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Application of Tris(1,10-phenanthroline-5,6-dione) Zinc(II)hexafluorophosphate Complex Modified Carbon Paste Electrode for Electrocatalytic Determination of Penicillamine in Rheumatoid Patient Serum Samples

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Abstract- A novel Zn(II)-fluorophore complex, tris(1,10-phenanthroline-5,6-dione)zinc(II) hexafluorophosphate, has been synthesized and structurally characterized for modification of carbon paste electrode. Then the fabricated electrode was applied as a highly selective and sensitive sensor for determination of penicillinamine. Despite of featureless voltammetric response of this complex in the range of +0.4 to +1.0 V, it exhibits potent electrocatalytic activity toward D-PA oxidation at +0.8 V. Impedance Spectroscopy (EIS) was used for comparing the surface electron transfer resistance of the modified and bare electrode and demonstrate that modified electrode make the electron transfer easier. Under the optimum conditions (pH=4.0), the oxidation current of penicillinamine was linearly related to its concentration in the range of 0.1-95.0 µmol L^{-1} with a detection limit of 0.05 µmol L^{-1} at the signal to noise of 3. Satisfactory results were achieved for determination of penicillinamine in serum samples of patients with rheumatoid arthritis using standard addition method.

Keywords- D-penicillamine, Tris(1,10-phenanthroline-5,6-dione)zinc(II) hexafluoro phosphate, Carbon paste electrode, Electrochemical sensor

1. INTRODUCTION

D-penicillamine (D-PA), 2-amino-3-mercapto-3-methylbutanoic acid, is derived from hydrolytic degradation product of β-lactam antibiotics but it does not have antibiotic activity [1]. It used to aid the elimination of copper in the treatment of hepatolenticular degeneration (Wilson's disease) [2]. D-PA is also the drug of choice in the treatment of rheumatoid arthritis, primary biliary cirrhosis, scleroderma, fibrotic lung diseases, cystinuria, and progressive systemic sclerosis. However, penicillamine therapy can cause some side effects such as bone marrow suppression, neutropenia and kidney disease [3]. So, all patients who take penicillamine require monitoring of regular blood and urine analysis. Due to its biological importance D-PA role, there have been many efforts made to determine PA in biological samples. Various analytical techniques include high performance liquid chromatography [4-5], chemiluminescence [6-7], capillary electrophoresis [8-9], electrochemistry [10-12], spectrophotometry [13-14], and fluorimetry [15-16] have been reported for the determination of D-PA. Among these techniques, electrochemical determinations of D-PA have paid great attention due to the redox property of thiol groups. One of the serious problems related to the electrochemical determination of D-PA at the bare electrodes is slow kinetic electrooxidation of this drug. In recent years, chemically modified electrodes have attracted dramatic interests because of their potential applications in electroanalysis [17]. Chemically modified carbon paste electrodes are one of the popular types of modified electrodes and widely applicable in electroanalysis study. They possess many advantages such as rapid surface renewal, favorable signal-to-background, and, in particular, a wide variety in the way of how to effectively modify their surface. Various chemically modified carbon paste electrodes using electron transfer mediators such as benzoylferrocene [10], TiO_2 nanoparticles [11], cupric ions (Cu^{2+}) [18] and 1-[4-(ferrocenyl ethynyl) phenyl]-1-ethanone [19], have been used for the electrocatalytic detection of D-PA. The most important effect of mediators is a reduction of the overpotential required for electrochemical reaction, which lead to enhances the electrochemical response. 1 ,10-Phenanthroline-5,6-dione (phen-dione) ligand have been extensively studied because this ligand has the ability to form stable complexes with a wide variety of metal ions and carries an o-quinone moiety with pH-dependent electroactivity [20]. The ligand or the metal in these complexes can be varied in an easily controlled manner to facilitate an individual application [21]. Herein, we report the synthesis and characterization of $[Zn(phen\text{-}dione)_3](PF_6)_2$ complex as a mediator in carbon paste electrode for electrocatalytic determination of D-PA. Finally the analytical performance of this modified electrode was examined by the voltammetric determination of D-PA in patient serum samples. Table1 compares the analytical parameters of recently reported electrochemical methods for determination of D-PA with our proposed method for the determination of D-PA [3,10-12,18,19,22]. The results showed that the detection limit and linear dynamic range of pencillamine with [Zn(phen-

dione)$_3$]$(PF_6)_2$/ Carbon paste electrode are comparable, and in some cases better than, those recently voltammetric methods.

