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Notes

Silver nanoparticle modified Pt electrode as voltammetric and electrochemical impedance sensor for hydrogen peroxide in live biological cells

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A non-enzymatic electrochemical sensor for hydrogen peroxide based on silver nanoparticle (AgNP) modified platinum electrode has been fabricated by multiple cyclic voltammetric scan of platinum electrode in AgNP solution in aqueous medium. The modified electrode (Pt/AgNP) can detect hydrogen peroxide in aqueous medium, bovine serum albumin and live L6 rat myoblast cells with high sensitivity and selectivity by cyclic voltammetry and electrochemical impedance spectroscopy. The limit of detection of Pt/AgNP towards H_2O_2 is 5.4×10^{-7} M. The detection of H_2O_2 by Pt/AgNP is free of interference from Na^+, K^+, Ca^{2+}, Mg^{2+}, dopamine and ascorbic acid.

Keywords: Electroanalytical chemistry, Sensors, Nanoparticles, Silver nanoparticles, Hydrogen peroxide, Cyclic voltammetry, Electrochemical impedance spectroscopy

Reactive oxygen species (ROS) are important intracellular signalling molecules, which participate in several physiological events such as protein synthesis, DNA damage, cell apoptosis, signal transition and immunity activity etc.[1] However, excess of ROS present in cells leads to various diseases, sometimes even causing death. Hydrogen peroxide (H_2O_2) is one of the common ROS found in different biological segments having several harmful effects. Therefore, the accurate detection and determination of H_2O_2 in cells as well as in environment is very important in environmental, pharmaceutical, clinical and industrial research[2,3]. Among various analytical techniques such as titrimetry, spectrophotometry, fluorescence, chemiluminescence, and electrochemistry, the electrochemical sensing of H_2O_2 is especially attractive due to low detection limit, high selectivity, sensitivity, cost-effectiveness, fast response, high sensitivity, and ease of miniaturization[4-10].

Enzyme based biosensors for H_2O_2 have attracted considerable attention due to excellent substrate specificity and high efficiency[11-15]. Poor reproducibility, chemical and thermal instabilities and complicated immobilization procedures of these biosensors[16,17] have turned recent efforts of H_2O_2 determination towards enzyme-free sensors[18,19]. Metal nanoparticles (NPs), due to their high electrical conductivity, high surface area and chemical stability, have become attractive electrode materials in electrochemical devices[20]. Metal NPs have become one of the popular and efficient electrode modifying agents for developing electrochemical sensors[21]. Electrode modified with metal NPs of Co[22], Au[23], Pt[24], Pd[25] and Ag[26] are known for H_2O_2 reduction.

In this work, we report a Pt electrode modified with the AgNPs synthesized from a reported method, which can detect H_2O_2 by cyclic voltammetry, square wave voltammetry and electrochemical impedance spectroscopy. Dopamine (DA), ascorbic acid (AA), uric acid (UA) and various metal ions such as Na^+, K^+, Ca^{2+}, Cu^{2+} and Zn^{2+} do not interfere in the detection of H_2O_2. The modified electrode has been applied successfully for the detection of H_2O_2 in live rat myoblast cells.

Experimental

$AgNO_3$ and hydrogen peroxide (H_2O_2) were purchased from Loba Chemie. Bovine serum albumin was purchased from Himedia. The electronic spectra were recorded on a UV-1800 Shimadzu spectrophotometer. SEM images were obtained on a Zeiss SEM analyzer. Electrochemical experiments were carried out on a CHI 660D electrochemical analyzer (USA) with a three-electrode cell system. All reagents were of analytical grade and used as received. The 0.1 M phosphate buffer solution (PBS) was prepared by mixing 19.0 mL of 0.1 M NaH_2PO_4 and 81.0 mL of 0.1 M $Na_2HPO_4.7H_2O$ and was used as a supporting electrolyte in all electrochemical experiments. Fresh solution of H_2O_2 was prepared before each experiment. All electrochemical experiments were carried out under N_2 environment.

In the square wave voltammetry experiments, the square wave amplitude was kept as 25 mV, the frequency was 15 Hz and the potential height for base staircase wave front was 4 mV.

AgNPs were synthesized according to the reported procedure[27]. Briefly, 100 mL (1 mM) solution of $AgNO_3$ was taken in an Erlenmeyer flask and placed on a hot magnetic stirrer plate. *Azadirachta indica* plant leaves extract (1 mL) was added and the solution was stirred for 10 min. The colour of the solution changed from light yellow to brown supporting the formation of AgNP which was confirmed by UV/visible spectroscopy and scanning electron microscopic (SEM) studies.

