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Electrochemical nitric oxide sensor preparation: A comparison of two electrochemical methods of electrode surface modification

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Abstract

Platinum electrodes modified with Mn(II) 5-(*N*-(8-pyrrole-yl-3,6-dioxa-1-aminooctane)phenylamide-10,15,20-trimethoxyphenylporphyrin (Mn(II)triOMeTCPPyP) using multi-sweep cyclic voltammetry and differential pulse amperometry were evaluated as electrocatalytic surfaces for the oxidation of nitric oxide. The electrodes modified using the pulse amperometric approach were more sensitive towards the detection of nitric oxide. The increased sensitivity led to the attainment of a wider linear dynamic range for the quantification of nitric oxide.
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Keywords: Nitric oxide; Manganese porphyrin; Pulse amperometry; Electrocatalysis

1. Introduction

Nitric oxide is known to be an essential molecule in biological systems that plays important roles in neurotransmission, immune response and blood pressure regulation [1–5]. Abnormal concentrations of nitric oxide has been reported to be responsible for a number of pathological conditions such as Parkinson's disease, Alzheimer's disease, post-ischemic heart and brain injury and diabetes [6–10]. The close monitoring of the concentration levels of nitric oxide is of utmost importance. This necessitates the development of sensitive and selective methods for the detection and quantification of nitric oxide. Several indirect methods are available for the detection and determination of nitric oxide in biological systems [11]. These methods have been reported to have poor sensitivity and/or selectivity and can only be applied for ex-situ determination of nitric oxide [12]. Electrochemical techniques are most promising for their simplicity, high sensitivity (particularly with the use of modified electrodes), good selectivity, fast response, long-term stability, ease of handling and the possibility of fabricating electrodes small enough for direct implantation into biological systems without damage to the surrounding tissues for real-time in vivo measurements [12–14].

Different types of porphyrins [15–20] and phthalocyanines [21–23] have been used as electrocatalysts to modify electrode surfaces for improved sensitivity of the electrodes towards the oxidation of nitric oxide; usually by repetitive cyclic voltammetry [21,23–26] or by a dip-dry method [13,27]. To solve the problem of interference posed by nitrite and other anionic molecules, the addition of a layer of Nafion®, a polyanionic resin, on the modified electrode surfaces has been demonstrated to be effective [12,28–30]. While the mechanism of the electrocatalytic activity of the metalloporphyrins for the oxidation of nitric oxide is still under deliberations [14,30,31], the metallophthalocyaninnes are known to exhibit electrocatalytic activity with the variable oxidation state of the central metal [23]. Although NO can be oxidised on the surface of most conventional electrodes, electrode surface modification is generally required for increased sensitivity of the electrode for the

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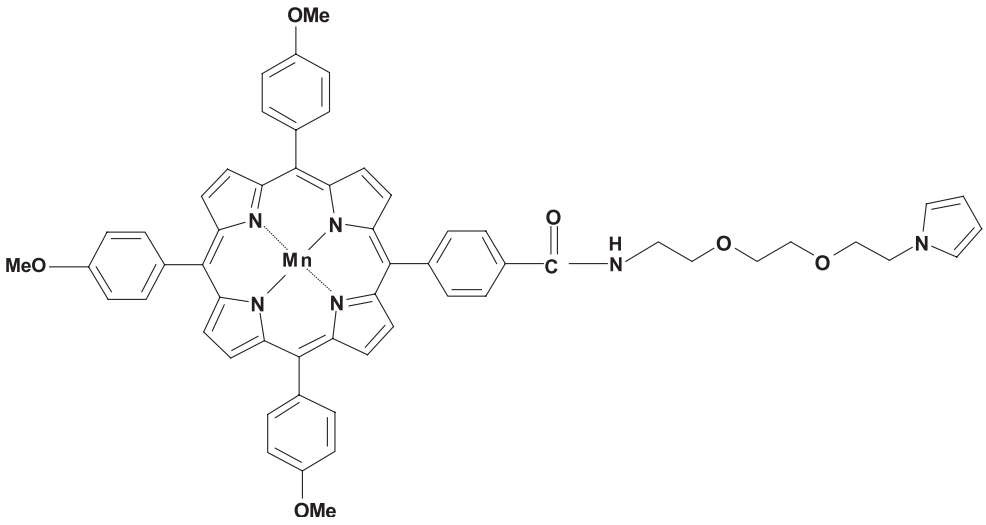

