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Improved Oxidation of Graphite by Optimizing the Oxidation Time and Concentration of Oxidizing Agent for Cellular Sensing

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Abstract— **In the present work, graphite has been oxidized by a modified oxidation method. Structural and optical properties of as prepared graphite oxide (GO) have been investigated for different oxidation time and for different concentrations of oxidizing agent ($KMnO_4$). GO samples were prepared by varying the oxidation time (6, 14 and 18 hours) and graphite: $KMnO_4$ wt.% ratios (1:3, 1:6 and 1:9). As-synthesized GO was characterized by XRD, FTIR, Zeta potential and UV-vis spectroscopic techniques. XRD results indicated oxidation of graphite through a shift in the position of (002) diffraction peak of graphite from 26° to 9 − 10° for GO. We observed maximum *d*-spacing (0.932 nm) for GO sample prepared for 6hrs with 1:6 wt.% of graphite: $KMnO_4$. GO sample prepared for 6 h showed excellent water dispersibility with zeta potential value of -43.35 mV. Present study suggests that GO sample prepared for 6 h oxidation time with 1:6 wt.% of graphite:$KMnO_4$ gives maximum exfoliation of graphite and is rich of oxygen functional groups. In comparison to the existing oxidation methods, our modified method could be suitable for large scale preparation of highly oxygen functionalized GO in a short time. As-prepared GO was further used for detection of cancer cells through electrochemical approach.**

Index Terms- **Graphite Oxide (GO), Oxidation, Cellular sensing, Electrochemistry**

I. INTRODUCTION

Graphite oxide (GO) was first prepared by Brodie in 1859 by mixing graphite, potassium chlorate and fuming nitric acid [1]. This process was found to be time consuming and hazardous. Further after 40 years, in 1898, Staudenmaier introduced a process which involved the addition of potassium chlorate to a mixture of graphite, concentrated sulphuric acid (H_2SO_4) and concentrated nitric acid (HNO_3) and the process was continued over a period of one week [2]. It was a hazardous method that evolved toxic gases such as chlorine dioxide, carbon dioxide and nitrogen during the reaction. In 1958, Hummers introduced a method for oxidation of graphite using $KMnO_4$, H_2SO_4 and HNO_3 for GO synthesis [3]. In this method, toxic nitrogen gas evolved during the synthesis. In 1995, another method was introduced in which oxidation time was varied from 24 − 240 h and the results were obtained in 120 h, which is very time consuming [4]. In 2010, GO was synthesized by the addition of H_2SO_4 and H_3PO_4 to the mixture of graphite and $KMnO_4$ keeping oxidation time as 12 h [5]. It has an advantage of being used for large scale production as it does not evolve any toxic gases but again it is a time consuming process. In a recent study, GO has been synthesized by a modified Hummer's method with few other modifications but still the problem of evolution of toxic gases remains the same [6]. In the present work we tried to establish a process for oxidation of graphite which is an easy, less time consuming method for synthesis of highly oxidized GO and also, does not produces any toxic gases. We studied the structural, and optical properties of as-prepared GO by varying the oxidation time and graphite:$KMnO_4$ wt.% ratio.

II. EXPERIMENTAL DETAILS

Pure graphite flakes (Sigma-Aldrich, 45 µm, 99.99%) were used as the base material for the preparation of GO. Firstly, graphite (3 g) and $KMnO_4$ (18 g) were mixed together in a round bottom flask. Then keeping temperature as 0˚C, 9:1 ratio of concentrated H_2SO_4 and H_3PO_4 (360:40 ml) was added to the above mixture which leads to exothermic reaction thereby rise of temperature up to 40 − 45 ºC. Further reaction was continued and stirred at 50˚C for 1 h. The reaction was cooled to room temperature and then transferred to an ice bath, where 30 vol.% of H_2O_2 solution was added into the mixture. Final product was centrifuged (at 4000 rpm for 4 h) to obtain the solid material. This was further washed in succession with 200 ml of water, 200 ml of 30% HCl and 200 ml of ethanol. It was finally vacuum dried at room temperature.

For the synthesis of other samples, keeping the oxidation time constant (6 h), the concentration of graphite:$KMnO_4$ was varied. Following the same experimental procedure as above, three samples were prepared with different graphite:$KMnO_4$ wt.% ratios of 1:3, 1:6 and 1:9. As-prepared GO samples were characterized by different characterization techniques and results were compared with the available literature. Further the as-synthesized functionalized GO was used for the detection of two different cancer cells e.g. cervical (HeLa) and breast cancer (MCF-7) cells. The GO was incubated with the cancer cells (separately for each cells) and then drop-casted on the glassy carbon electrode to check the electrochemical sensing behavior of GO.

