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# Direct Electron Transfer of Cytochrome C on Cadmium Oxide Nanoparticles Modified Carbon Paste Electrode

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In this study, the direct electrochemistry of cytochrome c immobilized on a modified carbon paste electrode with cadmium oxide nanoparticles was described. The prepared cadmium oxide nanoparticles were characterized by scanning electron microscope (SEM) and transmission electron microscope (TEM). The direct electron transfer of the immobilized cytochrome c exhibited two couples of redox peaks with the formal potentials of −0.305 V and -0.244 V (vs. SCE) in 0.1M (pH 7.0) PBS, respectively. The biosensor displayed an excellent electro-catalytical response to the reduction of $H_2O_2$. The linear range of this biosensor for $H_2O_2$ determination was from 20 to 210μM. The established biosensor exhibited fast response, high sensitivity, good reproducibility and stability.

**Keywords:** bioelectrochemistry, cytochrome c, cadmium oxide nanoparticles, carbon paste electrode

## 1. INTRODUCTION

Nanotechnology is the science of the small; the very small. It is the use and manipulation of matter at a tiny scale[1]. At this size, atoms and molecules work differently, and provide a variety of surprising and interesting uses. Nanotechnology should not be viewed as a single technique that only affects specific areas[2]. It is more of a 'catch-all' term for a science that is benefiting a whole array of areas, from the environment, to healthcare, to hundreds of commercial products[3]. Nanomaterials such as quantum dots, carbon nanotubes and fullerenes will have applications in many different sectors because of their new properties[4]. So quantum dots can be used in solar cells, but also in optoelectronics, and as imaging agents in medical diagnostics[5]. Electricity is a common phenomenon

in our modern world, powering everything from the lights in your room to the computer in front of you[6]. Electricity is the flow of electrons within a conductive material, such as metal wires. These electrons flow in bulk, meandering from atom to atom along the wire[7]. Cells also use electricity to power many processes, but the electrons move in a very different way[8]. The electrons do not flow smoothly along a cell-sized wire. Instead, electrons are transported one at a time, jumping from protein to protein[9-10]. In this way, the electrons may be picked up from one particular place and delivered exactly where they are needed[11]. Direct electron transfer of some proteins and enzymes for biosensors has received considerable attention in recent years[12]. The studies on direct electron transfer process between proteins or enzymes and electrodes cannot only provide us the information to elucidate their metabolic processes in the biological system, but also establish a foundation for constructing the third generation of electrochemical biosensors and a new kind of bioreactors[13]. Cytochrome c is a carrier of electrons. Like many proteins that carry electrons, it contains a special prosthetic group that handles the slippery electrons[14]. Cytochrome c contains a heme group with an iron ion gripped tightly inside. The iron ion readily accepts and releases an electron. The surrounding protein creates the perfect environment for the electron, tuning how tightly it is held[15]. A chemical biosensor is a sensor that produces an electric signal proportional to the concentration of biochemical analytes[16-17]. These biosensors use chemical as well as physical principles in their operation[18]. The body is composed of living cells. These cells, which are essentially chemical factories, the input to which is metabolic food and the output waste products, are the building blocks for the organ systems in the body. The functional status of an organ system is determined by measuring the chemical input and output analytes of the cells[3]. Therefore, the majority of tests made in the hospital or the physician's office deal with analyzing the chemistry of the body[19-21]. Cadmium oxide (CdO) is n-type semiconductor used as a transparent conductive material prepared as a transparent conducting film back. Cadmium oxide has been used in applications such as photodiodes, phototransistors, photovoltaic cells, transparent electrodes, liquid crystal displays, IR detectors, and anti-reflection coat[4].in this research we used of cadmium oxide nanoparticles as facile electron transfer between cytochrome c and carbon paste electrode. Carbon paste electrodes (CPEs) belong to promising electrochemical or bioelectrochemical sensors of wide applicability[22-23]. It is well accepted that $Cyt_c$ exhibits peroxidase activities, which can catalyze the reductive reaction of hydrogen peroxide. Determinations to $H_2O_2$ based on such catalytic interactions are widely reported [24]. Effective immobilization and maintenance to the bioactivity on a proper substrate are essential to get a stable and sensitive response signal. Many methods for protein immobilization are extensively investigated, such as physical and biophysical methods [25].

## 2. EXPERIMENTAL

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 and Ammonia solution was purchased 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 drop wise until pH 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 color, which confirmed the formation of CdO. Below equation shows the schematic diagram for the preparation of CdO nanoparticles.

