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Short Communication

Biomolecules Behavior on a Surface of Boron Doped/un-doped Graphene Nanosheets

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Recently, carbon-doped nanomaterials attract attention due to their unconventional properties as doping completely changes their properties. Herein, we try to compare the electrochemical response of the prepared boron-doped graphene nanosheets and un-doped graphene nanosheets modified glassy carbon electrodes toward some interesting biomolecules like ascorbic acid, uric acid, and dopamine. There are significant differences of prepared doped and un-doped graphene nanosheets toward the mentioned biomolecules. The resistance of surfaces toward fouling was studied on uric acid oxidation product in the long term. In the long term stability of the prepared materials, there are no significant differences found. As electrochemical materials, the differences between prepared materials will affect their applications.

Keywords: Biomolecules; Boron Doped graphene nanosheets; Biosensors

1. INTRODUCTION

We have done this research as an extension of our work previously published in other journals [1-7]. Nanomaterials attracted the attention of scientists in the last years due to their unique properties

[8-11]. Graphene-based nanomaterials are wonderful materials that have a unique effect in material science, chemistry, physics, and industrial processes [12-15]. Pristine graphene (non-doped) behave a zero-overlap semimetal because its conduction and valence bands drop at the point K in the Brillouin zone [16]. So, graphene applications in many fields including semiconductors electronics are limited as a result of this small bandgap. Consequently, it is important to adapt to graphene's electronic properties and ultimately be able to expose an electronic bandgap. Magnetic, chemical, and electronic properties of a material can be efficiently altered by chemical doping [17]. Boron and nitrogen rather than any other dopants attract the attention of chemists because they have similar C-atom sizes and can encourage graphene conduction. So, the concept of doping remains a new branch to study. Boron doped graphene studies [18] are very rare comparing to nitrogen-doped graphene [19, 20]. The electrochemical behaviors of biomolecules on the surface of boron-doped graphene are little. Since the replacement of carbon atom by another hetero atom changes the electrochemical properties of doped material, we decide to investigate the electrochemical behavior of biomolecules like uric acid, ascorbic acid, and dopamine on the surface of boron-doped graphene nanosheets that will give attention toward the interaction between these biomolecules and boron-doped graphene nanosheets.

2. EXPERIMENTAL

2.1. Materials

Sulfuric acid (H_2SO_4), hydrochloric acid (HCl), and ethanol were supplied from PENTA while sodium nitrate, potassium permanganate, ascorbic acid, dopamine, uric acid, and boric acid were supplied from Sigma Aldrich. Graphite was supplied from China Steel Corporation, Taiwan. N,Ndimethylformamide (DMF, 99.5%) was purchased from Aladdin. Hydrogen peroxide was supplied from Merck Millipore, Germany.

2.2. Preparation of boron-doped graphene nanosheets

In a round-bottomed flask, mix 23 ml sulfuric acid with 0.5 g graphite powder and 0.5 g sodium nitrate and stir for 4.5 h in the ice bath to avoid temperature increasing and explosion. During that add about 3.1 g $KMnO_4$ slowly to the above mixture. Then at 35^o C stirring was continued for 2 hours. Next to that, the flask was cooled to 20^{o}C and 45 ml distilled H_2O was added gradually. In a silicone oil bath at 98^{o}C, the mixture was stirred for 2 h under reflux. The mixture was kept to cool in room temperature then, 100 ml distilled H_2O was added and the mixture stirred for 1 h. Then 11 ml 30% hydrogen peroxide added to the mixture and the stirring process was continued for an additional 1 h. Water/hydrochloric acid mix was used to wash it numerous times until clear then the powder was dried to form dark brown graphene oxide [21]. Then, at 500^{o}C 0.5 g of produced graphene oxide was heated in a tubular furnace in an argon atmosphere for half-hour to perform the thermal reduction. After cooling in room temperature the reduced graphene nanosheets (GNS) are formed. To add boron as a doping material boric acid was used as a boron source and ethanol was used as a solvent. In a beaker mix 1 g of graphene nanosheets with 1.5 g boric acid and 51 ml ethanol solvent. Then the solution was sonicated to homogenize the components. After that, the solution transferred to an autoclave and heated for 12h at 150°C. The autoclave was kept to cool spontaneously and the obtained boron-doped graphene sheets (BGNS) washed with deionized H_2O and dried for 24h at 60°C. The synthesized nanostructures were characterized using various techniques included scanning electron microscope (SEM), transmission electron microscope (TEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FT-IR).

