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Natural polyhydroxyalkanoate–gold nanocomposite based biosensor for detection of antimalarial drug artemisinin

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ABSTRACT

The worrisome trend of antimalarial resistance has already highlighted the importance of artemisinin as a potent antimalarial agent. The current investigation aimed at fabricating a biosensor based on natural polymer polyhydroxyalkanoate–gold nanoparticle composite mounting on an indium-tin oxide glass plate for the analysis of artemisinin. The biosensor was fabricated using an adsorbing horse-radish peroxidase enzyme on the electrode surface for which cyclic voltammetry was used to monitor the electro-catalytic reduction of artemisinin under diffusion controlled conditions. Electrochemical interfacial properties and immobilization of enzyme onto a polyhydroxyalkanoate–gold nanoparticle film were evaluated, and confirmed by cyclic voltammetry, electrochemical impedance spectroscopy and scanning electron microscopy. The differential pulse voltammetric peak current for artemisinin was increased linearly (concentration range of 0.01–0.08 μg mL^{-1}) with sensitivity of 0.26 μA μg mL^{-1}. The greater sensitivity of the fabricated biosensor to artemisinin (optimum limits of detection were 0.0035 μg mL^{-1} and 0.0036 μg mL^{-1} in bulk and spiked human serum, respectively) could be of much aid in medical diagnosis.

1. Introduction

Malaria is a vector-borne infectious disease and continues to be a major health problem in the tropical and subtropical countries. Every year, almost 2 billion people tend to suffer from the risk of malaria infection and over 100 million people experience the illness [1]. Malaria infections are curable through the use of different antimalarial drugs, although the troublesome trend of antimalarial drug resistance is increasing at a phenomenal rate. A popular class of antimalarials which have successfully gathered the attention of the medical fraternity over the years are artemisinin and their derivatives.

Artemisinin or qinghaoshu (QHS), a naturally occurring sesquiterpene endoperoxide is a promising third generation antimalarial drug originating from the Chinese medicinal herb *Artemisia annua* L. Artemisinin has successfully been used in the treatment of growing resistance of *Plasmodium falciparum* for more than two decades [2,3]. In 2002, WHO recommended artemisinin and its derivatives especially artesunate, artemether along with dihydroartemisinin as antimalarial drugs.

The uptake and metabolism of artemisinin are important parameters in drug potency and therefore worthy of scientific investigation [4]. Marked similarity has been observed in the case of side effects from the artemisinin class of medications with those of malaria viz., nausea, vomiting, anorexia and dizziness [5,6]. As such the micro level detection of artemisinin in body fluids (serum, urine and plasma) via a simple, sensitive, fast, reliable and portable method is of utmost importance to aid medical diagnosis. Until now, various methods have been reported for the determination of artemisinin such as Liquid Chromatography–Mass Spectrometry [7–9], Spectrophotometry [10], High-Performance Liquid Chromatography (HPLC) [11–13], HPLC–ELSD (evaporative light scattering detector) and Gas Chromatography (GC)–FID [14], HPLC–PO-CL (peroxyoxalate chemiluminescence) [15] and HPLC–CL (Chemiluminescence) [16], Voltammetry [17,18] and Nano Au-Chitosan Modified Sensor [19]. However, to the best of our knowledge, electrochemical determination of artemisinin using a PHA/AuNP/HRP/ITO hybrid nanocomposite based biosensor has not been reported yet.

PHA on the other hand is known as biodegradable microbial polyester which provides a good alternative to petrochemical-derived plastics for different industrial purposes [20,21]. Ma et al. [22] reported that the electron transfer rate of hemoglobin (Hb) can be enhanced after the protein is incorporated in poly-3-hydroxybutyrate (P-3HB). Ma et al. [23] also reported that myoglobin immobilized in a P-3HB film provides a model for constructing a third generation H_2O_2 biosensor. In both of the above studies PHB could provide a desirable membrane environment for Hb to undergo facile electron-transfer reactions.

Among the inorganic nanoparticles, gold nanoparticles (AuNPs) have received worldwide interest due to several intriguing properties like high surface-to-volume ratio and high surface energy which provide stable immobilization of biomolecules with the retention of their

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bioactivity [24,25]. Moreover, AuNP is favorable for the immobilization of enzymes as the amine groups and cysteine residues in the enzyme are known to bind strongly with the gold colloids [26,27] and can maintain their enzymatic and electrochemical activity for a considerable long period [28,29]. AuNP was applied to construct different biosensors [30–32] as it possesses unique ability to promote faster electron transfer kinetics. AuNPs can also be incorporated with biopolymers to form biocomposites and applied for the different biosensor applications. An amperometric biosensor was prepared as the biocomposite of chitosan–glucose oxidase–AuNPs by direct and facile electrochemical deposition for the quantitative measurement of glucose [33,34]. Another disposable mediator-less glucose biosensor was reported by Jingjing et al. [35] composed of iron oxide and AuNPs in a chitosan matrix. The use for the composite film strongly increased the surface of the effective electrode for loading the enzyme glucose oxidase. Tangkuaram et al. [36] reported that HRP/AuNPs incorporated with chitosan [HRP/AuNPs/CHIT/SPCE] could exhibit the best performance. However, no attempt so far has been made towards the preparation of PHA/AuNP hybrid based biosensors.