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

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 by using of double distilled water. Phosphate buffer solutions (PBS) (0.1 M) were prepared form $H_3PO_4$, $KH_2PO_4$ and $K_2HPO_4$[25]. 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, made in Netherlands), driven with GPES software (Eco Chemie). A conventional three-electrode cell was used with a SCE (azar electrode co, made in Iran) as reference electrode, and a platine 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 Synthesized cadmium oxide nanoparticles was studied with scanning electron microscope (SEM) and transmission electron microscope (TEM) 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. The modified CPE with cadmium oxide nanoparticles was prepared by mixing 60% graphite powder and 30% paraffin wax with 1%, 5%, 10% and 15% of synthesized cadmium oxide nanoparticles. The surface of the electrode was polished with a piece of weighting paper and then rinsed with distilled water.

## 3. RESULTS AND DISCUSSION

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 [26] $D = K\lambda/(\beta \cos \theta)$ 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).

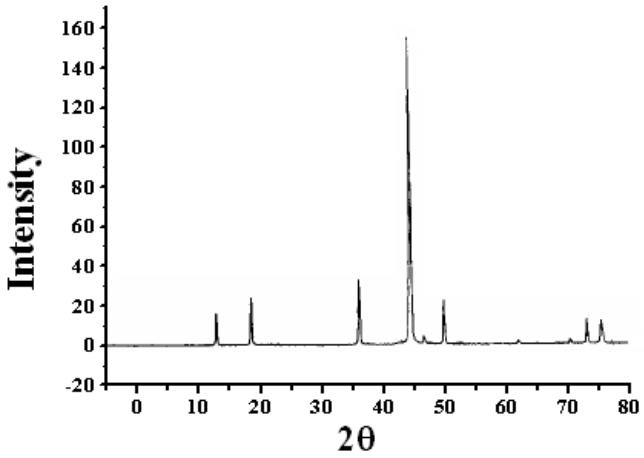



**Figure 1.** XRD pattern for CdO nanoparticles

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 in sample compare with bulk CdO particles. This optical phenomenon indicates that these nanoparticles show quantum size effect [27].

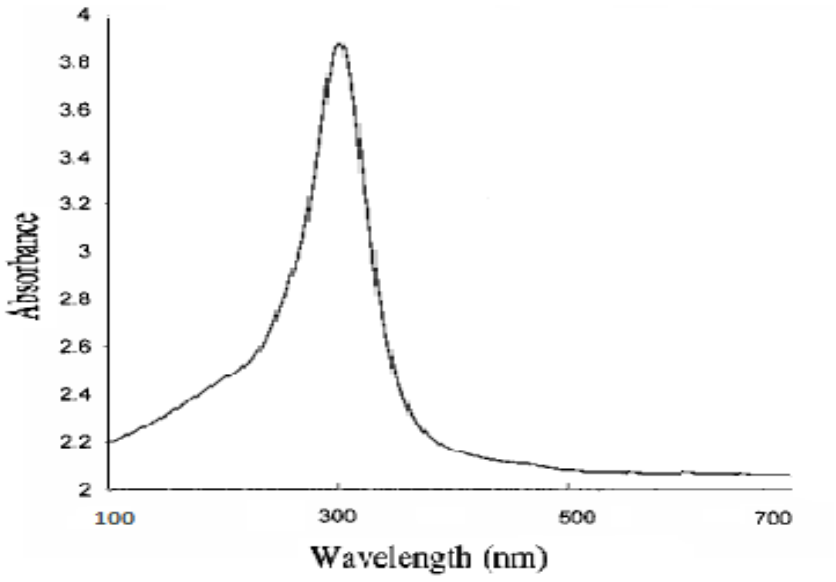



**Figure 2.** UV-Absorption spectra for CdO nanoparticles

The SEM and TEM images of cadmium oxide nanoparticles were captured at room temperature and are shown in fig.3 .The uniform porous structure cadmium oxide nanoparticles increased the homogeneous loading and affinity to the substrate of protein molecules, and provided good preparation reproducibility of the cytochrome c modified electrodes.

