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Polyglycine Modification of Glassy Carbon Electrode as a Sensor for Effective Sensing of Pyrazole Derivative and its Analytical Applications

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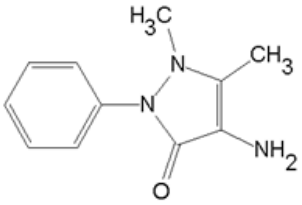
Abstract- Electrochemical investigations of 4-aminoantipyrine (4-AA) at polyglycine modified glassy carbon electrode (poly(glycine)MGCE) was carried out in acidic phosphate buffer medium. 4-AA exhibited a well-defined oxidation peak at a potential of 0.446 V with an oxidation current of 5.72 µA. Compared to the oxidation currents obtained by bare glassy carbon electrode (GCE) and modified electrode, poly(glycine)MGCE exhibited good catalytic activity towards the determination of 4-AA. Results of scan rate investigation indicate that electrode process was diffusion controlled. Effect of concentration of the analyte on current was studied. With the help of calibration curve, detection limit was calculated and was found to be 0.97 nM. Prepared electrode was applied for the recovery study of drug in urine with spiked as well as real samples.

Keywords- Poly(glycine) modification, Diffusion controlled, Calibration curve, Urine samples, Detection limit

1. INTRODUCTION

Pyrazole($C_3H_3N_2H$) is a characteristic heterocyclic organic compound having 5-membered ring of three carbon atoms and two adjacent nitrogen atoms.



Scheme 1. Chemical structure of 4-aminoantipyrine

4-Aminoantipyrine (4-AA), Scheme 1, is such a derivative of pyrazole having temperature reducing characteristic property [1]. It is used in the preparation of azo dyes [2]. 4-AA has also been used for many important directions in medical applications such as the protection against oxidative stress and defends of several diseases including cancer [3]. Derivatives of antipyrine were found to having an analgesic [4], anti-inflammatory [5], antimicrobial [6], and anticancer activity [7-9]. These are also strong inhibitors of cyclooxygenase enzymes, platelet thromboxane synthesis, and prostanoids synthesis [10]**,** which catalyze the rate-limiting step of prostaglandin synthesis. In view of medicinal application, hazardous effects of 4-AA on human body and environment issues its trace determination becomes important.

Literature exposes that there are many reports available for the determination of 4-AA in pure or pharmaceutical formulations including gas chromatography [11], Liquid Chromatography/Mass Spectrometry [12], Liquid/Solid Extraction followed by Liquid Chromatography/Mass Spectrometry [13], Capillary electrophoresis [14], solid phase spectroscopy [15], HPLC methods [16-18], kinetics methods [19], cloud point extraction [20] and electrochemical methods [21,22]. Although HPLC has been widely applied because of its high sensitivity, selectivity, the ability to minimize interferences but it is time consuming, solvent usage intensive and requires expensive devices and maintenance. Electrochemical detection of analyte is a very elegant method in analytical chemistry [23].

Electro-polymerization plays a major role in the field of electrochemistry. Now a days electropolymerized electrodes receiving wide range of applications in the detection of pharmaceutical compounds because of their high selectivity and sensitivity, uniformity in the electrochemical deposition with strong adherence to electrode surface and chemical stability of the films [24-26]. Different mechanisms such as size exclusion [27], ion exchange [28], hydrophobicity interaction [29] and electrostatic interaction [30,31] plays major role in the preparation of electropolymerized electrode. The present study is focusing to construct a stable as well as sensitive electrochemical sensor based on polyglycine modified glassy carbon electrode (poly(glycine)MGCE) and to extend the same for the analytical applications by using differential pulse voltammetric technique.

