

Electrocatalytic oxidation of NADH at gold nanoparticles loaded poly(3,4-ethylenedioxythiophene)–poly(styrene sulfonic acid) film modified electrode and integration of alcohol dehydrogenase for alcohol sensing

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Abstract

A new modified electrode is fabricated by dispersing gold nanoparticles onto the matrix of poly(3,4-ethylenedioxythiophene)–poly(styrene sulfonic acid), PEDOT–PSS. The electrocatalytic activity of the PEDOT–PSS–Au_{nano} electrode towards the oxidation of β -nicotinamide adenine dinucleotide (NADH) is investigated. A substantial decrease in the overpotential (>0.7 V) has been observed for the oxidation of NADH at the PEDOT–PSS–Au_{nano} electrode in comparison to the potential at PEDOT–PSS electrode. The Au nanoparticles dispersed in the PEDOT–PSS matrix prevents the fouling of electrode surface by the oxidation products of NADH and augments the oxidation of NADH at a less positive potential ($+0.04$ V vs. SCE). The electrode shows high sensitivity to the electrocatalytic oxidation of NADH. Further, the presence of ascorbic acid and uric acid does not interfere during the detection of NADH. Important practical advantages such as stability of the electrode (retains $\sim 95\%$ of its original activity after 20 days), reproducibility of the measurements (R.S.D.: 2.8%; $n = 5$), selectivity and wide linear dynamic range (1–80 μ M; $R^2 = 0.996$) are achieved at PEDOT–PSS–Au_{nano} electrode. The ability of PEDOT–PSS–Au_{nano} electrode to promote the electron transfer between NADH and the electrode makes us to fabricate a biocompatible dehydrogenase-based biosensor for the measurement of ethanol. The biosensor showed high sensitivity to ethanol with rapid detection, good reproducibility and excellent stability.

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1. Introduction

The electrochemical detection of β -nicotinamide adenine dinucleotide (NADH) is of considerable interest since a number of dehydrogenases require NADH as a cofactor for the enzymatic reaction. NADH is the terminal electron donor moiety in the mitochondrial electron transport chain [1]. However, direct electrochemical oxidation of NADH at a bare platinum electrode requires a high (>1 V) overpotential. The high overpotential for the oxidation of NADH results in interferences from easily oxidizable species present in the real samples. The overpotential for

NADH oxidation could be reduced by using a mediator. Also, electrode fouling is one of the main concerns as radical intermediates are generated during the one-electron oxidation of NADH. The subsequent polymerization products also foul the electrode [2]. Hence, considerable efforts have been devoted to modify the electrode surface with an adequate material to diminish the overpotential for the oxidation of NADH and to minimize the surface passivation effects.

Various methodologies have been adopted to immobilize the mediator on the surface of electrode [3–6]. Different types of modification were performed on the surface of the electrode to maintain satisfactory operational time for the mediator in the electrode [7]. The electrodes modified with carbon nanotubes were used for the oxidation of NADH [8–13]. For instance, a hybrid thin film from multi-wall carbon nanotubes dispersed in nafion with electrochemically generated redox mediator was

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developed for the sensitive detection of NADH [14]. However, the stability of the modifications limits the reproducibility and the operational lifetime of such modified electrodes. On the other hand, modified electrodes were also fabricated with CdS nanoparticles [15], nanostructured TiO₂ [16], boron-doped diamond [17], exfoliated graphite [18], single crystal gold [19] and conducting polymer nanotubules [20] for the electrochemical detection of NADH. However, the selective electrochemical detection of NADH is still a challenging task.

Gold nanoparticles display unparalleled catalytic activity for a number of reactions [21–24] that include oxidation of carbon monoxide [25]. Au nanoparticles have also been widely used to construct biosensors due to their excellent ability to immobilize biomolecules and at the same time retain the biocatalytic activities of those biomolecules. Recently, electrodes based on Au nanoparticles self-assembled on a thiol-terminated, sol–gel-derived silicate network from 3-(mercaptopropyl) trimethoxysilane were developed for the selective and sensitive detection of NADH [26], glucose [27] and L-lactate [28]. Many kinds of biosensors, such as enzyme sensor [29–31], immunosensor [32] and DNA sensor [33] were developed based on Au nanoparticles to obtain better analytical performances.

Poly(3,4-ethylenedioxythiophene), PEDOT doped with an excess of poly(styrene sulfonic acid) (PSS) has been attracting interest due to its film forming properties, high stability in water as well as in air and high conductivity. PSS plays the dual role of charge balancing and stabilizing the aqueous dispersion. The matrix of PEDOT–PSS is highly porous that facilitates the electrochemical redox reactions [34]. Further, the solubility of PEDOT–PSS in organic solvent is adequate enough to form thin films by many kinds of conventional techniques, such as solvent-casting, spin-casting and dip-coating methods. The presence of negatively charged electrolyte ions in PEDOT–PSS provides good redox activity even in neutral medium. This is attributed to the effective doping (protonation) of PEDOT by the trapped polyelectrolyte ions over a broad range of pHs.

In the present investigation, an electrocatalytic electrode was fabricated by dispersing Au nanoparticles onto PEDOT–PSS coated indium-doped tin oxide glass (ITO) plate and used for the detection of NADH. The PEDOT–PSS–Au_{nano} electrode exhibited excellent electrocatalytic activity towards the oxidation of NADH at a low potential in the phosphate buffer, PBS (pH 7.2). Further, an ethanol biosensor was developed by immobilizing alcohol dehydrogenase enzyme into the PEDOT–PSS–Au_{nano} matrix. The results are presented herein and discussed.