Table 1. Comparison of limit of detection and linear range of the proposed method with recently published voltammetric methods for the determination of D-PA

Electrode	**Limit of detection (μmol L^{-1})**	**Linear range (μmol L^{-1})**	**Ref.**
GCE	0.08	0.1-2.5	[12]
Benzoyl ferrocene-modified carbon nanotube paste electrode	0.13	1-800	[10]
Carbon paste modified 1-[4-(Ferrocenyl Ethynyl) Phenyl]-1-Ethanone	3.9	7-230	[19]
2,7-bis(ferrocenyl ethyl) one modified CPE-fluoren-9	6.8	7.0-200	[22]
Carbon paste modified- TiO_2 nanoparticles	0.76	0.8-140.0	[11]
Carbon paste electrode modified cupric ions	0.1	1-200	[18]
Tyrosinase immobilized on GCE	0.007	0.02-80	[3]
[Zn(phen-dione)$_3$](PF6)$_2$ modified carbon paste electrode	0.05	0.2-95	This work

2. EXPERIMENTAL

2.1. Apparatus and reagents

Voltammetric experiments were performed using an electrochemical system comprising the Metrohm instrument (Herisau, Switzerland), Model 797 *VA*, and three-electrode cell assembly containing an Ag/AgCl reference electrode, a platinum wire counter electrode and [Zn(phen-dione)$_3$]$(PF_6)_2$ modified electrode as the working electrode. Corning pH-meter (model 146) was controlled the pH of the solutions.

Pure graphite powder (particle size <50 μm) was prepared from Iran's Research Institute of Petroleum Industry. Spectroscopic grade paraffin oil was obtained from Merck and used for the preparation of carbon paste electrodes. D-PA was purchased from Sigma. Stock solution of D-PA (1×10^{-2} mol L^{-1}) was prepared daily by dissolving suitable amounts of D-

PA in water and store in 4 ^{o}C before using. Other standard solutions were prepared by dilution of the stock solution with buffer. A series of phosphate buffer solutions (0.1 mol L^{-1}) with different pH values (3-8) were used.

2.2. Preparation of the modified electrode

Tris(1,10-phenanthroline-5,6-dione)zinc(II)hexafluorophosphate complex was synthesized according to Rezvani et al. [21]. For preparation of the modified electrode about 0.01 g of synthesized $[Zn(phen\text{-}dione)_3](PF_6)_2$, and 0.5 g graphite powder were mixed together. Then diethyl ether was added and the solids were mixed to form a uniform mixture. After evaporation of diethyl ether, 0.2 g paraffin oil was added and the mixture was gently ground by mortar and pestle to obtain a uniformly wetted paste. The resulted paste was packed into the end of the Teflon cylinder hole (internal radius 2.2 mm) containing a copper wire for electrical connection. New surface was obtained after pushing an excess the paste and polishing it on a smooth paper. The unmodified carbon paste electrode was prepared in the same way, but without adding complex.

2.3. Serum samples preparation

Serum samples were obtained and stored frozen until the analysis. Proteins from human serum were precipitated by the rapid addition of two volumes of acetonitrile containing 0.1% of trifluoroacetic acid and immediately mixed by vortexing. Samples were at 5000 rpm for 5 min. The supernatant was filtered using a 0.45 μm pore size filter. For preparation of serum samples, 1.0 mL of each sample was diluted to 10.0 mL in voltammetric flask by phosphate buffer solution (pH 4.0). Then, 5 ml of this solution was transferred to the voltammetric cell, were diluted to 10 mL with phosphate buffer solution.

3. RESULTS AND DISCUSSION

The characteristic of synthesized complex was confirmed by ^{1}H NMR spectra, X-Ray diffraction and IR which in agreement with tris(1,10-phenanthroline-5,6-dione)zinc(II) hexafluorophosphate structure [21].

3.1. Characterization of the modified electrode surface

Cyclic voltammetry was used to examine the electrochemical behavior of this complex. Previous studies have shown that the phen-dione ligand is electrochemically active due to the presence of the *o*-quinone moiety [23]. It should be mentioned that the 1,10-phenanthroline (starting material for synthesis of phen-dione ligand) is also electroactive. As with quinines in general, the electrochemical behavior is strongly dependent on whether an aprotic solvent

such as acetonitrile or an aqueous solvent is employed. In aqueous solvent, the process is the "two-electron/two-proton" reduction of the quinone to the hydroquinone [24].

