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# Preparation of a New Electrochemical Sensor Based on Cadmium Oxide Nanoparticles and Application for Determination of Penicillamine

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Novel cadmium oxide nanoparticles based sensor for the detection of penicillamine (PA) in aqueous solution has been developed. Cyclic voltammetry at potential range -1.1 to 1.1V from aqueous buffer solution (pH 7) containing CdO produced a well-defined cadmium oxide nanoparticles deposited on the surface of glassy carbon electrode. The Differential pulse voltammetry detection of penicillamine is carried out at 0.451V in buffer solution with pH 7. Cadmium oxide nanoparticles have many advantageous such as remarkable catalytic activity, simple preparation procedure and long-term stability of signal response during of penicillamine oxidation.

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**Keywords:** Penicillamine, Cadmium oxide nanoparticles, Differential pulse voltammetry, Sensor

## 1. INTRODUCTION

Sulfhydryl compounds (thiols, R-SH) are known to possess a multitude of roles within physiological systems. As such, depletion of cysteine and glutathione has been noted to accompany premature arteriosclerosis, leukemia and cervical cancer [1]. In contrast, abnormally high levels of these compounds have been recorded in patients suffering from AIDS related dementia, Alzheimer's and Parkinson's disease and in the immediate aftermath of strokes [2]. Penicillamine is a medication that has been used for many years in the treatment of various rheumatic diseases, most commonly rheumatoid arthritis. It is also classified as a metal binding (or chelating) agent used in the treatment of Wilson's disease, a genetic disease that results in excessive copper deposits in the body tissues. Increased amounts of penicillamine can cause rashes early in treatment. Rashes may be associated with itching, which can often be controlled by simply adding antihistamine medication. Rashes associated with fever and joint pain usually require discontinuation of the penicillamine. It can also cause loss of appetite, nausea, abdominal pain, and loss of the sense of taste. Penicillamine can also cause bone marrow suppression and serious kidney disease. All patients who take penicillamine required regular blood and urine testing for monitoring.

Nanometer size materials have been used successfully for a wide range of technological applications including, bioelectronics, catalysis, optics, biomedical and chemical or biological sensors and biosensors [3-8], due to their catalytic properties and roughening of the conductive sensing interface. Although modified electrodes have shown interesting ability toward some of biological molecules detection, they also display many problems related to the immobilization of the mediator and its toxicity, low sensitivity and stability and mediator leakage. In addition, high cost, low reproducibility and poor repeatability are disadvantage of these sensors. Cadmium oxide nanoparticles are known to be highly reactive and they have been employed in processes such as energy storage system [9], electrochromic thin films [9] magnetoresistive devices [10] and heterogeneous catalysis [11]. Furthermore, Cadmium oxide and other cadmium-based oxides continue to attract considerable interest, mainly due to their excellent electrocatalytic activity toward various compounds, ozone and oxygen evolution [12, 13]. The electrocatalytic property of this film has depending on deposition method. For this purpose several methods such as spray pyrolysis [14] plasma sputtering [15] thermal salt decomposition [16] powder immobilization [17] irradiation [18] and sol-gel technique [19] have been used so far. Thin film with specific composition, morphology and good adhesion between the deposited film and the substrate can be easily prepared by electrochemical techniques. Glassy carbon and gold electrodes modified with Cadmium oxide have been used for electrocatalytic processes using different organic molecules such as glucose, cysteine, propylamine [20-22] hydroquinone [23] and methanol [24] as model compounds. Due to excellent electrocatalytic activity of the electrodeposited cadmium oxide films, following our previous works for electroanalytical application of metal oxide nanoparticles [26-30] in this study, cadmium oxide film is electrodeposited on the surface of GC electrode using cyclic voltammetry technique. In this work, a glassy carbon electrode coated with cadmium oxide film has been developed as a simple, sensitive, rapid and new assay method for penicillamine.

## 2. MATERIALS AND METHODS

### *2.1. Chemicals and reagents*

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 $K_2HPO_4$. The pH of buffer solutions was adjusted with HCl and KOH solutions.

### *2.2. Apparatus*

Electrochemical experiments were performed with a computer controlled Autolab modular electrochemical system (Eco Chemie Ultecht, The Netherlands), driven with GPES software (Eco Chemie). A conventional three-electrode cell was used with a SCE (Methrom, The Netherlands) as reference electrode, and a Pt wire as counter electrode. The working electrode was made by cadmium oxide nanoparticles modified glassy carbon electrode. Voltammetry on electrodes coated with cadmium oxide was done in buffers containing no cadmium salt. The electrochemical determination of penicillamine (PA) concentration in real samples was performed via the standard addition protocol. The procedure involves spiking the analyte solution of unknown concentration with analyte solution of known concentration; the analyte concentration can then be determined by reading the peak current increase with each addition. All measurements were conducted in a thermo stated temperature of 20±1 °C. The surface morphology of modified electrodes was studied with a Vega-Tesacn electron microscope.

