

Amperometric determination of nitric oxide at a carbon nanotube modified electrode with redox polymer coating

Junjie Fei · Shengshui Hu · Kwok-Keung Shiu

Received: 16 December 2009 / Revised: 29 April 2010 / Accepted: 31 May 2010 / Published online: 16 June 2010
© Springer-Verlag 2010

Abstract A novel chemically modified electrode based on an osmium complex-containing redox polymer film coated on single-walled carbon nanotube (SWNT) modified glassy carbon electrode (GCE) has been described for the determination of nitric oxide. The results showed that the oxidation current increased significantly at the SWNT/redox polymer coated GCE, as compared to that observed on a bare GCE- and SWNT-modified GCE. Amperometric measurement was carried out at the potential of +0.80 V (vs. Ag|AgCl) and the current response to NO was found to be directly proportional to its concentration in the range from 2.0×10^{-7} to 4.0×10^{-5} M, and the detection limit was estimated to be 5.0×10^{-8} M.

Keywords Nitric oxide · Osmium complex · Redox polymer · Carbon nanotubes

Introduction

Nitric oxide has been found to be released by many cells in mammalian systems to play a number of important biological roles such as neurotransmitter, cytostatic agent,

blood-pressure regulator [1]. The deficiency or excess concentration of NO would lead to the pathogenesis of several diseases [2]. There have been increasing interest in NO determination with the discovery that NO is the endothelium-derived relaxing factor in vivo [3]. The measurement of NO content is important in view of its essential function in physiological processes, including the neuro-transmission, platelet aggregation, macrophage function and vasodilation [4]. Many methods such as chemiluminescence [5], diazotization [6], electron paramagnetic resonance [7], and electrochemical methods [8, 9] have been proposed for NO determination. Among them, electrochemical methods are considered to be direct, simple and rapid, and are applicable in vivo. Although NO can be reduced at conventional electrode surfaces, it generally requires large overpotentials, which might lead to unexpected interference. Therefore, some chemically modified electrodes have been proposed to overcome such difficulties [10–17].

Osmium complex-containing redox polymers with high electron diffusion coefficient played important roles in developing electrochemical sensors and biosensors [18–23]. It has been reported that osmium complex-containing redox polymers could undergo inner-sphere chloride exchange with strongly coordinating pyridine or imidazole groups on polymer backbones upon electro-reduction, and were irreversibly cross-linked onto the electrode surface [24, 25]. The resulting films were hydrophilic and were permeable to the substrates and the products of the electrode reaction. Compared with chemical cross-linking, electrodeposition took place faster and provided a better control on the sensor construction through a simple one-step procedure [26].

This paper reports possibly the first successful preparation of a redox polymer containing Os-bipyridine complex and poly(4-vinylpyridine) (PVP) partially quaternized with 2-bromoethylamine (EA) functionalities (PVP–Os–EA)

J. Fei · K.-K. Shiu (✉)
Department of Chemistry, Hong Kong Baptist University,
Kowloon Tong,
Kowloon, Hong Kong, China
e-mail: kkshiu@hkbu.edu.hk

J. Fei
Key Laboratory of Environmentally Friendly Chemistry and
Applications, Ministry of Education, College of Chemistry,
Xiangtan University,
Xiangtan, 411105 Hunan, China

S. Hu
Department of Chemistry, Wuhan University,
Wuhan 430072, China

formed directly on the single-walled carbon nanotubes (SWNT) modified electrode employing double-potential step chronoamperometry and the application for the determination of nitric oxide. The modified electrode offered a significant improvement in the current response at +0.80 V when compared to that of bare glassy carbon and SWNT-modified electrodes.

Experimental

Reagents and chemicals

The synthesis of electron-conducting redox polymer (PVP–Os–EA) is described elsewhere [27]. In this preparation, PVP was partially coordinated to $[\text{Os}(\text{bpy})_2\text{Cl}_2]$ ($\text{bpy}=2,2'$ -bipyridine) and partially quaternized with 2-bromoethylamine (EA) with a PVP/Os/EA ratio of 6.2:1:1.2. The degree of quaternization was usually controlled by the amount of EA reagents used in the synthesis and was expected to be about 20% [24–27]. The chemical structure of the osmium complex-containing redox polymer is shown in Fig. 1. A phosphate buffer solution (pH 7.4) was used as the supporting electrolyte. All other chemicals were of analytical grade and were used as received. All solutions were prepared using doubly distilled water.

