



Article

Simultaneous Electrochemical Sensing of Dopamine, Ascorbic Acid, and Uric Acid Using Nitrogen-Doped Graphene Sheet-Modified Glassy Carbon Electrode

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Abstract: Nitrogen (N) doping is a well-known approach that can be effectively used to tune the properties of graphene-supported materials. The current attempt followed a simple hydrothermal protocol for the fabrication of N-doped graphene sheets (N-GSs). The N-GSs were subsequently applied to modify the surface of a glassy carbon electrode (GCE) for a dopamine (DA) electrochemical sensor (N-GSs/GCE), tested on the basis of differential pulse voltammetry (DPV). The findings highlighted a limit of detection (LOD) as narrow as 30 nM and a linear response in the concentration range between 0.1 and 700.0 μ M. The modified electrode could successfully determine DA in the co-existence of uric acid (UA) and ascorbic acid (AA), the results of which verified the potent electrocatalytic performance of the proposed sensor towards AA, DA, and UA oxidation, and three distinct voltammetric peaks at 110, 250, and 395 mV via DPV. The practical applicability of the as-developed N-GSs/GCE sensor was confirmed by sensing the study analytes in real specimens, with satisfactory recovery rates.



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Keywords: nitrogen-doped graphene sheets; glassy carbon electrode; dopamine; uric acid; ascorbic acid

1. Introduction

3,4-dihydroxyphenethylamine, or dopamine (DA), as a key neurotransmitter, is positioned in the family of excitatory chemical neurotransmitters. It is capable of regulating human cognition and emotions, and exists as a main catecholamine in the central nervous system (CNS). The mean DA content in blood and serum samples of human beings can range from 10^{-6} to 10^{-9} mol/L [1,2]. DA has an extensive presence in mammalian brain tissues and body fluids. It is significantly involved in some processes performed in the CNS, metabolic system, kidneys, cardiovascular system, and hormonal actions. Hence, any change in the level of DA in different parts of the body can be indicative of diverse neurological problems such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and addiction. DA can be a biochemical precursor for catechol amine neurotransmitters, epinephrine, and norepinephrine [3–5]. Accordingly, there is a need for a sensitive, simple, and selective approach to determine and quantify the DA for early diagnosis of relevant medical conditions.

There are some analytical methods for determination of DA, some of which include chemiluminescence [6], chromatography [7], colorimetry [8], fluorescence [9], and capillary electrophoresis [10]. In spite of the valuable advantages of these techniques, they suffer from some shortcomings such as long testing time, high cost, and tedious pretreatment processes. Hence, considerable attention has been recently focused on electrochemical approaches in determination of electroactive analytes because of their commendable merits such as high sensitivity, low cost, and fast reaction [11–21]. Nevertheless, the presence of some interferants such as uric acid (UA) and ascorbic acid (AA) can disrupt the detection

of DA [22,23]. This problem can be particularly aggravated with the use of bare electrodes due to some deficiencies such as overlapping oxidation potentials and marked electrode fouling, commonly leading to poor selectivity and reproducibility. This bottleneck can be circumvented by modifying the electrode surface using different modifiers [24,25]. In general, chemically modified electrodes lead to an increase in sensitivity and selectivity for the determination of various analytes [26–33].

The unique chemical and physical properties of nanomaterials make them extremely convenient for applications in various fields [34–40]. Nanomaterials are among the best candidates to create ultra-sensitive and ultra-selective electrochemical sensors due to their appreciable electro-conductivity and huge surface area [41–49].

Graphene with a 2D layer of C atoms in a honeycomb configuration have shown unparalleled electrical, mechanical, chemical, and optical properties. They can act as potent electrode modifiers due to huge surface area, high electro-conductivity, great chemical and thermal stability, and mechanical strength [50–52]. Reportedly, chemical doping of graphene can trigger semiconducting properties; thus, N-doped or P-doped graphene can experience high charge conductivity and chemical reactivity [53]. N-doping can be highly effective in the regulation of electronic activity and mechanical properties and in the elevation of the electrocatalytic performance of carbon materials [54]. N-doped graphene sheets (N-GSs) have been promising options in the fabrication of electrochemical (bio)sensors, supercapacitors, batteries, and electrocatalysis, because they possess huge surface area, great electric conductivity, subtle electronic traits, a large number of edge sites to adsorb electroactive molecules, catalytic capacity, and appreciable electron density [55–57].

The current attempt followed a simple hydrothermal protocol for the fabrication of N-GSs. The N-GSs was subsequently applied to modify the surface of a GCE to create a DA electrochemical sensor (NGSs/GCE), even in the co-existence of UA and AA, tested on the basis of DPV. The practical applicability of the as-developed NGSs/GCE sensor was examined by sensing the study analytes in real specimens.

