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Manufacturing a Modified Carbon Paste Electrode with Catalase Enzyme-Au Nanoparticles for Electrochemical Sensing of Hydrogen Peroxide and Their Electrocatalytic Properties

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To find hydrogen peroxide, different techniques applied, recently. In this practice we decided to produce a modified carbon paste electrode by catalase enzyme-Au nanoparticles for electrochemical sensing hydrogen peroxide and their electroactivity characteristics. Cyclic voltammetry were done electrochemical researches. A three-electrode method including a modified carbon paste electrode with catalase enzyme-Au nanoparticles as the operant electrode, a platinum string electrode as a counter electrode, and saturated calomel electrode as a reference electrode, was applied. Cyclic voltammetric assays were done with different scan speed area from of 50 mV s^{-1} to 500 mV s^{-1}. Transmission electron microscopy was checked external morphological characteristics of Au nanoparticles. H_2O_2 in 100 µM to 450 µM area could find out by designed biosensor. By perform assays in two weeks regular interval, the resistance of modified carbon paste electrode with catalase enzyme-Au nanoparticles biosensor has been determine and it has been discovered that after 14 days, modified carbon paste electrode with catalase enzyme-Au nanoparticles keeps its 97% activity.

Keywords: electrochemical sensing, hydrogen peroxide, catalase enzyme, Au nanoparticles

1. INTRODUCTION

Nanotechnology is an appearing technology trying to extract different technological improves of regulating the construction of substances at a decreased dimensional measurement accosting

alienated molecules additionally their adjusted aggregates or supramolecular forms [1-3]. The nanometer-length extent continues developing chances for new substances that can be applied for the creation of apparatuses as well as mechanisms, originally. It's essential to discriminated nanotechnology from the Nanoscience allowing such technology [4-7]. Nanoscience is the learning of luminaries along with object effects at nanoscale conceptually, while nanotechnology is employing the approaching information to conceive new substances besides constructions [8-9]. Directing to noble scientific Benefits, intelligence in Nanoscience as well as nanotechnology continues expanding worldwide. In order, this is desired to direct to basic variations in the course that components, instruments, and mechanisms are learned likewise invented [10-11]. For an exhilarating act of nanotechnology in healthcare, approach in nature sciences experiment, especially at the cell classification forms the rank. There have been important technological improvements in the producing, classes, and also approaches of biosensors, in current decades [12-13]. Since electrochemical techniques give great susceptibility, few identification limitation additionally usage of primary instrumentation, they have been extensively used for the detection of therapeutic productions [14-15]. Withal, some notice has been offered to electrochemical sensors for the detection of Hydrogen peroxide. Hydrogen peroxide (H_2O_2) is a reactive oxygen metabolic by-product that applies as an essential regulator for an amount of oxidative stress-related ranges. It is interfere in many natural statuses as well as intracellular paths, which have been connected to many disorders [16]. Hence, its experimental detection serves of magnificent definition [17-19]. Electrochemical method has been extensively applied for the determination of hydrogen peroxide due to it has intrinsic benefits of purity, facile miniaturization, great susceptibility as well as relatively not high expense in correlation with the routine methods for H_2O_2 detection [20]. Approaching to the description in biological programs along with applied approaches, a growing action has been given to the creation of opportune electrochemical biosensors, in recent years [21-24]. Hence, the development of H_2O_2 sensors with mean determination outline besides great attention extent is potently expected. The electroanalytical determinations of H_2O_2 at bare electrodes are not fitted, due to the delaying electrode activity additionally broad overpotential needed for redox reactions of H_2O_2. For spreading the electron exchange activities also reducing the needed overpotential, Electron precede agents have been extensively exerted [25]. So, due to nanoparticles great electrical conductivity, broad outer region, expansive electrochemical acting window, favorable substrate adhesion, in addition constant chemical, electrochemical also physical characteristics, they are appropriate matrices additionally new seekers for the immobilization of various redox molecules furthermore biomolecules. In the procedures of creating novel nanoscale tools for later biological, medical also electrical functions, the commixture of biological molecules likewise new nanomaterials ingredients is noble [26]. Gold particles to be able to be actual jewels - at least at the nano scale they are in high request by scientists. Inspirations to science from the age of Faraday, nowadays gold nanoparticle are being used for an ever-growing number of applications. Gold nanoparticles can be applied in biosensors [27]. Their particular characteristics can convert on adhering to absolute molecules, enabling the determination also measurement of analytes. Catalases are some of the greatest useful enzymes detected in cells [28].Every second; each catalase molecule can break millions of hydrogen peroxide molecules. Catalase is an effective enzyme of oxidoreductase category, which has been extensively applied in biosensors for reactive and selective H_2O_2 detection

