ELSEVIER

Nitric oxide sensor based on carbon fiber covered with nickel porphyrin layer deposited using optimized electropolymerization procedure

Jan Hrbáč [a,*], Čeněk Gregor [a], Markéta Machová [a], Jana Králová [b], Tomáš Bystroň [c], Milan Číž [b], Antonín Lojek [b]

[a] Department of Physical Chemistry, Palacký University, Faculty of Science, tř. Svobody 26, 771 46 Olomouc, Czech Republic
[b] Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic
[c] Department of Inorganic Technology, Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic

Abstract

Electropolymerization regime of meso-tetrakis(3-methoxy-4-hydroxyphenyl) porphyrin is optimized to yield films possessing both electrocatalytical and permselective properties towards nitric oxide oxidation. The sensor composed of electrochemically oxidized carbon fiber, covered solely with nickel porphyrin derivative layer electropolymerized using our method, is characterized by high selectivity towards nitrite (1:600), ascorbate (1:8000) and dopamine (>1:80), determined by constant potential amperometry at 830 mV (vs. Ag/AgCl). Selectivity for ascorbate and dopamine as well as detection limit for NO (1.5 nM at S/N=3) is 5-10 times better than parameters usually reported for Nafion® coated porphyrinic sensors. Nafion coating can further enhance selectivity properties as well as aids to the stability of the sensors' responses.
© 2006 Elsevier B.V. All rights reserved.

1. Introduction

Since the pioneering work of Malinski and Taha [1,2], the use of electropolymerized nickel tetrakis(3-methoxy-4-hydroxyphenyl) porphyrin (NiTMHPP) and similar porphyrinic compounds has become popular in the construction of nitric oxide sensors. Carbon fiber microelectrode, covered with electropolymerized nickel porphyrin derivative followed by Nafion layer - "porphyrinic sensor", is among the most widely used electrochemical sensors for the detection and monitoring of nitric oxide both in vitro and in vivo. The construction of porphyrinic sensor consists of three steps:

1. electrochemical oxidation of carbon fiber ("pretreatment"),
2. electropolymerization of porphyrinic catalyst layer,
3. Nafion coating - usually by using "dip-dry" method.

Pretreatment is a procedure aimed not only at increasing the sensor's sensitivity, but also at obtaining a stable baseline for long term experiments. The essence of the pretreatment is electrooxidation/electroreduction of the fiber's surface, yielding surfacial oxygen containing functional groups (carbonyl, carboxyl, quinone, ether, ester and hydroxyl), often denoted as carbon or graphitic oxide [3]. These moieties can modulate electron transfer rates for many electroactive species. Artificially oxidized surface eliminates the problem with gradual oxidation of the sensor's surface, potentially taking place while operating the sensor at relatively high potentials used in NO sensing. The increase in surface area (typically about five-fold [4]), accompanying the process, is also beneficial for higher current densities are achieved on "pretreated" fibers; nevertheless, capacitance is also increased, therefore higher background currents are inevitable for pretreated electrodes.

Electropolymerized NiTMHPP grants the sensor electrocatalytical properties towards nitric oxide oxidation, typically reducing its overpotential by some 50-100 mV [5]. It is known that the electrocatalytical action of NiTMHPP and similar compounds cannot be ascribed to easier oxidation of NO bound

---

* Corresponding author. Tel.: +420 585 634 755; fax: +420 585 634 425.
E-mail address: hrbac@aix.upol.cz (J. Hrbáč).

to nickel central atom of the porphyrin complex through axial coordination. This mechanism would require counterbalancing of the charge induced by Ni(II) oxidation to Ni(III) by diffusion of anions into the film. The electrocatalytic activity persists in the Nafion coated porphyrinic sensor, although Nafion effectively suppresses the diffusion of anions and thus blocks nickel redox transitions [1,6]. Moreover, the electrocatalytical action of porphyrine film remains exactly the same after removal of nickel from the film [7]. It was speculated that electrocatalysis might be caused by electrochemical oxidation (pretreatment) of the fiber in an alkaline solution used during TMHPP deposition process rather than by the poly-NiTMHPP layer itself [8,9] but nowadays the view that poly-NiTMHPP represents 3D porous conductive organic layer modifying the electrode surface without any specific chemical interaction with NO seems to be well established [7,10]. The electropolymerization procedures, used and recommended by majority of the authors working in the field, rely on constant potential electrolysis or cyclic potential scanning of NiTMHPP solution in 0.1 M NaOH. Although constant potential electropolymerization provides surfaces with good electrocatalytical properties, cyclic scanning method of poly-NiTMHPP layer preparation is reported to give layers which have 2-5 times higher electrocatalytical activities [6]. Most frequently used protocols for NiTMHPP electropolymerization are summarized in Table 1.

Selectivity of poly-NiTMHPP layer is the task which remains unaddressed in the literature, though at least in two cases some information can be extracted. Friedemann et al. [15] report that selectivity ratios for NiTMHPP-Nafion coated electrodes, compared to electrodes coated solely by Nafion, are 1.6 times higher for ascorbate and two times higher for dopamine, while selectivity for nitrite is slightly lowered at their NiTMHPP-Nafion coated electrodes. Even more convincing results were achieved by Ciszewski and Milczarek [12] who replaced the Nafion layer with polyeugenol and presented selectivity data (for ascorbate, nitrite, dopamine and several other interferents) for carbon fibers covered with polyeugenol

itself and for carbon fibers equipped with poly-NiTMHPP layer below the polyeugenol. The selectivities were much better in the presence of poly-NiTMHPP - 15.5 times for nitrite, 3.6 times for ascorbate and 6.2 times for dopamine, compared to sensors without poly-NiTMHPP. These findings encouraged us to find optimum conditions of NiTMHPP electropolymerization in terms of resulting sensor selectivity.