2. Experimental

2.1. Instruments and Chemicals

An Autolab PGSTAT 320N Potentiostat/Galvanostat Analyzer ((Eco-Chemie, Utrecht, The Netherlands)) with GPES (General Purpose Electrochemical System-version 4.9) software was applied for all electrochemical determinations at ambient temperature. A Metrohm 713 pH-meter with glass electrodes (Metrohm AG, Herisau, Switzerland) was used to determine and adjust the solution pH.

All solvents and chemicals applied in our protocol were of analytical grade from Merck and Sigma-Aldrich. Phosphate buffer solution (PBS) was prepared by phosphoric acid and adjusted by NaOH to the desired pH value.

2.2. Synthesis of N-GSs

The synthesis of N-GSs was based on the hydrothermal protocol using the base material graphene oxide (GO) and the reducing and doping agent urea. Thus, 100.0 mg of exfoliated GO was dissolved in 100 mL of deionized water under ultra-sonication, with the solution pH adjusted to 10.0 using $\text{NH}_3 \cdot \text{H}_2\text{O}$ (30%), followed by adding 6.0 g of urea under 3 h ultra-sonication. Next, the resultant mixture was placed in a Teflon-lined autoclave at 180 °C for 12 h, followed by cooling to room temperature. After collecting N-GSs, washing was done thoroughly with water and the pH was adjusted to a neutral value. Eventually, the freeze-drying process was done.

2.3. Preparation of the N-GSs/GCE Sensor

A drop-casting technique was followed to fabricate the N-GSs/GCE. Thus, a certain amount of as-prepared N-GSs (1.0 mg) was subsequently dispersed in deionized water (1.0 mL) under 25-min ultra-sonication. Then, the prepared suspension (4.0 μL) was coated on the GCE surface dropwise and dried at the laboratory temperature.

The surface areas of the N-GSs/GCE and the bare GCE were obtained by CV using 1.0 mM $K_3Fe(CN)_6$ at different scan rates. Using the Randles–Sevcik equation [58] for N-GSs/GCE, the electrode surface was found to be 0.119 cm^2 , about 3.8 times greater than bare GCE.

3. Results and discussion

3.1. Characterization of N-GSs

Figure 1 shows the characterization of GO and N-GSs using XRD. According to the XRD pattern for GO (Figure 1a), a peak can be seen at the 2θ value of 11.5° (inter-planer space = 7.69 \AA). A larger inter-planer space can be observed for GO in comparison to graphite, which can be attributed to the presence of H_2O molecules and diverse oxygen groups on its surface. The XRD pattern captured for the N-GSs highlighted a high degree of reduction (Figure 1b). Following the hydrothermal reactions and together with the N-doping process, the disappearance of the peak at $2\theta = 11.5^\circ$ confirmed the successful GO reduction, but the N-GSs pattern created a wide peak at 24.7° [59], related to the inter-planar distance of 3.602 \AA , which provides π – π stacking between graphene sheets [60].

