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Electrocatalytic oxidation of nitric oxide at nano-TiO₂/Nafion composite film modified glassy carbon electrode

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Abstract A novel nanocrystalline TiO₂ (nano-TiO₂) and Nafion composite film modified glassy carbon electrode has been developed for the determination of nitric oxide (NO) radical in an aqueous solution. This modified electrode can be employed as a NO sensor with a low detection limit, fast response, high sensitivity and selectivity. Two apparent anodic peaks were observed at 0.67 and 0.95 V at the nano-TiO₂ modified glassy carbon electrode by differential pulse voltammetry (DPV). After further modification with a thin film of Nafion, which was capable of preventing some anionic interference such as nitrite and ascorbic acid, only one peak appeared and the peak current enhanced greatly. The chronocoulometric experimental results showed NO was oxidized by one-electron transfer reaction at the composite film modified electrode. The amperometric responses increased linearly with the concentrations of NO ranging from 3.6×10^{-7} mol/L to 5.4×10^{-5} mol/L. The detection limit was estimated to be 5.4×10^{-8} mol/L. In this sensor system, the modification film provides complete selectivity for NO over nitrite anions (NO₂⁻).

Keywords Nitric oxide radical · Nanocrystalline TiO₂ · Chemically modified electrode · Nafion

Introduction

Since the discovery of the important role of nitric oxide radical in the biological system, such as endothelium-

derived relaxing factor (EDRF) [1], there has been an explosive growth in the study of direct and selective methods for the determination of NO. Nitric Oxide is an extremely important messenger molecular in the biological system. It has also been found that NO plays many other significant physiological functions in the vascular relaxation, platelet adhesion and activation, neurotransmission and immune response [2]. Based on its importance in the physiological process, there has been an increasing interest in the development of methods for detecting this compound. As a matter of fact, it has appeared that quantitative measurement of NO in biological models is very difficult because of its low concentration and relatively short lifetime (with a half-life ranging from 6 s to 50 s) [1], due to its high spontaneous chemical reactivity by reactions with endogenous O₂ or hemoglobin to form nitrite (NO₂⁻) or nitrate (NO₃⁻). Thus, most methods of NO measurements are indirect ones such as Griess assay for nitrite and detection of methemoglobin after NO reaction with oxyhemoglobin [3]. Current techniques for direct methods include spectroscopic methods (e.g. chemiluminescence and UV-visible spectroscopy) and electrochemical methods [3, 4].

Of all these techniques mentioned above, electrochemical techniques appeared as a very attractive alternative for monitoring NO concentration in situ as well as in vitro because of its high speed, good sensitivity and long-term calibration stability. In addition, electrochemical sensors can be fabricated to extremely small dimensions and thus are ideal for placement directly into biological preparations with minimal damage to tissues. Recently, several types of NO electrodes have been reviewed [3, 5]. The major goal of the reported studies has been to improve the sensitivity and selectivity of the electrochemical detection of NO through electrode surface modification. Although chemical modification of electrode surfaces with metalloporphyrin [6], metallophthalocyanine [7], and *o*-phenylenediamine [8] films has expanded the scope of application of such new designed electrodes, it is

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significant to look for new materials with high sensitivity and selectivity for the detection of NO.

Nanocrystalline materials with a particle size smaller than 100 nm exhibit many surprising properties, which are not found in conventional materials [9]. Due to its small particle size and large inner surface area, nanocrystalline materials have excellent catalytic activity. At present, nano-gold colloid [10] and carbon nanotubes [11] have been employed as a modifier to fabricate NO electrochemical sensor.

In this study, a novel nanocrystalline TiO₂ and Nafion composite film modified glassy carbon electrode was developed. The nano-TiO₂/Nafion film modified electrode was found to display good electrocatalytic activity toward NO oxidation with high selectivity and sensitivity. The electrode responses linearly to NO over the concentration range of 3.6×10^{-7} to 5.4×10^{-5} mol/L, and the detection limit is 5.4×10^{-8} mol/L by an amperometric method.

Materials and methods

Reagents and chemicals

Nanocrystalline TiO₂ used in this work (obtained from IR Materials Lab, Department of Chemistry, Wuhan University) was synthesized according to the literature [12]. Nafion solution was prepared by dilution, with ethanol, of a 5% solution w/v (Sigma) to a final concentration of 1% w/v. Dopamine (DA) and ascorbic acid (AA) (Sigma, Corporation), and other reagents were of analytical grade. Aqueous solutions were prepared with redistilled water. High purity nitrogen gas was used for deaeration.