2. EXPERIMENTAL

2.1. Reagents

Stock solution (1.0 mM) of 4-AA (Sigma–Aldrich) was freshly prepared in double distilled water. The supporting electrolyte was phosphate buffer solution (PBS) of 0.2 M with pH 3.0. PBS of other pH values (3.-9.2) was prepared by mixing the corresponding quantities of KH_2PO_4, Na_2HPO_4, $Na_3PO_4.12H_2O$ and sodium bicarbonate (s-d FINE CHEM limited). Other reagents used were of analytical grade, and all solutions were prepared using ultra-purified water.

2.2. Apparatus

All voltammetric experiments were performed using CH Instrument (Model CHI1112C (Version 9.03) USA) connected to a personnel computer. A conventional three-electrode cell was used, including a saturated calomel electrode (SCE, 3.0 M KCl) as reference electrode, platinum wire as counter electrode and poly(glycine) MGCE as working electrode. The pH measurements were carried out using Elico LI120 pH meter (Elico Ltd., India).

2.3. Preparation of polyglycine coated glassy carbon electrode

Before the modification procedure, polishing of electrode was done on micro cloths (Buehler) glued to flat mirrors. Different micro cloths were used for each size of alumina.

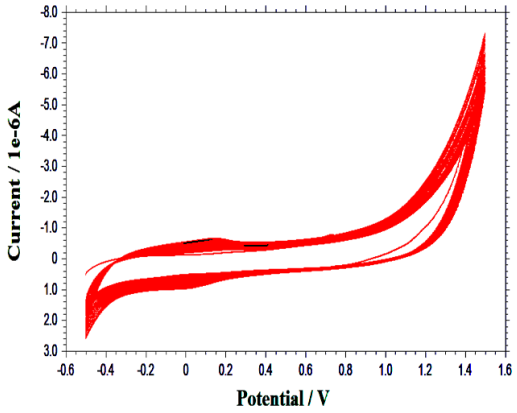


Fig. 1. Cyclic voltammograms for preparation of poly (glycine) modified glassy carbon electrode (0.05 mM aqueous solution of glycine in pH 3.0 of PBS at 20 cycles at a scan rate of 100 mVs^{-1})

The particle sizes used were 0.3, 0.1, and 0.05 μm. After polishing the electrode, it was thoroughly rinsed with water and sonicated in distilled water. It was then placed in 0.05 M glycine solution (pH 3.0 PBS). Poly(glycine)MGCE was prepared according to the literature [32]. GCE was firstly treated by cyclic scanning between -0.5 V and 1.5 V at a sweep rate of

0.1 V/s for twenty cycles as shown in Figure 1. Blue uniform polymer layer was noticed by careful observation on the GCE surface. After modification, the electrode was electro-activated by cyclic voltammetry from 0.0 to 0.8 V at 0.1 V/s in pH 3.0 PBS. Finely, modified electrode was washed with distilled water.

2.4. The surface area of GCE and poly(glycine)MGCE

The surface area for GCE and poly(glycine)MGCE were obtained by the cyclic voltammetric method using 1.0 mM $K_4Fe(CN)_6$ as a probe at different scan rates. For a reversible process following Randles-Sevcik formula was used [33]:

$$i_{pa} = (2.69 \times 10^{-5})n^{3/2}AD_0^{1/2}C_0v^{1/2}$$

where, i_{pa} refers to the anodic peak current, A is surface area of the electrode, C_0 concentration of $K_4Fe(CN)_6$ and υ is scan rate. In 1.0 mM $K_4Fe(CN)_6$, n=1 for electron transfer and the diffusion coefficient, D =7.60×10^{-6} cm^2 s^{-1}. Thus, from the slope of anodic peak current, I_{pa} *vs.* $\upsilon^{1/2}$ graph, electro-active surface area for GCE and poly(glycine)MGCE were calculated to be 0.0452 and 0.109 cm^2 respectively.

2.5. Determination of 4-AA in spiked human urine samples

4-AA in human urine also investigated using calibration curve. The urine sample was diluted 100 times with 0.2 M PBS (pH=3.0) to fit the calibration curve and reduced the matrix effect. No other pre-treatment process was performed.

