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The effect of gamma radiation on the structure of graphene oxide and graphene oxide functionalized with amino-PEG

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ABSTRACT

Covalent functionalization of graphene oxide (GO) with polyethylene glycol (PEG) has been widely used in drug delivery systems. This nanocomposite exhibits excellent stability in the presence of high concentrations of salts and proteins and shows low toxicity compared to its raw form. However, it must be sterilized prior to use in medical devices, and for this purpose, the gamma irradiation shows a promising option. Sterilization by ionizing energy through gamma rays, generated by Cobalt-60 self-disintegration, consists in exposing the materials to short electromagnetic waves. The irradiation process provides substantial advantages when compared to thermal and chemical processes, such as, more precise control of the process, lower energy consumption, and less environmental pollution. In this work the effects of gamma radiation on GO and GO functionalized with amino-PEG (GO-PEG-NH_2) irradiated with doses (15, 25, 35 and 50 kGy) at rate dose of 7.3 kGy.h^{-1} were evaluated. The analyses were performed by Fourier-transform infrared spectroscopy (FT-IR) and Raman spectroscopy. The results showed that gamma radiation up to 50 kGy did not cause any defects on the nanomaterials.

Keywords: **Functionalization, nanocomposite, graphene oxide.**

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1. INTRODUCTION

Graphene oxide (GO) is one of the precursors of graphene, a flat monolayer of carbon atoms arranged in a two-dimensional (2D) network [1]. This nanomaterial has been widely used in nanoscience and nanotechnology fields, especially as a component of new conductive nanocomposites and biomaterials [2].

The GO has a high dispersibility, hydrophilicity, large surface area and surface oxygen functional groups that promote biological interactions [3]. Despite the advances in its medical applications, there are some concerns about the potential biocompatibility and toxicity of graphene-based nanomaterials [4].

On the other hand, if the GO surface is chemically modified, there can be an increment in its compatibility with the physiological environment [5]. For instance, its functionalization with polyethylene glycol (PEG). The PEG has been used in medical applications due to its physicochemical and biological properties, such as minimal toxicity, solubility in water and in organic solvents. Furthermore, the terminal hydroxyl groups of PEG can be converted into reactive primary amino groups (PEG-NH_2) increasing their biocompatibility [6].

The GO functionalized with amino-PEG (GO-PEG-NH_2) is stable in the presence of high concentrations of salt and protein. The one appears to be less toxic than its raw form *in vitro* and *in vivo* [7].

For medical applications, materials need to be sterilized and an effective method is the gamma radiation. Ionizing radiation can affect DNA either directly, by energy deposition in this critical target; or indirectly, by the interaction of radiation with other atoms or molecules in the cell or surrounding it. Particularly, radiation interacts with water leading to the formation of free radicals that can damage the DNA [8].

In the present work, the effects of gamma radiation GO and GO-PEG-NH_2 surface were evaluated. The samples were irradiated with different doses of radiation, that have been usually used to sterilize materials for medical devices, according to ISO 11137 [9].

2. MATERIALS AND METHODS

2.1 Materials

Graphene oxide was synthesized from purified natural graphite, potassium permanganate ($KMnO_4$), sodium nitrate ($NaNO_3$), concentrated sulfuric acid (H_2SO_4), sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2). Amino-PEG was prepared from polyethylene glycol (PEG 4.000), diethyl ether, potassium phthalimide, pyridine, tosyl chloride, hydrazine sulfate, N,N-dimethylformamide, hydrochloric acid, dichloromethane, anhydrous potassium carbonate (K_2CO_3) and anhydrous sodium sulfate (Na_2SO_4). For the preparation of graphene oxide functionalized with amino-PEG these following reagents were used: amino-PEG, N-(3-dimethylaminopropyl-N'-ethylcarbodiimide) hydrochloride (EDC), and Amicon centrifugal filter device, 15 mL, 10 kDa.

