

A multiwall carbon nanotubes film-modified carbon fiber ultramicroelectrode for the determination of nitric oxide radical in liver mitochondria

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Abstract

A novel chemically modified electrode based on the multiwall carbon nanotubes (MWNTs) film-coated carbon fiber ultramicroelectrode (CFUE) has been described for the determination of nitric oxide radical ($\cdot\text{NO}$). The electrochemical behaviors of MWNTs-modified CFUE have been characterized in $0.2 \text{ mmol L}^{-1} \text{ K}_4\text{Fe}(\text{CN})_6$ and $0.1 \text{ mol L}^{-1} \text{ KCl}$ solution. The Nafion film was used to avoid some electroactive interferences. The amount of Nafion was optimized, and some possible interferents [such as nitrite (NO_2^-), nitrate (NO_3^-), ascorbate, dopamine (DA), L-arginine (L-Arg), etc.] were tested and evaluated. The oxidation peak current of $\cdot\text{NO}$ increases significantly at the MWNT/Nafion-modified CFUE, in contrast to that at the bare and the Nafion-modified CFUE, and the oxidation peak potential is at 0.78 V (vs. SCE), which can be used for the detection of $\cdot\text{NO}$. The oxidation peak current is linearly with the concentration of $\cdot\text{NO}$ from 2×10^{-7} to $8.6 \times 10^{-5} \text{ mol L}^{-1}$, and the detection limit is $2 \times 10^{-8} \text{ mol L}^{-1}$. The liver mitochondria in *Carassius auratus* were isolated and $\cdot\text{NO}$ release from mitochondria was monitored by using this ultramicroelectrode system.

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1. Introduction

Nitric oxide radical ($\cdot\text{NO}$) is a free radical molecule generated in biological systems by nitric oxide synthases (NOS). Because of its effect on neurotransmission, vasodilation, and immune response [1], $\cdot\text{NO}$ plays an important role in physiology, pathology, and pharmacology, which has stimulated extensive research interest. It is known that one of the main difficulties associated with its study and determination is the fact that free radical $\cdot\text{NO}$ has a high spontaneous chemical reactivity, with a half-life ranging from 6–50 s [2], and it can be easily oxidized by O_2 to nitrite (NO_2^-) and nitrate (NO_3^-) [3]. In a recent review, Taha [4] has summarized some procedures for the measurement of $\cdot\text{NO}$.

The most widely used techniques include electron spin and paramagnetic resonance spectroscopy, chemiluminescence, UV-visible spectroscopy, fluorescence, and electrochemical methods.

Of all these techniques mentioned above, electrochemical methods are most advantageous because they are simple, speedy and sensitive, and it can be performed in real time. 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. Bedioui and Villeneuve [5] also have made a global description of the various approaches that have involved chemically modified microelectrodes specially designed for the electrochemical detection of $\cdot\text{NO}$ in biological media. The first electrochemical approach is based on the direct oxidation of $\cdot\text{NO}$ on platinum electrode coated by gas permeable or anion membranes [1,6] which allowed the permeation of gases while excluding other materials.

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Recently, various interesting molecular materials are now being studied extensively with the aim of investigating the electrocatalytic oxidation of $\cdot\text{NO}$ in aqueous solutions. The second approach is based on the electrocatalytic oxidation of $\cdot\text{NO}$ on the electrode surface modified with electropolymerised films of metalloporphyrin [7,8] and metallophthalocyanine [9], modified with nanogold colloid and Nafion [10] and by the deposition of *o*-phenylenediamine layers [11].

Carbon nanotubes have attracted more and more attention due to their unique characteristic such as high electrical conductivity, high chemical stability, and high mechanical strength and modulus [12]. They were widely used as scanning probes [13], batteries [14], electron field emission sources [15], nanoelectronic devices [16], and chemical sensors [17]. Generally, carbon nanotubes exist in two forms: single-wall carbon nanotubes (SWNTs) and multi-wall carbon nanotubes (MWNTs). The subtle electronic properties of carbon nanotubes suggest that they have the ability to promote electron transfer reaction when used as an electrode in electrochemical reaction, which provides a new application in the electrode surface modification to design new electrochemical sensors and novel electrocatalytic materials [18]. Zhao et al. [19] found that $\cdot\text{NO}$ could be electrooxidized at MWNTs-modified normal platinum electrode. However, it cannot be applied to $\cdot\text{NO}$ determination in biological systems due to its large size and bad biocompatibility.