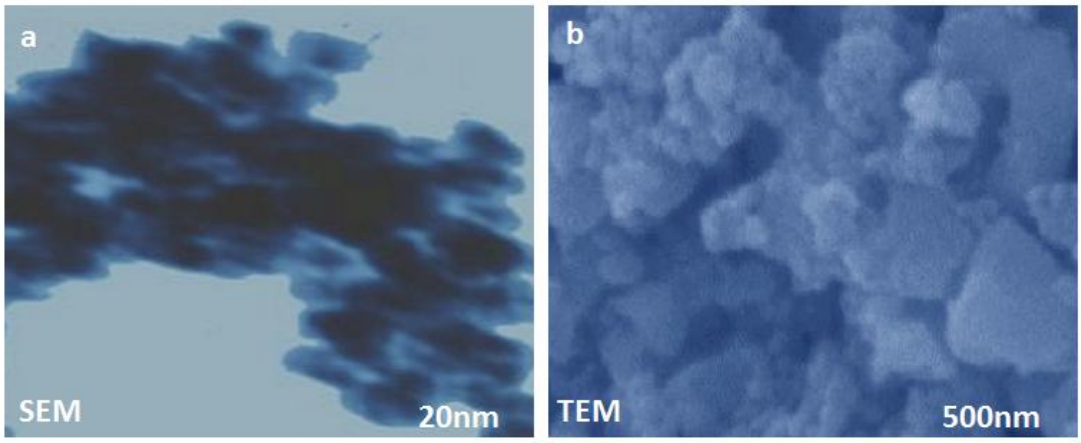



**Figure 3.** (a) SEM image of cadmium oxide nanoparticles, (b) TEM image of cadmium oxide nanoparticles

*3.1. Direct electrochemistry of cyt c/cdo NPs/ carbon paste electrode*

The cyclic voltammogram of cyt c/cdo nanoparticles/ carbon paste electrode displayed a couple of stable and well-defined redox peaks at −0.305 and −0.244 V at 200mVs$^{-1}$(Fig. 4 b), while no obvious electrochemical response was observed at bare carbon paste electrode (Fig. 4 a). The improvement in direct electrochemistry in presence of cytochrome c and cadmium oxide nanoparticles was due to the increase of the electron transfer between cytochrome c and modified electrode.

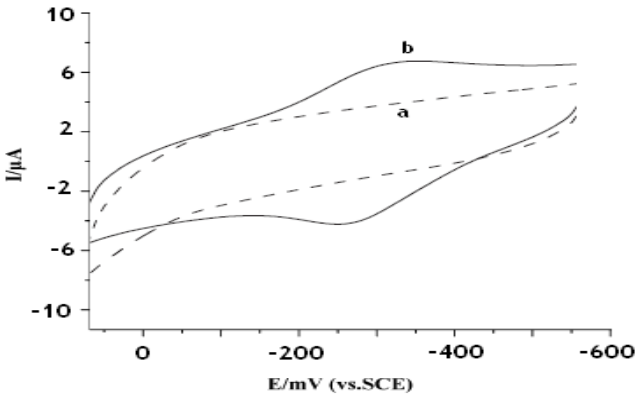
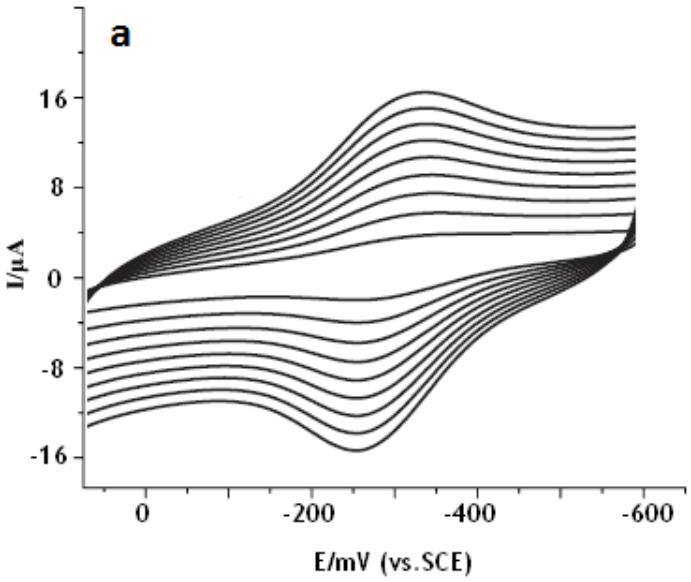



**Figure 4.** (a) CVof bare carbon paste electrode, (b) CV of carbon paste electrode that modified by cadmium oxide nanoparticles & cytochrome c in phosphat buffer solution 0.1 M & pH . 7 ( the scan rate is 200mv/s)

The further increase of the peak currents at different scan rates, indicated cadmium oxide nanoparticles was very important for facilitating the electron exchange. Figure 4 indicate CV of bare carbon paste electrode with no 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.