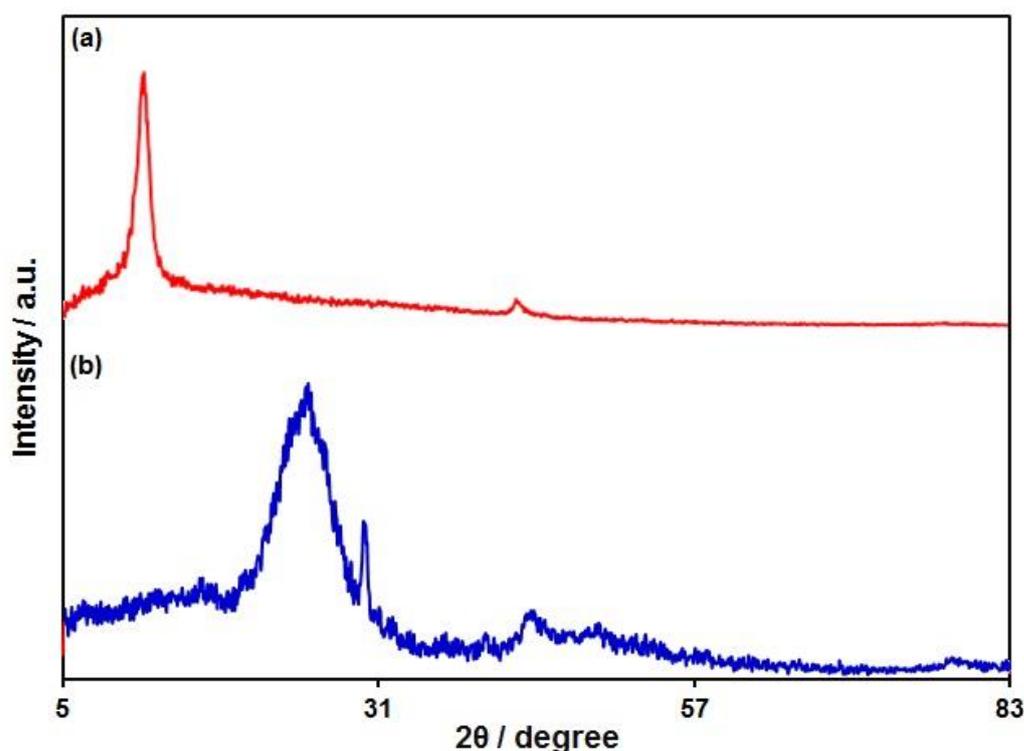


Figure 1. XRD pattern of (a) GO and (b) N-GSs.

Figure 2 compares the FT-IR spectra captured from GO and N-GSs. In the FT-IR spectrum of GO, there are different functional groups that are shown in Figure 2a. On account of these characteristic peaks, multiple oxygen (O)-containing functional groups, such as epoxy, hydroxyl, and carboxyl, were present on the GO surface. The lack of absorption peaks of oxygen groups in the N-GSs showed the effective reduction of GO during the hydrothermal process. Two peaks appeared at 1184 and 1562 cm^{-1} related to C–N and C=N (occasionally, C=C and C=N bonds are stretched at the identical wavelength), respectively. Therefore, the graphene was N-doped successfully.

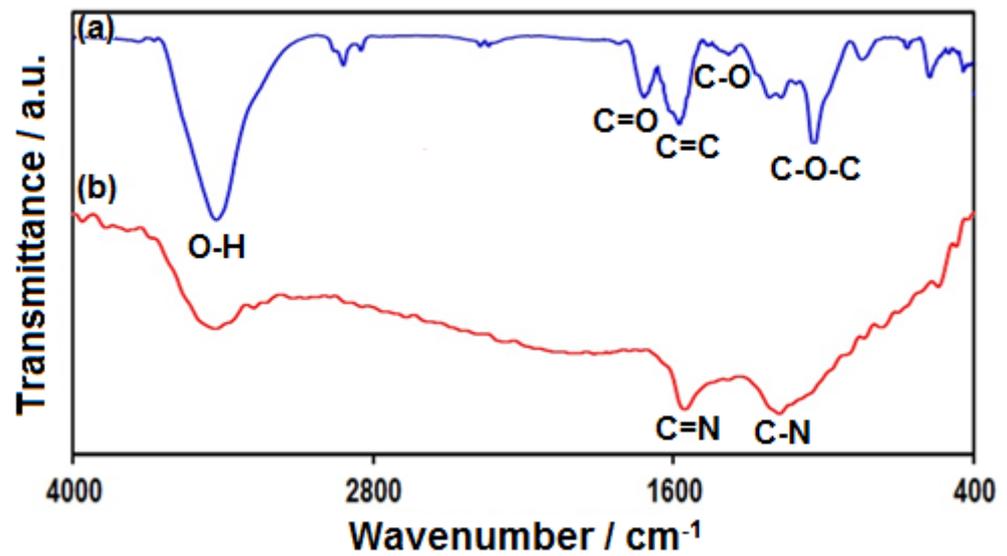


Figure 2. FT-IR spectra of (a) GO and (b) N-GSs.

As shown in Figure 3 depicting the FE-SEM image captured for the N-GSs, the N-GSs maintained the ultrathin flexible 2D structure of pristine graphene; and the N-GS sheets possessed a wave structure similar to thin wrinkled paper, representing the successful N-doping process for the graphene.