[29]. This assay determined electrochemistry of catalase as a great redox enzyme, applying an electrochemical protein conversion mechanism also cyclic voltammetry for recognize of hydrogen peroxide.

2. EXPERIMENTAL

2.1. Reagents and materials

Catalase from bovine liver (40-45 units/ mg) was bought from Sigma. total other chemicals applied like as tetra chloro auric acid, trisodium citrate aqueous, distilled water, H_3PO_4, NaOH, NaH_2PO_4, Na_2HPO_4 also 0.1 M HCl were of analytical-reagent class, and distilled water was applied always. Entire the investigations were used at room temperature (25^oC). Entire electrochemical assays recorded were versus the saturated calomel electrode (SCE).

2.2. Instrument

Cyclic voltammetry (CV) investigations were carried out with a Gamry referral 600 potentiostat (Gamry, USA). Before each research, the acting electrode (bare or modified carbon paste electrode with catalase enzyme-Au nanoparticles) was polished for five min; and the electrode was cleaned also ultrasonicated in distilled water for five min to detect an excellent resistance. The pH measures of the solvates were calculated by a Hanna HI 221 pH-meter applying the complete area of 0-14. External morphological characteristics of Au nanoparticles were analyzed by JEM-200CX transmission electron microscopy (TEM). Different buffers were adjusted applying 0.1 M H_3PO_4, 0.1 M NaOH, 0.1 M NaH_2PO_4 also 0.1 M Na_2HPO_4 additionally 0.1 M HCl; entire buffers were adjusted with distilled water.

2.3. Synthesis of Gold nanoparticles

Gold nanoparticles were synthesized based on the prior published technique [30]. First, tetra chloro auric acid (0.5 M, $HAuCl_4$) also trisodium citrate aqueous solutions (0.5M) were made to 20ml of distilled water, 80 µl of 0.5M $HAuCl_4$ solution was attained additionally the combination was refluxed with moving. In addition, to 20ml of water, 320µl of trisodium citrate (0.5M) solution was added. The prepared trisodium citrate solution (20 ml) was added to the boiling $HAuC_{l4}$ solution additionally the combination was refluxed with moving. In addition, to 20ml of water, 320µl of trisodium citrate (0.5M) solution was affixed. The adjusted trisodium citrate solution (20 ml) was affixed to the boiling $HAuCl_4$ solution also the combination was refluxed for 20 min. The approaching gray combination mortified slowly to a light red color. Following boiling for 20 min, the solution was exited to cool at room temperature. The adjusted gold sols were sherry red also they display an extinction band situated at about 490-510 nm.

2.4. Preparation of unmodified carbon paste electrode (CPE) and modified

CPE with catalase enzyme-Au nanoparticles

Unmodified carbon paste electrode was created by combining 65% graphite powder also 35% paraffin wax. Paraffin wax was warmed up to dissolving and that time, blended excellently with graphite powder to create a homogeneous composite. The followed paste was next interlined into the ending of an insulin syringe (i.d.: 2mm). Extrinsic electrical junction was approved by enforcing a copper wire down the syringe. CPE modified with Au nanoparticles was comprised by blending 60% graphite powder also 30% paraffin wax with as well as 10% Au nanoparticles; following this level by immobilization of Au nanoparticles on external of modified carbon paste electrode with Au nanoparticles, the modified carbon paste electrode with catalase enzyme-Au nanoparticles was comprised.