The present investigation reports for the time development of a PHA/AuNP/HRP/ITO hybrid nanocomposite based biosensor for the quantitative detection of artemisinin in body fluids. A better response towards the artemisinin detection was seen without the treatment of glutaraldehyde which is in general used as a cross linker. Many researchers presented the various applications of glutaraldehyde [37–40]. However, the disadvantage is that such cross-linkers built into the biomaterial could be reactive, or even toxic compounds may leach from them into the body upon biodegradation [41]. Murayama et al. [42] introduced a new cross-linking reagent polyepoxy compound to overcome the disadvantage of glutaraldehyde. The hydrophobic property as well as toxicity of glutaraldehyde treated tissues could turn out to be obstacles in the migration of host cells into the matrix of the materials, interdict their proliferation and as such could become less adaptable to the host tissue. In the present study, the PHA film is expected to act as a better matrix as compared to glutaraldehyde. Accordingly, the proposed biosensor is likely to have potential application in *in-vivo* usages.

2. Materials and methods

2.1. Reagents and materials

The anti-malarial drug artemisinin (99% purity) was a gift by Dr. N.C. Barua, Natural Product Chemistry Division, CSIR-NEIST, Jorhat, India. Horseradish peroxidase (HRP, 200 units/mg; Sigma-Aldrich, Germany) was freshly prepared in 0.05 M phosphate buffer solution (PBS; pH 7.0 ± 0.1) prior to use. $HAuCl_4$ (Sigma-Aldrich, Germany) was used without further purification. Serum sample was provided by CSIR-NEIST, Jorhat, Clinical Centre from healthy volunteers and kept frozen at −20 °C for further use. After gentle thawing, the serum sample was spiked with appropriate concentrations of the drug for testing. Standard working solutions were prepared by diluting the stock solution. Potassium ferrocyanide $[Fe(CN)_6]^{3-/4-}$ in PBS 200 ml with ionic strength 0.05 M in the pH range 5.5–8.5 was prepared in deionized water (NaH_2PO_4, 0.05 M and Na_2HPO_4, 0.05 M, pH adjustment with 1 M HCl and 0.1 M NaOH). All reagents used in the investigation were of analytical and molecular biology grade and obtained from Sigma Aldrich.

2.2. Production of polyhydroxyalkanoate (PHA)

The bacterial strain *Pseudomonas aeruginosa* BPC2 was isolated from the crude oil contaminated soil of Assam Asset, ONGC, Assam, India for the extraction of PHA. The bacterium was cultured in specific PHA detection medium [43] with minor modifications containing glucose as the carbon source for the production of biopolymer. The cultures were kept at 37 °C for 72 h in an incubator shaker at 180 rpm. The increase in turbidity of the culture medium signified bacterial growth. The shaken flask cultures were harvested and assayed for PHA production.

2.2.1. Polymer extraction, purification and characterization

The broth culture obtained after 72 h was harvested by centrifugation (8000 ×*g*) for 15 min at 4 °C and lyophilized. The PHA was extracted from the lyophilized biomass using chloroform by soxhlet extraction for 24 h. The polymer was collected with the drop wise addition of 10 volumes of ice-cold methanol and repeated three times to get the purified polymer [44]. The precipitated polymer was collected by air drying and the total PHA content measured gravimetrically. To confirm the chemical structure of the isolated PHA, ^{1}H and ^{13}C NMR (JEOL JNN-ECS 400) spectra were measured.

2.3. Preparation of PHA–AuNP nanocomposite film

The isolated polymer from *P. aeruginosa* BPC2 was used to prepare the composites; PHA–Au nanocomposite was prepared by mixing 20 μL PHA solution (3 mg mL^{-1}) and 80 μL $HAuCl_4$ in aqueous solution (0.0088 M) and sonicated until the color turned red [45]. The mixture was cast over an ITO glass plate (0.25 cm^2), one with normal casting and one with deep coating in a specialized chamber.

2.4. Immobilization of HRP on PHA–Au nanocomposite film

The bio-electrode for the detection of artemisinin was prepared by immobilizing HRP enzyme (1 mg mL^{-1} solution in phosphate buffer, pH 7.0 ± 0.1) on the PHA–Au nanocomposite by adsorption technique (in a specially assembled cell) and incubated overnight at 4 °C. In one experiment, glutaraldehyde was used as a cross linker, but none in the other. The condition used for the immobilization of the enzyme was based on prior studies [46–49]. To remove loosely-bound materials, the developed biosensors were rinsed with a phosphate buffer saline and preserved at 4 °C with pH 7.0 ± 0.1 in the buffer solution for further use.

2.5. Preparation of real samples

Artemisinin solution was prepared in water (0.0011 mg/100 mL) by sonication for 3 h (sparingly soluble in water). The stock solution of artemisinin was supplemented with the freshly prepared 0.05 M PBS (pH 7.0 ± 0.1) to make the desired concentrations. Prior to measurements, the human serum samples under use were centrifuged and diluted 10 times with the buffer solution. The standard addition method was used for analyzing the pharmaceutical samples and artemisinin-spiked human serum samples for the validation of the modified electrode.

