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Highly sensitive electrochemical determination of nitric oxide using fused spherical gold nanoparticles modified ITO electrode

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ABSTRACT

This paper describes the highly sensitive electrochemical determination of nitric oxide (NO) using the fused spherical gold nanoparticles (FAuNPs) modified ITO electrode. The FAuNPs were self-assembled on a (3-mercaptopropyl)-trimethoxysilane (MPTS) sol-gel film, which was preassembled on ITO electrode. The attachment of FAuNPs on MPTS sol-gel film was confirmed by UV-vis absorption spectroscopy, atomic force microscopy (AFM) and cyclic voltammetry (CV). The AFM image shows that the AuNPs retain their fused morphology after immobilized on MPTS sol-gel film. The FAuNPs modified ITO electrode shows an excellent electrocatalytic activity towards the oxidation of NO. Using FAuNPs modified electrode, the detection of 12 nM NO was achieved for the first time by amperometry method. Further, the current response was increased linearly with increasing NO concentration in the range of 1.2×10^{-8} to 7×10^{-4} M and the detection limit was found to be 3.1×10^{-10} M (S/N=3). The FAuNPs modified ITO electrode displays an excellent selectivity towards the determination of 12 nM NO even in the presence of 1000-fold excess common interfering agents.

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1. Introduction

Huge research interest in the chemical and physiological functions of nitric oxide (NO) has been aroused since it was involved in the endothelium-derived relaxing factor (EDRF) in cardiovascular systems [1–5]. NO plays an important role in various important physiological processes, serving as a neurotransmitter [6], and an immune system mediator [7]. It can also act as a marker for tissue damage [8]. Further, NO regulates the blood pressure via its vasodilating activity and inhibits platelet aggregation, leukocyte adherence, and vascular muscle cell proliferation [1,2,9]. It is an active molecule with a short half-life time of ~6 s in physiological solution, and can be easily oxidized by O_2 to form NO_2^- or $NO_3^$ ions [10], which makes NO detection difficult. Thus, the accurate measurement of NO is very important to unravel the action of this key molecule.

Several modified electrodes have been reported for the determination of NO including porphyrin [11,12] and phthalocyanine [13], vinylterpyridine complexes [14], multi-walled carbon nanotubes [15] and gold nanoparticles (AuNPs) [16–18]. In recent years, AuNPs modified electrodes have received much attention owing to their fascinating electrocatalytic properties [19-25]. Among the different AuNPs modified electrodes for the determination of NO, the AuNPs modified on indium tin oxide (ITO) electrode has several advantages over carbon based electrodes [26,27]. Since ITO can be used in a wide potential window and possesses stable electrochemical property, it can be served as an excellent working electrode substrate for the fabrication of electrochemical sensors [28]. Only few reports are available in the literature related to the electrochemical determination of NO using AuNPs modified ITO electrodes [29-31]. For instance, Caruso and co-workers prepared polyelectrolyte (PE)/AuNPs hybrid films by incorporating 4-(dimethylamino) pyridine-stabilized AuNPs into PE multilayers preassembled on ITO electrode and used for the electrochemical determination of NO [29]. Zhang and Oyama reported that AuNP arrays directly grown on nanostructured ITO electrode can be used for the determination of NO [30]. While Marken and coworkers demonstrated that TiO₂ nanoparticles deposited with a Nafion polyelectrolyte showed the electrocatalytic activity towards NO [31]. However, the sensitivity obtained at these modified electrodes for NO is relatively poor. Thus, the main objective of the present investigation is to improve the sensitivity of NO detection by employing the fused gold nanoparticles (FAuNPs) modified ITO electrode. The FAuNPs modified ITO electrode showed excellent electrocatalytic activity towards NO and a detection limit of 12 nM NO was achieved by amperometry method.





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Scheme 1. Schematic representation for the immobilization of FAuNPs on MPTS sol-gel film modified ITO electrode.

