

Covalent Functionalization of Graphene Oxide with Cisplatin

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The objective of this study was to obtain graphene oxide starting from graphite and to covalently functionalize this nanomaterial with Cisplatin. The presence of the drug was pointed out using different methods like FTIR Spectroscopy, X-Ray Photoelectron Spectroscopy (XPS), Thermogravimetric analysis (TGA), RAMAN Spectroscopy, X-Ray Diffraction and Scanning electron microscopy (SEM).

Keyword: Graphene oxide, covalent activation, Cisplatin

For many years cancer disease has been a major problem for medical science. Recently many researchers have been focused on discovery of new materials that can control and stop the evolution of cancer diseases. Thus for improving drug delivery and decreasing drug's side effects, drug-loaded vehicle were developed to overcome the difficulties [1- 3].

Graphene and graphene oxide (GO) have attracted much interest in recent years because of their important properties like electronic, mechanical, thermal, optoelectronic, magnetic and biological [4-10].

The applications of graphene for biosensors [11], nanocomposites [12], electronics [13], have been extensively studied. Also its potential for biomedical applications like drug loading and release has also been studied and reported by many researchers [14-15].

Cisplatin or cis-diammineplatinum(II) dichloride (CDDP) is an antitumor chemotherapeutic agent that is widely used in the treatment of many cancers [16-17]. However, to minimize the side effects of cisplatin a targeted drug delivery system or drug controlled release system is considered as one of the most effective approaches.

Y. Ni et al. [18] reported an investigation of interaction between GO and a medicinal drug (10-hydroxy camptothecin (HCPT)). They demonstrated that HCPT was easily loaded on GO via the $\pi - \pi$ stacking interaction, and the delivery of HCPT was improved in the presence of GO.

D. Depan et al. [19] described a method of non-covalent type of functionalization by attaching doxorubicin (an antitumor medicinal drug (DOX)) to graphene oxide via $\pi - \pi$ stacking interaction, followed by encapsulation of graphene oxide with folic acid conjugated chitosan.

The aim of our study was to obtain GO from graphite via chemical oxidation and to covalently functionalize the obtained GO with CDDP, using an activating system based on 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and N-Hydroxysuccinimide (NHS).

Experimental part

Materials and methods

Graphite powder ($M_w = 12.01$ g/mol) was purchased from Acros Organics. Oxidation process was done using H_2SO_4 95-98%, H_2O_2 30%, HCl 37% and $KMnO_4$ which were received from Sigma Aldrich.

Chloroacetic acid ($ClCH_2COOH$), NaOH and dialysis bag having 12000 Da purchased from Sigma Aldrich were

employed to transform the hydroxyl and epoxy groups from the material surface to carboxylic ones.

Activation process was made using EDC, NHS and Cisplatin purchased from Sigma Aldrich.

PBS (phosphate buffer solution) having $pH=5.5$ was obtained from 2 solutions. First solution contains 13.61 g potassium dihydrogen phosphate, purchased from Sigma Aldrich, diluted in 1000 mL flask containing distilled water. Second solution includes 35.81 g disodium hydrogen phosphate, received from Sigma Aldrich, diluted in 1000 mL flask containing distilled water. To obtain PBS with $pH=5.5$, 96.4 ml of solution I and 3.6 mL of solution II were mixed.

Methods

Oxidation of graphite

The oxidation of graphite was made using a modified Hummers method [20] presented by G.I. Titelman et al [21]. Briefly 200 mL H_2SO_4 and 10 g of graphite powder were placed in an iced bath at 0 °C. After mixing for 30 min, 1.5 g of $KMnO_4$ was added in small portions to keep the temperature less than 10 °C. It was observed the change of the color in dark blue. After other 30 min, 30 g of $KMnO_4$ were added in small portions to keep temperature less than 20°C. After the $KMnO_4$ was completely added the temperature was increased to 35 °C and kept 30 min. As the reaction progressed, the suspension became brownish in color and pasty. At the end 450 mL of distilled water were added slowly to avoid effervescence. The temperature increases to 95 °C after the water was added. The diluted suspension exhibiting a brown color was maintained at this temperature for 15 min. The suspension was then further treated with a mixture of 35 ml hydrogen peroxide (30%) and 265 mL of distilled water to reduce the residual permanganate. Upon treatment with the H_2O_2 , the suspension turned yellow. The warm suspension was filtered and washed 3 times with 500 mL of a 3% HCl solution at 45 °C. After this the filter cake was dispersed in 4000 mL distilled cold water and separated by centrifugation (20 min at 4000 rpm). The obtained suspension was dispersed in 2000 mL distilled water and centrifuged in the same conditions. The obtained graphite oxide was dried at 45 °C and the obtained films were milled in a ball mill at 400 rpm.

