PHARMACIA

Pharmacia 70(4): 993–998
DOI 10.3897/pharmacia.70.e110336

Research Article

Determination of dopamine in blood serum and urine samples by differential pulse voltammetry at an iodine-coated platinum electrode

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Received 31 July 2023 ♦ **Accepted** 9 September 2023 ♦ **Published** 5 October 2023

Citation: Hourani W, Amayreh M, Hourani MK (2023) Determination of dopamine in blood serum and urine samples by differential pulse voltammetry at an iodine-coated platinum electrode. Pharmacia 70(4): 993–998. https://doi.org/10.3897/pharmacia.70.e110336

Abstract

While platinum electrode shows hyperactivity towards adsorption and surface processes, iodine-coated platinum electrode offers remarkable inertness toward them. Therefore, iodine-coated platinum electrodes lend themselves to probe chemical species in the bulk solution without intervention from adsorption and surface processes. The current work presents utilization of iodine-coated polycrystalline platinum electrode as a voltammetric sensor for determination of dopamine in serum and urine samples. Differential pulse voltammetry (DPV) at iodine coated polycrystalline platinum electrode is the technique of choice whenever higher sensitivity is sought. DPV with a scan rate of 5 mV/s was applied for determination of dopamine in PBS at pH 7. The anodic peak related to dopamine oxidation in the above-mentioned solution was centered at ~0.1V vs. Ag/AgCl quasi reference electrode. The linear range for the developed method was between 1.0 and 100 µM. The anodic peak current showed excellent linearity with dopamine concentration (R^2 =0.9977) over the above-mentioned range. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.29 µM and 0.96 µM respectively which attests to the high sensitivity of the developed method. The proposed method was successfully applied to the analysis of dopamine in serum and urine samples. The percent recovery values ranged from 94.4 to 104.5% attesting to the accuracy of the developed method and absence of determinate errors.

Keywords

Dopamine, differential pulse voltammetry, iodine-coated platinum, platinum electrodes, blood analysis, urine analysis

Introduction

Dopamine is a well-known neurotransmitter of prime physiological and medical importance (Webester 2001). It plays a vital role in the functions of central nervous system, cardio vascular, renal and hormonal systems) Armando et al. 2011) and it has a relation with movement disorders and Parkinson disease (Ulas et al. 1994). This renders analysis of dopamine critically important because of its connection with public health. Many methods have been reported for analysis of dopamine in biological samples. These methods include flow injection analysis (Montenegro and Sales 2000), spectrophotometry (Guo et al. 2009), HPLC (Sultan and Bulduk 2020) and electrochemical methods (Ferapontova 2017). The quest for accurate, robust, selective, precise, simple, fast and reliable method for analysis of dopamine is a priority in medical applications.

Electrochemical methods have attracted the attention of developers of analytical methods due to their simple instrumentation, simple procedures and simple sample pretreatment and moderate demand for well-trained personnel (Hoyos-Arbelaez et al. 2016). The direct use of plain metallic electrodes is usually associated with interference from surface processes, adsorption and desorption phenomena and matrix effects (Bard and Faulkner 2001). These effects are overcome by surface modification. Surface modification with adatoms, organic molecules and polymeric moieties opened new horizons for electrochemical analytical methods (March et al. 2015). Modification of platinum electrodes with a monolayer of adsorbed iodine turns the hyperactive highly catalytic platinum into a real polarizable electrode over a reasonable potential range (Amayreh and Hourani 2017). The surface processes, surface adsorption and desorption are almost totally suppressed (Amayreh et al. 2021a). Within this limited range, a limited number of organic and inorganic molecules respond to the change in potential which imparts a kind of selectivity towards certain ions and compounds (Amayreh et al. 2021b).

In our laboratory, we have extended application of iodine-coated platinum electrode to analysis of many molecules of biological importance (Amayreh and Hourani 2019). The present work is devoted to explore the applicability of iodine-coated platinum electrode to analysis of dopamine in serum and urine.