Nafion layer is reported to be casted on NiTMHPP layer in order to grant the sensor selectivity against anionic interferents (e.g. ascorbate, nitrite, urate etc.). Coating of fibers by Nafion is a delicate procedure, because discontinuities in the Nafion layer could severely impair the selectivity of the resulting sensor. Original sensors by Malinski and Taha [1] used thermally sharpened carbon fibers, protruding only some 30 μm from the electrode bodies. Immersion of such electrode tip into the Nafion solution must have led to a relatively thick coating. To achieve a uniform coating of sufficient thickness on longer fibers is a much more difficult task and many authors resort to producing multiple coats on their electrodes. Heat treatment of Nafion layer is necessary to achieve selective coating: 80 °C is the most widely used temperature [7,16–18], though temperatures around 200 °C, close to glass transition of Nafion, are also recommended [15,19,20]. Selectivities and detection limits of porphyrinic as well as various other polymer modified nitric oxide microelectrode sensors are summarized in the review by Ciszewski and Milczarek [21]. Selectivities of electrodes coated by NiTMHPP and eight coats of Nafion, given by Friedemann [15], are 1:181±28 for nitrite, 1:986±12 for ascorbate and 1:4±1 for dopamine with detection limit 76±12 nM NO. These parameters were determined using constant potential amperometry (CPA) and appear to be typical. The same authors report that in few cases they obtained "outlier" electrodes with ascorbate selectivity around 1:20,000. An attempt to improve the selectivity of porphyrinic sensor was presented by Mitchell and Michaelis [22] who equipped their poly-NiTMHPP/Nafion electrodes with additional coatings of ascorbic acid oxidase and "polypyridinium" layers in order to improve selectivity towards ascorbate and dopamine respectively. They reached selectivities of 1:297 for nitrite, 1:9050 for ascorbate and 1:5191 for dopamine. The detection limit is not specified, but selectivity improvement achieved by the additional coatings strategy is usually at the expense of sensitivity. The aim of this work was to improve porphyrinic electrodes in terms of lowering their detection limit while keeping high selectivity. We focused on finding the conditions of reproducible preparation of sensors with high selectivity towards ascorbate, similar to those which were only occasionally obtained by Friedemann [15].

2. Experimental

2.1. Reagents

Nafion 117, 5% solution in lower aliphatic alcohols and water, Fluka (cat. No. 70160), nickel II tetrakis(3-methoxy-4-hydroxyphenyl) porphyrin, Interchim (cat. no. UP266260), ascorbic acid, Sigma, sodium nitrite, Lachema Brno, Czech Republic, 3-hydroxytyramine hydrochloride (dopamine), Fluka.

Table 1
Protocols used for NiTMHPP electrodeposition

Solution composition	Method and parameters	Deposition time	Reference
Ni-THMPP (mM)/ NaOH (M)			
0.5/0.1	CP, 0.8 V (vs. SCE)	N/A [a]	[1]
0.5/0.1	CP, 1.2 V (vs. SCE)	10 min	[11–14]
2.0/0.1	CP, 1.2 V (vs. SCE)	10 min	[5]
0.5/0.1	CV, −0.2 V to 1 V (vs. SCE), scan rate N/A	N/A [a]	[1]
0.05/0.1	CV, −0.2 V to 1 V (vs. SCE), 300 mV/s	5.5 min (50 cycles)	[15]
0.5/0.1	CV, −0.2 V to 1.2 V (vs. SCE), 100 mV/s	23 min (50 cycles)	[10,16]
0.5/0.1	CV, −0.2 V to 1.2 V (vs. SCE), 100 mV/s	N/A [a]	[6]

[a] Finished after defined surface coverage (0.9–1.4 nM cm^{-2}) is achieved. CP - constant potential, CV - cyclic voltammetry.

All products were of reagent grade and were used as received. Stock solutions were made using double distilled water. Nitric oxide saturated aqueous solutions were prepared by bubbling NO gas from commercial cylinder (Linde, Czech Republic) through deionized water carefully deaerated with argon. The whole procedure was done in a glass gasometric burette, equipped with a silicone rubber septum, through which aliquots of NO solution were collected using gastight Hamilton microsyringe. Phosphate buffered saline (PBS), in which the majority of measurements was carried out, contained 23.9 g $Na_2HPO_4 \cdot 12H_2O$, 2.5 g $NaH_2PO_4 \cdot 2H_2O$ and 8 g NaCl/l, the pH was adjusted to 7.4 by small additions of NaOH or HCl solutions as required.

2.2. Microelectrode fabrication

Carbon fiber (diameter 7 μm, Ten Cate Advanced Composites, Amsterdam, The Netherlands) is glued using conductive silver epoxy (Epotek H20E, Polytec, Germany) onto copper wire, the junction is then cured at 170 °C for 10 min. The fiber with copper contact attached is fitted into the glass capillary, about 5 mm of the fiber is left protruding from its contracted end. Both sides of the capillary (fiber and wire) are glued using epoxy resin (CHS Epoxy 1200, Sindat Pilsen, Czech Republic). Prior to use, the protruding fiber is cut to the length of approx. 0.5 mm by lancet, the fiber end of the electrode is briefly sonicated in dichloromethane in order to remove grease. The fiber is then electrochemically oxidized ("pretreated") in PBS. After the pretreatment the electrodes are covered with poly-NiTMHPP, electropolymerized from NiTMHPP solutions containing 0.1 M NaOH. Nafion coating is made from 5% Nafion solution using a "dip-dry" method. Detailed description of pretreatment and coating procedures is given in the Results and discussion section of this paper.

