior of the Au/(PMSA1:PQQ-GDH) electrode. A clear redox couple at $E_f = 0.18 \pm 0.01 \text{ V vs. Ag/AgCl}$ might be attributed to the polymer conversion from the emeraldine to pernigraniline state.

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 the active enzyme in the film (Fig. 1A-curve 2). The current starts from a potential of about $E = -0.025 \text{ V vs. Ag/AgCl}$ and reaches at 0.35 V a change of $\Delta I = 57 \pm 6 \text{ nA}$, $n = 3$. The bioelectrocatalysis demonstrates 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 efficient electron withdrawal from the reduced enzyme. However, investigation by varying the polymer/enzyme ratios shows that there is only a limited concentration range, in which

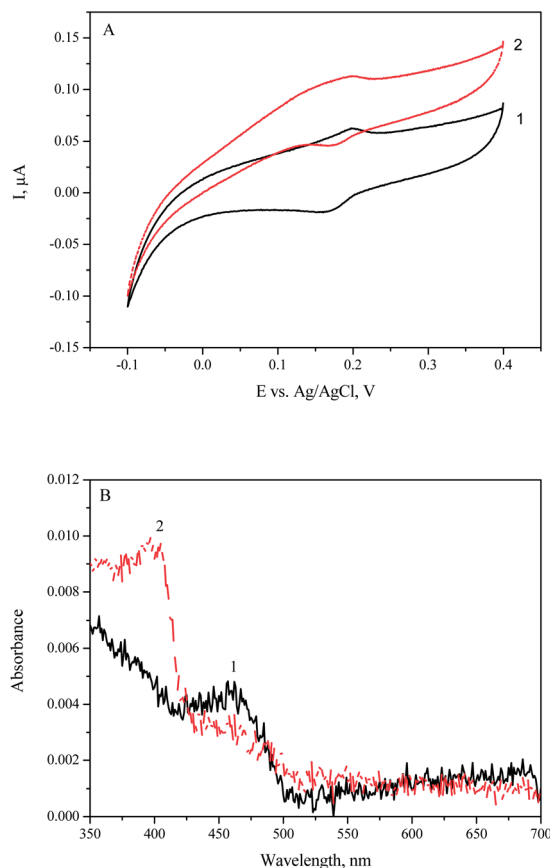


Fig. 1 (A) CV for the Au/(PMSA1:PQQ-GDH) electrode without (1) and with (2) glucose (scan rate = 5 mV s^{-1}); (B) UV-Vis spectra of the ITO electrode covered with (PMSA1:PQQ-GDH) without (1) and with (2) glucose. Measurements are taken in $20 \text{ mM MES} + 5 \text{ mM CaCl}_2$ buffer pH 6, $[\text{glucose}] = 5 \text{ mM}$.

bioelectrocatalysis occurs. At 4 mg ml^{-1} PMSA1/ $5 \mu\text{M}$ PQQ-GDH concentration ratio precipitation of the polymer occurs. At polymer concentrations $1.5 \text{ mg ml}^{-1} < \text{PMSA1} < 4 \text{ mg ml}^{-1}$ rather smaller catalytic currents can be detected. Thus, all further measurements were performed with this medium concentration ratio of 1.5 mg ml^{-1} PMSA1/ $5 \mu\text{M}$ PQQ-GDH (see the Experimental section).