### *2.3. Preparation of cadmium oxide modified glassy carbon electrode*

Glassy carbon electrode (2 mm diameter) was carefully polished with alumina on polishing cloth. The electrode was placed in ethanol container and used bath ultrasonic cleaner in order to remove adsorbed particles. Cadmium oxide film was electrodeposited on the surface of glassy carbon electrode from 1mM cadmium chloride pH 7 PBS, using repetitive potential cycling (30 cycles at 100 $mVs^{-1}$) between -1.1V and 1.1. Modified electrode was eventually washed with double distilled water and stored at ambient temperature (25°C) before being used in experiments.

## **3. RESULTS AND DISCUSSION**

### *3.1. Characterizations of cadmium oxide nanoparticles*

Fig. 1 shows a typical image of an electrodeposited cadmium oxide film on glassy carbon electrodes. It can be seen that small particles with average size of less than 100 nm is almost uniformly distributed on the surface of electrode forming a thin layer. The inset of this figure shows SEM image of the same sample with lower magnification (the scale bar is about 1μM). A large agglomerated particle with the average size varies from under 70 nm to slightly less than 150 nm are also observe on the image.

The surface morphology of the Cd (II) oxide film is shown in Fig. 2. The AFM (Atomic Force microscopy) image shows that the GC surface was incompletely covered with the Cd (II). A Cd (II) modified electrode was prepared by cycling the potential once in the Cd (I) complex solution.

The formation of cadmium oxide layer on the electrode surface was checked by recording cyclic voltammograms of the modified electrode in buffer solution without cadmium ions (Fig. 3A).

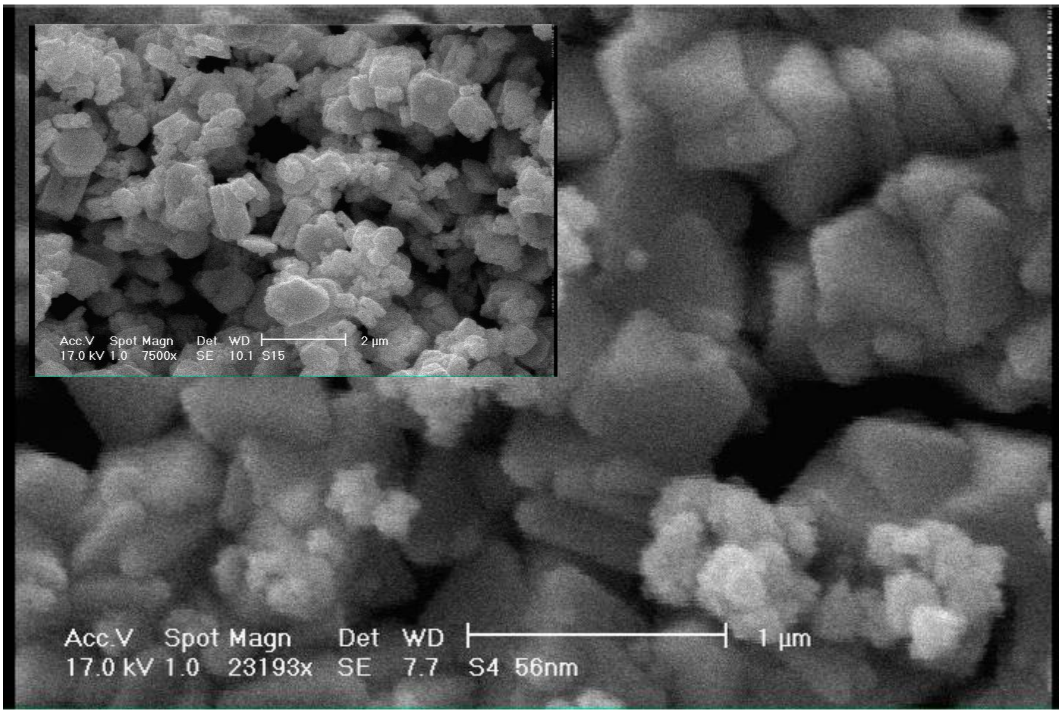
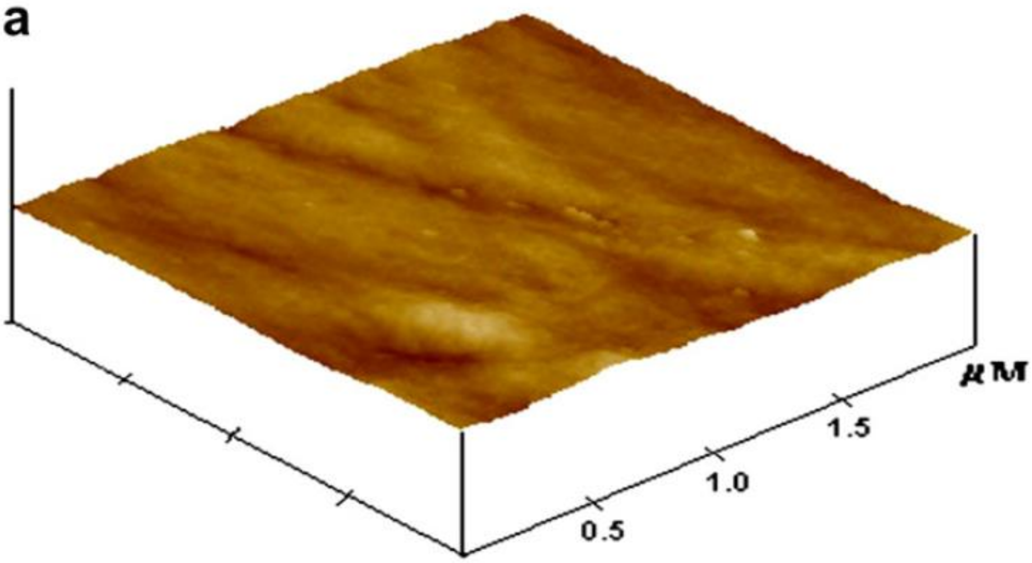


