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Electrochemical Sensing of Ascorbic Acid on ZnO-decorated Reduced Graphene Oxide Electrode

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Abstract

A voltammetric sensor using differential pulse voltammetry (DPV) was developed for the detection of Vitamin C (ascorbic acid). The sensing platform was ZnO-decorated reduced graphene oxide on glassy carbon electrode (ZnO-RGO-GCE). Graphene oxide, synthesized by an improved Hummers method, was reduced with zinc powder under ultrasonication, followed by washing with HCl. X-ray diffraction and scanning electron microscopy showed ZnO nanoparticles decorating the reduced graphene oxide sheets. ZnO-RGO-GCE showed reversible behaviour with ferricyanide system, had about 3.5 times more surface area than GCE, and exhibited higher currents for ascorbic acid oxidation compared to bare GCE. Ascorbic acid was sensed over a wide range of 1 μM to 5000 μM (R^2=0.9899) with a sensitivity of 0.178 μA/μM-cm^2 and detection limit of 0.01 μM, with good reproducibility (RSD=2.02%; n=5). The recovery of Vitamin C from pharmaceutical formulations, lemon juice, and gooseberry (amla) extract was also studied and compared against results from colorimetric methods. These results indicate that the developed ZnO-RGO-GCE platform could be used for voltammetric determination of Vitamin C in food samples.

Keywords: Zinc Oxide; Reduced graphene oxide; Ascorbic acid; Differential pulse voltammetry; Lemon juice; Gooseberry extract

Introduction

Ascorbic acid (AA), also known Vitamin C, is an essential nutrient that helps the human body in forming collagen, a protein which gives structure to bones, muscles teeth, cartilages and blood vessels. It is a water-soluble antioxidant that donates hydrogen to prevent oxidation process, thereby preventing tissue damage [1-5]. The human body is unable to synthesize ascorbic acid on its own. Ascorbic acid is found in variable quantities in fruits and vegetables. Rich sources of ascorbic acid include gooseberry (amla), citrus fruits, black currant, leafy vegetables, green and red peppers. Diets rich in Vitamin C are even prescribed as supplements to patients with cancer and AIDS [6]. It is therefore important to detect and quantify ascorbic acid in food samples, products, and nutraceuticals. Various methods have been employed for the determination of ascorbic acid in pharmaceutical compounds, vegetables, fruits and juices [7,8].

Ascorbic acid is conventionally determined using colorimetric methods [9] using either bromine/water or dichlorophenol-indophenol dye method. Electrochemical determination of ascorbic acid is gaining interest due to its fast analysis, ability to detect lower concentrations, and simplicity of operation. Further, a lab-on-a-chip electrochemical sensing platform has the potential to simultaneously determine multiple antioxidants such as polyphenols as well as other micronutrients in a food sample [5,10-17]. Therefore, the development of electrochemical sensing platforms that have the ability to determine multiple analytes relevant in nutritive food formulations and nutraceuticals is of paramount interest. A brief summary of salient works on the determination of ascorbic acid in food samples is provided in Table 1.

References	System	Technique	Linear Range (mM)	Samples
[2]	GCE	CV	5-Jan	Tropical fruit
[3]	GCE	DPV	0.01-0.6	Tablets, Fruit juice
[6]	Platinum	DPV	0.31-20	Fruit juice, wine
[6]	Carbon paste electrode	DPV	0.07-20	Fruit juice, wine
[7]	GCE	CV	0.06-2.10	Fruits
[11]	GCE	CV	0.0051-0.51	Citrus Limon
[14]	Cellulose Acetate film-GCE	CV	0.1-6	Beverages, Tablets
[16]	MWCNT	SWV	0.25-S5	Orange Juice

[17]	Chitosan-graphene-GCE	DPV	0.02-3	Tablets, orange juice
This work	ZnO-RGO-GCE	DPV	0.001-5	Lemon juice, Amla

Table 1: Electrochemical sensing of Ascorbic Acid in pharmaceutical tablets and fruit samples.