Fig. 1. Molecular structure of Mn(II) 5-(*N*-(8-pyrrole-yl-3,6-dioxa-1-aminooctane)phenylamide-10,15,20-trimethoxyphenylporphyrin (Mn(II)triOMeTCPPyP).

detection of NO at the low concentration levels at which it exists in the biological system. The success of electrochemical techniques for the detection of NO therefore depends on factors such as the electrode material and the electrocatalyst employed as well as the method adopted to anchor the electrocatalyst on the electrode surface.

In this work, we report on the use of Mn(II) 5-(*N*-(8-pyrrole-yl-3,6-dioxa-1-aminooctane)phenylamide-10,15,20-trimethoxyphenylporphyrin (Mn(II)triOMeTCPPyP) to modify platinum electrodes by repetitive cyclic voltammetry and differential pulse amperometry for the oxidation of nitric oxide. The molecular structure of the porphyrin used in this investigation is shown in Fig. 1.

2. Experimental

2.1. Chemicals

Trimethoxyphenylcarboxyphenylporphyrin (triOMePCPP) was synthesised following standard procedures. $NaNO_2$, $Na_2HPO_4 \cdot 7H_2O$, $NaH_2PO_4 \cdot 2H_2O$, H_2SO_4 and CH_3CN were obtained from Riedel-de-Haen Laborchemikalien (Seelze, Germany). Tetrabutylammoniumhexafluorophosphate was purchased from Fluka (Buchs, Switzerland) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) was obtained from Sigma (Steinheim, Germany).

The pyrrole substituted Mn(II)triOMeTCPPyP was synthesised by carbodiimide activated reaction of the carboxylic side chains of triOMePCPP with the amino group of 8-pyrrole-1-yl-3,6-dioxaoctyl amine as described previously [17,32].

NO standard solution was prepared in a chamber designed for complete exclusion of oxygen using a vacuum/argon line where H_2SO_4 was slowly dropped into a solution of $NaNO_2$ by means of a dropping funnel. The nitric oxide gas evolved was bubbled into triply distilled water to obtain a saturated, oxygen-free nitric oxide solution with an approximate concentration of 2 mM at 20 °C [17,32,33].

Electrochemical measurements were carried out with a model 273 potentiostat/galvanostat (EG&G Princeton Applied Research, Bad Wildbad, Germany) which was controlled by the M270 software package. A three-electrode electrochemical cell comprising of a Ag/AgCl reference electrode, a Pt wire counter electrode and a Pt disk working electrode (0.5 mm diameter) was used. Constant-potential amperometry for the detection of NO was performed at a working potential of 750 mV.

Pt working electrodes were cleaned by polishing with alumina suspension of decreasing particle sizes of 1.0, 0.3 and 0.05 μm on a polishing cloth (Technotron, Wehrheim, Germany) followed by washing in an ultrasonic bath with H_2SO_4, NaOH and triply distilled water.

Mn(II)triOMeTCPPyP was deposited on the electrode surface from a solution containing 3 mM of the porphyrin and 100 mM tetrabutylammoniumhexafluorophosphate as supporting electrolyte by repetitive cycling in the cyclic voltammetry mode between −400 and +1300 mV vs. Ag/AgCl or by pulse amperometry with pulse potentials of 0 and +1200 mV vs. Ag/AgCl. After modification, the electrode was rinsed thoroughly with acetonitrile and an additional thin coating of Nafion® was formed on the electrode by dropping 10 μl of a 3% solution of Nafion® in ethanol/water on the surface and allowing the solvent to dry.