AgNP modified Pt electrode was prepared as follows: Prior to the modification, Pt electrode was cleaned as per reported procedure[28] followed by sonication first in CH_3OH and then in water. The electrode was dried under a stream of N_2 gas. The cleaned electrode was placed in the AgNPs solution containing 0.1 M $NaNO_3$ as the supporting electrolyte. Cyclic voltammetric runs were carried out for 100 scan segments at a scan rate 0.09 V s^{-1}. The electrode was then gently washed with water and dried under a stream of N_2 gas and stored overnight in a refrigerator for use. The modified electrode is designated as AgNP/Pt henceforth in this report.

For the detection of H_2O_2 in living Cells, rat L6 myoblasts cells were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and maintained at 37 °C in a humidified atmosphere with 5% CO_2. For the electrochemical study, the cells were seeded in a 6-well (35 mm) culture dish with a seeding density of 3×10^5 cells per dish. After reaching 80% confluence, cells were washed with PBS (0.02 M, pH = 7.4) three times. Then 3 mL of PBS (0.02 M, pH = 7.4) was added to the plates for real sample measurements. The modified AgNP/Pt electrode, auxiliary and reference electrode were placed on the plate. Upon addition of AA to the system, the cells released H_2O_2 which was measured electrochemically.

Results and discussion

UV/visible spectrum of the aqueous solution of Cu NPs show a peak at 440 nm, which is the characteristic peak for AgNPs[27]. The morphology of the synthesized AgNPs was studied by scanning electron microscopy. Spherical nanoparticles of average size 100 nm were observed in SEM image (Supplementary data Fig. S1).

Figure 1 shows the cyclic voltammograms (100 scans) during the modification process of Pt electrode by Ag NPs. The increase in current indicates formation of AgNPs film on Pt electrode surface. The AgNP/Pt electrode was further characterised by recording double potential step chronocoulometry of AgNP/Pt in PBS containing 0.1 M $NaNO_3$ (Fig. 1, inset). The sharp decrease in charge versus time is indicative of the formation of electroactive film on the surface of Pt electrode.

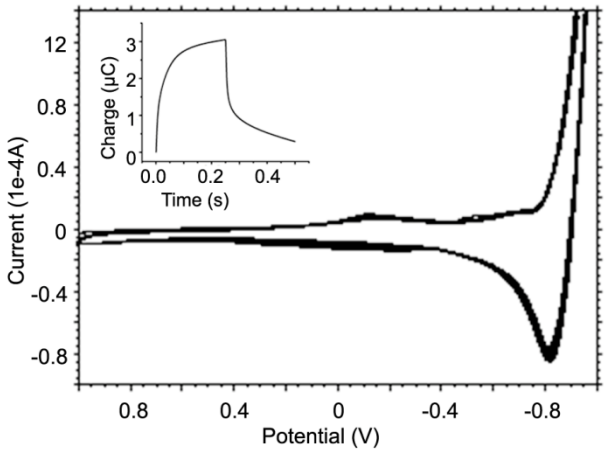

Fig. 1 – Cyclic voltammograms of Pt electrode in Ag nanoparticle solution in water containing 0.1 M $NaNO_3$ as the supporting electrolyte for 100 scans at scan rate 0.09 V s^{-1}. [Inset: Chronocoulogram of the modified electrode in water containing 0.1 M $NaNO_3$ as supporting electrolyte].

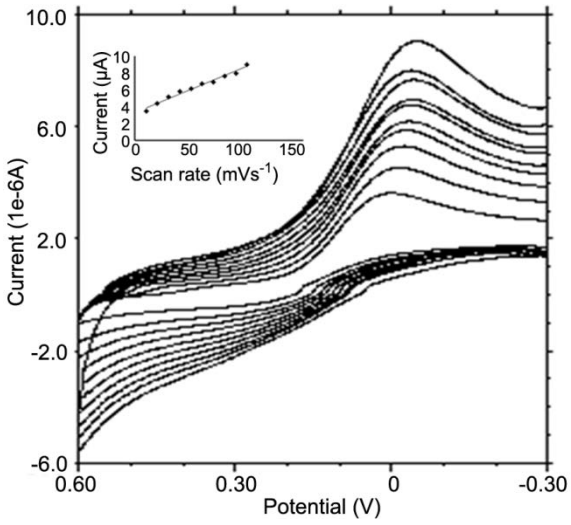

Fig. 2 – Cyclic voltammograms of AgNP/Pt at different scan rates in PBS containing 0.1 M $NaNO_3$. [Inset: Plot of cathodic peak current versus scan rate].