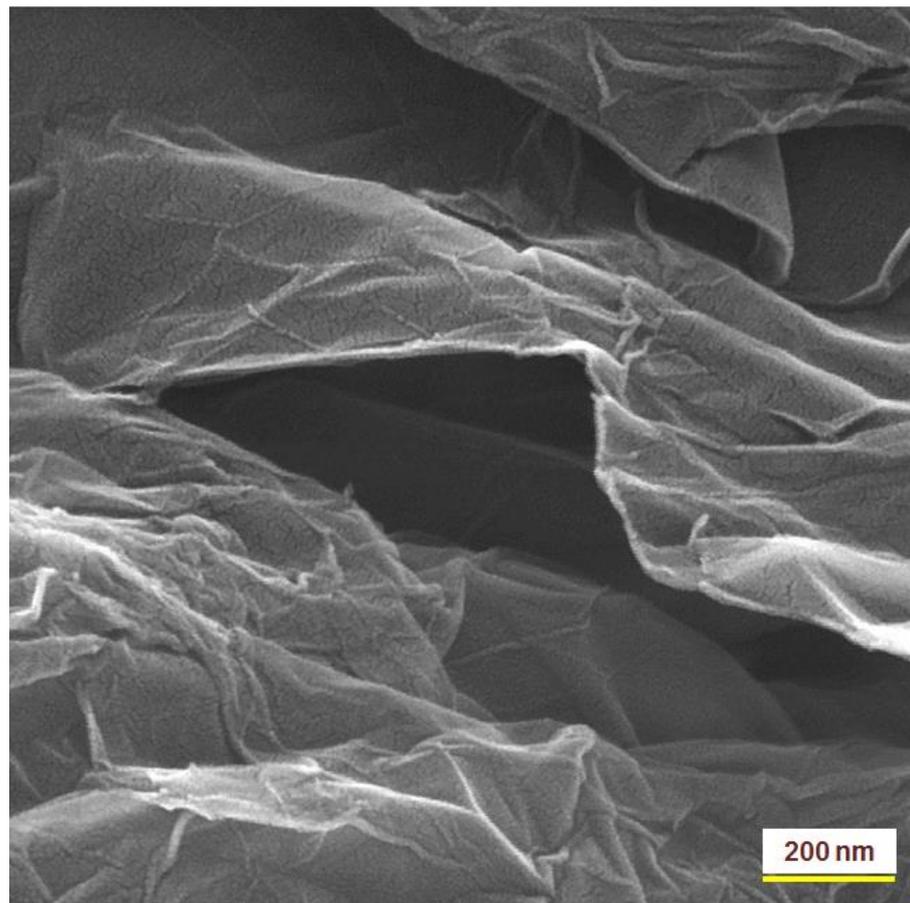


Figure 3. The FE-SEM image of the NGSs.

Figure 4 shows the energy dispersive X-ray spectroscopy (EDS) analysis in determining the chemical composition of the N-GSs, the findings of which showed C (80.4%), N (16.3%), and O (3.3%) present in the prepared sample.

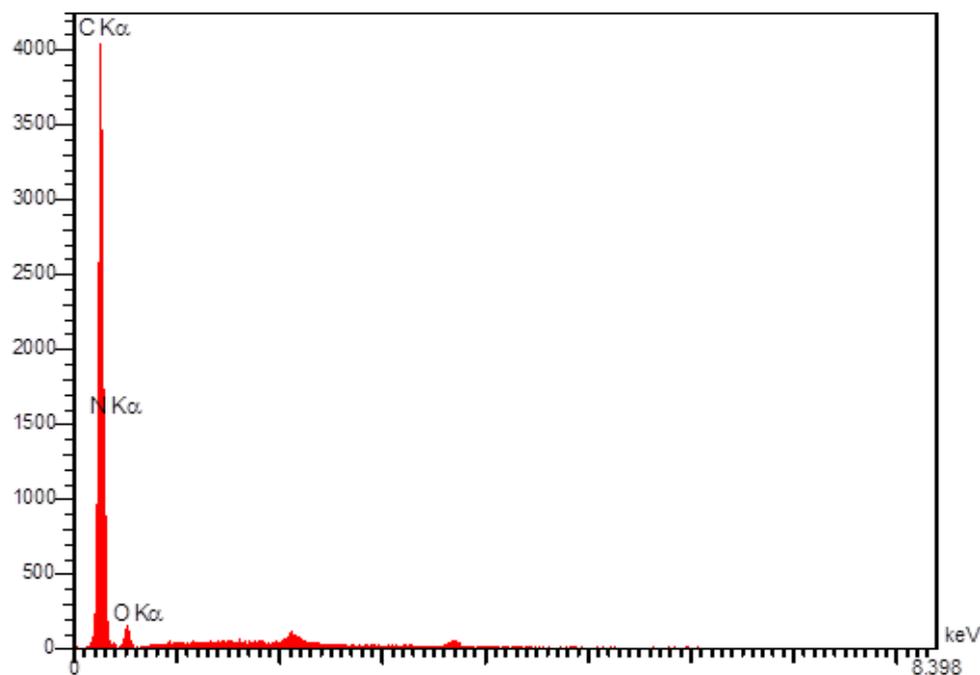
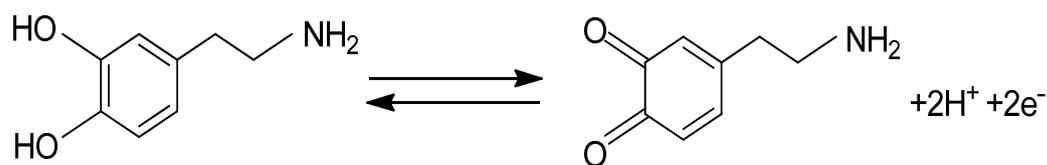


Figure 4. EDS spectrum of N-GSs.