2.3. Instruments

Carbon fiber pretreatment, electropolymerization coating and voltammetric measurements were performed using a homemade potentiostat, built according to Refs. [23] and [24], controlled by National Instruments PCI-6023E multifunction card using routines programmed in LabView™ programming environment. UV/VIS spectra were recorded on Helios Alpha (Thermo Spectronics, U.S.A.) spectrometer in thin layer cuvettes (0.25 mm optical path). EQCM measurements were performed on gold coated quartz crystals using EQCN-700 apparatus (Elchema, U.S.A.) equipped with Elchema Model PS-205B potentiostat. Silver-silver chloride electrode (MF-2052, Bioanalytical Systems, U.S.A.) was used as a reference electrode. Platinum wire served as an auxiliary electrode. Gold electrode was purchased from Polaro Sensors (Prague, Czech Republic).

2.4. Sensor testing

For testing our sensors, we chose CPA at 830 mV (vs. Ag/AgCl), which is the peak potential of nitric oxide oxidation (measured by differential pulse voltammetry) on bare carbon fiber. After immersion of the tested electrode into a beaker containing 25 ml of PBS, the potential was set up and the background current was allowed to decay until stable baseline was achieved (usually approx. 10-15 min). The solution was stirred using a magnetic stirrer at 300 r.p.m. and the stirring bar length was chosen to fit beaker's diameter, so that stable hydrodynamic conditions were kept throughout the measurement. NO and interferents were injected using Hamilton microsyringes. Selectivities were expressed as ratios between concentrations of nitric oxide and interferent, which gave rise to the same current response. These ratios were determined using final concentrations of 800 nM for NO solution and 20 μM for nitrite, ascorbate and dopamine respectively (concentration ratio 1:25). Thus, if the squirt of NO yielded current 0.5 nA and the squirt of nitrite 0.05 nA, the resulting selectivity ratio was 1:(25×10)=1:250. When high selectivities (>ca 1:2000) were to be determined, 200 μM ascorbate (final concentration) was used. Tested compounds were added into the stirred PBS solution in the order of NO, nitrite, ascorbate and dopamine, best electrodes were tested in clean PBS solutions for each interferent separately. Typically, five electrodes were used at each experiment.

3. Results and discussion

3.1. Effect of pretreatment

The bare carbon fibers we used are more sensitive to nitric oxide than to nitrite and ascorbate when used as received - selectivity ratios are 1:30±12 for nitrite and 5.8±3.5 for ascorbate. On the other hand the fiber is less sensitive to NO than to dopamine (1:0.51±0.32). A typical sensitivity of sensor equipped with 0.5 mm fiber is 0.044 nA/μM NO. This unfavorable parameter improves after electrochemical pretreatment is performed on the electrodes. Obviously, the pretreatment protocols (typically, microelectrode is cycled in PBS between 0 and 2.9 V (vs. Ag/AgCl) for 20 s, 50 Hz triangle wave, followed by 5 s at constant potential -0.8 V and 5 s at 1.5 V), used almost exclusively for the construction of NO sensors [5,8,10,14], match the pretreatments which can be found in the literature dealing with dopamine and ascorbic acid in vivo measurements [4,25–27]. In this particular application, surface carbon-oxygen functionalities, prepared by cycling the potential from 0 to 3 V (vs. SCE) in neutral solution (1% NaCl, phosphate buffer or PBS), make the surface capable of binding cationic compounds (dopamine) while ascorbate is oxidized only on dislocations ("cracks"), uncovered by oxidic layer [4]. As a result, an apparent electrode area is much larger for dopamine than for ascorbate and, in extreme cases, relative sensitivities for dopamine and ascorbate can reach values up to 1000:1 [28]. This makes dopamine and similar compounds very difficult interferents to cope with. Our results indicate that pretreatment protocol according to Refs. [5,8,10,14] (in further text we use the term "conventional" for the above procedure) leads to a 5.7±1.8-fold increase in the current for NO, compared to untreated fiber; the sensitivity increase for nitrite
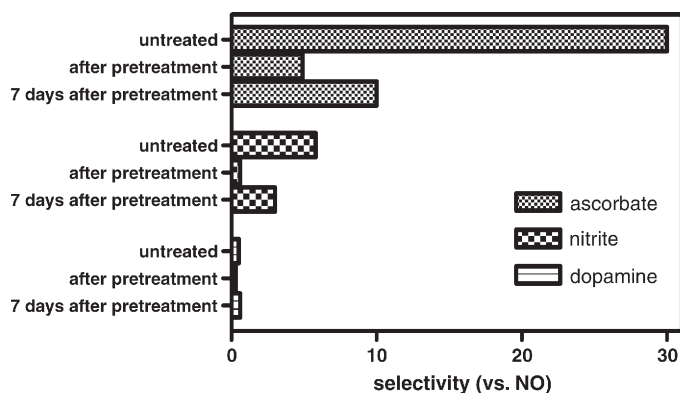
Fig. 1. Selectivity characteristics of uncoated carbon fiber before and after the pretreatment procedure.

is 35 ± 14, ascorbate 53.5 ± 12 and dopamine 4.5 ± 2.2. These data were obtained immediately after pretreatment. On one week standing on air the electrodes retain their current response almost unaltered for NO, but the sensitivity to nitrite and dopamine drops down to approx. one half of its original value. An almost five-fold (4.8 ± 0.4) decrease in sensitivity is observed for ascorbate after seven days. The selectivities observed immediately after pretreatment/after one week air equilibration were 1:4.9/1:10 for nitrite, 1:0.6/1:3 for ascorbate and 1:0.28/1:0.6 for dopamine. The influence of pretreatment and electrode aging on selectivity parameters are visualized in Fig. 1.

