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Design a Hydrogen Peroxide Biosensor by Cytochrome c and Cadmium Oxide Nanoparticles and its Application in Diagnostic Cancer Cells

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Abstract

Direct electrochemical of cytochrome c in carbon paste electrode is easily obtained and a reversible pair of peaks that are similar, of Fe (II) and Fe (III) with the formal potential (E°) about - 0 /295 volts, appears. This electrochemical reaction occurred in phosphate buffer solution 0.1 M (PBS) at PH = 7. Bio-modified electrode that has been produced by our group has a good ability to redox H_2O_2 that is promising for the mass production the third generation of biosensors. Accumulating evidence suggests that hydrogen peroxide (H_2O_2) plays an important role in cancer development. Experimental data have shown that cancer cells produce high amounts of H_2O_2. An increase in the cellular level of H_2O_2 has-been linked, to key alteration in cancer. in this project we design a biosensor that can determine hydrogen peroxide produced white cancer cells. In overall This Biosensor diagnostic process, can be very important in cancer.

Keywords: biosensor, hydrogen peroxide, cancer, cytochrome c, cadmium oxide nanoparticles

1. Introduction

A biosensor is composed of two elements: a biological recognition unit able to interact specifically with a target, and a transducer able to convert a change in property of the solution or surface, due to complex formation, into a recordable signal. In contrast with conventional bioassays, biosensors allow the detection of molecular interactions as they take place, without requiring auxiliary procedures, making them highly attractive for biotechnological applications. Initially, biosensor recognition elements were isolated from living systems[1-3]. However, many biosensor recognition elements now available are not naturally occurring, but have been synthesized in the laboratory. Biosensors can also meet the need for continuous, real-time in vivo monitoring to replace the intermittent analytical techniques used in industrial and clinical chemistry[4-5]. The sensing of targets, i.e. analytes of interest, is already being influenced by the emergence of engineered binding proteins.With the advent of nanostructures and new interface materials, these recognition elements will be major players in future biosensor development. Atoms and molecules are the essential building blocks of every object. The manner in which things are constructed with these basic units is vitally important to understand their properties and their reciprocal interactions. An efficient control of the synthetic pathways is essential during the preparation of Nano building blocks with different sizes and shapes that can lead to the creation of new devices and technologies

with improved performances[6]. To do this, two opposite, but complementary approaches are pursued. One is a top-down strategy of miniaturizing current components and materials, while the other is a bottom-up strategy of building ever-more-complex molecular structures atom by atom or molecule by molecule[7-8]. These two different methods highlight the organization level of nanosystems as the crossing point hanging between the worlds of molecular objects and bulk materials[9]. The top-down approach has been advanced by Richard Feynman in his often-cited 1959 lecture stating that "there is plenty of room at the bottom" and it is ideal for obtaining structures with long-range order and for making connections with macroscopic world[10-11]. Conversely, the bottom-up approach was pioneered and it is best suited for assembly and establishing short-range order at the nanoscale. The integration of the two techniques is expected to provide, at least in principle, the widest combination of tools for nanofabrication. Carbon-paste electrodes (CPEs), due to their ease of construction, renewability, and compatibility with various types of modifiers, have been widely used as a suitable matrix for preparation of modified electrodes. Further, they show rather low background current compared to solid graphite or noble metal electrodes [12]. We too used carbon paste electrode in this project and the modified electrode, is characterized based on scanning electronic microscopy (SEM) and cyclic voltammetry (CV). This modified electrode is applied for the electrochemical investigations. Many redox proteins are located on or in the biological membranes of living cells as a part of the electron transport chain [14-15]. Of these proteins, cyt c is a basic redox metalloprotein and Basic lysine residues are clustered around its active site, which is a heme iron porphyrin. The direct electron transfer reaction of cyt c has been the most widely investigated among all redox proteins. Since the first quasi-reversible electrochemistry of cyt c at a gold electrode was reported [16], many other compounds have been used to promote the electron transfer of cyt c on promoter modified electrodes [17]. More recently, lipids [18] and nucleic acid [19] have also been used as an electrode modifier to observe the direct electron transfer process of cyt c. The cyt c adsorbed in this way remained in its native form and gave well-defined redox peaks (11 ± 14). Beside this, cyt c could be covalently linked to the electrode surface and forms a more stable protein layer on the electrode surface [19]. One part of my research project was devoting to diagnostic hydrogen peroxide that product by cancer cells. Most of the energy that our cells need to live depends on a mitochondrial process that requires oxygen (O_2). In this process, called oxidative phosphorylation, ATP generation is coupled with a reaction in which O_2 is reduced to H_2O. Under certain conditions, O_2 can also be reduced to H_2O via the ROS superoxide anion (O^{-2}) and hydrogen peroxide (H_2O_2) [20-21]. It is recognized that the cellular production of O^{-2} and H_2O_2 favors the formation of other reactive oxygen and nitrogen species such as hydroxyl radical (OH^-) and peroxynitrite ($ONOO^-$) – and that an excessive production of these species causes oxidative stress and may play an important role in carcinogenesis [22]. Accumulating