Phosphate-buffered saline (PBS), containing 137 mmol/L NaCl, 2.7 mmol/L KCl, 8.0 mmol/L Na₂HPO₄ and 1.5 mmol/L KH₂PO₄, was prepared in water and adjusted to pH 7.4.

Nitric Oxide saturated solution was prepared as described previously [11], using a value of 1.8 mmol/L for its concentration at saturation [2]. Shortly, NO gas was generated by slowly dropping a 2 mol/L H₂SO₄ into a glass flask containing saturated NaNO₂ solution, NO being formed in this disproportional reaction. The gas generated was forced to twice bubble in a 30% NaOH solution and once in water in order to trap any NO₂ formed as a result of oxidation of NO from traces of oxygen. Before the addition of H₂SO₄, all apparatus were degassed meticulously with nitrogen for 30 min to exclude O₂, as NO is rapidly destroyed by O₂. Nitric Oxide saturated solution was obtained by bubbling NO pure gas through 10 mL deoxygenated distilled water for 30 min and kept under NO atmosphere until use. Nitric Oxide gas is toxic at concentration higher than 100 ppm, so the bubbling procedure was carried out in a fume hood. Nitric Oxide standard solutions were prepared by making serial dilutions of the saturated NO solution as previously reported [13]. Nitric Oxide solu-

tions were made fresh and kept in a glass flask with a rubber septum, stored in a light-free place.

Apparatus

All the electrochemical experiments were performed with a CHI660A electrochemical workstation (CH Instruments, Shanghai, China) in conjunction with an IBM-compatible PC. The three-electrode system consisted of the modified glassy carbon electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the auxiliary electrode. During NO determination, a semi-hermetic electrochemical cell was employed to ensure the experiment's progressing under nitrogen atmosphere. The whole cell was placed in a Faraday cage to avoid electromagnetic interference from the surroundings. All electrochemical experiments were performed at room temperature.

Preparation of the modified electrode

A total of 0.5 mg nano-TiO₂ was dispersed into 1 mL redistilled water with the aid of ultrasonic agitation to give a 0.5 mg/mL milk white suspension. The glassy carbon electrode (3 mm in diameter) was polished to mirror finish with polish paper and 0.3~0.05 μm alumina slurry, then cleaned thoroughly in an ultrasonic cleaner with 1:1 nitric acid solution, alcohol and redistilled water, sequentially. Then, 4 μL of nano-TiO₂ suspension was pipetted onto the freshly cleaned surface of the glassy carbon electrode and air-dried until a uniform film formed to give the nano-TiO₂ film electrode. Coverage of the nano-TiO₂ film electrode further with Nafion was obtained by depositing 3 μL 1% Nafion solution on the surface of the electrode and then placing the electrode in air to allow the ethanol to evaporate, denoted as nano-TiO₂/Nafion film electrode.

Procedures of electrochemical measurement

Differential pulse voltammetry (DPV) was used for NO determination in a deoxygenated PBS. Prior to NO measurements, the electrode was placed in a blank PBS and the potential was cycled between 0.0 V and 1.2 V at a scan rate of 50 mV/s until a steady cyclic voltammogram was obtained (about continuously scanning for ten cycles). To determine the hydrodynamic curve and linearity of the electrode to NO, a conventional amperometric method was used. Amperometric measurements of NO were performed at a constant potential of 0.67 V under a stirred condition. After allowing the electrode to reach a steady baseline, a gas-tight syringe was used to inject NO solutions into the semi-hermetic electrochemical cell containing 10 mL deoxygenated PBS under unaerated conditions using a nitrogen gas.

Chronocoulometry (pulsed from 0.4 V to 0.8 V) was used to confirm one-electron transfer process of NO oxidation at the nano-TiO₂/Nafion film electrode.