Giulivi et al. [20] reported the production of $\cdot\text{NO}$ by rat liver mitochondria by electron paramagnetic resonance using the spin-trapping technique and by the oxidation of oxymyoglobin, which indicated the presence of NOS in the mitochondria. At the same time, he and his coworkers [21] identified, purified, and characterized NOS from rat liver mitochondria by biological assays. In this paper, we report the modification of a carbon fiber ultramicroelectrode (CFUE) by depositing MWNTs dispersed in water in the presence of hydrophobic surfactant, achieving a MWNTs film-modified carbon fiber ultramicroelectrode (denoted as CFUE/MWNTs). The CFUE/MWNTs electrode exhibits an electrocatalytic property for $\cdot\text{NO}$ oxidation and high sensitivity. The electrode responses linearly to $\cdot\text{NO}$ over the concentration range of 2×10^{-7} to 8.6×10^{-5} mol L⁻¹, and the detection limit is 2.0×10^{-8} mol L⁻¹ by an amperometric method. Thus, to test its ability to measure $\cdot\text{NO}$ in biological medium, we first report the measurement of $\cdot\text{NO}$ release from fish liver mitochondria by using our modified CFUE.

2. Experimental

2.1. Reagents and chemicals

The MWNTs used in this work (obtained from the Institute of Nanometer, Central China Normal University)

were synthesized by the catalytic pyrolysis method and then refluxed for 10 h in HNO₃ [22]. It is well known that this treatment causes segmentation and carboxylation of MWNTs at their terminus.

Five percent Nafion solution w/v, dopamine (DA), and ascorbic acid (AA; Sigma), dihexadecyl hydrogen phosphate (DHP; Aldrich), L-arginine (L-Arg; Shanghai Reagent Factory, Shanghai, China), and other reagents were of analytical grade. Aqueous solutions were prepared with redistilled water. High purity nitrogen gas was used for deaeration.

$\cdot\text{NO}$ gas was generated by slowly dropping a 2 mol L⁻¹ H₂SO₄ into a glass flask containing saturated NaNO₂ solution, $\cdot\text{NO}$ being formed in this disproportional reaction [23]. 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 $\cdot\text{NO}$ from traces of oxygen. Before the addition of H₂SO₄, all apparatus was degassed meticulously with nitrogen for 30 min to exclude O₂, as $\cdot\text{NO}$ is rapidly destroyed by O₂. To produce a saturated $\cdot\text{NO}$ solution (at 20 °C, NO \approx 1.8 mmol L⁻¹ [24]), 10 mL deoxygenated distilled water was bubbled with pure $\cdot\text{NO}$ gas for 30 min and kept under $\cdot\text{NO}$ atmosphere until use. $\cdot\text{NO}$ gas is toxic at concentration higher than 100 ppm, so the bubbling procedure was carried out in a fume hood. $\cdot\text{NO}$ standard solutions were prepared by making serial dilutions of a saturated $\cdot\text{NO}$ solution, as previously reported [8]. $\cdot\text{NO}$ solutions were made fresh and were kept in a glass flask with a rubber septum, stored in a light-free place.

Carassius auratus was supplied by the Institute of Life Science, Wuhan University.

2.2. 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 a modified carbon fiber ultramicroelectrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the auxiliary electrode. During $\cdot\text{NO}$ determination, a hermetic electrochemical cell was employed to ensure no $\cdot\text{NO}$ leakage and avoid O₂ permeation. The whole cell was placed in a Faraday cage to avoid electromagnetic interference from the surroundings. Scanning electron microscopy (SEM) was done with a Hitachi X-650 microscope. All electrochemical experiments were performed at room temperature.

2.3. Fabrication and modification of carbon fiber ultramicroelectrode

The method to fabricate carbon fiber ultramicroelectrode was referred to the method that Cheng et al. described [25].

First, a single cleaned carbon fiber (7.8 μm diameter, Kureha Chemical Industry, Tokyo, Japan) was connected to the copper wire with homemade graphite-epoxy paste. After a glass capillary (0.9 mm inner diameter) was pulled on the flame of the gas lamp to obtain a tip size of 10–20 μm , the carbon fiber–copper wire was inserted from the other end into the tip and a 1-cm length of carbon fiber was exposed from the tip. Then, fixing the copper and glass capillary with epoxy, the capillary tip was fused on the flame to seal the carbon fiber. Following this process, the protruded carbon fiber was etched carefully on the flame to the desired length (about 300–500 μm), and the electrodes were tested for electrical contact and potential leaks at the glass–fiber interface in 20 mmol L^{−1} K₄Fe(CN)₆ and 0.1 mol L^{−1} KCl solution. Finally, the carbon fiber ultramicroelectrode was rinsed with redistilled water and followed by further surface treatment described below.