3.2. Electrochemical Response of DA at the Various Electrodes Surfaces

The electrochemical response of DA in the 0.1 M PBS adjusted to variable pH values (2.0 to 9.0) was explored to determine the influence of the electrolyte solution pH. The results showed that the redox peaks of DA depended on the pH value, with these reaching a maximum with increasing pH up to 7.0 and then decreasing with greater pH values. Hence, the pH value of 7.0 was considered to be the optimum for subsequent electrochemical determinations. The reaction mechanism of DA is shown in Scheme 1.



Scheme 1. Electrochemical oxidation mechanism of DA at the surface of NGSs/GCE.

The cyclic voltammograms (CVs) were captured for the DA (200.0 μ M) on the bare GCE and NGSs/GCE to explore the electrocatalytic performance of the N-GSs (Figure 5). Figure 5 illustrates two weak oxidation and reduction peaks on the bare GCE with oxidation peak current (I_{pa} = 4.6 μ A) and reduction peak current (I_{pc} = -0.8 μ A), whereas the NGSs/GCE had a significant improvement in the currents (I_{pa} = 13.9 μ A and I_{pc} = -4.8 μ A). This significant improvement in the redox peaks may have appeared because of the appreciable catalytic impact of NGSs for the DA oxidation and reduction.

3.3. Effect of Scan Rate

The CVs were recorded for DA (150.0 μ M) on the NGSs/GCE under variable scan rates (Figure 6). There was an apparent gradual elevation in the redox peaks achieved by raising the scan rate range from 10 to 500 mV/s. As seen in Figure 6 (inset), the anodic peak current (I_{pa}) and cathodic peak current (I_{pc}) had a linear association with the scan

rate square root ($v^{1/2}$). This result indicates that a diffusion-controlled DA redox reaction occurs on the NGSs/GCE.

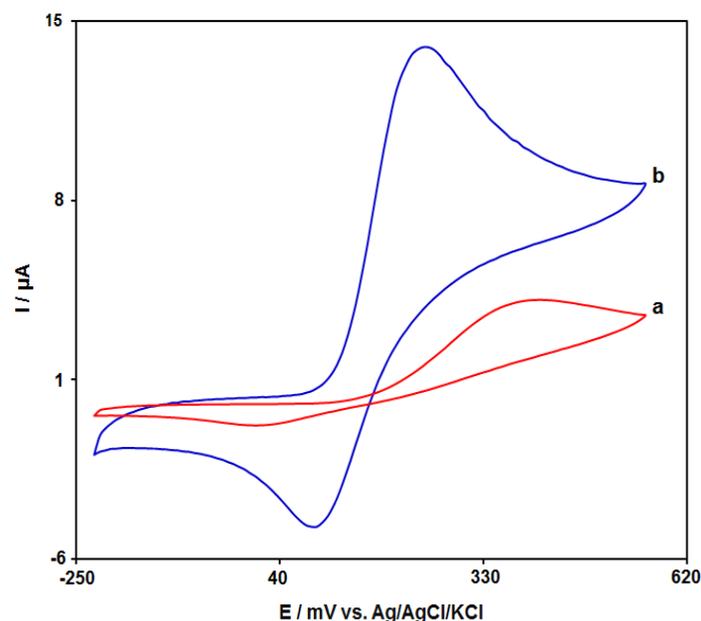


Figure 5. CVs captured for DA (200.0 μM) in PBS (0.1 M; pH = 7.0) on (a) bare GCE and (b) NGSs/GCE with a scan rate of 50 mV/s.