2.6. Instrumentation

Electrochemical measurements were performed using Autolab Potentiostat/galvanostat (Eco Chemie, Netherlands) with NOVA software and potentiostat/galvanostat/ZRA (Gamry Reference 3000, USA) with Gamry Echem Analyst Software with the replacement of the working electrode by PHA/ITO, PHA/AuNPs/ITO and PHA/AuNPs/HRP/ITO. Platinum wire and Ag/AgCl (3 M KCl) were used as counter and reference electrodes, respectively. CV and EIS were carried out in a 20 mL Dr Bob's electrochemical cell stand. The surface topology of respective films was studied by using a Scanning Electron Microscope (SEM, JEOL-JSM-6390LV).

3. Results and discussion

3.1. Characterization of PHA by NMR analysis

After the extraction of purified PHA from *P. aeruginosa* BPC2, ^{1}H NMR and ^{13}C NMR of the polymer sample in $CDCl_3$ solution were done to illustrate the structure (Fig. 1). In the ^{1}H NMR chemical shift (δ) values ranging from 0.91 to 0.99 ppm attributed to the protons of methyl terminal groups, while δ values at 1.22–1.93 ppm corresponded to all internal $-CH_2$ groups in the polymer. The peak at 4.29–4.72 ppm was due to the presence of ester (−O−CH) proton and at 5.25–5.68 ppm clearly indicated −CH═CH moiety in the polymer chain. Likewise, ^{13}C NMR peaks appearing at 10–30 ppm represented methyl and methylene carbons. The ^{13}C signal at 124.32–139.31 ppm indicated −CH═CH− carbons and that at 173.38–173.66 ppm were the representative peaks for two types of C═O (carbonyl) carbons of the copolymer composition. Presence of −CH═CH moiety in the polymer chain indicates unsaturation in one monomer of the polymer. The spectrum closely resembles the spectra of the PHA isolated from *P. putida* cultivated on glucose [50] and PHA isolated from *P. aeruginosa* MTCC 7925 with different carbon sources [51]. From the NMR information, it can be inferred that the polymer isolated from *P. aeruginosa* BPC2 contains 3-hydroxyvalerate (3HV), 5-hydroxydecenoate (5HDE) and 3-hydroxyoctadecenoate (3HODE).

3.2. CV study

Reversibility in the reduction process and the electrochemical behavior were investigated by using CV. Fig. 2 shows the cyclic voltammetric behavior of the (A) PHA/ITO (B) PHA/AuNPs/ITO and (C) PHA/AuNPs/HRP/ITO electrodes studied at different scan rates (5 to 40 mV s^{-1}) in PBS (50 mM, pH 7.0 ± 0.1) containing 5 mM $[Fe(CN)_6]^{3-/4-}$. No significant redox peak was observed [Fig. 2(A)] which indicated that the biopolymer PHA is not electro-active in nature. In the CV curves of PHA/AuNPs/ITO and PHA/AuNPs/HRP/ITO [Fig. 2(B), (C)] the peaks shifted towards more positive potential. The improved electrochemical behavior of the AuNP modified electrode can be attributed to the improved charge propagation within the electrode on incorporation of conducting Au nanoparticles. The effect of pH on the peak current was investigated in the pH range from 5.5 to 8.5 in the presence of 0.01 μg mL^{-1} artemisinin Fig. 2(D). It was observed that the electrodes showed a maximum peak current as well as good redox behavior at pH 7.0 ± 0.1 of PBS. Therefore, in order to maximize the biosensor current signal, a PBS solution (pH 7.0 ± 0.1) was optimized as the optimum solution for all further experiments.

The change in redox current with scan rate represented that with increasing scan rate, both peak current and peak potential increased steadily. Also good uniformity of current function, $ip/AC\upsilon^{1/2}$ was observed from plot. The plot of ip against $\upsilon^{1/2}$ evidenced significant straight line relationship of Randles–Sevcik nature with a positive correlation coefficient of 0.99 supporting mass transport as a means of diffusion.

$$i_p(\mu A) = 8.3 \times \upsilon^{1/2}\left(mV\ s^{-1}\right) + 9.1; r^2 = 0.992; n = 8(PHA/AuNPs/ITO)$$

$$i_p(\mu A) = 0.12 \times \upsilon^{1/2}\left(mV\ s^{-1}\right) + 9.8; r^2 = 0.996; n = 8(PHA/AuNPs/HRP/ITO)$$