experimental data suggest that an increase in the cellular concentrations of H_2O_2 can explain all these hallmarks of cancer.

2. Materials and methods

In the present work, we have synthesized cadmium oxide (CdO) nanoparticles by simple and low cost precipitation method using cadmium acetate and ammonium hydroxide as starting materials. All chemicals used in the experiment were of analytic reagent (AR) grade. Cadmium acetate was purchased from merck. (99.0%) and ammonia solution 25% GR too from Merck. All chemicals were used as received without further purification. Deionized water was used during the experiment. Cadmium acetate (6.66 g, 0.5 M) was dissolved in 100 ml water and ammonia solution was added to above solution dropwise until *p*H value of about 8 was reached with constant stirring. The white precipitate was formed and it was allowed to settle for 5-6 hour and then filtered and washed 3-4 times with water. It was dried at 100°C and then grinded. The resulting powder was calcined at 400°C for 2 hour. It turned into yellowish colour which confirmed the formation of CdO. Below equation shows the schematic diagram for the preparation of CdO nanoparticles.

$$(CH_3COO)_2\,Cd{\cdot}H2O + 2NH_4OH \rightarrow Cd\,(OH)_2 + 2H_2O + 2CH_3COONH_4$$

During calcinations as prepared powder loses H_2O, which is as follows:

$$Cd\,(OH)_2 \rightarrow CdO + H_2O$$

The temperature is 400 °C

We received cytochrome c from sigma. Solutions were prepared from analytical reagent grade chemicals without further purification using double distilled water. Phosphate buffer solutions (PBS) (0.1 M) were prepared form H_3PO_4, KH_2PO_4 and K_2HPO_4[23]. The pH of buffer solutions was adjusted with HCl and KOH solutions. Electrochemical experiments were performed with a computer controlled Autolab modular electrochemical system (palm sens, The Netherlands), driven with GPES software (Eco Chemie). A conventional three-electrode cell was used with a SCE (azar electrode co, The iran) as reference electrode, and a Pt wire as counter electrode. The working electrode was made by cadmium oxide nanoparticles modified carbon paste electrode. All measurements were conducted in a thermo stated temperature of 25±1 ˚C. The surface morphology of modified electrodes was studied with a scanning electron microscope (SEM) in Tehran University. Unmodified carbon paste electrode was prepared by mixing 65% graphite powder and 35% paraffin wax. Paraffin wax was heated till melting and then, mixed very well with graphite powder to produce a homogeneous paste. The resulted paste was then packed into the end of an insulin syringe (i.d.: 2mm). External electrical contact was established by forcing a copper wire down the syringe. CPE modified with cadmium oxide nanoparticles complex was prepared by mixing 60% graphite powder and 30% paraffin wax with 1, 5, 10 and 15%

synthesized cadmium oxide nanoparticles. The surface of the electrode was polished with a piece of weighting paper and then rinsed with distilled water thoroughly.