Ultrasonication agitation for 20 min was used to disperse 5 mg MWNTs and 5 mg DHP into 5 mL of redistilled water to give a black suspension [26]. The MWNTs suspension was deposited onto the tip of the carbon fiber ultramicroelectrode, which was set on the planar glass, making the protruded carbon fiber tip immersed in MWNTs suspension and then evaporating the solution under an infrared lamp to obtain MWNTs-modified carbon fiber ultramicroelectrode (denoted as CFUE/MWNTs). The modified carbon fiber ultramicroelectrode was then coated with 3% (w/v) Nafion four times each for 10 s and then allowing the electrode to dry in air to obtain MWNTs/Nafion-modified carbon fiber ultramicroelectrode (denoted as CFUE/MWNTs/Nafion). The Nafion-modified CFUE was prepared by the same procedure explained above without MWNTs (denoted as CFUE/Nafion).

2.4. Procedures of electrochemical measurement

Square wave voltammetry (SWV) was used for $\cdot\text{NO}$ determination in 0.1 mol L^{−1} phosphate buffer (pH 7.4). Prior to $\cdot\text{NO}$ measurements, the electrode was placed in a blank 0.1 mol L^{−1} phosphate buffer, and the potential was cycled between 0.2 and +1.2 V at a scan rate of 50 mV s^{−1} until a steady cyclic voltammogram was obtained (about continuously scanning for 10 cycles). To determine the linearity and sensitivity of the electrode to $\cdot\text{NO}$, a conventional chronoamperometric method was used. Chronoamperometric measurements of $\cdot\text{NO}$ were performed operating at 800 mV, with the electrolyte solution under a stirred condition. After allowing the electrode to reach a steady baseline, a gas-tight syringe was used to inject $\cdot\text{NO}$ solutions into the hermetic electrochemical cell containing 10 mL deoxygenated 0.1 mol L^{−1} phosphate buffer (pH 7.4). All calibrations were performed using chronoamperometry, unless otherwise indicated.

2.5. Direct measurement of $\cdot\text{NO}$ release from liver mitochondria

2.5.1. Isolation of fish liver mitochondria

Liver mitochondria were isolated from *C. auratus* by differential centrifugation, essentially as described in Ref. [27]. First, removing liver from the *C. auratus* and washing with sterile isolating medium (0.25 mol L^{−1} sucrose, 1 mmol L^{−1} EDTA, 10 mmol L^{−1} Tris with pH buffered to 7.4, using HCl. Prior to use, the medium was sterilized at 120 °C for 30 min). The liver was then weighed, homogenized, and centrifuged at 4000 rpm for 15 min. The clear supernatant was centrifuged again for 15 min at 4000 rpm. The sediment was discarded after each step. The clear liquid was then centrifuged twice at high speed (10 000 rpm) for 15 min each time to deposit the mitochondria as sediment. This was resuspended in the isolating medium to give a protein concentration of about 4.0 mg mL^{−1}. Protein was determined by using UV spectrophotometry and biuret reagent. All the above operations were performed aseptically at 273–277 K.

The isolated particles we obtained can exhibit characteristic green color of mitochondria under phase contrast microscope after being stained with Janus green. This indicates that the mitochondria still have biological activity.

2.5.2. Direct measurement of $\cdot\text{NO}$ release from mitochondria

Prior to $\cdot\text{NO}$ measurement, the MWNTs/Nafion-modified CFUE was also scanned potentially between 0.2 and 1.0 V in a deoxygenated 0.1 mol L^{−1} phosphate buffer (pH 7.4) until the stable response. One milliliter isolated sample was transferred to the electrochemical cell. The electrode was inserted into the mitochondria, and chronoamperometric measurements were started under nonstirring condition. During the experiment, 0.1 mmol L^{−1} L-Arg was added, and the chronoamperometric curve was recorded continuously.

3. Results and discussion

3.1. SEM characterization

The SEM images of the surface of a bare CFUE and CFUE/MWNTs are shown in Fig. 1. In SEM image, Fig. 1a gives the general view of a CFUE and shows that a smooth carbon fiber is tightly sealed in the glass capillary. Comparing the surface of the bare CFUE with that of the CFUE/MWNTs, it can be seen that carbon fiber is wrapped with MWNTs sheets (Fig. 1c and d) while the surface of the bare CFUE is quite smooth (Fig. 1b). It suggests that MWNTs display stereo porous interfacial layer, and the CFUE/MWNTs have much larger internal surface per external geometric area.