2. Experimental

2.1. Chemicals

PEDOT–PSS was obtained from Sigma–Aldrich (1.3 wt.% suspension in water; a composition of 0.5 wt.% PEDOT and 0.8 wt.% PSS). Sulfuric acid, auric acid, ascorbic acid and uric acid of analytical grade from Aldrich were used as received. Baker's yeast alcohol dehydrogenase (alcohol: NAD⁺ oxidoreductase, E. C. 1.1.1.1) (ADH), β -nicotinamide adenine

dinucleotide (NADH) and β -nicotinamide adenine dinucleotide (reduced form, NAD⁺) were obtained from Sigma chemicals. Double-distilled water was used throughout the experiments. Aqueous solutions of NADH were prepared in PBS (pH 7.2), afresh at the time of experiments.

2.2. Fabrication of PEDOT–PSS–Au_{nano} electrode

PEDOT–PSS matrix electrode was prepared by spin coating (2000 rpm for 1 min) on ITO substrate using SPIN-1200, MIDAS spin coater system. A very thin film of PEDOT–PSS was casted over a cleaned ITO electrode (1.0 sq. cm). Conductivity of the PEDOT–PSS coated ITO was found to be 5 mS cm^{−1} and the average particle size was $\sim$ 200 nm. Before each experiment, ITO coated glass was cleaned in an ultrasonic bath using double distilled water and acetone, then dried with a dry nitrogen flow.

Au particles were electrochemically deposited onto spin casted PEDOT–PSS electrode from 0.5 M H₂SO₄ solution of HAuCl₄ (2.0×10^{-4} M) by employing a repetitive potential scan from 1.1 to 0.0 V (vs. SCE) at a scan rate of 50 mV s^{−1} and thus, the PEDOT–PSS–Au_{nano} modified electrode was fabricated. Before subjecting to electrochemical experiments, the PEDOT–PSS–Au_{nano} modified electrode was washed extensively with distilled water.

2.3. Electrochemical measurements

Electrochemical measurements were done with EG & G PAR Electrochemical Analyzer in a standard 10 mL cell containing the modified electrode, a SCE reference electrode and a Pt wire auxiliary electrode. Prior to all electrochemical measurements, the solutions were purged with nitrogen for 10 min. The amperometric response of PEDOT–PSS–Au_{nano} electrode was recorded under steady-state conditions in the PBS (pH 7.2) by applying a constant potential (+0.04 V) to the working electrode. The background response at PEDOT–PSS–Au_{nano} electrode was allowed to decay to a steady-state under stirring. When the background current became stable, the aliquot amount of the analyte was injected into the electrolytic cell, and its response was measured. In the case of square wave voltammetry, a 10 mL aliquot of buffer solution with NADH of a definite concentration was placed in the voltammetric cell and the solution was purged with nitrogen for 10 min. Then, a potential scan was initiated at a scan rate of 10 mV s^{−1} and the resulting voltammogram was recorded. Optimum conditions (pulse amplitude, frequency, step potential and quiet time) were established by measuring the peak currents in dependence on all parameters. The electrochemical sensing of ethanol was carried out in PBS (pH 7.2) in the presence of NAD⁺ (0.2 mM) with ADH (7 U mL^{−1}) using the PEDOT–PSS–Au_{nano} electrode.

2.4. Characterization

The surface topography of PEDOT–PSS–Au_{nano} electrode was examined using atomic force microscopy, AFM (Digital Instruments; Nanoscope Multimode) in the tapping mode with silicon nitride tip (tip height: 4 μ m and the tip radius is of 15 nm).

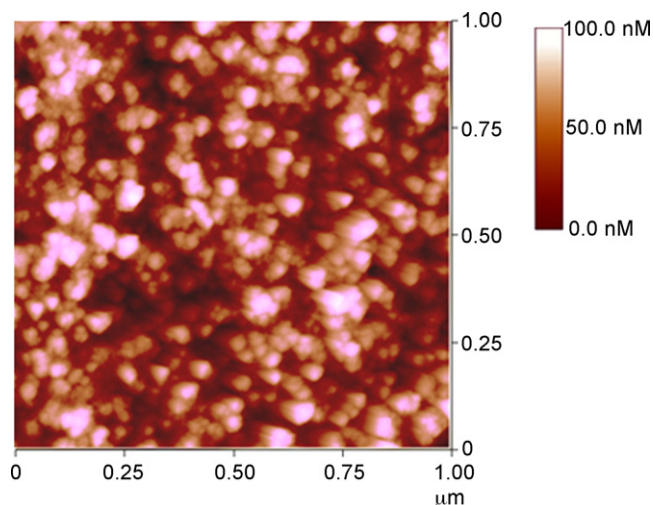


Fig. 1. AFM image of Au nanoparticles deposited on PEDOT-PSS spin coated ITO glass plate.

X-ray diffraction pattern of the sample was collected by employing a D8-Advanced Bruker AXS diffractometer using Cu K α radiation.

3. Results and discussion

3.1. Fabrication and morphology of PEDOT-PSS-Au_{nano} modified electrode

The PEDOT-PSS-Au_{nano} modified electrode was fabricated by two steps. Firstly, film of PEDOT-PSS was formed on the surface of ITO glass plate through spin coating. Subsequently, Au nanoparticles were deposited by the reduction of H₂AuCl₄ from an electrolyte solution consisting of H₂AuCl₄. In the typical fabrication of modified electrode, Au nanoparticles were deposited onto PEDOT-PSS coated ITO electrode from 0.5 M H₂SO₄ solution containing H₂AuCl₄ (2.0×10^{-4} M) by applying a repetitive potential scan from 1.1 to 0.0 V (vs. SCE) at a scan rate of 50 mV s⁻¹ as described in the literature [25]. Cyclic voltammogram (CV) shows two cathodic peaks at ~ 0.75 and ~ 0.53 V (vs. SCE). The peak at 0.53 V represents the reduction of solution bound Au^{III} to Au⁰ and the wave at 0.75 V is attributed to the reduction of adsorbed AuCl₄⁻ ions to Au⁰ [25].