**Figure 1.** SEM image of the electrodeposited cadmium oxide on glassy carbon, scale bare 150 nm. Inset: The SEM image with lower magnification for the same sample, scale bar is 5.0µm.



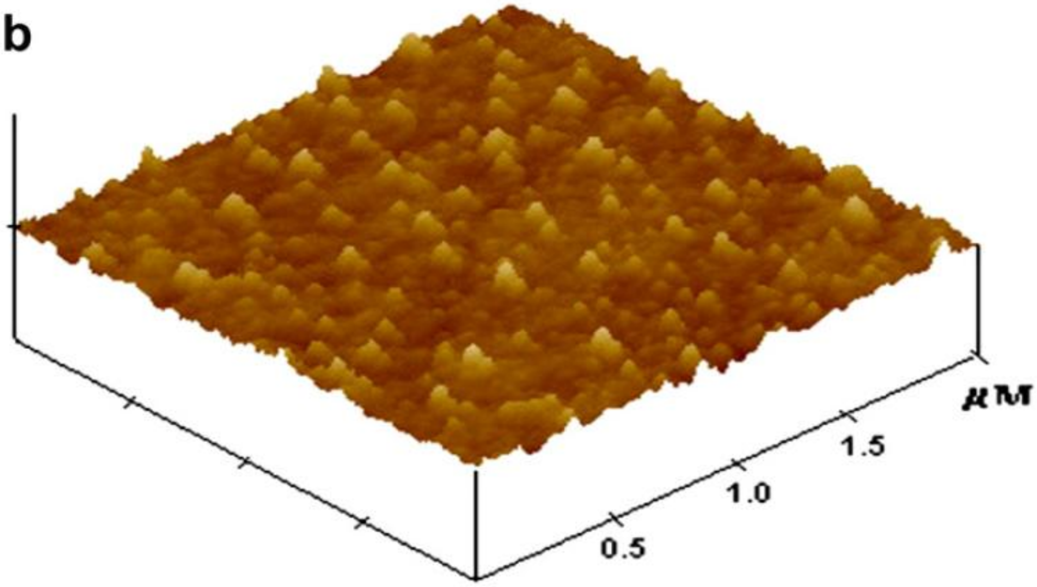
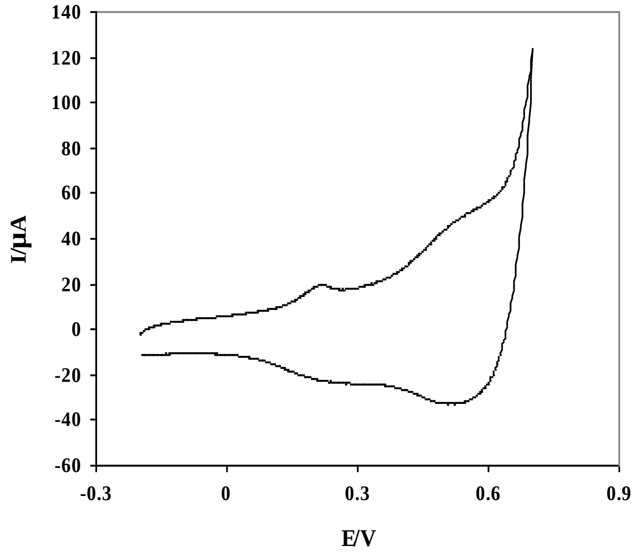
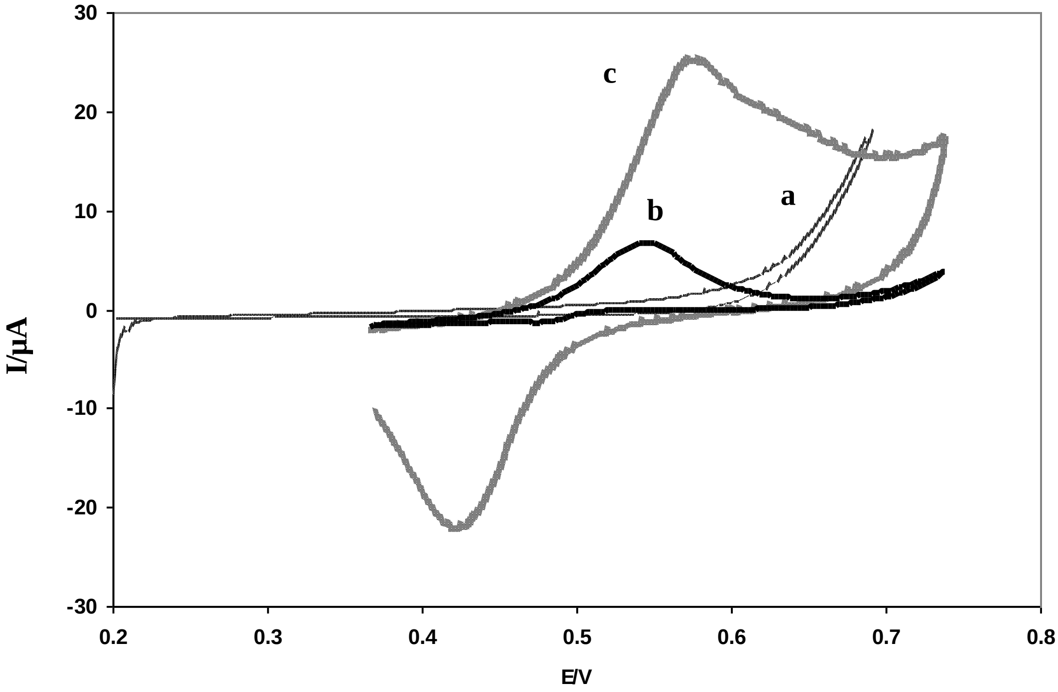