The cyclic voltammograms of AgNP/Pt electrode in PBS at different scan rates are shown in Fig. 2. The reduction peak current was found to increase with scan rates. The plot of reduction current against scan rate was linear, confirming the formation of AgNPs film on the Pt electrode surface (Fig. 2, inset).

Figure 3 shows the cyclic voltammogram of AgNP/Pt in PBS at different added concentrations of H_2O_2 (0.33–3.27 mM). In the absence of H_2O_2, an irreversible cyclic voltammogram was obtained with reduction peak at +0.048 V. Addition of H_2O_2 shifted this reduction peak of AgNP/Pt to –0.035 V with gradual increase in the cathodic current. A good linear plot of the reduction peak current versus concentration of H_2O_2 was observed with R^2 = 0.97. Cyclic voltammograms AgNPs/Pt was recorded at different scan rates in presence of 3.27 mM of H_2O_2 (Fig. 4). The current was found to increase with the scan rate. The linearity of the cathodic current versus square root of scan rates plot (R^2 = 0.99) indicates that the H_2O_2 reduction process at AgNPs/Pt surface is a diffusion controlled process.

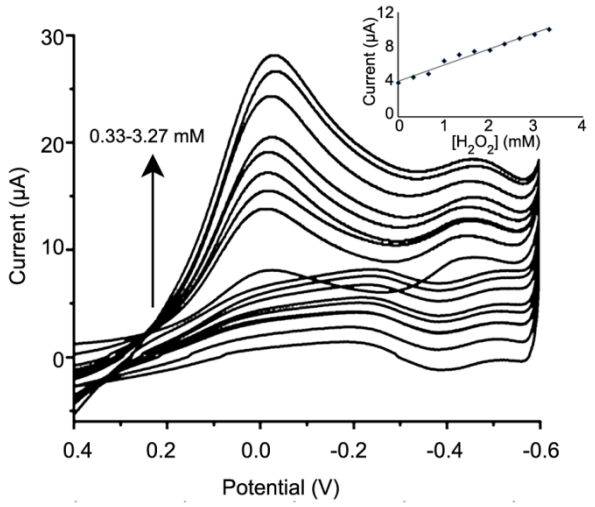

Fig. 3 – Cyclic voltammograms of AgNP/Pt in PBS containing 0.1 M $NaNO_3$ at different added concentration of H_2O_2. [Inset: Plot of the cathodic current versus H_2O_2 concentration].

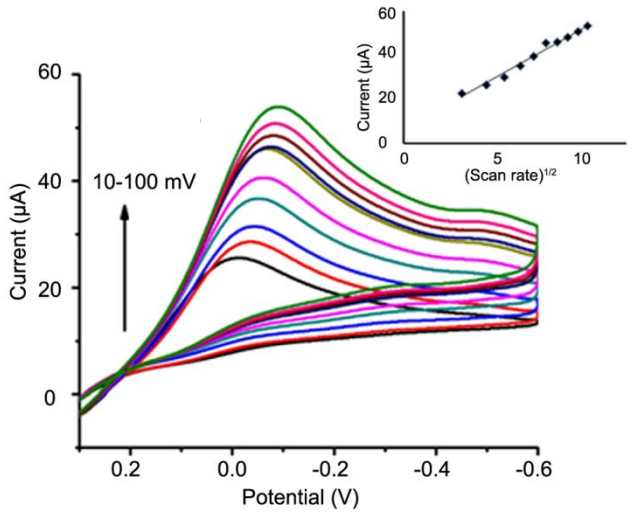

Fig. 4 – Cyclic voltammograms of AgNP/Pt in PBS containing 0.1 M $NaNO_3$ at different scan rates containing 3.27 mM H_2O_2. [Inset: Plot of cathodic current versus square root of scan rates].

The mechanism of electrocatalytic reduction of H_2O_2 by AgNP/Pt can be shown to follow C_iE_i mechanism as shown below. Here C_i stands for irreversible chemical reaction while E_i stands for irreversible electrochemical reaction.