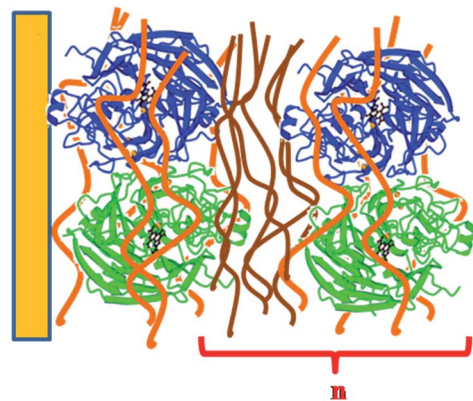
To determine whether it is possible to verify the biochemical PMSA1 polymer reduction spectrophotometrically ITO/PMSA1:PQQ-GDH electrodes have been prepared in the same way as on gold. Fig. 1B demonstrates the spectral changes of the mixed polymer–enzyme film on ITO in the absence (curve 1) and in the 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 a 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 (Fig. 1B), but nevertheless, 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) were 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 an additional negatively charged PABMSA1 polymer layer to the Ca²⁺-containing PMSA1 conformer *via* electrostatic binding. Since the coordination of Ca²⁺ to sulfonate ring substituents in emeraldine salt-polyanilines is known^{52,53} and expected to stabilize their more planar conformers, PMSA1 is applied here in a Ca²⁺-containing buffer. The Ca²⁺ ions can compensate the negative charge at the SO₃⁻ 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*), the 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 *R*_{ct} to characterize the modification steps of the electrodes. Fig. 2A shows the EIS spectra presented as the Nyquist plot of electrodes modified with different numbers of



Scheme 3 Schematic representation of the multilayered enzyme network.

(PABMSA1/PMSA1:PQQ-GDH)_n bilayers. Significant differences after each layer deposition were 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 Fig. 2A.

In order to demonstrate the ability of the PABMSA1 polymer to enhance the formation of the desired multilayered assembly, EIS measurements were performed on electrodes, which were repeatedly incubated in a PMSA1-GDH solution without intermittent PABMSA1 depositions. No change in impedance spectra with the 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 (Fig. 2B). In addition, no change in impedance spectra is detectable, when both incubation steps and measurements are performed in buffer solutions containing no Ca²⁺ ions, confirming the formation of multilayers *via* electrostatic interactions discussed before. Also cyclic voltammetry and UV-Vis

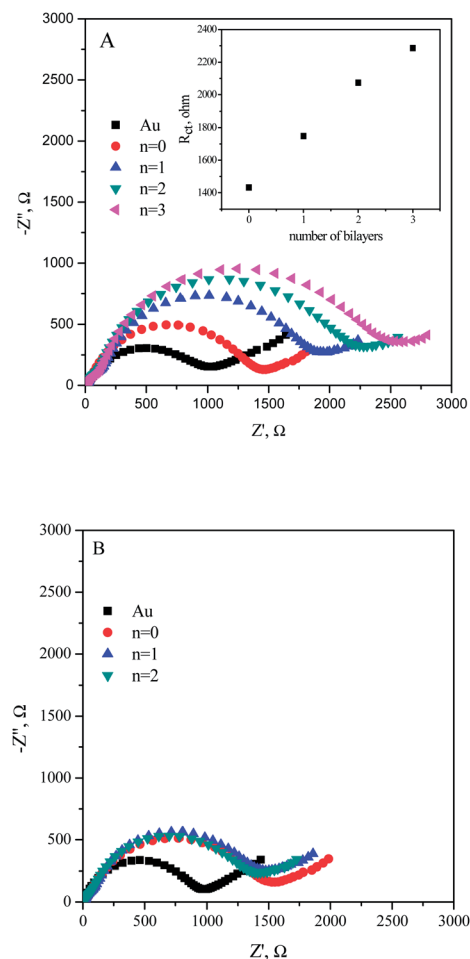


Fig. 2 Impedance spectra of the (A) Au/(PMSA1:PQQ-GDH)/(PABMSA1/PMSA1:PQQ-GDH)_n electrodes with different numbers of layers (*n* = 0 to 3). Inset: the relationship of *R*_{ct} vs. the number of bilayers; (B) Au/(PMSA1:PQQ-GDH)_n electrodes (*n* = 0 to 3). Measurements are taken in 5 mM K₃[Fe(CN)₆]/5 mM K₄[Fe(CN)₆] solutions of 20 mM MES + 5 mM CaCl₂ buffer, pH 6.