Cyclic voltammetry was performed on an acetonitrile solution of $[Zn(phen\text{-}dione)_3](PF_6)_2$ with 0.1 mol L^{-1} tetrabutylammonium-hexafluorophosphate as a supporting electrolyte. In this complex, two reversible reduction couples at -0.450 and -0.740 V are assigned to the reduction of phen-dione ligand to phen-semiquinonate and phen-diolate, respectively by analogy to other phen-dione complexes. In comparison to free phen-dione, the reduction couples shift to more positive potentials due to the coordination of phen-dione ligand to the zinc(II) center [25]. Electrochemical Impedance Spectroscopy (EIS) was used for measuring the impedance value of various properties at the interface of the electrode and the solution. Fig. 1A illustrates the Nyquist diagrams of CPE and $[Zn(phen\text{-}dione)_3](PF_6)_2$/CPE in the presence of 1.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ (1:1)+0.1 mol L^{-1} KNO_3 in phosphate buffer solution (pH 4.0). In the Nyquist diagram, the semicircle diameter of EIS is equal to the surface electron transfer resistance (R_{ct}) of the $[Fe(CN)_6]^{3-/4-}$ redox reaction. It can be seen that R_{ct} value for CPE is 1450 Ω. The modification of CPE with $[Zn(phen\text{-}dione)_3](PF_6)_2$ decrease R_{ct} to 500 Ω suggesting a significant acceleration for the $[Fe(CN)_6]^{3-/4-}$ redox reaction. The CV responses of $[Fe(CN)_6]^{3-/4-}$ at the bare GCE and modified electrode also confirmed the EIS results (Fig. 1B).

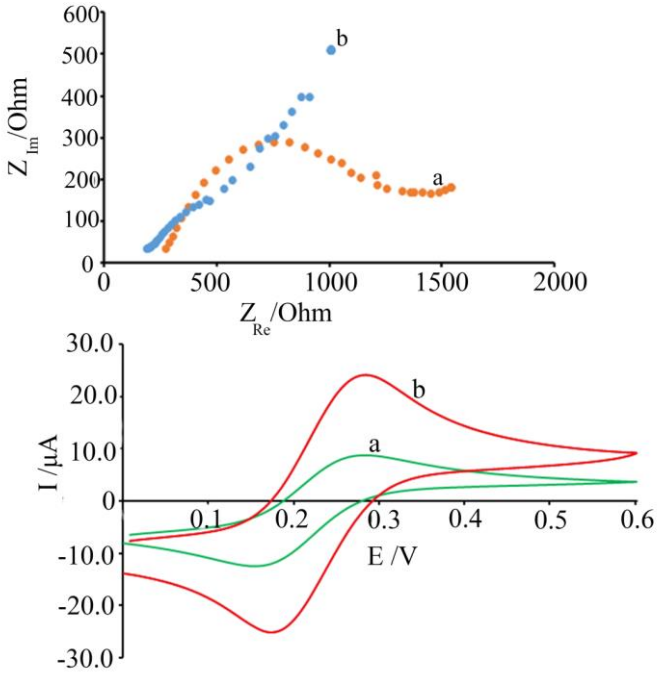

Fig. 1. A) Impedance spectra; and B) CVs of a) CPE and b) $[Zn(phen\text{-}dione)_3](PF_6)_2$ modified electrode in 5.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ containing 0.1 mol L^{-1} KNO_3. Conditions: polarization potential: 0.15 V, frequency: 5.0×10^{-3} to 10^5 Hz. CV measurement at scan rate of 100 mV s^{-1} between 0.0 and +0.60 V.

3.2. Electrochemical behavior of D-PA on $[Zn(phen\text{-}dione)_3](PF_6)_2$ /CPE

Cyclic voltammetry of 50 µM PA (Fig. 2a) shows that there is no obvious peak in the range of 0.2 to +1.0 V (*vs.* Ag/AgCl) on the CPE because of large over voltage on the bare CPE. However, at modified electrode D-PA in solution, give considerable anodic current at 0.78 V (Fig. 2c). Fig. 2b show that $[Zn(phen\text{-}dione)_3](PF_6)_2$/CPE has a featureless voltammetric response in the range of 0.2 to +1.0 V in pH=4.0.

