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Simple immobilization of pyrroloquinoline quinone on few-walled carbon nanotubes

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Abstract

Pyrroloquinoline quinone (PQQ) was immobilized on glassy-carbon electrodes (GCE) modified with single-walled carbon nanotubes (SWCNT), few-walled carbon nanotubes (FWCNT) and carbon black (Vulcan XC72R). Modified electrodes were prepared by drop-casting. Immobilization was achieved with an extremely simple dipping procedure and without any further modification to the electrodes. Electrochemical performance of the electrodes was studied by cyclic voltammetry and spectroelectrochemistry. FWCNT adsorbed 30 times more PQQ than the other carbon materials. Compared to more complicated immobilization methods, PQQ/FWCNT/GCE showed well-defined electrochemistry in a considerably wide pH area from 2 to 12. The dipping process is affected by pH and electrostatic forces. At dipping pH 9.5, where both FWCNTs and

PQQ have strong negative charge, the adsorption was halved compared to dipping pH 2, where the charges are smaller.

Keywords

Pyrroloquinoline quinone; carbon nanotubes; immobilization; pH; electrochemistry

1. Introduction

Quinoproteins are a group of enzymes which contain a quinone species as the cofactor. They catalyze the oxidation of many organic molecules, like alcohols, sugars and primary amines [1]. One of these cofactors is pyrroloquinoline quinone (PQQ), *e.g.* in alcohol and glucose dehydrogenase (EC 1.1.98.8 and 1.1.5.2). PQQ is tightly bound to the enzyme, which makes the enzymes attractive for simple biosensors as the cofactor does not need to be present in the bulk solution. Furthermore, PQQ-dependent enzymes do not require oxygen as the electron acceptor making them applicable to non-oxygenous environments. Thus, these enzymes have been immobilized on electrodes and used as biosensor candidates for glucose and alcohol detection [2-6]. PQQ itself is also a good catalyst for oxidation and reduction of another common cofactor, nicotinamide adenine dinucleotide (NAD^+), which exhibits very irreversible electrochemistry [7,8].

However, the electrochemistry of PQQ is not straightforward. In neutral conditions, it reacts very irreversibly on many solid electrode materials, like platinum, gold and glassy carbon [9]. This could be due to strong adsorption of PQQ on solid materials [10]. The problem has been circumvented by modifying electrode surface with an organic layer, which should inhibit strong interaction. Shinohara *et al.* [9] used polypyrrole on platinum and the modified electrode showed reversible oxidation and reduction of PQQ in pH 7. Katz *et al.* [11] modified gold with cystamine and observed quasi-reversible reactions in neutral and acidic conditions.

PQQ can also react reversibly if it is immobilized on the electrode surface. This has been achieved for example with didodecyldimethylammonium bromide on glassy carbon [7], polypyrrole on platinum [9], ZrO₂ modified silica gel carbon paste electrode [12] and by carbodiimide coupling to cystamine groups on gold electrode [11]. Carbon nanotubes (CNT) have also been reported as immobilization substrates for PQQ. Han and Tachikawa [13] used single-walled CNTs when they prepared a PQQ-modified electrode for thiol detection. Sun *et al.* [14] used multi-walled CNTs with chitosan, when they studied PQQ-dependent glucose dehydrogenase. However, little focus was given to the pH dependency of the modified electrodes.

In the present work the electrochemistry of PQQ adsorbed on glassy-carbon electrodes (GCE) modified with different nanocarbon materials is compared. We show that adsorption represents a simple immobilization strategy of PQQ on CNT-modified GCE and explore its pH dependent electrochemical response.

2. Experimental details

All the chemicals excluding the carbon materials were purchased from Sigma-Aldrich and used without further purification. Carbon black was Vulcan XC72R (Cabot Corporation). Few-walled carbon nanotubes (FWCNT) with 2-5 walls (< 6 nm diameter, ~ 1 μm length) were obtained by catalytic pyrolysis of CH₄ diluted with 80% H₂ at 950° C according to a method reported elsewhere [15]. The catalyst is a mixture of Co-Mo oxides (5 at.%) supported on MgO. After the synthesis, residual catalyst was removed (< 1% w.) from the FWCNTs by washing in HCl, followed by rinsing with deionized water and drying in vacuum, a treatment that did not alter nanotube size but introduced –COOH groups. Single-walled carbon nanotubes (SWCNTs) were purchased from Cheap Tubes (> 90%wt purity, 1-2 nm diameter, 5-30 μm length, with –COOH functionalities) dispersed in aqueous solutions containing polyvinylpyrrolidone (PVPD) surfactant.