The surface topography of PEDOT-PSS-Au_{nano} electrode was analyzed through atomic force microscope (AFM). Fig. 1 shows the AFM image of PEDOT-PSS-Au_{nano} electrode. A relatively high-coverage of ordered monolayer of Au nanoparticles without agglomeration was found on the surface of PEDOT-PSS with an average size of Au as 10–15 nm. Further, it can be seen that Au nanoparticles were homogeneously distributed onto the surface of the modified electrode. PEDOT-PSS with its network structure acts as a three-dimensional, random and electronically conducting background (micro-reactor) and provides the matrix for the distribution of Au nanoparticles [35].

Crystalline structure and size of the Au nanoparticles present in PEDOT-PSS-Au_{nano} were examined by XRD analysis (Fig. 2). Peaks observed around 38.0°, 47.9°, 64.1° and 76.1°

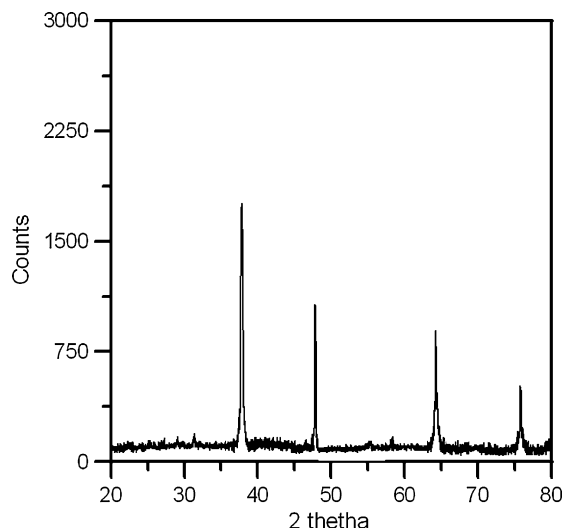


Fig. 2. XRD spectrum of PEDOT-PSS-Au_{nano}.

are attributed to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) facets of the fcc crystal structure of Au [20]. From the full width measured at the half-maximum of the peak at $2\theta = 38^\circ$, the average crystallite size of the Au particles was evaluated to be ~ 10 nm using Scherer's equation [36].

Real surface area, rugosity factor, specific surface area and the amount of Au nanoparticles deposited on the PEDOT-PSS-Au_{nano} were determined. Fig. 3 shows the CV recorded at PEDOT-PSS-Au_{nano} in aq. 1 M H₂SO₄ solution at a scan rate of 50 mV s⁻¹. A clear oxidation peak around 1.48 V and a sharp reduction peak at 1.0 V are observed, due to the reduction of the surface oxide monolayer on Au nanoparticles [37]. The specific surface area, S (cm² μ g⁻¹) of the catalyst particles was calculated by using the relation,

$$S = \frac{100A_{\text{rsa}}}{WA_{\text{gsa}}}$$

where A_{rsa} is the real surface area (as estimated from the charge consumed for the reduction process of the surface oxide monolayer (the peak at ~ 1.0 V in Fig. 3 and using a reported value of 400 μ C cm⁻² [38–40], A_{gsa} is the geometric surface area ($A_{\text{gsa}} = 0.0707$ cm²) and W (in μ g cm⁻²) is the amount of Au loading. The real and specific surface areas were estimated to

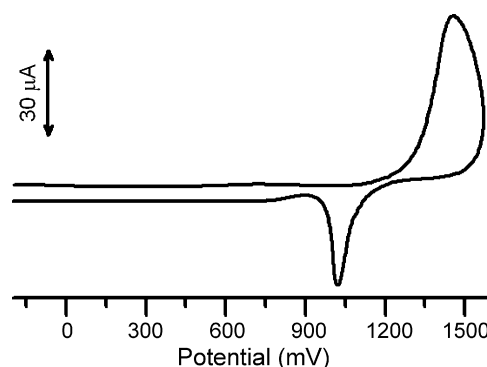


Fig. 3. CV of PEDOT-PSS-Au_{nano} in aq. 1 M H₂SO₄ solution; scan rate: 50 mV s⁻¹.

be 1.52 cm^{-2} and $28.23 \text{ cm}^2 \mu\text{g}^{-1}$, respectively. The rugosity factor is the ratio of A_{rsa} to A_{gsa} , assuming spherical particles of similar radius and found to be 19.56. The amount of Au nanoparticles deposited onto the PEDOT–PSS–Au_{nano} was determined [41] by using the charge (Q_{dep}) (obtained through graphical integration of cyclic voltammetric curves) utilized for the deposition of Au nanoparticles and was found to be $88 \mu\text{g cm}^{-2}$.