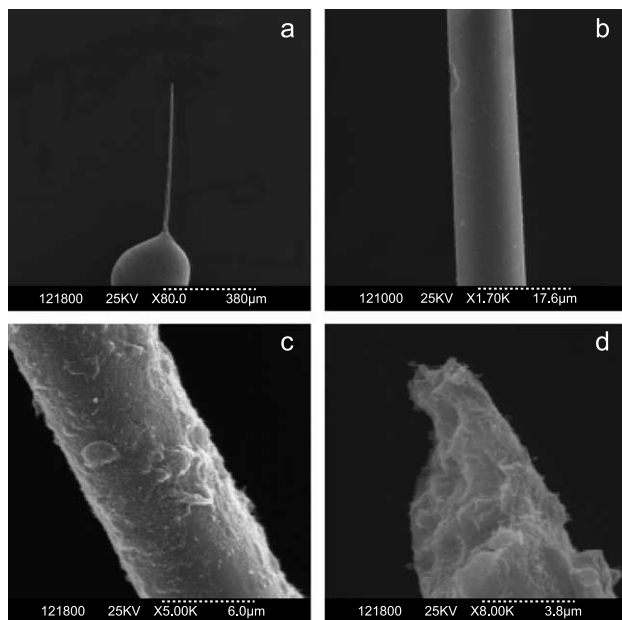


Fig. 1. SEM images of (a) the general view of CFUE, (b) the midsection of bare CFUE, (c) the midsection of CFUE/MWNTs, and (d) the tip and cross-section of CFUE/MWNTs.

3.2. Cyclic voltammetric behavior of the CFUE/MWNTs

Fig. 2 shows cyclic voltammograms of the bare CFUE and CFUE/MWNTs with the potential range of -0.4 – 0.2 V in a 0.1 mol L^{-1} phosphate buffer (pH 7.4) at a scan rate of 50 mV/s . A couple of redox peaks was observed at -0.13 and -0.25 V at the CFUE/MWNTs, which corresponded to the reduction and the reoxidation of carboxylic acid group on the surface of MWNTs [28]. The background current of the modified CFUE is much larger than that of the bare CFUE, which indicates that MWNTs could reliably increase

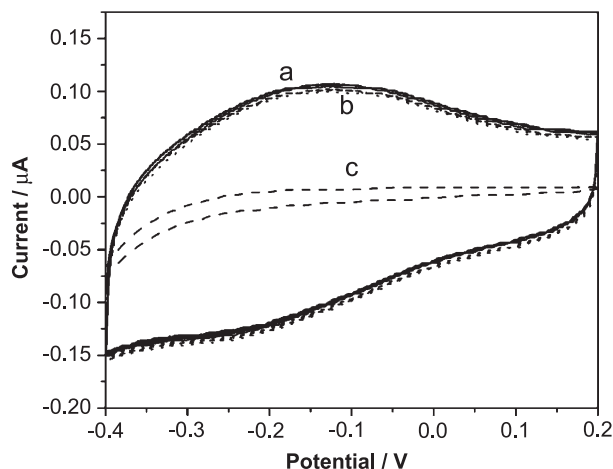


Fig. 2. Cyclic voltammetric behaviors of bare CFUE and its CFUE/MWNTs in 0.1 mol L^{-1} phosphate buffer (pH 7.4). (a) 10 cycles successively scanned at the CFUE/MWNTs; (b) 10 cycles successively scanned after the CFUE/MWNTs was placed in air for 1 week; (c) 1 cycle at its corresponding bare CFUE.

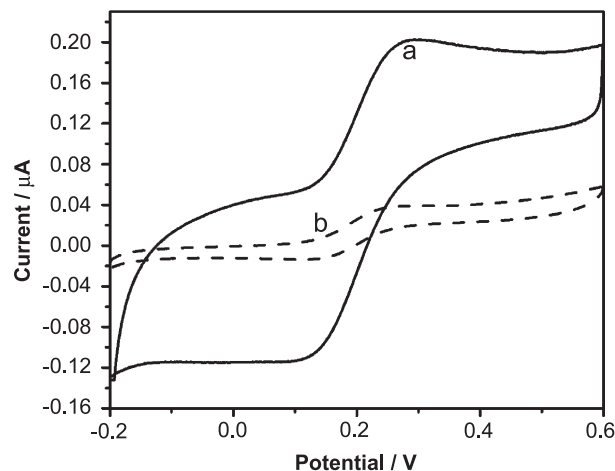


Fig. 3. Cyclic voltammograms of $0.2 \text{ mmol L}^{-1} \text{ K}_4\text{Fe(CN)}_6$ in $0.1 \text{ mol L}^{-1} \text{ KCl}$ at a CFUE/MWNTs (a) and its bare CFUE (b). The potential scan rate is 10 mV/s .

the inner surface area of the CFE. The response of the modified CFUE almost remained constant for 1 week. It suggests that the MWNTs-modified CFUE is very stable.