2. Experimental

2.1. Chemicals

Hydrogen tetrachloroaurate trihydrate (HAuCl₄·3H₂O), (3-mercaptopropyl)-trimethoxysilane (MPTS) and 2-mercapto-4-methyl-5-thiazoleacetic acid (TAA) were purchased from Sigma–Aldrich and were used as received. Indium tin oxide (ITO) plates were purchased from Asahi Beer Optical Ltd., Japan. Sodium nitrite (NaNO₂) was purchased from Merck (India). 0.2 M phosphate buffer (PB) solution (pH 2) was prepared by using Na₂HPO₄–NaH₂PO₄ and phosphoric acid. All other chemicals were of analytical reagent grade and were used as received without any further purification. Double distilled water was used to prepare all the experimental solutions in the present work.

2.2. Synthesis of TAA-AuNPs

All glassware was thoroughly cleaned with freshly prepared aquaregia (3:1; HCl/HNO₃) and rinsed comprehensively with double distilled water prior to use. We have prepared the fused TAA–AuNPs based on our recent report [32]. TAA–AuNPs were prepared by adding 0.50 ml of HAuCl₄·3H₂O and 0.25 ml of TAA to 44.25 ml of water in a round bottom flask with constant stirring. To this solution, 5 ml of (0.125%) NaBH₄ was added and the stirring was continued for another 30 min. The color of the solution turns wine-red immediately after the final addition of NaBH₄, indicating the formation of AuNPs. The prepared AuNPs were kept at 4 °C and remained stable for several months. The UV–vis spectrum of colloidal TAA–AuNPs showed an absorption band at 508 nm and the HR-TEM images of TAA–AuNPs showed that most of the nanoparticles were in fused spherical shape with a size range of ~7 nm diameters (Fig. S1; supporting information).

2.3. Preparation of MPTS sol-gel and FAuNPs/MPTS/ITO electrode

The MPTS sol-gel was prepared by dissolving MPTS, methanol, and water (as 0.1 M HCl) in a molar ratio of 1:3:3 and stirring the mixture vigorously for 30 min [33]. The ITO electrodes (exposed geometry area 0.24 cm²) were sonicated for 10 min in each of the following solvents: water, acetone, ethanol in an ultrasonic bath. Then, the electrodes were rinsed with double distilled water and dried with a stream of nitrogen gas. To obtain the –SH functionalized ITO surfaces, the pretreated ITO electrode was immersed into MPTS for 20 min. Then the MPTS/ITO electrode was washed with methanol followed by water to remove the physically adsorbed

silane molecules on the ITO electrode surface. Finally, the ITO/MPTS modified ITO electrode was immersed in a colloidal solution of FAuNPs (TAA–AuNPs) for 6 h (Scheme 1). The FAuNPs were strongly bonded with –SH functional groups of MPTS sol–gel film. This electrode was referred as ITO/MPTS/FAuNPs electrode.

2.4. Instrumentation

UV–vis spectra were recorded with a Perkin-Elmer Lambda 35 Spectrophotometer. The atomic force microscopy (AFM) image of FAuNPs was obtained from digital instrument, Nanoscope IV, Veeco. The electrochemical measurements were carried out with CHI electrochemical workstation Model 643B, Austin, TX, USA. The ITO electrode with an exposed geometric area of ca. 0.24 cm² was employed as a working electrode; a platinum wire electrode as counter electrode and an Ag/AgCl (saturated NaCl) electrode as a reference electrode. All the electrochemical measurements were performed at room temperature in the presence of nitrogen gas atmosphere.

3. Results and discussion

3.1. Spectral and AFM characterization of FAuNPs modified ITO substrate

The FAuNPs on MPTS sol-gel film modified ITO electrode was characterized by UV-vis absorption spectroscopy and AFM. Fig. 1 shows the UV-vis spectra obtained for bare ITO, ITO/MPTS and ITO/MPTS/FAuNPs modified plates. No absorption band was observed for both bare ITO (curve a) and ITO/MPTS (curve b) plates in the region of 400–1000 nm. On the other hand, ITO/MPTS/FAuNPs plate showed an absorption band at 550 nm, corresponding to the surface plasmon resonance (SPR) band of AuNPs (curve c). The appearance of a SPR band at 550 nm for FAuNPs on MPTS modified ITO plate confirmed that they were successfully immobilized on the MPTS sol-gel film. The thiol group of MPTS has a strong affinity to FAuNPs and is able to chemisorb on the surface of AuNPs through the cleavage of the S-H bond. Since the thiol groups are distributed throughout the MPTS sol-gel film, it is likely that the FAuNPs were self-assembled both inside and on the surface of the MPTS sol-gel film. The observed 40 nm red shift of SPR band in contrast to colloidal solution is likely due to the electromagnetic interaction between the assembled FAuNPs and the ITO substrate [34]. Further, the size and surface morphology of the FAuNPs on the MPTS sol-gel film ITO plate were examined by AFM. Fig. 2A shows the AFM image obtained for the FAuNPs on MPTS modified