3.2. Voltammetric studies on NADH oxidation

3.2.1. Cyclic voltammetry

Fig. 4 compares the CVs for 1 mM NADH in PBS (pH 7.2) recorded at ITO/PEDOT–PSS and ITO/PEDOT–PSS–Au_{nano} electrodes, respectively, at a scan rate of 50 mV s^{-1} . Oxidation of NADH (1 mM) occurs at PEDOT–PSS and PEDOT–PSS–

Au_{nano} electrodes with an oxidation peak current at 0.2 V (Fig. 4a-inset) and 0.04 V (Fig. 4a) (vs. SCE), respectively. A large negative shift in the oxidation potential was noticed at PEDOT–PSS–Au_{nano} electrode. Unlike at the PEDOT–PSS modified ITO electrode (Fig. 4a-inset), a stable oxidation peak, without change in position in the subsequent sweep of potentials was noticed for PEDOT–PSS–Au_{nano} electrode (Fig. 4a). This observation informs that the electrode is not influenced by fouling effect of the oxidation products. The large decrease in the overpotential for the oxidation of NADH at a PEDOT–PSS–Au_{nano} electrode is ascribed to the high specific surface area provided by the Au nanoparticles and the synergistic effects between the dispersed Au nanoparticles and the PEDOT–PSS matrix, which facilitates direct electron transfer between NADH and the electrode surface. Furthermore, a higher current was noticed at the PEDOT–PSS–Au_{nano} electrode in comparison to PEDOT–PSS modified electrode. The magnitude of the oxidation peak current is proportional to the concentration of NADH (Fig. 4b)—a factor which is important for the development of a biosensor. The interesting aspect is that oxidation of NADH at PEDOT–PSS–Au_{nano} electrode occurs with a low positive potential without having an additional redox mediator.

In general, the formal potential of the NADH/NAD⁺ couple in neutral pH at 25 °C is estimated to be -0.56 V vs. SCE, and an over potential of $>1.0 \text{ V}$ is often required for the direct oxidation of NADH at the bare electrode. However, in the present study, oxidation of NADH occurs with a large decrease in the overpotential ($>0.70 \text{ V}$) at PEDOT–PSS–Au_{nano} electrode. On comparative literature, oxidation potential of $\sim 0.075 \text{ V}$ for NADH was reported at an electrode modified with polyaniline-doped mercaptosuccinic-acid-capped Au nanoparticles [42]. In another study, oxidation of NADH has been reported around 0.07 V at the Au nanoparticles self-assembled 3-mercaptopropyltrimethoxy silane modified gold electrode [43].

The scan rate dependence of the voltammetric response at the PEDOT–PSS–Au_{nano} electrode was explored. The peak current for the oxidation of NADH increases linearly ($R^2 = 0.995$) with the square root of scan rate between 10 and 200 mV s^{-1} (Fig. 4b-inset), suggesting that the overall oxidation of NADH at the electrode is controlled by the diffusion of NADH in solution. Further, the oxidation peak potential shifts towards positive potentials with increasing scan rates informing the electrochemical irreversibility of the electrochemical process. The diffusion coefficient (D) was determined by the equation;

$$I = (2.99 \times 10^5) AC_0 D^{1/2} \nu^{1/2} n(\alpha n_a)^{1/2}$$

where n is the number of electrons involved for the oxidation of NADH, which is 2 (as determined from Tafel slope), C_0 is the bulk concentration, A is the electrode area, ν is the scan rate, with values for α and n_a which are 0.48 and 1, respectively. The D value was found to be $2.98 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$.

3.2.2. Square wave voltammetry

The voltammetric response of NADH at the PEDOT–PSS–Au_{nano} electrode was studied by square wave voltammetry since this technique could provide a better resolution and signal-to-noise ratio. The influence of square wave voltammetric

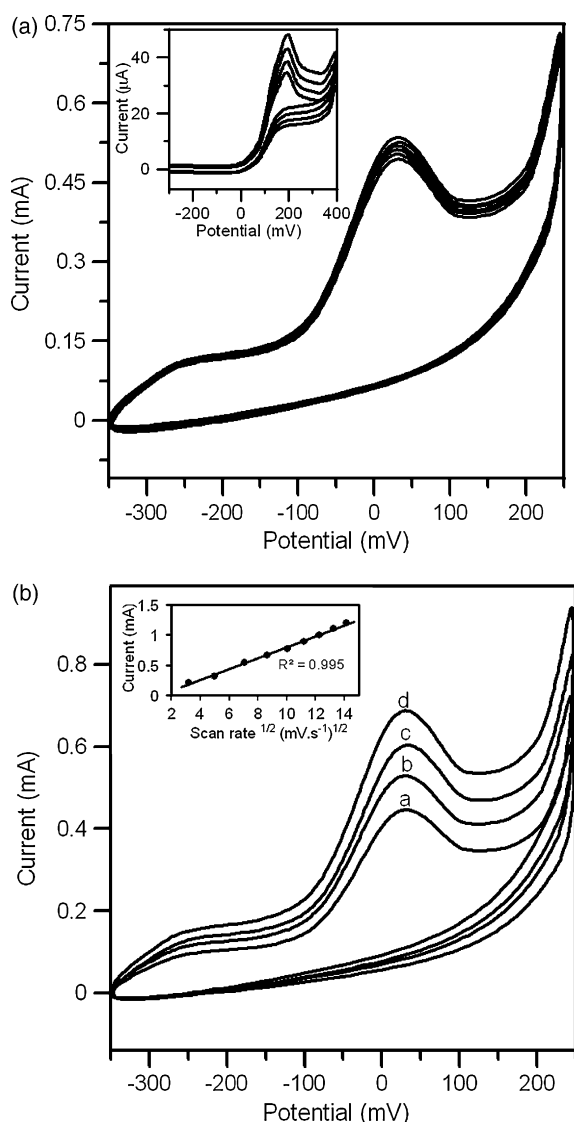


Fig. 4. (a) CVs recorded at PEDOT–PSS–Au_{nano} and PEDOT–PSS (inset) electrodes in the presence of 1 mM NADH in PBS (pH 7.2); scan rate: 50 mV s^{-1} (b) CVs of PEDOT–PSS–Au_{nano} electrode in the presence 0.8 (a), 1.0 (b), 1.2 (c) and 1.4 (d) mM NADH in PBS (pH 7.2); scan rate: 50 mV s^{-1} . Inset shows the plot of scan rate with peak current.