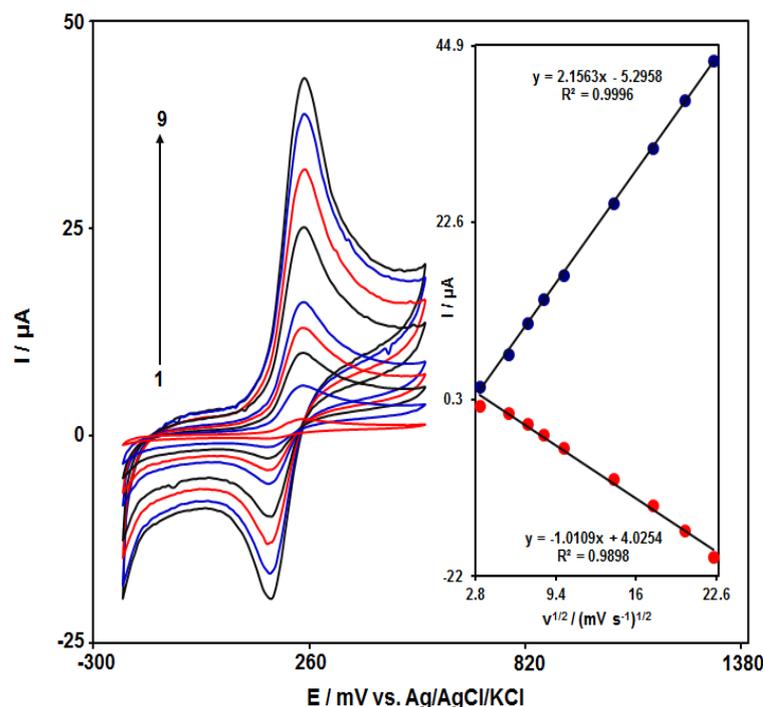


Figure 6. CVs captured for the DA (150.0 μM) on the NGSs/GCE under variable scan rates (1:10, 2:30, 3:50, 4:70, 5:100, 6:200, 7:300, 8:400, and 9:500 mV s^{-1}). Inset: the correlation of I_{pa} and I_{pc} of DA with $v^{1/2}$.

A Tafel plot (Figure 7 (inset)) was created on the basis of data related to the rising domain of the current–voltage curve from the linear sweep voltammogram at a low scan rate (10 mV/s) for DA (150.0 μM) to explore the rate-determining step. The linearity of the E vs. $\log I$ plot clarifies the involvement of electrode-process kinetics. The slope from this

plot presents the count of transferred electrons during the rate-determining step. Based on Figure 7 (inset), the Tafel slope was estimated to be 0.091 V for the linear domain of the plot. The Tafel slope value reveals that the rate-limiting step is a one-electron transfer process considering a transfer coefficient (α) of 0.35.

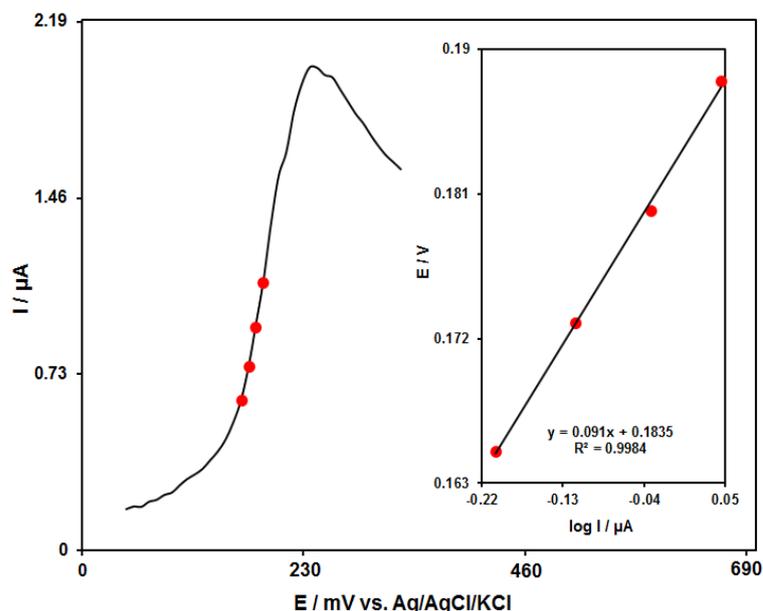


Figure 7. LSV for DA (150.0 μM) at the scan rate of 10 mV/s. Inset: the Tafel plot from the rising domain or the respective voltammogram.

3.4. Calibration Curve (DPV Analysis of DA)

DPV analysis was performed for variable DA contents to explore the linear dynamic range, LOD, and sensitivity of the N-GSs/GCE under optimized experimental circumstances (Figure 8). As expected, the elevation in DA level enhanced the peak current. Figure 8 (inset) shows a linear proportional of the oxidation peak currents to variable DA contents (0.1 μM to 700.0 μM), with the linear regression equation of $I_{pa} (\mu\text{A}) = 0.0625C_{DA} + 1.1884$ ($R^2 = 0.9996$) and a sensitivity of 0.0625 $\mu\text{A}/\mu\text{M}$. The LOD was estimated at 30.0 nM for DA determination on N-GSs/GCE. Table 1 compares the efficiency of the DA sensor prepared by the N-GSs-modified GCE and other reported works.