Fig. 1. UV-vis spectra obtained for (a) bare ITO, (b) ITO/MPTS and (c) FAuNPs on MPTS modified ITO plates.

ITO plate. It showed that most of the nanoparticles are fused spherical in shape. Further, the nanoparticles are uniformly distributed throughout the MPTS sol-gel film. The three-dimensional view of AFM image clearly showed that the immobilized AuNPs were fused spherical shape and were not aggregated (Fig. 2B). These results indicated that the TAA capped AuNPs retain their fused spherical morphology on MPTS sol-gel film modified ITO surface.

3.2. Electrochemical characterization of FAuNPs modified ITO electrode

The attachment of AuNPs on ITO electrode was also confirmed by cyclic voltammetry (CV). CVs obtained for a bare ITO, ITO/MPTS and ITO/MPTS/FAuNPs modified electrodes in 0.2 M PB solution (pH 2) are shown in Fig. 3. No electrochemical response was observed for both the bare and MPTS sol–gel film modified ITO electrodes in the potential region from -0.20 to 1.5 V (curves a and b). On the

Fig. 3. CVs obtained for (a) bare ITO, (b) ITO/MPTS and (c) ITO/MPTS/FAuNPs modified electrodes in 0.2 M PB solution (pH 2) at a scan rate of 50 mV s⁻¹.

other hand, the FAuNPs modified ITO electrode shows an oxidation peak at 1.15 V and a corresponding reduction peak at 0.1 V (curve c). The redox peaks are attributed to the oxidation and subsequent reduction of surface gold oxide (AuO_x) formation of the FAuNPs, as a result of the positive potential polarization of the FAuNPs modified ITO electrode. The amount of Au oxide exposed to the solution was calculated by integrating the charge under the Au oxide reduction peak and it was found to be $63.84 \,\mu$ C without subtracting the roughness factor of 1.95 [35]. We have calculated the particle coverage (θ_n) of FAuNPs on the MPTS sol-gel film modified ITO electrode using Eq. (1). The particle coverage is defined as the ratio between the electrochemically accessible AuNPs area and the geometric area of the ITO electrode in contact with the electrolyte solution [36]. The particle coverage of FAuNPs modified ITO electrode was calculated by using the charge involved in the reduction of electrochemically formed Au oxide from the CV recorded, between the potential window from 0.5 to -0.2 V in 0.2 M PB solution, by assuming the charge



Fig. 2. AFM images obtained for FAuNPs on MPTS sol-gel film modified ITO plate (A) 2D and (B) 3D views.



Fig. 4. CVs obtained for (a) bare ITO, (b) ITO/MPTS and (c) ITO/MPTS/FAuNPs modified electrodes in 0.2 M PB solution (pH 7.2) containing 1 mM K_3 [Fe(CN)₆] at a scan rate of 50 mV s⁻¹.

density for the reduction peak of the Au oxide is $723 \,\mu\text{C/cm}^2$ [36].

$$\theta_{\rm p} = \frac{\text{Au oxide reduction charge } (\mu C)/723 \ (\mu C/cm^2)}{\text{ITO geometric area } (cm^2)} \times 100 \quad (1)$$

The particle coverage of FAuNPs on MPTS modified ITO electrode was found to be 36.79%. Previously, the particle coverage of 25–30% was reported for citrate-stabilized gold nanoparticles (C-AuNPs) immobilized on amine functionalized ITO substrate [36] while 10% particle coverage was reported for C-AuNPs immobilized on a monolayer of 1-mercapto-11-aminoundecane on a polycrystalline Au electrode surface [37]. In the present case, the FAuNPs showed relatively higher particle coverage on the MPTS modified ITO electrode when compared to reported papers [35–37].