3. Results and discussion

Porphyrin films on electrodes are most often formed by repetitive cyclic voltammetry or by “dip-dry/drop-dry” methods in order to improve the sensitivity of the electrode towards nitric oxide oxidation. The “dip-dry/drop-dry”

method is largely not reproducible and the thickness of the modifier on the electrode cannot be accurately controlled, whereas the electrochemical approach to film formation on the electrode is known to be reproducible as well as allowing for the control of the thickness of the film by controlling the film formation conditions such as the scan rate, the potential range, the film formation time and the type of supporting electrolyte employed [34].

A film of Mn(II)triOMeTCPPyP was formed on a Pt electrode surface by repetitive cyclic voltammetry, cycling the potential between −400 and +1300 mV. The cyclic voltammograms recorded during the film formation process are shown in Fig. 2.

During the first scan of the voltammogram (curve a) the pyrrole residue at the porphyrin is oxidized to the radical cation leading to a polymerization of the modified porphyrin on the electrode surface by altering the accessible active electrode surface. Thus, in subsequent scans (curve b) the radical cation formation is less pronounced; however, a gradual increase in both the anodic and cathodic currents was observed with the scan number, signifying the formation of a conductive polymer on the electrode surface. However, after the 14th scan, there was no further noticeable increase in current, indicating that the film growth has stopped. Thus, a total of 14 cycles was applied to modify the electrodes used in this work.

A pulse amperometric technique was equally used to form the Mn(II)triOMeTCPPyP film on Pt electrodes. Two pulse potentials were applied: a resting potential of 0 mV for 1 s and a polymerisation potential of 1200 mV for 1 s in 14 cycles. In other words, the potential value was changed from a value at which no pyrrole oxidation occurs at the electrode surface to a value slightly higher than the oxidation potential of the monomer. The current–time plot obtained during the polymer film formation process is shown in Fig. 3.

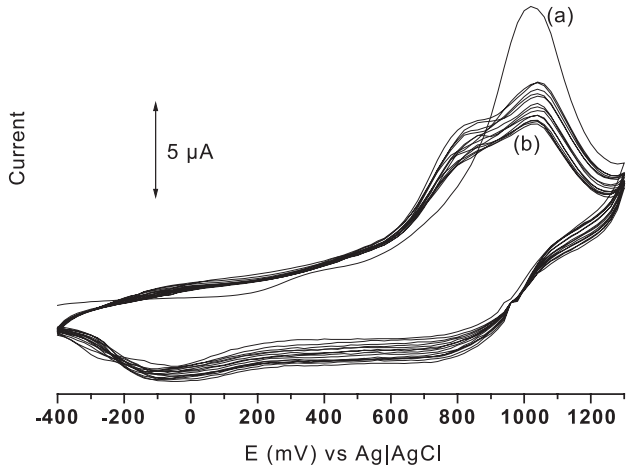

Fig. 2. Increase in both anodic and cathodic currents observed during the electropolymerisation of Mn(II)triOMeTCPPyP at a Pt electrode by repetitive cyclic voltammetry. (a) First scan and (b) second scan.

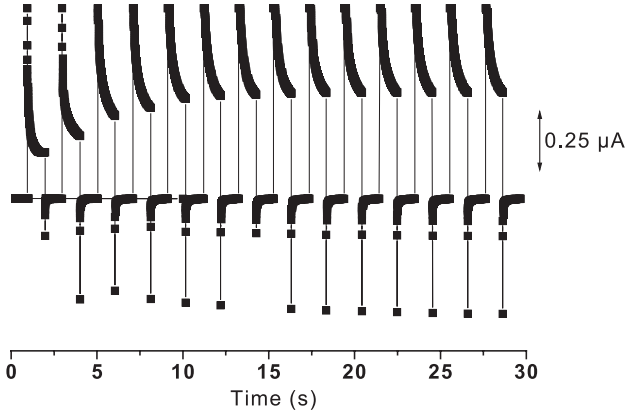

Fig. 3. Current–time plot obtained during the electropolymerisation of Mn(II)triOMeTCPPyP at a Pt electrode using differential pulse amperometry.