Single-walled carbon nanotube materials were obtained from the Chengdu Organic Chemicals Co., Ltd, (Chinese Academy of Sciences, China). According to the supplier, the SWNT material (with a purity >90%) has an internal diameter of 1–2 nm and a length of 5–30 μm . The nanotube materials were purified by refluxing in 2.6 M HNO_3 for

$m=1, n=4, p=1.2$

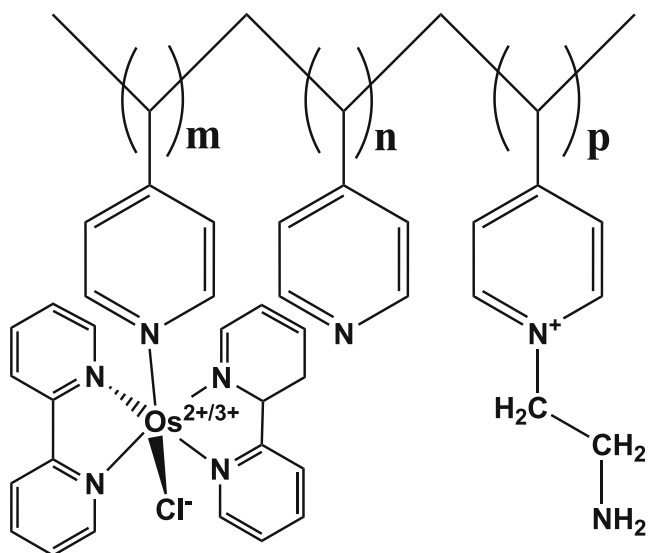


Fig. 1 Structure of the osmium complex-containing redox polymer

24 h, and then exposed to a $\text{HNO}_3 + \text{H}_2\text{SO}_4$ (1:3) mixture in a sonicating bath for 4 h [28].

NO-saturated solutions were prepared as described in the literature [29]. The phosphate buffer solution was purged with high purity nitrogen gas for 30 min to remove oxygen and then was bubbled with pure NO gas for 30 min. Standard NO solutions were prepared by diluting the saturated solution with doubly distilled water, assuming the NO-saturated solution contained 1.9 mM of NO [30].

Apparatus

All electrochemical measurements were carried out with a computer-controlled model CHI630C electrochemical Analyzer (CH Instruments, Austin, USA) at room temperature. A conventional three-electrode cell was employed with a Pt wire counter electrode, an Ag|AgCl reference electrode and a glassy carbon working electrode. All potentials were quoted versus the Ag|AgCl reference.

Preparation of the GC/SWNT/PVP–Os–EA film modified electrode

Glassy carbon electrode (Bioanalytical Systems, Inc., USA) of 3 mm in diameter was used. The electrode was polished with 0.3 μm and 0.05 μm alumina slurry successively. The electrode was then rinsed with doubly distilled water and cleaned in sonicating bath for 5 min.

Purified SWNT materials (2.5 mg) were dispersed in 5-mL doubly distilled water under ultrasonication agitation for 30 min. Eight microliters of the SWNT suspension was added to the electrode surface and was allowed to dry in air. Pretreatment of the freshly prepared SWNT-modified electrode was conducted by cyclic voltammetry in phosphate supporting electrolyte between -1.0 to $+1.0$ V until stable voltammograms were obtained. The PVP–Os–EA film was then electrodeposited onto the SWNT surface from a 0.5 mg/mL PVP–Os–EA solution with double-potential step for 100 cycles. The applied potential was set alternately at $+0.70$ V for 2 s and then switched to -0.40 V for another 2 s [24–27]. The resulting SWNT/PVP–Os–EA modified electrode was then rinsed thoroughly with doubly distilled water.

Amperometric measurement

All electrochemical measurements were performed in a deoxygenated phosphate buffer (pH 7.4). Prior to NO measurements, the electrode was placed in a blank 0.1 M phosphate buffer, and the potential was cycled between zero and $+1.0$ V at a scan rate of 50 mV/s until stable voltammograms were obtained. Amperometric measurements of NO were performed in a 0.1-M phosphate buffer

solution (pH 7.4) at an applied potential of +0.80 V under steady-state conditions. The background current was allowed to decay to a constant level before aliquots of analyte sample were added to the stirred buffer solution with a gas-tight syringe.

