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Electrochemical impediometric detection of anti-HIV drug taking gold nanorods as a sensing interface

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ABSTRACT

In present work, gold nanorods were used for amplification of electrochemical sensing of anti-HIV replication drug i.e. deferiprone. Gold nanorods (nano Au) deposited onto pencil graphite electrode (PGE) has been utilized for covalent immobilization of horse radish peroxidase (HRP), via glutaraldehyde (Glu), for deferiprone detection using impedimetric technique. Gold nanorods (nano Au) prepared were characterized by TEM and XRD. The resulting nano Au sensor exhibited a good response to deferiprone with a wide linear range (0.005–1000 μM) and a low detection limit 0.005 μM. The biosensor also showed a short response time (within 15 s). In addition, the biosensor exhibited high reproducibility, good storage stability and anti-interference ability. The applicability of the nano Au sensor is to determine deferiprone level in spiked urine and serum samples.

1. Introduction

Nanomaterials based novel biosensing systems with enhanced performance of bioanalytical assay makes them promising material for fabricating devices. Among them, gold nanoparticles have many unique properties which make them suitable for use in biological applications. Budding interest in nanotechnology has inspired many studies on the use of different shapes of gold nanoparticles in many applications. The morphologies of interest include nanospheres, nanoshells, nanocages, and nanorods (Daniel and Astruc, 2004; Liao and Hafner, 2005; Pissuwan et al., 2006; Skrabalak et al., 2007). Among them nanorods are considered exceptional candidates for biological electrochemical sensing (Pérez-Juste et al., 2005). Gold nanorods are biocompatible as they have a strong binding affinity to thiol groups which make them to efficiently bind with biomolecules like enzymes for better electrochemical sensing in diagnostic application (Liao and Hafner, 2005). In the present report we used gold nanorods as an immobization matrix for binding of horse radish peroxidase (HRP) for electrochemical sensing of anti-HIV drug i.e. deferiprone (DEF).

HIV/AIDS is a global deadly disease (Cohen et al., 2008). There were about 1.8 million deaths from AIDS in 2010, down from 2.2 million in 2005 (Grady and Giardina, 2000).Various pharmaceutical drugs are used for controlling this disease. Among them, deferiprone (DEF) drug is most prominent drug used for curing this disease as it blocks replication of HIV-1 by triggering apopotosis (Sappey et al., 1995). In order to curtail the side effects related to drug, analysis is very important parameter in monitoring the drug sample. Overdose or under dose of drug can cause ailment or serious disease or may cause great impact on health. So monitoring of drug plays pivotal role in clinical applications. Various methods are available for analysis of drug like chromatography (Epemolu et al., 1990), HPLC (Goddard and Kontoghiorghes, 1990) and liquid chromatography coupled with mass spectrometry (LC/MS) (Biancamano et al., 2009). But these methods have many setbacks like time consuming preparation, skilled person to operate, bulky instruments and high cost. There is extensive need for developing the easy approach technique for drug analysis. Biosensor/electrochemical technique is best alternative for drug analysis as it does not require any sample pre-treatment, easy to operate and can be available at on site of analysis.

Among electrochemical technique, electrochemical impedance spectroscopy (EIS) shows potential in examination of the electrode electrolyte interface and the binding kinetics of molecules. EIS is nowadays a powerful technique for electrochemical sensing because of its distinct advantages such as allocating an avenue to interface biorecognition events and signal transduction (Qi et al., 2013). Additionally, it is simple, sensitive and selective.

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In current method, EIS technique was also used for selective, sensitive electrochemical sensing of DEF. We describe herein construction of anti-HIV drug sensor based on gold nanorods-chitosan (CHIT) decorated onto pencil graphite electrode (PGE) for electrochemical sensing. The exploitation of gold nanorods provides high sensitivity and selectivity for electrochemical sensing of DEF. The novel anti-HIV drug-detection podium provides excellent potential applicability in fast and sensitive electrochemical sensing of DEF in spiked serum and urine samples.