**Figure 2.** AFM images of the GC electrode surface (a) before and (b) after modification with the cadmium oxide film. The images were obtained using tapping mode AFM, and the cadmium oxide film was electrodeposited by cycling the potential once between 1.2 and-1.1V at a scan rate of 100 $mVs^{-1}$

As shown two oxidation peaks were observed at 225 and 550 mV/SCE during the positive potential scan which were attributed to Cd (I)/ Cd (II) and Cd (II)/ Cd (III) redox transition associated with different cadmium oxide species on the electrode surface. These results can be attributed to the conversion between four different cadmium oxidation phases of $Cd(OH)_2$, CdOOH and CdO which are stable at pH 13. The cathodic peaks at 186 and 522 mV correspond to the reduction of various cadmium oxide species formed during the anodic sweep. The electrochemical properties, stability and pH effect on the behavior of the modified electrode was investigated in previous report.



A



B

**Figure 3**. **A:** Cyclic voltammetry response of GC electrode modified with cadmium oxide nanoparticles in pH 7 buffer solutions at scan rate of 20 mV s$^{-1}$. **B:** Cyclic voltammograms of cadmium oxide nanoparticles modified GC electrode in buffer solution pH 7 at scan rate of 20 mVs$^{-1}$ in the absence (c) and presence of 70μM PA (d). (a and b) are same results as (c) for bare GC electrode.

### *3.2. Electrocatalytic oxidation of penicillamine (PA) on cadmium oxide nanoparticle -GC electrode*

In order to verify the electrocatalytic activity of the modified electrode for PA oxidation, the electrochemical experiments in the presence of PA was carried out. Fig. 3B shows cyclic voltammograms of modified and unmodified electrodes in the absence and presence of PA (70μM) at pH 7 buffer solution. In the bare glassy carbon electrode, no redox response of PA can be seen in the potential range from 0.2 to 0.8V. However, at the cadmium oxide modified GC electrode, the oxidation current of cadmium oxide film was greatly increased due to catalytic oxidation of PA, while the reduction peak has largely disappeared. Decreasing of overvoltage and increased peak current of PA oxidation confirmed that cadmium oxide nanoparticles have high catalytic ability for PA oxidation. Therefore, cadmium oxide nanoparticles are suitable as mediators to shuttle electron between PA and working electrode and facilitate electrochemical regeneration following electron exchange with PA. In order to evaluate the electrocatalytic activity of cadmium oxide film, the cyclic voltammograms of the modified electrode in the presence of different concentrations of PA were recorded (Fig. 4). With increasing PA concentration the anodic peak currents increased and cathodic peaks disappeared. The catalytic peak currents are proportional to the concentration of PA in the range of 1 to 50 μM. The linear regression equation for the concentration range from 1 to 50 μM is obtained as I (μA) = 2.9793C (μM)-5.5306, $R^2 = 0.995$. The detection limit is estimated to be 0.6μM when the signal to noise ratio is 3. It can be inferred from these results that the presence of the electrodeposited cadmium oxide nanoparticles on the surface of GC electrode facilitates the detection of PA at low concentration level.