Results and discussion

Electrochemical behavior of NO at the nano-TiO₂ film electrode

The differential pulse voltammetric response of 7.0×10^{-5} mol/L NO at the nano-TiO₂ film electrode is shown in Fig. 1. At the bare glassy carbon electrode, almost no apparent peaks are observed in the PBS containing 7.0×10^{-5} mol/L NO (curve a). No peak current was obtained at the nano-TiO₂ film electrode in the absence of NO (not shown). However, two conspicuous and sharp peaks, the first peak (i_{p1}) at 0.67 V and the second peak (i_{p2}) at 0.97 V, can be clearly observed at the nano-TiO₂ film electrode in the presence of NO (curve b). Both peaks increase in height with increase of NO concentration. These indicated that the nano-TiO₂ film electrode display good electrocatalytic activity for NO oxidation. From SEM images (not shown), it could be concluded that this is mainly due to highly porous structure and larger ratio surface area of nano-TiO₂ film electrode. Small molecule NO could freely diffuse from the nano-TiO₂ membrane into the surface of the electrode and become mobile/desorbed within accessible potential range.

The electrochemical behavior of NO at polycrystalline platinum electrode has been reported [14]. It was proposed that oxidation of NO at platinum electrode proceeds via a two-step mechanism: a one-electron oxidation of NO to NO⁺ or its hydrated species (NO₂⁻)

followed by a two-electron oxidation of NO₂⁻ to NO₃⁻. On the basis of these studies, it could be speculated that the first peak corresponds to the one-electron oxidation of NO to NO⁺ or NO₂⁻, and the second peak corresponds to the two-electron oxidation of NO₂⁻ to NO₃⁻. This speculation appears to be further validated by the observation that the first peak is not influenced by the addition of NO₂⁻ but the second peak is, as shown in Fig. 2. The NO₂⁻ oxidation has very similar potential as the second peak of NO oxidation at the nano-TiO₂ film electrode (curve c). After addition of NO in PBS containing NO₂⁻, the first peak current and potential almost remain unchangeable, but the second peak current increase remarkably (curve b), comparing with in PBS only containing NO (curve a). These suggest that the second peak really corresponds to the two-electron oxidation of NO₂⁻ to NO₃⁻. After further coated with 1 % Nafion to give a nano-TiO₂/Nafion film electrode, the second peak of NO oxidation disappeared and the first peak current increases and peak potential shifts negatively to 0.64 V (curve d). Nafion is a cationic exchanger with polyfluorinated polyacid. Nafion film could stabilize NO⁺ formed upon the oxidation of NO and prevent a complicated pattern of reactions that could lead to the formation of NO₂⁻ and NO₃⁻ [6]. Therefore, NO can only be oxidized to NO⁺ by a one-electron transfer reaction at the nano-TiO₂/Nafion composite film electrode due to Nafion's coating. On the other hand, Nafion film can accumulate NO, which makes the oxidation current increase for NO. Therefore the mechanism of NO oxidation at the nano-TiO₂ film electrode could be described as:

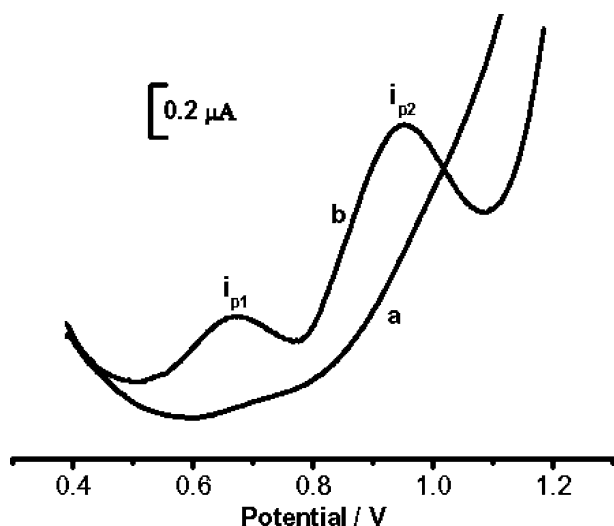
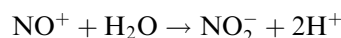


Fig. 1 Differential pulse voltammograms of the bare GCE (a) and nano-TiO₂-modified GCE (b) in a deoxygenated PBS containing 7.0×10^{-5} mol/L NO