Further, the $[Fe(CN_6)]^{3-/4-}$ redox probe was used to access the electronic communication between the immobilized FAuNPs and the electrode [38]. Fig. 4 shows the CVs of bare ITO, ITO/MPTS and ITO/MPTS/FAuNPs modified electrodes in 0.2 M PB solution (pH 7.2) containing 1 mM K₃[Fe(CN)₆]. The bare ITO electrode shows a redox response for $[Fe(CN)_6]^{3-/4-}$ with a peak separation of 330 mV indicating the characteristics of a diffusion-controlled redox process (curve a), whereas its redox reaction was blocked completely at the ITO/MPTS electrode, indicating that MPTS forms a relatively compact three-dimensional monolayer on ITO electrode (curve b). However, after the attachment of FAuNPs on the MPTS sol-gel film, not only the peak currents were increased but also the peak separation (220 mV) was decreased (curve c) when compared to bare ITO electrode [39]. This reveals that the FAuNPs electrode improved the electron transfer between $[Fe(CN_6)]^{3-/4-}$ and the electrode surface. Here, the FAuNPs can act as "electron antennae" and effectively facilitating the electron transfer on the MPTS sol-gel film. These results once again confirmed the successful immobilization of FAuNPs on the MPTS sol-gel film.

The electrochemical impedance spectrum (EIS) is an efficient tool for studying the interface properties of surface modified electrodes. The electron-transfer resistance (R_{et}), the semicircle diameter at the higher frequencies in the Nyquist plot of impedance spectroscopy, controls the interfacial electron-transfer rate of the redox probe between the solution and the electrode. Therefore,

 $R_{\rm et}$ can be used to describe the interface properties of the electrode [40]. The EIS measurements in this work were carried out in a background solution of 0.2 M PB solution (pH 7.2) containing $1 \text{ mM } K_3[Fe(CN)_6]$ as a redox probe using a frequency range between 0.01 Hz and 100 kHz. The EIS-Nyquist plots obtained for bare ITO, ITO/MPTS and ITO/MPTMS/FAuNPs modified electrodes are shown in Fig. 5. The EIS includes a semicircle portion at higher frequencies, corresponding to the electron-transfer (R_{et}) limiting process, and a linear part at lower frequencies, resulting from the diffusion limiting step of the electrochemical process. It is observed that a semicircle of about 2750Ω (curve (a)) diameter with an almost straight tail line is observed for the bare ITO, demonstrating low-electron-transfer resistance to the redox probe dissolved in the electrolyte solution. It also can be seen that a much greater semicircle diameter appeared for MPTS sol-gel film ($R_{et} = 42,000 \Omega$), modified ITO electrode (inset, Fig. 5), suggesting that the resistance of the MPTS sol-gel film was increased significantly when compared to bare ITO electrode. The self-assembly of MPTS sol-gel film on the electrode surface generated an insulating layer on the electrode that functioned as a barrier to the interfacial electron transfer. Interestingly, the Ret value of FAuNPs modified ITO electrode enormously decreased to about 600Ω (curve (b)) after the immobilization of FAuNPs on the MPTS sol-gel film. The decrease of Ret at FAuNPs modified ITO electrode is due to the homogeneous distribution of the FAuNPs within the MPTS sol-gel film and thus accelerated the electron transfer between the $[Fe(CN)_6]^{3-/4-}$ and ITO electrode. The results extracted from EIS measurements connected with each step of the electrode modification are in good agreement with the results obtained from CVs (Fig. 4). It has been shown that if electron tunneling across an insulating barrier is implicated in the electron-transfer process, the redox current at any potential should decrease exponentially with the barrier thickness according to the following expression [41]:

$$I = I_0 e^{-\beta d} \tag{2}$$

where I_0 is the current measured at the bare electrode, β is the potential independent electron tunneling coefficient, and *d* is the



Fig. 5. EIS–Nyquist plots obtained for bare ITO (curve a), ITO/MPTS (inset) and ITO/MPTS/FAuNPs (curve b) modified electrodes in 0.2 M PB solution (pH 7.2) containing 1 mM K_3 [Fe(CN)₆]. The frequency range for impedance measurements was from 0.01 to 100,000 Hz.