As no significant peak was observed in the case of PHA, therefore no slope was assigned to it. But after addition of AuNPs into PHA sharp anodic and cathodic peaks were observed with slopes of 0.122 μA (mV^{-1}) 1/2 and 8.31 μA $(mV^{-1})1/2$, in Fig. 3(a) & (b) respectively. This is because highly conducting AuNPs facilitate the oxidation–reduction process inside the electrodes. After immobilization of HRP, slopes were decreased to 0.106 μA $(mV^{-1})^{1/2}$ and 0.168 μA $(mV^{-1})^{1/2}$ respectively which was due to the successful immobilization of (HRP) retaining their bioactivity onto the PHA/AuNPs/ITO. Further, Fig. 4(A) shows relative variation in CV at a constant scan rate (5 mV s^{-1}) in PBS (50 mM, pH 7.01) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ for three electrodes (a) PHA/ITO (b) PHA/AuNPs/ITO and (c) PHA/AuNPs/HRP/ITO. In the PHA/ITO electrode, no significant redox peak was observed which was due to the fact that PHA acts as a barrier in electron transport. CV curve of PHA/AuNPs/ITO showed significant redox peaks with nearly rectangular shape. This improved electrochemical behavior of the electrode was due to the addition of AuNPs which facilitates the charge transfer within the system. However, in PHA/AuNPs/HRP/ITO, the peak current decreased in comparison to PHA/AuNPs/ITO [Fig. 4A (c)] which may be due to a

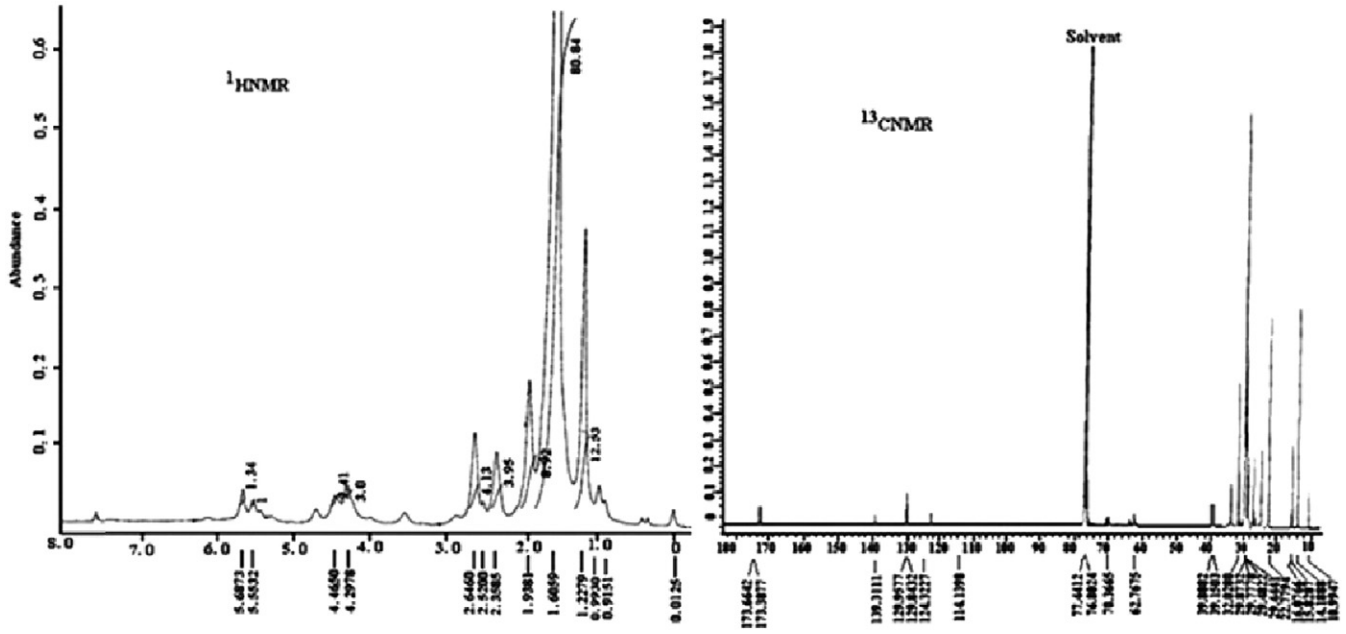

Fig. 1. ^{1}HNMR and ^{13}CNMR spectra of PHA.