The effects of electrochemical pretreatment are reported to be pH dependent [29,30] and from the point of view of NO sensor construction it is noteworthy that the surface oxidic film, responsible for dopamine adsorption does not form when anodization is carried out in an alkaline solution - it is even removed if the electrode pretreated in neutral pH is transferred into alkaline solution [31]. This is exactly the case which should occur during construction of porphyrinic NO sensor, because after pretreatment the electrode is anodized in 0.1 M NaOH solution in the course of porphyrin electropolymerization. A four-fold increase in current due to NO oxidation was observed by Allen and Piantadosi [32] who anodized carbon composite material by cycling the potential between -0.2 and 1.2 V (vs. Ag/AgCl) in 0.1 M NaOH, unfortunately the changes in the responses of the interferents were not determined. Our results indicate that there is no advantage to conventional procedure as pretreatment in NaOH enhances the electrode response to ascorbate and dopamine to a greater extent than to nitric oxide, compared to conventional pretreatment. In another set of experiments we found out that electrodes, on which conventional pretreatment was performed prior to anodization in NaOH, behave as if anodization in NaOH was not done - selectivity as well as sensitivity parameters remained virtually unaltered. This observation justifies the utility of pretreatment prior to NiTMHPP deposition. In addition to anodic pretreatment protocols, cathodization of carbon surfaces was also described [33] and shown to improve redox characteristics of certain redox couples attached to carbon electrode surface. During cathodic pretreatment procedure the electrode potential

is set to -1.5 V (vs. SCE) in 0.1 M H_2SO_4 for 2 min [34]. Interestingly, for our electrodes the cathodic pretreatment selectively increased current response to nitrite (4.7×), while responses to ascorbate and dopamine remained at the level of untreated fiber and response to NO was even lowered (0.4×).

3.2. Coating by electropolymerized NiTMHPP

During our efforts to develop selective nitric oxide microelectrode we first tested poly-NiTMHPP covered electrodes prepared by electropolymerization from 0.5 mM NiTMHPP, dissolved in 0.1 M NaOH at constant potential 1.2 V (vs. Ag/AgCl) for 10 min, according to Refs. [11–14]. Oxygen saturated solutions were used, since polymer layers prepared under both aerobic and anaerobic conditions possesses the same properties. Some authors prefer deoxygenated solutions for electropolymerization [35], but in most papers this piece of information is not provided. We found these electrodes slightly selective towards both ascorbate (1:37) and nitrite (1:20). As expected, cyclic scanning method gives much better results not only from the point of view of electrocatalytical activity, but also from selectivity. We have found that selectivity of the resulting poly-NiTMHPP coating strongly depends on monomer concentration in the coating solution. Selectivity ratios for poly-NiTMHPP deposited on pretreated carbon fibers from NiTMHPP solutions diluted in 0.1 M NaOH by fifty CV cycles (0 to 1.2 V (vs. Ag/AgCl), 100 mV/s) are given in the Table 2.

Selectivities of the sensors dramatically improved when going from the usually used 0.5 mM to 0.4 mM solutions. This finding was reproduced for more than fifty electrodes. Increasing the number of scans to 100 leads to further enhancement of the coating selectivity performance. Typical ratios were 1:600 for nitrite and 1:8000 for ascorbate with 30% variation. At this point, selectivities towards dopamine were determined and were found to be 1:80-1:120. Additional coating steps (up to four, using the same method) for 0.2 and 0.3 mM NiTMHPP solutions also led to selectivity improvement - after a total of four coating steps these electrodes attained selectivity parameters found for electrodes prepared from 0.4 mM NiTMHPP, 100 CV cycles. For more concentrated NiTMHPP additional coating did not lead to significant selectivity improvement. Detection limit for sensor coated from 0.4 mM by 100 CV cycles was found to be 1.5 nM NO

Table 2
Selectivities for nitrite and ascorbate achieved at pretreated carbon fibers covered with poly-NiTMHPP layers in dependence on NiTMHPP monomer concentration

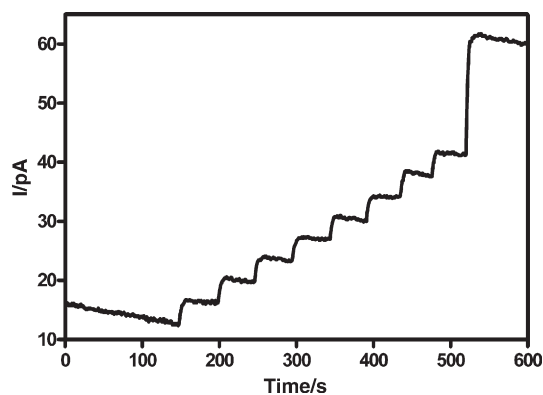| NiTMHPP (mM)	Nitrite	Ascorbate
0.2	1:58	1:7
0.3	1:66	1:49
0.4	1:300	1:2300
0.5	1:39	1:300
0.6	1:63	1:23
0.75	1:120	1:5.5
1	1:64	1:67
1.5	1:66	1:16
2	1:12	1:10

Fig. 2. Carbon fiber sensor's performance at nanomolar NO concentrations (CPA at 830 mV (vs. Ag/AgCl)). Eight additions of NO into aerated PBS, each resulting in 4 nM NO concentration, followed by response for 20 nM NO are shown. After pretreatment the electrode was coated with poly-NiTMHPP, electropolymerized from 0.4 mM NiTMHPP by 100 cycles from 0 to 1200 mV (vs. Ag/AgCl), scan rate 100 mV/s.

(signal to noise ratio S/N=3) and the performance of the sensor in nanomolar NO concentrations is shown in Fig. 2.