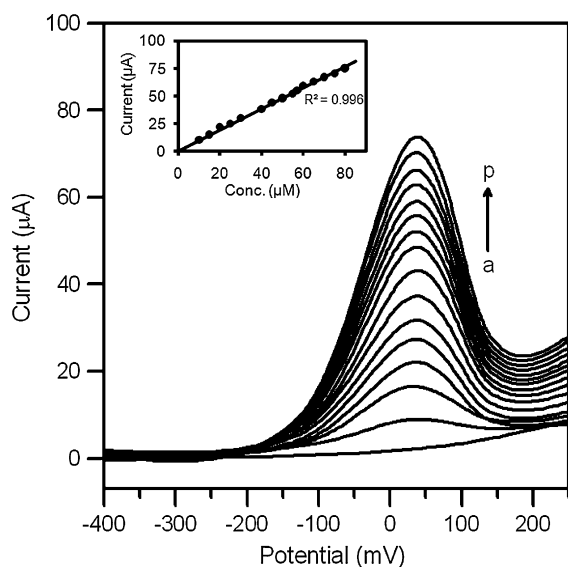


Fig. 5. SWVs of NADH on PEDOT-PSS-Au_{nano} electrode at different concentrations of NADH: (a) 0, (b) 10, (c) 15, (d) 20, (e) 25, (f) 30, (g) 40, (h) 45, (i) 50, (j) 55, (k) 57.5, (l) 60, (m) 65, (n) 70, (o) 75 and (p) 80 μM in PBS (pH 7.2).

parameters on the analytical signal was monitored to understand the kinetics of NADH oxidation, as well as to optimize the parameters for analytical application. Square wave voltammograms (SWVs) were recorded by changing the pulse amplitude (ΔU), frequency (f) and step potential in the range 10–100 mV, 10–100 Hz and 1–10 mV, respectively. Conditions such as 1.0 μM , pH 7.2, $t = 3$ s, step potential = 3 mV gave optimized response to oxidation of NADH. SWVs of PEDOT-PSS-Au_{nano} electrode (condition: $t = 3$ s, step potential = 3 mV, $f = 90$ Hz, and $\Delta U = 50$ mV) recorded for different concentration of NADH and the SWVs are shown in Fig. 5. A peak corresponding to the oxidation of NADH was observed at 0.04 V and the peak intensity was found to increase with concentration of NADH. Fig. 5 (inset) depicts the typical calibration curve by plotting the peak current, I_{cat} at 0.04 V, with the concentration of NADH. The I_{cat} linearly increases with concentration of NADH in the range of 1–80 μM (Fig. 5-inset). The linear regression equation is $y = 0.958x$, with a correlation coefficient of 0.996. A detection limit of 0.1 μM was calculated as the NADH concentration from the signal to the blank signal y_B (intercept) for three standard deviations of y -residuals $s_{y/x}$ [44]. The experimentally determined detection limit is in close agreement with the calculated detection limit.

3.3. Constant potential amperometry

The voltammetric results detailed above suggest that the PEDOT-PSS-Au_{nano} electrode facilitates a stable and low potential amperometric detection of NADH. Further, the sensitivity was examined by recording the amperometric response of oxidation of NADH at PEDOT-PSS-Au_{nano} electrode in a stirred solution, but with smaller addition of NADH over the concentration range of 0.1 to 2.2 μM . Fig. 6 shows an amperometric trace recorded at the PEDOT-PSS-Au_{nano} electrode ($E = +0.04$ V) during the successive addition of NADH aliquots into a stirred PBS (pH 7.2). The PEDOT-PSS-Au_{nano} electrode

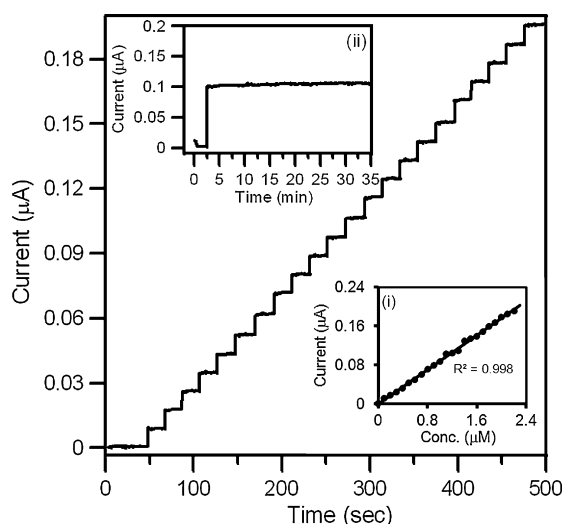


Fig. 6. Amperometric response of PEDOT-PSS-Au_{nano} electrode for the oxidation of NADH at +0.04 V in PBS (pH 7.2). Each addition increased the concentration of NADH by 0.1 μM . (i) Plot of [NADH] vs. catalytic peak current; (ii) Stability of the current response of 1 μM NADH at PEDOT-PSS-Au_{nano} electrode at the applied potential of +0.04 V in PBS (pH 7.2).

responds effectively to the NADH spikes, yielding steady-state signals within 2 s. Fig. 6(i) shows a calibration curve for the NADH. The calibration plot over the concentration range of 0.1–2.2 μM has a slope of $88 \pm 2 \text{ mA M}^{-1} \text{ cm}^{-2}$ with a correlation coefficient $R^2 = 0.9981$. The electrocatalytic behavior was highly reproducible, as reflected by a relative standard deviation of 1.2% estimated from the slopes of the calibration plots for six freshly prepared samples at PEDOT-PSS-Au_{nano} electrode.