To obtain graphene oxide (GO) 100 mg of the graphite oxide previously obtained were sonicated in 100 mL

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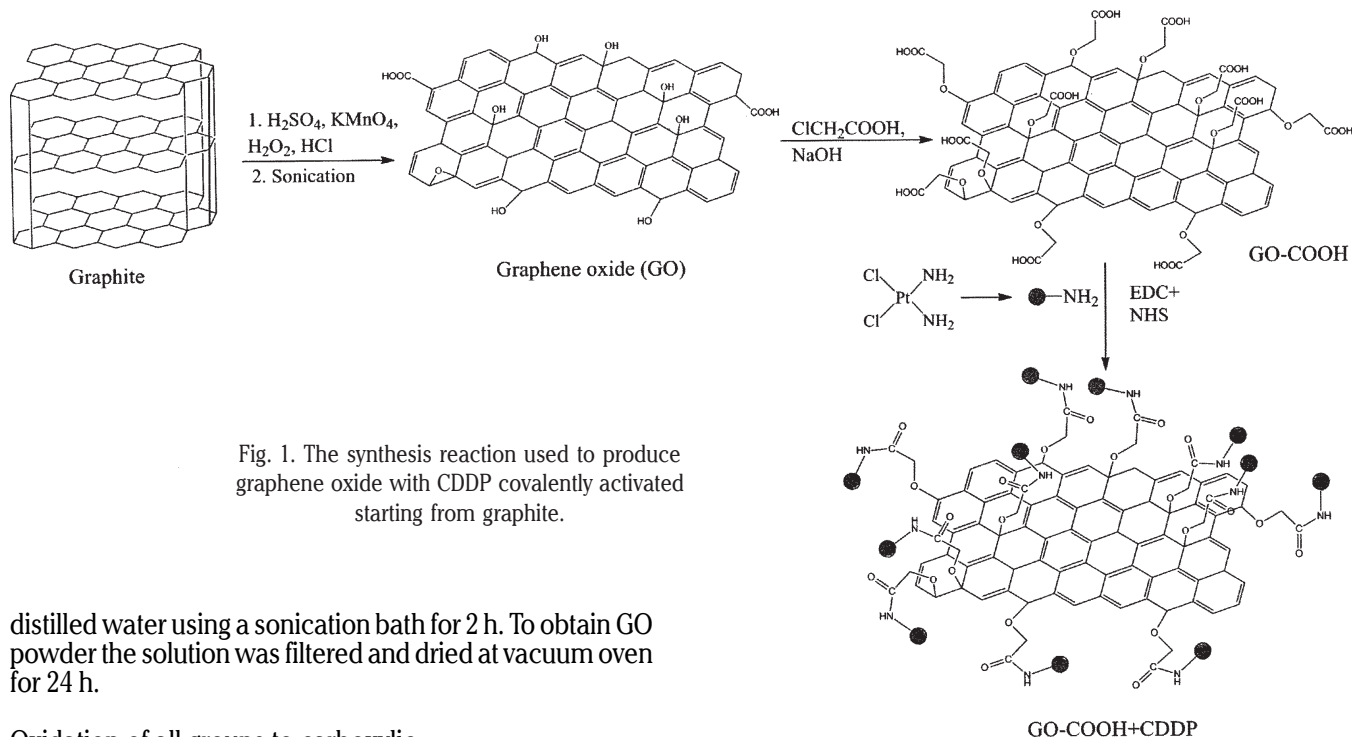


Fig. 1. The synthesis reaction used to produce graphene oxide with CDDP covalently activated starting from graphite.

distilled water using a sonication bath for 2 h. To obtain GO powder the solution was filtered and dried at vacuum oven for 24 h.