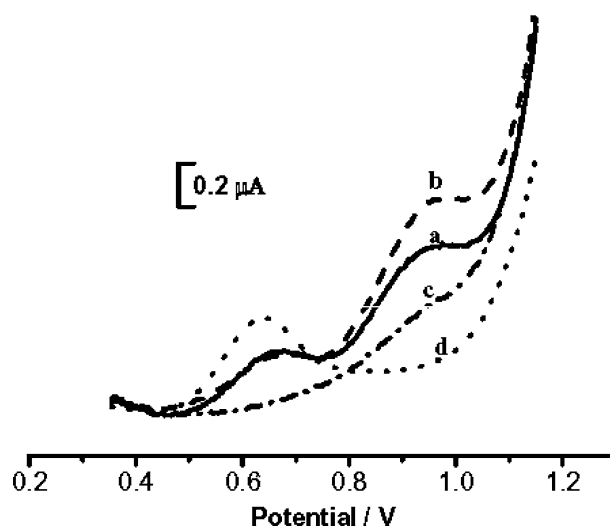


Fig. 2 Differential pulse voltammograms of the nano-TiO₂ film electrode (a, b, c) and the nano-TiO₂/Nafion composite film electrode (d) in a deoxygenated PBS containing 3.5×10^{-5} mol/L NO (a, d), 3.5×10^{-5} mol/L NO + 5×10^{-5} mol/L NO₂⁻ (b) and 5×10^{-5} mol/L NO₂⁻ (c), respectively

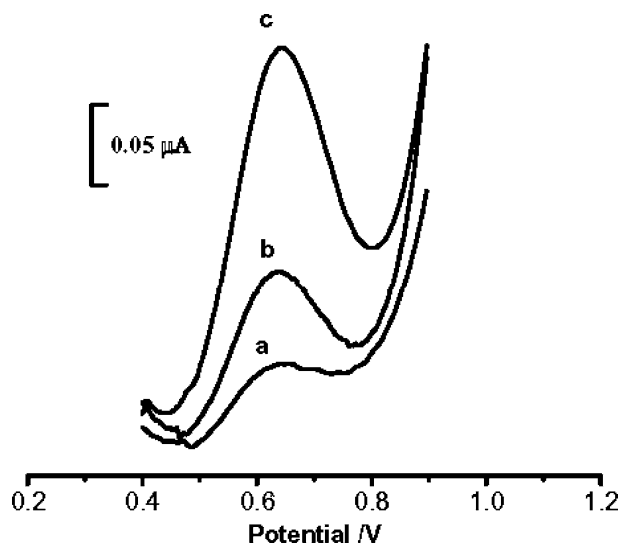


Fig. 3 Differential pulse voltammograms of the nano-TiO₂/Nafion composite film electrode in a deoxygenated PBS containing 1.8×10^{-5} mol/L NO (a), 3.5×10^{-5} mol/L NO (b) and 7.0×10^{-5} mol/L NO (c)



And NO oxidation at the composite film electrode proceeds only by a one-step and one-electron mechanism.

For sensing purposes, this peak of 0.64 V at nano-TiO₂/Nafion composite film electrode is most useful, and therefore the rest of this work was concentrated on this peak. Figure 3 shows the differential pulse voltammetric response of NO at this composite film electrode. Obviously, the peak current is largely increased with the adding of NO, which indicates that the composite film electrode is very sensitive to NO.

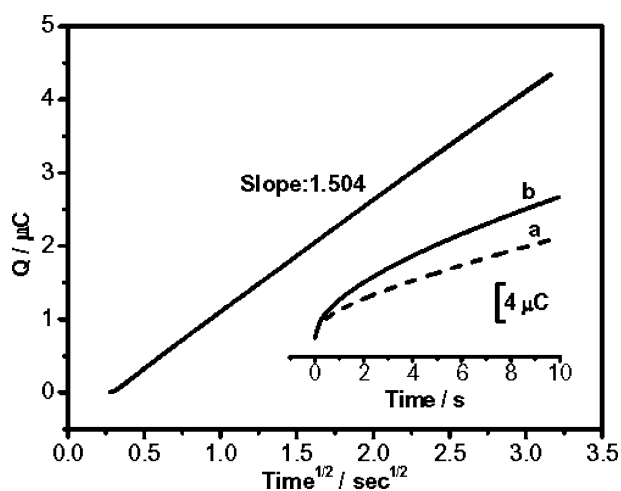


Fig. 4 Linear plot of chronocoulometric response for oxidation of NO at the nano-TiO₂/Nafion composite film electrode. Inset Chronocoulometric curves in a deoxygenated PBS containing a 0 mol/L NO (the blank for b) and b 3.5×10^{-5} mol/L NO