The cyclic voltammograms measured using a bare CFUE and MWNTs-modified electrode in $20 \text{ mmol L}^{-1} \text{ K}_4\text{Fe(CN)}_6$ and $0.1 \text{ mol L}^{-1} \text{ KCl}$ solution are presented in Fig. 3. The cyclic voltammograms take on a sigmoid shape, which indicates the nonlinear diffusion behaviors of the electrodes. The background current and limiting current at the MWNTs-modified CFUE (curve a) are much higher than that of the bare CFUE (curve b), which also indicates the larger surface area of the MWNTs-modified CFUE. For the CFUE/MWNTs, at low scan rates ($<100 \text{ mV/s}$), the cathodic peak currents are in linear relationship with $v^{1/2}$ (see inset in Fig. 4), indicating that a diffusion-controlled reaction was obtained.

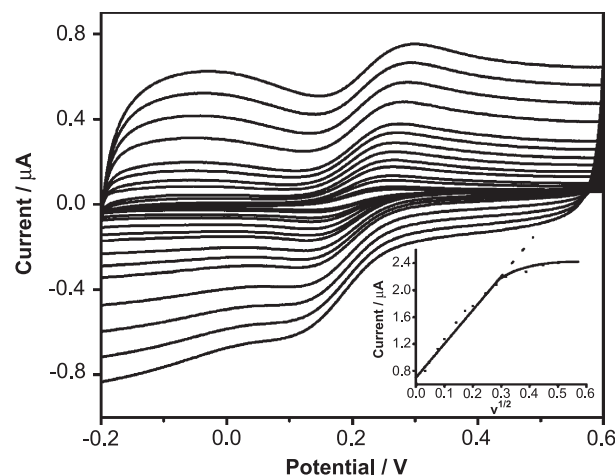


Fig. 4. Cyclic voltammograms of $0.2 \text{ mmol L}^{-1} \text{ K}_4\text{Fe(CN)}_6$ in $0.1 \text{ mol L}^{-1} \text{ KCl}$ at the CFUE/MWNTs. Scan rates (from inner to outer): $1, 2, 6, 10, 20, 40, 60, 80, 100, 150, 200, 250$, and 300 mV/s . Inset: the relationship between the cathodic peak current and the scan rate.

3.3. Electrooxidation behavior of $\cdot\text{NO}$ at the CFUE/MWNTs/Nafion

Fig. 5 shows the square wave voltammograms of $\cdot\text{NO}$ at CFUE/Nafion and CFUE/MWNTs/Nafion in deoxygenated 0.1 mol L^{-1} phosphate buffer (pH 7.4), respectively. At the CFUE/Nafion, a small anodic peak is observed (curve c). However, at CFUE/MWNTs/Nafion, the peak current increases significantly with increasing $\cdot\text{NO}$ concentration, and the oxidation peak potential shifts negatively to ca. 0.78 V (curve a, b). These results suggest that MWNTs exhibit the catalytic activity for the electrooxidation of $\cdot\text{NO}$. This could mainly be attributed to the subtle electronic properties and the larger surface area of CFUE/MWNTs/Nafion, which results in the remarkable enhancement of the peak current for $\cdot\text{NO}$ oxidation.

3.4. Effects of Nafion coating

The modified CFUE was fabricated to determine $\cdot\text{NO}$ in biological samples or in vivo. Therefore, it was necessary to remove the interference from other electroactive substances coexisting in biological system, such as ascorbate and some neurotransmitters. Nitrite is also a main source of interference in the biological detection of $\cdot\text{NO}$ because it is one of the main decomposed products of $\cdot\text{NO}$ oxidation and can be oxidized at nearly the same potential.

Nafion, which is a polyfluorinated polyacid with cation exchange properties, can not only exclude the anionic interfering analytes such as nitrite, nitrate, and AA, diminish the electrode fouling due to nonspecific adsorption of proteins and other macromolecule in biological samples, but also enhance the mechanical strength of the film [29]. In addition, Nafion film should also stabilize NO^+ formed