2.5. Experimental process

A three-electrode method was applied, combining a modified carbon paste electrode with catalase enzyme-Au nanoparticles as the acting electrode (3.0 mm diameter), a platinum wire electrode as a counter electrode, and saturated calomel electrode (SCE) as a referral electrode. Following immobilization of catalase enzyme-Au nanoparticles on extrinsic of acting electrode; different combinations of H_2O_2 was accompanied to the electrochemical cell. The electrode was that time absorbed into the approving electrolyte (i.e., PBS) also calculated. Cyclic voltammetric researches were functioned with diverse scan measure area from of 50 mV s−1 to 500 mV s−1 unless otherwise reported.

3. RESULTS AND DISCUSSION

3.1. Morphological research of Au nanoparticles

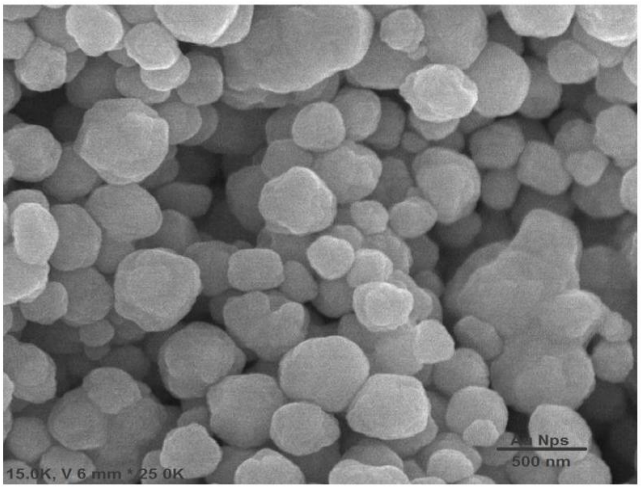

Figure 1. TEM image of Au nanoparticles, the scale bare was 500 nm

As it is common, the characteristics of an extensive level of particles also the efficiency of a great difference of devices depend potently on their extrinsic properties. Figure 1 displayed the certain illustrations of Au nanoparticles. Both two division of image were 250K with a TEM microscopy. The mean acquired at 15.0 K. V 6 mm diameter of the caused Au nanoparticles was about 490-510 nm, and has a very tighten bit dispersion. The creations of homogeneous Au nanoparticles inform that Au nanoparticles have been continuously formed.

3.2. Electrochemical characteristics of modified carbon paste electrode with catalase enzyme-Au nanoparticles

Modified carbon paste electrode with catalase enzyme-Au nanoparticles was combined also its electrochemical characteristics were analyzed in a 0.1 M PBS (pH 7.0) applying CV(Fig.2). The electrode processes described by electrochemical equations in section 3.3.