2. Experimental

2.1. Materials

Deferiprone was procured from vendors of Pharmaceautical Company. Chloroauric acid trihydrate and chitosan were procured from Sigma. All other chemicals were of analytic reagent grade. Double distilled water (DW) was used throughout the experiments.

2.2. Construction of working electrode for impedimetric sensing of DEF

Gold nanorods were prepared by the seed-mediated method (Chen et al., 2007). Gold seeds (5 ml) were prepared by reducing $HAuCl_4$ (250 μM) with freshly prepared ice-cold $NaBH_4$ (3 mM) in the presence of cetyl trimethylammonium bromide (CTAB; 75 mM). After mixing, the mixture developed into light-brown color solution. The prepared nanoseeds were used for subsequent preparation of nanorods after 2 h. For nanorods preparation, growth solution (10.0 mL) was prepared by the reducing $HAuCl_4$ (0.2 mM) with freshly prepared ascorbic acid (L-AA; 7 mM) in the presence of CTAB (1.6 mM). The solution was mixed by inversion and color changes from orange to colorless. Prepared gold seed solution was added and mixed gently. The color of mixture became red gradually which confirm the formation of gold nanorods.

For fabrication of working electrode, the gold nanorods, enzyme, chitosan and glutraldehyde were decorated on pencil graphite electrode (PGE) for electrochemical sensing of anti-HIV drug. Firstly, chitosan was dropped onto PGE and was kept overnight. Then PGE was washed thoroughly with DW to remove unbound matter. After that gold nanorods were decorated on the chitosan modified electrode. Gold nanorods (10 μl) solution was poured onto chitosan modified electrode and was kept overnight for physical adsorption. For cross-linking of enzyme, modified electrode (nano Au/CHIT/PGE) was dipped into 2.5% glutaraldehyde (1 ml) at room temperature (RT). Then this electrode was dipped into 10 μl of HRP (40 mg ml^{-1} protein) and kept for 24 h at 4 °C. The electrode was finally washed with 0.1 M phosphate buffer (pH 8.5) to remove unbound enzyme. The resulting HRP/nano Au/CHIT/PGE was used as working electrode and stored at 4 °C, when not in use. This working electrode was characterized by SEM and CV at various stages of its fabrication (Scheme 1).

2.3. Preparation of anti-HIV replication drug solution

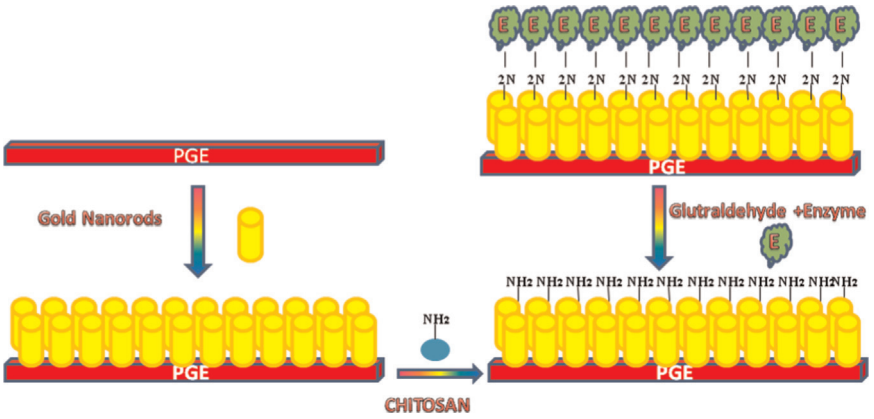
The standard solution of authentic deferiprone was prepared in phosphate buffer solution (pH 7.0). Solutions of different concentrations of deferiprone ranging from 0.005 to 1000 μM was prepared in 0.1 M sodium phosphate buffer (pH 7.0) and stored at 4 °C until use.