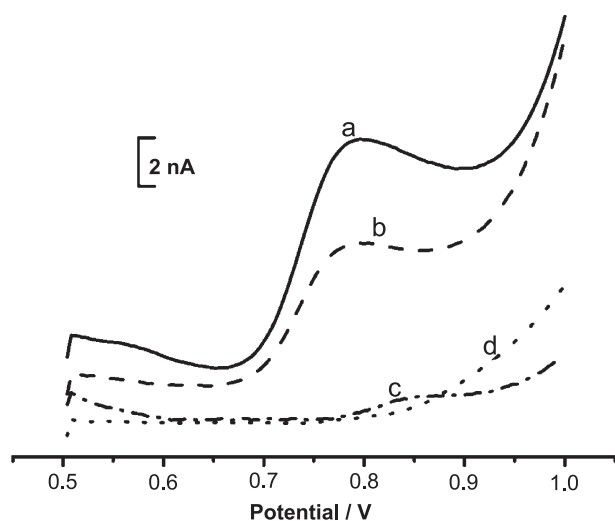


Fig. 5. Square wave voltammograms at CFUE/MWNTs/Nafion (a, b, d) and the CFUE/Nafion (c) in the presence of $0 \text{ } \mu\text{mol/L } \cdot\text{NO}$ (d), $20 \text{ } \mu\text{mol L}^{-1} \cdot\text{NO}$ (c, b), and $40 \text{ } \mu\text{mol L}^{-1} \cdot\text{NO}$ (a) in a deaerated 0.1 mol L^{-1} phosphate buffer (pH 7.4).

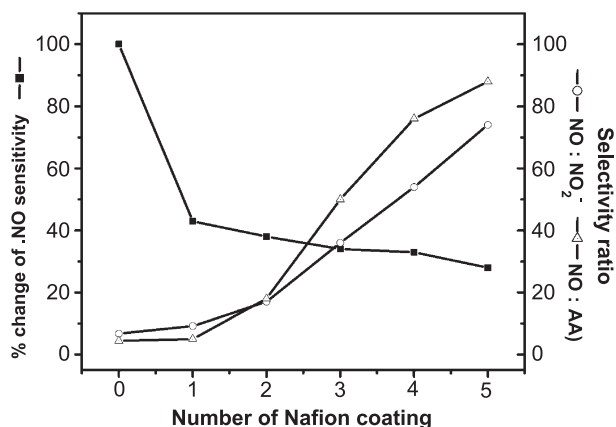


Fig. 6. Effect of Nafion coating on the $\cdot\text{NO}$ sensitivity and the $\cdot\text{NO}$ selectivity ratio against nitrite and AA.

upon the oxidation of $\cdot\text{NO}$ and prevent a complicated pattern of reactions that could lead to the formation of NO_2^- and NO_3^- [7]. However, it is equally important to optimize the coverage of Nafion. A thicker film implies a slowdown in the response time and lowering of electrode sensitivity. As we know, the amount of Nafion coated onto the electrode surface depends on the concentration of Nafion used and the immersion time of the electrode in the Nafion solution.

Fig. 6 shows the effect of Nafion coating on the $\cdot\text{NO}$ sensitivity and the $\cdot\text{NO}$ selectivity ratio against nitrite and AA. Here, each sensitivity measurement obtained from an individual calibration curve was expressed as a change of current per μM concentration. The selectivity ratio was calculated as the ratio of [$\cdot\text{NO}$ sensitivity/interferent sensitivity] [30]. The sensitivity of each electrode varies according to the length of the carbon fiber. The signal is increased as the carbon fiber is lengthened. This suggests that the identical electrode is used to test the sensitivity and selectivity under the same conditions. In Fig. 6, it is seen that the signal from CFUE/MWNTs with Nafion farther modification is decreased to 43% of the original response of CFUE/MWNTs after just one coating with Nafion, while the $\cdot\text{NO}$ selectivity ratio against nitrite and AA are increased, which indicates that the electrode needs at least four or more coatings to get the desired selectivity ratio against nitrite and AA. Therefore, the electrode was selected to dip into 3% Nafion solution four times and each for 10 s.

3.5. Amperometric detection of $\cdot\text{NO}$ at the CFUE/MWNTs/Nafion

Amperometric detection of $\cdot\text{NO}$ in deaerated and unaerated 0.1 mol L^{-1} phosphate buffer (pH 7.4) was undertaken. According to the comparison of the performances of the different CFUE/MWNTs/Nafion, we found that the current response of the modified electrode is precise. The amperometric response in a deaerated solution containing $7.2 \times 10^{-6} \text{ mol L}^{-1} \cdot\text{NO}$ is very stable (RSD=4.7%, $n=12$). Fig. 7 shows a typical experimental amperogram