thickness of the monolayer. For an electrochemical reaction at equilibrium, Eq. (3) can be obtained using Eq. (2):

$$k = k_0 e^{-\beta d} \tag{3}$$

where k_0 and k are the electron-transfer rate constants at the bare and ITO/MPTS modified electrodes. Heterogeneous electron-transfer rate constant (k_{et}) at the bare and modified electrodes can be calculated using Eq. (4) [42]:

$$k_{\rm et} = \frac{KI}{n^2 F^2 A R_{\rm CT} C_0} \tag{4}$$

where *R* is the gas constant, *T* is temperature (K), *F* is the Faraday constant, *A* is the electrode area (cm²), *R*_{CT} is the charge transfer resistance, *C*₀ is the concentration of the redox couple in the bulk of solution $(1 \times 10^{-3} \text{ mol/cm}^3)$, and *n* is the number of transferred electrons per molecule of the redox probe (*n*) one in the case of [Fe(CN)₆]^{3-/4-} redox couple. The apparent charge transfer resistance values were obtained from fittings of the EIS spectra. The heterogeneous electron-transfer rate constant, *k*_{et}, estimated using Eq. (4) was found to be $4.0323 \times 10^{-7} \text{ cm s}^{-1}$ for bare ITO electrode, $2.6402 \times 10^{-8} \text{ cm s}^{-1}$ for MPTS sol–gel film modified ITO electrode and $1.8481 \times 10^{-6} \text{ cm s}^{-1}$ for FAuNPs modified electrode indicated that the fastest electron transfer takes place at the FAuNPs modified electrode.

3.3. Electrocatalytic oxidation of NO at FAuNPs modified electrode

The prime objective of the present study is to utilize the FAuNPs modified ITO electrode for the determination of NO. Sodium nitrite (NaNO₂) was used as a precursor to produce NO in acidic solution. It has been well established that NaNO₂ can generate free NO by the disproportionation reaction in acidic solution (pH \leq 4) (Eqs. (5) and (6)) [43]. Addition of a known amount of NaNO₂ into the bulk electrolyte solution at pH \leq 4 generates a series of concentrations of NO.

$$2NaNO_2 + H_2SO_4 \rightarrow 2HONO + Na_2SO_4$$
(5)

$$3HONO \rightarrow H^+ + NO_3^- + 2NO + H_2O$$
 (6)

Fig. 6A shows the CVs obtained for bare ITO, ITO/MPTS and ITO/MPTS/FAuNPs modified electrodes in 0.2 M PB solution (pH 2) containing 0.5 mM NO. The bare ITO electrode does not show any oxidation wave for NO (curve a, dotted line), Similar response was also observed at ITO/MPTS modified electrode (curve b) due to the formation of a compact MPTS sol-gel film on ITO electrode. On the other hand, FAuNPs modified electrode showed the oxidation peak with higher oxidation current for NO at 0.87 V (curve c). When compared to bare and MPTS sol-gel modified ITO electrodes (curves a and b) and the NO oxidation current was 15 times higher at FAuNPs modified electrode (curve c). The FAuNPs modified ITO electrode does not show any oxidation response in the absence of NO (curve d, dashed line). These results indicated that the presence of nanostructured FAuNPs modified ITO electrode in the MPTS sol-gel film mediates the electrochemical oxidation of NO. Since the FAuNPs attached on the MPTS sol-gel film are in good electrical communication with each other, an efficient electron-transfer process is observed at the FAuNPs modified ITO electrode in the absence of any other mediator in the film. The fused gold nanostructures provide a large surface area with specific interaction towards the substrate, resulting an improved electron-transfer kinetics and higher enhancement in the oxidation of NO. Further, the oxidation peak of NO at FAuNPs modified ITO electrode was found to be highly stable. Fig. 6B shows the CVs recorded for the oxidation of NO after every three continuous potential cycles. It can be seen from Fig. 6B, the oxidation potential of NO remained stable even