3. Results

The XRD pattern Fig. 1 for CdO nanoparticles, the diffraction peaks are absorbed at 2θ values. The prominent peaks have been utilized to estimate the grain size of sample with the help of Scherrer equation [24] $D = K\lambda/(\beta \cos \theta)$ where K is constant(0.9), λ is the wavelength(λ = 1.5418 A°) (Cu Kα), β is the full width at the half-maximum of the line and θ is the diffraction angle. The grain size estimated using the relative intensity peak for CdO nanoparticles was found to be 30 nm and increase in sharpness of XRD peaks indicates that particles are in crystalline nature. The reflections are clearly seen and closely match the reference patterns for CdO (Joint Committee for Powder Diffraction Studies (JCPDS) File No. 05-0640).

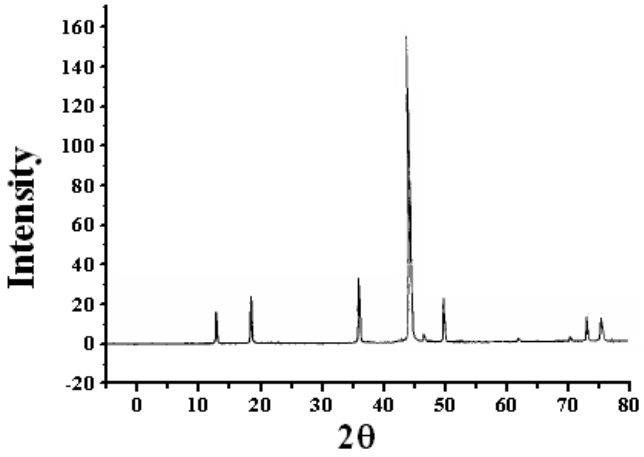


Figure 1. XRD pattern for CdO nanoparticles

The UV–visible absorption spectra of CdO nanoparticles are shown in Fig. 2 although the wavelength of our spectrometer is limited by the light source, the absorption band of the CdO nanoparticles have been shows a blue shift due to the quantum confinement of the excitons present in the sample compare with bulk CdO particles. This optical phenomenon indicates that these nanoparticles show the quantum size effect [24].

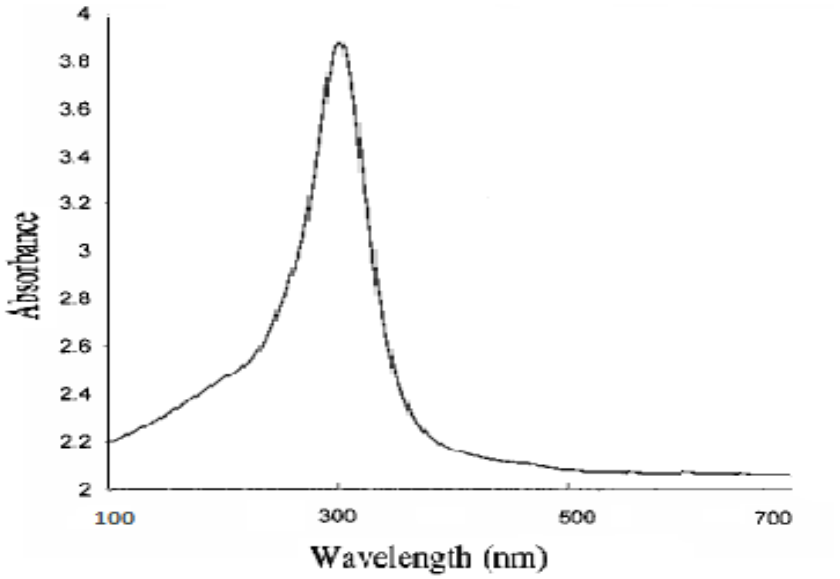


Figure 2. UV-Absorption spectra for CdO nanoparticles

The SEM image of cadmium oxide nanoparticles with ×13000 magnification at room temperature are shown in fig.3 that indicate Diameter of synthesized nanoparticles is about 30 nanometer.