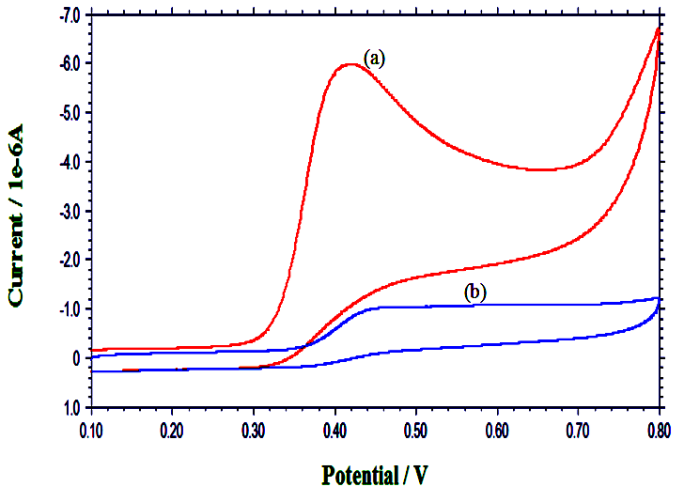


Fig. 2. Electrocatalytic response of 4-AA at (a) poly(glycine) modified glassy carbon electrode; (b) at bare glassy carbon electrode

3. RESULT AND DISCUSSION

3.1. Electro catalytic response of 4-AA at the poly(glycine)MGCE

The CVs of 0.1 mM of 4-AA in PBS of pH 3.0 at poly(glycine)MGCE is compared with the bare GCE as shown in Figure 2. At unmodified GCE, the oxidation of 4-AA result in broad wave with the peak potentials of 0.454 V whereas poly(glycine)MGCE shows significantly enhanced peak current with slightly decrease in peak potential to 0.446 V. This is because the poly(glycine)MGCE has large surface area, excellent conductivity and a large capacitive current, hence fast electron kinetics [34].