III. RESULTS AND DISCUSSION

Fig.1 (a) represents the XRD patterns of GO samples prepared for different oxidation times of 18, 14 and 6 h. The

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peak shift in the XRD patterns confirmed the graphite oxidation to a great extent [5, 7]. It infers that sample with 6 h oxidation time is very well exfoliated and hence more functional groups are attached on the basal plane [8]. Fig.1 (b) represents the FTIR spectra of GO samples (6, 14 and 18 h) recorded using KBr as reference material. For sample with 6 h oxidation time, O − H stretching vibrations (~3450 cm^{-1}), C − H stretching vibration (~2920 cm^{-1}), CO$_2$ stretching (~2349 cm^{-1} for asymmetrical C−O bond), ketone group i.e. C=O stretching vibrations (~1720 cm^{-1}), O − H bending (~1637 cm^{-1}), carbonyl groups (C-O stretching, ~1100 cm^{-1} and ~1420 cm^{-1}), epoxy group (C−O−C stretching, ~1220 cm^{-1}) were observed in accordance with the literature [8, 9, 10]. In the sample with 14 h of oxidation time, all the functional groups were seen but the intensity of C − O stretching was slightly lower than the sample with 18 and 6 h oxidation time. The intensity of C − H stretching is high as compared to sample with 6 h oxidation time and also the intensity of other peaks are not prominent. Similarly, almost all the functional groups present in sample with 6 h oxidation time were obtained for sample with 18 h oxidation time.

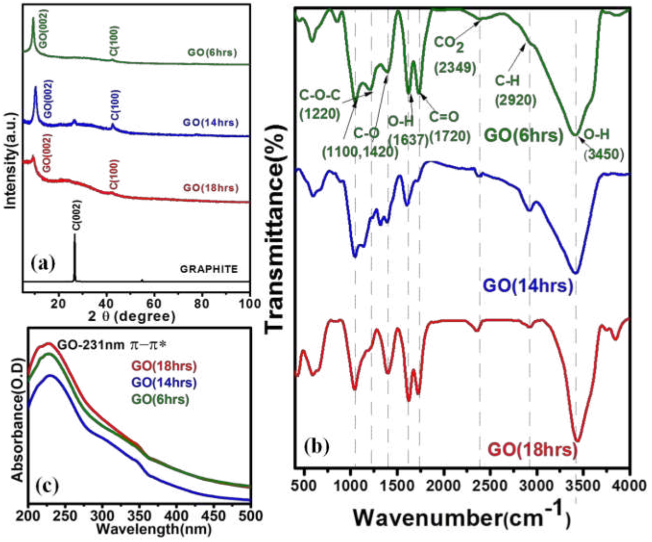

Fig. 1 (a) XRD pattern, (b) FTIR spectra and (c) UV-Vis spectra of different GO samples synthesized for different oxidation time.

The UV–Vis spectra (Fig.1 (c)) for GO samples showed absorption peak at ~210 −240 nm, which is attributed to the π→π* transition of the C−C bonds. This peak corresponds to the confinement of carbon rings on the basal plane. It gives the amount of energy required by the electrons for π→π* transitions [10, 11].

The zeta potential gives the information about the dispersion stability of the material. The zeta potential obtained for 6, 14 and 18 h samples were -43.5, -31.87 and -45.70 mV, respectively. From the results we concluded that the sample with 6 h oxidation time is rich of oxygen functional groups and is having the best aqueous dispersibility among all GO samples.

Further, to study the effect of the concentration of oxidizing agent on properties of GO, three different GO samples were prepared with variation in the concentration of graphite and KMnO$_4$ ratio of 1:3, 1:6 and 1:9 (in wt.%) keeping oxidation time as constant (6 h).

The XRD patterns of as-synthesized GO samples are shown in Fig. 2(a). Results are indicated that the *d*-spacing for GO sample prepared with 1:6 ratio of graphite:KMnO$_4$ is 0.932 nm which is higher as compared with other samples e.g. 0.908 nm for 1:3 ratio and 0.863 nm for 1:6 ratio.

Fig. 2(b) presents the FTIR spectra of GO samples prepared from three different graphite:KMnO$_4$ weight ratios (1:3, 1:6 and 1:9 respectively). For the sample with 1:6 graphite:KMnO$_4$, O−H stretching vibrations (~3450 cm^{-1}), C−H stretching vibration (~2920 cm^{-1}), asymmetrical C − O stretching (~2349 cm^{-1}), C=O stretching (~1720 cm^{-1}), O−H bending (~1637 cm^{-1}), carbonyl groups that is C−O stretching (~1100 cm^{-1} and ~1420 cm^{-1}), epoxy group that is C−O−C stretching (~1220 cm^{-1}) were obtained. In sample with 1:3 ratio of graphite:KMnO$_4$, all the above functional groups were obtained except CO$_2$ stretching vibration. Though the intensity of others groups were high, the intensity of C−H stretching (~2920 cm-1) and O-H stretching are weak due to the small amount of oxidizing agent used during synthesis. Similarly, for the GO sample with 1:9 ratio, all major oxygen functional groups were obtained with significant intensity (carbonyl and ketone), except the intensity of C-H stretching (2920 cm^{-1}) and of O-H stretching (~3450 cm^{-1}). In GO sample with 1:9 graphite:KMnO$_4$, amount of oxidizing agent used was more as compared with GO sample with 1:3 and 1:6 ratios. Due to this some functional groups were highly enhanced e.g. carbonyl, ketone and hydroxyl groups.