Fig. 6. (A) CVs obtained for 0.50 mM NO at (a) bare ITO, (b) ITO/MPTS and (c) ITO/MPTS/FAuNPs modified electrodes in 0.2 M PB solution (pH 2). CV obtained at ITO/MPTS/FAuNPs modified electrode in the absence of NO (d) in 0.2 M PB solution (pH 2). Scan rate = 50 mV s⁻¹. (B) CVs obtained for 0.50 mM NO at ITO/MPTS/FAuNPs modified electrode in 0.2 M PB solution (pH 2) after 1st (solid line), 5th (dotted line) and 9th (dashed line) continuous potential cycles at a scan rate of 50 mV s⁻¹.

after nine repeated potential cycles indicating that the oxidation of NO was highly stable at FAuNPs modified ITO electrode.

3.4. Effect of scan rate

Fig. 7 shows the effect of scan rate on the electrochemical oxidation of NO at ITO/MPTS/FAuNPs modified electrode in 0.2 M PB solution (pH 2) containing 0.5 mM NO. The oxidation peak currents of NO increased linearly with the scan rates in the range of 5–100 mV/s with a correlation coefficient of 0.9984 (inset, Fig. 7). This suggests that the oxidation of NO at FAuNPs modified ITO electrode is diffusion-controlled process. We presumed that the oxidation mechanism of NO oxidation at FAuNPs modified ITO electrode as the NO in solution first loses an electron to form NO⁺ at the FAuNPs modified electrode. The formed NO⁺ can then be further oxidized to form other more stable nitrogen products [44].

3.5. Amperometric determination of NO

Amperometric method was used to examine the sensitivity of FAuNPs modified ITO electrode towards the detection of NO in acidic solution (pH 2). Fig. 8A shows the amperometric i-t curve obtained for NO at FAuNPs modified ITO electrode in a homoge-



Fig. 7. CVs obtained for 0.50 mM NO at ITO/MPTS/FAuNPs modified electrode in 0.2 M PB solution (pH 2) at different scan rates: (a) 5 mV s^{-1} ; (b) 25 mV s^{-1} ; (c) 50 mV s^{-1} ; (d) 75 mV s^{-1} and (e) 100 mV s^{-1} . Inset shows the square root of the scan rate vs. NO peak current.

neously stirred 0.2 M PB solution (pH 2) at an applied potential of 1.0 V. The FAuNPs modified ITO electrode shows the initial current response due to 12 nM NO and further addition of 12 nM NO in each step with a sample interval of 50 s, the current response increases and a steady state current response was attained within 2 s. The amperometric current response was increased linearly with increasing NO concentration on the addition of 12 nM NO in each step (inset, Fig. 8A) with a correlation coefficient of 0.9989. We have estimated 30 nA for 100 nM NO at FAuNPs modified ITO electrode. Further, the amperometric current response was increased linearly with increasing NO concentration in the range of 1.2×10^{-8} to 7×10^{-4} M with a correlation coefficient of 0.9986 and the detection limit was found to be 3.1×10^{-10} M (S/N=3). The dynamic range and the lowest detection limit of the FAuNPs modified ITO electrode towards NO oxidation were compared with the recently reported ITO and other modified electrodes [15-18,29,30,45,46] and are given in Table 1. As can be seen from Table 1, the present modified electrode showed the lowest detection limit for the NO $(3.1 \times 10^{-10} \text{ M})$. We have also studied the determination of NO in the presence of common physiological interferents such as ascorbic acid, uric acid, urea and cysteine and common metal ions such as Na⁺, K⁺, Mg²⁺ and Cu²⁺. Fig. 8B shows the amperometric i-t curve obtained for NO at FAuNPs modified ITO electrode in the presence of several interferences in a homogeneously stirred 0.2 M PB solution. The increased initial current response was due to the addition of 12 nM NO (a) and further addition of 12 nM NO in each step with a sample interval of 50 s, the current response increases and a steady