The formal potential $E^o$ of the heme FeIII/II couple in cyt c/cdo nanoparticles/ carbon paste electrode, estimated as the midpoint of reduction and oxidation potentials, was −(0.295±2) V (versus SCE) in 0.1M pH 7.0 PBS. The difference of anodic and cathodic peak potential values was $\Delta E$ = 0.061 V. The collected voltammograms in Fig. 5 a, substantiated a statement that the nanometer-scale cadmium oxide particles could play a key role in the observation of the cytochrome c CV response. On the grounds that the surface-to-volume ratio increases with the size decrease and because of the fact that the protein size is comparable with the nanometer-scale building blocks, these nanoparticles displayed a great effect on the electron exchange assistance between cytochrome c and carbon paste electrode. To further investigate the cytochrome c characteristics at the Cyt c/Cdo NPs/CPE electrode, the effect of scan rates on the cytochrome c voltammetric behavior was studied in detail. The baseline subtraction procedure for the cyclic voltammograms was obtained in accordance with the method reported [28]. The scan rate (ν) and the square root scan rate ($\nu^{1/2}$) dependence of the heights and potentials of the peaks are plotted in Fig. 5b and Fig. 5c respectively. It can be seen that the redox peak currents increased linearly with the scan rate, the correlation coefficient was 0.9972 (ipc = 0.0173ν + 2.441) and 0.9972 (ipa = -0.017ν -2.6967), respectively. This phenomenon suggested that the redox process was an adsorption-controlled and the immobilized cytochrome c was stable. It can be seen that the redox peak currents increased more linearly with the ν in comparison to that of $\nu^{1/2}$. However, there is clearly a systematic deviation from linearity in this data, i.e. low scan rates are always on one side of the line and the high scan rate points are on the other.