Electrodes were prepared by pipetting 5.5 μl of each carbon solution (0.5 mg ml⁻¹ in 2-propanol) on a GCE (5 mm diameter). In the case of SWCNT, surfactant PVPD was

removed by soaking the electrode in water for 2 hours. After drying, the modified electrode was dipped in 0.14 mM PQQ solution in 0.2 M phosphate buffer for 2 hours. Finally, the PQQ-modified electrode was washed with phosphate buffer and used as the working electrode of a 3-electrode electrochemical cell. The counter electrode (CE) was a platinum plate and the reference electrode (RE) a saturated calomel electrode (SCE). Measurements were performed in 0.2 M phosphate buffer solution under N₂ atmosphere.

The spectroelectrochemical measurements were conducted in reflection mode with an optical fiber set-up consisting of a bifurcated reflection probe (FCR-7UV200, Ocean Optics) connected on one end to a deuterium-halogen light source (DH-2000, Ocean Optics) and on the other end to a fiber optic spectrometer (QE-65000, Ocean Optics). The working electrode was the PQQ-modified GCE and a Pt wire and Ag/AgCl (KCl 3 M) were the CE and RE, respectively. The supporting electrolyte was 0.2 M phosphate buffer at pH 9.5 bubbled with N₂.

3. Results and discussion

First, the adsorption of PQQ on different carbon supports (the bare GCE, Vulcan/GCE, SWCNT/GCE and FWCNT/GCE) was studied by cyclic voltammetry. CVs for PQQ adsorbed on electrodes with different carbon materials are shown in Fig. 1. It can be seen that while PQQ did not adsorb at all on the bare GCE, the electrode modified with FWCNTs showed by far the strongest electrochemical response indicating a good adsorption of PQQ.

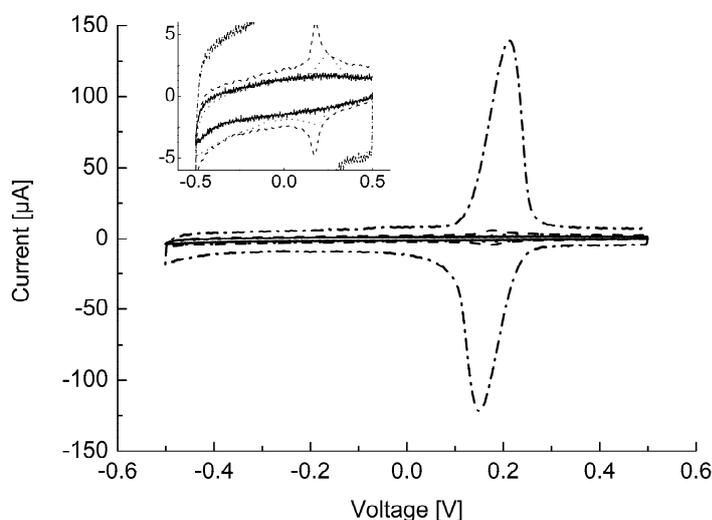


Figure 1. CVs of GCE modified with PQQ (—), Vulcan/PQQ (— —), SWCNT/PQQ (·····) and FWCNT/PQQ (— · —). Measurements done at pH 2, scan speed 50 mV/s. The inset shows a zoom for the electrodes with poorer adsorption.

The amount of adsorbed PQQ per gram of carbon material was calculated from the area of the oxidation and reduction peaks assuming the following two-electron transfer reaction: [1]



This yields the values (in g of PQQ per g of carbon): 0.1364 for the case of FWCNTs, 0.0044 for Vulcan and 0.0041 for SWCNTs. This means roughly 30 times larger adsorption for PQQ on FWCNTs than on the other carbon materials. The formal potential of PQQ on FWCNTs and Vulcan was estimated from the mid-point of the cathodic and anodic peaks to be around +180 mV, in agreement with the value reported by Jao *et al* [7]. For the SWCNT/GCE, the formal potential was +210 mV. Peak currents were linearly dependent on the scan speed in all cases indicating a surface-confined electrochemical process. The peak spacing was small, with only 11 mV for Vulcan/GCE indicating nearly ideal nernstian behavior (peak spacing 0 mV for a surface confined reaction), 67 mV for FWCNT/GCE and 82 mV for SWCNT/GCE. The CV of PQQ at each electrode was stable for at least 100 scans.

PQQ electrochemistry was also followed by in situ UV-Vis absorption spectroscopy. Fig. 2 shows the current/charge together with the absorbance at λ_{\max} for PQQH₂ (310 nm) [9] measured during a CV (Fig. 2a) and a double potential step chronocoulometry (Fig. 2b). There is a clear synchronization between electrical and optical responses, with the absorbance due to the generation(depletion) of the reduced form of PQQ increasing(decreasing) concurrently with the current/charge during the cathodic(anodic) sweep in the CV or step in the chronocoulometry.