Chronocoulometry

The one-electron oxidation of NO at the nano-TiO₂/Nafion composite film electrode could be confirmed by chronocoulometry. Figure 4 depicts the linear plot of charge Q versus $t^{1/2}$ after the subtraction of the background charge while adding $35 \mu\text{mol/L}$ NO to deoxygenated PBS. According to Cottrell equation [15], the number of electron involved in the process of NO oxidation could be estimated from the Cottrell slope of this plot (Q vs. $t^{1/2}$):

$$\text{Slope} = 2nFAD^{1/2}C^*\pi^{-1/2}$$

Where n is the number of electron, A is the surface area of the electrode, C^* is the bulk concentration of NO and D is the diffusion coefficient of NO in aqueous solution, t is the time of electrolysis and other symbols have their usual significances. The following values were used: $A = 0.071 \text{ cm}^2$, $C^* = 3.5 \times 10^{-5} \text{ mol L}^{-1}$, $D = 2.5 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (based on the value reported by Colucci [16]) and slope = $1.504 \times 10^{-6} \text{ C s}^{-1/2}$. Thus, the calculated value of n is equal to 1.08; this is in agreement with the value reported by Dutta [14].

Optimization of experimental parameters

The effects of the nano-TiO₂ film thickness on the voltammetric response were examined. Variable concentrations and volumes of nano-TiO₂ suspension were chosen to investigate the relationship between the peak current and the film thickness. It was found that a maximum peak current was obtained when $4 \mu\text{L}$ of 0.5 mg/mL suspension was used. However, the peak current decreased when nano-TiO₂ suspension was more than $6 \mu\text{L}$. This is due to the fact that the film is thicker, which results in blocking of the mass transfer.

We also observed that the supporting electrolyte used influenced the second peak. If the supporting electrolyte does not contain Cl^- , e.g. only phosphate buffer solution, i_{p2} decreases and the shape of i_{p2} becomes bad, whereas i_{p1} does not show any change with or without the presence of Cl^- . Therefore, PBS was selected as the supporting electrolyte. The other reason to choose PBS as the supporting electrolyte is that it is closer to the nature environment of NO in biological system. At the same time, the experimental results indicated that the pH values of solutions have little effect on the peak current for the determination of NO, but it influences the peak potential. Since our goal is to develop the NO sensor for biological applications, we are more interested in NO detection at pH = 7.4.

Effects of Nafion and its amount

The use of Nafion film as a barrier to discriminate against anions such as nitrite, nitrate and AA has been

well established in the literature [6]. Furthermore, it can diminish the electrode fouling due to non-specific adsorption of macromolecule in biological sample and enhance the mechanical strength of the film [17]. In this study, Nafion can not only exclude interference, but also play the role of accumulating NO and stabilizing NO^+ . So it is very important to optimize the coverage of Nafion. A series of experiments have been carried out in the identical concentration of NO solution at the nano-TiO₂ electrode, further coated with 0-5 μL 1% (w/v) Nafion solution.

Figure 5 shows the effect of Nafion's amount on differential pulse voltammetric response to NO at the nano-TiO₂-modified GCE. With increasing the volume of Nafion, the peak current increases gradually and reaches a maximum at the point of 3 μL 1% Nafion. However, the peak current decreased when 1% Nafion was more than 3 μL . This is due to the fact that the thicker film can block the electron transfer and lead to the decline of the peak current. Therefore, 3 μL of 1% Nafion was suitable as modification concentration.

Stability and reproducibility

It was found that Nafion coating helped to enhance the stability and extend the lifetime of the electrode. Without the Nafion coating, the nano-TiO₂ film electrode can only be used for 3-4 days. Beyond 4 days, the response current would decrease dramatically. With the Nafion coating, the stability of the modified electrode was found to be very ideal. No significant change in the peak current was observed even if it was immersed in PBS or stored at room temperature for 10 days. The reproducibility was investigated on the same nano-TiO₂/Nafion composite film electrode in a deoxygenated PBS containing 3.5×10^{-5} mol/L NO. After each measurement, the working electrode was taken out of the electrolyte