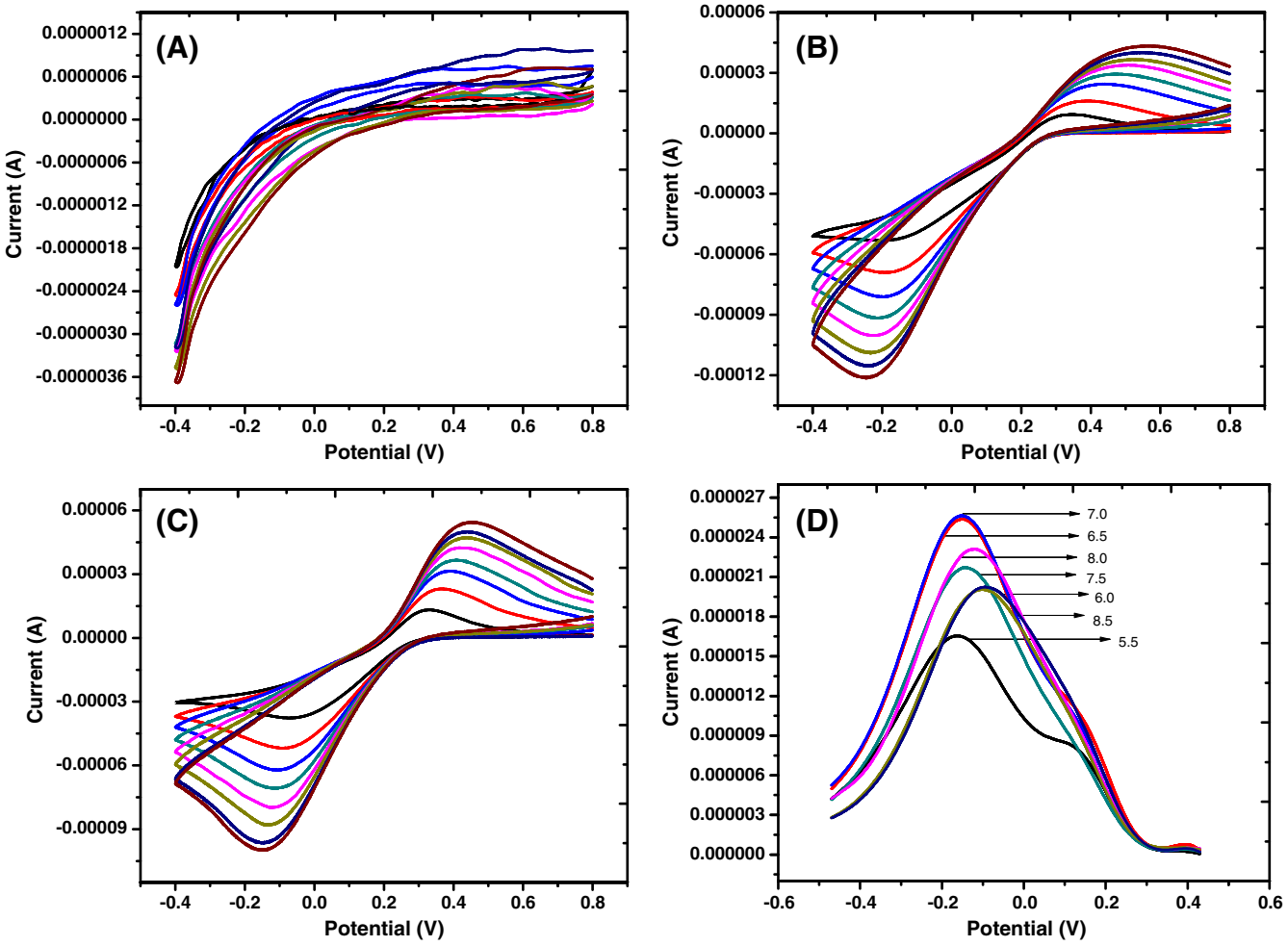

Fig. 2. Cyclic voltammograms of (A) PHA/ITO (B) PHA/AuNPs/ITO and (C) PHA/AuNPs/HRP/ITO electrodes in PBS (50 mM, pH 7.0 ± 0.1) containing (5 mM) $[Fe(CN)_6]^{3-/4-}$ at scan rates from 5 mV s^{-1} to 40 mV s^{-1} and (D) study of pH.

slow redox process after immobilization of HRP. The value of the Michaelis–Menten constant $Km_{(app)}$ and $I_{(max)}$ have been estimated using the Lineweaver–Burke plot as 0.07 μM and 0.0361 μA min^{-1} respectively. The low $Km_{(app)}$ value indicates the strong affinity of immobilized enzymes towards artemisinin. This result can be assigned to the uniform distribution of HRP molecules that retained native characteristics onto the PHA/AuNP surface.

3.3. EIS study

To investigate the effect of surface charge modulation for the determination of relative change in surface resistance at zero potential, EIS was carried out on (a) PHA/ITO, (b) PHA/AuNPs/ITO and (c) PHA/AuNPs/HRP/ITO electrodes in Fig. 4(B). R_{CT} is the surface charge transfer resistance, R_p, the polarization resistance obtained at zero potential and