2.3. Electrochemical measurements

To prepare the working electrode 0.5 mg of prepared boron-doped graphene nanosheets or graphene nanosheets were dissolved in DMF solvent and sonicated for 7 min. Then 1 ml of the suspension was distributed on a clean glassy carbon surface that polished using alumina. The DMF solvent was evaporated under a tungsten lamp to obtain a uniform distributed boron-doped graphene nanosheets layer on the glassy carbon surface. This prepared modified glassy carbon electrode acts as a working electrode while Pt is acting as a counter electrode and Ag/AgCl is acting as a reference electrode. The electrodes are immersed inside the electrolytic solution to form an electrochemical cell for cyclic voltammetry study. The electrochemical behavior of biomolecules uric acid, ascorbic acid, and dopamine was studied over glassy carbon (GC), un-doped graphene nanosheets modified glassy carbon (GNS) and boron-doped graphene nanosheets modified glassy carbon (BGNS) in phosphate buffer electrolytic solution (pH=7.1) at 100 mVs^{-1} scan rate and biomolecules concentration of 12 mM. To study the resistance to fouling of the GC, BGNC, and GNS surfaces, 20 successive cyclic voltammetry scans of uric acid were performed inside the phosphate buffer solution at the scan rate 100 mVs^{-1}.

3. RESULTS AND DISCUSSION

3.1. Characterization of doped and un-doped graphene nanosheets

The prepared graphene nanosheets and boron-doped graphene nanosheets were characterized using different techniques transmission electron microscope (TEM), scanning electron microscope (SEM), Fourier-transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD). The SEM and TEM images are presented in Fig.1 in which the morphology of thermally formed nanosheets and boron-doped nanosheets are clear.

The GNS appears in SEM image to have parallel nanosheets with a few nanometers between each other's while BGNS has morphology like a flower with the same structure of GNS. XRD and FT-IR spectroscopy of GNS and BGNS were presented in Fig.2. According to FT-IR of GNS, the principal bands appear at frequencies 1195 cm^{-1} that represents C-O stretching vibrations, 806 cm^{-1} that represents C-H out of plane bending vibration, and 882 cm^{-1} that represents C-H in plane bending vibration while the principal bands of BGNS appear at 1185 cm^{-1} that represents B-C bending vibrations, 1121 cm^{-1} represents B-C stretching vibrations, 1065 cm^{-1} B-O stretching vibrations, 888 cm^{-1} represents C-H in plane bending vibrations, and 788 cm^{-1} represents epoxy.