To explain the enhanced selectivity properties of poly-NiTMHPP deposited from solutions containing less than 0.5 mM NiTMHPP, we measured the concentration dependence of NiTMHPP UV/VIS spectra in thin layer cell and EQCM profiles of NiTMHPP deposition on gold surface for 0.2 and
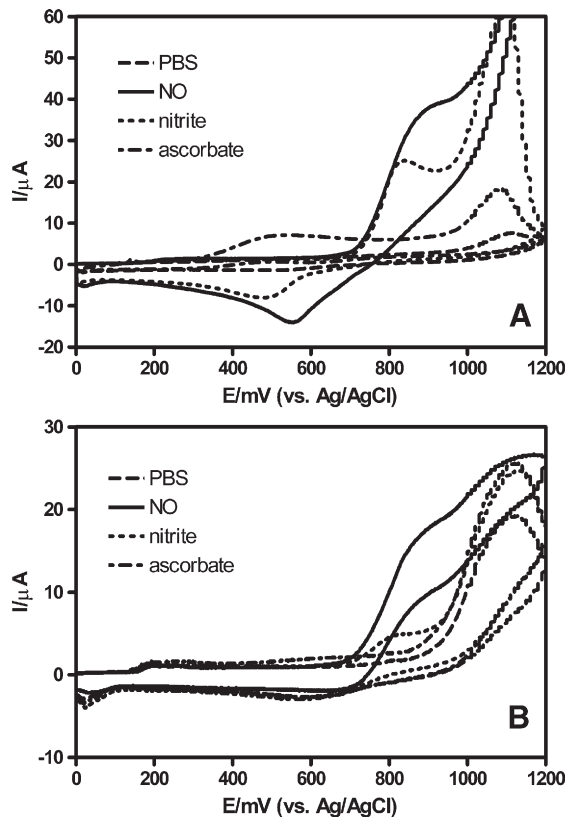

Fig. 3. Cyclic voltammograms of NO (1 mM), nitrite (5 mM) and ascorbate (5 mM) at bare gold electrode (panel A) and gold electrode coated with poly-NiTMHPP, deposited from 0.4 mM solutions (panel B). The geometric area of the electrode was 0.25 cm^2; scan rate 100 mV/s. Deaerated PBS solutions.
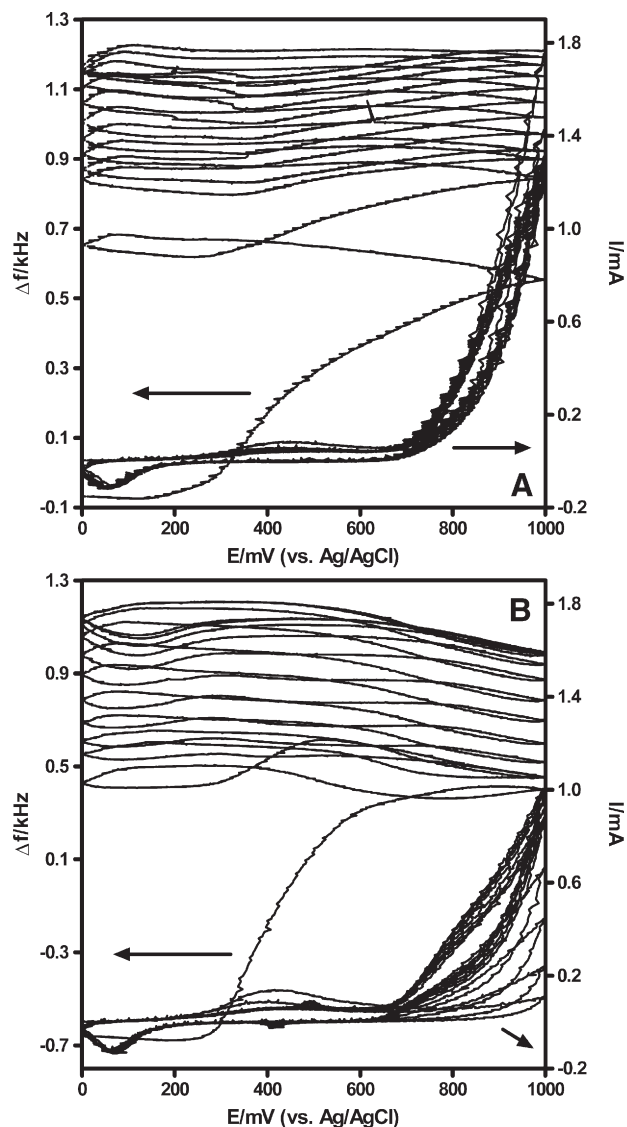

Fig. 4. EQCM profiles and corresponding voltammograms of NiTMHPP deposition at gold coated quartz crystals for two different NiTMHPP concentrations: 0.2 mM (panel A) and 1 mM (panel B); 0.1 M NaOH; scan rate 100 mV/s. Δf Δf=f−f(ref), i.e. frequency change relative to reference crystal. An increase in Δf indicates the increase in mass of the material deposited on the electrode. Arrows in the figures indicate the curves' corresponding axes.

1 mM NiTMHPP concentrations. Lambert-Beer's law was strictly obeyed within the concentration range of 0.05-2 mM NiTMHPP in the region of wavelengths 300-700 nm (not shown); there is no indication of NiTHMPP aggregation which could be responsible for a different surface morphology of poly-NiTMHPP. While we did not find any changes in the positions and heights of peaks in the UV/VIS spectra, distinct differences were found in EQCM profiles. Prior to EQCM experiments, cyclic voltammograms of nitric oxide, nitrite and ascorbate were measured on gold electrode with and without poly-NiTMHPP. They are shown in Fig. 3.