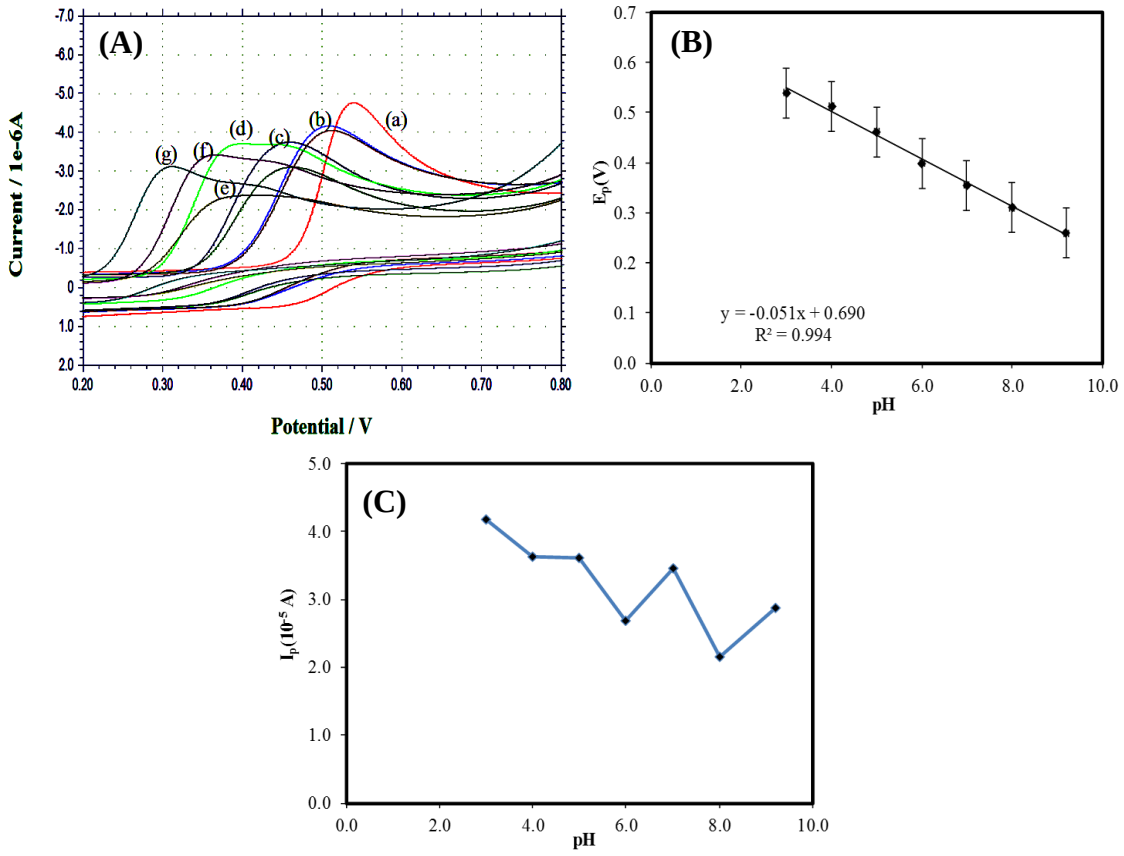


Fig. 3. A) CVs of 1.0 ×10^{-4} M 4-AA at different pH:(a) 3.0 (b) 4.2 (c) 5.0 (d) 6.0 (e) 7.0 (f) 8.0 (g) 9.2; **B)**Influence of pH on potential of 1.0 ×10^{-4} M 4-AA on poly(glycine)MGCE at the scan rate of 50 mV s^{-1} in phosphate buffer; **C)** Variation of current with pH of 0.1 mM 4-AA on poly(glycine)MGCE at the scan rate of 50 mV s^{-1} in phosphate buffer

3.2. Effect of medium pH

The pH of an experimental solution markedly influences the electrochemical process at the electrode surface. Hence, to know the pH effect on electro-oxidation of 4-AA, phosphate buffer solution of different pH ranging from 3.0 to 9.2 was prepared. Figure 3A shows the CVs of 1.0 ×10^{-4} M 4-AA at different pHs. Voltammograms indicates the effect of solution

pH on oxidation peak current as well as on potential. By the plot of E_p against pH (Figure 3B) it was noticed that peak potential decreases with increase of pH with following linear equation:

E_p= -0.0513 pH/V+0.6903; R^2=0.9941

The slope of the linear equation is -51.3 mV/pH. By the slope value, it has been proven that during the electrochemical process at electrode surface, the number of electrons and protons transferred are equal, which is verified by the closeness in the slope value with the theoretical value of -59 mV/pH [35, 36]. Looking into the dependence of peak current on pH of the medium (Figure 3C), maximum oxidation current was observed for pH 3.0 of PBS in the prescribed experimental conditions; hence, it was selected for further study.

3.3. Effect of scan rate and determination of n and k^0

Effect of variation of scan rate on oxidation of analyte plays a crucial role in the investigation of many of electro-kinetic parameters. Figure 4A is the linear sweep voltammograms of 4-AA at different scan rates. Peak current gradually increases and the peak potential shifted slightly towards more positive value by increasing the scan rate. A linear relationship between current and square root of scan rate in the range of 50 to 250 mV/s indicates the typical diffusion controlled electrode process. There was a linear relation between log of scan rate and log of peak current (Figure 4B) with slope value of 0.57. Obtained slope value well agreed upon the theoretical value of 0.5 for purely diffusion controlled electrode process [37], which in converse, diffusion controlled electrode process was there. Peak potential changed positively by changing the sweep rate from 50–250 mV/s (Figure 4C) with following linear equation:

E_p=0.0539 log υ+0.4829; R^2=0.9856

For irreversible electrode process, E_p can be defined as below by Laviron formula [38],

$$E_{pa} = E^{0'} + \left(\frac{2.303RT}{\alpha n F}\right)\log\left(\frac{RTk^0}{\alpha n F}\right) + \left(\frac{2.303RT}{\alpha n F}\right)\log \nu$$