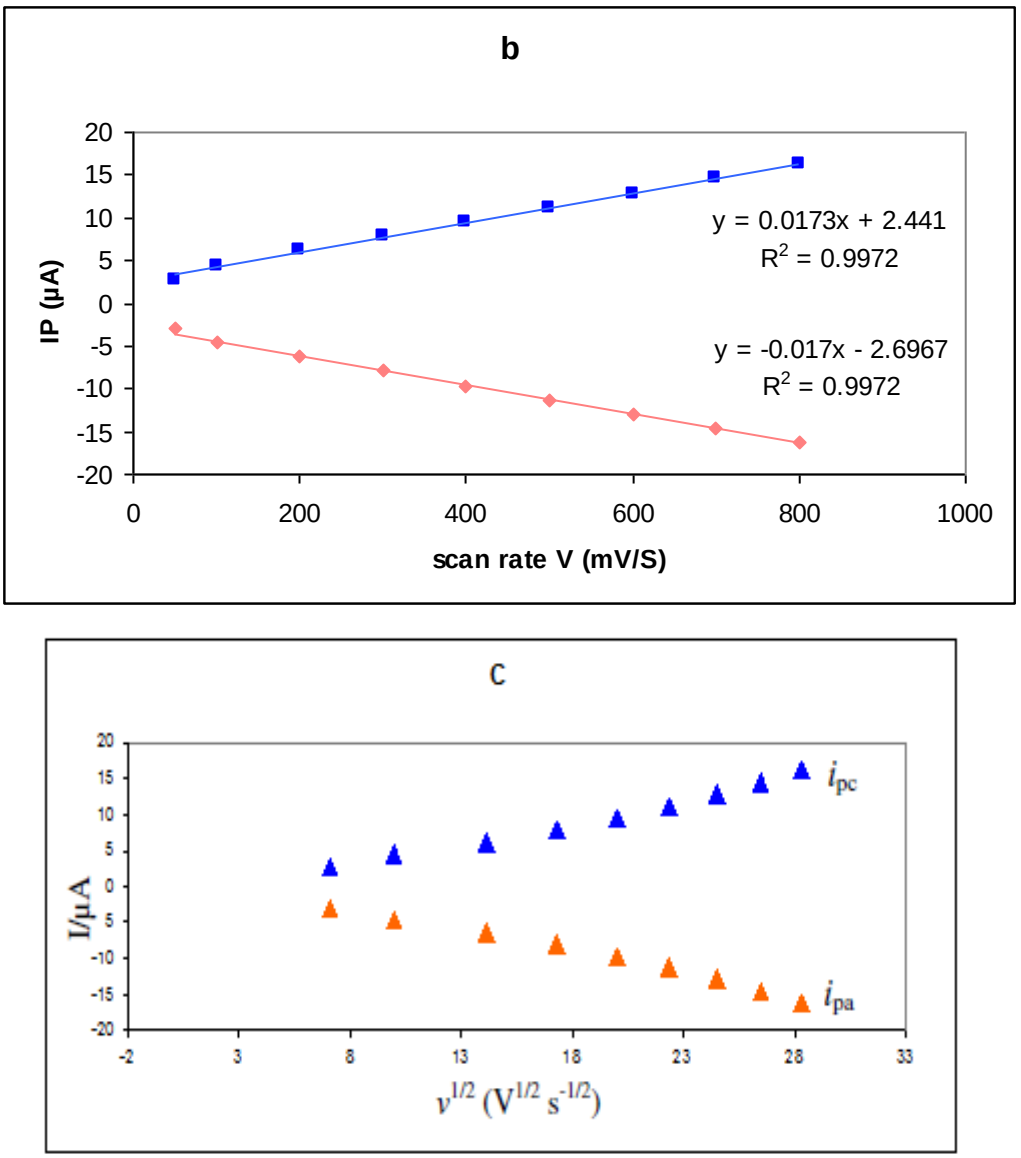



**Figure 5.** (a) CVs of Cyt c/cdo NPs/CPE electrode in PBS at various scan rates, from inner to outer; 50, 100, 200, 300,400, 500, 600, 700 and 800 mV $s^{-1}$, the relationship between the peak currents (ipa, ipc) vs., (b) the sweep rates and (c) the square root of sweep rates.

The anodic and cathodic peak potentials are linearly dependent on the logarithm of the scan rates (ν) when ν > 1.0 V $s^{-1}$, which was in agreement with the Laviron theory, with slopes of -2.3RT/αnF and 2.3RT/ (1 -α) nF for the cathodic and the anodic peak, respectively [29]. So, the charge-transfer coefficient (α) was estimated as 0.47.

Furthermore, the heterogeneous electron transfer rate constant (ks) was estimated according to the following equation [30-31]:

$$[\log ks = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nF\nu} - \frac{\alpha(1-\alpha)nF\Delta EP}{2.3\,RT}] \quad (1)$$

Here, n is the number of transferred electrons at the rate of determining reaction and R, T and F symbols having their conventional meanings. $\Delta E_p$ is the peak potential separation. The $\Delta E_p$ was equal to 0.230, 0.350 and 0.542 V at 0.7, 1 and 2 V $s^{-1}$, respectively, giving an average heterogeneous transfer rate constant (ks) value of 0.84 $s^{-1}$.

*Cyt c/cdO NPs/CPE to reduction of $H_2O_2$*

Upon addition of $H_2O_2$ to 0.1M pH 7.0 PBS, the cyclic voltammogram of the Cyt c/CdO NPs/CPE electrode for the direct electron transfer of cyt c changed dramatically with an increase of reduction peak current and a decrease of oxidation peak current (Fig. 6a), while the change of cyclic voltammogram of bare or cdo Nps/ CPE was negligible (not shown), displaying an obvious electrocatalytic behavior of the cyt c to the reduction of $H_2O_2$. The decreases of the oxidative peak current together with the increases of the reductive Cyt c/ cdo NPs/CPE. The electro-catalytic process could be expressed as follows:

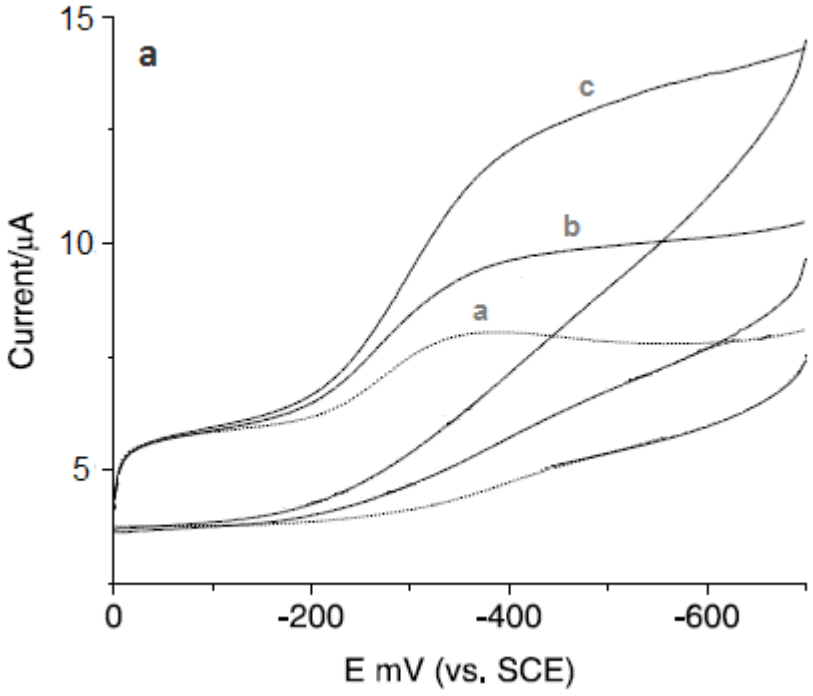
$$\text{Cyt c Fe (III)} + H_2O_2 \rightarrow \text{Compound I} + H_2O \quad (2)$$

$$\text{Compound I} + H_2O_2 \rightarrow \text{cyt c Fe (III)} + O_2 + H_2O \quad (3)$$

$$\text{Cyt c Fe (III)} + H^+ + e^- \rightarrow \text{cyt c Fe (II) (at electrode)} \quad (4)$$

$$\text{Cyt c Fe (II)} + O_2 \rightarrow \text{cyt c Fe (II)-}O_2\text{ (fast)} \quad (5)$$

$$\text{Cyt c Fe (II)-}O_2 + 2\,H^+ + 2\,e^- \rightarrow \text{Cyt c Fe (II)} + H_2O_2\text{ (at electrode)} \quad (6)$$



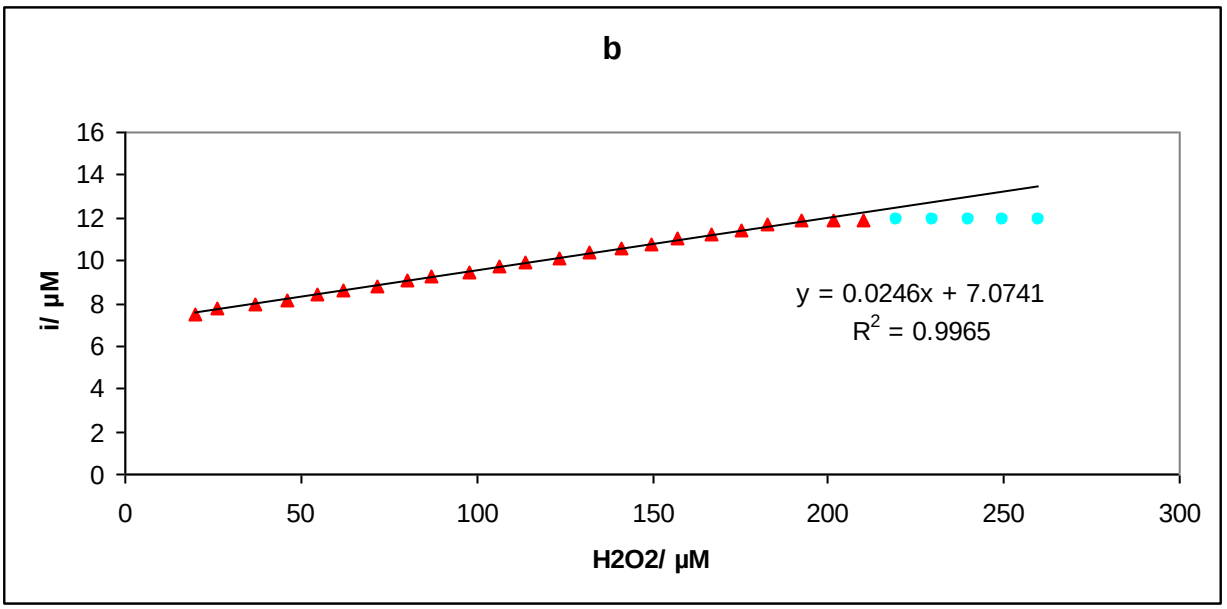



**Figure 6.** (a) Cyclic voltammograms obtained at an Cyt c/ CdO NPs/CPE in 0.1M phosphate buffer solution (pH 7.0) for different concentrations of and (b) the relationship between cathodic peak current of cyt c and different concentrations of $H_2O_2$ (scan rate: 200 $mVs^{-1}$).