Graphene is a single-to-few-atoms-thick planar sheet of carbon atoms arranged in a honeycomb two-dimensional lattice. It exhibits excellent electrical, thermal, optical properties and biocompatible properties. It also has high surface to volume ratio, abundant defect sites (when synthesized by chemical or electrochemical reduction), and fast electron transfer rates, and therefore is a suitable material for electrochemical sensing [18-21]. Graphene-based electrochemical sensors have been developed for ascorbic acid by modification of graphene with chitosan, MnO_2 nanorods, palladium nanoparticles, and polyinylpyrolidone (Table 2). Electrochemical techniques such as cyclic voltammetry, differential pulse voltammetry and amperometry have been used. A summary of literature on graphene-based electrochemical sensing of ascorbic acid is provided in Table 2 [16-52]. Most of the literatures have been reported that being defective, Graphene based sensors is very promising in sensing applications [53,54].

References	System	Technique	Linear Range (μM)	Sensitivity 2	Detection Limit (μM)
0	MWCNT-GCE	SWV	4.7 – 5000	0.471 μA/μM-cm^2	1.4
0	Chitosan-graphene	DPV	20 – 3000	0.247 mA/μM-cm^2	0.013
0	MnO2-Graphene	DPV	0.05 – 400	2.40 μA/μM- cm^2	0.01
0	MWNT-Silica-AuNP-GCE	CV	1000 – 5000	8.59 μA/mM	-
0	PdNP-GCE	Amperometry	20 – 2280	-	-
0	Chitosan-graphene	CV	50 – 1200	-	50
0	N2-doped graphene	DPV	5 – 1300	0.39 μA/μM- cm^2	2.2
0	Graphene-doped carbon paste electrode	Amperometry	0.17 – 106	0.47 μA/μM- cm^2	0.07
0	Graphene-Cu-phthalocyanine-PANi	CV	0.5 – 12	24.46 μA/mM	0.063
0	Tryptophan functionalized graphene	DPV	200 – 12900	0.0818 μA/mM	10.09
0	Graphene-Nickel hydroxide composite	DPV	150 – 300	0.99 μA/μM- cm^2	30
0	ERGO-Carbon fiber electrode	DPV	8 – 2016.45	0.066 μA/μM	4.5
0	Graphene flowers-Carbon modified fibers	DPV	73.52 – 2305.53	-	24.7
0	PdNPs-Graphene-Chitosan-GCE	DPV	100 – 4000	20	
0	Screen printed graphene electrode	DPV	4 – 4500	0.25 μA/mM	0.95
0	Graphene- size selected Pt-GCE	DPV	0.15-34.4	0.3457 μA/μM	0.15
0	Fe3O4@ AuNPs-graphene sheet-chitosan-GCE	DPV	4-400	7.86 μA/μM	0.3
0	SWCNT-ZnO-GCE	CV	200-10000	0.196 μA/μM- cm^2	85
[38]	Cu-Zeolite A-Graphene-GCE	DPV	20-200	-	1.1
[39]	Poly(acridine orange) film-RGO-GCE	DPV	0.8 – 5000	-	0.3
[40]	Graphene ceramic composite	CV	3 – 84	6.06 μA/μM cm^2	0.82
[41]	Double-walled nanotubes-choline-GCE	DPV	0.10-777	0.199 μA/μM-cm^2	0.03
[42]	Chitosan-Graphene-GCE	DPV	0.6-1678.1	9.344 μA/μM-cm^2	0.1
[43]	GO-Templated Polyanline Microsheets	DPV	150-1050	10.88 μA/μM	50
[44]	PVP-stabilized graphene electrode	CV	10-500	-	1
[45]	Graphene doped carbon paste electrode	DPV	0.1-106	0.033 μA/μM	0.07