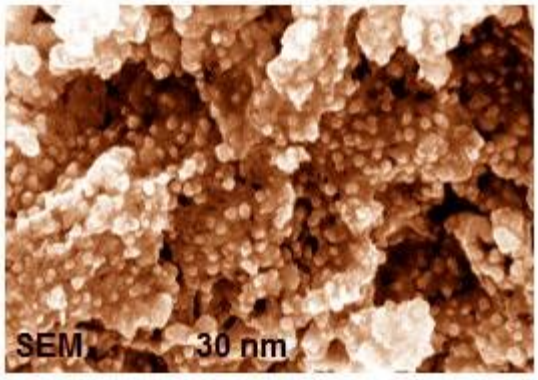


Figure 3. SEM image of cadmium oxide nanoparticles with ×13000 magnification

The electrochemical behaviors of cyt c/CPE/cdoNps molecules were examined by cyclic voltammetry and the results were shown in Figure 4.A pair of well-defined quasi-

reversible redox peaks appeared with the Epa = −0.244 V and Epc = −0.305 V (*vs*. SCE) and the ratio of Ipa/Ipc = 0.994. The formal potential (E0'), which is calculated by the midpoint of Epa and Epc, was got as −0.295 V (*vs*. SCE) and the peak-to-peak separation (ΔEp) was got as 145 mV at the scan rate of 200 mV s^{-1}, which indicated a fast heterogeneous electron transfer process The results are in good agreement with the characteristics of the heme Fe(III)/Fe(II) redox couples. While no voltammetric peak appeared at bare carbon paste electrode. These results indicated that the CPE modified by cadmium oxide nanoparticles and cytochrome c provided a suitable microenvironment for the cytochrome c molecules and the presence of cadmium oxide nanoparticles in the electrode could enhance the electron transfer rate for cytochrome c. Then the direct electron transfer of cytochrome c in the modified carbon paste electrode was achieved successfully. Figure 4 indicate CV of bare carbon paste electrode with any peak (a) and too in this figure, part b indicate CV of modified carbon paste electrode with cadmium oxide nanoparticles and cytochrome c. in this measurement, the scan rate was 200 mv/s.

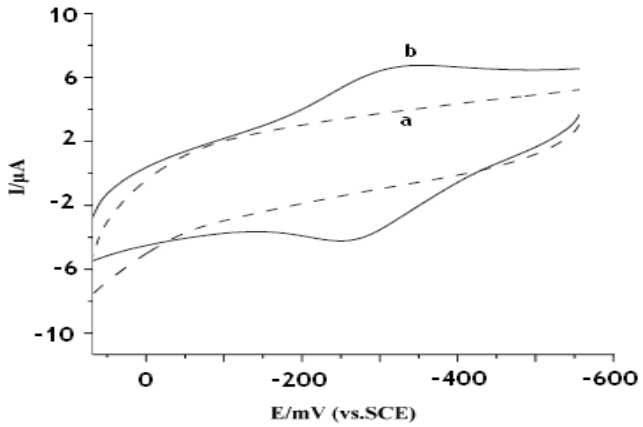


Figure.4. (a) CVof bare carbon paste electrode, (b) CV of carbon paste electrode that modified by cadmium oxide nanoparticles & cytochrome c in 0.1 M phosphat buffer solution& pH . 7 (the scan rate is 200mv/s).

In the next study of electron transfer properties of cytochrome c on modified carbon paste electrode with cadmium oxide nanoparticles were investigated and the effect of scan rates on a cyclic voltammogram of cytochrome c was studied.in Figure 5 (a & b) a linear range between the cathodic and anodic flows of protein myoglobin with different scan rates is observed. Correlation coefficients were equal to: 0.9972 for cathodic peak and 0.9972 too for anodic peak. This phenomenon suggested that the redox process was an adsorption-controlled one and the immobilized cytochrome c was stable.