$$AgNP + H_2O_2 + 2H^+ \longrightarrow Ag^+NP + H_2O \quad (Ci)$$

$$Ag^+NP + e^- \longrightarrow AgNP \quad (Ei)$$

The number of electrons involved in the overall reduction process of H_2O_2 by AgNP/Pt can be calculated from the plot of current versus (scan rate)$^{1/2}$. For an irreversible diffusion controlled process the relation between current (I) and scan rate (v) is given by $I = 3.01\times10^5\ n[(1-\alpha)n_\alpha]^{1/2}ACD^{1/2}v^{1/2}$. Here, diffusion co-efficient (D) was calculated as 8.07×10^{-6} cm^2 s^{-1} with area of the electrode (A) as 0.91 cm^2 and concentration (C) of H_2O_2 as 0.34×10^{-3} mol cm^{-3}. $[(1-\alpha)n_\alpha]$ was considered as 0.7 as reported in literature[1]. The number of electrons involved (n) has been calculated as 1.97 (~2.0). This value is in conformity with the value reported for electrocatalytic reduction of H_2O_2. The limit of detection (LOD) of H_2O_2 was found as 0.541 μM and calculated by using the formulae, LOD = 3 (RSD/slope), where RSD = standard deviation for the average measurement of the blank sample and slope = calibration plot slope value[29].

The H_2O_2 sensing ability of AgNPs/Pt electrode was further studied by square wave voltammetry. Figure 5 shows the square wave voltammograms of AgNP/Pt electrode at different added concentration of H_2O_2 in the electrolytic medium. With the addition of H_2O_2 (0.33–3.27 mM) the peak current at potential +0.006 V increases from 3.909 μA to 10.09 μA. The relationship between peak current and concentration of H_2O_2 is linear with R^2 = 0.98. The potential of AgNP/Pt electrode shifted from +0.058 V in absence of H_2O_2 to +0.010 V when concentration of H_2O_2 becomes 3.27 mM.

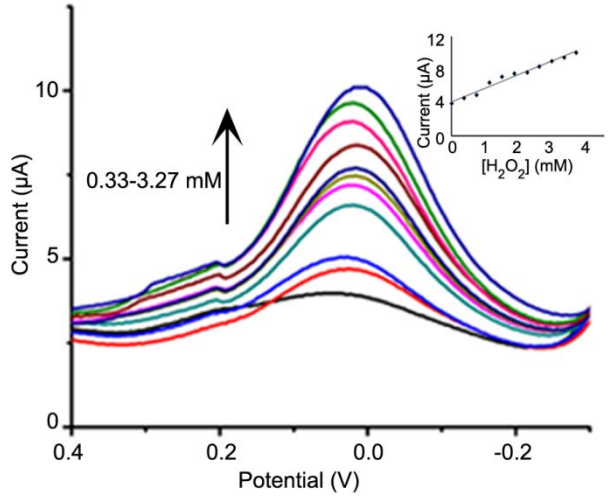

Fig. 5 – Square wave voltammograms of AgNP/Pt in PBS containing 0.1 M $NaNO_3$ at different added concentration of H_2O_2. [Inset: Plot of the cathodic current versus H_2O_2 concentration].

The EIS measurements of interaction between AgNPs/Pt and H_2O_2 were studied at E_{DC} = 1.5 V. The EIS Nyquist plot of AgNP/Pt at different added concentration of H_2O_2 in PBS shows the charge transfer resistance (R_{CT}) to increase with increasing H_2O_2 concentration (Fig. 6). The difference in charge transfer resistance (ΔR_{CT}) was found to increase linearly with increasing H_2O_2 concentration (Fig. 6, Inset.

The selectivity of AgNPs/Pt electrode towards H_2O_2 in the presence of biologically important metal ions and molecules has also been established. For this purpose, cyclic voltammograms of AgNP/Pt were recorded in PBS at different added concentration of H_2O_2, in presence of Na^+, K^+, Ca^{2+}, Mg^{2+}, DA and AA in the electrolytic medium. The nature of the cyclic voltammograms remained same and their peak positions were within ±0.010 V, as compared to those recorded without the above-mentioned interfering agents. This confirms the selectivity of AgNPs/Pt towards H_2O_2 in the presence of Na^+, K^+, Ca^{2+}, Mg^{2+}, DA and AA.

Studies were carried out for the detection of H_2O_2 in living cells and in bovine serum albumin (BSA): We detected current of H_2O_2 released by the L6 rat myoblast cells lines stimulated by 1 μM AA. The cells were first washed with PBS solution (0.02 M; pH = 7.4) three times. AgNP/Pt in PBS (0.02 M, pH = 7.4) in presence of L6 rat myoblast cells did not show any specific current (Fig. 7, black line). Cyclic voltammogram recorded after addition of 1 μL AA to the cell solution showed the reduction peak

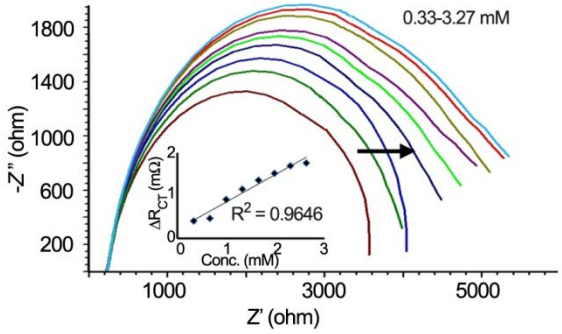

Fig. 6 – Nyquist plot of AgNP/Pt in PBS containing 0.1 M $NaNO_3$ at different added concentration of H_2O_2. [Inset: Plot of ΔR_{CT} versus H_2O_2 concentration].