Table 1. Comparison of the efficiency of the NGSs/GCE sensor with other reported modified electrodes for DA determination.

Electrochemical Sensor	Electrochemical Method	Linear Range	LOD	Ref.
N-doped graphene quantum dots–chitosan nanocomposite/screen printed carbon electrode	DPV	1–100 and 100–200 μM	0.145 μM	[61]
Au-Cu ₂ O/reduced graphene oxide/glassy carbon electrode	DPV	10–90 μM	3.9 μM	[62]
Au nanoparticle–ZnO nanocone arrays/graphene foam electrode	DPV	-	0.04 μM	[63]
Ni-based metal–organic framework/GCE		0.2–100.0 μM	60 nM	[64]
Graphene quantum dots /glassy carbon electrode	DPV	0.4–100 μM	0.05 μM	[65]
Polypyrrole–mesoporous silica molecular sieves (MCM-48)/Au electrode	Square-wave voltammetry	2–250 μM	0.7 μM	[66]
Hierarchical MnO ₂ nanoflower/multiwalled carbon nanotube nanocomposite/glassy carbon electrode	DPV	0.5–30.0 μM	0.17 μM	[67]
NGSs/GCE	DPV	0.1–700.0 μM	30.0 nM	This work

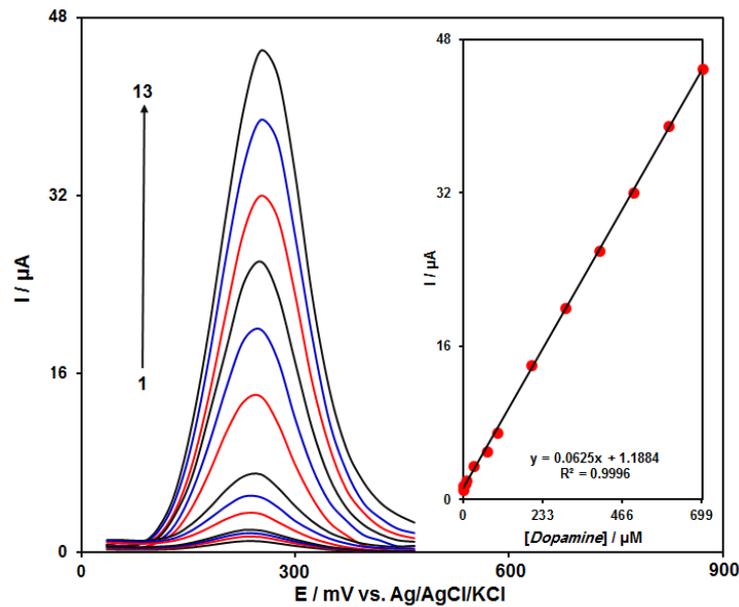


Figure 8. DPVs captured for the variable DA contents on the N-GSs/GCE (1–13: 0.1, 1.0, 5.0, 10.0, 30.0, 70.0, 100.0, 200.0, 300.0, 400.0, 500.0, 600.0, and 700.0 μM); Inset: calibration curve of voltammetric response against DA level.

3.5. DPV Analysis of DA in the Presence of AA and UA

Electrochemical detection of DA concurrent with AA and UA on the surface of bare electrode entails interference due to the comparatively close oxidation potentials of the three substances. Hence, the analyte concentration needed to be changed simultaneously to obtain the DPVs (Figure 9). There were three well-separated signals with the potential differences of 140 mV for AA and DA as well as 145 mV for DA and UA. Therefore, oxidation of these compounds on NGSs/GCE occurred independently, i.e., it is possible to simultaneously determine these analytes with no significant interference.