Calibration curve (Figure 6b) shows the linear dependence of the cathodic peak current on the H2O2 concentration in the range of 20 to 210 µM. In Figure 6 b, at higher concentration of H2O2, the cathodic peak current decreased and remains constant. Upon addition of an aliquot of H2O2 to the buffer solution, the reduction current increased steeply to reach a stable value (Fig 6 b). This implies electrocatalytic property of electrode. Thus, this experiment has introduced a new biosensor for the sensitive determination of H2O2 in solution.

*3.2. Influence of pH and applied potential on biosensor response*

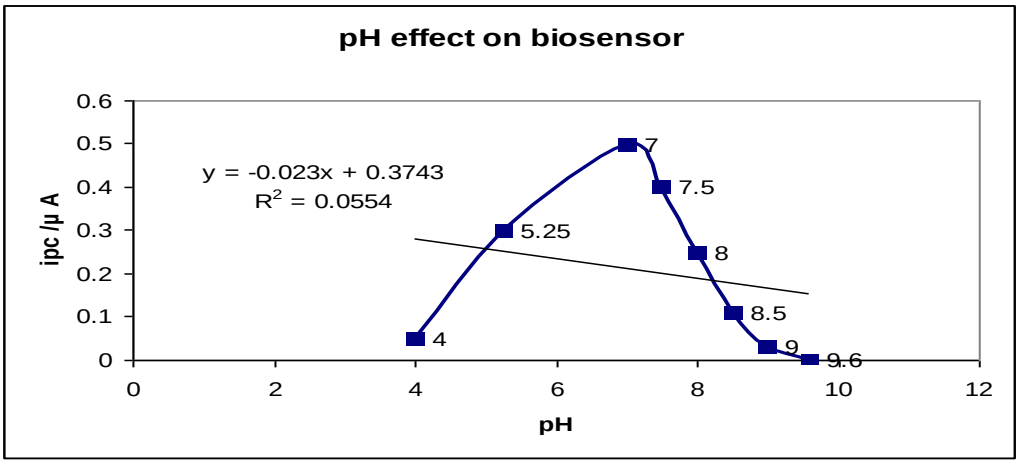



**Figure 7.** Dependence of the current response of Cytc/CdONPs/CPE electrode to 50.0µM $H_2O_2$ on the pH of buffer solutions.

In order to obtain an efficient biosensor for $H_2O_2$, the influence of pH and applied potential on the response of Cytc/CdONPs/CPE electrode were investigated. The change of chronoamperometric current with the pH under constant hydrogen peroxide concentration (50.0μM) is shown in Fig. 7. As can be seen, the maximum response appears at pH 7.0. So the buffer solution of pH 7.0 was selected for experiments. This designed biosensor possesses good stability and reproducibility, and achieves 94% of the steady-state current in less than 10 s.

### *3.3. Stability and reproducibility of the $H_2O_2$ biosensor*

The Cyt c/ CdONPs/CPE could retain the direct electrochemistry of the immobilized cytochrome c at constant current values in 0.1M pH 7.0 PBS upon the continuous cyclic voltammetric sweep over the potential range from −600 to +25mV at 200mV$s^{-1}$. After cyclically swept at 200mV$s^{-1}$ for 30 times the immobilized cytochrome c lost only 6% of its initial activity. When the sensor was not in use, it was stored in 0.1M pH 7.0 PBS at 4 ˚C. A storage period of a week almost did not change the currents of the direct electron transfer and the responses to $H_2O_2$. The sensor could retain 94% of its initial response to $H_2O_2$ after 45 days.

## 4. CONCLUSION

Direct electron transfer reaction of cytochrome c has been obtained at a cadmium oxide nanoparticles modified electrode. These nanoparticles helped cytochrome c to have a favored orientation and reduce the effective electron transfer distance. Direct electron transfer of the cytochrome c immobilized in modified carbon paste electrode was easily achieved. A pair of well-defined and quasi-reversible redox peaks appeared at the modified electrode with the cathodic and anodic peak potential of −0.305 V and -0.244 V (vs. SCE) respectively, indicating that direct electrochemistry of cytochrome c had occurred. As a result, this novel biosensor showed high sensitivity, a wide linear range, low detection limit and good stability for electrochemical detection of $H_2O_2$. This work may represent a facile and promising approach for the fabrication of various electrochemical biosensors.