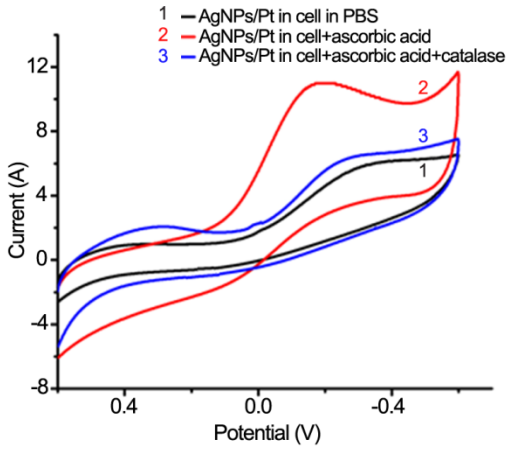

Fig. 7 - Cyclic voltammograms of AgNP/Pt in rat L6 myoblasts cells (1), rat L6 myoblasts cells+AA (2), rat L6 myoblasts cells+AA+catalase (3).

corresponding to H_2O_2 (Fig. 7, red line). In order to verify whether the current response after addition of AA was due to the H_2O_2 produced from the living cells or not, 40 μL of catalase was added into PBS and cyclic voltammogram was recorded. The reduction peak was absent due to the metabolism of H_2O_2 by catalase to water and oxygen (Fig. 7, blue line). These results suggest that the reduction current peak observed was only due to H_2O_2 released from the living cells.

In order to investigate any damage to the cells due to the release of H_2O_2 the optical morphology of cells before and after the addition of AA. The optical microscopy during the measurements showed that all the cells kept their regular shapes and there were no difference before and after the addition of AA (Supplementary data, Fig. S2).

The AgNPs/Pt electrode was also tested to detect H_2O_2 in aqueous solution of bovine serum albumin (BSA).

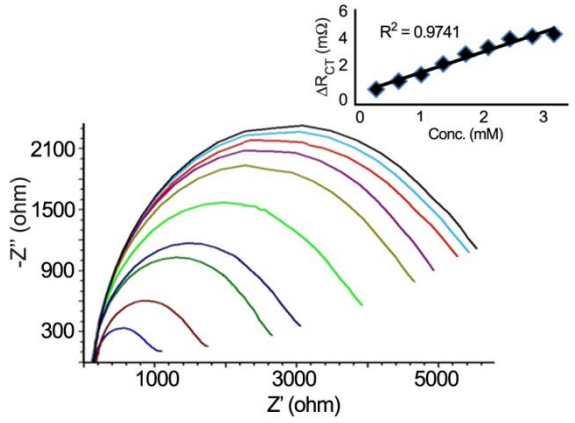

Fig. 8 – Nyquist plot of AgNP/Pt in bovine serum albumin in PBS containing 0.1 M $NaNO_3$ at different added concentration of H_2O_2. [Inset: Plot of ΔR_{CT} versus H_2O_2 concentration].

Figure 8 shows the Nyquist plots of AgNPs/Pt when H_2O_2 was added in the BSA medium up to 3.27 mM. The plot of the difference in charge transfer resistance (ΔR_{CT}) in the absence of H_2O_2 in BSA medium is shown in Fig. 8 (Inset). A linear plot was obtained similar to that for aqueous medium.

In summary, AgNPs were prepared and characterized with the help of UV/visible spectroscopy and SEM analysis. The modified AgNPs/Pt electrode acts as an electrochemical sensor for H_2O_2 by cyclic voltammetry, square wave voltammetry and electrical impedence spectroscopy. The AgNPs/Pt electrode can detect H_2O_2 in presence of Na^+, K^+, Ca^{2+}, Mg^{2+}, DA and AA in aqueous medium. This electrode can also detect H_2O_2 in living cells and in BSA-water medium by CV, SWV and EIS.

Supplementary data

Supplementary data associated with this article are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_57A(04)485-489_SupplData.pdf.

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