Oxidation of all groups to carboxylic

The obtained GO surface exhibits hydroxyl, epoxy and carboxyl groups. To convert the hydroxyl and epoxy groups to carboxyl, 50 mg of GO were dispersed in 25 mL distilled water. Then 2.5 g of ClCH_2COOH and 3.2 g of NaOH were added. The suspension was sonicated for 3 h at room temperature. Further the suspension was neutralized with 2.7 mL HCl. The suspension was filtered and washed with distilled water. The obtained filter cake was dispersed in 25 mL distilled water and immersed in a dialysis bag to remove the residual ions from NaOH. The dialysis bag was introduced in a beaker with 75 mL distilled water. Then the solution from dialysis bag was filtered, washed with distilled water and dried in vacuum oven [22].

Activation of GO-COOH with Cisplatin

To activate the GO having COOH groups on the surface with CDDP, 20 mg of EDC were dissolved in 2 mL of PBS with $\text{pH}=5.5$. Then 20 mg of GO-COOH and 60 mg of NHS were added. The suspension was sonicated for 30 min at room temperature. Further 20 mg of CDDP were added and the suspension was sonicated for another 90 min at room temperature in the dark. Finally the suspension was filtered, washed with PBS 5.5 and dried at vacuum oven for 48 h. All the reactions employed are shown in figure 1.

Advanced characterization

FTIR spectra were registered on an Bruker Vertex 70 equipment in $400 \div 4000 \text{ cm}^{-1}$ range with 4 cm^{-1} resolution and 32 scans. The samples were analyzed in KBr pellets.

Raman spectra of all the materials were recorded on a DXR Raman Microscope (Thermo Scientific) by 532 nm laser line. The 10x objective was used to focus the Raman microscope.

Thermogravimetry analysis (TGA) of the samples was done on Q500 TA equipment, using nitrogen atmosphere from 20 to 900 °C with 10 °C/min heating rate.

The X-ray photoelectron spectroscopy (XPS) spectra were recorded on a Thermo Scientific K-Alpha equipment, fully integrated, with an aluminum anode monochromatic source. Survey scans (0-1200 eV) were performed to identify constitutive elements.

The XRD measurements have been performed on a BRUKER D8 ADVANCE type X-ray diffractometer, in focusing

geometry, with a vertical theta–theta goniometer and horizontal sample carrier.

Scanning electron micrographs were obtained using a Zeiss EVO 50 SEM having LaB6 cathode with Bruker EDX system.

Results and discussion

XPS Analysis

The XPS analysis was employed to analyze the chemical composition of graphite, graphene oxide, GO-COOH and GO-COOH covalently activated with CDDP. Thus after the oxidation process is completed the content of O 1s increased from 2.7% to 34.4 % demonstrating that oxidation process has been successfully carried out [6, 23]. The presence of Na 1s in XPS spectrum of GO-COOH is explained by the use of NaOH to the transformation reaction of hydroxyl and epoxy groups in carboxylic ones. Also the presence of K 2s and S 2p is explained by the use of the PBS at activation reaction (table 1). The XPS spectrum of GO-COOH+CDDP shows the presence of Pt 4f indicating that CDDP was attached to the GO-COOH surface (fig. 2.) [24]

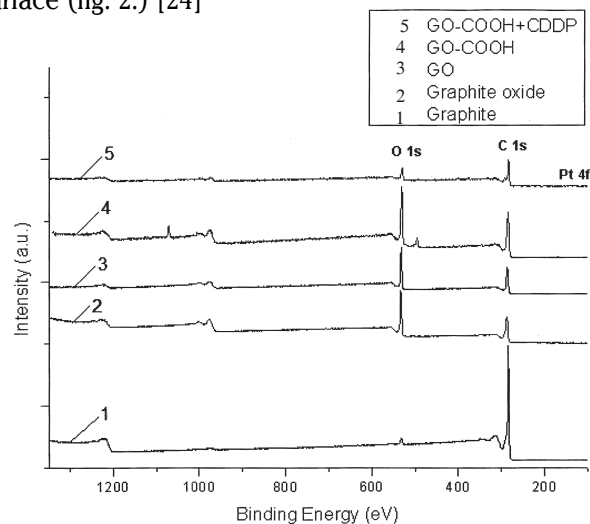


Fig. 2. XPS spectra of Graphite, GO, GO-COOH and GO-COOH activated with CDDP

Samples	Graphite	GO	GO-COOH	GO-COOH+CDDP
C 1s	97.3	65.5	71.1	73.6
O 1s	2.7	34.5	25.4	22.4
Na 1s	0	0	3.5	0
Pt 4f	0	0	0	0.3
S 2p	0	0	0	1.3
K 2s	0	0	0	2.4