Experimental

Instruments and materials

A modified 174 polarographic analyzer (EG & G, USA) was used for application of the cyclic voltammetric and differential pulse voltammetric excitation potential regimes and measurement the resulting current. Data acquisition was performed through a GPIB (IEEE, USA) interface interfaced to a computer and controlled by an in-house modified LabVIEW (IEEE, USA) software. All the experiments were performed using a home-made 3-electrode H-shape electrochemical cell where the working electrode compartment was separated from the reference and auxiliary electrode compartment by a fine glass frit. The two compartments of the cell were equipped with inlets and outlets for purging and blanketing with oxygen-free nitrogen gas, rinsing, furnishing the cell with pure supporting electrolyte solution and introduction of the standards and dopamine samples. A 0.5 mm polycrystalline platinum wire was used as a working electrode (Aldrich 99.99% minimum purity certified reagent, USA). The electrode surface area was controlled by rendering the immersed part of the electrode had a U-shape and the shorter part of the electrode was touching the surface of the solution from underneath the level of the solution. A silver/silver chloride was used as a quasi-reference electrode (QRE) and all the reported potentials in this work are referenced to this electrode. The auxiliary electrode was a 0.5 mm polycrystalline platinum wire (Aldrich, certified 99.99% minimum purity, USA). The wire was spiral-shaped to provide a large surface area. All chemicals used were of analytical grade (AR) and used as received without further purification. Sulfuric acid (95–97%) was supplied from Merck (USA), Dopamine (99% pure) was purchased from Across Organics (USA.USA), potassium iodide was purchased from Sigma-Aldrich (USA). Milli-Q water (Millipore, Aldrich, USA) was used for preparation of all solutions. Nitrogen gas (5G grade, 99.999% minimum purity) was supplied by The International Jordanian Gas Company (Jordan).

Phosphate buffer solutions (PBS) with different pH values were prepared by mixing a 0.1M of Na_2HPO_4 with 0.1M of NaH_2PO_4. The pH was measured using (Jenway 3030 pH meter, USA) and the pH was adjusted to the desired value using 0.100 M HCl solution. A 0.100 mM dopamine stock solution was prepared from dopamine hydrochloride (Thermo Fisher Scientific, USA, 99% pure) and the above-mentioned PBS solution. Standard solutions of lower concentrations of dopamine were prepared by successive dilution from the stock solution.

Preparation of iodine-coated platinum electrode

Preparation of iodine-coated platinum electrode was described elsewhere (Hourani and Amayreh 2018). Briefly, the platinum working electrode was cleaned by immersion in a hot freshly prepared chromic acid solution followed by extensive rinsing with Milli-Q water. The electrode was transferred to an electrochemical cell containing 1.0 M H_2SO_4 solution where the electrode potential was scanned between the cathodic and anodic potential limits as an "electrochemical cleaning". The potential cyclization was continued until the well-known cyclic voltammogram of polycrystalline platinum was reproduced (Bard and Faulkner 2001).

The potential scan was stopped in the double layer region on the positive-going scan where we believe that the electrode is not loaded with hydrogen or oxygen in this region. The electrode was exposed to $1.0x10^{-2}$ M potassium iodide solution for 5 minutes. The electrode potential was cycled in a 0.5M H_2SO_4 solution between -0.2V and +0.85V at 50 mv/s to verify the completeness of the coating process. The cyclic voltammogram of the iodine-coated platinum electrode between the cathodic potential limit and about 0.85 V shows complete absence of all the characteristic voltammetric features of platinum electrode. The absence of oxygen and hydrogen adsorption-desorption peaks from the recorded cyclic voltammograms of an iodine-coated platinum electrode is an indication of complete coverage of platinum electrode surface with a monolayer of iodine(Hourani and Amayreh 2018). The electrode potential was maintained between the cathodic potential limit at -0.2 V and the onset of iodine desorption from the platinum surface at ca. 0.85 V. The iodine-coated platinum electrode acts as a real polarizable electrode in the potential window between ~-0.2 and ~ +0.85 V. Validation of the electrochemical system cleanliness and

coating of the platinum electrode with iodine was ensured before any experiment. It is also worth mentioning that scanning of the electrode potential was limited to the potential range from -0.2 V to 0.85 V at most to avoid desorption of iodine from the platinum surface.

Sample preparation and measurement

Blood serum and urine samples from various healthy volunteers were collected by The University of Jordan Hospital personnel. The samples were stored in a freezer at -20 °C until the time of analysis which didn't exceed one week. A volume of 50 µL of human blood serum was diluted to the mark in a 20.00 ml volumetric flask with pH 7 PBS solution. The solution was introduced to the working electrode compartment in the electrochemical cell. The differential pulse voltammograms were recorded by application of a potential scan between -0.2 and 0.80 V vs. QRE.