2.4. Study of electrochemical behavior of nano Au biosensor

To study the electrochemical impedance spectra (EIS), electrochemical cell composed of HRP/nano Au/CHIT/PGE as working electrode, Ag/AgCl as reference electrode and Pt wire as auxiliary electrode was recorded in PBS (50 mM, pH 6.5, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$. Frequency range 0.01 Hz–10 KHz. Then EIS spectra of different stages of electrode fabrication were taken. The semicircle diameter in the curve of spectrum related to the resistance in electron transfer kinetics, resistance charge transfer (Rct). This resistance controls the electron-transfer kinetics of the redox probe at the electrode interface (Liu et al., 2003). Rct can be defined as electron transfer resistance corresponds to the impediment croping up from diffusion of the electro active species to the electrode. It is only considerable at low frequencies. Rct is due to the redox reactions at the electrode surface and the energy blockade of the redox species reaching the electrode due to steric hindrance (Daniels and Pourmand., 2007). Various variable conditions like pH, incubation temperature and time were optimized. For evaluation of functioning of biosensor, parameters like precision and accuracy were also studied.

2.5. Applicability of nano Au sensor in real and pharmaceutical samples

Serum and urine samples were procured from University of Health Sciences (UHS), Rohtak. Different concentrations of deferiprone drug were spiked into serum and urine samples. The measurements were performed after successive additions of spiked samples. After each addition, was recorded by cycling the potential between 0.0 and +1.75 V at a scan rate of 100 mV s^{-1}. Deferiprone content was determined by the present biosensor by replacing deferiprone with spiked serum/urine samples under its optimal working conditions.



Scheme 1. A schematic showing the stepwise fabrication of the nano Au sensor biosensor.

2.6. Storage stability of nano Au sensor

Storage stability of the bioconjugated electrode and its electrochemical sensing toward deferiprone was examined over a period of 100 days at 4 °C.

3. Results and discussions

3.1. Evidence of preparation and characterization of working electrode

Fig. 1(A) shows TEM images, which was done to confirm the formation of gold nanorods. Microscopic illustration was reflecting the formation of nanorods with size 80 nm. Fig. 1(B) revealed the X ray diffraction (XRD) pattern of gold nanorods which is also confirming the formation of nanorods. All four peaks were consistent with the plane for instance (111), (198), (222), (300), (311). All peaks can be indexed to face center Au Nanomaterials. No other peaks were observed for other impurities. This proved that the prepared gold nanorods were pure.

Evidence which proved the modification of electrode was the surface morphology of electrode. The surface morphologies of the (a) bare PGE (b) nano Au/CHIT/PGE (c) and HRP/nano Au/CHIT/PGE were also observed. Fig. 2A shows SEM image of the bare electrode, which shows stripped surface. Nanorods were seen in SEM image (Fig. 2A) of nano Au/CHIT/PGE. Upon bioconjugation of enzyme, morphology of electrode was changed into the spongy form (Fig. 2C).