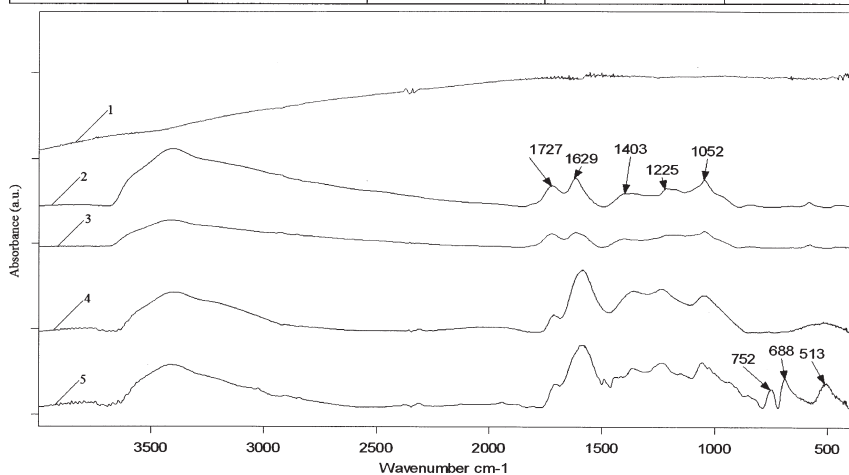


Fig. 3. FT-IR spectra for (1) Graphite, (2) Graphite oxide, (3) Graphene oxide (GO), (4) GO-COOH and (5) GO-COOH+CDDP.

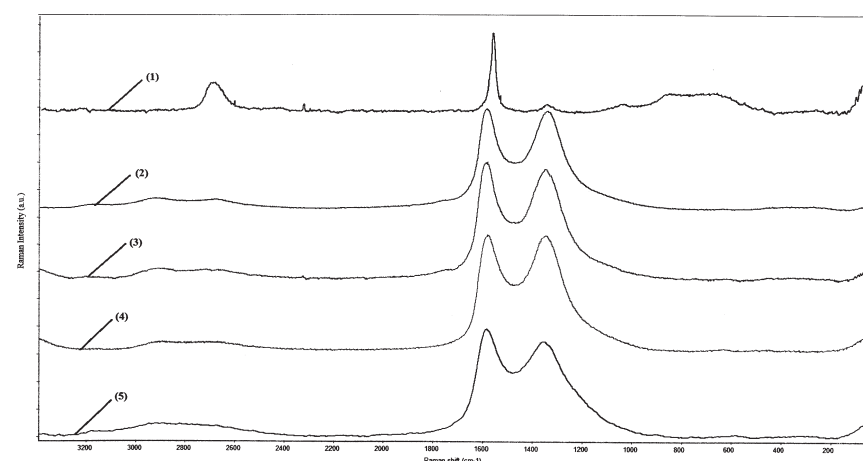


Fig. 4. RAMAN spectra for (1) Graphite, (2) Graphite oxide, (3) Graphene oxide (GO), (4) GO-COOH and (5) GO-COOH+CDDP.

FT-IR Analysis

The FT-IR spectra of graphite, graphite oxide, GO, GO-COOH and GO-COOH+CDDP are presented in figure 3. The large absorption from 3450 cm^{-1} is assigned to the -OH stretching vibrations. The absorption peaks from 1727 and 1629 cm^{-1} can be assigned to C=O stretching vibrations of carboxylic functional groups. The two absorption peaks from 1225 cm^{-1} and 1052 cm^{-1} are assigned the C-O stretching vibrations. The peak centered at 1403 cm^{-1} is attributed to the deformation vibration of C-OH bonds [19, 26]. From spectrum (5) the absorption peaks at 752 cm^{-1} and 688 cm^{-1} are assigned to deformation vibrations of NH_2 groups and the peak from 513 cm^{-1} is attributed to stretching vibrations of C-NH-Pt groups [26].

This leads to the conclusion that not all the -NH_2 groups from CDDP have reacted with carboxyl groups from GO-COOH and also that the covalently attachment of CDDP on GO-COOH surface was successfully done.