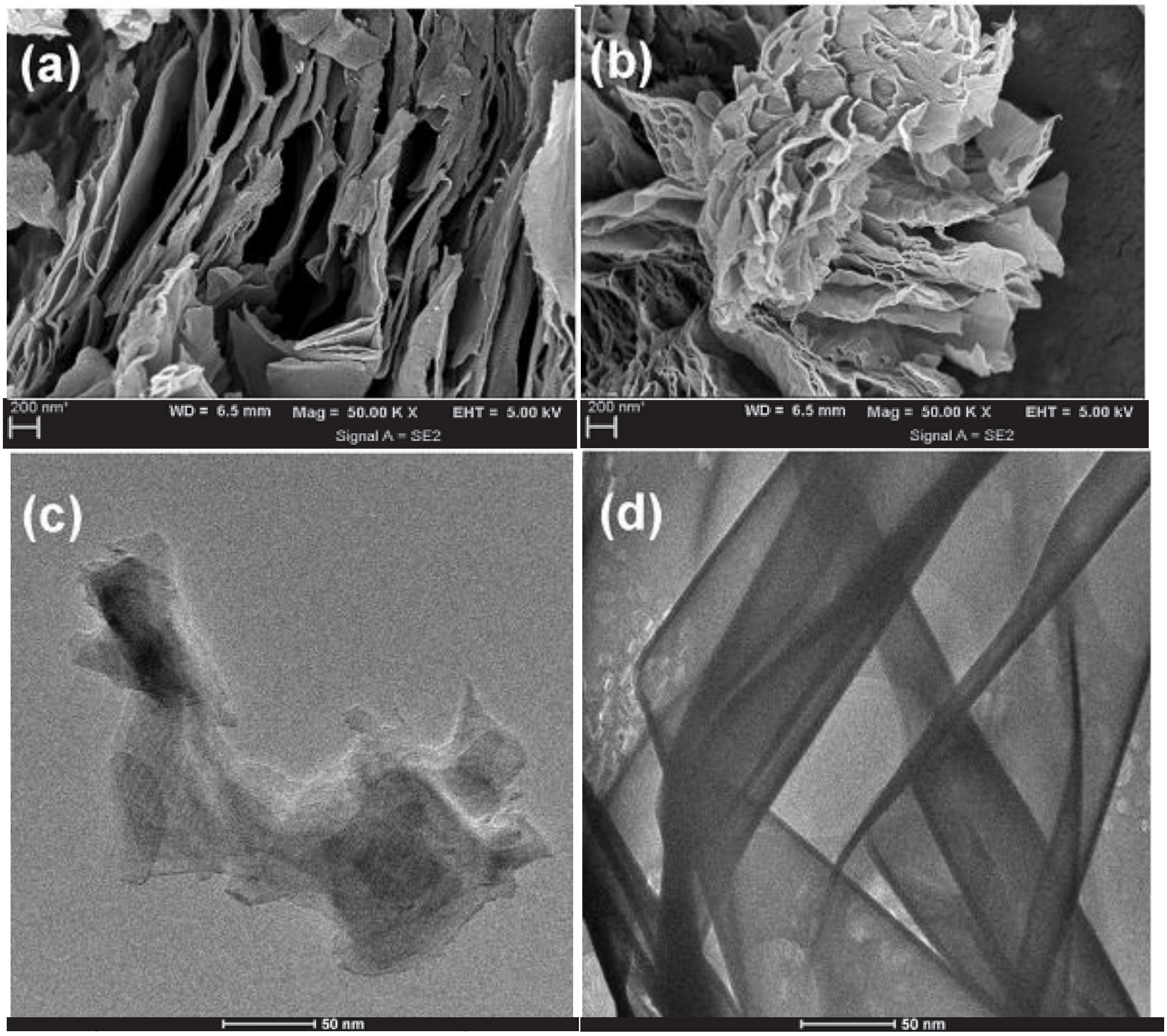
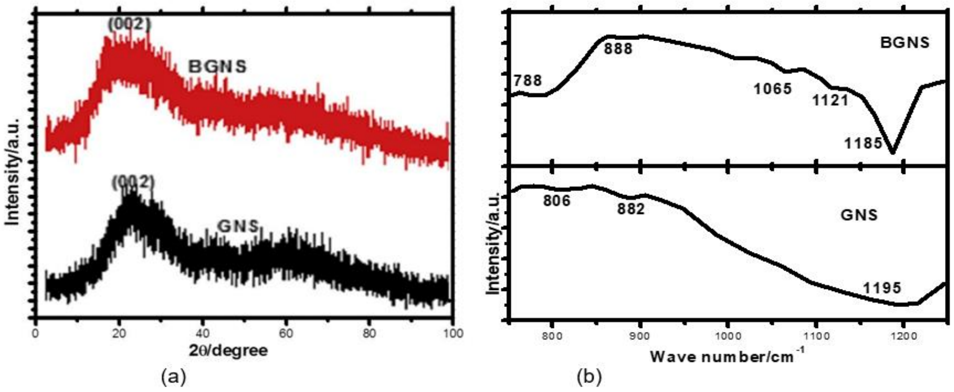


Figure 1. SEM image of GNS (a), BGNS (b) and TEM image of GNS (a), BGNS (b)



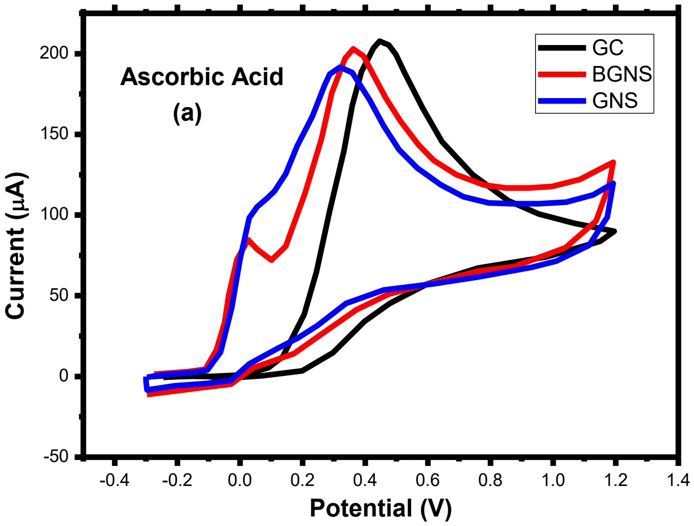
(a) (b)