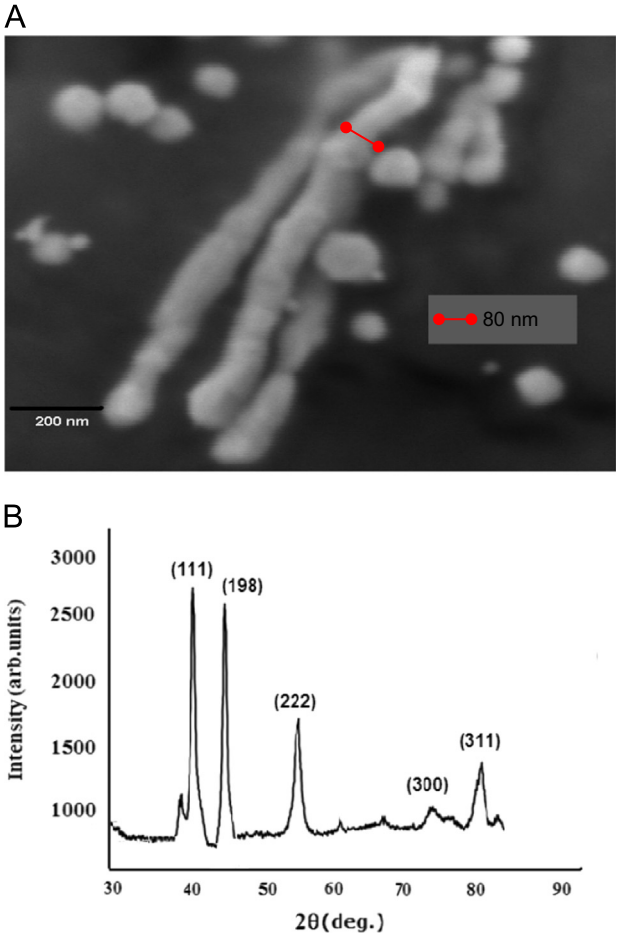


Fig. 1. (A) Transmission electron microscope (TEM) image of gold nanorods and (B) XRD pattern of gold nanorods.

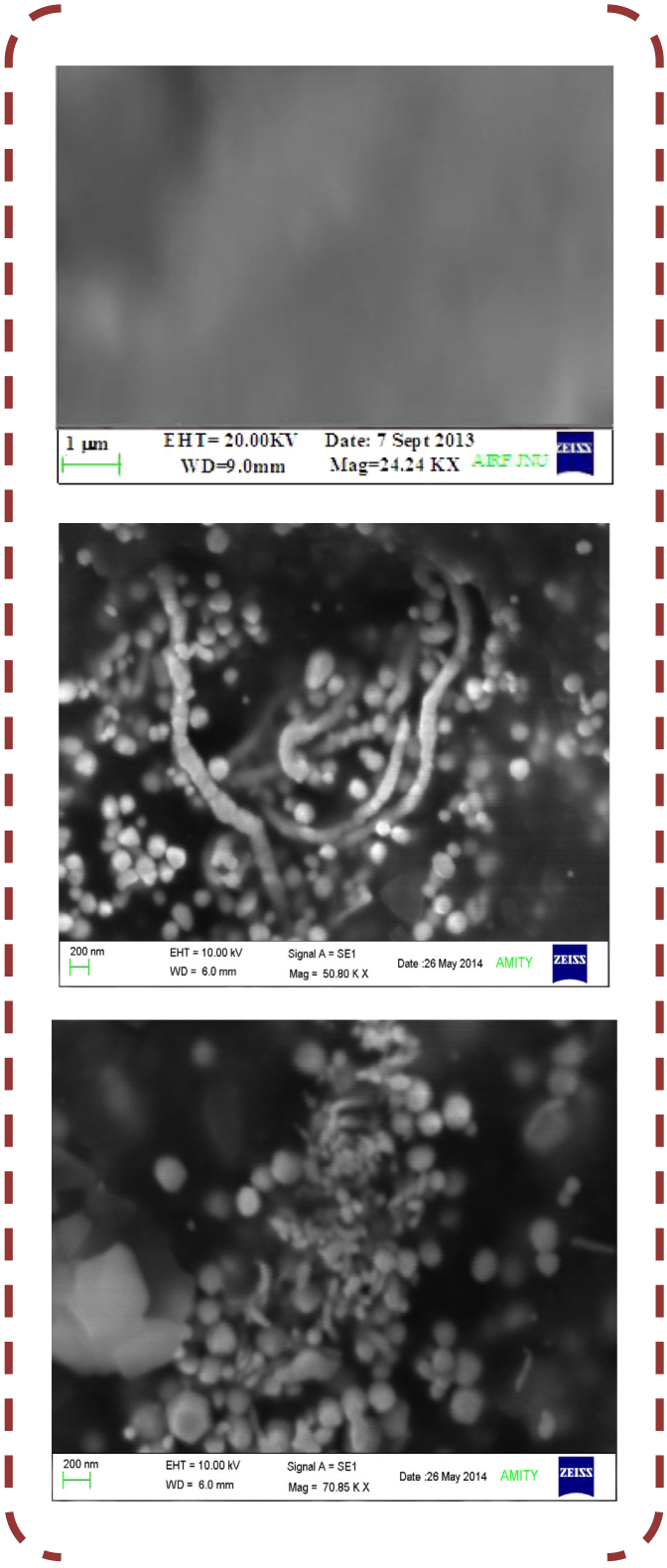


Fig. 2. SEM image of (a) bare PGE (a), nano Au/CHIT/PGE (b) and HRP/nano Au/CHIT/PGE.