Here, α, k^0, n, υ and $E^{0'}$ are represented as transfer coefficient, standard heterogeneous rate constant, transferred electrons during the process, sweep (scan) rate and standard redox potential respectively. Other symbols are used with their usual meaning. From the slope of E_p *vs*. log υ, the value of αn can easily be calculated and was found to be 1.11 (T=298 K, R=8.314 JK/mol, and F=96480 C/mol). On the basis of previous reports [39] by taking α is as 0.5 for irreversible electrode processes, number electrons transferred was calculated to be 2.23 (≈2.0).

Intercept of E_p *vs.* log υ plot was employed to determine the heterogeneous rate constant. In Leviron equation, the value of $E^{0'}$ was obtained from the intercept of E_p *vs.* υ curve by extrapolating to the vertical axis at υ=0 [40]. Using all the parameters, calculated heterogeneous rate constant was found to be 858.28 s^{-1}.

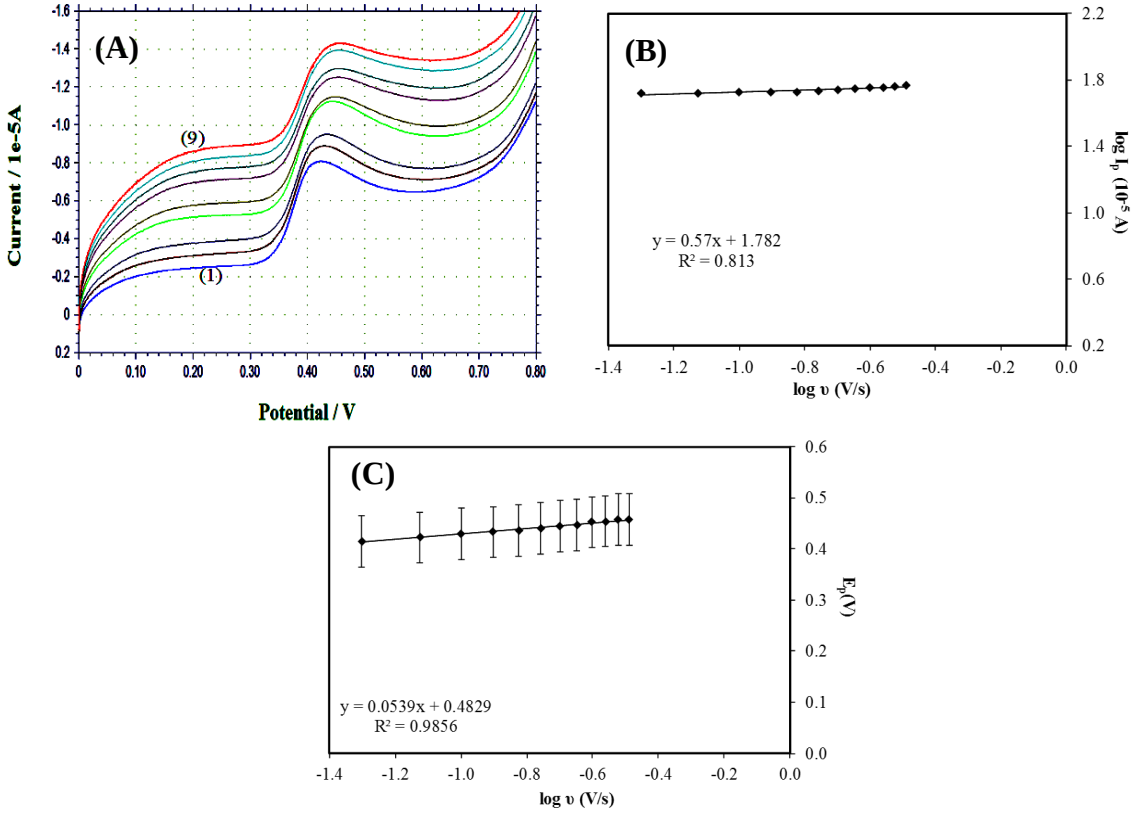


Fig. 4. A) Linear sweep voltammograms of 1.0×10^{-4} M 4-AA at various scan rates (1–9; 50-250 mV s^{-1}) in 0.2 M PBS of pH 3.0; **B**) Dependence of the of peak current on scan rate; **C**) Graph of peak potential vs. logarithm of scan rate