Fig. 8. (A) Amperometric *i*-*t* curve for the determination of NO at FAuNPs modified ITO electrode in a homogeneously stirred solution of 0.2 M PB solution (pH 2). Each addition increases the concentration of 12 nM of NO, $E_{app} = 1.0$ V. (B) Amperometric *i*-*t* curve for the determination of (a–d) 12 nM NO in the presence of various interferents such as 12 μ M each (e) ascorbic acid, (f) uric acid, (g) dopamine, (h) DL-cysteine, (i–l) 12 nM NO, (m) Na⁺, (n) K⁺, (o) Mg²⁺, (p) Ni²⁺ and (q–t) 12 nM NO at FAuNPs modified ITO electrode in a homogeneously stirred 0.2 M PB solution (pH 2). $E_{app} = 1.0$ V.

state current response was attained within 2 s (b–d). After four steps, the addition of 12 μ M of each ascorbic acid (e), uric acid (f), urea (g) and cysteine (h), separately with a sample interval of 50 s to the same solution no change in current response was observed. However, addition of 12 nM NO to the same 0.2 M PB solution (pH 2) solution, the current response was again increased (i–l). After four steps, the addition of 12 μ M Na⁺ (m), K⁺ (n), Mg²⁺ (o) and Cu²⁺ (p), separately with a sample interval of 50 s to the same solution caused no change in current response. While 12 nM of NO was added to the same 0.2 M PB solution (pH 2) solution, an increase in current response was observed and it is similar to the current

Table 1

Linear range and detection limit of different modified electrodes for the determination of NO.

Electrode	Linear range	Detection limit	Reference
Multi-walled carbon nanotubes modified electrode	2×10^{-7} to $1.5\times 10^{-4}M$	$8.0\times 10^{-8}\ M$	15
Mono-/bi-layer Au nanoparticle films modified electrode	5×10^{-8} to $1\times 10^{-5}~M$	$2.7 imes 10^{-8} \text{ M}$	16
Nafion polymer/gold nanostructures film modified GC electrode	5×10^{-5} to $5\times 10^{-4}~M$	$1 imes 10^{-9} \ M$	17
Electrodeposition of Pt-Fe(III) nanoparticle on glassy carbon electrode	8.4×10^{-8} to $7.8\times10^{-4}M$	$1.8 imes 10^{-8}$ M	18
Polyelectrolyte(PE)/gold nanoparticle hybrid films modified electrode	5×10^{-5} to $5\times 10^{-4}~M$	$1 \times 10^{-5} \ M$	29
Gold nanoparticle arrays directly grown on nanostructured indium tin oxide electrodes	1×10^{-6} to $5\times 10^{-4}~M$	$6.5 imes 10^{-7} \text{ M}$	30
Carbon fiber covered with nickel porphyrin layer	-	$23\times10^{-9}M$	45
Nano-Au colloid and Nafion film modified Pt electrode as a microsensor	$1 imes 10^{-7}$ to $4 imes 10^{-5}$ M	$5.0 imes 10^{-8}$ M	46
Fused gold nanoparticles modified electrode	1.2×10^{-8} to $7\times10^{-4}M$	$3.1 imes 10^{-10} \ \text{M}$	This work
	Electrode Multi-walled carbon nanotubes modified electrode Mono-/bi-layer Au nanoparticle films modified electrode Nafion polymer/gold nanostructures film modified GC electrode Electrodeposition of Pt–Fe(III) nanoparticle on glassy carbon electrode Polyelectrolyte(PE)/gold nanoparticle hybrid films modified electrode Gold nanoparticle arrays directly grown on nanostructured indium tin oxide electrodes Carbon fiber covered with nickel porphyrin layer Nano-Au colloid and Nafion film modified Pt electrode as a microsensor Fused gold nanoparticles modified electrode	ElectrodeLinear rangeMulti-walled carbon nanotubes modified electrode 2×10^{-7} to 1.5×10^{-4} MMono-/bi-layer Au nanoparticle films modified electrode 5×10^{-8} to 1×10^{-5} MNafion polymer/gold nanostructures film modified GC electrode 5×10^{-5} to 5×10^{-4} MElectrodeposition of Pt-Fe(III) nanoparticle on glassy carbon electrode 8.4×10^{-8} to 7.8×10^{-4} MPolyelectrolyte(PE)/gold nanoparticle hybrid films modified electrode 5×10^{-5} to 5×10^{-4} MGold nanoparticle arrays directly grown on nanostructured indium tin oxide electrodes 1×10^{-6} to 5×10^{-4} MCarbon fiber covered with nickel porphyrin layer-Nano-Au colloid and Nafion film modified Pt electrode as a microsensor 1×10^{-7} to 4×10^{-5} MFused gold nanoparticles modified electrode 1.2×10^{-8} to 7×10^{-4} M	ElectrodeLinear rangeDetection limitMulti-walled carbon nanotubes modified electrode 2×10^{-7} to 1.5×10^{-4} M 8.0×10^{-8} MMono-/bi-layer Au nanoparticle films modified electrode 5×10^{-8} to 1×10^{-5} M 2.7×10^{-8} MNafion polymer/gold nanostructures film modified GC electrode 5×10^{-8} to 5×10^{-4} M 1×10^{-9} MElectrodeposition of Pt-Fe(III) nanoparticle on glassy carbon electrode 8.4×10^{-8} to 7.8×10^{-4} M 1.8×10^{-8} MPolyelectrolyte(PE)/gold nanoparticle hybrid films modified electrode 5×10^{-5} to 5×10^{-4} M 1.8×10^{-8} MGold nanoparticle arrays directly grown on nanostructured indium tin oxide electrodes 1×10^{-6} to 5×10^{-4} M 6.5×10^{-7} MCarbon fiber covered with nickel porphyrin layer $ 2-3 \times 10^{-9}$ MNano-Au colloid and Nafion film modified Pt electrode as a microsensor 1×10^{-7} to 4×10^{-5} M 5.0×10^{-8} MFused gold nanoparticles modified electrode 1.2×10^{-8} to 7×10^{-4} M 3.1×10^{-10} M