CV pattern was done in order to confirm the fabrication of working electrode by using the pencil graphite electrode (PGE) as the working electrode in the scanning potential range of 0.0 to $+0.175$ V s^{-1} at the scan rate 20 mV s^{-1} in 0.1 M phosphate buffer pH 7.5 (Fig. 3A). A bare PGE, nano Au/CHIT/PGE and HRP/nano Au/CHIT/PGE were studied for the relative study. For bare electrode,

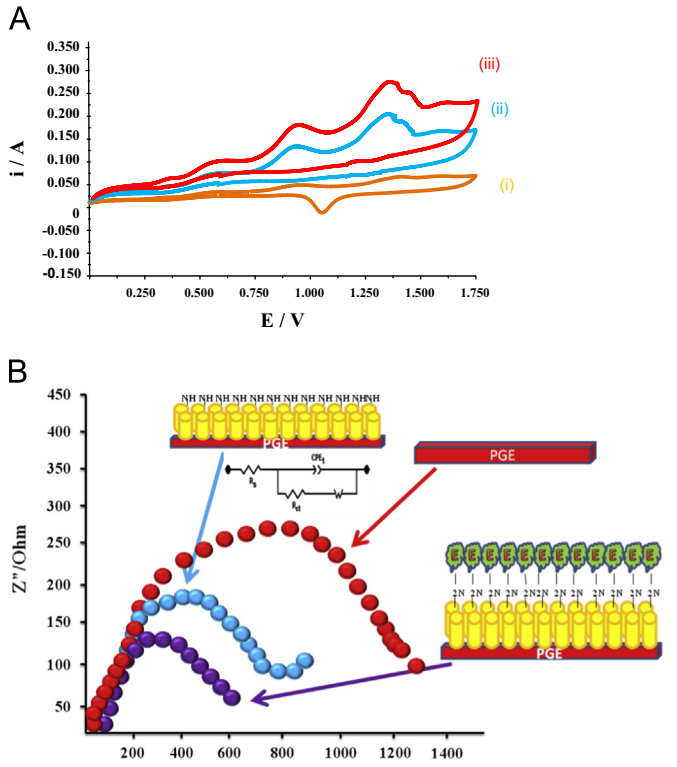


Fig. 3. (A) CV pattern of bare PGE (i), nano Au/CHIT/PGE (ii), HRP/nano Au/CHIT/PGE (iii) in the scanning potential range of 0.0 to +0.175 V s^{-1} at the scan rate 20 mV s^{-1} in 0.1 M phosphate buffer pH 7.5 in the presence of DEF. (B) EIS of bare PGE (a), nano Au/CHIT/PGE (b), HRP/nano Au/CHIT/PGE containing 1 mM $[Fe(CN)_6]^{3-/4-}$ with 0.1 M KCl at 0.20 mV s^{-1} (frequency range of 0.01 Hz–10 kHz) in the presence of DEF.