These experiments were aimed at testing whether selectivity properties of poly-NiTMHPP deposited on gold and carbon fiber coincide. Voltammetric response of ascorbate almost completely disappeared on coated electrode and considerable
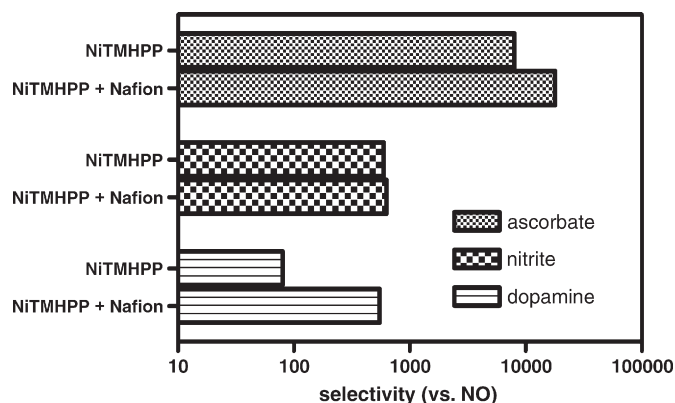
Fig. 5. Selectivity characteristics of poly-NiTMHPP and poly-NiTMHPP-Nafion coated carbon fiber based NO sensors. Pretreated electrodes were coated with poly-NiTMHPP, electropolymerized from 0.4 mM NiTMHPP by 100 cycles from 0 to 1200 mV (vs. Ag/AgCl), scan rate 100 mV/s. Nafion coating was prepared by dipping the carbon fiber into Nafion solution for 15 min and the sensor was dried for 1 h at 45 °C.
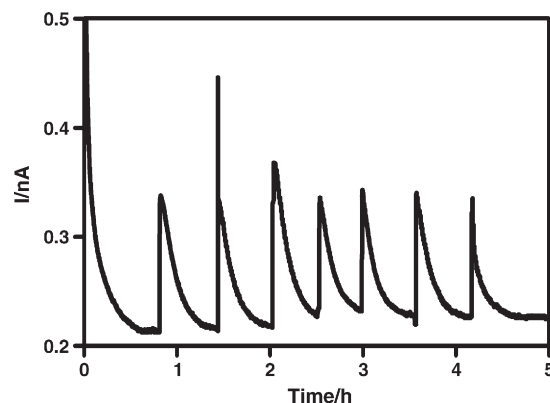

Fig. 6. Short term sensor stability for repeated additions of NO. Saturated aqueous solution of NO was injected every approx. 30 min. into stirred PBS solution. Each addition corresponded to 200 nM NO (final concentration).

selectivity towards nitrite was also achieved. Based on these observations it can be assumed that results obtained on gold surfaces can be, with some caution, related to carbon fiber. EQCM profiles of NiTMHPP deposition from 0.2 and 1 mM NiTMHPP (10 cycles) are shown along with corresponding voltammograms in Fig. 4.

A rough estimation of NiTMHPP surface coverage indicates around 18 monolayers are deposited from 0.2 mM and 30 monolayers from 1 mM NiTMHPP during the first voltammetric scan, assuming that effective area of porphyrine molecule is 1×10^{-18} m^2. During the first scan, approx. 40% of the total amount of poly-NiTMHPP is deposited from 0.2 mM and 60% from 1 mM NiTMHPP. While in the case of 0.2 mM NiTMHPP the weight increase begins at approx. 160 mV (vs. Ag/AgCl), which is some 80 mV before the NiTMHPP oxidation wave appears on the voltammogram (its onset at 240 mV (vs. Ag/AgCl) is accompanied by steeper weight increase), for 1 mM NiTMHPP the onset of weight increase occurs at ca. 240 mV and coincides with the voltammogram. Furthermore, a small weight loss in the poly-NiTMHPP deposited from 1 mM solution occurs between 750 and 1000 mV (vs. Ag/AgCl) during anodic scan which can be explained as a loss of material weakly attached to the electrode. Similar weight loss is not observed for polymer deposited from 0.2 mM solution - the weight increase is spread over the potential region 80-1000 mV instead. Cyclic voltammograms of NiTMHPP prepared from 1 mM solution contain reversible pair of peaks with $E' = 472$ mV (vs. Ag/AgCl), indicating that Ni(II)/Ni(III) redox transitions take place, while voltammograms of poly-NiTMHPP deposits prepared from 0.2 mM solution do not exhibit any indication of redox transition in this potential region. This observation can imply that either demetalation of porphyrin may occur during the deposition, which is improbable in an alkaline solution [36], or the polymer prepared from 0.2 mM solution is so compact that diffusion of anions (OH$^-$) into it cannot take place and therefore Ni(II)/Ni(III) redox transition is blocked, similar to counteranion diffusion suppression by Nafion layer occurring in conventional porphyrinic NO

sensor. On the other hand, the hydrophobic characteristic of poly-NiTMHPP [36,37] permits an access of small, uncharged NO molecule to the electrode surface.

3.3. Coating with Nafion, storage and operational stability of poly-NiTMHPP-Nafion modified NO sensor

A sensitivity loss is observed, if the poly-NiTMHPP modified electrode is left standing in dry state for longer periods. Within several days (probably depending on air humidity) the sensor's sensitivity drops down to one half to one third of the original value. Although most of the sensitivity is restored after few hours in the solution, the selectivities are found to be decreased, typically to 60-70% of their original values. We observed almost unaltered sensitivities for sensors coated with Nafion (the sensor was immersed into Nafion solution for 15 min and dried for 1 h at 45 °C). This coating has a protective effect on poly-NiTMHPP and further improves its selectivity especially towards ascorbate and dopamine. No loss in sensitivity and selectivity is observed after storage of the sensors in dry state for at least two weeks. Typical selectivity ratios are 1:600 for nitrite, 1:18,000 for ascorbate and 1:550 for dopamine (Fig. 5).
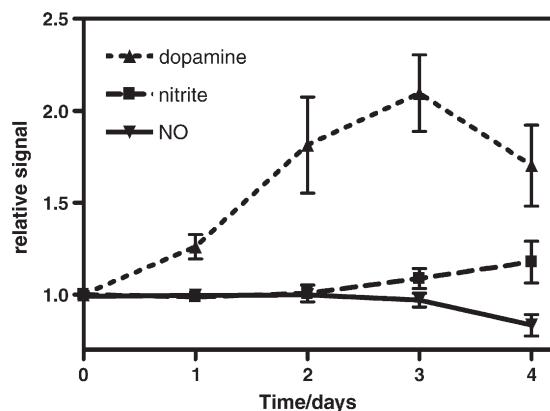