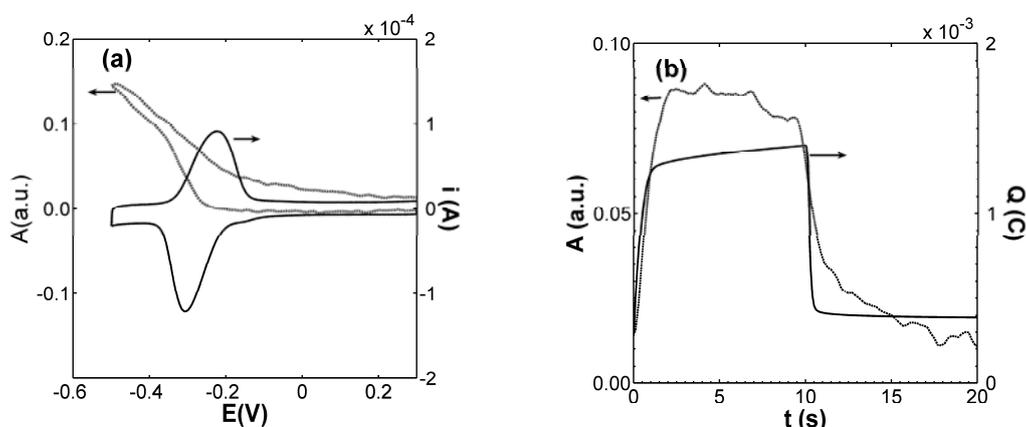


Figure 2. a) Simultaneous CV and absorbance (310 nm) of PQQ/FWCNT/GCE electrode, b) electrical charge and absorbance of the same electrode during a double potential step experiment at -0.5 and +0.1 V

As PQQ has many protonatable groups, its electrochemistry can be expected to vary with the pH of the solution. The influence of pH on PQQ electrochemistry was studied at the FWCNT/GCE due to the higher currents. CVs corresponding to different pH values (between 2 and 12) are shown in Fig. 3a. Formal potentials were determined as +180 mV (pH 2), +33 mV (pH 4), -90 mV (pH 6), -234 mV (pH 8) and -361 mV (pH 10). The potential change over a pH unit is approximately 67 mV, which suggests a two-proton, two-electron transfer according to Eq. 1.