Results and discussion

Analytical parameters of the developed method

Fig. 1 shows a representative cyclic voltammogram for the iodine-coated platinum electrode in 0.10 M PBS containing 1.00 mM dopamine. The recorded cyclic voltammogram shows a prominent anodic peak for dopamine oxidation at a potential of~ 0.4 V with a counter peak centered at 0.2 V.

The effect of pH on the oxidation peak current and peak potential of dopamine at iodine-coated platinum electrode was investigated within the pH range 3.0–7.0. Fig. 2A, B show the effect of pH on the peak potential and peak current respectively. Fig. 2A shows a sharp change in peak potential upon changing the pH from 4 to 7.

The balanced equation for the oxidation is

$$C_8H_{11}NO_2 \rightarrow C_8H_9NO_2 + 2H^+ + 2e^-$$

Which dictates a shift towards less positive potential with increasing pH as manifested in Fig. 2A. The explanation relies on the fact that increasing the pH of the solution decreases the concentration of hydrogen ions which shifts the equilibrium in the forward direction and stabilizes the products. Thus the increase in the pH makes The results here coincide with others' work on electrooxidation of dopamine (Barham et al. 2009). Peak current (Fig. 2B) shows a more complicated trend between the pH and the anodic peak current. Seemingly, there is more than one factor affecting the rate of the oxidation of dopamine. At low pH values, the dopamine exists in the protonated form which is more stable and less reactive towards oxidation. With increasing pH, the easily oxidizable deprotonated dopamine form prevails over the protonated form. This is reflected in observation of higher peak current at higher pH because the current is an index of the rate of the electrochemical reaction. The highest peak current was recorded at pH 7 and higher pH values were avoided because of the

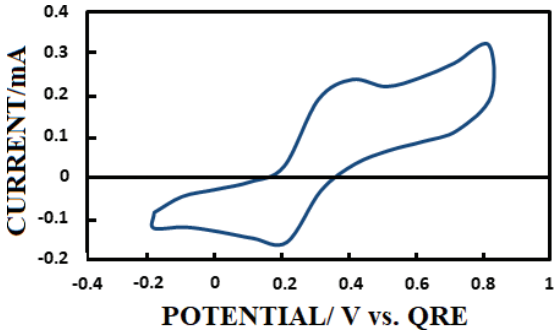

Figure 1. Cyclic voltammogram of an iodine-coated platinum electrode in 0.1 M PBS solution containing 1.0 mM dopamine. Experimental conditions: dE/dt = 100 mV/s, pH = 7. This anodic peak was considered for quantification of dopamine in urine and serum samples.

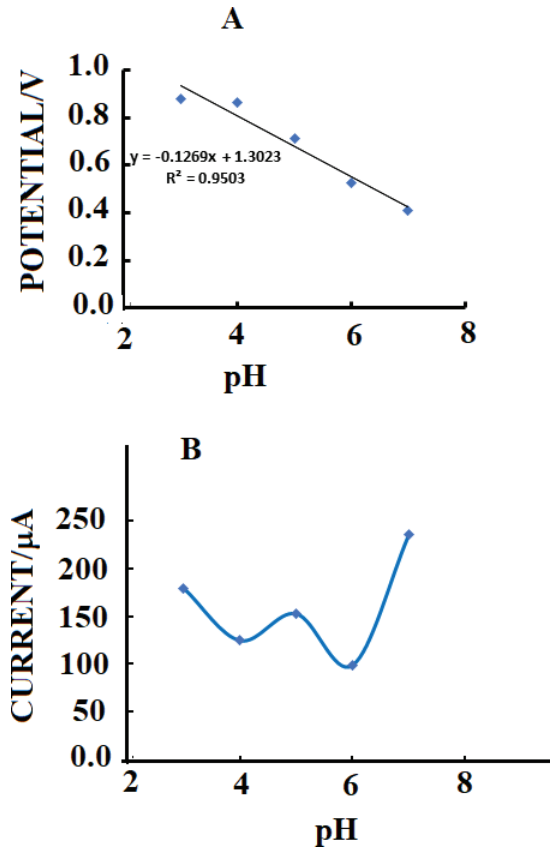

Figure 2. The effect of pH on (**A**) anodic peak potential and (**B**) anodic peak current. Anodic peak potentials and currents were extracted from cyclic voltammograms of iodine-coated platinum electrode in 0.1 M PBS solution containing 1.0 mM dopamine at different pH values. dE/dt = 100 mV/s.

instability of iodine coating in basic solutions (Hourani and Hijaz 2019). Thus, the optimal pH value is 7 and all of our experiments were performed at this pH for this reason.