Results and discussion

Electrochemical behavior of the GC/SWNT/PVP–Os–EA modified electrode

The synthesis and electrochemical characterization of the PVP–Os–EA redox polymer has been reported elsewhere [24, 27]. The PVP–Os–EA film was electrodeposited onto the SWNT surface with double-potential step between +0.70 and –0.40 V [24–27]. Cyclic voltammograms of the GC/SWNT/PVP–Os–EA modified electrode in pure supporting electrolyte are shown in Fig. 2. The cyclic voltammograms showed that the GC/SWNT/PVP–Os–EA electrode exhibited reversible voltammetric response. A pair of redox peaks with nearly equal charges of about $6.7 \mu\text{C}$ for the reduction and the oxidation responses was observed at a scan rate of 25 mV s^{-1} . The formal potential (taken as the average value of anodic and cathodic peak potential) was about +0.33 V with a peak separation of 47 mV, corresponding to the redox switching of the Os(III)/(II) redox couple in the SWNT/PVP–Os–EA film. The electrode exhibited the classical features of a kinetically fast redox couple bound to an electrode surface. Figure 2 also shows that the peak currents increased linearly with increasing scan rate and the difference between the reduction and the oxidation peak potential remained roughly unchanged, indicating fast charge-transfer from the film to the electrode [31].

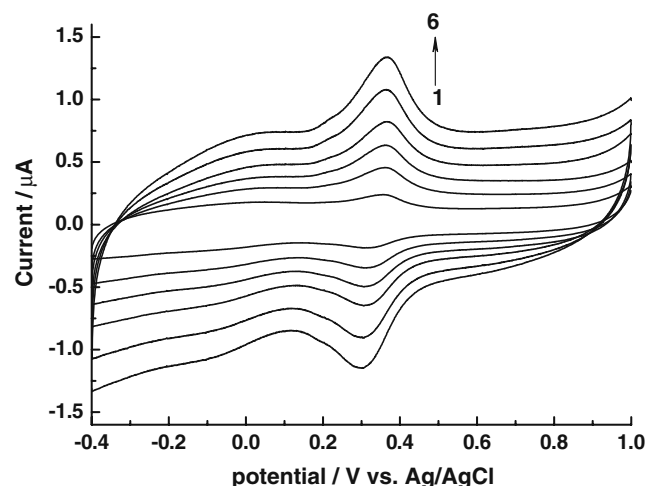


Fig. 2 Cyclic voltammograms of the GC/SWNT/PVP–Os–EA electrode in phosphate buffer at different scan rates from 25 to 250 mV/s

Electrochemical behavior of NO at the GC/SWNT/PVP–Os–EA modified electrode

Figure 3 shows the cyclic voltammograms of the bare glassy carbon, GC/SWNT and GC/SWNT/PVP–Os–EA modified electrodes in deoxygenated 0.1 M phosphate buffer (pH 7.4). In the presence of NO, a small increase in the anodic current was observed for the bare GCE at potentials higher than +0.8 V (Curve 2), indicating that NO oxidation was inefficient at bare electrode (Curve 1). NO oxidation was observed at the SWNT-modified electrode at around +0.8 V, as shown in Curve 4. For the GC/SWNT/PVP–Os–EA modified electrode, the anodic peak current at around +0.80 V increased significantly with the same NO concentration (Curve 6). There were no obvious changes in the peak current for the osmium peaks at around +0.4 V. These results suggested that the SWNT/PVP–Os–EA film exhibited enhanced catalytic activity for the electrooxidation of NO.

Since the NO oxidation did not occur at the redox potential windows of the Os(III)/(II) redox couple, the catalytic activity for the electrooxidation of NO was not resulted from the Os(III)/(II) redox couple itself. Probably, the PVP–Os–EA film might act as a three-dimensional catalyst for the NO oxidation [28]. On the other hand, the PVP–Os–EA possessed a positively charged central metal ion and negatively charged ligand ring with expanded conjugated p-electron system between the ligand ring and the central metal ions. Moreover, chloride was a much weaker ligand and could be readily replaced by other stronger ligands [24]. NO is well known as an extremely powerful ligand for its high electron density.