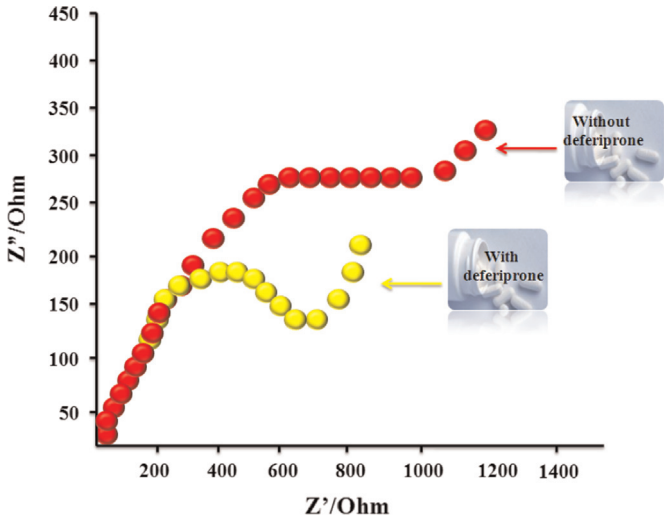


Fig. 4. Impedimetric response of nano Au sensor (a) with deferiprone (b) without deferiprone.

no electrochemical signal was observed, while electrochemical signal was observed when nanorods got decorated (nano Au/CHIT/PGE) confirming that gold nanorods produces fast electron transfer. But upon bioconjugation of enzyme (HRP/nano Au/CHIT/PGE), electrochemical signal was amplified because of catalyzed redox reactions. Oxidation peaks observed were 0.125, 0.200 and 0.300 mA which are higher than that of nano Au/CHIT/PGE (0.099, 0.150 and 0.225 mA). So enzyme initiates the reaction and produces amplified signal for electrochemical sensing of drug. From cyclic voltammograms, it can be observed that two peaks are associated with diffusion-controlled processes while other peaks are corresponded to surface-confined processes and can be related to the adsorption of produced reaction products. It is well known that the adsorption of reaction product on the electrode surface represents a pre-peak (Bard and Faulkner, 2001). So, two peaks are due to diffusion-controlled processes and other peaks are their corresponding pre-peaks.

Electrochemical impedance spectroscopy (EIS) is an effectual means for inquiring the properties of a surface modified electrode. Semi-circle portion at higher frequencies corresponds to the electron transfer limited process and the linear portion at lower frequencies may attribute to diffusion process. Nyquist diameter (real axis value at lower frequency intercept) gives value of charge transfer resistance (RCT) i.e. hindrance provided by the electrode material to transfer of charge from solution to the electrode that can be correlated with the modification of surface. EIS were done at various stages of working electrode. Fig. 3B shows EIS of bare PGE (a), nano Au/CHIT/PGE (b), HRP/nano Au/CHIT/PGE (c) in the presence of deferiprone. Modified nano Au/CHIT/PGE exposed a semicircle region (curve a), entailing small Rct value of the redox probe, showing that the nano Au/CHIT/PGE accelerate electron transfer. After the immobilization of enzyme in the presence of deferiprone, the impedance was decreased (Fig. 4a, b) and the semicircle diameters of nano Au/CHIT/PGE and HRP/nano Au/CHIT/PGE were about 800 and 600 Ω respectively. The increasing of mass transfer can be explained that after bioconjugation of enzyme redox reaction was catalyzed and reaction rate become faster therefore more redox probe transformed electrochemically and more electrons occurred. These results confirmed the bioconjugation of enzyme on the PGE surface. EIS studies are matching with the CV pattern of modified electrode (Supplementary information).

3.2. Principle behind electrochemical sensing of anti-HIV drug

Principle behind electrochemical sensing of anti-HIV drug is that reaction was catalyzed by HRP which caused the oxidation of deferiprone to corresponding dione. The dione formed undergoes a anodic hydroxylation of the methyl side chain (Yadegari et al., 2008a, 2008b, 2008c). It is expected that because of the participation of proton(s) in the oxidation reaction of deferiprone within a quasi-reversible two-electron process. To prove the above mechanism the effect of pH was studied using 0.1 M buffer at various pH values ranging from 1 to 12 (Supplementary Fig. 1). Depiction of figure indicated that there is change in the potential peak with change in pH of solution. The plot shows that at potential peak remains constant at range of pH less than 3.0 and above than 10.0 values. While in the pH range of 3.0–10.0, the peak potential shifted to less positive values. Consequently, it is possible to estimate two pKa values for deferiprone, as pKa, 1 = 3.0 and pKa, 2 = 10.0, it is same as reported in previous literature (Motekaitis and Martell, 1991). On the basis of our results, we have depicted a mechanism for the oxidation of deferiprone (Supplementary Scheme A) and HRP is involved in the catalysis of the oxidation of deferiprone to corresponding dione.