[46]	Mesopore-rich active carbon-modified pyrolytic graphite electrode	DPV	0.5-2000	2.27 μA/mM	0.3
[47]	3D graphene foams- CuO-GCE	CV	0.43-240	2.06 μA/mM-cm^2	0.43
[48]	AgNPs-RGO-GCE	CV	10-1000	1.64 μA/μM -cm^2	10
[49]	ERGO-GCE	DPV	300-2000	0.171 μA/μM-cm^2	0.5
[50]	CTAB-GO-MWNT-GCE	DPV	5-300	401.64 μA/μM-cm^2	1
[51]	Solar graphene-GCE	CV	400-4000	0.00049 μA/μM-cm^2	-
[52]	CuNPs/PSA-GCE	CV	0.30-730	-	0.15
This work	ZnO-RGO-GCE	DPV	Jan-00	0.178 μA/μM-cm^2	0.01

Table 2: Graphene-based electrochemical sensing of ascorbic acid (CV: Cyclic Voltammetry; DPV: Differential Pulse Voltammetry; SWV: Square Wave Voltammetry)

The purpose of this work is to investigate the state of the art of applying graphene based sensor in determination of ascorbic acid in food. In this work, we have developed an electrochemical sensor based on ZnO-decorated reduced graphene oxide on glassy carbon electrode (ZnO-RGO-GCE) for the determination of ascorbic acid. Using this system, we have been able to detect ascorbic acid over a wide range of concentrations (1 μM to 5000 μM) with good sensitivity (0.178 μA/μM-cm^2) and a low detection limit of 0.01 μM. We have further used this system to successfully determine the amount of ascorbic acid in pharmaceutical formulations, lemon juice, and gooseberry (amla) extract. We have also compared our results with the standard colorimetric method.

Materials and Methods

Reagents and apparatus

Graphite powder and ascorbic acid were purchased from Sigma Aldrich; Zinc dust, potassium dihydrogen phosphate (KH_2PO_4) and hydrogen peroxide (H_2O_2) were purchased from Rankem. All chemicals used were of analytical grade and ultrapure water (Millipore) was used throughout the experiments for preparing working solutions.

All electrochemical experiments were performed using a three-electrode setup from Pine Instrumentation Limited (WaveNow potentiostat /galvanostat). Glassy carbon electrodes (3 mm dia), with or without modification by ZnO-decorated RGO, were used as working electrodes. Platinum wire was used as counter electrode and an Ag/AgCl (in 3M KCl) was used as reference electrode. All electrodes were procured from CH Instruments, Inc.

Preparation of graphene oxide

Graphene oxide (GO) was synthesized by Improved Hummers method [55-57]. Briefly, 3 g of graphite was added to a 9:1 mixture of H_2SO_4:H_3PO_4, followed by stirring for 30 min in an ice bath. 18 g of $KMnO_4$ was then slowly added into the mixture under vigorous stirring. The ice bath was removed and the mixture was kept for stirring for 12 h at 50°C until it turned light brown in color. The mixture was then cooled to room temperature and poured onto 400 ml of ice. Then 3 ml of 30% H_2O_2 was added. The solution was then repeatedly washed with 200 ml of water, 30% HCl, and ethanol by centrifugation. Finally, the graphene oxide synthesized was kept in oven for drying at 60°C for 2-3 days.

Preparation of ZnO-decorated reduced GO

The reduction of graphene oxide was done by the following approach, similar to the procedure described elsewhere [58]: To 100 mg of GO, 100 ml of distilled water was added and sonicated for 5 min. The pH was adjusted to 2 by adding 37% HCl solution. 200 mg of zinc powder was added and sonicated for 15 min until black precipitate appeared at the bottom. Excess zinc was dissolved by adding 5 ml of 37% HCl. The reduced graphene oxide (RGO) was collected by filtration after rinsing with distilled water for several times. The resultant product was kept at room temperature for 2-3 days for drying.