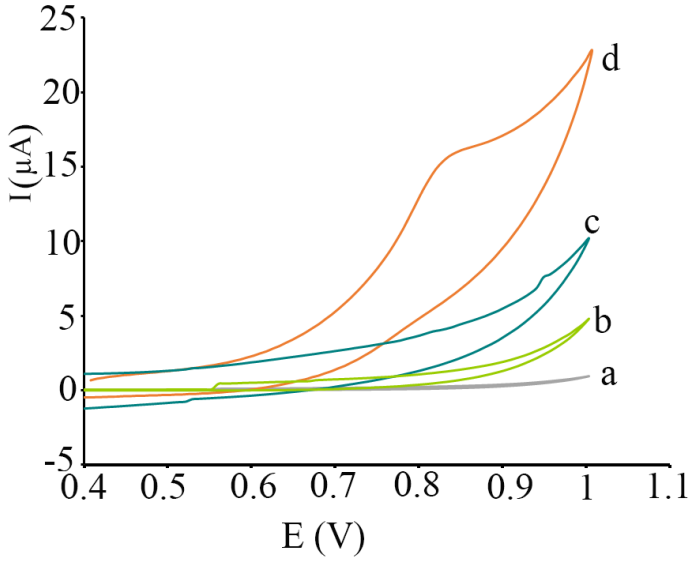

Fig. 2. Cyclic voltammograms of the blank solution at (a) CPE, and (b) $[Zn(phen\text{-}dione)_3](PF_6)_2$ modified electrode. (c) as (a) and (d) as (b) in a solution containing 50 µmol L^{-1} D-PA. Condition: scan rate of 50 mVs^{-1}

Due to the electrochemically innocent behavior of the Zn(II) ion in comparison to other phen-dione transition metal complexes, there is not any reduction couple associated to metal center. D-PA has three pK_a values related to –COOH (1.8), $–NH_2$ (7.9) and –SH (10.5) groups. At pH=4.0 D-PA has negative charges due to carboxylate anion. Since zinc complexes are labile and rapidly exchange its ligands for others, the carboxylate can compete with phen-dione ligand for exchange. The mechanism seems to be dangling bond rupture. Fig. 3 show the influence of pH solution on the oxidation peak potential of D-PA in the pH range 3.0–8.0.As shown in Fig. 3, the oxidation peak potential of D-PA shifts negatively with rising pH confirmed that deprotonation occurs during the oxidation process. As the value of proton in the electrode reaction could be estimated by $E_p=E_0$-0.059p/and pH, the slope of the/DPH indicates the number of electron and poroton involved in the electrochemical reaction. The plot of E_p versus pH give a linear equation as: $E_p(V)$=-0.054 pH+0.4347 imply the number of electron and poroton are equal. The oxidation mechanism of D-PA has been previously proven that contain two electrons and two protons [26].

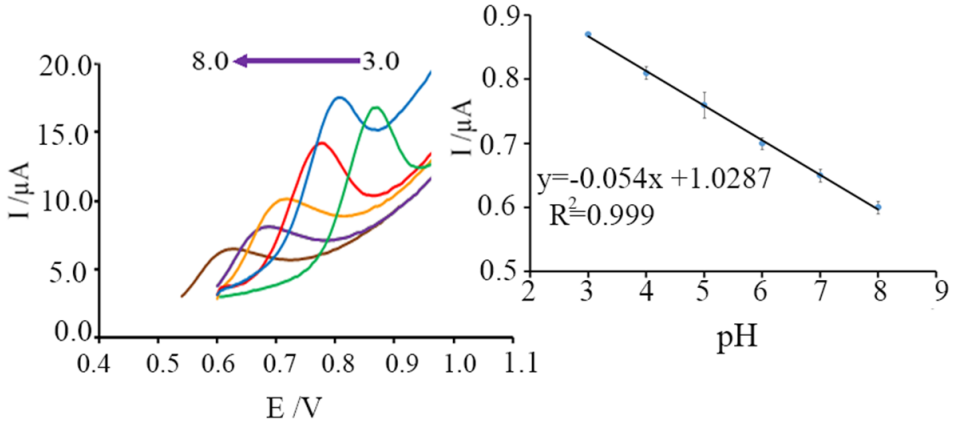

Fig. 3. Dependence of oxidation peak potential of 45 µmol L^{-1} D-PA with pH at [Zn(phen-dione)$_3$](PF$_6$)$_2$ modified CPE. Pulse amplitude of 100 mV, Pulse time of 50 ms, Sweep rate of 50 mV s^{-1}

Cyclic voltammetry was used for the relationship between the anodic peak current (I_{pa}) and the scan rate in the range of 10 mV s^{-1} to 100 mV s^{-1} for 40 µmol L^{-1} of D-PA. The regression equation is I_P=146.81 υ+3.198 with R^2=0.9962. The results confirm that the anodic peak currents increase linearly with the increasing of the scan rate, suggesting that the adsorption controlled.