RAMAN spectroscopy

The Raman spectrum of graphite (fig. 4) shows characteristic peaks at 1569 cm^{-1} and 1345 cm^{-1}

corresponding to G and D band respectively. The intensity of G band is higher than for D band. After the oxidation process the D band increases due to the functionalization of graphite surface with carboxylic, epoxy and hydroxyl groups and the G' band or 2D band from 2700 cm^{-1} disappears due to the extreme oxidation conditions. The D band slightly decreases after sonication because of the graphite oxide dispersion (spectrum 3) but increases again when the epoxy and hydroxyl groups were transformed to carboxylic groups (spectrum 4). The spectrum (5) corresponding to GO-COOH covalently activated with CDDP shows a lower I_D/I_G ratio due to the introduction of CDDP on the GO-COOH surface which may count for a more ordered structure (table 2) [27].

Thermogravimetric analysis (TGA)

The as-received graphite exhibits a good thermal stability; it starts to decompose around 600°C . The graphite oxide curve exhibits different shape compared with the graphite curve. This is explained by the presence of carboxylic, epoxy and hydroxyl groups. Therefore between $25 - 200^\circ\text{C}$ the degradation of carboxylic, epoxy and hydroxyl groups introduced by oxidation process occurs.

Samples	I_D	X_D, cm^{-1}	I_G	X_G, cm^{-1}	I_D/I_G
Graphite	4	1345.3	45.7	1569	0.09
Graphite oxide	91.1	1343.2	93.3	1591	0.98
Graphene oxide (GO)	65.8	1353.8	70	1591	0.94
GO-COOH	68.2	1350	68.3	1582.3	1
GO-COOH+CDDP	68.9	1355.7	78.1	1586.1	0.88

Table 2
 I_D / I_G RATIO OF RAMAN SPECTRA

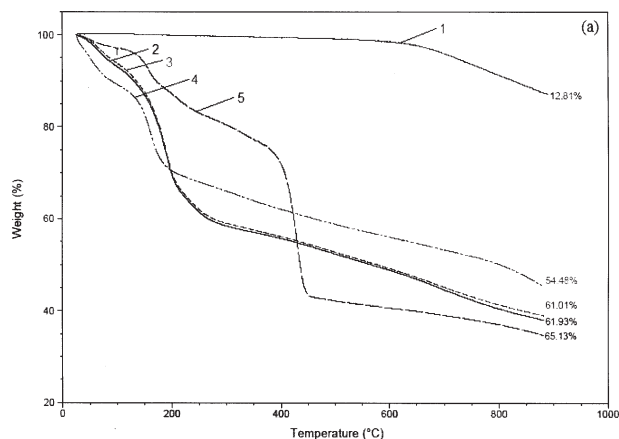


Fig. 5. TGA curves (a) and derivatives (b) for (1) Graphite, (2) Graphite oxide, (3) Graphene oxide (GO), (4) GO-COOH and (5) GO-COOH+CDDP.

Samples	Total Mass loss (%)	Loss temp at maximum degradation temperature
Graphite	12.8	
Graphite oxide	61.9	24.8% at 189.8 °C
Graphene oxide (GO)	61	25.3% at 195.1 °C
GO-COOH	54.5	20.4% at 162 °C
GO-COOH+CDDP	65.1	7.44% at 157.4 °C; 13.7% at 210.5 °C; 45.8% at 430.1 °C

Table 3
MASS LOSS FROM OF TGA CURVES

Sample	2θ (°)	d_{002} : Layer dist. (nm)
Graphite	26.5	0.33
Graphite oxide	10.6	0.81
Graphene oxide (GO)	10.9	0.79
GO-COOH	10.8	0.81
GO-COOH-CDDP	10.4	0.84

Table 4
XRD DATA FOR GRAPHITE-GRAPHITE OXIDE, GRAPHENE OXIDE (GO), GO-COOH AND GO-COOH+CDDP

From table 3 and figure 5 it can be observed that graphite oxide starts to decompose near 600 °C (curve 1).

The graphite oxide and graphene oxide exhibit similar maxima for the degradation temperature close to 189 °C with a mass loss of 25% meaning that the GO structure was not modified by sonication. These decompositions are assigned to the hydroxyl releasing from the graphite oxide and graphene oxide surfaces [28].

The GO-COOH curve (4) starts to decompose at lower temperature (160°C) because the number of carboxyl groups on GO surface is higher. The curve 5 representing GO-COOH covalently activated with CDDP exhibits three maximum degradation temperatures. The first one is at 156°C which represents the degradation of carboxylic, epoxy and hydroxyl groups, the second one is at 210°C assigned to the dissociation of amino bonds and the last one at 430°C which is attributed to the the degradation of CDDP.