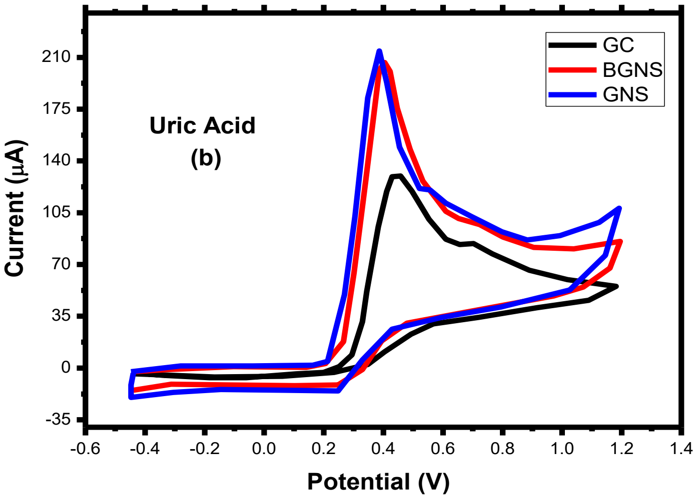
Figure 2. XRD of GNS and BGNS (a), and FT-IR of GNS and BGNS (b)

Boron doping inside graphene multilayers is indicated by the frequency of B-C stretching. According to XRD in Fig.2, the amorphous nature is indicated due to the appearance of strong broad bands at 24.1 for BGNS and 26 for GNS which corresponding to the plane 002. The basal spacing d_{002} is found to be 0.340 nm for GNS and 0.335 nm for BGNS which indicating a higher inter-planar spacing for BGNS resulted from the doping of boron that is bigger in size than carbon between graphene multilayers.

3.2. Electrochemical behavior of biomolecules

The electrochemical behaviors of biomolecules ascorbic acid (vitamin C), uric acid and dopamine were determined using the cyclic voltammetry technique and their voltammograms on the surfaces of glassy carbon (GC), un-doped graphene nanosheets modified glassy carbon (GNS) and boron-doped graphene nanosheets modified glassy carbon in phosphate buffer electrolytic solution (pH=7.1) are shown in Fig.3. For ascorbic acid, there are two anodic peaks on the surface of modified glassy carbon the first at the potential of 31 mV while the second peak at 357 mV in the case of BGNS and 324 mV in case of GNS. Ascorbic acid has only one anodic peak appear at potential 442 mV. The difference between the two cases is the presence of graphene on the surface of modified glassy carbon which makes electrostatic attraction with ascorbic acid and speeds up the charge transfer rate between graphene and ascorbic acid and causes the appearance of an additional anodic peak. Different heights of the first peaks are indicating different adsorption power between ascorbic acid and different graphene materials. Peak height is higher in the case of GNS than that of BGNS indicating well-stacked crystals due to the absence of boron atom between graphene nanosheets that is bigger in size than the carbon atom. According to the voltammograms of uric acid on the surfaces of three electrodes, the peak potentials in the case of GNS and BGNS are overlapped indicating that the morphology is not essential in the uric acid oxidation process. Uric acid anodic peaks appear at 454 mV on GC surface, 404 mV on BGNS, and 392 mV on the GNS surface.