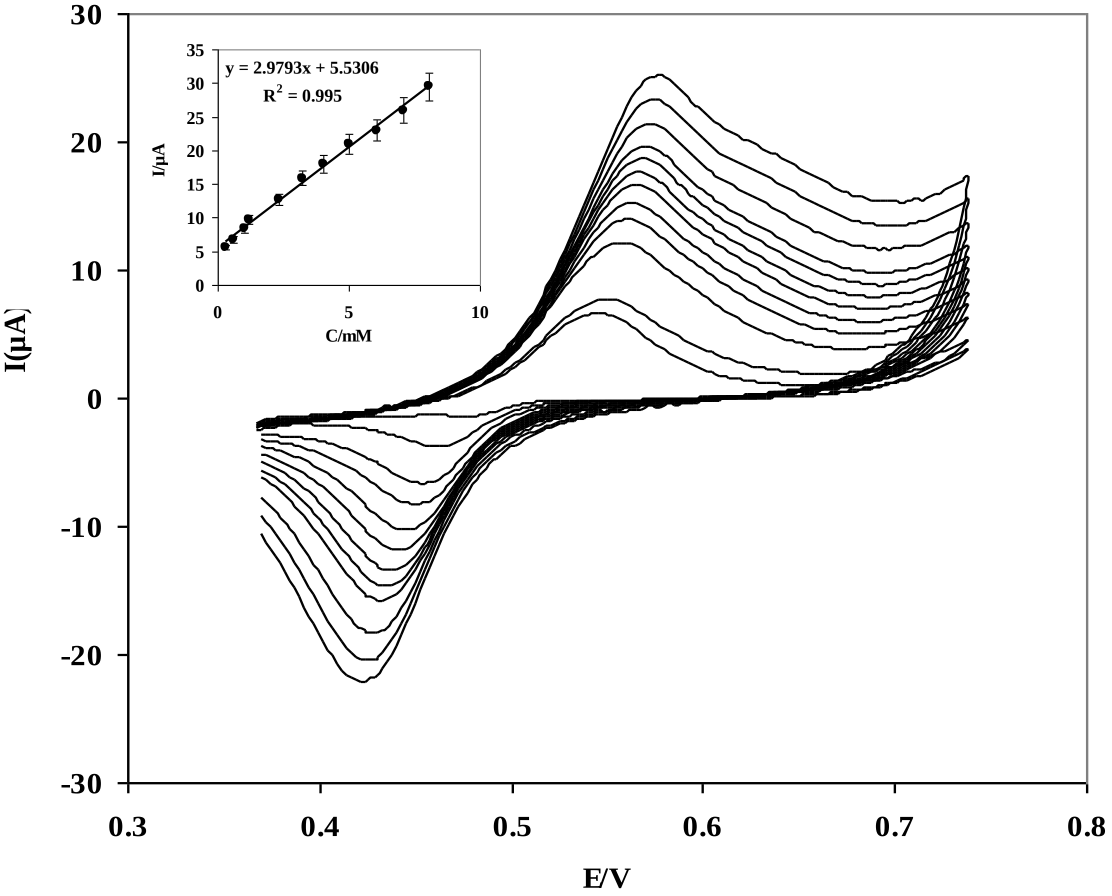


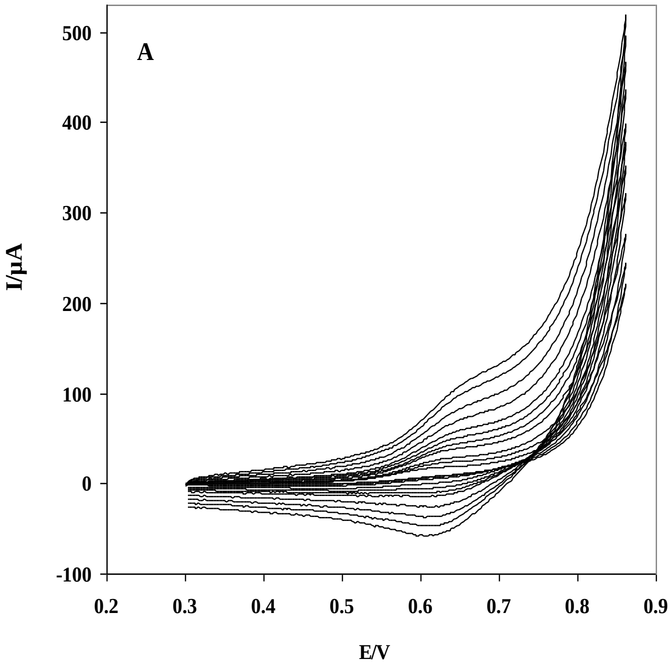
**Figure 4**. Cyclic voltammograms of cadmium oxide nanoparticles modified GC electrode in buffer solution pH 7 at scan rate 20 $mVs^{-1}$ with increasing PA concentration (from inner to outer) 1,2,3,5,7,10,12,15,20,25,30,35,40,45and 50μM. Inset: plot of peak current vs. PA concentrations.

Fig. 5A shows the cyclic voltammograms of 100 μM of PA solution at different scan rates. The peak current for the anodic oxidation of PA is proportional to the square root of the scan rate, at scan rate 10-150 $mVs^{-1}$, suggesting that the process is controlled by diffusion as expected for a catalytic system.

In order to gain some of information about the rate-determining step a Tafel plot was drawn. The Tafel slope (b) was obtained from the linear relationship observed for $E_p$ versus log v (Fig. 5B) by using the following equation [30].

$$Ep = \left(\frac{b \log v}{2}\right) + cons\tan t \qquad (1)$$

The resulting b value was 162.4 mV, indicating that one electron process was involved in the rate limiting step, assuming a charge transfer coefficient of $\alpha = 0.38$. Under the above conditions for an EC′ mechanism, the Andrieux and Saveant theoretical model [31] can be used in order to calculate the catalytic rate constant. According to the Andrieux and Saveant approach and using Fig. 1 the average value of $k_{cat}$ is $(2.33\pm0.2)\times10^{4}$ $M^{-1}$ $s^{-1}$. These results indicate high catalytic activity of the sensor for PA detection.