Besides good sensitivity and linearity for the detection of NADH, an extremely attractive feature of the PEDOT-PSS-Au_{nano} electrode is the stable amperometric NADH response. Fig. 6(ii) shows the amperometric responses of 1 μM NADH at PEDOT-PSS-Au_{nano} electrode recorded over a continuous period of 35 min, when the potential was held at +0.04 V. It is clear that the current response to NADH oxidation at PEDOT-PSS-Au_{nano} electrode is stable over the entire period. However, it must be noted that a decaying of current signal up to 58.2% was observed at PEDOT-PSS modified electrode (figure not shown). For the PEDOT-PSS modified electrode, surface passivation by the radical intermediates may limit the stability and cause the loss of analytical sensitivity and reproducibility over the operational time. On the other hand, the formation of radical intermediates and subsequent reaction at PEDOT-PSS-Au_{nano} electrode are minimum, probably due to the lower oxidation potential (0.04 V). Hence, PEDOT-PSS-Au_{nano} electrode is free from the fouling effect by the oxidation products. These results demonstrated that PEDOT-PSS-Au_{nano} electrode greatly minimizes the surface passivation effects which generally give hindrance to amperometric detection of NADH.

3.4. Influence of interferences

Ascorbic acid (AA) and uric acid (UA) generally interference with the electrochemical determination of NADH, since

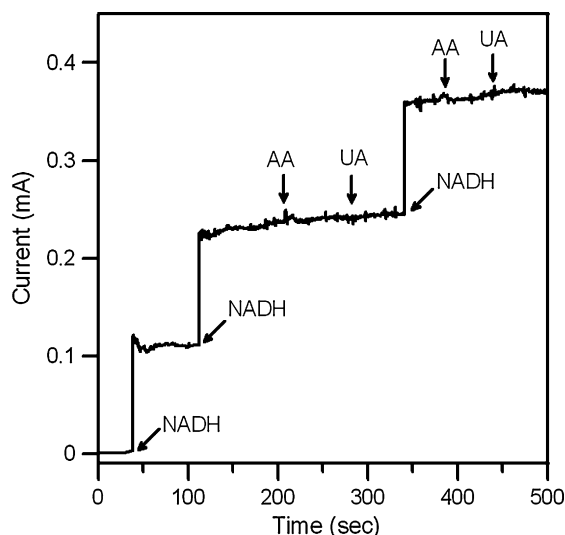


Fig. 7. Amperometric responses of PEDOT-PSS-Au_{nano} electrode to NADH, UA and AA (each of 1 mM concentration) at +0.04 V in PBS (pH 7.2).

AA and/or UA are/is commonly oxidized at potentials nearer to oxidation potential of NADH. Hence, cyclic voltammetry experiments were performed to examine the influence of UA and AA on the oxidation of NADH at PEDOT-PSS-Au_{nano} electrode. A broad anodic peak at 0.185 V and a shoulder around 0.04 V were observed for the oxidation of AA and NADH, respectively. On the other hand, two well defined oxidation peaks, one at 0.038 V and another at 0.256 V, due to the oxidation of NADH and UA, respectively, were observed at PEDOT-PSS-Au_{nano} in the PBS (pH 7.2) containing NADH and UA of equal concentration. In order to authenticate the influence of AA or UA on the oxidation of NADH, amperometric measurements were made at the PEDOT-PSS-Au_{nano} electrode with the intermittent addition of NADH, AA and UA at equal concentrations. Fig. 7 represents the

amperometric response for NADH, AA and UA of 1 mM concentration at the PEDOT-PSS-Au_{nano} electrode in PBS (pH 7.2). A well-defined NADH response was observed at the potential of +0.04 V. The subsequent injection of AA and UA did not show any additional signal or disturb the current response indicating the absence of interference in the signal.

3.5. Development of ethanol biosensor

To validate the usefulness of the described electrocatalytic system, an ethanol biosensor was developed. The biosensor was prepared by incorporating a model enzyme, alcoholdehydrogenase (ADH), into the PEDOT-PSS-Au_{nano} electrode. Au nanoparticles are convenient scaffold for enzyme immobilization and have been used previously for the immobilization of other dehydrogenases in enzymatic reactors [26,45]. In general, dehydrogenase requires NAD⁺ as a cofactor for enzymatic reaction due to the fact that NAD⁺ can be reduced to NADH simultaneously with oxidation of analyte. In the present study, alcohol dehydrogenase enzyme catalyzes the oxidation of ethanol, and simultaneously the cofactor NAD⁺ gets reduced to NADH. The enzymatically formed NADH undergoes electrochemical oxidation to NAD⁺ at the PEDOT-PSS-Au_{nano} electrode. Thus, NADH produced at the PEDOT-PSS-Au_{nano} electrode can be quantitatively correlated to the amount of ethanol.