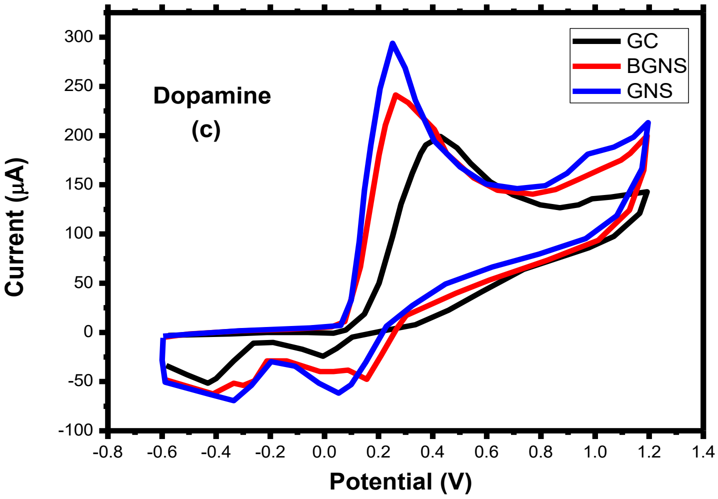


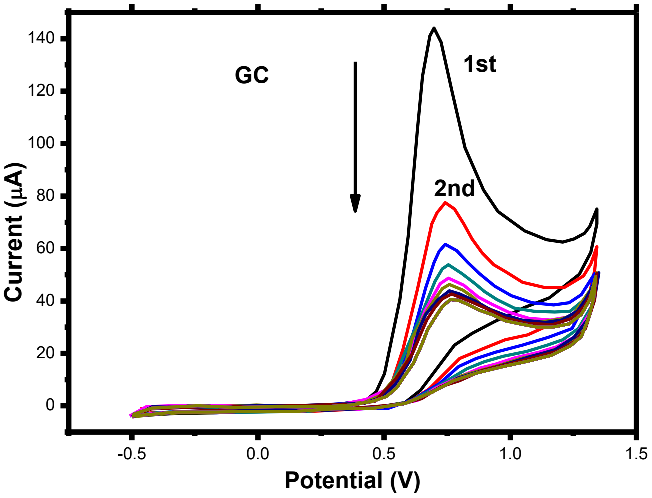
Figure 3. Cyclic voltammograms of 12mM ascorbic acid (a), 12mM uric acid (b), and 12 mM dopamine (c) at 100 mVs^{-1} scan rate on the surface of GC, GNS, and BGNS.

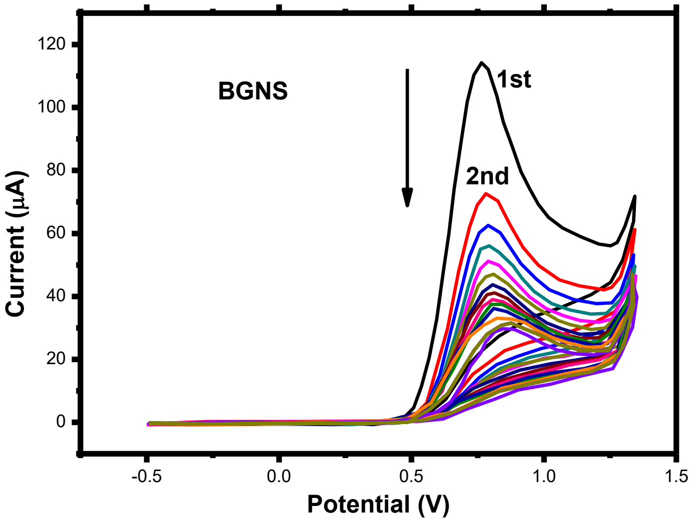
The difference in oxidation current between three-electrode surfaces is explained to depend on the surface area so the peak current in the case of modified glassy carbon is higher than that of glassy carbon [22]. Also, the peak current differences may explain on the basis of graphene defect sites [23] or according to the presence of impurities that enhance the oxidation of the analyte [24-27]. The last examined biomolecule on the surface of GC, GNS, and BGNS is the dopamine that exist two cathodic peaks and one anodic peak as shown in Fig.3. The anodic peak potential appears at the potential of 408 mV for GC, 273 mV for BGNS, and 258 mV for GNS. The two cathodic peaks appear at 5 mV and 425 mV for GC, 64 mV and, 343 mV for GNS and 200 mV and 600 mV for BGNS. The same behavior of dopamine on the three surfaces indicates that the response of graphene toward dopamine doesn't originate from the GC surface.

3.3. Study of long term stability on uric acid

In several electrochemical studies, the fouling of the electrode surface is considered to be a severe problem that has a bad effect on the sensor and its reliability, reproducibility, detection limit, and sensitivity and therefore reduces the performance of the sensor in different applications [28, 29]. Fouling of the electrode surface is meaning the adsorption of unwanted material over the electrode [30] that hinders the electron transfer process that stimulates an electrochemical response [31, 32].

In order to study the resistance to fouling of the GC, BGNC, and GNS surfaces, 20 successive cyclic voltammetry scans of uric acid were performed inside the phosphate buffer solution at 100 mVs^{-1} scan rate and cyclic voltammograms were introduced in Fig.4. The passivation resistance by oxidation product of uric acid was studied on the three surfaces and compared in Fig.4. The relation between the number of cycles and normalized current that gets from cyclic voltammograms was drawn and shown in Fig.4. In this plot, the higher the normalized current at cycle end is the higher resistance. According to scans, it is noticed that BGNS modified glassy carbon has better resistance than GNS modified glassy carbon to passivation by the oxidation product of uric acid.