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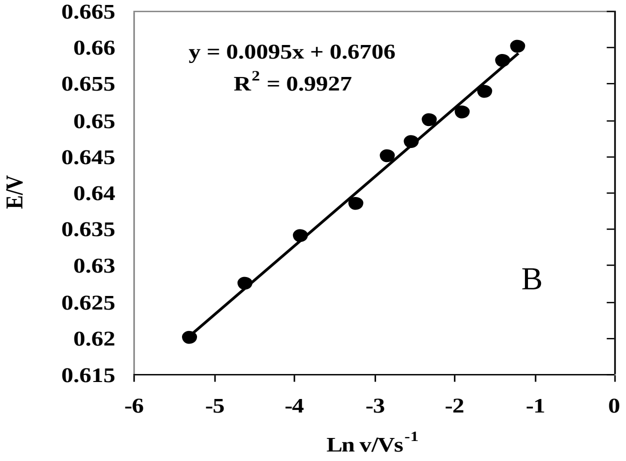

B

**Figure 5**. (A) Cyclic voltammograms response modified GC electrode in pH 7 buffer solution containing 100 µM of PA at different scan rates (from inner to outer) 10-150 $mVs^{-1}$. (B) Plot of Ep vs. log *v*.

### *3.3. Differential pulse voltammetry, analytical performance characteristics*

Differential pulse voltammetry (DPV) was used as a very highly sensitive and rapid electrochemical method for the detection of trace amounts of PA. Fig. 6 shows DPVs for buffered solutions of PA at pH 7 in the concentration range of 1-50 μM. The results of the voltammetric calibration curves are shown in Table 1 (row A).

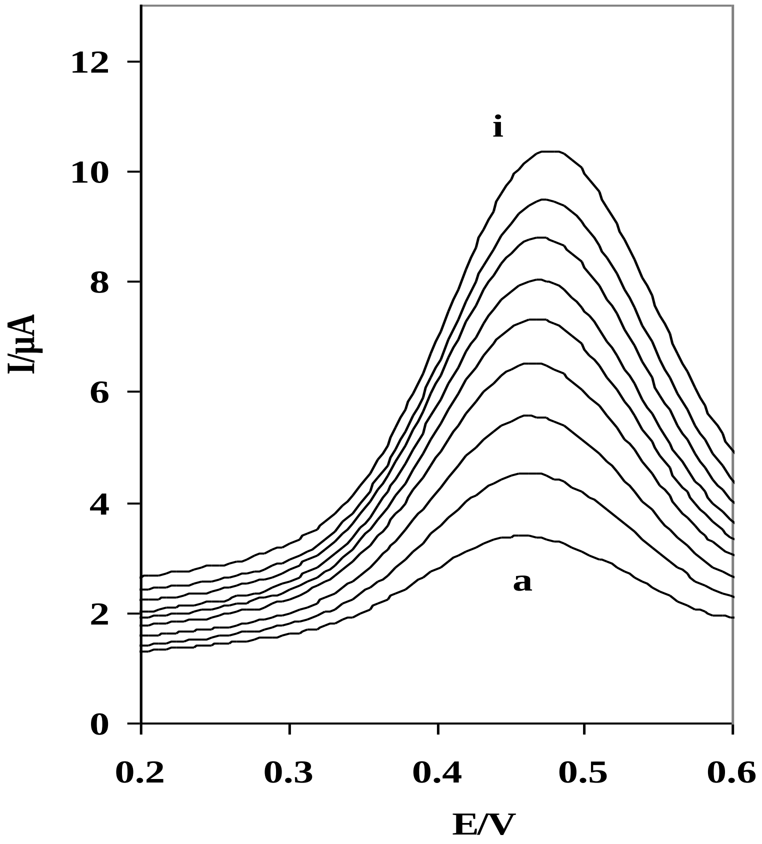

**Figure 6**. Differential pulse voltammograms of PA at the modified electrode with increasing PA concentration 1,3,5,10,20,30,40 and 50 μM PA in pH 7 buffered solutions. Pulse amplitude was 50 mV.