Preparation of modified electrode

Prior to modification, a glassy carbon electrode was polished using 0.1 μm alumina slurry and washed, until it showed excellent reversibility to ferricyanide redox system. The prepared ZnO-RGO was dispersed in distilled water (2 ml) and sonicated for 10 mins. 10 μL of RGO was drop cast on the surface of the polished glassy carbon electrode and dried under an IR lamp to obtain the modified glassy carbon electrode (ZnO-RGO-GCE). All solutions for electrochemical studies were prepared with a pH of 7.4 (phosphate buffer).

Colorimetric analysis of vitamin C in tablets and food samples

Analysis of vitamin C in fresh samples such as amla, lemon juice and Vitamin C tablets was performed according to the standard procedure of NIN [0]: To 10 ml of a sample extract, few drops of bromine water was added until the solution turned yellow color (indicates oxidation of ascorbic acid to dehydroascorbic acid). 1 ml of 2% thiourea solution was added to remove the excess bromine water and thus clear solution was obtained. Then 1 ml of 2, 4-Dinitrophenyl hydrazine dye solution was added to the mixture and incubated for 3 h. Then 4 ml of 85% sulphuric acid was added and the red color developed was read against at 620 nm.

Results and Discussion

Characterization of GO and ZnO-RGO

The synthesized GO and ZnO-RGO were characterized for functional groups using Fourier Transform Infrared Spectroscopy (FTIR, Thermo Nicolet iS10), crystallinity using X-ray Diffraction (XRD, Bruker aXS KAPPA APEX-II), morphology using scanning electron microscopy (HRSEM, FEI Quanta FEG 200 High Resolution Electron Scanning Microscope), and composition using thermogravimetric analyzer (TGA, PerkinElemer/TGA400).

Figure 1 shows the FTIR spectra of GO and ZnO-RGO. The spectrum of GO indicates the presence of -OH (3386 cm^{-1}), -CH (2853 cm^{-1}), -C=O (1732 cm^{-1}), C=C (1625 cm^{-1}), and C=O epoxide (1000 cm^{-1}). Reduction of GO by zinc resulted in significant reduction of the hydroxyl, carbonyl/carboxyl, and epoxide groups. Additionally, the spectrum for ZnO-RGO showed a distinct peak at around 469 cm^{-1}, which corresponds to ZnO.

The characteristic peak of GO at 10.25° was observed that indicates the presence of the larger interlayer distance between GO due to oxidation of graphite and formation of the functional groups such as carboxyl, hydroxyl and epoxy, as indicated in the FTIR results. The peak around 10° was reduced in intensity significantly upon reduction of graphene oxide with zinc, indicative of the formation of reduced graphene oxide.

Also, it is of interest to note that graphite powder was not fully oxidized, resulting in the presence of graphite peaks in the GO sample. Reduction of GO with zinc resulted in an additional ZnO peak at 32.36°. Along with the FTIR results and SEM image, this confirms the presence of ZnO in the reduced graphene oxide.

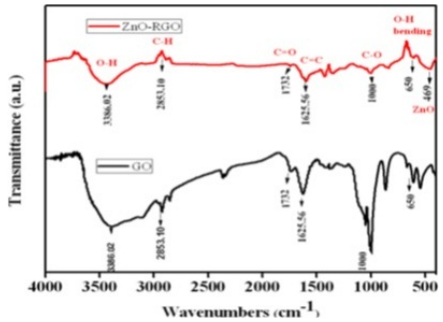

Figure 1: FT-IR spectra of the synthesized GO and ZnO-RGO

The X-ray diffraction spectra of GO and ZnO-RGO are shown in Figure 2.

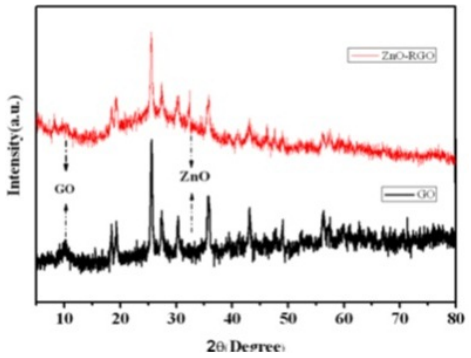

Figure 2: XRD spectra of the synthesized GO and ZnO-RGO

Morphology of the reduced graphene oxide obtained through zinc reduction was investigated by scanning electron microscopy. Figure 3 shows the HRSEM image of reduced graphene oxide synthesized by the reduction of zinc.