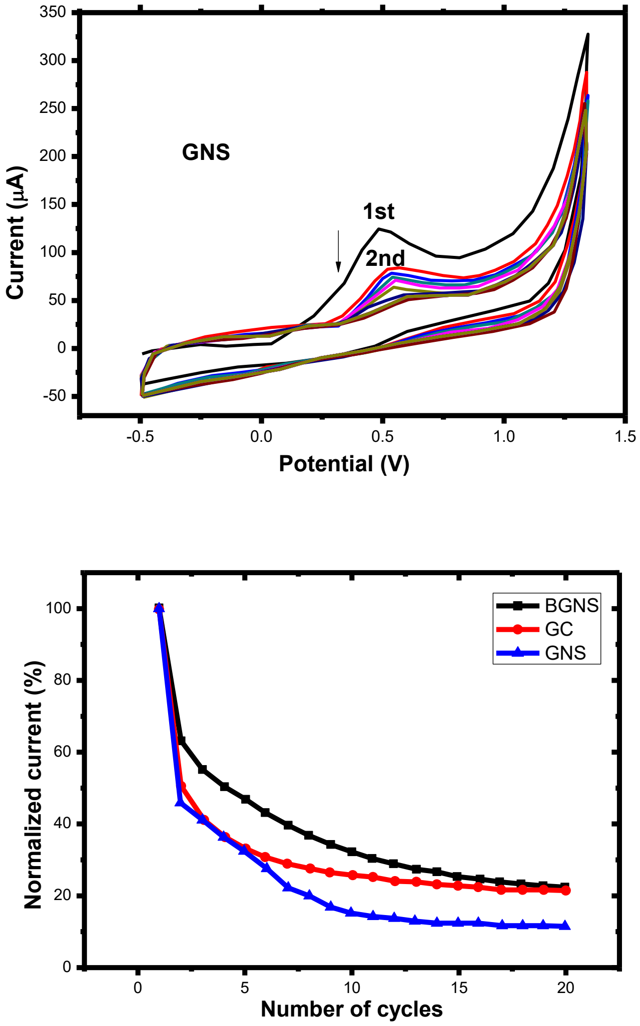


Figure 4. Series of 20 successive cyclic voltammograms of 12 mM uric acid on surfaces of GC, BGNS, and GNS in 55 mM phosphate buffer at 100 mVs^{-1} scan rate

For the three-electrode surfaces, the peak current of the 2nd cycle was decreased to 53% with GC, 47% with GNS, and 64% with BGNS of the peak current of the 1st cycle. According to the 1st and 2nd cycle's peak current, the resistance to fouling has the order: BGNS> GC> GNS. So the best resistance is showed by BGNS modified electrode with no long term significant differences.

The BGNS better resistance than GNS indicates the advantages of graphene nanosheets doping with boron. The current results approve the unique property of graphene electrodes that is the ability to resist fouling. The results approve the reports indicated that modification of electrodes with nanomaterials is the best strategy to increase the fouling resistance of electrode [33-34] due to their large surface area and their anti-fouling properties [35]. The fouling resistance of BGNS modified glassy carbon electrode arises from extensive structure resulted from doping and the low surface of polar functional groups that prevent the adsorption of fouling agents [36]. The un-doped GNS glassy carbon modified electrode has less fouling resistance than doped one due to its complete structure that enhances the adsorption of fouling agents [36]. Many studies have been reported the use of graphene-based nanomaterials to modify bare electrodes that reduce the fouling of electrode surfaces [37-39]. Thus, the electrochemical behavior of studied biomolecules over this modified electrode as well as anti-fouling properties make it promising for the application as a biomolecules sensor in the real samples that must be studied in the future.

4. CONCLUSION

The graphite powder was used for the synthesis of graphene oxide powder that was used as a raw material for the synthesis of graphene nanosheets via thermal reduction. Then the graphene nanosheets prepared were doped with boron via the solvothermal method by using boric acid as boron source and ethanol as solvent. The prepared graphene nanosheets and boron-doped graphene nanosheets were characterized using different techniques like XRD, SEM, TEM, and FT-IR. Doped and un-doped graphene nanosheets were used to modify the glassy carbon electrode. The electrochemical behavior of different biomolecules ascorbic acid, uric acid, and dopamine was studied over three electrodes (GC, BGNS, and GNS) surfaces. The higher anodic potential was noticed with boron-doped graphene nanosheets glassy carbon modified electrode for biomolecules. The results support the use of boron-doped graphene nanosheets for chemical and biochemical sensing applications.

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