It is known that in the dehydrogenase-based amperometric biosensor, pH, concentration of the enzyme and cofactor influence the response of biosensor. Hence, experiments were performed in order to study the influence of pH, ADH (enzyme) and NAD⁺ (cofactor) concentration on the current response at PEDOT-PSS-Au_{nano} electrode. The study of pH influence was conducted in the range of 6.0–9.0 (Fig. 8(i)). The current response increased almost linearly from 6.0 to 7.2 and at pH 7.2, the largest response was observed and the current response

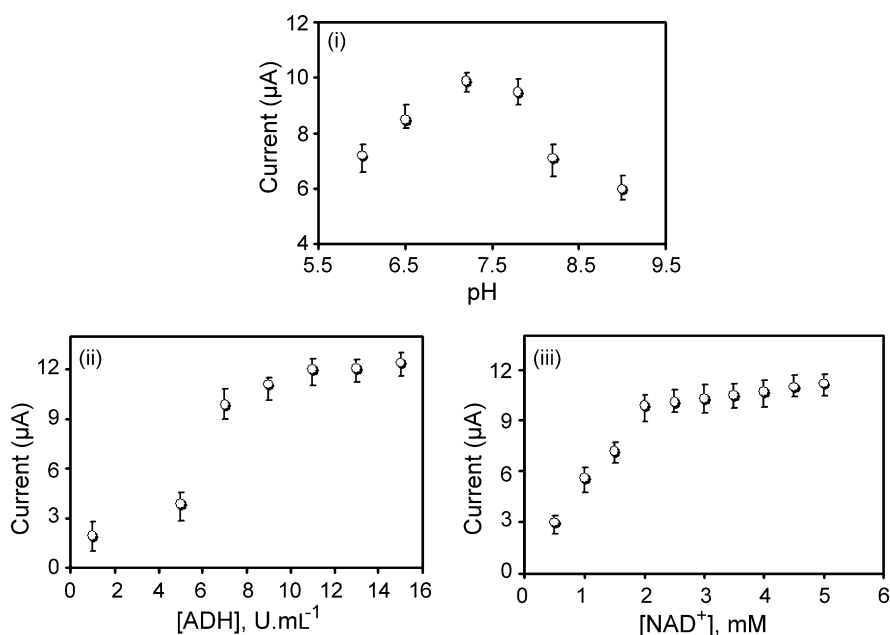


Fig. 8. Current responses at PEDOT-PSS-Au_{nano} electrode as a function of pH (i), ADH (ii) and NAD⁺ (iii); potential: +0.04 V, [ethanol]: 10 μM.

decreases appreciably in the alkaline pHs. The unstable nature of NAD^+ in alkaline solution is the reason for the decrease in current response. Hence, pH 7.2 buffer was used in all experiments.

The current response of the PEDOT–PSS– Au_{nano} electrode was studied by varying the amount of ADH used for the fabrication of modified electrode. Seven electrodes were prepared with different amount of ADH in the range between 1 and 15 U mL^{-1} , while the amount of other components were kept constant. It can be seen from Fig. 8(ii) that the current response at PEDOT–PSS– Au_{nano} electrode was dependent on the amount of enzyme incorporated into the electrode. An increase in the current was observed up to 7 U mL^{-1} , while no significant increase in the response current was observed for higher loading of the enzyme. Experiments were also performed to study the influence of NAD^+ concentration. Fig. 8(iii) shows the current response vs. concentration of NAD^+ recorded at the PEDOT–PSS– Au_{nano} electrode in PBS (pH 7.2) containing $10 \mu\text{M}$ ethanol. A largest response current was observed at PEDOT–PSS– Au_{nano} electrode with the $[\text{NAD}^+] = 0.2 \text{ mM}$ (Fig. 8(iii)). As the concentration kept increasing, the current response increases faintly. The increase in response is due to the higher conversion efficiency with higher concentration of NAD^+ in the enzyme-catalyzed reaction. Nevertheless, by considering the high cost of cofactor, NAD^+ , 0.2 mM was kept as optimum.

After being optimized the conditions, square wave voltammograms were recorded ($t = 3 \text{ s}$, step potential = 3 mV , $f = 90 \text{ Hz}$, and $\Delta U = 50 \text{ mV}$) for the different concentration of ethanol at PEDOT–PSS– Au_{nano} electrode (Fig. 9a). A voltammetric peak at 0.04 V was observed upon the addition of ethanol and the peak corresponds to the oxidation of NADH at the PEDOT–PSS– Au_{nano} electrode. It is to be noted that the peak 0.04 V did not appear in the absence of either ethanol or the co-factor, NAD^+ . This observation clearly supports that the peak observed at 0.04 V is due to the oxidation of enzymatically produced NADH. Fig. 9a also informs that the peak current at 0.04 V increases with increasing the concentration of ethanol.

A steady-state amperometric response of the PEDOT–PSS– Au_{nano} electrode to the addition of ethanol aliquots to a stirred PBS (pH 7.2) is shown in Fig. 9b. Upon addition of an aliquot of ethanol, the current increased steeply to 97% of the stable value within 5 s, indicating a fast response at the electrode. A calibration plot was drawn between the response current and the concentration of ethanol ($1\text{--}100 \mu\text{M}$) (Fig. 9b-bottom inset). The calibration plot is found to be linear ($R^2 = 0.9995$). It is interesting to note that the sensitivity toward ethanol ($97 \text{ mA M}^{-1} \text{ cm}^{-2}$) is comparable to the sensitivity to NADH ($95 \text{ mA M}^{-1} \text{ cm}^{-2}$), and this is an indication of efficient signal transduction at the PEDOT–PSS– Au_{nano} biosensor. A plateau current was observed while increasing the concentration of ethanol beyond $75 \mu\text{M}$ showing the characteristics of the Michaelis–Menten kinetics [46]. The apparent Michaelis–Menten constant (K_M) was estimated from the slope and intercept values of the plot of the reciprocals of the steady-state current vs. ethanol concentration (Fig. 9b-top inset) and a value of $K_M = 13 \text{ mM L}^{-1}$ was obtained.

