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ARTICLES

Electrocatalytic Determination of Penicillamine Using Multiwall Carbon Nanotubes Paste Electrode and Chlorpromazine as a Mediator[1]

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Abstract—In this study, we describe the application of carbon paste electrode modified with multiwall carbon nanotubes as a voltammetric sensor for determination of penicillamine (**PA**) in the presence of chlorpromazine as a mediator. This modified electrode showed very efficient electrocatalytic activity for the anodic oxidation of PA. The peak current of linear sweep voltammograms of PA increased linearly with it's concentration in the range of 0.5–500 μM PA. The detection limit for PA was 0.2 μM. The RSDs for 1.0 and 10.0 μM PA were 1.1 and 1.7%, respectively. The proposed sensor was successfully applied for the determination of PA in human urine and tablet.

Keywords: penicillamine, carbon nanotubes paste electrode, sensor, voltammetry

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Penicillamine is a pharmaceutical of the chelator class. The pharmaceutical form is D-penicillamine, whereas its L-penicillamine form is toxic(because it inhibits the action of pyridoxine). Despite having no antibiotic effects, penicillamine is a metabolite of penicillin. It is the drug of first choice for patients with Wilson's disease [1], an autosomal recessive disorder of copper transport [2]. It is able to enhance the urinary excretion of others heavy metals such as lead, arsenic, mercury and zinc and therefore, is used as an oral chelating agent to treat conventional heavy metals intoxication [3]. It is also used as antifibrotic agent to treat scleroderma [4] and as antirheumatic drug to treat patients with active rheumatoid arthritis [5]. PA reduces excess of cystine excretion in cystinuria, another rare inherited disease affecting the active transport of the diamino acids cystine, ornithine, lysine and arginine across the renal tubule and the small intestine [6]. It may also inhibit the replication of the human immunodeficiency virus, the cause of acquired immune deficiency syndrome (AIDS) [7]. The typical dose administered to humans is 0.5–2.0 g daily. Several methods have been proposed for the determination of PA in biological specimens and pharmaceutical formulations. Up to now, PA has been determined by HPLC with fluorescence [8], and mercury-based electrochemical [9] and other electrochemical methods [10] have also been proposed for the determination of PA. Carbon nanotubes are a new kind of nanostructuredinorganic material promising as an immobilization substance for different electron transfer mediators [11, 12]. The electronic properties of these nanomaterials have been exploited as a mean of promoting the electron transfer reaction for a wide range of molecules and biological species [13–20].

In this study we describe application of multiwall carbon nanotubes paste electrode (**MWCNTPE**) in the presence of chlorpromazine (**CHP**) as a suitable mediator for sensitive and selective determination of PA in aqueous solution.

EXPERIMENTAL

Apparatus and reagents. For electrochemical investigation we used a potentiostat/galvanostatAutolab PGSTAT 302N, (Utrecht, The Netherlands) connected to a three-electrode cell, Metrohm (Herisau, Switzerland) Model 663 VA stand, linked with a computer (Pentium IV, 1.200 MHz) with Autolab software. A platinum wire was used as the auxiliary electrode. MWCNTPE and $Ag/AgCl/KCl_{sat}$ were used as the working and reference electrodes, respectively. The electrode prepared with carbon nanotubes was characterized by scanning electron microscopy (**SEM**) (Seron Tech. AIS 2100). A digital pH/mV-meter (Metrohm model 710) was applied for pH measure-

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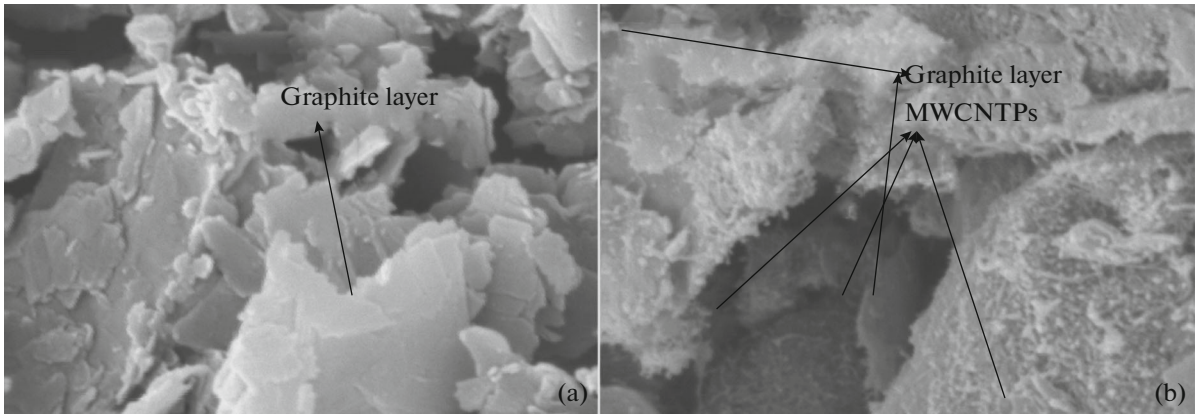