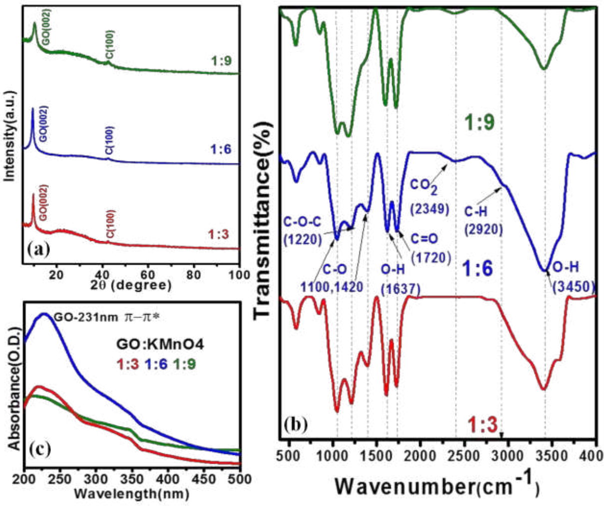

Fig. 2 (a) XRD patterns, (b) FTIR spectra and (c) UV-Vis spectra of different GO samples synthesized for different concentration of oxidizing agent with graphite:KMnO$_4$ ratio as 1:3, 1:6 and 1:9 wt.%.

The UV-absorption peaks obtained for all the three samples (1:3, 1:6 and 1:9 ratios) are in the range of 228−231 nm which is shown Fig. 2(c) and the results are in well-accordance with the available reports [12, 13, 14]. The zeta potential values of the as-synthesized GO samples with 1:3, 1:6 and 1:9 wt. ratio of graphite:KMnO$_4$ are -26.74, -43.35 and -32.46 mV, respectively. Here 1:6 gives the excellent dispersibility and highly functionalized GO in comparision to the other GO samples (1:3 and 1:9 wt. ratio).

The cyclic voltammetry (CV) was performed to examine the electrochemical behavior of GO (prepared for 6 h with 1:6

graphite:$KMnO_4$ wt.%) which is shown in Fig. 3(a). Furthermore, the as-synthesized GO was used for the detection of MCF-7 and HeLa cells as shown in Fig. 3(b) and Fig. 3(c), respectively. CV was carried out with 0.1M PBS and 0.1M KCl as electrolyte at 50 mV/s scan rate. The cathodic peaks for GO were obtained at -0.3 and 0.5V and the current obtained was of 2μA. For MCF-7 cells, anodic and cathodic the above peaks were shifted to 0.6 and -0.5 V respectively while for HeLa cells, these are observed at 0.4 and -0.4 V. Such a peak-shifting in the CV of GO upon incubation with cancer cells by -0.2 V (cathodic peak) and 0.1 V (anodic peak) for MCF-7 cells and by -0.1 V (cathodic peak) and 0.1 V (anodic peak) for HeLa cells indicated the possibility using of GO as a potential cellular sensing material. The current was found to increase from 2 μA to 10 μA due to the binding of GO with the cancer cells.

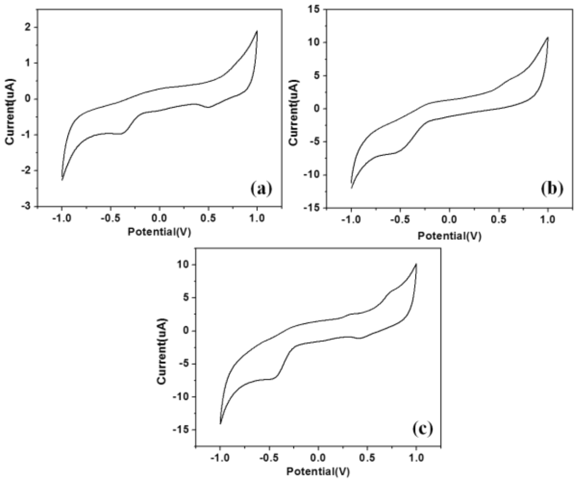

Fig. 3 Cyclic voltammograms of (a) GO, (b) GO/MCF-7 cells and (c) GO/HeLa cells in the potential range -1.0 to 1.0V versus Ag/AgCl at a scan rate of 50 mV/s.

IV. CONCLUSION

In summary, GO prepared by this improved method has various advantages over other existing methods. We observed the sample with 6 h of oxidation time gives the best aqueous dispersibility and also showed major oxygen functional groups as compared with the other GO samples (prepared for 18 and 14 h of oxidation time). Further keeping fixed 6 h of oxidation time, the sample with 1:6 wt. ratio in term of graphite:$KMnO_4$ shows better oxygen functionalization and aqueous dispersion than the samples with 1:3 and 1:9 wt. ratio. From this, we conclude that GO synthesis for 6 h of oxidation time with 1:6 wt. ratio of graphite:$KMnO_4$ gives the excellent aqueous dispersibility among all the other GO samples. The as-prepared GO was further used for the detection of cancer cells. On the basis of shifting in cathodic and anodic peak positions during CV measurements, we conclude that the optimized GO material could be used for the detection of different cancer cells.

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