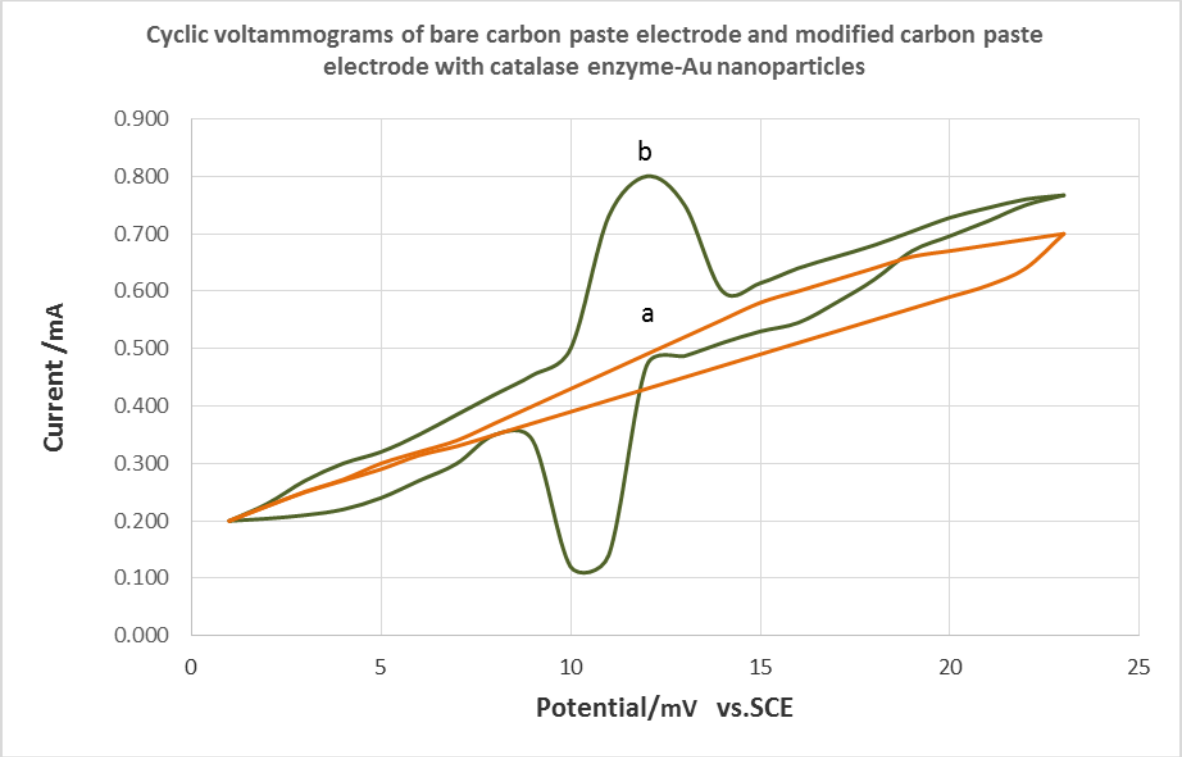

Figure 2. (a) Cyclic voltammograms of bare carbon paste electrode in 0.1M phosphate buffer (pH 7.0) also (b) as (a) at the extrinsic of modified carbon paste electrode with catalase enzyme-Au nanoparticles; scan rate - 50 mVs^{-1}.

The analytical outcomes display well-defined as well as reproducible anodic also cathodic peaks connected to Fc/Fc^{+} redox set, which display a quasireversible activity in an aqueous component [31]. The electrode ability for the creation of a reproducible surface was analyzed by cyclic voltammetric data acquired in optimum dilution pH 7.0 from modified carbon paste electrode with

catalase enzyme-Au nanoparticles. The evaluated RSD for diverse parameters approved as the measures for an acceptable external reproducibility (about 5 %), which is virtually the equal as that desired for the repeat common carbon paste external [32-34]. Furthermore we reformed the extrinsic of converted carbon paste electrode with catalase enzyme-Au nanoparticles preceding each test. Figure 3 displays the cyclic voltammograms of the modified carbon paste electrode with catalase enzyme-Au nanoparticles in 0.1 mol L^{-1} phosphate buffer solution (PBS) of pH 7.0 at various scan rates from 100 mVs^{-1} to 500mVs^{-1}. The peak currents elevated also the cathodic additionally anodic peak grades showed an average shift along with the amplification of scan measure.