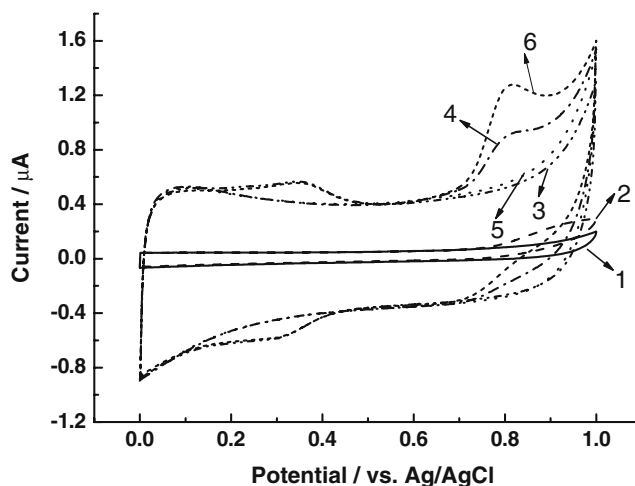


Fig. 3 Cyclic voltammograms of difference electrodes to NO at a scan rate of 50 mV/s. (1) bare glassy carbon electrode without NO; (2) bare GCE in 50 μM NO; (3) GC/SWNT-modified electrode without NO; (4) GC/SWNT-modified electrode in 50 μM NO; (5) GC/SWNT/PVP–Os–EA electrode without NO; (6) GC/SWNT/PVP–Os–EA electrode in 50 μM NO

Hydrodynamic amperometry

Figure 4 shows the hydrodynamic voltammograms obtained for 5 μM NO at the GC/SWNT/PVP-Os-EA modified electrode. The current increased with increasing potential and finally reached a steady response at about +0.80 V. Thus, the applied potential of the electrochemical sensor was kept at +0.80 V in all experiments.

A typical hydrodynamic amperometric experiment of NO was carried out in a well-stirred solution at an applied potential of +0.80 V. Amperometric results showed that the modified electrode offered a good response for sequential addition of NO, as shown in Fig. 5. The oxidation current increased with addition of NO and reached the steady-state quickly. The inset figure shows the corresponding calibration plot of the amperometric responses. The current response of NO was found to be directly proportional to its concentration in the range from 2.0×10^{-7} to 4.0×10^{-5} M. The precision was estimated from ten repetitive measurements of 2 μM NO solution, and the relative standard deviations (RSD) were found to be 4.8%. These results suggested that the GC/SWNT/PVP-Os-EA modified electrode had a good accuracy for the determination of NO. The limit of detection (LOD) was found to be 5×10^{-8} M for a signal-to-noise ratio of 3:1. The GC/SWNT/PVP-Os-EA modified electrode offered lower detection limit for NO determination as compared to modified electrode systems reported in the literature [32–36], including those employing carbon nanotube materials [35, 36].

As nitrite anion is usually present at a much higher concentration than that of NO in the real system and their oxidation potential is very close, the interference from nitrite anion has to be considered. Nafion as a cation-exchange membrane was usually employed to discriminate