EIS also observed for with and without addition of drug. Fig. 4 reveals curve (i) with addition of drug (ii) without addition of drug. Small Rct value (620 Ω) observed after addition of substrate while Rct value observed without addition of substrate was 1100 Ω, showing that the nano Au sensor is specific toward oxidation of drug.

3.3. Impedimetric detection of anti-HIV drug

The relationship between the electron transfer resistance and the anti-HIV drug concentrations was studied in the range of

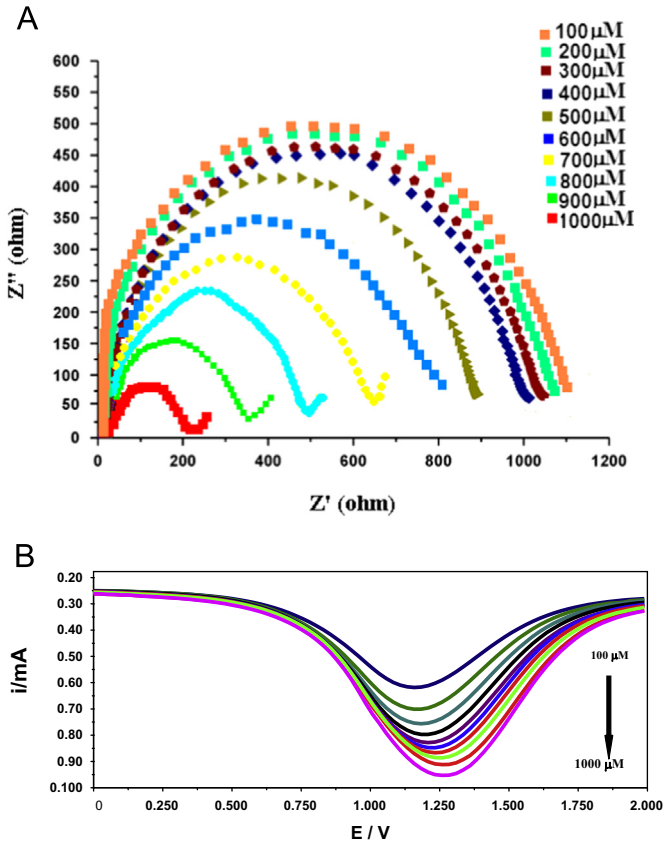


Fig. 5. (A) Impedimetric response of nano Au sensor for deferiprone detection. (B) SWVs of modified electrode using different concentrations of substrate.

0.005–1000 µM. EI spectra were recorded in 5 mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ containing 0.1 M KCl with incubation time of 15 s. (Fig. 5A). The incubation time was kept 15 s, which shows a quick response. It can be found that the electron transfer resistance decreased rapidly within the first 15 s of incubation time The faster response was mainly due to the fact that gold rods as a sensing interface is providing favorable orientation and conductive pathway to transfer electrons. The electrocatalytic mechanism initiated by HRP makes the process fast i.e. oxidation of drug. EIS also observed for with and without enzyme. EIS curve observed for (i) with enzyme (ii) without enzyme. Small Rct value (600 Ω) observed after introduction of enzyme on modified surface while Rct value observed without introduction of enzyme was 800 Ω, showing that the nano Au sensor showed enhanced electrochemical response after introducing enzyme (Supplementary Fig. 2).