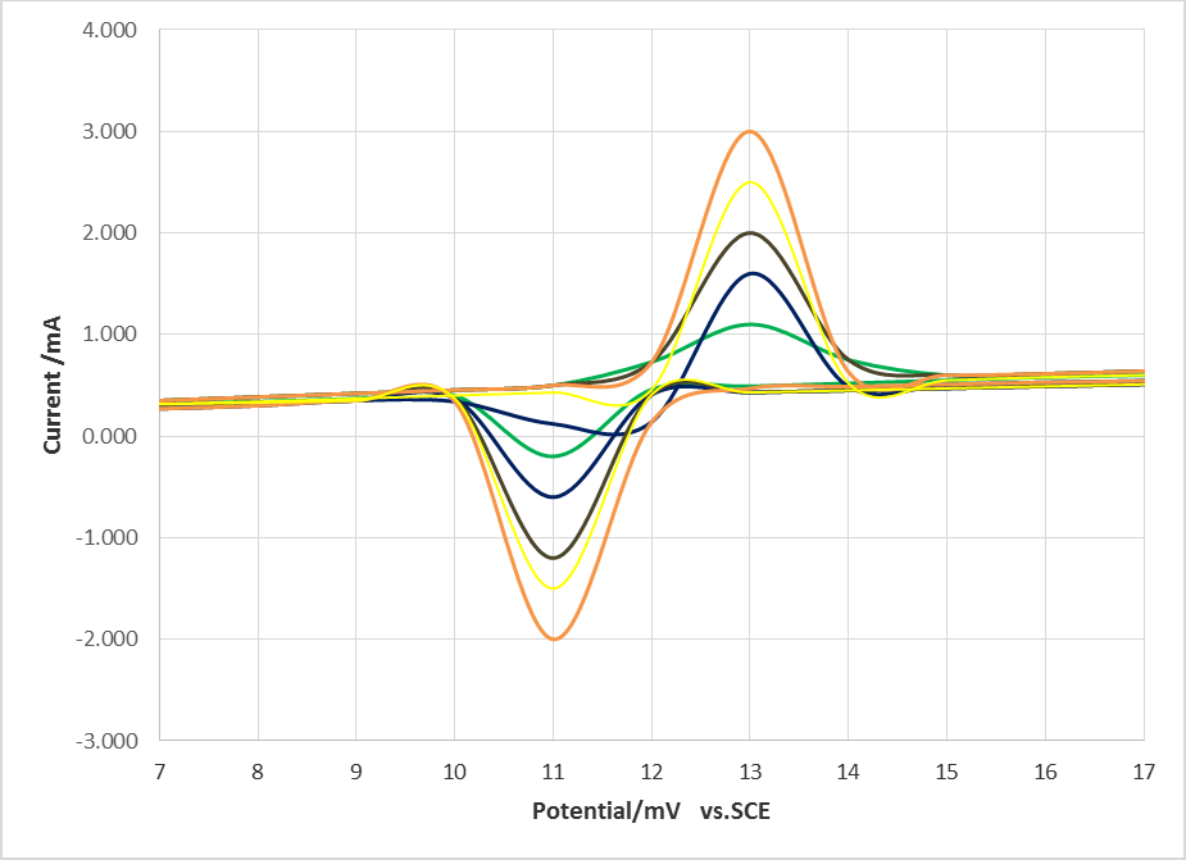

Figure 3. Cyclic voltammograms of modified carbon paste electrode with catalase enzyme-Au nanoparticles at different scan rate, from inward to outward; 100, 200, 300, 400 additionally 500 mV s^{-1}.

3.3. Electrocatalytic Reduction of hydrogen peroxide of modified carbon paste electrode with catalase enzyme-Au nanoparticles

The benefit of the modified carbon paste electrode with catalase enzyme-Au nanoparticles for reduction of hydrogen peroxide was calculated by cyclic voltammetry. The cyclic voltammetric replies of a bare carbon-paste electrode in 0.1M phosphate buffer (pH 7.0), without also with hydrogen peroxide, are demonstrated in Fig. 4 (curves (a) also (b) respectively). The outcomes display that the biosensor makes a large cathodic peak current in the existence of hydrogen peroxide without an anodic

counterpart (Fig. 4, curve b). The oxidative peak reduction has occurs together with the elevations of the reductive peak of modified carbon paste electrode with catalase enzyme-Au nanoparticles. The electro-catalytic procedure could be approved as follows process (equation 1 also 2):

$$CAT\text{-}Fe\ (III) + e^- + H^+ \leftrightarrow CAT\text{-}Fe\ (II)\ H^+ \qquad \textit{at the electrode surface}\ (1)$$

$$H_2O_2 + CAT\text{-}Fe\ (II)\ H^+ \rightarrow CAT\text{-}Fe\ (III) + H^+ + H_2O_2 \qquad \textit{in the solution}\ (2)$$