Fig. 7. Time dependence of NO, dopamine and nitrite relative signal. Number of electrodes=8; 800 nM NO, 20 μM dopamine and nitrite, stirred PBS solutions.

The detection limits remain low, ranging from 2 to 3 nM NO (S/N=3). To assess the sensors' operational stability, two kinds of tests were performed on the sensors. A short term stability of NO response was checked in experiment, during which 1 μL of saturated NO solution was injected into 25 mL PBS every 30 min. A typical result is shown in Fig. 6. Next, a four-day test was performed for eight sensors coated with poly-NiTMHPP and Nafion. The sensors were stored in PBS and tested each day in PBS solutions for NO response stability and ascorbate, dopamine and nitrite selectivity characteristics. As shown in Fig. 7, only a very slight decrease of nitric oxide signal and a slight decrease in selectivity towards nitrite are observed after three days.

The response of the sensor to ascorbate did not change during the testing (not shown). On the other hand, the currents due to dopamine electrooxidation gradually increased during the test - a 50% decrease in dopamine selectivity occurred after three days. The observed stability characteristics should be sufficient for most applications of NO sensor, including measurements in cell cultures or in vivo.

4. Conclusions

We have shown that nickel tetrakis(3-methoxy-4-hydroxyphenyl) porphyrin at concentrations slightly lower than usually used can be electropolymerized by cyclic potential scanning to give compact films, acting as efficient barrier against ascorbate, nitrite and dopamine - these compounds were used as model anionic and cationic interferents. The films were studied on gold surfaces by voltammetry and EQCM. Selectivities achieved at carbon fibers coated solely with nickel porphyrin were comparable or better than selectivities achieved by others at fibers covered with heat-treated Nafion. Nafion layer, cured at low temperature (45 °C), prevents the sensor from gradual selectivity and sensitivity loss which occurs when the sensor is stored in dry state and further improves its selectivity especially towards ascorbate and dopamine. The sensors' operational characteristics are appreciably stable within several days with the exception of dopamine (sensor's selectivity towards dopamine is approximately halved after three days in solution).

Acknowledgement

References

[1] T. Malinski, Z. Taha, Nitric oxide release from a single cell measured in situ by a porphyrinic-based microsensor, Nature 358 (1992) 676–678.

[2] T. Malinski, D.A. Wink, J. Younathan, R.W. Murray, M. Sullivan, T.J. Meyer, D.D. Christodulou, Nitric oxide sensor. US patent No. 5.603.820, 1997.

[3] L.J. Kepley, A.J. Bard, Ellipsometric, electrochemical, and elemental characterization of the surface phase produced on glassy carbon electrodes by electrochemical activation, Anal. Chem. 60 (14) (1988) 1459–1467.

[4] P. Kovach, M.R. Deakin, R.M. Wightman, Electrochemistry at partially blocked carbon fiber microcylinder electrodes, J. Phys. Chem. 90 (19) (1986) 4612–4617.

[5] M. Pontié, F. Bedioui, J. Devynck, New composite modified carbon microfibers for sensitive and selective determination of physiologically relevant concentrations of nitric oxide in solution, Electroanalysis 11 (12) (1999) 845–850.

[6] T. Malinski, Z. Taha, S. Grunfeld, A. Burewicz, P. Tomboulian, F. Kiechle, Measurements of nitric oxide in biological materials using a porphyrinic microsensor, Anal. Chim. Acta 279 (1993) 135–140.

[7] A. Ciszewski, G. Milczarek, E. Kubaszewski, M. Lozynski, Oxidation of nitric oxide at a porphyrinic-based sensor. New results from rotating disk experiments, Electroanalysis 10 (9) (1998) 628–632.

[8] B. Fabre, S. Burlet, R. Cespuglio, G. Bidan, Voltammetric detection of NO in the rat brain with an electronic conducting polymer and Nation® bilayer-coated carbon fibre electrode, J. Electroanal. Chem. 426 (1997) 75–83.

[9] B.W. Allen, L.A. Coury, C.A. Piantadosi, Electrochemical detection of physiological nitric oxide: materials and methods, Methods Enzymol. 359 (2002) 125–134.

[10] S. Trévin, F. Bedioui, J. Devynck, Electrochemical and spectrophotometric study of the behavior of the electropolymerized nickel porphyrin films in the determination of nitric oxide in solution, Talanta 43 (1996) 303–311.

[11] N. Villeneuve, F. Bedioui, K. Voituriez, S. Avaro, J.P. Vilaine, Electrochemical detection of nitric oxide production in perfused pig coronary artery: comparison of the performances of two electrochemical sensors, J. Pharmacol. Toxicol. Methods 40 (2) (1998) 95–100.

[12] A. Ciszewski, G. Milczarek, A new Nafion-free bipolymeric sensor for selective and sensitive detection of nitric oxide, Electroanalysis 10 (11) (1998) 791–793.