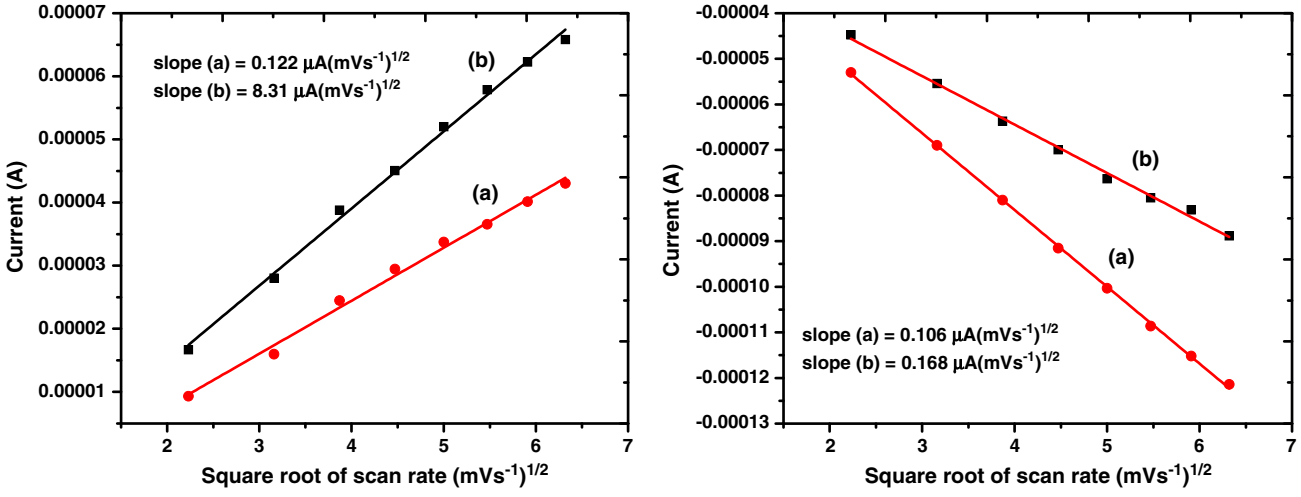

Fig. 3. Picture showing the PHA/AuNPs/ITO and PHA/AuNPs/HRP/ITO reduction and oxidation peak current with square root of scan rate.

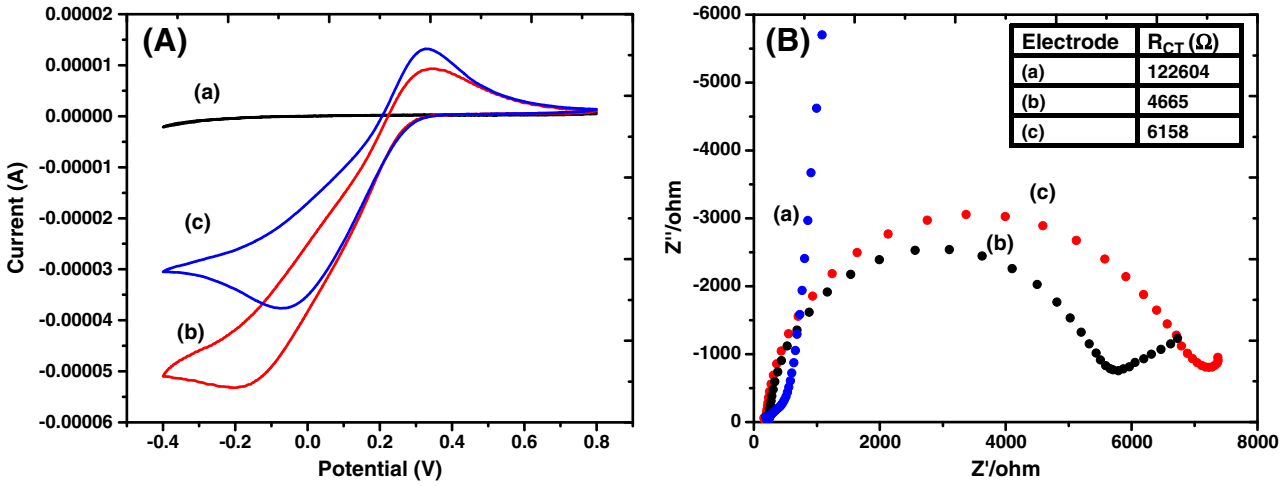

Fig. 4. Cyclic voltammograms of [A] (a) PHA/ITO (b) PHA/AuNPs/ITO (c) PHA/AuNPs/HRP/ITO with the scan rate at 5 mV s^{-1} and electrochemical Nyquist plots of [B] (a) PHA/ITO (b) PHA/AuNPs/ITO (c) PHA/AuNPs/HRP/ITO electrodes in PBS (50 mM, pH 7.0 ± 0.1) containing 5 mM $[Fe(CN)_6]^{3-/4-}$.

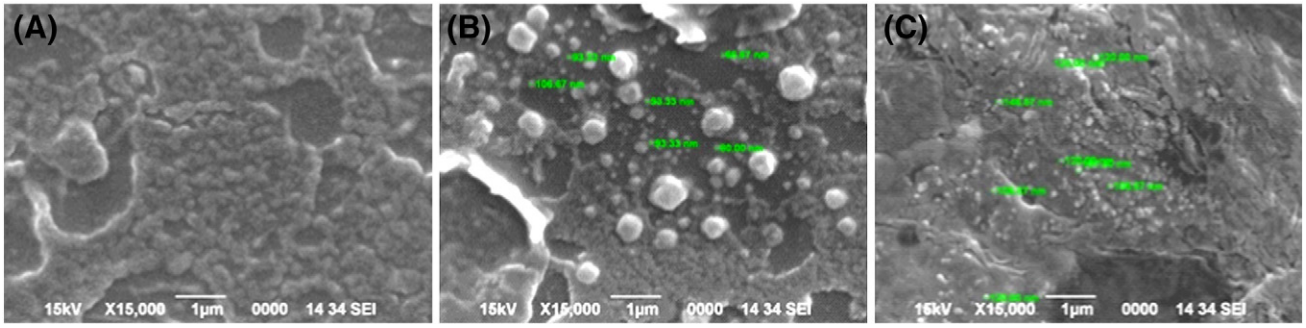

Fig. 5. SEM micrograph of the surface of (A) PHA/ITO (B) PHA/AuNPs/ITO and (C) PHA/AuNPs/HRP/ITO.