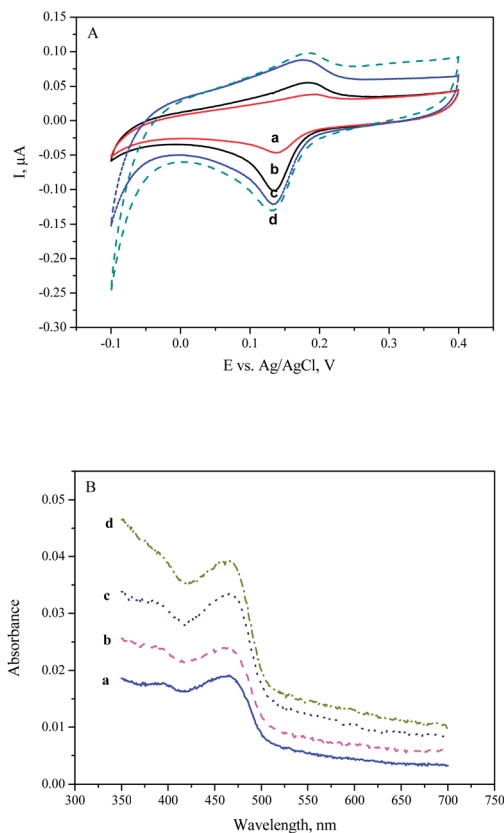


Fig. 3 (A) Cyclic voltammograms and (B) UV-Vis spectra of Au/(PMSA1:PQQ-GDH)/(PABMSA1/PMSA1:PQQ-GDH)_n multilayer electrodes in the order of the increasing number of bilayers ((a) $n = 0$, (b) $n = 1$, (c) $n = 2$ and (d) $n = 3$). Scan rate -5 mV s^{-1} . Measurements are taken in 20 mM MES + 5 mM CaCl_2 buffer pH 6.

spectroscopy were carried out to characterize the formation of the multilayered structure. Fig. 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 $E = +0.18 \text{ V vs. Ag/AgCl}$ 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. Fig. 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 the 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 (Fig. 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 were prepared on Au and their bioelectrocatalytic characteristics were investigated. Fig. 4A represents cyclic voltammetric responses obtained from electrodes modified

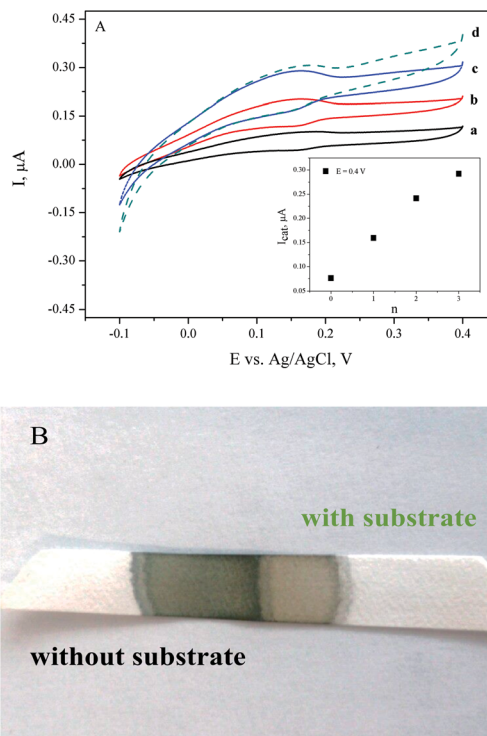


Fig. 4 (A) Catalytic response of Au/(PMSA1:PQQ-GDH)/(PABMSA1-PMSA1:PQQ-GDH)_n multilayer electrodes in the presence of 5 mM glucose ((a) $n = 0$, (b) $n = 1$, (c) $n = 2$ and (d) $n = 3$). Inset: the anodic currents vs. the number of layers at $E = +0.4 \text{ V vs. Ag/AgCl}$. (B) Colour change of the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