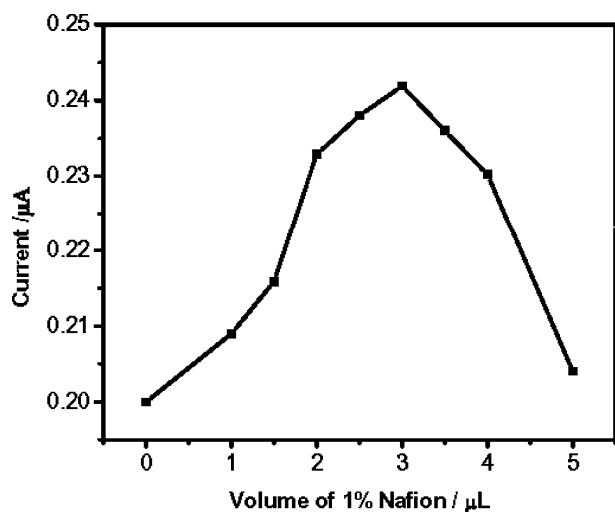


Fig. 5 Effect of Nafion coating on differential pulse voltammetric response to NO at the nano-TiO₂-modified GCE

solution and rinsed with water, then re-inserted into a new deoxygenated PBS for the next measurement. The relative standard deviation of eight parallel detections is 6.3%.

Interference

Generally, there are mainly two sources of interference for NO determination. The first interfering source (such as NO_2^- , AA and DA) is due to the direct oxidation of the interfering species at the same potential at which NO is oxidized, which would give rise to a higher current and an overestimation of NO concentration. Another interfering source like oxygen is due to a chemical reaction between the interfering species and NO, which would consume NO and make the response current of NO decrease. Here, a study of species interfering with the current response indicates that the nano-TiO₂/Nafion composite film provides higher selectivity for NO determination. The NO_2^- anion is known to be the main source of interference in biological detection of NO, since it has a similar oxidation potential as NO at the electrode [6]. However, in this electrode, even 2.0×10^{-3} mol/L NO_2^- did not disturb the determination of 1.8×10^{-6} mol/L NO by amperometric method, which is due to the fact that NO_2^- does not oxidize at the potential of 0.67 V. 1.0×10^{-6} mol/L DA and 1.0×10^{-5} mol/L AA did not cause interference with accurate NO measurements. Superoxide and oxygen interferes the NO detection by the second mechanism. However, this interference is remarkable only when the NO concentration is higher than 0.1 mmol/L. When the NO concentration is lower than 10^{-5} mol/L, the existence of oxygen in the solution does not influence the NO measurement. Taha and his coworkers have also observed the same phenomenon [18]. Butler also found by calculations that even in an aqueous medium saturated with oxygen, NO at a concentration of 10^{-8} mol/L has a half-time of approximately 3 h [2].

Amperometric detection of NO at the nano-TiO₂/Nafion composite film electrode

Hydrodynamic voltammetric experiment and amperometric measurements were carried out in a deoxygenated PBS. The solution was magnetically stirred and the whole cell was protected under nitrogen atmosphere during the experiment. Figure 6 shows the hydrodynamic voltammograms obtained for 7.0×10^{-5} mol/L NO at a nano-TiO₂/Nafion composite film electrode. The current increases with increasing potential, and it finally reaches a steady response at about 0.67 V. Thus, the nano-TiO₂/Nafion composite film electrode could be used as an effective amperometric sensor for the determination of NO with high sensitivity. From this gram, a small inflexion is appeared at 0.45 V. This is probably due to NO low stability and high fugacity. Figure 7

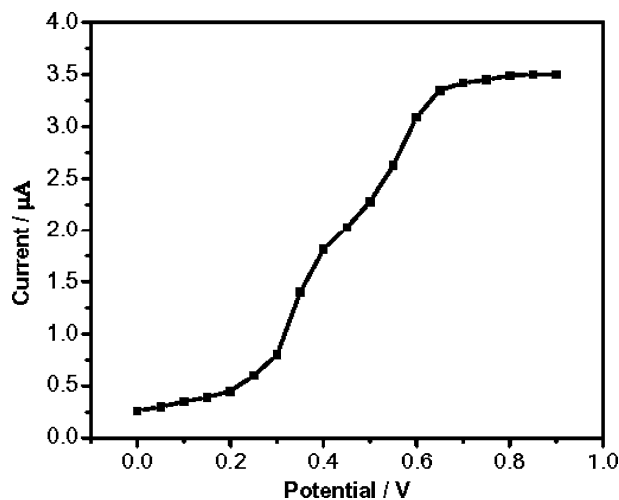


Fig. 6 Hydrodynamic voltammograms of 7.0×10^{-5} mol/L NO at the nano-TiO₂/Nafion-modified GCE