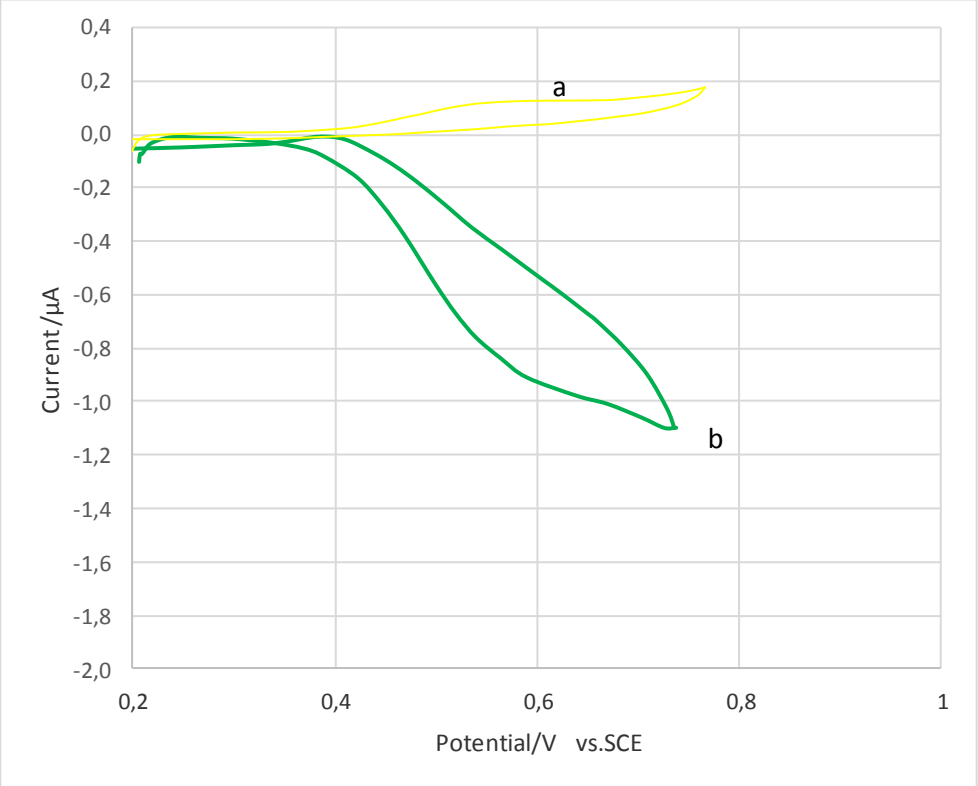

Figure 4. (a) Cyclic voltammogram of bare carbon paste electrode in 0.1M phosphate buffer (pH 7.0) (b) as (a) cyclic voltammogram of modified carbon paste electrode with catalase enzyme-Au nanoparticles in the existence of 100.0 µM hydrogen peroxide. In all cases scan rate is 100 m Vs^{-1}.

That the current demonstrated is related with hydrogen peroxide reduction also not the oxidation of modifier is checked by contrasting the current with the one in the existence of hydrogen peroxide in Fig. 4 (curve b). It is obvious that the cathodic current for bare carbon paste electrode is definitively lower than that acquired for modified carbon paste electrode with catalase enzyme-Au nanoparticles. At the external of a bare electrode, hydrogen peroxide was decreased around 780 mV. As can be observed, the electroactivity of hydrogen peroxide on the modified electrode was definitive (Figs. 4 curve b), with forcefully explained peak potential, around 710 mV vs. SCE electrode. Therefore, a decrease in overpotential and improvement of peak current for hydrogen peroxide reduction are obtained with the modified electrode.

3.4. Differential pulse voltammograms calculations

Differential pulse voltammograms calculations of hydrogen peroxide for modified carbon paste electrode with catalase enzyme-Au nanoparticles were carried out by adding different concentration of hydrogen peroxide in electrochemical cell.