Fig. 1. SEM images of CPE (a) and MWCNTPE (b).

ments. Spectrally pure graphite powder (particle size <50 μm) from Merck and multiwall carbon nanotubes (>90% MWCNTs basis, *dl* = (90–70 nm)(5–9 μm) from Fluka were used as the substrate for the preparation of the carbon paste electrode.

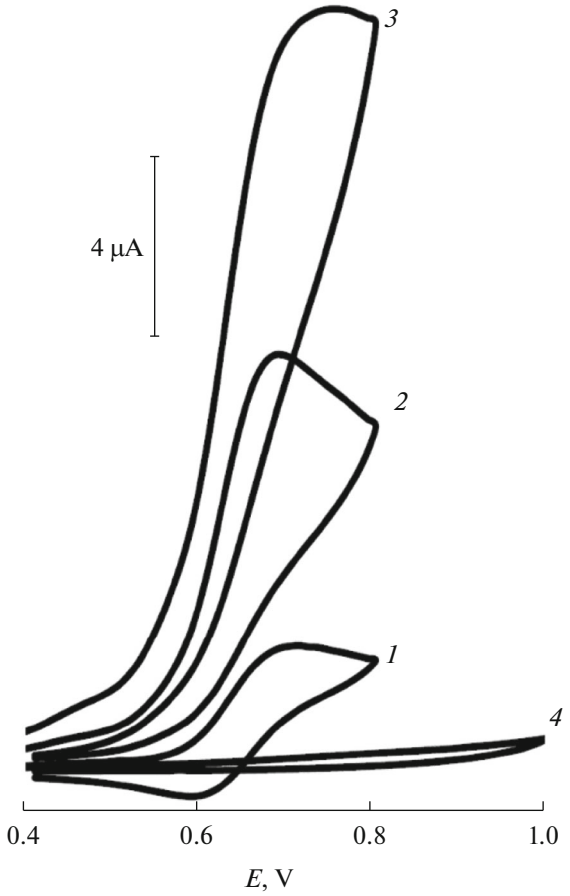


Fig. 2. Cyclic voltammograms in 0.04 M universal buffer (pH 4.0) at a scan rate of 10 mV/s. (*1*) 300 μM CHP at the surface of MWCNTPE, (*2*) 500 μM PA and 300 μM CHP at the surface of CPE, (*3*) 500 μM PA and 300 μM CHP at the surface of MWCNTPE, (*4*) 500 μM PA at the surface of MWCNTPE.

Preparation of the electrode. Graphite powder (0.900 g) was dissolved in diethyl ether and hand mixed with 0.100 g carbon nanotubes with a mortar and pestle. The solvent was evaporated by stirring. A syringe was used to add paraffin to the mixture, which was mixed well for 50 min until a uniformly oiled-pastee, was obtained. The paste was then packed into a glass tube.The electrical contact was made by pushing a copper wire down the glass tube from the back of the mixture. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing it on weighing paper.

Preparation of real samples. For the determination of PA in pharmaceuticals, five capsules of PA labeled 250 mg per capsule were completely ground and homogenized. Then, 150 mg of the powder was accurately weighed and dissolved in 25 mL of water then the mixture was filtered through a 0.45 μm filter. The resulted solution was diluted 20 fold with the buffer (pH 4.0) and used for the determination of PA.

Urine samples were stored in a refrigerator immediately after collection. Ten milliliters of the sample solution was centrifuged for 10 min at 2000 rpm. The supernatant was filtered using a 0.45 μm filter and diluted seven fold with the buffer solution (pH 4.0). The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. Standard addition method was used for the determination of PA in real samples.