C_d is the double layer capacitance at the electrode/solution interface. The frequency associated with maximum Z″ (imaginary impedance) and R_{CT} were used to calculate C_d using the following equation [52]

$$R_{CT}C_d = 1/2pf_{max}$$

where f_{max} is the frequency at which maximum Z″ is obtained.

From the Nyquist plot it was seen that the Nyquist diameter of the electrode deposited with PHA [Fig. 4B (a)] is much larger than that of the PHA/AuNP electrode [Fig. 4B (b)], which manifested the formation of a PHA layer on the electrode surface, and it in some degree blocked the redox probe of $[Fe(CN)_6]^{3-/4-}$ contacting with the electrode surface. This clearly indicated that the AuNPs were not only self-assembled on the outer surface of the PHA, but also partly distributed within the film as tiny conduction centers which facilitated the

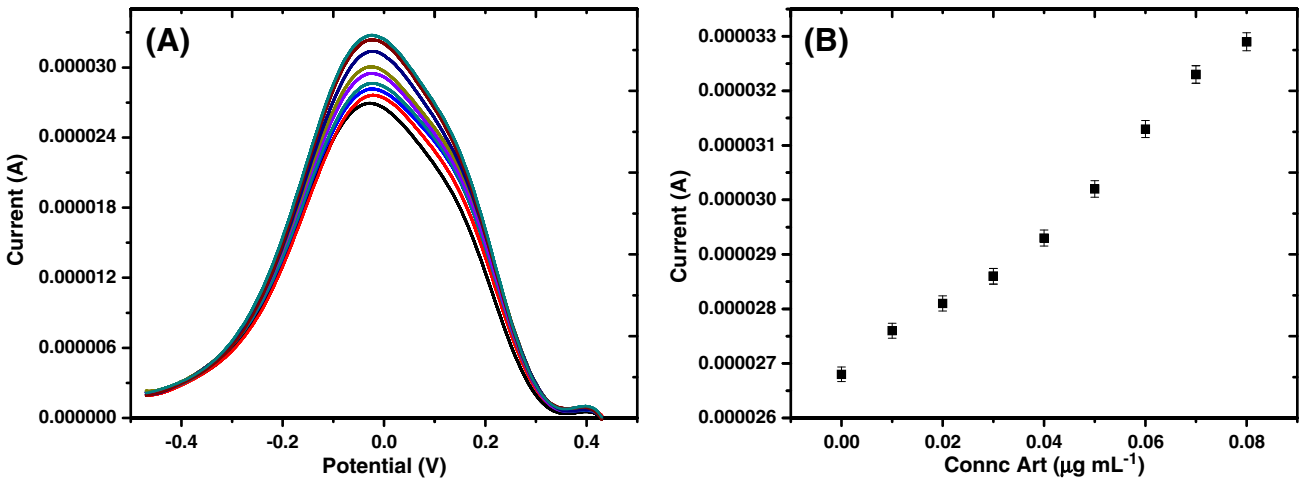

Fig. 6. (A) Shows biosensor response with increases in concentration of artemisinin and (B) calibration curve showing linearity for artemisinin as 0.01 to 0.08 μg mL^{-1}.

Table 1
Regression data of the calibration lines for quantitative determination of Artemisinin by organic–inorganic hybrid nanocomposite PHA/AuNPs/HRP based biosensor in bulk form and spiked human serum using DPV waveform.

Parameters	Bulk form	Human serum
Linearity range (μg mL^{-1})	0.01–0.08	0.01–0.06
Slope (A/μg mL^{-1})	8.8641×10^{-6}	9.1050×10^{-6}
Intercept (A)	2.6222×10^{-5}	2.6172×10^{-5}
Correlation coefficient (r^2)	0.985	0.984
RSD of the intercept	1.0438×10^{-8}	1.0762×10^{-8}
RSD of the slope	3.8728×10^{-7}	4.6491×10^{-7}
Reproducibility (%RSD)	1.01	1.13
Repeatability (%RSD)	0.66	1.01
LOD (μg mL^{-1})	0.0035	0.0036
LOQ(μg mL^{-1})	0.0110	0.0121

electron-transfer [53] and evidenced more conductive. However, AuNPs act as an electron-conducting tunnel which can greatly promote the direct electron transfer [54]. This change in the capacitance suggested that AuNPs were successfully incorporated into the PHA films and this is further supported by CV study which shows drastic enhancement of current in PHA/AuNPs as compared to PHA in Fig. 4(A). It has also been noticed in the impedance spectrum of the resulting electrode immobilized with HRP [Fig. 4B (c)] that the increase in R_{CT} was due to the dielectric and insulating features at the electrode electrolyte interface and was associated with successive immobilization of HRP. The change in R_{CT} after HRP immobilization might also be due to the hindrance of the macromolecular structure of HRP to the electron-transfer, and it also confirms the successful immobilization of HRP [53].