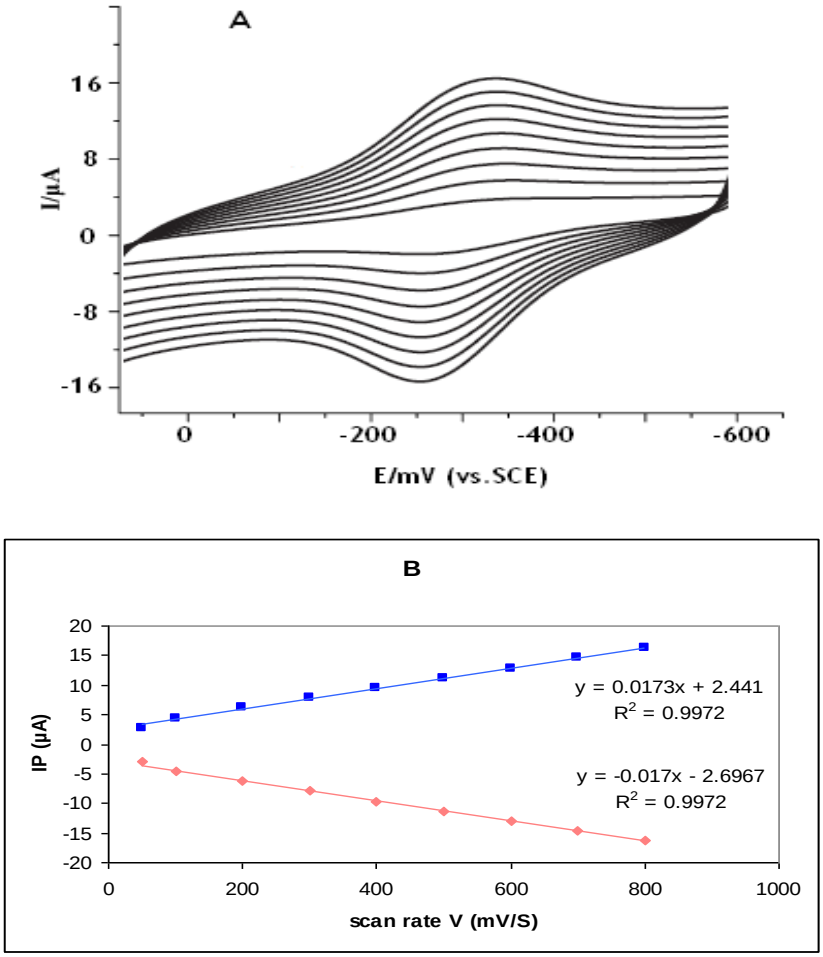


Figure.5. (a) Typical cyclic voltammograms of cytochrome c/ cadmium oxide nanoparticles /carbon paste electrode at different scan rates. The voltammograms (from inner to outer) designate scan rates of 50, 100, 200, 300, 400, 500, 600, 700 and 800 mV s^{-1}, respectively. (b) Dependence of the anodic (with red color) and cathodic (with blue color) peak currents on the scan rates. All the data were obtained at pH=7 and in 0/1M phosphate buffer solution (PBS).

The relationships of the anodic and cathodic peak currents with the scan rate were investigated. In Both the redox peak currents increased with the scan rate and two linear plots could be obtained, which was the typical behaviors of modified carbon paste electrode electrochemistry. The results suggested that all the electroactive cytochrome c Fe (III) in the electrode was converted to cytochrome c Fe (II) on the forward cyclic voltammetric scan and then transferred back to cytochrome c Fe(III) on the reverse scan.. With the increase of the scan rate, the reduction peak moved negatively and the oxidation peak moved positively, and the value of ΔE was less than 200 mV. According to the Laviron's equation for quasi-reversible modified carbon paste electrode

electrochemistry when the n × ΔE < 200 mV, the apparent heterogeneous electron transfer rate constant (*ks*) can be calculated with the following equations:

$$Epc = E^{0'} - \frac{2.3\,RT}{\alpha\, nF}\log v \quad (1)$$

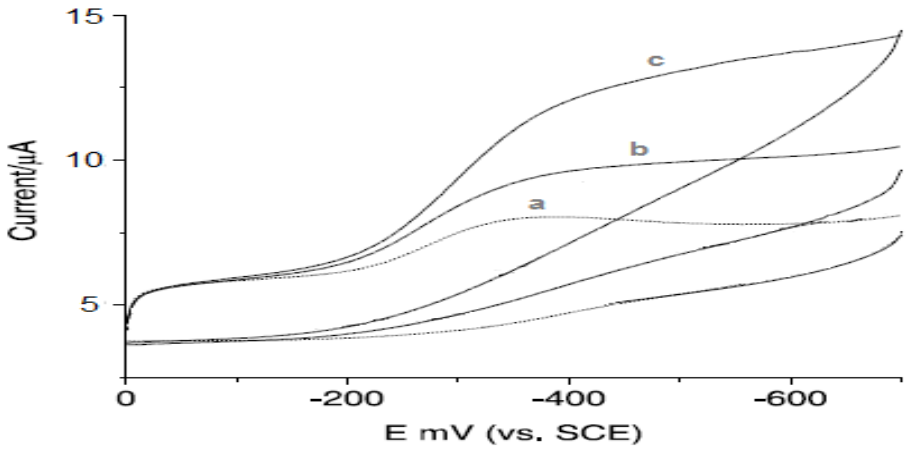
$$Epa = E^{0'} + \frac{2.3RT}{(1-\alpha)\, nF}\log v \quad (2)$$

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nF\nu} - \frac{nF\Delta Ep\alpha(1-\alpha)}{2.3RT} \quad (3)$$

where α is the charge transfer coefficient, n is the number of electron transfer and v is the scan rate. The relationships of Ep with log*v* were calculated. Form the slope and the intercept the value of n and α were got as 0.97 and 0.34, respectively. From the equation 3, the value of *ks* was calculated as 1.013 s^{-1}, which was also proved as a quasi-reversible redox process of cyt c. The most important part of this research project is to measure the hydrogen peroxide. Sensitive determination of H_2O_2 is of great significance in biological, clinical and environmental and many other fields. Many analytical methods have been developed for this reason, such as electrochemistry, photometry. Among these methods, amperometric enzyme-based biosensors have received significant concentration due to its expediency, high selectivity and sensitivity [24-25]. As mentioned before a significant confront to development of sensitive and stable sensors comes from the effective immobilization of enzyme to solid electrode surface [12]. There are many materials have been used to immobilize enzyme on the electrode surface toward H_2O_2 sensing, such as quantum dots [1], polymers [7-8], mesoporous materials [11] and nanomaterials [7]. Among these, nanomaterials have attracted great research interest in biosensor because of their flexibility of the physical and chemical properties [26-27]. In addition, in order to retain the electrocatalytic activity and further modification onto the electrode surface, various methods have been used to enhance the electron transfer, such as electropolymerization [28], sol–gel [29], layer by layer assembly [30], covalent bonded immobilization [31] and direct embedded biocompatible membrane [32]. You can see the cyclic voltammograms of the cytochrome c-cadmium oxide nanoparticles modified electrode in PBS, at pH 7.0, containing different concentrations of H_2O_2 are shown in Figure 6. Upon the addition of H_2O_2 to the electrochemical cell, the reduction peak current of the immobilized cyt c

increased, indicating a typical electro-catalytic behavior to the reduction of H_2O_2. The calibration curve (Figure 6b) shows the linear dependence of the cathodic peak current on the H_2O_2 concentration in the range of 20-100 μM. The recent experiment has introduced a new biosensor for the sensitive determination of H2O2 in the solution and this important result, will have many applications in cancer therapy.

