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In-situ functionalization of aniline oligomer onto layered graphene sheet and study of its application on electrochemical detection of ascorbic acidin food samples

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Aniline oligomers are considered as one of the electron transfer mediators for the electrochemical oxidation of ascorbic acid. The electrochemical oxidation of ascorbic acid was investigated using aniline oligomer-functionalized polymer modified electrode. In the present investigation, we demonstrated a novel methodology for the in-situ modification of aniline oligomer onto the layered graphene sheet by using diazonium salt form as precursor molecule. An enhanced electrocatalytic current was obtained for the oxidation of ascorbic acid using aniline pentamer-functionalized reduced graphene oxide (AP-rGO). Detailed studies have been carried out to study the surface modified rGO by FTIR spectroscopy. A linear relationship between peak current against the concentration of ascorbic acid was observed within the ranges from 1 μM to 10 μM. The detection limit was measured at signal/noise (S/N) of 3. The present method can be utilized for the electrochemical detection of ascorbic acid present in food products like fruit juices.

Keywords: graphene oxide, pentamer, voltammetric method, ascorbic acid, food samples.

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1. Introduction

Ascorbic acid is one of most important biological compounds involved in various human metabolisms. It is used to ameliorate a number of illnesses, such as the scurvy, common cold, cancer and AIDS [1]. Moreover it is present in fresh fruits, vegetables and also available in pharmaceutical products and foodstuffs as an oxidant as well as stabilizer. Thus, the development of inexpensive and easy methods for the determination of ascorbic acid is particularly important in the pharmaceutical and food industries. There are several reported methods for the detection of ascorbic acid in foodstuffs, such as chromatography [2], spectrophotometry [3], capillary electrophoresis [4] and most recently, electrochemical methods [5–7].

The amperometric determination of ascorbic acid is based on its electrochemical oxidation, which occurs at high potential at carbon or metal electrodes, however, fouling by oxidation products leads to poor reproducibility [8]. Numerous attempts to decrease the high working electrode potential and improve reproducibility have been made by modifying the electrode surface with various active mediators for the electrochemical oxidation of ascorbic acid. Attempts have been made to develop a chemically-modified electrode with various redox mediators like 7,7,8,8-tetracyanoquinodimethane, osmium 2,2-bipyridyl poly-4-vinyl pyridine chloride complex, lanthanum 2,6-dichlorophenolindophenol, manganese dioxide and a cobalt (II) phthalocyanine and Prussian blue was also used to oxidize ascorbic acid [9, 10]. The electrocatalytic oxidation of ascorbic acid on conducting polymer modified electrodes, in particular on polyaniline, has also been studied. Recently, aniline oligomer-functionalized polymers have been used for the electrochemical oxidation of ascorbic acid has been developed that has both a low applied operating potential and a low detection limit.

In the present investigation, we demonstrated the preparation of an aniline oligomer-modified graphene oxide based on chemical reductive binding of diazonium salt form of aniline oligomer in presence of graphene oxide. The electrocatalytic behavior of an aniline oligomer-functionalized graphene oxide-modified glassy carbon electrode was tested against the electrochemical oxidation of ascorbic acid in phosphate buffer solution. The greater sensitivity and low detection limits were achieved by the differential pulse voltammetry method. Thus, the present method can be considered as an efficient one for electrochemical detection of ascorbic acid in food products.

2. Experimental Section

2.1. Chemicals

Graphite powder, Ascorbic acid and N, N-diphenylamine were received from Sigma Aldrich, USA. Ammonium hydroxide and sodium nitrite were purchased from Fisher Scientific Pvt. Ltd. India. DMF, HCl, H_2SO_4 and ethanol were received from SRL Pvt. Ltd. India. Ethanol and $KMnO_4$ were procured from Merck, India. All reagents and chemicals were used an analytical grade and without further purification.

2.2. Preparation of pentamer

2.28 g amino capped aniline trimer and 3.04 g N, N-diphenylamine were dissolved with 80 mL of DMF. 20 mL of water and 10 mL of 36 % of HCl were then added to the above solution with vigorous stirring at 4 hrs for room temperature. The HCl-doped aniline pentamer was obtained by filtration and then washed by a mixture of DMF/water. The product was doped in 100 mL of 1 ML^{-1} ammonium hydroxide for 30 m to produce aniline pentamer in emeraldine state. The emeraldine aniline pentamer was reduced by phenylhydrazine and was precipitated in a H_2O/ethanol mixture. The leucomeraldine aniline pentamer was collected by filtration and washed thoroughly with H_2O/ethanol mixture. The product was finally dried in vaccum oven.