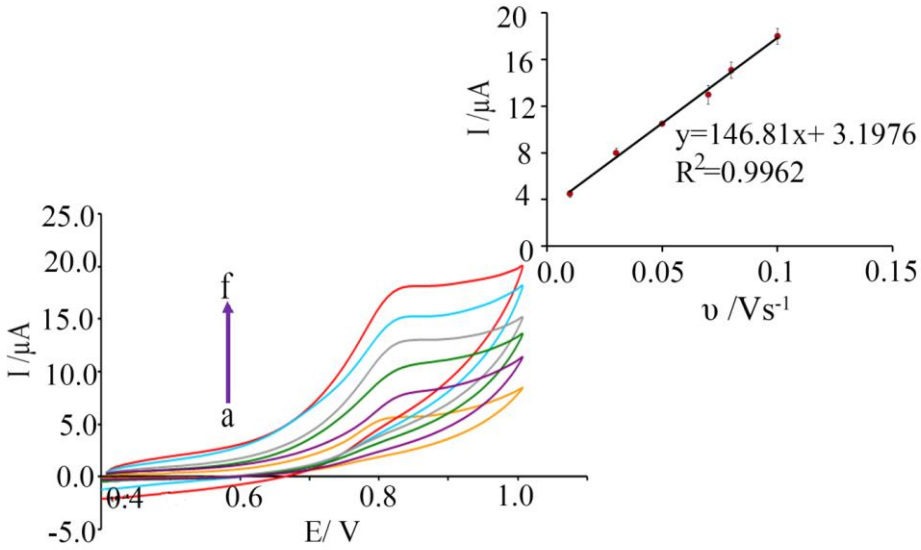

Fig. 4. Cyclic voltammograms of 40 µmol L^{-1} D-PA at [Zn(phen-dione)$_3$](PF$_6$)$_2$ modified electrode with various scan rates as: a) 10; b) 30; c) 50; d) 70; e) 80.0;, and f) 100 mV s^{-1}

Accumulation potential and time are two crucial conditions for the accumulation step. The influence of the accumulation potential on the oxidation peak current was evaluated over

the potential range of 0.2 to 0.75 V. The results indicated that, an accumulation potential of +0.70 V gives the best sensitivity and was selected for the study. The DPV peak current of D-PA increases with accumulation time increasing from 0 to 100 s indicated that D-PA was adsorbed onto the modified electrode surface. Further increment of the accumulation time, the oxidation peak current increased slightly. This phenomenon may be due to the electrode surface becomes saturated. Hence, an accumulation time of 100 s was chosen for the best conditions for the method proposed.

3.3. Stability and reproducibility of the sensor

The stability of $[Zn(phen\text{-}dione)_3](PF_6)_2$ /CPE was checked out over a four-week period using 50 µmol L^{-1} of D-PA. The cyclic voltammetry of D-PA at the surface of the modified electrode (stored in the laboratory at room temperature) shows that the oxidation peak potential of D-PA was retained without any alteration in the peak potential and the anodic peak current is only decreased less than 4.3% of the initial oxidation peak current. The reproducibility of the modified electrode was investigated by comparing the current of DPV response to D-PA at six modified electrodes prepared independently. The relative standard deviation (RSD) of 3.1% was obtained for D-PA concentration of 50 µmol L^{-1}.