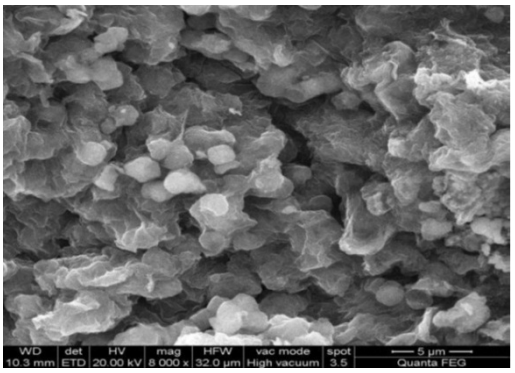

Figure 3: SEM image of ZnO-RGO

Wrinkled sheets of graphene decorated with bright, nearly spherical zinc oxide particles are observed. Thermogravimetric analyses of GO and ZnO-RGO were also carried out (Figure 4). The results indicate that the functional groups in GO are significantly reduced during Zn reduction.

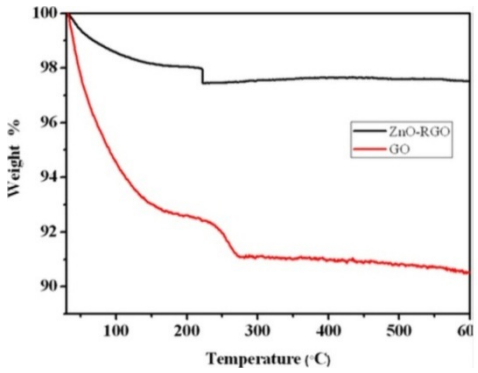

Figure 4: Thermogravimetric analysis of GO and ZnO-RGO

Electrochemical behaviour of ZnO-RGO-GCE electrode

The electrochemical behaviour of GCE and ZnO-RGO-GCE was studied using cyclic voltammetry in 0.5 mM ferricyanide solution at a scan rate of 50 mV/s. It was found that both electrodes displayed reversible behaviour. Using a diffusion coefficient of 6.8×10^{-6} cm^2/s from the literature [58] and Randles-Sevcik equation, it was seen that the electroactive surface area for ZnO-RGO-GCE was found to be 3.51 times higher than that of the bare electrode. The effect of scan rate on oxidation and reduction currents on ZnO-RGO-GCE was investigated by cyclic voltammtery in 0.5 mM ferricyanide at different scan rates (5-500 mV/s) in the potential range -0.2 to 0.8 V (Figure 5a).

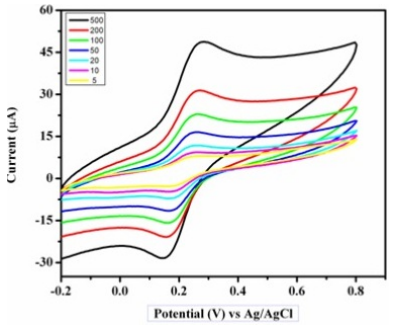

Figure 5a: Effect of varying Scan rate for 0.05 mM ferricyanide solution at ZnO-RGO-GCE

The oxidation and reduction peak currents linearly increased with the square root of scan rate (Figure 5b), while the anodic and cathodic peak potentials varied linearly with the logarithm of scan rate (Figure 5c). This further confirmed that ZnO-RGO-GCE displayed reversible behavior in known reversible redox systems such as the ferricyanide system.