**Table 1.** Differential pulse voltammetric parameters and response properties for the determination of PA in pure buffered solutions and standard addition to tablet samples

| Experiment | $E_p$/mV | Pulse amplitude (mV) | Linear range (μM) | Detection limit (μM) | Slope variation (%RSD, n= 6) |
|---|---|---|---|---|---|
| A | 0.439 | 50 | 0.3-150 | 0.14 | 3.2 |
| B | 0.451 | 50 | 0.2-135 | 0.42 | 4.0 |
| C | 0.448 | 50 | 0.25-140 | 0.42 | 3.8 |

**Table 2.** Results of analysis of PA tablets and recovery of PA added to tablet preparations and human synthetic serum samples.

| Experiment | Sample preparation | Labeled value of PA (mg/100 mg)[a] | Amount found for PA (mg/100 mg)[b] | Recovery (%) |
|---|---|---|---|---|
| 1 | PA tablet dissolved in buffer solution, pH 3.0 | 60.03 | 58.6 (±0.05) | |
| 2 | Sample 1 +10 mg PA | | 69.2(±3.7) | 93.0 |
| 3 | Sample1+20 mg PA | | 78.2 (±1.97) | 98.5 |
| 4 | Sample1+50 mg PA | | 107.1(±2.1) | 100.3 |
| 5[c] | 10 ml serum+1.0 mg PA | | 0.88 (±0.25) | 101.2 |
| 6[c] | 10 ml serum+4.0 mg PA | | 2.85 (±0.41) | 92.0 |
| 7[c] | 10 ml serum+10.0 mg PA | | 8.11(±0.4) | 96.3 |

a Amount of PA (mg) in 100 mg of powdered samples of PA tablets containing 250 mg PA per tablet.
b Values in parenthesis give the standard deviation based on five replicates.
c Human synthetic serum samples containing buffer solution at pH 7.

A linear range is obtained from 0.3-150 μM of PA with a detection limit of 0.14 μM (SN=3). The DPV method in standard addition mode was also used for the determination of the amounts of PA in buffered solutions that were prepared by dissolution of pharmaceutical samples (250 mg D-penicillamine tablets, Table 1; row B). The correlation coefficients of the calibration plots in the standard addition method were in the range of 0.9992-0.9996 (n=6).

The precision of the method was assessed by six repetitions of the analyses of the pharmaceutical preparations. In these experiments, the RSD was 3.3% (n=6, mean=233.2 mg PA/tablet) and when spiked with PA solution, was between 4.1% and 4.2% (Table 2).

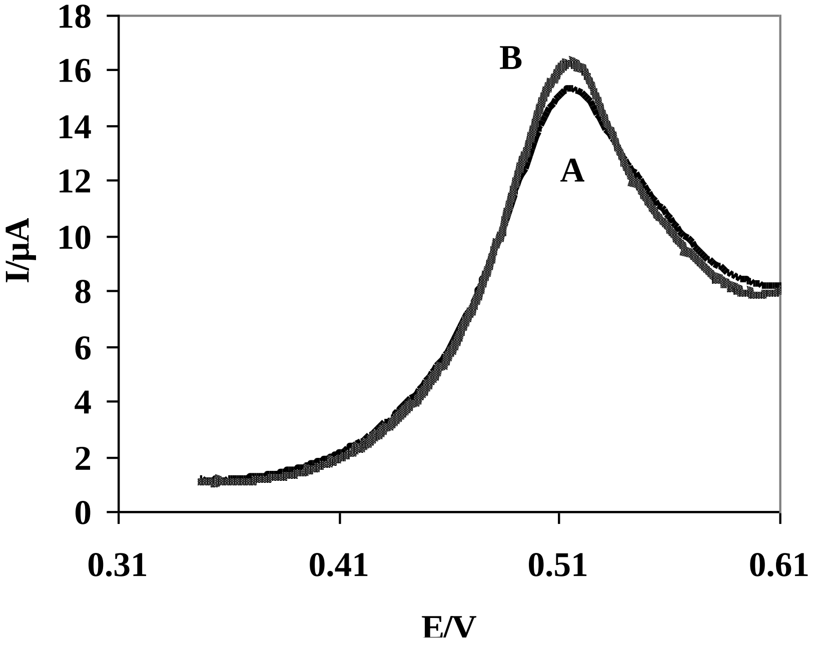

**Figure 7**. Differential pulse voltammograms of a modified electrode in a solution prepared by the addition of 1.00 ml of 0.001 M of PA to; (A) 10.0 ml of pH 7 buffer solution, and (B) 10.0 ml human synthetic serum sample (buffered to pH 7). Pulse amplitude was 50 mV.

Results of the recoveries of the PA added to 10.0 ml of human synthetic serum are shown in Table 2. In these measurements, the composition of the synthetic serum was chosen near to its normal level in real human serum [32]. Fig. 7 also shows the differential pulse voltammograms for 10.0 ml phosphate buffer solution spiked with 1.00 ml+1mM PA and 10.0 ml of synthetic serum when spiked with the same amount of 1mM solution of PA. In both series of quantitative experiments, the peak potential for determination of the peak current was 450 mV (vs. SCE). Under the same conditions, the potential peaks for anodic oxidation of some biological thiols (e.g., L-cysteine and glutathione) were obtained at higher positive potentials (>300 mV versus PA) with considerably less sensitivity (peak current at the same concentration of PA). The excellent recovery results indicate that the constituents in the complex matrix of the serum sample do not interfere with the detection of PA and this voltammetric method can be used as a very highly sensitive detection device for PA in biological and pharmaceutical preparations.