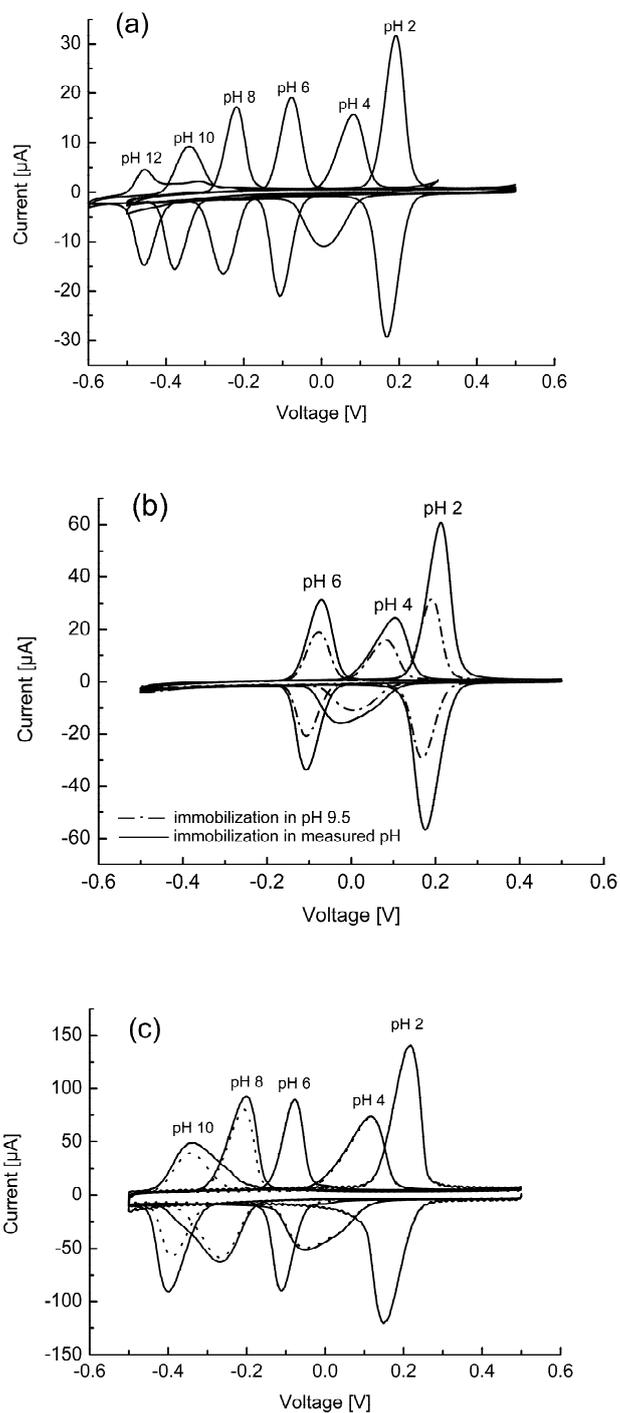


Figure 3. CVs of PQQ/FWCNT/GCE electrode in phosphate buffer solutions with different pH. a) PQQ immobilization performed at pH 9.5 (10 mV s^{-1}). b) PQQ immobilization performed in two dipping solutions with different pH and measured in pH 2, 4 and 6 (10 mV s^{-1}). c) Before (solid) and after (dotted) 100 consecutive CVs (50 mV s^{-1}).

The optimum pH seems to be in the acidic region at around pH 2, where the highest reversibility and currents are noted. However, the CV is very well defined up to pH as high as 12 where the oxidation peak starts to be suppressed and a shoulder emerges. These broad peaks are probably related to a two-step mechanism for the two-electron oxidation and reduction of PQQ, since at sufficiently high scan rates two peaks started to be clearly resolved in the anodic scan at pH 4 and 10 and also in the cathodic scan at pH 8. This could be due to increased charge density at the surface. As pH rises, the $-\text{COOH}$ groups at the surface of FWCNTs resulting from the purification process [16] will deprotonate and FWCNT surface will become more negatively charged. Similarly, the negative charge of PQQ increases with pH: -1 ($1 < \text{pH} < 4$), -2 ($4 < \text{pH} < 5$) and -3 ($\text{pH} > 5$) [7]. The charged groups have repulsive interactions, which can distort the shape of the peaks [17]. When cystamine and carbodiimide coupling was used for immobilization, the reversibility of the reaction increased towards alkaline conditions, where the surface also has less charge [11]. Thus, a possible way to increase the reversibility of the PQQ/FWCNT electrodes would be the use of carbon nanotubes modified with amine groups. These effects, as well as some irreversible processes noted at very low potentials (< -0.5 V), are currently under further investigation. Overall, PQQ adsorbed on FWCNTs exhibits a better-resolved electrochemistry in a wider pH window than PQQ immobilized using more complex strategies such as with didodecyldimethylammonium bromide [7] or ZrO_2 modified silica gel [12].

The pH of the dipping solution was also found to have an influence on the extent of PQQ adsorption on FWCNT/GCE and on its electrochemistry. Since both the FWCNTs and PQQ have negative charge at high pH, the adsorption is less favored due to electrostatic repulsion. This effect is illustrated by CVs of PQQ adsorbed on FWCNT/GCE from two dipping solutions with different pH and measured also in different buffers (Fig. 3b). For all the investigated pH, lower pH dipping solution promotes PQQ adsorption resulting in higher peak currents (at pH 2, the peak area doubles when the pH dipping solution is dropped from 9.5 to 2.0). On the other hand, lowering the pH increases the peak spacing slightly suggesting that the larger amount of adsorbed PQQ has a negative effect on the

reversibility of the reaction. This could be because of the repulsive interaction between PQQ molecules, which is enhanced when the surface concentration increases.

The solution pH also had an effect on the stability of PQQ, with the electrical charge hardly decreasing (<5 %) after 100 consecutive potential cycles at pH between 2 and 6 but dropping a 30 and 40% at pH 8 and 10, respectively. At pH 12, the drop was apparent from the initial scans. The stability of PQQ on FWCNT/GCE is comparable to that exhibited by PQQ immobilized with cystamine, poly(ethylamine) and triethylenetetraamine on gold [18]. The electron transfer rates, k_s , determined from Laviron's theory [19] were 0.58 s^{-1} (pH 2) and 0.66 s^{-1} (pH 6), which are in the same range as the values obtained by Katz *et al.* [11]. k_s has not been estimated at higher pH values where peak splitting starts to occur but we can conclude that k_s seems fairly constant in the acidic range.

4. Conclusions

PQQ was successfully adsorbed and immobilized on GC electrode modified with FWCNTs, SWCNTs and Vulcan XC72R with a simple dipping procedure. FWCNT/GCE had the largest adsorption of PQQ by a factor of 30. FWCNT/GCE performed best at pH 2 and had well-defined electrochemistry up to pH 12. The electrochemical reaction was also followed by monitoring the absorbance of the reduced form of PQQ generated at the electrode. The electrode was very stable during 100 consecutive CVs in $\text{pH} \leq 6$ solutions. Lowering the pH of the immobilization solution was also found to result in better adsorption, highlighting the importance of electrostatic forces in the adsorption process. Overall, we have prepared and characterized a stable PQQ-electrode that exhibits very reversible electrochemistry in a considerably wide pH range using a very simple preparation procedure.

Acknowledgements

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