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 (Fig. 4A), starting from the potential at about $E = -0.025 \text{ V vs. Ag/AgCl}$. The anodic response exhibits a nearly linear dependence on the number of (PABMSA1/PMSA1:PQQ-GDH)_n bilayers (inset in Fig. 4A) due to the increased amount of the enzyme embedded in the layers. The bioelectrocatalysis demonstrates that efficient electron transfer between the redox centre of the enzyme and the electrode through different polymer/enzyme layers is 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 the redox state of the polymer after the enzymatic reaction, such a layered deposition of PQQ-GDH was also performed on a paper material. Fig. 4B demonstrates the change in colour 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 colour characteristic of highly conducting polyanilines

in the emeraldine salt redox state starts to disappear. This shows that an easy read out by the naked eye is feasible, exploiting 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

Fig. 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 was measured as a function of glucose concentrations at $E = +0.4$ V vs. Ag/AgCl. It was found that the dynamic range is between 0.025 and 3 mM of glucose with a tendency to increase with increasing the 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 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 20 mM MES + 5 mM CaCl₂, pH 6 when not in use. Fig. 5B shows that the catalytic current response was 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 enzyme immobilization in one layer (PMSA1:PQQ-GDH) is stable (>90%) over the whole period of investigation (see Fig. 5A). This is not usual for PQQ-GDH, which often shows a stronger decrease in activity due to disassembly of PQQ from the enzyme.⁵⁵⁻⁵⁷ These results demonstrate that the entrapment of PQQ-GDH in 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 was 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 is 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⁻¹). This fact, together with the unusual stability of the enzyme electrode makes a hypothesis on potential mediation of electron transfer by some denaturated enzyme and liberated PQQ rather unlikely.

Based on these, and also on previously obtained results with polypyrrole-entrapped quino-hemo-protein alcohol dehydrogenase QH-ADH,^{4,5} which demonstrated direct electron transfer *via* the π -system of the polypyrrole network, it seems to be feasible to build reagentless biosensors when close contact between the polymer and enzymes 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 a significant colour change of the polymer without applying any potential. We demonstrate the same effect 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 matrices for the entrapment of 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 the increasing

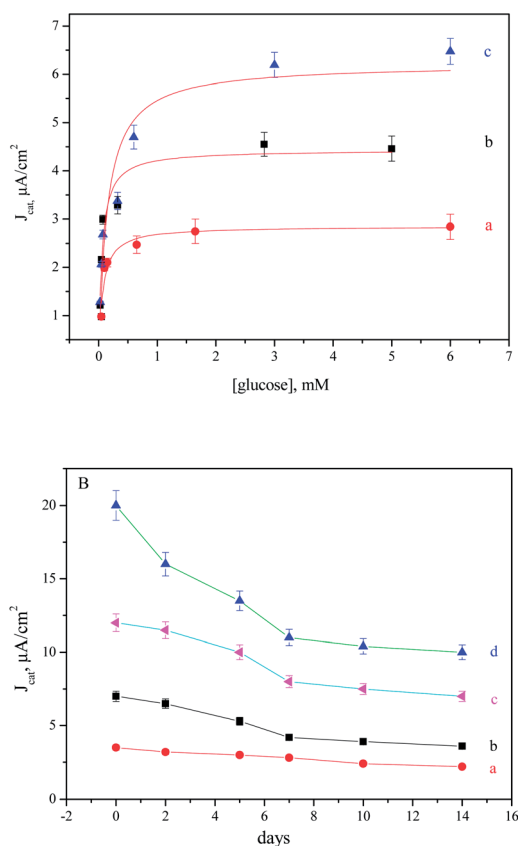


Fig. 5 (A) Change in catalytic current density of the Au/(PMSA1:PQQ-GDH)/(PABMSA1/PMSA1:PQQ-GDH)_n ((a) $n = 1$, (b) $n = 2$, and (c) $n = 3$) as a function of glucose concentrations. Bioelectrocatalytic signal values were registered from the respective cyclic voltammograms at $E = +0.4$ V; surface area used for the calculation – 0.065 cm² (B) stability of the multilayers: ((a) $n = 0$, (b) $n = 1$, (c) $n = 2$, and (d) $n = 3$).

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 systems for immune reactions based on label detection and miniaturized biosensors.

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