As can be seen in Fig. 5A, the resistance charge transfer decreased with increasing anti-HIV drug concentrations. This can be attributed to the fact that HRP caused the oxidation of deferiprone to corresponding dione. This oxidation reaction of deferiprone is two-electron process (Yadegari et al., 2008a, 2008b, 2008c). The generated electrons during the catalyzed redox reactions helps in enhanced charge transfer rate leading to decreased in Rct value resulting in increased sensitivity of the sensor. When increased concentrations of DEF the Rct value were decreased markedly, this enhanced the electron transfer kinetics. The electron transfer resistance decreased with the increasing concentrations of DEF, which gives rise to a linear-type detection response from 100 to 1000 µM, and the regression equation obtained with a correlation coefficient of $R^2=0.97$ (Supplementary Fig. 3). These results clearly elucidate the impedimetric detection of DEF using gold nanorods. From these observations, we can conclude that the gold nanorods modified electrode provides highly conductive and expected as a good platform for sensing applications. The results of experiments carried out in duplicate sets reveal reproducibility of the system within 2%. Limit of detection was found to be 0.005 µM. It is verified by the minimum concentration of anti-HIV drug at which decrease in Rct value as compared to bioelectrode is observed.

Calibration was also performed using square-wave voltammetry with increasing concentration of deferiprone (Fig. 5B). The peak current is linearly related to the various concentrations of deferiprone (Supplementary Fig. 4).

3.4. Optimization and analytical performances of nano Au sensor

The response of nano Au sensor has been investigated at different pH and temperatures. The effect of the pH value on the response current of the HRP/nano Au/CHIT/PGE was studied between 5.0 and 8.0 in 0.05 M PBS. The suitable pH with the maximum activity of the immobilized HRP was at pH 7.0. Effect of temperature on biosensor was also studied in order to ensure the optimization. The current response reaches a maximum at approximately 60 °C, and then goes down as the temperature turn higher. In order to maintain steady with the temperature of human body, 35 °C is selected for this work. The effects of serum interferents on the ant HIV drug measurement has been studied by taking physiological concentration of interferents such as glucose, uric acid, urea and cholesterol. None had any significant interference. So nano Au sensor is anti-interferants (Supplementary Table 1).

The analytic recovery of known amount of added deferiprone was determined by the present biosensor. The mean analytic recoveries of added 5 and 10 mmol L^{-1} deferiprone (final conc. in reaction mixture) were 99.5 ± 1.2 and 97.1 ± 1.9 respectively. To test the reproducibility and reliability of the present biosensor deferiprone content in ten spiked serum and urine sample from different persons and was estimated on single day (within batch) and five times again after storage at −20 °C (Supplementary Table 2). The accuracy of the proposed method was determined by spiking serum and urine samples with different concentrations of deferiprone. Accuracy of proposed method was 99%. To study the substrate specificity on the electrochemical response of nano Au sensor, the following analogs of deferiprone like deferasirox, desferrioxamine, mimosine and 1,2 diketones. All are hydroxy ketone molecules which were added in place of deferiprone in the reaction mixture individually at a final concentration of 1.0 mM and the electrochemical response was measured under the similar assay conditions. Results depicts that deferasirox was utilized as substrate, while desferrioxamine, mimosine and 1, 2 diketones were practically unutilized. This shows the absolute substrate specificity of the developed nano Au sensor (Supplementary Table 3).

The developed nano Au sensor shows excellent reusability. The long-term stability is also tested after a month. It is revealed that the increase in the value of Rct has been found to be about 10% after 1 week while Rct increases sharply resulting in about 50% loss in about 10 weeks.

A comparison of analytic parameters of various nanoparticles based biosensors for detection of deferiprone with the present biosensor is summarized in Supplementary Table 4.

4. Conclusions

Nano Au sensor was fabricated by using gold nanorods as sensing interface and its applicability is for detection of anti-HIV drug via EIS technique. The Nano Au sensor exhibits improved biosensing characteristics like linearity as 0.005–1000 µM, fast

response time of 15 s, specific and anti-interferants. The response of Nano Au sensor with serum and urine samples shows its implications towards the development of biosensor for commercial drug monitoring device.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.11.038.

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