3.4. Analytical application

Under the optimized experimental conditions, DPV technique was used to construct calibration curve by varying the analyte concentration at modified GCE. DPV voltammograms found in case are sharper and better defined at lower concentration of analyte than the other techniques with a lower background current, resulting in improved resolution. Voltammograms at different concentrations of 4-AA are as shown in Figure 5A. Calibration plot was constructed for varying concentrations of 4-AA in the range of 1.0×10^{-8} M to 10.0×10^{-6} M (Figure 5B) with the characteristics as: $I_{pa}(10^{-5}A)=0.053083 \times 10^{-6}$ [4-AA]+2.008 (R^2=0.993). Deviation from linearity has been observed for higher concentration which may be adsorption of 4-AA or its oxidation product on the electrode surface [41].

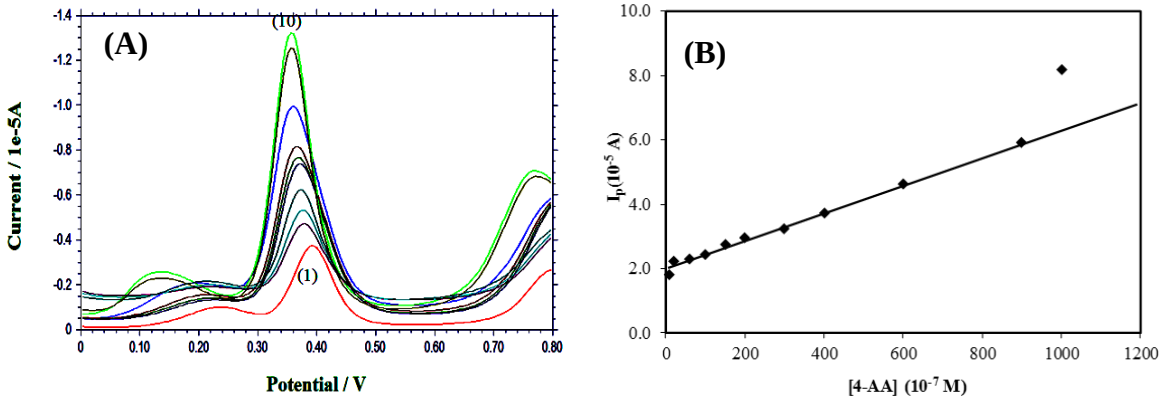


Fig. 5. A) Variation of concentration of 4-AA in pH 3.0 PBS on poly(glycine)MGCE by DPV; (1)-(10): 1.0×10^{-8} M to 10.0×10^{-6} M of 4-AA concentration; **B)** Plot of peak current against the concentration of 4-AA

Table 1. Comparison of linear range and limit of detection of developed technique with other reported analytical techniques for the determination of dipyrones

Dipyrones	**Methods**	**LOD**	**LOQ**	**Ref.**
Dipyrone	Nano-Riboflavin-Modified Glassy Carbon Electrode(voltammetry)	0 .0502 µM	--	[43]
	Titanium Phosphate/Nickel Hexacyanoferrate mod. graphite electrode(voltammetry)	3.75×10^{-4} M	--	[44]
4-aminophenazone (4-Aminoantipyrine)	Graphite pencil electrode(voltammetry)	0.45×10^{-7} M	1.52×10^{-7} M	[45]
4-Aminoantipyrine	Poly(glycine)MGCE	0.97 nM	0.032 nM	**Present work**

Limit of detection (LOD) and limit of quantification (LOQ) have been calculated to be 0.97 nM and 0.032 nM using following equation respectively [42].