(A)



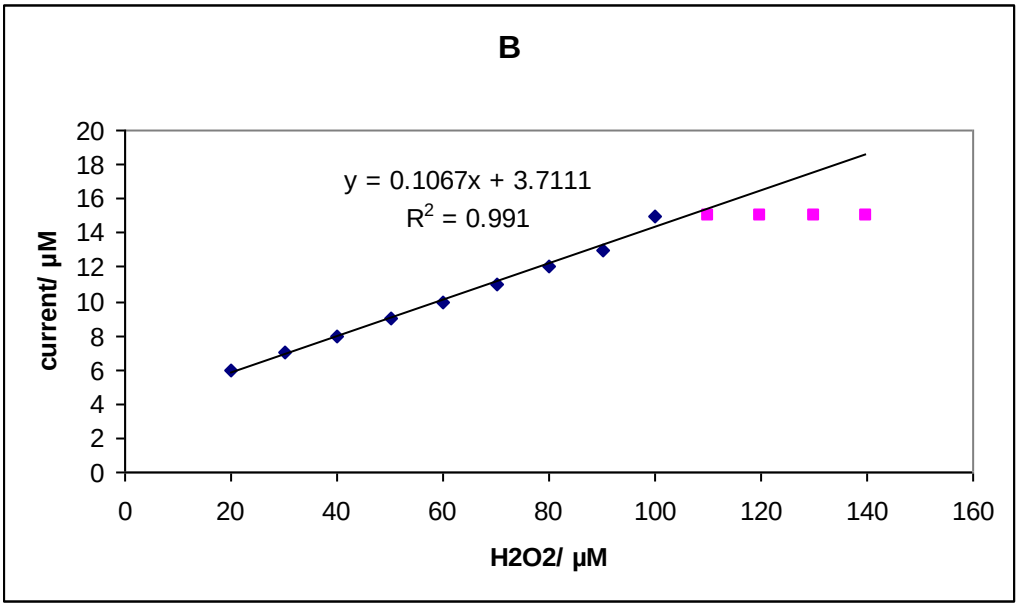


Figure 6. a) Cyclic voltammograms obtained at an cyt c-cdo nanoparticles modified carbon paste electrode in 0.1 M PBS (pH 7.0) for 0, 0.007 and 0.07 M concentrations of H_2O_2 and b) the relationship between cathodic peak current of cyt c and different concentrations of H_2O_2 (scan rate: 100 mVs^{-1}).

As discussed above, there is evidence that H_2O_2 may have an important function in cancer development. However, there is also compelling evidence that have shown that increasing the cellular levels of H_2O_2 may be an efficient way of killing cancer cells. Figure. 7 represents that different concentrations of H_2O_2 can produce different cellular effects; this may contribute to explain apparently controversial studies that have shown, for instance, that H_2O_2 can both produce apoptosis resistance [33] and be an efficient inductor of apoptosis in cancer cells [34]. Numerous reports have demonstrated that H_2O_2 can induce cell death in cancer cells. It has been observed, that a significant increase in the intracellular H_2O_2 production and downstream acidification provides an environment conducive for apoptotic cell death in tumor cells [35].

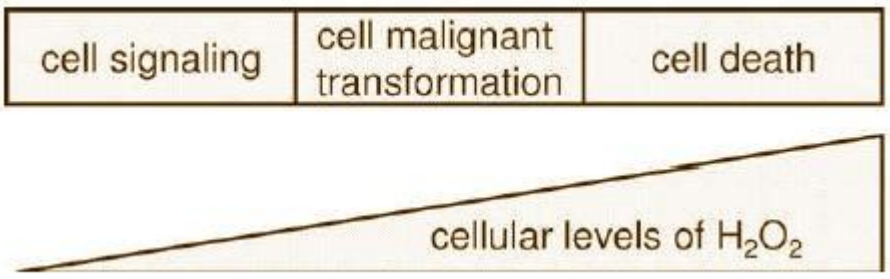


Fig. 7. Different cellular effects by different cellular levels of H_2O_2. Low levels of H_2O_2 have a physiological role in cell signaling. A constitutive increase in the cellular levels of H_2O_2 has been associated with the carcinogenesis process. Higher levels of H_2O_2 can produce cell death.