RESULTS AND DISCUSSION

SEM characterization. Typical SEM images of different electrodes are shown in Fig. 1. It can be seen that at the surface of carbon paste electrode (**CPE**) (Fig. 1a), the layer of irregular flakes of graphite powder was present and isolated with each other. By addition of MWCNTs to the carbon paste, it can be seen that MWCNTs were distributed on the electrode with

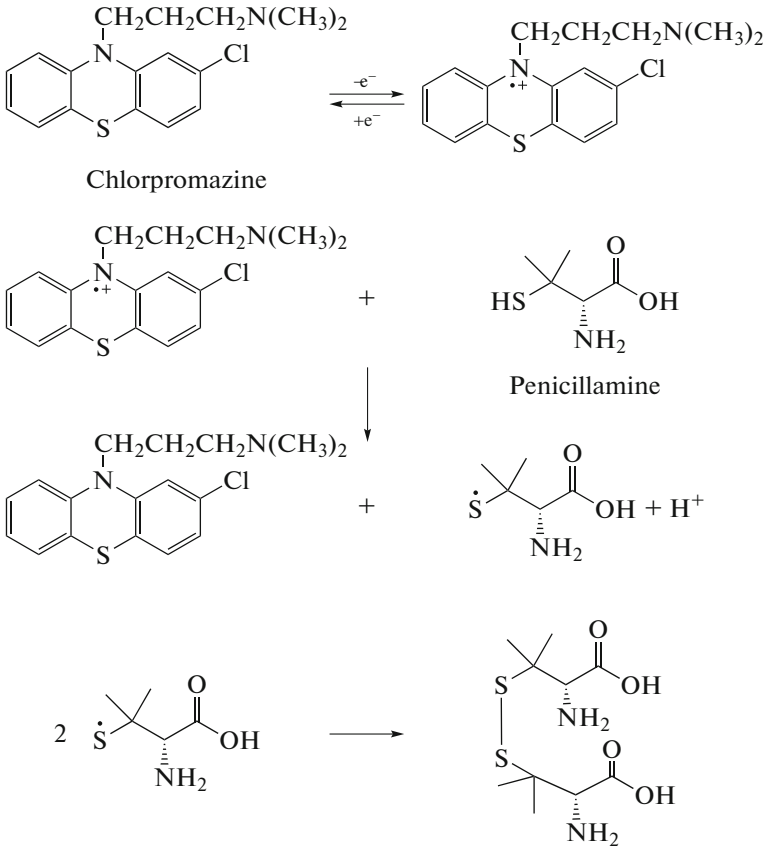
a special three-dimensional structure (Fig. 1b), indicating that MWCNTs were mixed well with graphite layer to make a homogeneous mixture on a MWCNTPE.

Electrocatalytic effect. An objective of the present work was to develop a modified electrode capable of electrocatalytic oxidation of PA. The cyclic voltammetric responses for the electrochemical oxidation of 500 μM of PA at MWCNTPE and at CPE in the presence of 300 μM CHP are shown in Fig. 2, curves *3* and *2*, respectively. Curve *4* (Fig. 2) is the same as curves *3*, but only without the mediator. Curve *1* (Fig. 2) show the cyclic voltammogram of CHP (300 μM) at a surface of MWCNTPE in the buffer solution (pH 4.0). As can be seen, the anodic peak potentials for the oxidation of PA in the presence of mediator at both MWCNTPE and CPE (curves *3* and *2*) are about 680 mV. On the other hand, PA oxidation (without the mediator) does not take place at the surface of MWCNTPE up to +1. 0 V. Similarly, when we compare the anodic peak current of PA with the mediator at MWCNTPE (Fig. 2, curves *3*) and at CPE (Fig. 2, curves *2*), an enhancement of found at the MWCNTPE.

In other words, the data obtained clearly show that the combination of carbon nanotubes and the CHP mediator definitely improve the characteristics of the electrode for the oxidation of PA, by increasing the sensitivity and decreasing its overpotential. Based on these results, the following catalytic diagram (EC' catalytic mechanism) [21–31] describes the voltammetric response of the electrochemical oxidation of PA in the presence of mediator at the surface of MWCNTPE (Scheme).