2.3. Preparation of graphene oxide

20 g of graphite powder stirred with 500 mL of conc. H_2SO_4 in 1L beaker for 30 min. Then, 60 g of $KMnO_4$ was gradually added to this solution (added 20 g/30 minutes). The solution was then stirred for an additional 5 to 8 h and then added one l L water (900 mL) to the above mixture. Then, the reaction was terminated by addition of 50 mL H_2O_2 solution. Repeated centrifugation was done by using 5 % HCl aqueous solution followed by washed with deionized water until the pH of the solution reaches neutral. Then 160 mL of water was added resulting in the formation of precipitate. A uniform suspension of grapheme oxide (GO) nanoparticles was obtained after sonication [11].

2.4. Preparation of AP-rGO

The modification of GO was accomplished by dispersing GO (10 mg) in DI water (5 mL) to which aniline pentamer (10 mg) in 2 mL water was added. After complete dispersion of the reagent 0.011 g sodium nitrite in 1 ml of conc. HCl was added drop wise. The mixture was stirred for 4 h and then filtered, washed successively with deionized water followed by methanol. Finally, the powdered form of the product was isolated and then dried under nitrogen atmosphere in overnight.

2.5. Instrumentation

FTIR spectrum was recorded in the range of 400 to 4000 cm^{-1} FTIR spectrum was collected against the background spectrum of KBr. The cyclic voltammetric experiment was carried out using CHI 660A electrochemical instrument, USA and Gamry model 330, USA. A conventional three electrode system comprising of glassy carbon electrode (GCE) of 3 mm of geometrical surface area was purchased from BAS. Pvt. Ltd., USA. The Ag/AgCl and platinum wire were used as a reference electrode and counter electrode, respectively. The working electrode was polished using Bioanalytical system (BAS, USA) polishing kit.

3. Results and discussion

3.1. Characterization of AP-rGO

The FTIR spectrum of pentamer and AP-rGO are shown in Fig. 1. The absorption of aniline pentamer showed main bands at 1590 cm^{-1} and 1485 cm^{-1} assigned to the absorption of benzene ring and the quinoid ring. The 1273 cm^{-1} band was attributed to C-N stretching in the proximity of quinoid rings as shown in Fig. 1 [12]. After modification of AP-rGO, the benzenoid and quinoid peaks are bounded in the surface of graphene oxide. So these results confirm that the pentamer modified on graphene oxide surface.

The electrochemical behavior of AP-rGO was studied and the resulting cyclic voltammetry responses are shown in Fig. 2. As seen from Fig. 2(A), the typical cyclic voltammogram of AP-rGO in presence of 0.1 M H_2SO_4. With increase of scan rate the electrocatalytic oxidation peak current also increased gradually in the range from 5–100 mV/s. A linear relationship exists between peak current (I_p) versus scan rate (ν) and a linear regression equation of y = 0.406 – 5.685 with correlation coefficient (R^2) of 0.9959 as shown in Fig. 2(B), which illustrates a reversible electron transfer process with adsorption controlled one.