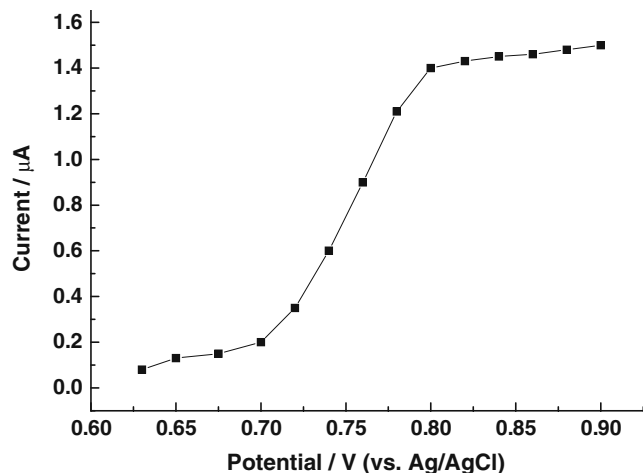


Fig. 4 Hydrodynamic voltammograms of the GC/SWNT/PVP-Os-EA electrode in 5 μM NO

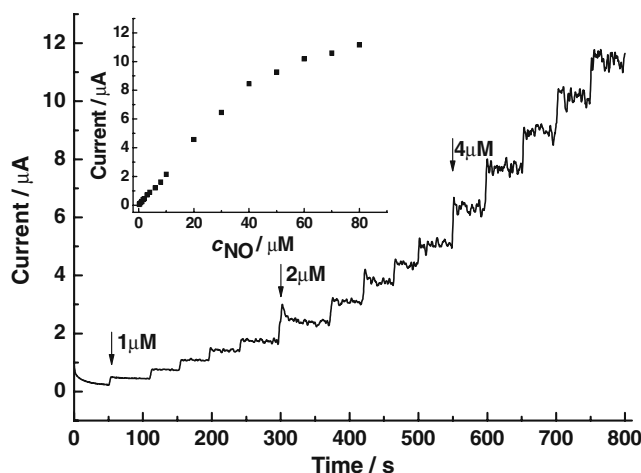


Fig. 5 Amperometric responses of the GC/SWNT/PVP-Os-EA electrode to NO at an applied potential of +0.80 V. The inset figure shows the corresponding calibration curve for NO determination

the interference of anions such as nitrite and ascorbic acid in electrochemical biosensors [37]. When the GC/SWNT/PVP-Os-EA electrode was further coated with a Nafion film [26], the cyclic voltammograms of the Nafion-coated GC/SWNT/PVP-Os-EA electrode were similar to that of the GC/SWNT/PVP-Os-EA electrode. Figure 6 shows the amperometric responses of the GC/SWNT/PVP-Os-EA electrode to 2 μM NO in the presence of 100 μM nitrite ions at an applied potential of +0.80 V. The amperometric response of NO at the Nafion-coated electrode showed little changes, indicating that the existence of Nafion film had little effect on the electrochemical characteristics of the SWNT/PVP-Os-EA film. The presence of nitrite

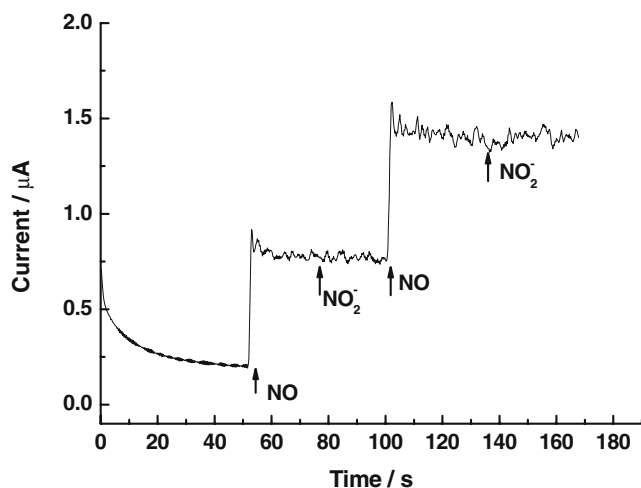


Fig. 6 Amperometric responses of the GC/SWNT/PVP-Os-EA electrode to 2 μM NO in the presence of 100 μM NO_2^- at an applied potential of +0.80 V

anion of 50-fold higher concentration did not influence the amperometric detection of NO.

Furthermore, the modified electrode possessed a good stability. Stability tests of the redox-polymer-modified electrode were carried out by measuring the current response at room temperature. It was shown that the GC/SWNT/PVP–Os–EA modified electrode maintained its initial sensitivity after 64 h.

Conclusions

A highly sensitive NO sensor based on osmium complex-containing redox polymer (PVP–Os–EA) coated on single-walled carbon nanotube modified glassy carbon electrode was obtained. Experimental results suggested that the SWNT/PVP–Os–EA film exhibited catalytic activity for the electrooxidation of nitric oxide. This electrode offered high sensitivity, good selectivity, and fast stability for the determination of nitric oxide.

Acknowledgment This work was partially supported by the Research Grants Council of Hong Kong (HKBU 201804P). The authors thank Dr. Zhiqiang Gao (Institute of Microelectronics, 11 Science Park Road, Singapore 117685) for providing the PVP–Os–EA material.