## 4. CONCLUSIONS

The glassy carbon electrode modified with electrodeposited cadmium oxide nanoparticles showed excellent electrocatalytic activity toward oxidation high PA over the pH range 5-11 and a wide range of concentration. This electrode can be use for determination of micromolar or nanomolar concentration ranges of PA using, voltammetry or DPV, respectively. The electrochemical system will be applicable for analysis of PA in real samples containing different reducing compounds and other interferences exists in sample matrix. Furthermore, the modification procedure offers considerable simplicity and economy of electrode preparation as compared to other electrochemical methods for PA detection. Due to low detection limit of this system, it can be used for determination of PA in real samples.

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## References

1. L.G. Rodrigues, A.G. Wedd and A.M. Bond, *J. Electroanal. Chem.* 312 (1991) 131
2. W.A. Kleiman and J.P. Richie, *Biochem. Pharm.* 60 (2000) 19.
3. Y.W. Jun and J.T. Jan, J. Cheon, *Bull. Korean Chem. Soc.* 27 (2006) 961
4. C.M. Welch and R.G. Compton, *Anal. Bioanal. Chem.* 384 (2006) 601
5. A.N. Shipway and I. Willner, *Chem. Commun.* 20 (2001) 2035
6. M. Yang, Y. Yang, Y. Liu, G. Shen and R. Yu, *Biosens. Bioelectron*. 21 (2006) 1125
7. I.Willner and E. Katz, Bioelectronics, Wiley VCH, Verlag Gmbh&Co. KGaA, Weinheim, (2005).
8. C.S.S.R. Kumar, J. Hormes and C. Leuschner, Nanofabrication Toward Biomedical Applications, Wiley VCH, Verlag GmbH & Co KGaA, Weinheim, (2005).
9. V. Srinivasan and J. W. Weidner, *J. Power Sources*, 108 (2002) 15

10. L.D. Kadam, S.H. Pawar and P.S. Patil, *Chem. Phys.* 68 (2001) 280
11. H. Okabe, J. Akimitsu, T. Kubodera, M. Matoba, T. Kyomen and M. Itoh, *Phys. B Condens. Matter*, 863 (2006) 863
12. J. Tyczkowski, R. Kapica and J. Lojewska, *Thin Solid Films*, 515 (2007) 6590
13. M. Longhi, L. Formaro, *J. Electroanal. Chem.* 464 (1999) 149
14. S. Trasatti, in: J. Lipkowski and N. Ross (Eds.), Transition Metal Oxides: Versatile Materials for Electrocatalysis, VCH Publishers Inc. New York (1994).
15. V.R. Shinde, S.B. Mahadik, T.P. Gujar and C.D. Lokhande, *Appl. Surf. Sci.* 252 (2006) 7487
16. W. Estrada, M.C.A. Fantini, S.C.D. Castro, C.N.P.D. Fonseca and A. Gorenstein, *J. Appl. Phys.* 74 (1993) 5835
17. L.M. Dasilva, J.F.C. Boodts and L.A.D. Faria, Electrochim. Acta, 46 (2001) 1369
18. S.P. Jiang, A.C.C. Tseung, J. Electrochem. Soc. 137 (1990) 3442-3447.
19. Y. Ni, X. Ge, Z. Zhang, H. Liu, Z. Zho and Q. Ye, *Mater. Res. Bull.* 36 (2001) 2383
20. F. Svegl, B. Orel, I.G. Svegl and C.V. Kaucic, *Electrochim. Acta*, 45 (2000) 4359
21. I.G. Casella and M.R. Guascito, *J. Electroanal. Chem.* 476 (1999) 54
22. I.G. Casella and M. Gatta, J. Electroanal. Chem. 534 (2002) 31
23. I.G. Casella, *J. Electroanal. Chem.* 520 (2002) 119
24. L.F. Fan, X.Q. Wu, M.D. Guo and Y.T. Gao, *Electrochim. Acta*, 52 (2007) 3654
25. A.Rusmidah, Wan Azelee Wan Abu Bakar and T. Lee Kin, *Modern Appl. Sci.* 4 (2010) 56
26. L.A. Saghatforoush, M. Hasanzadeh, N. Shadjou and B. Khalilzadeh, *Electrochimica Acta*, 56 (2011) 1051
27. I.Kazeman, M. Hasanzadeh, M. Jafarian, N. Shadjou and B. Khalilzadeh, *Chin. J. Chem.* 28 **(**2010) 504
28. M. Hasanzadeh, G. karimnezhad, M.G. Mahjani, M. Jafarian, N. Shadjou, B. Khalilzadeh and L.A. Saghatforoush, *Catal. Commun.* 10 (2008) 295
29. M. Hasanzadeh, B. Khalilzadeh, N. Shadjou, G. Karim-Nezhad, L.A. Saghatforoush, I. Kazeman, M.H. Abnosi, *Electroanalysis*, 22 (2010) 168
30. M. Hasanzadeh, N. Shadjou, L.A Saghatforoush, B. Khalilzadeh and I. Kazeman, *Bull. Korean Chem. Soc.* 30 (2009) 2943.
31. J.A. Harrison and Z.A. Khan, *J. Electroanal. Chem.* 28 (1970) 131
32. C.P. Pau and G.A. Rechnitz, *Anal. Chim. Acta*, 160 (1984) 141