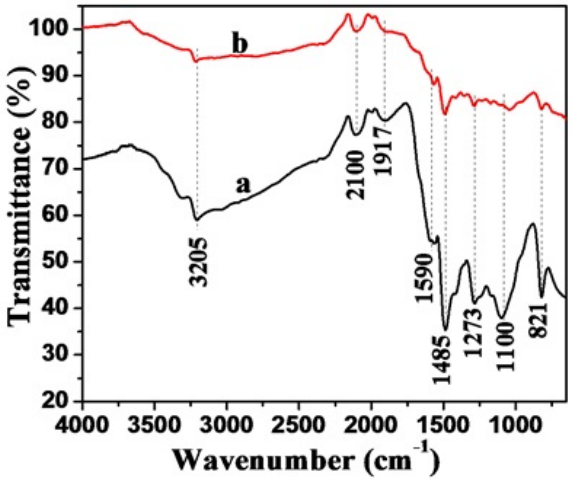

FIG. 1. FTIR spectrum of a) pentamer and b) AP-rGO

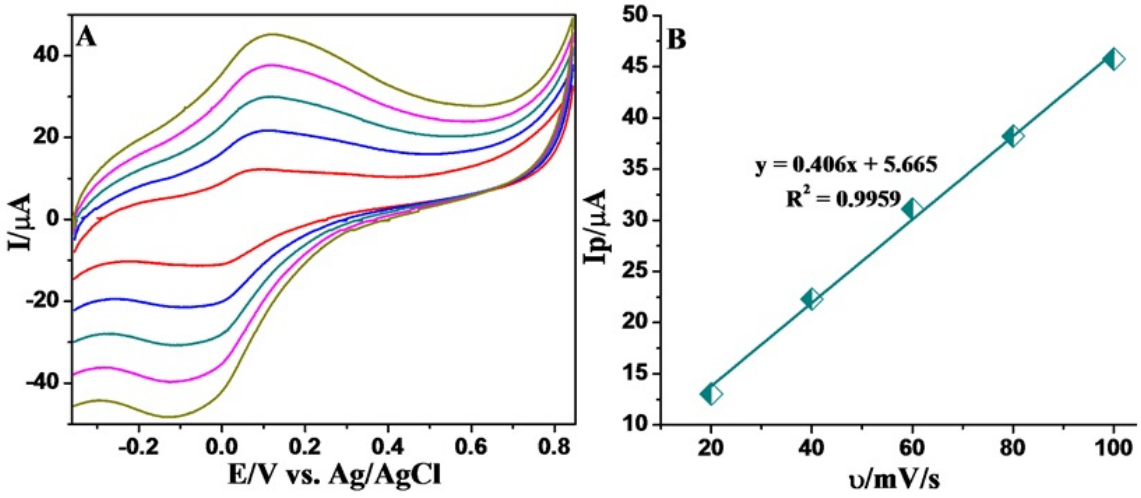

FIG. 2. A) CV of AP-rGO modified GCE at different scan rates (5-100 mV/s) in presence of 0.1 M H_2SO_4. B) Linear plot of Ip vs. υ

3.2. Electrochemical oxidation of ascorbic acid

The electrochemical behaviors of a) bare, b) bare-with ascorbic acid and c) AP-rGO modified GCE in presence of AA are shown in Fig. 3.(A) poor anodic current response was observed for the AA oxidation on bare GCE where as a well define oxidation peak was observed at +0.05 V (vs. Ag/AgCl) in the case of AP-rGO modified GCE.

The electrochemical behavior of AP-rGO/GCE at different concentrations of ascorbic acid (1×10^{-4} – 7×10^{-4} M) were investigated in 0.1 M pH 7.0 PBS by cyclic voltammetry (CV). As can be seen in Fig. 4(A), the AP-rGO/GCE after addition of AA to the buffer solution, an irreversible oxidation peak was observed at a scan rate of 50 mV/s with a anodic oxidation peak potential of +0.05 V (vs. Ag/AgCl). In Fig. 4(B) shows a linear regression equation of $y = 0.092x - 0.14$ with correlation coefficient is 0.9994.

The influence of peak potential scan rate against the electrochemical oxidation of ascorbic acid was investigated using AP-rGO/GCE in presence of 1mM of ascorbic acid at 0.1 M PBS (pH 7) with various scan rates as shown in Fig. 4(C). From AP-rGO modified GCE, the oxidation peak current of AA was increased by increasing the scan rates (10–120 mV/s) and linearity was observed by plotting the peak current values versus the square of scan rates with correlation coefficient of 0.9920 (Fig. 4(D)), suggesting that the electrochemical oxidation of AA is diffusion controlled electron transfer process.

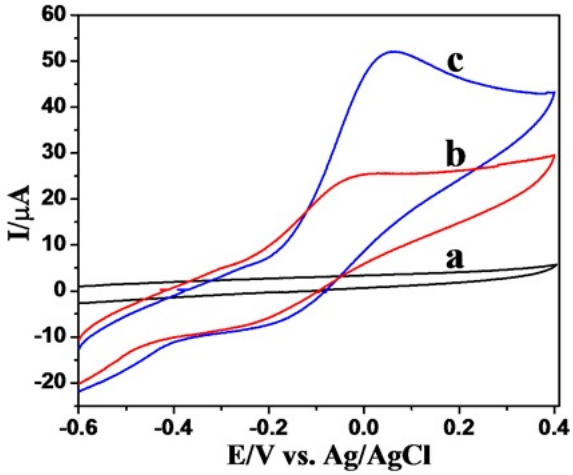

FIG. 3. Electrocatalytic behavior of a) bare, b) bare-with AA (1 mM) and c) AP-rGO/GCE with 1 mM of AA in 0.1 M PBS (pH 7) at scan rate 50 mV/s