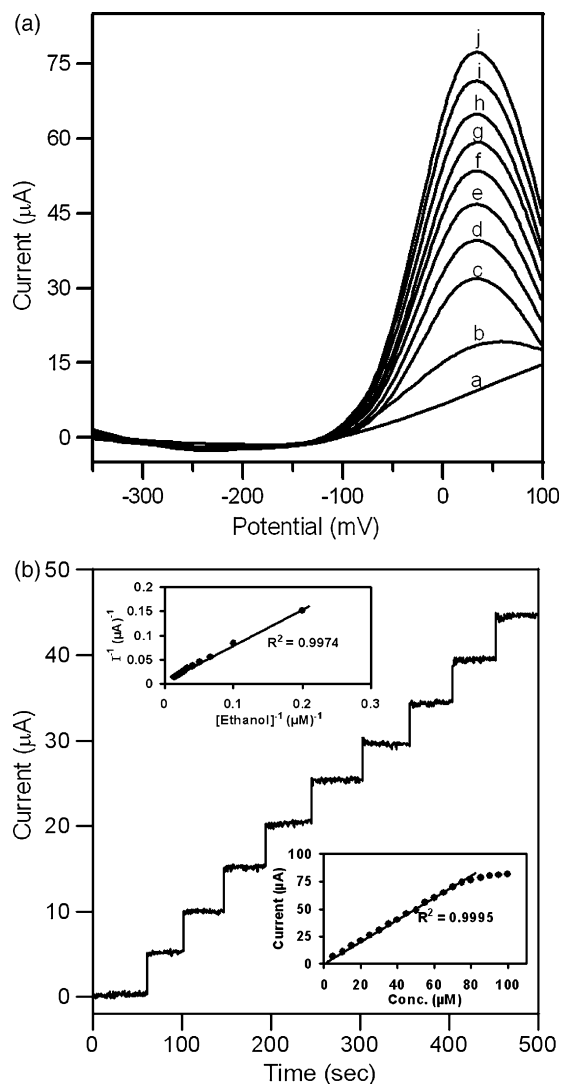


Fig. 9. (a) SWVs recorded at PEDOT–PSS– Au_{nano} electrode in PBS (pH 7.2) for the different concentration of ethanol in the presence of NAD^+ (0.2 mM), [ethanol]: (a) 10, (b) 20, (c) 30, (d) 40, (e) 50, (f) 55, (g) 60, (h) 65, (i) 70 and (j) $75 \mu\text{M}$ (b) Amperometric responses of PEDOT–PSS– Au_{nano} electrode to successive additions of $5 \mu\text{M}$ ethanol in PBS (pH 7.2) at $+0.04 \text{ V}$. Inset (bottom) shows the calibration plot of the concentration of ethanol with current at PEDOT–PSS– Au_{nano} electrode ($1\text{--}100 \mu\text{M}$); Inset (top) shows the Lineweaver–Burk plot.

3.6. Stability and reproducibility of PEDOT–PSS– Au_{nano}

The stability of the PEDOT–PSS– Au_{nano} electrode was determined for a period of operation. The performance of PEDOT–PSS– Au_{nano} electrode for the detection of NADH was tested in PBS (pH 7.2) for a period of 20 days. The first 2 days, a 2.3% decrease of the initial over a response current signal was noticed. After 15 days, the current response decreased by 4.8% of initial current. After 20 days, the decrease in current signal was 6.2% of the initial value. PEDOT–PSS– Au_{nano} electrode retains $\sim 94\%$ of its original activity after 20 days and continued to exhibit excellent response to NADH.

The repeatability of the current response of the PEDOT–PSS– Au_{nano} electrode was examined in the presence of $1 \mu\text{M}$ NADH in PBS (pH 7.2). R.S.D. was found to be 2.3% for ten succes-

sive assays. The electrode-to-electrode reproducibility was also determined in the presence of 1 μ M NADH in PBS (pH 7.2) by comparing the performance of five freshly prepared electrodes. The reproducibility is revealed with a R.S.D. of 2.8%. The good reproducibility might be due to the homogenous distribution of Au nanoparticles throughout the PEDOT–PSS matrix.

The experimental results show that Au nanoparticles dispersed in the PEDOT–PSS matrix have larger specific surface area and good biocompatibility and suited for immobilization of biomolecules or enzymes. PEDOT–PSS matrix provides an efficient electron-conducting tunnel for NADH oxidation. It is well documented in the present study that the PEDOT–PSS–Au_{nano} electrode is an excellent electrocatalytic material for the preparation of sensitive, reproducible and stable electrochemical biosensors for dehydrogenase substrates.

4. Conclusions

Au nanoparticles are uniformly dispersed into PEDOT–PSS matrix and an electrocatalyst electrode was fabricated for the detection of NADH. PEDOT–PSS–Au_{nano} electrode provides an enhanced electrocatalytic response to the oxidation of NADH. The response time for NADH detection is low and the performance of the electrode is stable. Stability of the electrode, reproducibility of the measurements, selectivity and wide linear response for NADH are in favor of fabricating an enzymatic sensor for the determination of ethanol at PEDOT–PSS–Au_{nano} electrode. A biosensor has thus been successfully assembled by immobilizing alcohol dehydrogenase enzyme into the PEDOT–PSS–Au_{nano} matrix and the functioning of sensor electrode is satisfactory. The present study forms a platform for the development of modified electrodes suited for the combined usage of electrocatalysis and electrochemical biosensing.

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