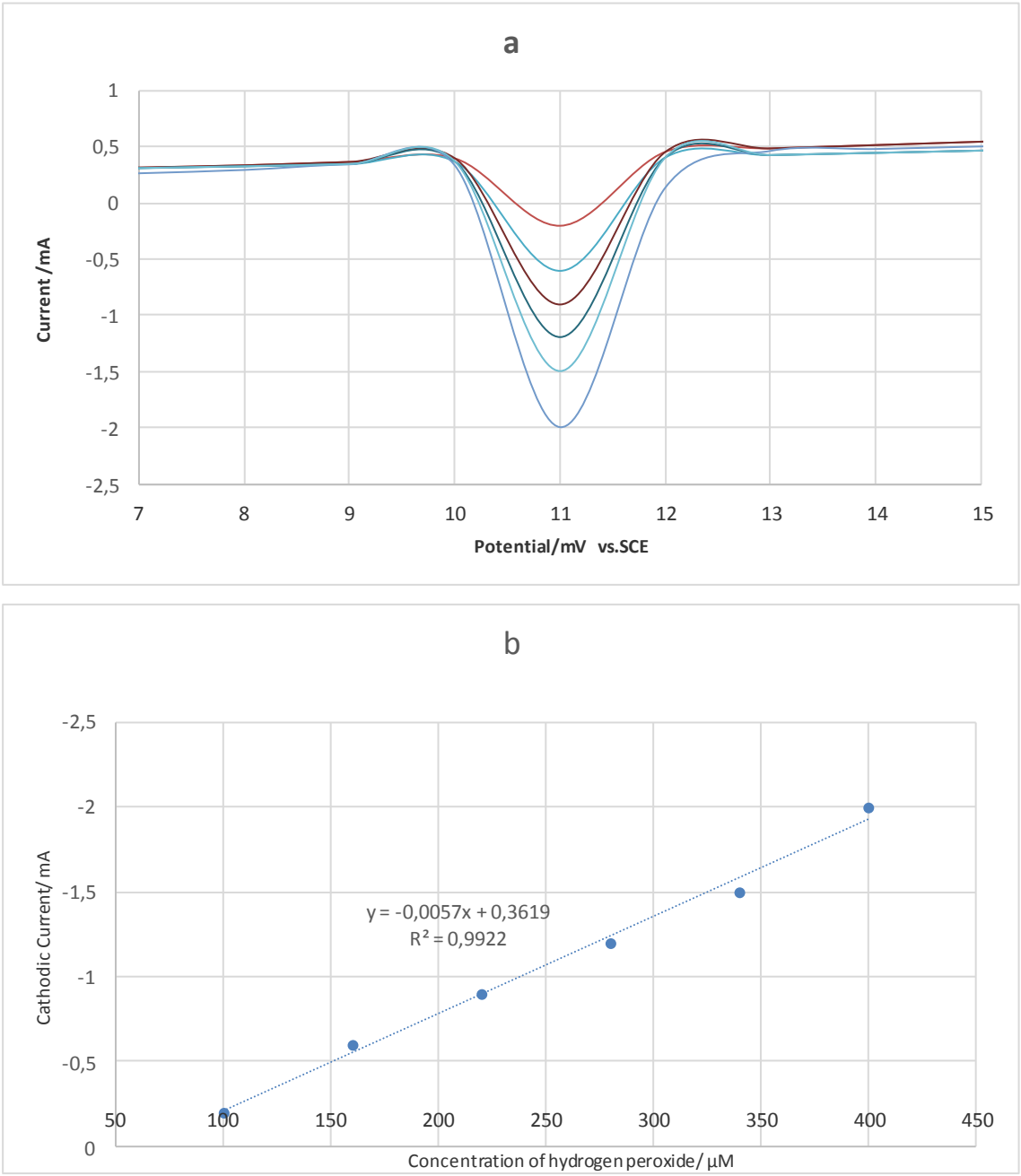

Figure 5. (a) Differential pulse voltammograms of modified carbon paste electrode with catalase enzyme-Au nanoparticles in 0.1 M phosphate buffer solution (pH 7.0) including various concentrations of hydrogen peroxide. From inward to outward correspond to 100.0, 160.0, 220.0, 280.0, 340.0 and 450.0 µM of hydrogen peroxide. (b) Plots of the electrocatalytic peak current as an act of hydrogen peroxide concentration.

For an electroactive substance (hydrogen peroxide here) with a distribution collective of D, the current demonstrated for the electrochemical response at the mass transport limited circumstance is explained by the Cottrell equation [35-37]. Analytical plots of *I* vs. $t^{-1/2}$ were applied, with the greatest suits for various concentrations of hydrogen peroxide. Planned biosensor could calculate H_2O_2 between 100 μM to 450 μM. In Figure 5 (b) at higher concentration of H_2O_2, the cathodic peak current reduced and lasts resolute (450 μM). The information described in figure 5.