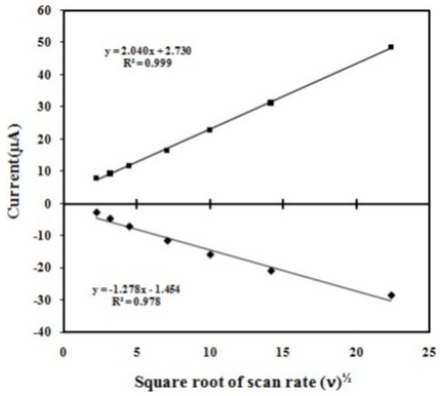

Figure 5b: Reversibility of ZnO-RGO-GCE: Variation of anodic and cathodic peak currents with the square root of scan rate, in 0.5 mM ferricyanide solution.

Electrochemical impedance spectrum (EIS) used to study the electrochemical properties at interface of ZnO-RGO-GCE. Figure 6 shows the electrochemical impedance spectra on the bare GCE (a), ZnO-RGO-GCE (b) in the presence of 0.5 mM ferricyanide solution. As, it is shown from the graph, the electron transfer resistance of the electrode decreased after modification. GCE shows higher electron transfer resistance. The ZnO-RGO-GCE shows a depressed semicircle which indicates low interfacial electron transfer resistance and excellent conductivity.

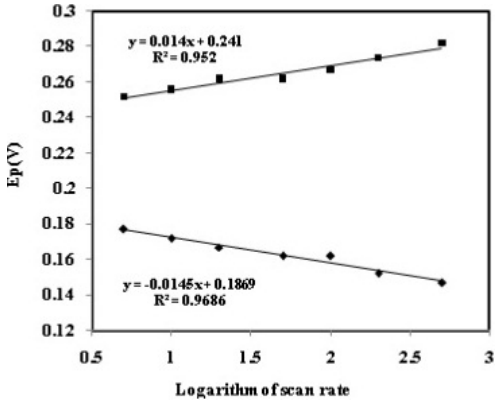

Figure 5c: Reversibility of ZnO-RGO-GCE: Variation of anodic and cathodic peak potentials with logarithm of scan rate, in 0.5 mM ferricyanide solution

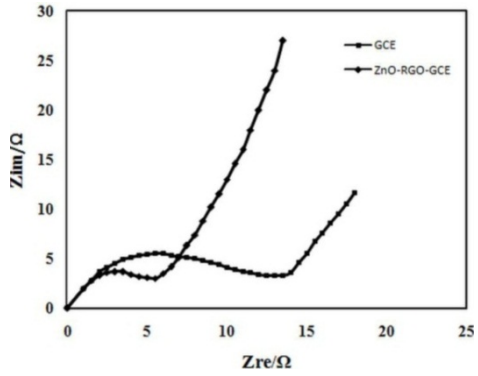

Figure 6: Cyclic voltammetry of 500 µM ascorbic acid in 0.1 M phosphate buffer solution at a scan rate of 10 mV/s at bare GCE (black) and at ZnO-RGO-GCE (blue)

Electrochemical determination of ascorbic acid

Cyclic voltammograms were obtained for 500 µM ascorbic acid (AA) in phosphate buffer at pH 7.4 at bare GCE and ZnO-RGO-GCE at scan rate 10 mV/s in the potential range -0.2 to 0.6V. The sensing mechanism involves that the AA molecules in the solution were adsorbed onto the surface of ZnO-RGO-GCE. Then the AA molecules is hydrolyzed with water and oxidized to dehydroascorbic acid. This oxidation process of AA can release electrons that correspond to current which can be detected. It was observed, in Figure 7, that the oxidation of the ascorbic acid is an irreversible process due to the absence of a reduction peak.