X-Ray Diffraction

The gradual process of graphite oxidation into graphite oxide, graphene oxide and GO-COOH induces an increasing of d-spacing of layer-layer distance (table 4). This process is described by the shift of the peak from 26.5 ° to 10.6 ° resulting in the increase of d-spacing from 0.33 to 0.81 due to the OH-bonding and the presence of a small amount of residual oxygen-containing functional groups or other structural defects [29].

For GO-COOH+CDDP it can be observed a slightly increase of distance between layers due to the covalent functionalization of GO-COOH with CDDP.

Scanning electron microscopy

In order to evaluate the morphological changes during the reactions the nanocomposites were examined by SEM (fig. 6). The SEM images are in agreement with the evolution of chemical processes occurred. Thus from big

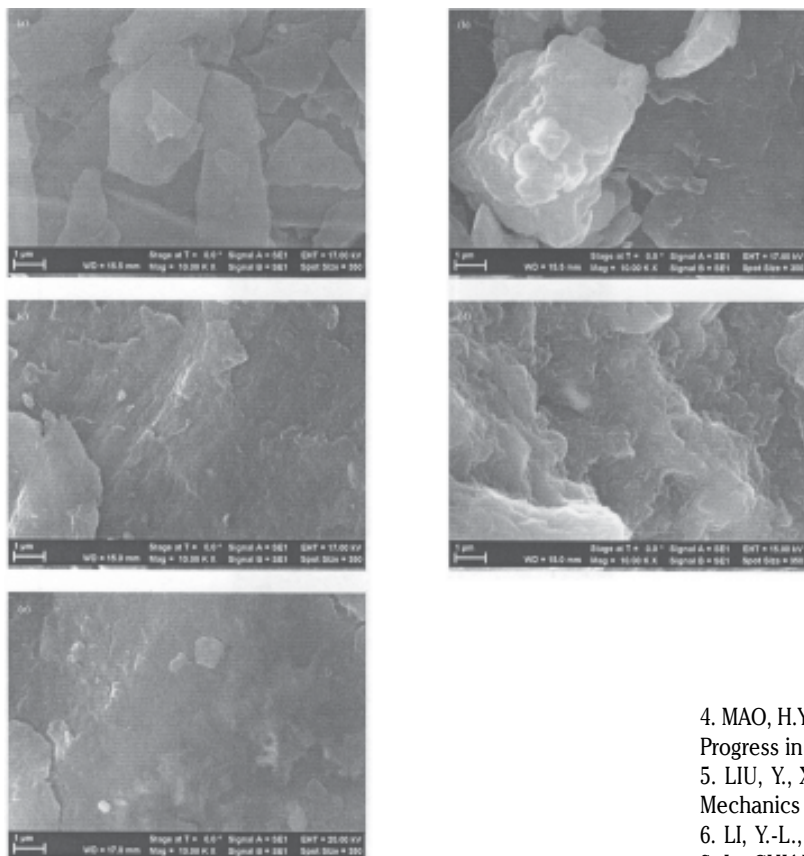


Fig. 6. SEM images of Graphite (a), Graphite oxide (b), Graphene oxide (c), GO COOH(d) and GO-COOH+CDDP (e).

agglomerates for graphite and graphite oxide, the structure of graphene oxide is rather smooth and finally the morphology of GO-COOH+CDDP shows a quite homogeneous structure formed probably by the numerous amidic bonds.

Conclusions

Graphite was successfully oxidized to finally synthesize the carboxylated graphene which was further linked to CDDP. The XPS spectra proved the attachment of CDDP molecules on GO-COOH. However as it was showed by the FTIR spectroscopy that not all the $-NH_2$ groups from CDDP have reacted with the carboxylic groups from GO-COOH

The entire process of oxidation and coupling with CDDP may be followed by Raman spectroscopy. Thus the D band decrease and increase give information about dispersion of graphite oxide by sonication and further oxidation of epoxy and hydroxyl groups to carboxylic ones.

The oxidation process is better described by the increase of d-spacing of layer-layer distance noticed from XRD tests. Also the covalent modification of GO-COOH with CDDP can be pointed out by an increase of distance between the layers.

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