References

- Palmer RMJ, Ashton DS, Moncada S (1988) *Nature* 333:664–666
- Malinski T (2002) In: Wilson GS (ed) *Encyclopedia of electrochemistry: bioelectrochemistry*. Wiley-VCH, Weinheim, pp 231–256
- Jia L, Bonaventura C, Bonaventura J, Stamler JS (1996) *Nature* 380:221–226
- Eich RF, Li T, Lemon DD, Doherty DH, Curry SR, Aitken JF, Mathews AJ, Johnson KA, Smith RD, Phillips GN Jr, Olson JS (1996) *Biochemistry* 35:6976–6983
- Aoki T (1990) *Biomed Chromatogr* 4:128–130
- Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G (1987) *Proc Natl Acad Sci USA* 84:9265–9269
- Arroyo CM, Kohno K (1991) *Free Radic Res Commun* 14:145–155
- Shibuki K (1990) *Neurosci Res* 9:69–76
- Malinski T, Taha Z (1992) *Nature* 358:676–678
- Younathan JN, Wood KS, Meyer TJ (1992) *Inorg Chem* 31:3280–3285
- Xian Y, Sun W, Xue J, Luo M, Jin L (1999) *Anal Chim Acta* 381:191–196
- Malinski T, Radomski MW, Taha Z, Moncada S (1993) *Biochem Biophys Res Commun* 194:960–965
- Pariante F, Alonso JL, Abruna HD (1994) *J Electroanal Chem* 379:191–197
- Maskus M, Pariante F, Wu Q, Toffanin A, Shapleigh JP, Abruna HD (1996) *Anal Chem* 68:3128–3134
- Chen J, Ikeda O (2001) *Electroanalysis* 13:1076–1081
- Roh SW, Stetter JR (2003) *J Electrochem Soc* 150:H266–H271
- Wang Y, Hu S (2009) *Bioelectrochemistry* 74:301–305
- Doherty AP, Vos JG (1993) *Anal Chem* 65:3424–3429
- Doherty AP, Stanley MA, Vos JG (1995) *Analyst* 120:2371–2376
- Heller A (1990) *Acc Chem Res* 23:128–134
- Danilowicz C, Cortón E, Battaglini F, Calvo EJ (1998) *Electrochim Acta* 43:3525–3531
- Forzani ES, Otero M, Perez MA, Teijelo ML, Calvo EJ (2002) *Langmuir* 18:4020–4029
- Willner I, Heleg-Shabtai V, Blonder R, Katz E, Tao G, Bückmann AF, Heller A (1996) *J Am Chem Soc* 118:10321–10322
- Gao ZQ, Binyamin G, Kim HH, Barton SC, Zhang YC, Heller A (2002) *Angew Chem Int Ed* 41:810–813
- Fei JJ, Wu YH, Ji XB, Wang J, Hu SS, Gao ZQ (2003) *Anal Sci* 19:1259–1263
- Fei JJ, Wu KB, Wang F, Hu SS (2005) *Talanta* 65:918–924
- Gregg BA, Heller A (1991) *J Phys Chem* 95:5970–5975
- Tsang SC, Chen YK, PHarris PJF, Green MLH (1994) *Nature* 372:159–162
- Ciszewski A, Milczarek G, Kubaszewski E, Lozynski M (1998) *Electroanalysis* 10:628–632
- Gerrard W (1980) *Gas solubilities widespread applications*. Pergamon, Oxford
- Murray RW (1984) In: Bard AJ (ed) *Electroanalytical chemistry*, vol. 13. Marcel Dekker, New York, pp 191–368
- Liu HH, Tian ZQ, Lu ZX, Zhang ZL, Zhang M, Pang DW (2004) *Biosens Bioelectron* 20:294–304
- Lu Q, Hu SS (2006) *Chem Phys Lett* 424:167–171
- Jia SS, Fei JJ, Deng JJ, Cai YL, Li JN (2009) *Sens Actuat B* 138:244–250
- Du FY, Huang WH, Shi YX, Wang ZL, Cheng JK (2008) *Biosens Bioelectron* 24:415–421
- Wu FH, Zhao GC, Wei XW (2002) *Electrochem Commun* 4:690–694
- Bindra DS, Wilson GS (1989) *Anal Chem* 61:2566–2570