Fig. 3 shows the effect of scan rate on the anodic peak current in the linear sweep voltammograms. . An excellent linearity between anodic peak current and the square root of scan rate ((scan rate)$^{1/2}$) has been indicated (R^2 = 0.9977). This indicates that the oxidation of dopamine at iodine-coated platinum electrode over the investigated range (10–100

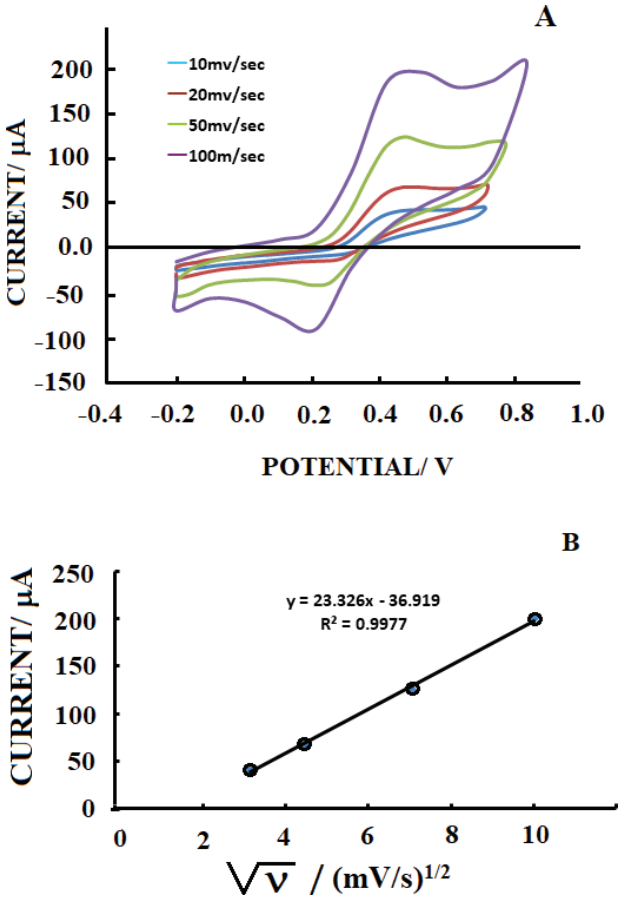

Figure 3. The effect of scan rate on the anodic peak current for oxidation of dopamine at iodine-coated platinum electrode. **A.** Cyclic voltammograms of dopamine electrooxidation at iodine-coated electrode in 0.1 M PBS (pH 7) solution containing 1.00 mM dopamine. Scan rate = 100 mV/s **B.** A plot of anodic peak current vs. (scan Rate)$^{1/2}$, where n is the scan rate of the recorded voltammograms in (A).

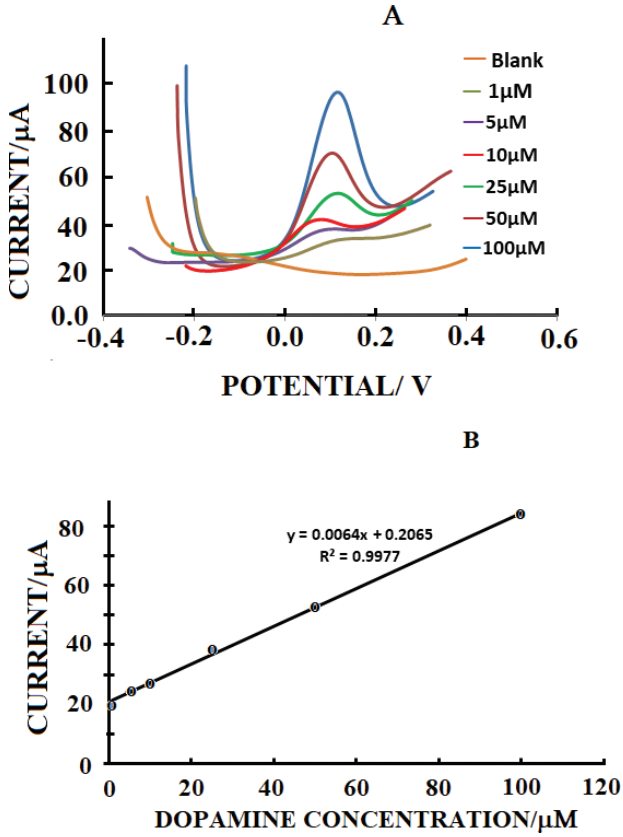

Figure 4. **A.** Differential pulse voltammograms of an iodine-coated platinum electrode in 0.1 M PBS (pH 7) containing different concentrations of dopamine. scan rate: 5 mV s^{-1}, modulation amplitude = 50 mV. pulse repeat time: 1 s. **B.** A plot of the concentration of dopamine vs. the peak currents extracted from the corresponding voltammograms (in Fig. 4A).