Recent data support that increasing the cellular levels of H_2O_2 by using H_2O_2-generating drugs may be an efficient way of killing cancer cells. Thus, the anticancer effect of various chemotherapeutic agents currently used in the clinic (e.g., paclitaxel, cisplatin, arsenic trioxide, etoposide, doxorubicin) is mediated, at least in part, by an increase in the cellular levels of H_2O_2 [36–46]. There is experimental evidence that cancer cells are more susceptible to H_2O_2-induced cell death than normal cells [43–46]. Using several cancer and normal cell lines, it was observed that pharmacologic ascorbic acid concentrations selectively killed cancer cells; this effect was mediated by H_2O_2. Researchers showed, for instance, that a concentration of 50lM of H_2O_2 induced more percentage of cell death in Burkitt's lymphoma cells than 250lM in normal lymphocytes and normal monocytes [44]. It is not clear why specific concentrations of H_2O_2 can kill cancer cells selectively. It has been proposed that, in normal cells, ROS are at low levels, originate from NADPH oxidase and the concentration of H_2O_2 is regulated by the glutathione system. By contrast, in tumor cells, high levels of ROS close to the threshold of cytotoxicity are produced through the mitochondrial respiratory chain, and H_2O_2 concentration is controlled by catalase [45]. On the other hand, Researchers found no correlation between H_2O_2-mediated selective cell death of cancer cells and intracellular glutathione concentrations, catalase activity, or glutathione peroxidase activity [44]. A possible explanation to the high susceptibility of cancer cells to H_2O_2 is represented in Fig. 8.

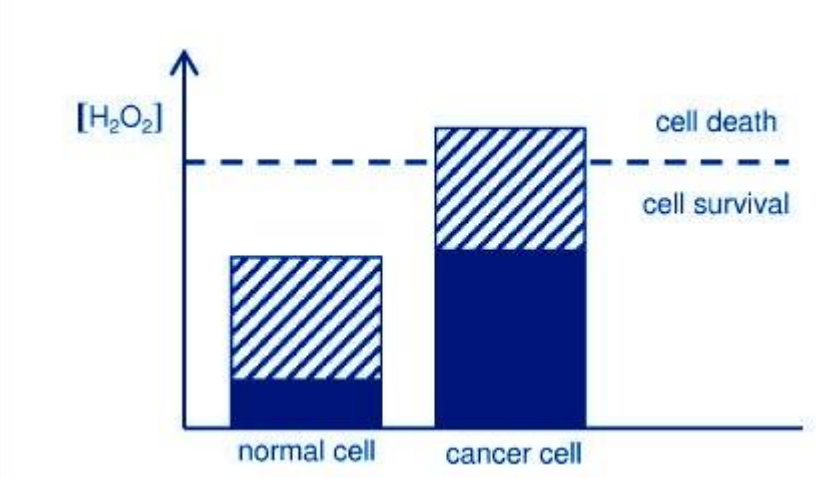


Fig. 8. Selective killing of cancer cells by H_2O_2. There is evidence that cancer cells have higher levels of H_2O_2 than normal cells (represented in black) and that there is a threshold of H_2O_2 above, which cells, cannot survive. This might explain why specific concentrations of H_2O_2 (represented in striped black) can produce selective death of cancer cells.

4. Conclusion

A new biosensor for H_2O_2 was prepared based on carbon paste electrode and cytochrome c. in this project the electrode was modified by cadmium oxide nanoparticles as facilitor. The amperometric experiments showed excellent electrocatalytical activity of the biosensor for H_2O_2. Direct electrochemical of cytochrome c in carbon paste electrode is easily obtained and a reversible pair of peaks that are similar, of Fe (II) and Fe (III) with the formal potential (E°) about - 0 /295 volts, appears. We hope with this designed new biosensor, we provide a new way to detect cancer cells.

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