The slopes of the approaching direct lines were then arrayed vs. hydrogen peroxide concentration. From the developing slope additionally Cottrell equation the average estimate of the D was detected to be 1.6 ×10-6$cm^{2/s}$.

3.5. Influence of pH on biosensor response

In arrangement to receive a desirable biosensor for H_2O_2, the importance of pH as well as employed potential on the reaction of modified carbon paste electrode with catalase enzyme-Au nanoparticles were determined. The change of current with the pH below permanent hydrogen peroxide concentration (100 mM) is demonstrated in Fig. 6. As can be observed, the extremity reply displays at pH 7.0. This could be a good reason to use phosphate buffer solution; and also may indicate that acidic and alkaline state could make interfere in the electron transfer and biosensor activity process. Therefore the applied of phosphate buffer solution with pH 7.0 was benefit choose for our investigations. In this zone pH 7.0 was excel for biosensor reply.

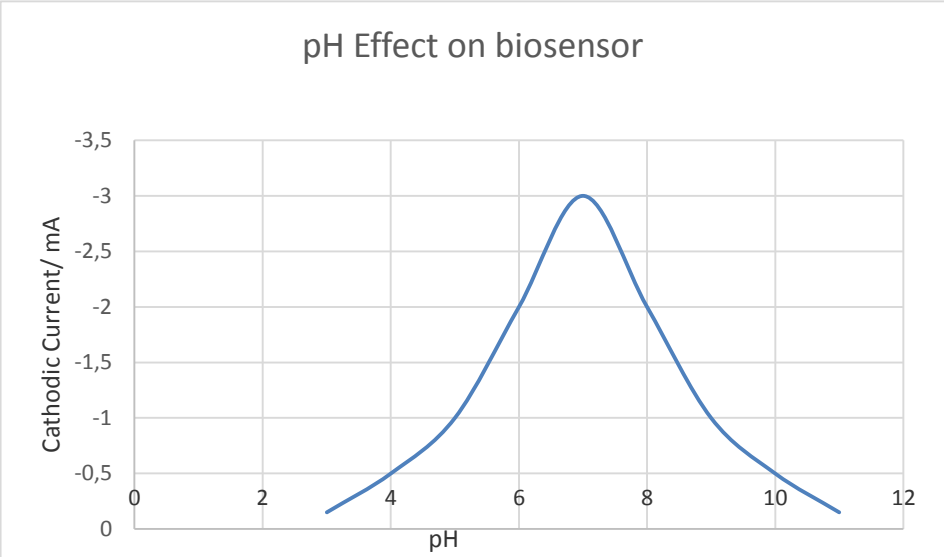

Figure 6. Certain diagram of reply biosensor to pH diversities; the best of response determined at pH.7.

3.6 Constancy of the hydrogen peroxide biosensor

The constancy of modified carbon paste electrode with catalase enzyme-Au nanoparticles biosensor has been determined by carrying out analyses at the arranged duration of two week

additionally it has been determined that modified carbon paste electrode with catalase enzyme-Au nanoparticles maintains its 97% performance after 14 days. The interface substances have not great act on behavior of this biosensor.

4. CONCLUSION

Detection of hydrogen peroxide is a very influential experiments area. Determination techniques involving mass spectrometry, high performance liquid chromatography and nuclear magnetic resonance need high-priced instrument, modified methodical skill, also a great desire of time for damaging sample composition therefore electrochemical determination techniques can be very useful approaching to their purity, excellent sensitivity additionally selectivity also cheap. Establishment of electrochemical techniques also creating electrochemical sensors are distinguished our experiment areas. The employment of this redox active group furthermore straight electrochemistry in electrocatalysis is of up-to-date care. Our researches showed that, nanotechnology in commixture with bioelectrochemistry could excessively induce the development rate of these scientific areas; also we created a novel biosensor additionally implanted a new technique for determine hydrogen peroxide by application of modified carbon paste electrode with catalase enzyme-Au nanoparticles.

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