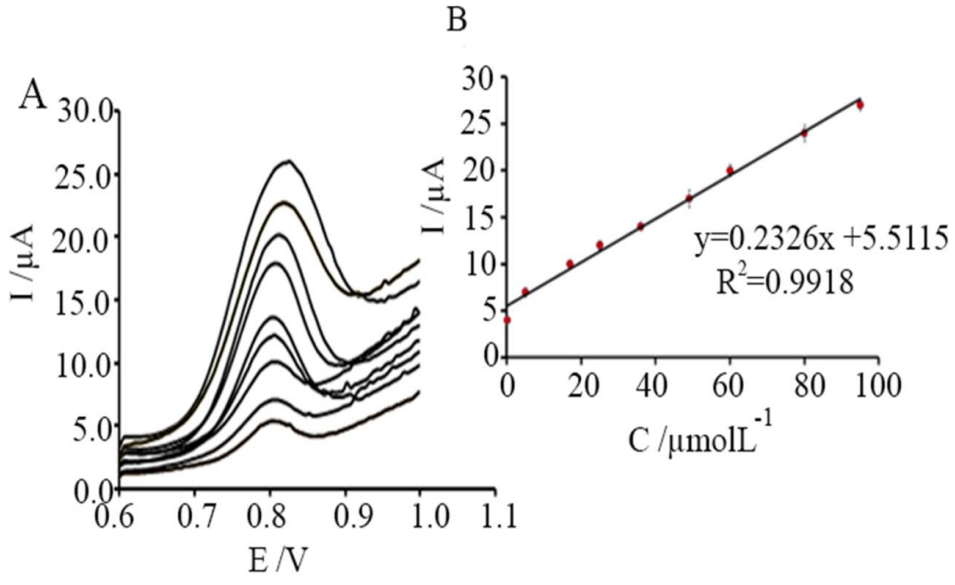

Fig. 5. A) DPVs of 0.1, 5.0, 17.0, 25.0, 36.0, 49.0, 60.0, 80.0 and 95.0 µmol L^{-1} D-PA at $[Zn(phen\text{-}dione)_3](PF_6)_2$ modified electrode, B) The calibration curve for the determination of D-PA on modified electrode. DPV experimental conditions: Pulse amplitude of 100 mV, Pulse time of 50 ms, Sweep rate of 50 mV s^{-1}

3.4. Calibration curve and detection limit

The differential pulse voltammetry method was used for determination of the D-PA concentration in the aqueous solutions with optimum conditions. The plot of peak current *vs.* the D-PA concentration give linear segment with the regression equation of I_p (μA)=(0.2326±0.0030) C+(5.5115±0.5000) (R^2=0.9918, n=5), where C is concentration of D-PA in the ranges of 0.1 to 95.0 μmol L^{-1}. The detection limit of the method was found to be 0.05 μmol L^{-1} D-PA.

3.5. Interferences

In order to investigate the analytical selectivity of the proposed method, the response of the modified electrode in 10 μmol L^{-1} D-PA was evaluated in the presence of various foreign species. The tolerance limit was defined as the extreme amount of the foreign ion yielding a relative error less than 5% in the oxidation signal of D-PA. The results are presented in Table 2. As the results show, cysteine and other thiol compounds such as glutathione at more than 20-fold interfered. However, despite their interference, they aren't present at significant levels in the plasma samples [27].

Table 2. Tolerance limit of interfering ions for the determination of 50.0 μmol L^{-1} D-PA under the optimized conditions

Species	**Tolerance limit ($mol_{Substance}/mol_{D-PA}$)**
Na^+, Cl^-, Ca^{2+}, K^+, NO_3^-, Mg^{2+}, ClO_4^-, SO_4^{2-},	800
Fructose, Sucrose, Lactose, Urea, Aspartic acid, Glycine, Glucose	500
Tryptophane, Citric acid	300
Uric acid, Ascorbic acid	30
Gluthathione, Cysteine	20

3.6. Serum samples analysis

In order to validate the proposed method and to more reveal its features, D-PA was determined in serum of patients with rheumatoid arthritis receiving 250 mg D-penicillamine. The samples were collected after 4 hours and D-PA contents were determined using the standard addition method. The result listed in Table3. As the results show, the recovery are between 95.3 to 104.3% indicate that serum matrix do not interfere with the detection of D-PA.

Table 3. Determination of D-PA in patient serum samples (n=3)

Real sample	Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)
Serum 1	-	44.2±2.0	-
	10.0	53.4±2.7	98.5
Serum 2	-	35.5±3.1	-
	5.0	38.6±3.0	95.3
Serum 3	-	40.0±2.6	-
	15.0	57.1±4.0	96.3
Serum 4	-	38.7±1.9	-
	10.0	50.8±2.3	104.3