mV s^{-1}) is controlled by diffusion. In absence of surface processes and adsorption and at one hand and the diffusion-controlled oxidation of dopamine at the iodine-coated platinum electrode on the other hand makes this electrode nearly ideal for voltammetric analysis of dopamine.

Fig. 4A shows the differential pulse voltammograms of the iodine-coated platinum electrode at different concentrations ranging from 1.00 µM to 100 µM dopamine solutions. The calibration curve for the analysis (Fig. 4B) shows excellent linearity with a coefficient of determination, $R^2 = 0.9977$. The calibration equation is

$$i_p(\mu A) = 0.0064C_{dopamine} + 0.2064$$

where $C_{dopamine}$ is in micromolar units.

The limit of detection (LOD) and the limit of quantitation (LOQ) for determination of the dopamine by differential pulse voltammetry at iodine coated platinum electrode were estimated based on the equations $LOD=3s_b/m$, and $LOQ = 10s_b/m$ respectively where s_b is the standard deviation for 20 measurements of the anodic peak current for a blank solution and m is the slope of the calibration curve (calibration sensitivity). The estimated LOD and LOQ are 0.29 µM and 0.96 µM respectively. The values of LOD and LOQ attest to the sensitivity of the developed method.

The interday and intraday precision were estimated by extracting the peak currents from the differential pulse voltammograms for electrooxidation of dopamine at the iodine-coated electrodes for the 25.0 µM dopamine standard solution. Ten differential pulse voltammograms were reproduced for the above-mentioned solution within one day for the intra-day and within ten days for the inter-day precision evaluation. The measurements were equally spaced by one hour for the intraday precision evaluation and 24-hour for the interday precision evaluation. The coefficient of variation was 2.66% and 4.34% for the intra-day and the inter-day respectively. The measured values of standard deviation confirm the high precision of the developed method.

Interferences

Method selectivity is of prime importance in chemical analysis. The selectivity of the developed method was tested by application of the method to analysis of dopamine in presence of two potential interferences ascorbic acid and uric acid spiked in analyzed samples.

Fig. 5A shows the effect of presence of ascorbic acid on the voltammetric analysis of dopamine. The results indicate that there is no effect of presence of ascorbic acid even at concentrations of tenfold or higher of ascorbic acid. A little shift in the anodic peak potential is observed but it does not affect the accuracy of analysis.

Experimental conditions for all voltammograms: scan rate: 5 mV s-1, pulse repeat time: 1 s, modulation amplitude: 50 mV.

Similarly, presence of uric acid at large concentrations of uric acid has no effect on the peak current of dopamine. On the other hand, presence of relatively very large concentrations of uric acid leads to a shift in the anodic peak of dopamine electrooxidation towards higher positive potential. The peak current, however, is not affected by the presence of uric acid as shown in Fig. 5B. This indicates that quantification of dopamine in presence of a large concentrations of uric acid is still possible.

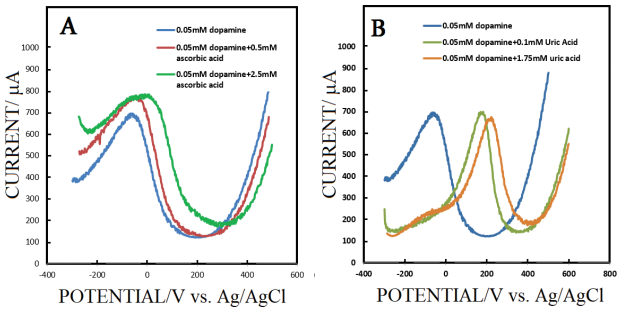

Figure 5. A. Differential pulse voltammograms for the iodine coated electrode in PBS containing (A) (blue) 0.05 mM dopamine, (red) 0.05 mM dopamine + 0.5 mM ascorbic acid, and (green) 0.05 mM dopamine + 2.5 mM ascorbic acid. **B.** (blue) 0.05 mM dopamine, (green) 0.05 mM dopamine + 0.1 mM uric acid and (orange) 0.05 mM dopamine + 1.75 mM uric acid.