[13] A. Brunet, M.A. Pailleret, J. Devynck, F. Bedioui, Electrochemical sensing of nitric oxide for biological systems: methodological approach and new insights in examining interfering compounds, Talanta 61 (2003) 53–59.

[14] F. Lantoine, S. Trévin, F. Bedioui, J. Devynck, Selective and sensitive electrochemical measurement of nitric oxide in aqueous solution: discussion and new results, J. Electroanal. Chem. 392 (1995) 85–89.

[15] M.N. Friedemann, S.W. Robinson, G.A. Gerhardt, O-phenylenediamine-modified carbon fiber electrodes for the detection of nitric oxide, Anal. Chem. 68 (15) (1996) 2621–2628.

[16] A.W. Allen, C.A. Piantadosi, L.A. Coury Jr., Electrode materials for nitric oxide detection, Nitric Oxide 4 (1) (2000) 75–84.

[17] M. Pontié, H. Lecture, F. Bedioui, Improvement in the performance of a nickel complex-based electrochemical sensor for the detection of nitric oxide in solution, Sens. actuators, B, Chem. 56 (1999) 1–5.

[18] H. Cai, Z. Li, A. Goette, F. Mera, C. Honeycutt, K. Feterik, J.N. Wilcox, S.C. Dudley Jr., D.G. Harrison, J.J. Langberg, Downregulation of endocardial nitric oxide synthase expression and nitric oxide production in atrial fibrillation: potential mechanisms for atrial thrombosis and stroke, Circulation 106 (2002) 2854–2858.

[19] F.O. Brown, J.P. Lowry, Microelectrochemical sensors for in vivo brain analysis: an investigation of procedures for modifying Pt electrodes using Nafion, Analyst 128 (6) (2003) 700–705.

[20] N.R. Ferreira, A. Ledo, J.G. Frade, G.A. Gerhardt, J. Laranjinha, R.M. Barbosa, Electrochemical measurement of endogenously produced nitric oxide in brain slices using Nafion/o-phenylenediamine modified carbon fiber microelectrodes, Anal. Chim. Acta 535 (2005) 1–7.

[21] A. Ciszewski, G. Milczarek, Electrochemical detection of nitric oxide using polymer modified electrodes, Talanta 61 (2003) 11–26.

[22] K.M. Mitchell, E.K. Michaelis, Multimembrane carbon fiber electrodes for physiological measurements of nitric oxide, Electroanalysis 10 (2) (1998) 81–88.

[23] H.J. Huang, P. He, L.R. Faulkner, Current multiplier for use with ultramicroelectrodes, Anal. Chem. 58 (13) (1988) 2889–2891.

[24] A. Economou, S.D. Bolis, C.E. Efstathiou, G.J. Volikakis, A "virtual" electroanalytical instrument for square wave voltammetry, Anal. Chim. Acta 467 (2002) 179–188.

[25] F.G. Gonon, C.F. Fombariet, M.J. Buda, J.F. Pujol, Electrochemical treatment of pyrolytic carbon fiber electrodes, Anal. Chem. 53 (9) (1981) 1386–1389.

[26] G.N. Kamau, W.S. Willis, J.F. Rusling, Electrochemical and electron spectroscopic studies of highly polished glassy carbon electrodes, Anal. Chem. 57 (2) (1985) 545–551.

[27] J.X. Feng, M. Brazzel, K. Renner, R. Kasser, R.N. Adams, Electrochemical pretreatment of carbon fibers for in vivo electrochemistry: effects on sensitivity and response time, Anal. Chem. 59 (14) (1987) 1863–1867.

[28] P.M. Kovach, A.G. Ewing, R.L. Wilson, R.M. Wightman, In vitro comparison of the selectivity of electrodes for in vivo electrochemistry, J. Neurosci. Methods 10 (3) (1984) 215–227.

[29] Y.W. Alsmeyer, R.L. McCreery, Surface enhanced Raman examination of carbon electrodes: effects of laser activation and electrochemical pretreatment, Langmuir 7 (10) (1991) 2370–2375.

[30] A.L. Bellby, T.A. Sasaki, H.M. Stern, Electrochemical pretreatment of carbon electrodes as a function of potential, pH, and time, Anal. Chem. 67 (5) (1995) 976–980.

[31] D.M. Anjo, M. Kahr, M.M. Khodabakhah, S. Nowinski, M. Wagner, Electrochemical activation of carbon electrodes in base: minimization of dopamine adsorption and electrode capacitance, Anal. Chem. 61 (23) (1989) 2603–2608.

[32] B.W. Allen, C.A. Piantadosi, Electrochemical activation of electrodes for amperometric detection of nitric oxide, Nitric Oxide 8 (2003) 243–252.

[33] G. Ilangovan, K. Chandarasekara Pillai, Unusual activation of glassy carbon electrodes for enhanced adsorption of monomeric molybdate(VI), J. Electroanal. Chem. 431 (1) (1997) 11–14.

[34] G. Ilangovan, K. Chandarasekara Pillai, Mechanism of activation of glassy carbon electrodes by cathodic pretreatment, J. Solid State Electrochem. 3 (6) (1999) 357–360.

[35] L.W. Dobrucki, A. Burewicz, T. Malinski, Porphyrinic nanosensor for measurement of nitric oxide in the cardiovascular system, Anal. Sci. 17 (2001) i583–i586.

[36] T. Malinski, A. Ciszewski, J.R. Fish, L. Czuchajowski, Conductive polymeric tetrakis(3-methoxy-4-hydroxyphenyl) porphyrin film electrode for trace determination of nickel, Anal. Chem. 62 (9) (1990) 909–914.

[37] J.E. Bennett, T. Malinski, Conductive polymeric porphyrin films: application in the electrocatalytic oxidation of hydrazine, Chem. Mater. 3 (1991) 490–495.