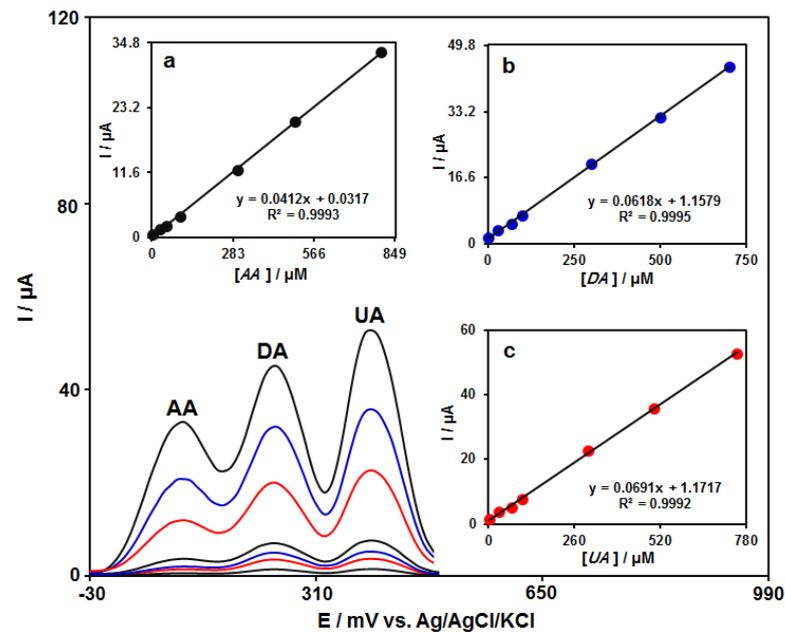


Figure 9. DPVs of NGSs/GCE in 0.1 M PBS (pH 7.0) with different contents of AA + DA + UA (bottom-up: 1.0 + 1.0 + 1.0, 30.0 + 30.0 + 30.0, 50.0 + 70.0 + 70.0, 100.0 + 100.0 + 100.0, 300.0 + 300.0 + 300.0, 500.0 + 500.0 + 500.0, and 800.0 + 700.0 + 750.0 μM). Insets: the plot of peak current versus AA concentration (a), DA concentration (b), and UA concentration (c).

3.6. Real Samples Analysis

The practical applicability of the N-GSs/GCE was tested by sensing AA, DA, and UA in a DA ampoule, a AA ampoule, and urine samples using the DPV procedure and standard addition method, the results of which can be seen in Table 2. The recovery rate was between 96.0% and 103.5% and all RSD values were $\leq 3.4\%$. According to the experimental results, the N-GSs/GCE sensor possesses high potential for practical applicability.

Table 2. Voltammetric sensing of AA, DA, and UA in real specimens using N-GSs/GCE. All concentrations are in μA ($n = 5$).

Sample	Spiked (μM)			Found (μM)			Recovery (%)			(% R.S.D.)		
	AA	DA	UA	AA	DA	UA	AA	DA	UA	AA	DA	UA
DA Ampule	0	0	0	-	5.0	-	-	-	-	-	2.9	-
	5.0	2.0	6.0	4.9	7.3	6.1	98.0	104.3	101.7	3.5	1.9	2.3
	7.5	3.0	7.0	7.6	7.9	7.3	101.3	98.7	104.3	1.7	3.4	1.6
	10.0	4.0	8.0	9.9	9.1	7.7	99.0	101.1	96.2	2.9	2.2	3.0
	12.5	5.0	9.0	12.8	9.6	8.9	102.4	96.0	98.9	2.8	2.4	2.3
AA Ampule	0	0	0	3.5	-	-	-	-	-	3.4	-	-
	2.0	4.5	4.0	5.6	4.4	4.1	101.8	97.8	102.5	2.0	1.9	3.4
	4.0	6.5	5.0	7.3	6.7	4.8	97.3	103.1	96.0	2.9	3.5	1.8
	6.0	8.5	6.0	9.4	8.4	6.1	98.9	98.8	101.7	2.4	2.7	2.5
	8.0	10.5	7.0	11.9	10.6	6.8	103.5	100.9	97.1	2.6	2.1	2.9
Urine	0	0	0	-	-	3.0	-	-	-	-	-	3.1
	5.0	6.0	3.0	4.9	6.2	5.9	98.0	103.3	98.3	3.4	2.3	1.7
	10.0	8.0	4.0	10.3	7.8	7.1	103.0	97.5	101.4	2.4	2.6	3.0
	15.0	10.0	5.0	14.9	10.1	7.7	99.3	101.0	96.2	1.6	3.1	2.1
	20.0	12.0	6.0	20.2	11.9	9.3	101.0	99.2	103.3	2.9	1.9	2.3

4. Conclusions

The present research developed a new sensor (N-GSs/GCE) for determination of DA. The analytical findings highlighted commendable electrocatalytic performance of the proposed sensor (N-GSs/GCE) for DA detection. The peak current of DA oxidation had a linear relationship with variable concentrations (0.1–700.0 μM), with a thin LOD (30.0 nM). The modified electrode could successfully determine the DA in the presence of UA and AA. The practical applicability of the as-developed N-GSs/GCE sensor was verified by sensing the study analytes in real specimens, with satisfactory recovery rates. These results suggest the proposed electrode has excellent performance and may be used for future DA detection probes based on electrochemical techniques.

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Data Availability Statement: The data presented in this study are available upon request from the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

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