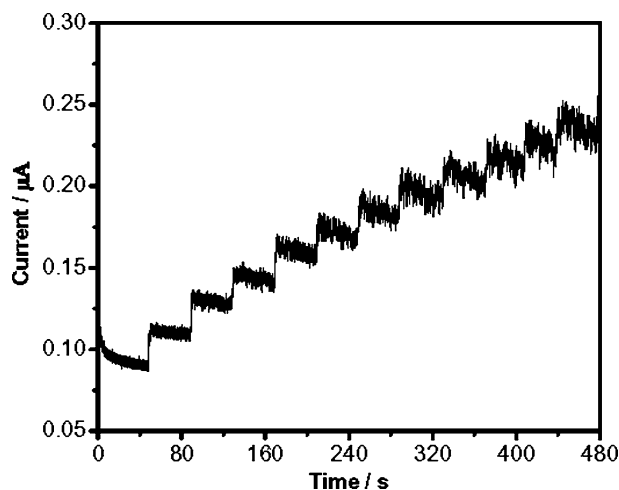


Fig. 7 Amperometric responses at 0.67 V of the nano-TiO₂/Nafion composite film electrode in a deoxygenated PBS with successive additions of 1.8×10^{-6} mol/L NO

displays the results of a typical hydrodynamic amperometric response of this electrode to the successive addition of NO at the applied potential of 0.67 V. As it can be seen from Fig. 7, the response is very fast and has good reproducibility. The response curve is gradually decreased with time after injections of NO standard solution, since the nitrite oxide radical could react with some species on the electrode surface. Previously reported papers dealing with polymer-modified electrode showed a similar declining pattern, while the solution was aerated using a nitrogen gas [19]. The current re-

sponses were found to be linear to the NO concentration over the range of 3.6×10^{-7} to 5.4×10^{-5} mol/L, and the detection limit is estimated to be 5.4×10^{-8} mol/L.

Conclusion

The nano-TiO₂ film electrode and its composite film electrode after coating with Nafion were studied for the catalytic oxidation of NO. A one-electron and one-step transfer mechanism of NO was proposed and validated by chronocoulometry at the composite film electrode. The stability and sensitivity of the nano-TiO₂ was enhanced remarkably with the coating of an appropriate amount of Nafion. The modification composite film provided complete selectivity for NO over NO₂⁻ (up to 2 mmol/L) due to relatively low oxidation potential. The current response exhibited a good linear relationship with the NO concentration in the range of 3.6×10^{-7} to 5.4×10^{-5} mol/L with a detection limit of 5.4×10^{-8} mol/L.

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References

- Shibuki K (1990) *Neurosci Res* 9:69
- Butler AR, Williams DLH (1993) *Chem Soc Rev* 22:233
- Taha TH (2003) *Talanta* 61:3
- Feelish M, Stamler J (1996) *Methods in nitric oxide research*. Wiley, Chichester
- Bedioui F, Villeneuve N (2003) *Electroanalysis* 15:5
- Manlinski T, Taha Z (1992) *Nature* 358:676
- Pontie M, Lecture H, Bedioui F (1999) *Sens Actuators B Chem* 56:1
- Friedemann MN, Robinson SW, Gerhardt GA (1996) *Anal Chem* 68:2621
- Siegel RW (1991) *Annu Rev Mater Sci* 17:559
- Zhu M, Liu M, Shi GY, Chen JS, Jin LT (2003) *Chem J Chin Univ* 24:245
- Wang YZ, Li Q, Hu SS (2005) *Bioelectrochemistry* 65:135
- Zan L, Zhong JC, Luo QR (1999) *J Inorg Mater* 14:264
- Trevin S, Bedioui F, Devynck J (1996) *J Electroanal Chem* 408:26
- Dutta D, Landolt D (1972) *J Electrochem Soc Electrochem Sci Technol* 119:1320
- Bard AJ, Faulkner LR (2001) *Electrochemical methods fundamentals and applications*. Wiley, New York, p 210
- Colucci JA, Foral MJ (1985) *Electrochim Acta* 30:521
- Moussy F, Harrison DJ (1994) *Anal Chem* 66:674
- Taha Z, Kiechle F, Malinski T (1992) *Biochem Biophys Res Commun* 188:734
- Park JK, Tran PH, Chao JKT, Qhodadra R, Rangarajan, Thakor NV (1998) *Biosens Bioelectron* 13:1187