To obtain further information on the rate determining step, a Tafel plot was developed for the PA at MWCNTPE in the presence of the mediator using the data derived from the rising part of the current–voltage curve (Fig. 3). The slope of the Tafel plot is equal to $n(1-\alpha)F/2.3RT$, which comes up to 7.7071 decade/V. We obtained n_α as 0.54. Assuming $n = 1$, then $\alpha = 0.54$.

The effect of scan rate on the electrocatalytic oxidation of PA at the MWCNTPE in the presence of the mediator was investigated by cyclic voltammetry (Fig. 4). As can be observed in Fig. 4, the oxidation peak potential shifted to more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, a plot of peak height (I_p) vs. the square root of scan rate ($v^{1/2}$) was found to be linear in the range of 2–20 mV/s, suggesting that, at sufficient overpotential, the process is diffusion rather than surface controlled (Fig. 4a).



The role of chlorpromazine in the oxidation of PA.

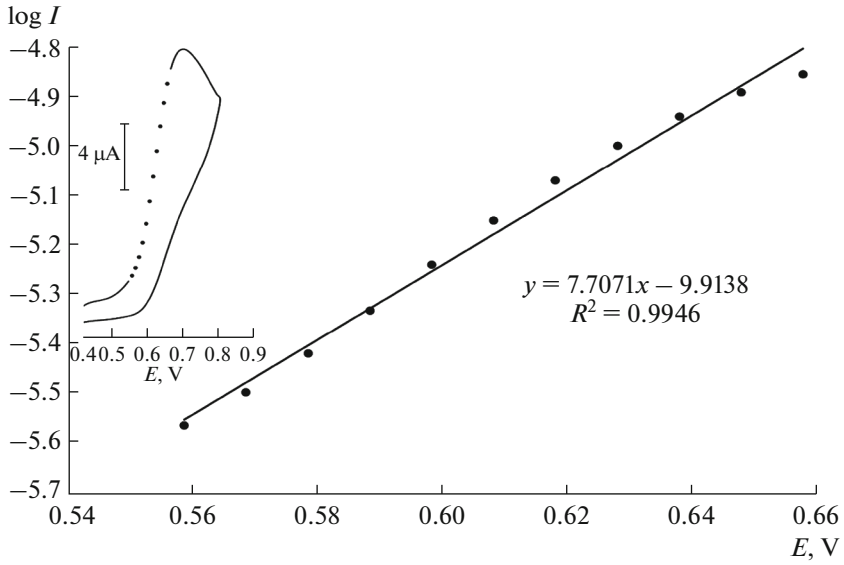


Fig. 3. Tafel plot for 200 μM PA at the surface of MWCNTPE in 0.04 M universal buffer (pH 4.0) at a scan rate of 20 mV/s in the presence of 300 μM CHP.

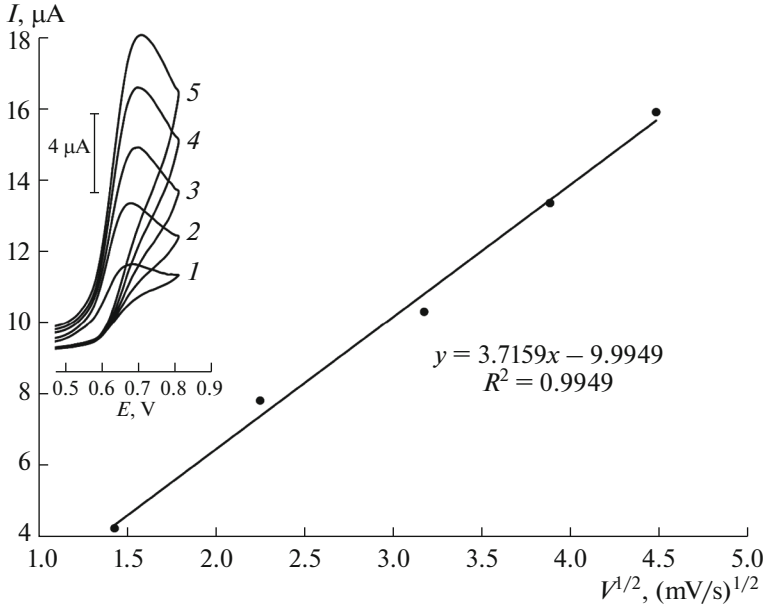


Fig. 4. Plot of I_{pa} versus $\nu^{1/2}$ for the oxidation of 200 μM PA in the presence of 300 μM CHP at the surface of MWCNTPE. Inset: cyclic voltammograms of 200 μM PA in the presence 300 μM CHP at scan rates as 2 (*1*), 5 (*2*),10 (*3*), 15 (*4*) and 20 (*5*) mV/s in 0.04 M buffer solution (pH 4.0).