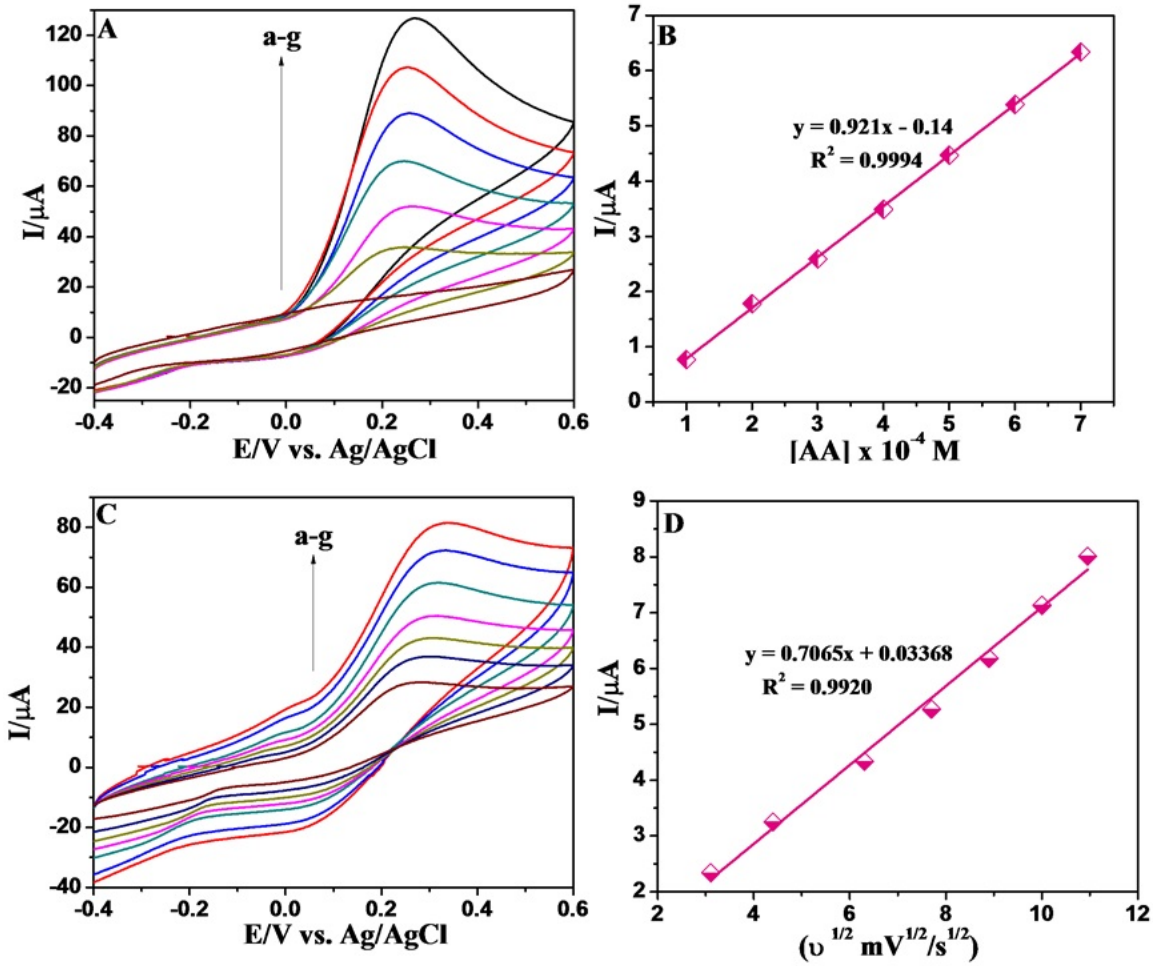

FIG. 4. A) AP-rGO/GCE in presence of AA in 0.1 M PBS (pH 7) at scan rate 50 mV/s and B) Linear plot of conc. vs. peak current. C) AP-rGO/GCE in presence of AA at different scan rates in 0.1 M PBS (pH 7) and D) Linear plot of Ip vs. $\upsilon^{1/2}$