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## References

1. J.M. Cooper, K.R. Greenough, C.J. McNeil, *J. Electroanal., Chem.* 347 (1993) 267.
2. G. Zhao, Z. Yin, L. Zhang, X. Wei, *Electrochem. Commun*., 7 (2005) 256.
3. C. M. Welch, C. E. Banks, *Anal. Bioanal. Chem.*, 12 (2005) 382.
4. S. Rezaei-Zarchi, A. A. Saboury, *J. Appl. Electrochem*., 37 (2007) 1021.

5. Willner, I.; Katz, E. Angew. *Chem. Int. Ed*., 39 (2000) 1181.
6. Sheng, Q.-L.; Luo, K.; Li, L.; Zheng, *J.-B. Bioelectrochemistry.,* 74 (2009) 246.
7. Tang, D.-Y.; Xia, B.-Y.; Zhang, Y.-Q. *Microchim. Acta*., 160 (2008) 367.
8. Wu, Y.-H.; Hu. S.-S, *Microchim. Acta*., 1 (2007) 159.
9. Lu, Z.; Huang, Q.; Rusling, J.-F. *J. Electroanal. Chem*., 59 (1997) 423.
10. Andolfi, L.; Bruce, D.; Cannistraro, S.; Canters, G.-W.; Davis, J.-J.; Hill, H.; Crozier, J.; Verbeet, M.-P.; Wrathmell, C.-L.; Astier, Y. *J. Electroanal. Chem*., 21(2004) 565.
11. A. Taleb, C. Petit, M. P. Pileni, *Chemistry of Materials*., 9, (1997) 950.
12. Antariksh Saxena, R.M. Tripathi, R. P. Singh, *Digest Journal of Nanomaterials and Biostructures*., 5 (2010) 427.
13. P. Mohanpuria, K. N. Rana, S. K. Yadav, *Journal of nanoparticle research.,* 10 (2008) 507.
14. J.L. Willit, E.F. Bowden, *J. Phys. Chem*. 94 (1990) 8241.
15. X. Yuan, F.M. Hawkridge, J.F. Chlebowski, *J. Electroanal. Chem*. 350 (1993) 29.
16. P. Bertrand, O. Mbarki, M. Asso, L. Blanchard, F. Guerleequin, M. Tegoni, *Biochemistry*., 34 (1995) 11071.
17. T. Pascher, J.P. Chesick, J.R. Winkler, H.B. Gray, *Science.,* 271 (1996) 1558.
18. P. Gru¨ ndler, T. Zerihun, A. Kirbs, H. Grabow, Anal. *Chim. Acta.,* 305 (1995) 232.
19. P. Gru¨ ndler, A. Kirbs, T. Zerihun, *Analyst.,* 121 (1996) 1805.
20. J. Osteryoung, M.M. Schreiner, CRC Crit. Rev. *Anal. Chem*. 19 (1988) 1.
21. H. Ju, S. Liu, B.Ge, F. Lisdat, F.W. Scheller, *Electroanalysis.,* 14 (2002) 141.
22. A. Salimi, E. Sharifi, A. Noorbakhsh, S. Soltanian, *Electrochem. Commun*., 8 (2006) 1499.
23. Y. Yang, H. Yang, M. Yang Y. liu, G. Shen, R. Yu, *Anal. Chim. Acta.,* 525 (2004) 213.
24. G. Chen, J. Zhao, X. Liu, G. Gao, J. Huang, G. Li, *J. Biotechnol*. 127 (2007) 653.
25. C. Nanjundiah, S. F. McDevitt, V. R. Koch, *J. Electrochem. Soc.,* 144 (1997) 3392.
26. S. Mikoshiba, S. Murai, H. Sumino, S. Hayase, *Chem. Lett*., 31 (2002)1156.
27. Haiming Fan, Lintao Yang, *Nanotechnology*., 15 (2004) 37.
28. E.A. Meulenkamp, *J. Phys. Chem.*, 102 (1998) 7764.
29. H.A.O. Hill, N.I. Hunt, A.M. Bond, *J. Electroanal. Chem*., 436 (1997) 17.
30. E. Laviron, *J. Electroanal. Chem*., 101 (1979) 19.
31. E. Laviron, *J. Electroanal. Chem*., 100 (1979) 263.