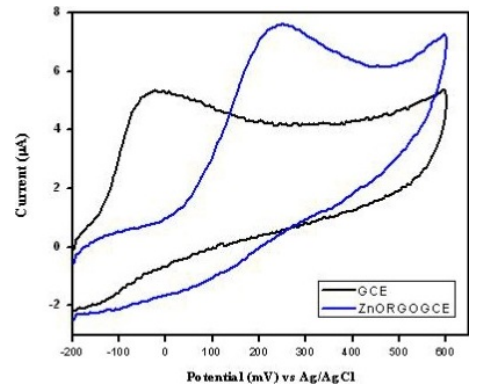

Figure 7: Cyclic voltammetry of 500 µM ascorbic acid in 0.1 M phosphate buffer solution at a scan rate of 10 mV/s at bare GCE (black) and at ZnO-RGO-GCE (blue)

The voltammograms further indicate that the ZnO-RGO-GCE showed higher currents for AA oxidation compared to bare GCE. The increased peak currents are largely the result of increased electroactive surface area of the ZnO-RGO-GCE. Both bare GCE and ZnO-RGO-GCE oxidized AA at low overpotentials. Therefore, both electrodes displayed catalytic activity for AA oxidation. However, it is known from literature that graphene-based electrodes display much better selectivity for AA in the presence of other analytes [58-61]. Therefore, ZnO-RGO-GCE was used to develop a voltammetric sensor for AA.

A voltammetric sensor using differential pulse voltammetry (DPV) was developed for the detection of AA. Figure 8a shows the obtained differential pulse voltammograms at ZnO-RGO-GCE for different concentrations of AA. The concentration was varied from 1 µM to 5000 µM in phosphate buffer of pH 7.4. The oxidation peak current obtained was directly proportional to the concentration of ascorbic acid. The calibration graph for peak current against concentration of ascorbic acid was plotted (Figure 8b), with the linear regression equation of with R^2=0.9899 (adjusted R^2=0.9894), and the sensitivity was found to be 0.178 µA/µM-cm^2.

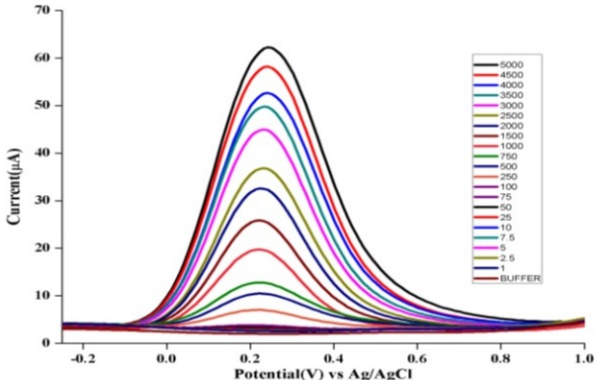

Figure 8a: Differential pulse voltammograms of oxidation of ascorbic acid in 0.1 M phosphate buffer solution at ZnO-RGO-GCE from 1 µM to 5000 µM

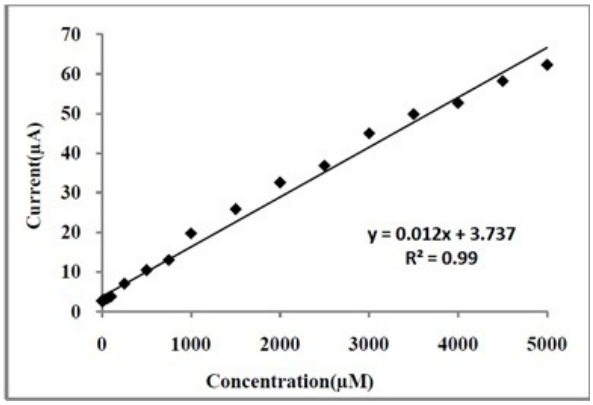

Figure 8b: Calibration graph of linear variation of DPV peak current as a function of concentration of ascorbic acid in 0.1 M phosphate buffer solution using ZnO-RGO-GCE

The limit of detection was found to be 0.01 µM. To estimate the limit of detection a statistically rigorous approach was adopted. Briefly, two sets of seven replicates were analyzed, one with the concentration of an estimated detection limit and another with blank (buffer alone). A null hypothesis was formulated that the mean currents of both systems were equal (i.e, the method used is unable to distinguish the estimated detection limit concentration and the blank). The validity of this hypothesis was tested using a one-sided Student's t-test (equal data with unequal variance) at the confidence level of 95% (α=0.05). If the null hypothesis could be rejected (i.e., the method is indeed able to

distinguish between the estimated detection limit and the blank) at this confidence level, then the estimated detection limit was accepted as the method's detection limit.