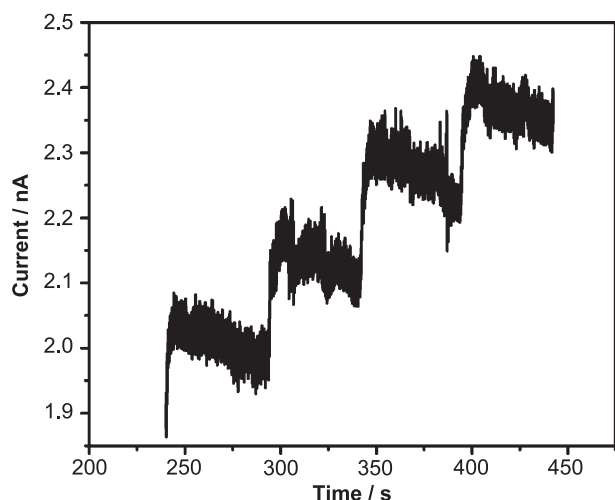


Fig. 7. Amperometric response of $\cdot\text{NO}$ on the CFUE/MWNTs/Nafion with successive injections of $7.2 \times 10^{-7} \text{ mol L}^{-1} \cdot\text{NO}$ in a deaerated 0.1 mol L^{-1} phosphate buffer (pH 7.4). Applied potential: 800 mV.

obtained at the CFUE/MWNTs/Nafion in deaerated 0.1 mol L^{-1} phosphate buffer (pH 7.4). The amperogram clearly shows an increase in anodic current with $\cdot\text{NO}$ injections. The response curve is gradually decreased after injections of $\cdot\text{NO}$ 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 [30]. Fig. 8 shows the linear variations of the current vs. $\cdot\text{NO}$ concentrations in deaerated and unaerated 0.1 mol L^{-1} phosphate buffer, respectively. As shown in Fig. 8, the sensitivity of the modified electrode in deaerated condition is higher than that in unaerated condition when $\cdot\text{NO}$ concentration was higher than $5.0 \times 10^{-6} \text{ mol L}^{-1}$. While $\cdot\text{NO}$ concentration is less

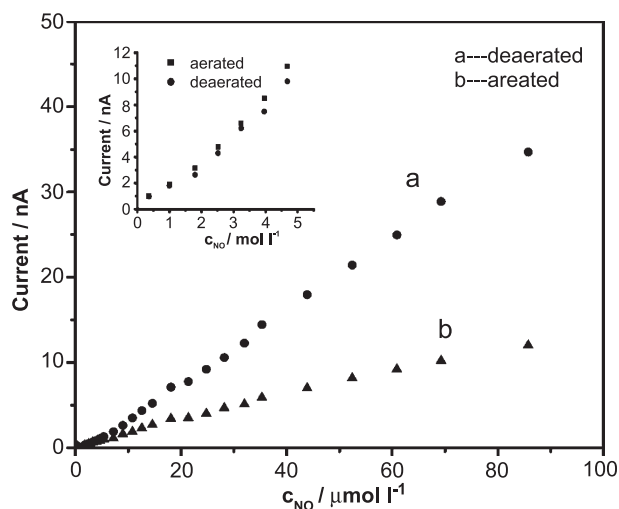


Fig. 8. Linear variations of a measured current by amperometry with $\cdot\text{NO}$ concentrations ranging from $1.8 \times 10^{-7} \text{ mol L}^{-1}$ to $8.6 \times 10^{-5} \text{ mol L}^{-1}$. (a) the deaerated condition; (b) the unaerated condition. Inset: $\cdot\text{NO}$ concentrations ranging from 1.8×10^{-7} to $5.0 \times 10^{-7} \text{ mol L}^{-1}$.

than $5.0 \times 10^{-6} \text{ mol L}^{-1}$, only a slight discrimination is observed in these two conditions (see inset in Fig. 8). These indicate that $\cdot\text{NO}$ determination in biological samples can be converted by the calibration curve in deaerated medium because the $\cdot\text{NO}$ concentration varies between 10^{-6} and $10^{-9} \text{ mol L}^{-1}$ in biological models.

The current response exhibits a good linear relationship with the $\cdot\text{NO}$ concentration in the range of 2.0×10^{-7} to $8.6 \times 10^{-5} \text{ mol L}^{-1}$. The sensitivity of the electrode tested is $2.291 \text{ nA } \mu\text{M}^{-1}$ at $\cdot\text{NO}$ concentration ranging from 1.0×10^{-7} to $5.0 \times 10^{-7} \text{ mol L}^{-1}$. The minimum detectable concentration of $\cdot\text{NO}$ is estimated to be $2.0 \times 10^{-8} \text{ mol L}^{-1}$ (based on a signal-to-noise of 3).

3.6. Stability of the CFUE/MWNTs/Nafion

Whether the electrode was used in vivo experiment or the test in biological sample, the electrode should be very stable. In our experiments, the modified electrode remains 90% ($n=9$) of the initial response after it was kept in air for 1 week. For the $\cdot\text{NO}$ detection in biological sample, the modified electrode could be used continuously for at least 8 h. The electrode response gradually decreased to approximate 14% ($n=3$) of the initial response after being used continuously for 8 h in biological sample.