The current is recorded only at the very end of the pulse time to allow for a decay of the capacitive charging current and hence for the visualization of the film growth as shown in Fig. 3. A gradual increase in the current was observed at the end of successive pulses. This increase in current can be explained in terms of a change in either the conductivity of the electrode surface caused by the deposition of porphyrin film or an increase in the electrode surface area due to the formation of the ramified polymer network.

The successful deposition of Mn(II)triOMeTCPPyP films on the electrodes, modified using both approaches of repetitive cyclic voltammetry and pulse amperometry, was verified by recording the cyclic voltammograms of the modified electrodes in solution of the supporting electrolyte in the absence of the porphyrin monomer in solution. An oxidation peak was obtained at +1200 mV (figure not shown) which is attributed to the oxidation of the porphyrin-linked pyrrole subunit. This oxidation wave was not observed when the cyclic voltammogram was recorded with an unmodified electrode.

The electrodes obtained from the two methods of modification were employed for the oxidation of nitric oxide using differential pulse voltammetry. The differential pulse voltammograms in presence of 10 μM nitric oxide recorded using a Pt electrode modified by pulse amperometry (curve a), a Pt electrode modified by cyclic voltammetry (curve b) and a bare Pt electrode (curve c) are shown in Fig. 4.

A feature common to both electrodes, when used to detect nitric oxide in solution, is the observation of electrocatalytic currents resulting from the oxidation of nitric oxide while the unmodified electrode could not detect nitric oxide at this concentration level. Although the same prophyrin molecule (Mn(II)triOMeTCPPyP) was used to modify both electrodes, a significant difference was observed in the current response obtained from both electrodes from a solution of the same concentration of nitric oxide. The electrode modified using the proposed

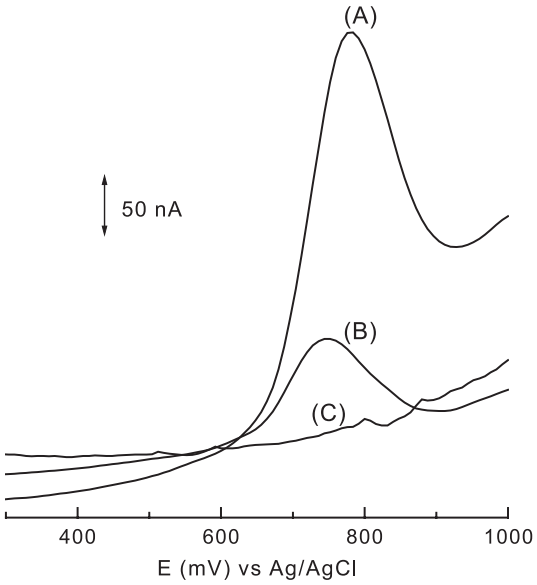

Fig. 4. Differential pulse voltammograms for the oxidation of a 10 μM nitric oxide solution at Pt electrodes modified by (A) differential pulse amperometry, (B) cyclic voltammetry and (C) unmodified platinum electrode.

pulse amperometric technique is more sensitive towards the detection of nitric oxide as shown by the higher current response. The response obtained from this electrode is three times the magnitude of that from the electrode modified using repetitive cyclic voltammetry. This difference in current response is obviously purely due to the difference in the way the Mn(II)triOMeTCP-PyP films employed as electrocatalyst were formed on the electrode.