3.3. Differential pulse voltammetry method

For the quantitative detection of ascorbic acid (AA) present in the pharmaceutical formulations and fruits samples at low concentration levels, the differential pulse voltammetry method was demonstrated using AP-rGO modified GCE under optimized experimental conditions. Fig. 5(A) shows differential pulse voltammogram for the electrocatalytic oxidation of AA using AP-rGO/GCE in PBS (pH 7) containing various concentrations of AA. The result shows the electrocatalytic peak current of AA oxidation at the AP-rGO/GCE was linearly dependent on the AA concentration and its ranges from 0.3 μM to 3 μM. As seen from Fig. 5(B), the calibration plot shows electrocatalytic peak current (I_p) versus AA concentrations with linear regression equation of $y = 0.494x - 0.212$ with correlation coefficient of 0.9923 and the detection limit (3σ/slope, σ is a standard deviation) is found to be 20 nM. These results strongly suggest that the oxidation of AA can be good selective and sensitive at aniline AP -rGO/GCE.

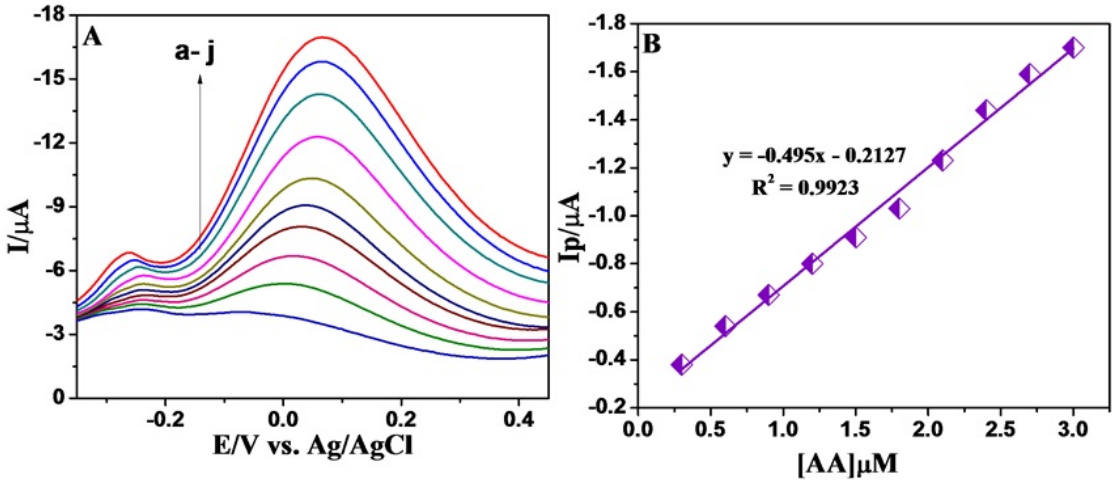

FIG. 5. A) DPV of AP-rGO/GCE in presence of AA at various concentrations (0.3–3.0 μM) in 0.1 M PBS (pH 7). B) Linear plot of concentration vs. peak current

3.4. Food sample analysis

In order to estimate the amount of AA present, fruit juice food samples were analyzed. Based on the repeated differential pulse voltammetric responses ($n = 5$) of the diluted analyte, samples were spiked with specified concentration of AA, measurements were made for determination of AA concentration in food samples. The results are listed in Table 1. The AP-rGO/GCE possessed reasonable selectivity and produced satisfactory recovery result with an average recovery of 100.3 % and the RSD was less than 5.3 %. The measured data of AA concentration was in good agreement with the results found in the titration measurements, suggesting the good accuracy and reliability.

TABLE 1. Determination of AA in fruit samples

Samples	Added (10^{-6} g/ml^{-1})	Found (10^{-6} g/ml^{-1})	Recovery (%)
Apple	0.50	1.024	99.3
Pineapple	0.70	1.208	100.6
Orange	1.50	2.133	101

4. Conclusion

A single step method was adapted to modified graphene sheet with aniline pentamer as electron transfer mediator. The surface modification of graphene was confirmed from FTIR and cyclic voltammetry studies. A simple differential pulse voltammetry method was developed to determine low concentration ranges of ascorbic acid using aniline pentamer grafted graphene sheet. Enhanced detection limits was achieved using this method. The present method can be utilized for the electrochemical detection of ascorbic acid in pharmaceutical and fruit samples.

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