LOD=3S/m; LOQ=10 S/m

Where, m and S are slope of the calibration plot and standard deviation of the peak currents of the blank (five runs) respectively. The limit of detection of present work was compared with the values of other reported classical analytical techniques for 4-AA and its derivatives as in Table 1. Present sensor shows a good sensitivity as compared to other reported analytical techniques [43-45].

Precision of the proposed method was examined by inter day and intra-day repeatability measurements. Inter-day repeatability of the technique was tested for 10 µM of 4-AA solutions during 3 days using the same poly(glycine)MGCE after washing with distilled

water thoroughly by DPV technique for five replicates. For the result, relative standard deviation of 2.85% was obtained for the peak current.

For intra-day repeatability, 10 μM 4-AA solutions were studied by successive measurements (five runs). The RSD of peak current obtained was 2.21%. Both the RSD values express the high-quality precision of the developed technique using poly(glycine)MGCE.

3.5. Interference studies

Selectivity of the developed technique was tested by the addition of possible interferents/excipients (Tartaric acid, Citric acid, Glucose, Gum acacia, Lactose, Dextrose, Sucrose, Starch) in a standard solution containing 1.0×10^{-5} M 4-AA, at the concentration ratios (standard solution: interferent) of 1:100, and the obtained current signals were compared with those of standard solutions in the absence of interfering substances. Percentage of relative signal change were found to be lower less than 5.0% for all the interferents in the proportion of 1:100. These results allow us to conclude that the tartaric acid, citric acid, glucose, gum acacia, lactose, dextrose, sucrose, starch etc did not have a significant interference in the 4-AA determination using poly(glycine)MGCE (Table 2).

Table 2. Influence of potential excipients on the voltammetric response of 1.0×10^{-5} M 4-AA

Excipients(1.0 mM) + Drug (1.0×10^{-5}M)	Potential observed (V)	Signal change (%)
Only 4-Aminoantipyrine	0.446	00
Tartaric acid + 4-AA	0.456	2.264
Citric acid + 4-AA	0.442	-0.890
Glucose + 4-AA	0.435	-2.466
Gum acacia + 4-AA	0.451	1.121
Lactose + 4-AA	0.439	-1.569
Dextrose + 4-AA	0.465	4.260
Sucrose + 4-AA	0.447	0.224
Starch + 4-AA	0.454	1.793

3.6. Detection of 4-AA in urine samples

To evaluate applicability of proposed method, a recovery of 4-AA was determined in urine samples by spiking 4-AA. Drug free urine samples were diluted 100 times with the PBS of pH 3.0 and spiked three different concentrations into urine samples before analysis. A quantitative analysis was carried out by calibration graph by measuring peak current of the

system (calculated by considering peak current for standard solution of 1.0×10^{-8} M of 4-AA is 1.921 µA). Recovery results of three urine samples with RSD values are listed in Table 3.

Table 3. Application of poly(glycine) MGCE to the determination of 4-AA in spiked human urine samples

Sample	Added ($\times10^8$ M)	Average peak current(µA)[a]	Found [a] ($\times10^8$ M)	Recovery (%)	R.S.D (%)
1	3	0.5729	2.982	99.41	0.14
2	6	1.1512	5.993	99.88	0.22
3	8	1.5342	7.986	99.83	0.24

[a]Average of five determinations

4. CONCLUSION

In present investigation, electrochemical oxidation of 4-AA by poly(glycine)MGCE by cyclic, linear sweep and differential pulse voltammetric techniques was studied. DPV method enabled a simple, inexpensive, rapid, selective, and accurate analysis of 4-AA. The improved analytical performance compared to other analytical techniques makes the proposed method is an excellent alternative means of analytical determination of 4-AA because of its low cost, repeatability and adequate precision. Moreover, because of the low LOD of the proposed method, it could be applied in clinical laboratories and pharmacokinetic studies.

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