A number of steps are involved in the electrochemical induced formation of polymeric films on electrode surfaces [35]. These are the transport of monomeric species to the electrode by diffusion, monomer oxidation at the appropriate potential to produce radical cations, radical–radical coupling, electrochemical oxidation of the formed oligomers and the precipitation of the polymer on the electrode surface as the last step. A limitation of repetitive cyclic voltammetry for the deposition of polymer films on electrode surfaces is the diffusion limited mass transport of the monomeric species to the electrode surface. Even when the rate of the electron-transfer reaction at the electrode is fast with respect to the scan rate of the experiment, the mass transport towards the electrode surface is mainly determined by diffusion. This effect is, as a matter of fact, more pronounced when the momomeric species in solution are large molecules. Thus, the diffusion limited mass transport may be responsible for the cessation of the growth of the film on the surface of the electrode modified using cyclic voltammetry after 14 scans. The termination of the film growth during repetitive potential cycling may be caused by the formation of only a low conducting polymer film which does not allow the formation of a sufficiently high radical cation concentration in the diffusion zone in front of the electrode during the relatively short time window for radical formation during scanning. In addition, during film formation by repetitive cyclic voltammetry, the potential is held at values at which radical cations are formed for only a very short fraction of the time and hence the concentration of the primarily formed radical cations reaches a maximum during each scan which leads to a continuous variation of the probability of chain propagation and thus a variation of the film morphology.

The differential pulse amperomeric approach for the formation of the porphyrin film easily overcomes this problem of diffusion limited mass transport as it re-establishes the bulk concentration of the monomers in solution in the vicinity of the electrode after the application of the deposition potential during the resting phase. At the no-effect potential applied during the resting phase, the concentration gradients established during the deposition pulse disappear by diffusion processes allowing the following deposition pulse to be applied to virtually the same concentrations of the monomer as the previous one. Thus, it can be anticipated that the uniformity of the films obtained using the differential pulse amperometric approach is better, which is also suggested by the higher current signal obtained for the oxidation of the same concentration of nitric oxide as compared with the current obtained using the electrode modified by repetitive cyclic voltammetry.

Calibration plots (Fig. 5) were derived from the current responses obtained from both electrodes following successive additions of aliquots of a standard nitric oxide solution and measuring the nitric oxide oxidation current by means of constant potential amperometry at a working potential of 750 mV vs. Ag/AgCl.

A significantly improved sensitivity was obtained at the electrode modified using differential pulse amperometry (Fig. 5a, i_p=3.83[NO]+4.76, R^2=0.998) as compared with the electrode modified by repetitive cyclic voltammetry (Fig. 5b, i_p=1.28[NO]+2.54, R^2=0.902), where i_p is

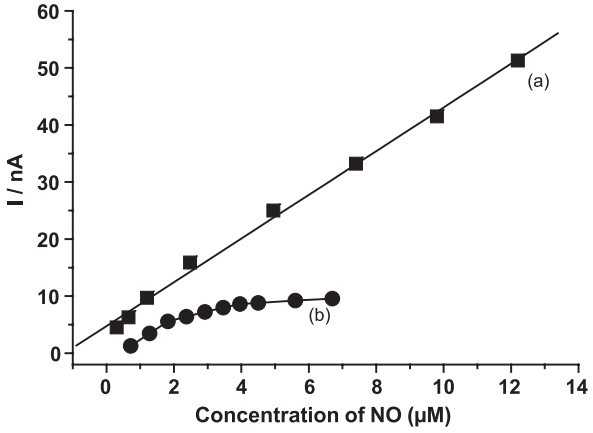

Fig. 5. Calibration plots derived from the oxidation currents of nitric oxide obtained at a Pt electrode modified with a Mn(II)triOMeTCPPyP polymer by (a) differential pulse voltammetry and (b) repetitive cyclic voltammetry.

in nA and [NO] is in μM. The higher sensitivity obtained with the electrode modified using pulse amperometry implies in addition the attainment of a wider linear dynamic range.

4. Conclusion

The method used to anchor an electrocatalyst onto the surface of a Pt electrode considerably modulates the sensitivity of the modified electrode so produced towards the target analyte. The problem of diffusion-limited mass transport encountered while modifying electrodes by repetitive cyclic voltammetry can be easily overcome by using differential pulse amperometry for the formation of the Mn(II)triOMeTCPPyP polymer film on the electrode surface. Although the same porphyrin molecule has been used to modify Pt electrode surfaces, the sensitivity of the electrode modified using a pulse profile is higher than the one modified by multi-sweep cyclic voltammetry. The increased sensitivity led to the attainment of a wider linear dynamic range for the determination of nitric oxide.

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