response observed in the earlier steps (q-t). Further, we have studied the effect of NO_2^- as an interferent for the determination of NO. It was found that no interference effect was noted even in the presence of $12 \mu M NO_2^{-}$. It is likely that the negatively charged TAA molecules present on the surface of the AuNPs repel the negatively charged NO₂⁻ ions while allow the NO molecules. This may be the possible reason for the observed selective determination of NO at FAuNPs modified electrode in the presence of NO₂⁻ ions. The above results indicate that the determination of 12 nM NO is possible even in the presence of 1000-fold excess of common interferences.

3.6. The stability and reproducibility of FAuNPs electrode

In order to investigate the stability of the FAuNPs modified ITO electrode, the CVs for 0.5 mM NO in 0.2 M PB solution (pH 2) were recorded for every 3 min interval. It was found that the oxidation current of NO remained same with a relative standard deviation of 1.8% for 15 times repetitive measurements indicating that this electrode has a good reproducibility. After voltammetric measurements, this electrode was kept in pH 2 PB solution at room temperature. The current response decreased to about 2.3% in 1 week and 6.1% in about 2 weeks. The above results showed that the present modified electrode was very much stable and reproducible towards the electrochemical oxidation of NO. The MPTS sol-gel film coating helped to increase the stability and extend the lifetime of this sensor. The above results suggested that FAuNPs modified ITO electrode can be effectively utilized as a new kind of electrode material for the determination of NO in 0.2 M PB solution (pH 2).

4. Conclusions

We have demonstrated the highly sensitive electrochemical determination of NO using FAuNPs modified ITO electrode. The attachment of FAuNPs on MPTS sol-gel film was confirmed by UV-vis absorption spectroscopy, atomic force microscopy and CV measurements. The AFM image clearly showed that the TAA capped AuNPs retained their fused morphology on MPTS sol-gel film. The FAuNPs showed an excellent electrocatalytic activity towards the oxidation of NO. We have achieved the lowest detection of 12 nM NO for the first time at FAuNPs on MPTS sol-gel film modified ITO electrode by amperometric method. The current response was increased linearly with increasing NO concentration in the range of 1.2×10^{-8} to $7\times 10^{-4}\,\text{M}$ and the detection limit was found to be 3.1×10^{-10} M (S/N = 3). Further, the FAuNPs modified ITO electrode displayed an excellent selectivity towards the determination of 12 nM NO even in the presence of 1000-fold excess common interfering agents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.electacta.2010.01.084.

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