3.7. Interference

Trace biological species can potentially contribute to the interference of the modified electrode detection scheme. For example, monoamine neurotransmitters such as DA, AA, and nitrite can be directly oxidized at the positive potential (800 mV). At this modified electrode, $5.0 \times 10^{-5} \text{ mol L}^{-1}$ AA and $2.0 \times 10^{-5} \text{ mol L}^{-1}$ nitrite did not disturb the determination of $3.6 \times 10^{-6} \text{ mol L}^{-1} \cdot\text{NO}$ with the use of

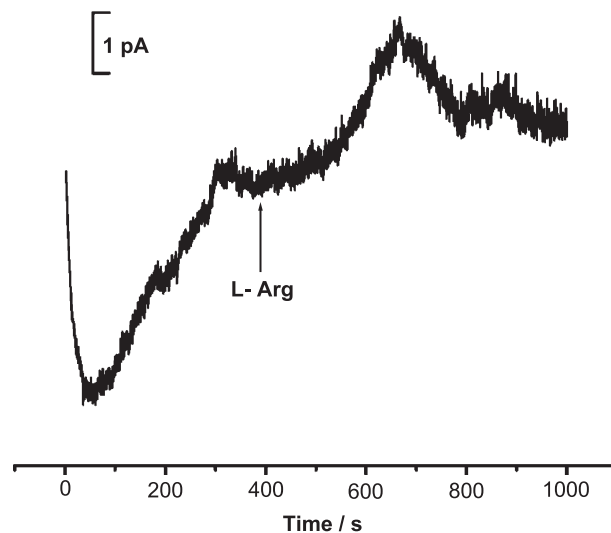


Fig. 9. Amperogram showing $\cdot\text{NO}$ release from fish liver mitochondria without L-Arg and adding $1.0 \times 10^{-4} \text{ mol L}^{-1}$ L-Arg, measured by the CFUE/MWNTs/Nafion. Applied potential: 800 mV.

Nafion. 1.0×10^{-6} mol L⁻¹ DA and 1.0×10^{-3} mol L⁻¹ solutions of nitrate, L-arginine, glucose, and sucrose did not cause interference with accurate $\cdot\text{NO}$ measurements. Superoxide and oxygen are another types of interference because they can react with $\cdot\text{NO}$ and decrease measurable $\cdot\text{NO}$, although they cause no response at the modified electrode themselves.

3.8. Measurement of $\cdot\text{NO}$ release from liver mitochondria of *C. auratus*

Fig. 9 shows the amperogram after the electrode was placed in mitochondria. A gradual increase of the response current is observed without any stimulator, which suggested an endogenous pool of L-Arg capable of sustaining $\cdot\text{NO}$ production through an NOS-catalyzed reaction in the mitochondria. This is in agreement with the result reported by Guilivi et al. [20]. While adding 0.1 mmol L⁻¹ L-Arg into the mitochondria, the amperometric current increased rapidly, which corresponds to the release of $\cdot\text{NO}$ from mitochondria. Subsequently, we successively injected the L-Arg for three times to the liver mitochondria, the similar response was observed. The RSD of the response current is about 6.7%. Measured current was equivalent to $\cdot\text{NO}$ concentration up to approximately 0.4 $\mu\text{mol L}^{-1}$. Of note, this level of $\cdot\text{NO}$ is biologically relevant because it is in the range of those concentrations reported to inhibit respiration in intact mitochondria [31]. Therefore, it could be hypothesized that this inhibition of mitochondria respiration by $\cdot\text{NO}$ may represent a novel biochemical pathway regulating the supply of O₂ and energy to tissues under dynamic conditions.

4. Conclusion

MWNTs dispersed into water in the presence of DHP were successfully coated onto the tip of carbon fiber ultramicroelectrode. MWNTs-modified CFUE exhibits an excellent electrocatalytic activity to $\cdot\text{NO}$ electrooxidation due to its the subtle electronic properties and stereo porous interfacial layer. The effects of the Nafion coating and the interference on the $\cdot\text{NO}$ detection have been examined. The linear range of $\cdot\text{NO}$ determination is from 2×10^{-7} to 8.6×10^{-5} mol L⁻¹, with a detection limit of 2×10^{-8} mol L⁻¹ using an amperometric method. Finally, we tested the performance of the modified electrode by detecting the $\cdot\text{NO}$ release from fish liver mitochondria. The result is satisfactory.

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