3.4. SEM study

In order to get insight into the surface morphology of the working electrodes, SEM micrograph was investigated. Fig. 5(A) shows non-porous and smooth surface of PHA which is in agreement with CV [Fig. 2(A)] studies confirming the non-conductivity of the PHA that is not electro-active in nature. Fig. 5(B) shows that the electrode surface consists of uneven porous matrix of PHA, embedded with AuNPs of 66.67 nm which increased the conductivity of the film. From Fig. 5(C) it can be assumed that PHA/AuNPs could provide a matrix for the successful immobilization of the HRP enzyme [55]. Lin et al. [55] have developed a disposable biosensor for rapid determination of H_2O_2 by incorporating HRP enzyme in Au–chitosan membrane to modify the ITO electrode. They reported that the presence of the Au–chitosan membrane played an important role in immobilization of the HRP enzyme and also facilitated immobilization to expose enzyme activity sites. This could make the substrate more easily accessible to the enzyme, resulting in a good amperometric response of the biosensor. The presence of the Au nanoparticles has attributed an increase in roughness of the surface area and demonstrated effective sensitivity of the biosensor.

3.5. Application to real samples

The applicability of the proposed PHA/AuNPs/HRP/ITO bio-electrode based electrochemical biosensor has been studied in bulk and spiked human serum. Representative voltammograms and calibration curve are shown in Fig. 6. The figure shows that the current is proportional to the concentration of artemisinin over a convenient range in Fig. 6(A). The precision was estimated for 0.01–0.08 μg mL^{-1} of the drug using the calibration curve and the standard addition method in Fig. 6(B). The calibration equation parameters and related validation data are presented in Table 1. Recovery experiments were performed using the standard addition method. The recovery value of artemisinin (0.01 μg mL^{-1}) in spiked human serum was 95.41% with % RSD of 1.13, n = 5. Moreover, it demonstrated considerable repeatability and reproducibility, along with accuracy and precision in experimental study and offers candidature as a promising alternative method for the direct determination of artemisinin in pharmaceutical formulations and spiked biological fluids. The advantages in the form of a comparative study on results obtained from the present approach with already existing ones are presented in Table 2.

4. Conclusion

A sensitive and selective electrochemical biosensor based on bacterial biopolymer with gold-nanoparticles and an enzyme (PHA/AuNPs/HRP/ITO) was developed for the determination of artemisinin in bulk and spiked human serum for the first time. The electrochemical behavior of artemisinin under the conditions described in the present work is an irreversible process controlled by diffusion. The proposed method has distinct advantages over the other existing methods regarding sensitivity, selectivity and minimum detectability. Furthermore, in some earlier HPLC and GC based methods [12,13] for the determination of artemisinin, the sensitivity and the lower limit of detection were

Table 2
Comparison of the different methods for artemisinin determination with organic–inorganic hybrid nanocomposite PHA/AuNPs/HRP based biosensor technique.

Method	Detection limit/bulk/ dosage form/plant extract	(Quantification limit)		Refs
		Human serum	Human plasma	
Non-biosensor				
UPLC–MS/MS	–	(4 ng m L^{-1})	–	7
LC–MS	0.1 ng mL^{-1}	–	–	8
LC–MS	–	–	0.257 ng mL^{-1}	9
Spectrophotometric	0.97 μg mL^{-1}	–	–	10
HPLC–DAD	0.1 μg mL^{-1} 0.5 μg mL^{-1}	–	–	11
HPLC–ED	5 ng mL^{-1}	–	–	12
HPLC–UV	–	–	10 ng mL^{-1}	13
HPLC–ELSD evaporative light scattering detector GC–FID	50 ng mL^{-1} 30 ng mL^{-1}	–	–	14
HPLC–PO-CL peroxyoxalate chemiluminescence	–	–	17.5 ng mL^{-1}	15
HPLC–CL chemiluminescence	–	10 ng mL^{-1}	–	16
Voltammetry–CPE	6.5×10^{-6} M (1.835 μg mL^{-1})	–	–	17
Voltammetry–CNT	10 μg mL^{-1}	–	–	18
Au–nanoparticles–chitosan sensor	1.7×10^{-9} mol L^{-1} (0.479 ng mL^{-1})	–	–	19
Biosensor				
PHA/AuNPs/HRP/ITO biosensor	0.0035 μg mL^{-1}	0.0036 μg mL^{-1}	–	Present study

found to be 5.0 ng mL^{-1}, 50 ng mL^{1} and 30 ng mL^{-1}, respectively; but in the present method it could be estimated up to a level of 0.0035 μg mL^{-1}, referring to much higher efficiency. In the case of spiked human serum, the method could detect up to 0.0036 μg mL^{-1}, which is much more sensitive to the HPLC method [15,16]. Consequently, the proposed method has a potential of a good analytical alternative and it is more environment friendly as compared to the chromatographic methods for the detection of the anti-malarial drug artemisinin in human body fluids.

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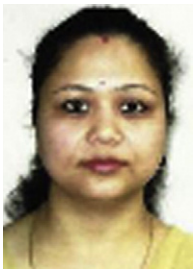

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