The reproducibility of ZnO-RGO-GCE was also investigated by performing DPV analysis in solutions containing 750 μM ascorbic acid in 0.1 M phosphate buffer. For five different runs, the standard deviation was 2.02%, which indicated that the electrode could reproducibly determine ascorbic acid.

Real sample analysis

The ability of the ZnO-RGO-GCE sensor to determine ascorbic acid in real samples was studied. To this end, Celine (Glaxo Smithkline Pharmaceuticals Ltd) tablets, labeled 500 mg of ascorbic acid, were taken, and the recovery of ascorbic acid by DPV analysis was examined. Five analyses were conducted, with three tablets used in each analysis. Colorimetry [62] was also used to determine the amount of ascorbic acid for each analysis.

Vitamin C Tablet Samples	Recovery (%)	
	Colorimetric method	Electrochemical method
1	98.91	102.06
2	99.08	103.57
3	97.61	94.4
4	98.69	94.71
5	99.81	105.31

Table 3: Recovery of ascorbic acid from Vitamin C tablets using ZnO-RGO-GCE by DPV and standard colorimetric analysis

A comparison of the results from DPV and colorimetry are presented in Table 3, along with % recoveries in each case. The results indicate that the ZnO-RGO-GCE electrochemical sensor is able to successfully determine ascorbic acid in tablet samples, comparable to the performance of a standard analytical technique such as colorimetry.

The developed ZnO-RGO-GCE platform was also used to determine the amount of ascorbic acid in food samples such as lemon juice (Citrus Limon) and amla (Phyllanthus Emblica). Briefly, lemon was hand-squeezed whereas amla was finely grated and crushed with mortar-pestle to obtain juice. Following centrifugation, the supernatant was collected for analysis.

DPV studies were carried out on ZnO-RGO-GCE and colorimetric analysis was also carried out. For each analysis, three fruits were used for extraction, and five different analyses each were carried out for lemon and amla. The amounts of ascorbic acid obtained through both methods are compared in Table 4. From the above results, it is clear that the ZnO-RGO-GCE platform developed in this study can be used for ascorbic acid determination in real samples such as lemon and amla.

Conclusions

An electrochemical sensing platform for ascorbic acid based on ZnO-decorated reduced graphene oxide was developed. In comparison to GCE, the ZnO-RGO-GCE electrodes showed better electrocatalytic activity towards the oxidation of ascorbic acid and was capable of sensing ascorbic acid over a wide range of concentrations (1 to 5000 μM) with good sensitivity (0.178 μA/μM-cm^2) and a low detection limit (0.01 μM). The ZnO-RGO-GCE platform could also successfully predict ascorbic acid concentrations in pharmaceutical formulations, lemon and gooseberry extracts.

Samples	Amt. of ascorbic acid obtained in amla /mg in 100g		Amt. of ascorbic acid obtained in lemon /mg in 100g	
	Colorimetric	Electrochemical	Colorimetric	Electrochemical
1	610.75	664.54	53.42	56.93
2	610.75	666.58	53.42	57.44
3	605.77	665.1	58.39	62.69
4	610.75	662.5	53.42	53.53
5	610.77	662.88	53.42	56.67

Table 4: Analysis of amount of ascorbic acid in lemon (Citrus Limon) and amla (Phyllanthus Emblica) samples using DPV on ZnO-RGO-GCE and colorimetry. (**Note:** NIN ICMR [63] Standard value of Vitamin C in amla has been reported to be around 600 mg/100 gm of edible portion, and in lemon it has been reported to be 53-64 mg/100 gm of edible portion)

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