Chronoamperometric measurements of PA at the MWCNTPE in the presence of the mediator were carried out by setting the working electrode potential at 0.5 V (at the first potential step) and at 0.8 V (at second potential step) vs. $Ag/AgCl/KCl_{sat}$ for the various concentrations of PA in universal buffer solution (pH 4.0) (Fig. 5a). For an electroactive material (PA in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [26]. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of PA (Fig. 5b). The slopes of the resulting straight lines were then plotted vs. PA concentration (Fig. 5b). From the resulting slope and Cottrell equation the mean value of D was found to be 2.5×10^{-4} cm^2/s.

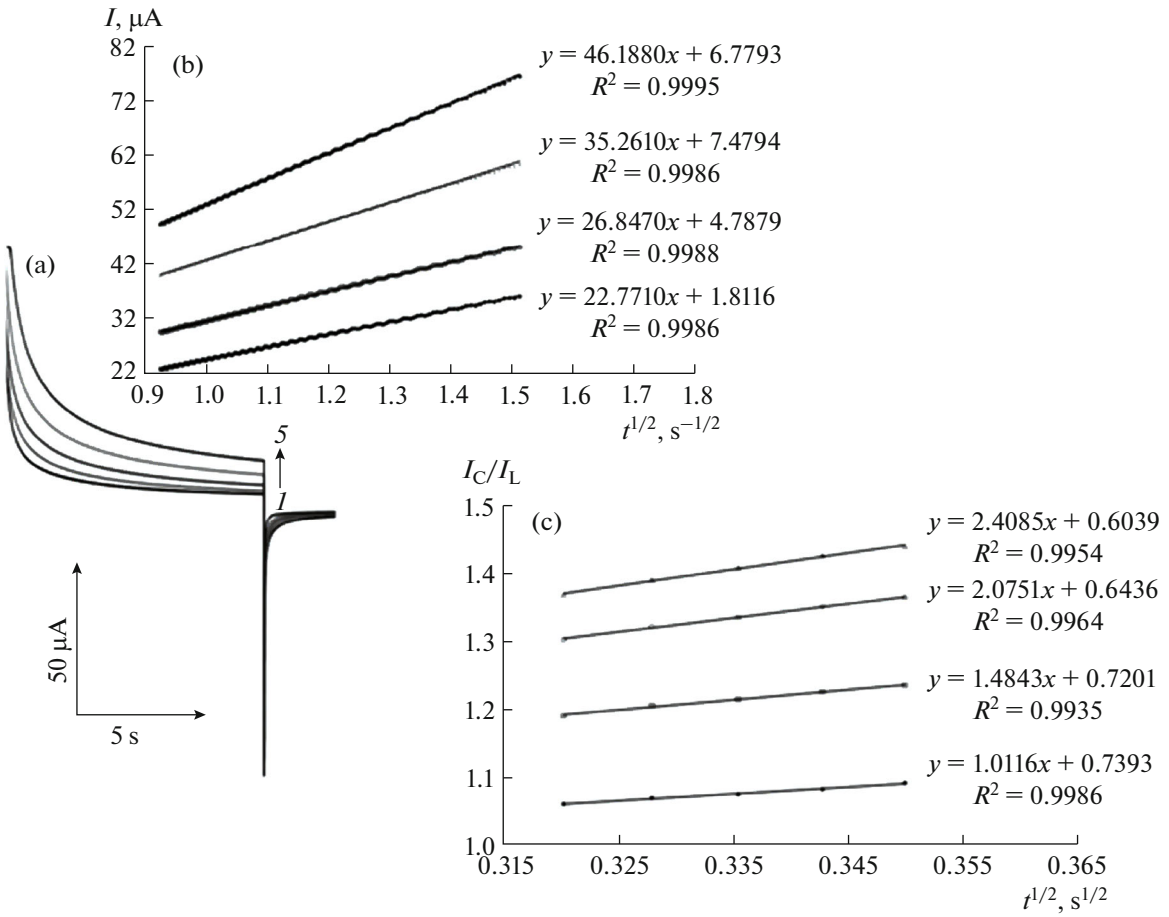


Fig. 5. (a) Chronoamperograms obtained for 300 μM CHP at the MWCNTPE in the absence (*1*) and in the presence of 200 (*2*), 300 (*3*), 400 (*4*) and 500 (*5*) μM PA in a buffer solution (pH 4.0). (b) Cottrell's plot for the data from the chronoamperograms. (c) Dependence of I_C/I_L on the $t^{1/2}$ derived from the chronoamperogram data.

However, we determined catalytic reaction rate constant k_h for PA using Galuse method. Based on the slope of the I_C/I_L (I_C is the catalytic current, I_L is the limited current in the absence of PA) versus $t^{1/2}$ plots, k_h can be obtained for a given PA concentration. From the values of the slopes an average value of k_h was found to be 4.29×10^3 L/ mol.

Dynamic range and limit of detection. Linear sweep voltammetry was used for determination of PA. The linear sweep voltammograms clearly showed two linear dynamic ranges. The plot of the peak current versus PA concentration was linear for 0.5–80 μM with a regression equation of I_p (μA) = $(0.0618 \pm 0.0021)c_{PA} + (6.8194 \pm 0.4122)$ ($r^2 = 0.9857$, $n = 8$) and for 80–500 μM of PA, the regression equation was I_p (μA) = $(0.0279 \pm 0.0011)c_{PA} + (10.1810 \pm 0.7351)$ ($r^2 = 0.9857$, $n = 7$). The detection limit was 0.2 μM PA according to the definition of $Y_{LOD} = Y_B + 3\sigma$.