Recovery tests

The recovery results can be taken as evidence for the absence of interference and absence of determinate errors upon analysis of the spiked samples.

Calculated volumes of 1.0 mM dopamine standard solution were spiked separately into the serum and urine samples (three of each) to produce solutions with nominal concentrations of 20 and 40µM dopamine in serum and urine matrices. The spiked samples were analyzed for their dopamine concentration and the recoveries were calculated. Fig. 6 shows representative differential pulse voltammograms for human urine samples before and after spiking the samples such that their nominal concentrations were 20.0 and 40.0 µM. The voltammograms unequivocally indicate an increase in the peak current with increasing dopamine spiked volume.

Table 1 shows the recoveries for 20 and 40µM dopamine spiked samples in urine and serum matrixes. The recoveries of the analyzed spiked samples ranged from 94.4 to 104.5 104.5%. Thus the recoveries lie within 5% of the nominal values which eliminates the possibility of determinate errors regardless of the complexity of urine and serum samples.

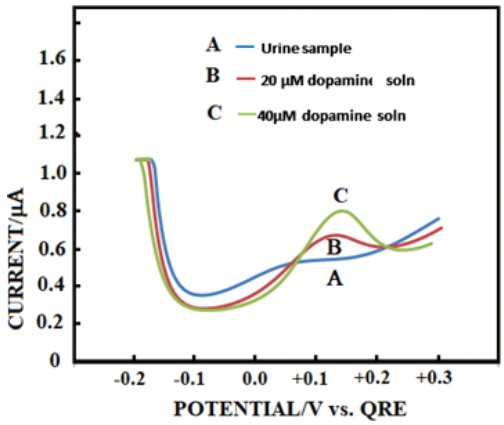

Figure 6. Differential pulse voltammograms for an iodine coated electrode in 0.1 M PBS (pH 7) of spiked with (A) spiked with urine sample only (B) spiked with 20 µl of dopamine (C) spike with 40 µl of dopamine. Scan rate = 5 mV s^{-1}, modulation amplitude = 50 mV, pulse repeat time = 1 s.

Table 1. The percent recovery results for urine and serum samples spiked with 400 and 800 µl of 1.0 mM dopamine standard solution such that the nominal final solutions of urine and serum are 20.0 and 40.0 µM.

Sample type and number	Concentration (µM)		Standard deviation*	CV	% Recovery
	Calculated	Found			
Urine 1	20.0	20.90	±1.0	4.8	104.5
	40.0	41.00	±0.21	0.5	102.5
Urine 2	20.0	19.70	±1.4	7.1	98.5
	40.0	39.68	±0.22	0.6	99.2
Urine 3	20.0	18.87	±0.58	3.1	94.4
	40	39.58	±1.0	2.5	99.0
Serum 1	20.0	20.56	±1.1	5.4	102.8
	40.0	40.53	±1.8	4.4	101.3
Serum 2	20.0	20.60	±1.2	5.8	103
	40.0	40.61	±0.49	1.2	101.5
Serum 3	20.0	19.82	±1.4	7.1	99.1
	40.0	38.08	±0.15	0.4	95.2

* based on three runs.

Conclusions

In the present work, iodine-coated platinum electrode was applied successfully to differential pulse voltammetric analysis of dopamine. The lowest detection limit of the developed method is 0.29 μM and the limit of quantitation is 0.96 μM. The dynamic range is 1–100 μM which extends over two orders of magnitude rendering the developed method suitable for routine analysis. The method was successfully applied to quantification of dopamine in three serum samples and three urine samples. The results show high precision where the coefficient of variation was limited to 7.1% and excellent percent recovery (94.4–104.5%).

Aside from the above-mentioned figures of merits, iodine-coated platinum electrodes have many tempting features. Iodine coated electrode shows superior performance in terms of simplicity of preparation, mechanical strength and durability, and inertness towards mineral acids. Moreover, iodine-coated platinum electrodes can be fabricated in any size and shape which presents an added value to the application of this electrode. The developed method can be considered a green method because it is based on minimal consumption and minimal waste of chemical reagents.

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