4. CONCLUSION

A new derivative of zinc complex, tris(1,10-phenanthroline-5,6-dione) zinc(II) hexa fluorophosphates, was synthesized and used for the modification of a carbon paste electrode. Despite of zinc complex exhibits no voltammetric response in the range of studied potential; the complex had a considerable electrocatalytic effect on the oxidation of D-PA. The modified electrode has been shown to be promising for the detection of D-PA with many desirable properties including good reproducibility, high sensitivity, wide dynamic range and excellent catalytic activity towards D-PA. Applicability of the proposed sensor was demonstrated by determination of D-PA in serum of rheumatoid arthritis patients with appreciable recovery values**.**

REFERENCES

[1] A. Deshpande, K. Baheti, and N. Chatterjee, Curr. Sci. 87 (2004) 1684.
[2] J. M. Walshe, Am. J. Med. 21 (1956) 487.
[3] A. A. Torriero, E. Salinas, E. J. Marchevsky, J. Rabaa, and J. J. Silber, Anal. Chim. Acta 580 (2006) 136.
[4] R. F. Bergstrom, D. R. Kay, and J. G. Wagner, J. Chromatogr. 222 (1981) 445.
[5] I. M. Merino, E. B. González, and A. Sanz-Medel, Microchim. Acta 107 (1992) 73.
[6] Z. D. Zhang, W. R. G. Baeyens, X. Zhang, Y. Zhao, and G. van der Weken, Anal. Chim. Acta 347 (1997) 325.
[7] F. E. Suliman, M. M. Al-Hinai, S. M. Al-Kindy, and S. B. Salama, Talanta 74 (2008) 1256.
[8] L. Song, Z. Guo, and Y. Chen, Electrophoresis 33 (2012) 2056.
[9] X. Yang, H. Yuan, C. Wang, X. Su, L. Hu, and D. Xiao, J. Pharm. Biomed. Anal. 45 (2007) 362.

[10] F. Mirrahimi, M. A. Taher, H. Beitollahi, and R. Hosseinzadeh, Appl. Organometal. Chem. 26 (2012) 194.
[11] M. Mazloum-Ardakani, H. Beitollahi, Z. Taleat, H. Naeimi, and N. Taghavinia, J. Electroanal. Chem. 644 (2010) 1.
[12] S. Shahrokhian, A. Souri, and H. Khajehsharifi, J. Electroanal. Chem. 565 (2004) 95.
[13] F. E. O. Suliman, H. A. J. Al Lawati, S. M. Z. Al Kindy, I. E. M. Nour, and S. B. Salama, Salama, Talanta 61 (2003) 221.
[14] S. Belal, O. Abdel-Razak, A. F. El-Walily, and R. Bakry, Int. J. Biomed. Sci. 7 (2011) 289.
[15] P. Wang, B. L. Li, N. B. Li, and H. Q. Luo, Spectrochim Acta 135 (2015) 198.
[16] R. A. A. Shaalan, Cent. Eur. J. Chem. 8 (2010) 892.
[17] M. Taei, F. Hasanpour, M. Movahedi, and S. Mohammadian, RSC Adv. 5 (2015) 37431.
[18] A. Ghaffarinejad, F. Hashemi, Z. Nodehi, and R. Salahandish, Bioelectrochemistry 99 (2014) 53.
[19] J. B. Raoof, R. Ojani, F. Chekin, and R. Hossienzadeh, Int. J. Electrochem. Sci. 2 (2007) 848.
[20] Y. Yamada, H. Sakurai, Y. Miyashita, K. Fujisawa, and K. Okamoto, Polyhedron 21 (2002) 2143.
[21] A. R. Rezvania, H. Saravani, and H. Hadadzadeh, J. Iran. Chem. Soc. 7 (2010) 825.
[22] J. B. Raoof, R. Ojani, M. Majidian, and F. Chekin, J. Appl. Electrochem. 39 (2009) 799.
[23] H. Hadadzadeh, M. M. Olmsted, A. R. Rezvani, N. Safari, and H. Saravani, Inorg. Chim. Acta 359 (2006) 2154.
[24] K. Kano, and B. Uno, Anal. Chem. 65 (1993) 1088.
[25] M. J. O'Neil, and S. Budavari, The Merck index: an encyclopedia of chemicals, drugs, and biologicals, Merck Whitehouse Station, NJ (2001).
[26] J. B Raoof, R. Ojani, and F. Chekin, J. Chem. Sci. 121 (2009) 1083.
[27] Z. D. Zhang, W. R. G. Baeyens, X. R. Zhang, and G. Van der Weken, Biomed. Chromatogr. 9 (1995) 287.