Interference study. The influence of various substances as compounds potentially interfering with the determination of PA was studied with 10.0 μM PA. The potentially interfering substances were chosen from the group of substances commonly found with PA in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5% for the determination of PA. We found that neither 1000-fold amounts of L-aspartic acid, glycine, urea, succinic acid, glucose, orinthrine, Ba^{2+}, Cl^-, fructose, Mg^{2+}, Na^+, SO_4^{2-}, Ca^{2+}, methionine, alanine, lactose, and sucrose, and nor 600-fold amounts of methanol, ethanol, saturated starch solution, 100-fold of benzoic acid interfered with the determination of PA. Although ascorbic acid show interference, it can be minimized by using ascorbic oxidase enzyme, which exhibits high selectivity to oxidation of ascorbic acid, if necessary.

Application of the method. In order to evaluate the applicability of the proposed modified electrode in real sample analysis, it was used for the determination of PA in urine and tablet samples using standard addition method. In addition, electrochemical methods [10] were used for the analysis of the analytes, to con-

Table 1. Determination of PA in pharmaceutical and urine samples ($n = 3$)

Sample	Added, μM	Expected, μM	Found, μM	Found by published method [10], μM	F_{ex}	F_{tab}	t_{ex}	t_{tab}
Tablet	–	5.00	4.9 ± 0.4	5.5 ± 0.7	5.0	19	1.3	3.8
	4.00	9.00	9.1 ± 0.3	9.4 ± 0.6	4.5	19	1.1	3.8
	11.00	20.00	20.5 ± 0.5	19.7 ± 0.8	8.5	19	2.0	3.8
Urine	–	–	<LOD	<LOD	–	–		–
	40.00	40.00	40.2 ± 0.2	39.9 ± 0.4	6.0	19	1.4	3.8
	20.00	60.00	59.8 ± 0.4	50.6 ± 0.8	6.6	19	1.8	3.8
Urine[a]	–	–	4.2 ± 0.1	4.5 ± 0.4	7.5	19	2.0	3.8
	2.78	7.00	7.3 ± 0.4	7.6 ± 0.6	8.5	19	2.5	3.8

[a]Sample was taken from a man in 2.0 h after used PA.

F_{ex} is calculated F-value; F_{tab} is the F value obtained from one-tailed table of F-test; t_{exp} is calculated value of Student's t-test; t_{tab} is the t-value obtained from the table of Student s t-test.

firm the accuracy of the proposed method. The results presented in the Table indicates that's the modified electrode retained its efficiency for the determination of PA in real samples with satisfactory results.

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