2.2 Equipment

Structural analysis of materials was performed in infrared spectrometry (FT-IR) Alpha model from Bruker. Raman spectroscopy was performed using a Renishaw in Via Reflex microscope equipped with a CCD camera (Renishaw, 600 x 400 pixels) thermally cooled and coupled to a Leica model DM2500M microscope. The lines of lasers used were 532 nm and 785 nm (diode laser, Renishaw) respectively for the GO and GO-PEG-NH_2 samples. The samples were irradiated at the Multipurpose Gamma Irradiation Facility at CTR/IPEN/CNEN-SP.

2.3 Statistical Analysis

The structural characterization was analyzed by the spectrum profile and performed in Origin 8 software.

2.4 Synthesis of Graphene Oxide (GO)

Graphene oxide was synthesized according to the Hummers method with modifications [10]. In details, 3 g of graphite and 3 g of $NaNO_3$ were mixed with 140 mL of H_2SO_4 and stirred in an ice

bath for 1 h. 18 g of $KMnO_4$ were slowly added maintaining the temperature at 10 °C and then, the solution was stirred for 2 h at room temperature. After 1h at 35 °C the temperature was raised and kept constant below 100°C for another hour. At the end of the reaction, 600 mL of deionized water were added, followed by 10 mL of H_2O_2 (30%) under constant stirring. The mixture was washed with NaOH [1.0 M], HCl [1.0 M] and deionized water. Every work up steps was centrifuged at 12000 rpm. The final product was dried and exfoliated by the ultrasonic equipment.

2.5 Synthesis of Amino-PEG (PEG-NH_2)

Amino-PEG (PEG-NH_2) was synthesized according to the method described by Mutter with modifications [11]. Briefly, 0.5 g of tosyl chloride and 1 g of polyethylene glycol (PEG 4000) were mixture using pyridine and dichloromethane as solvents. The solution was kept under stirring for 12 h at room temperature at the end of this reaction HCl [1M] was added. The organic phase was washed with distilled water and dried over Na_2SO_4. The solution was filtered and to the white solid an aprotic solvent (N,N-dimethylformamide) and 1.5 g of potassium phthalimide solid were added. The mixture was stirred under N_2 at 120 °C for 4 h. At room temperature, diethyl ether was added and the mixture was filtered. To the white solid compound, 0.5 g of hydrazine sulfate and 0.5 g of K_2CO_3 were added and the mixture was kept in reflux for more 4 h. At room temperature, ethyl ether was added to precipitate the final product.

2.6 Synthesis of Graphene Oxide Functionalized with Amino-PEG (GO-PEG-NH_2)

Graphene oxide functionalization was performed by amidation employing the amino-PEG (PEG-NH_2) as described by Yang et al [12]. Briefly, 100 mL of dispersed GO (1mg/mL), 100 mg of PEG-NH_2 and 20 mg of EDC were kept stirring vigorously overnight at room temperature. The product obtained was centrifuged at 12000 rpm and the supernatant was washed using DI water and centrifuged on 15 mL Amicon filter (MWCO=10kDa).

2.7 γ – Irradiation

Samples of GO and GO-PEG-NH_2 were irradiated in the Multipurpose Gamma Irradiation Facility at CETER/IPEN/CNEN-SP, a category IV gamma irradiator by the International Atomic Energy Agency classification, in stationary mode, under the dose rate of 7.31 kGy.h^{-1}. The doses that have been applied were the same used to sterilize medical devices: 15, 25, 35 and 50 kGy, according to ISO 11137-2 [9]. Non-irradiated samples were used as the control group.

3. RESULTS AND DISCUSSION

3.1 The Structures of GO and GO-PEG-NH_2

The synthesis of GO-PEG-NH_2 was carried out using N- (3-dimethylaminopropyl-N'-ethylcarbodiimide) hydrochloride (EDC) as catalyst. The nitrous [13] acid and ninhydrin tests [14] were positives for primary amine groups in PEG-NH_2 samples.

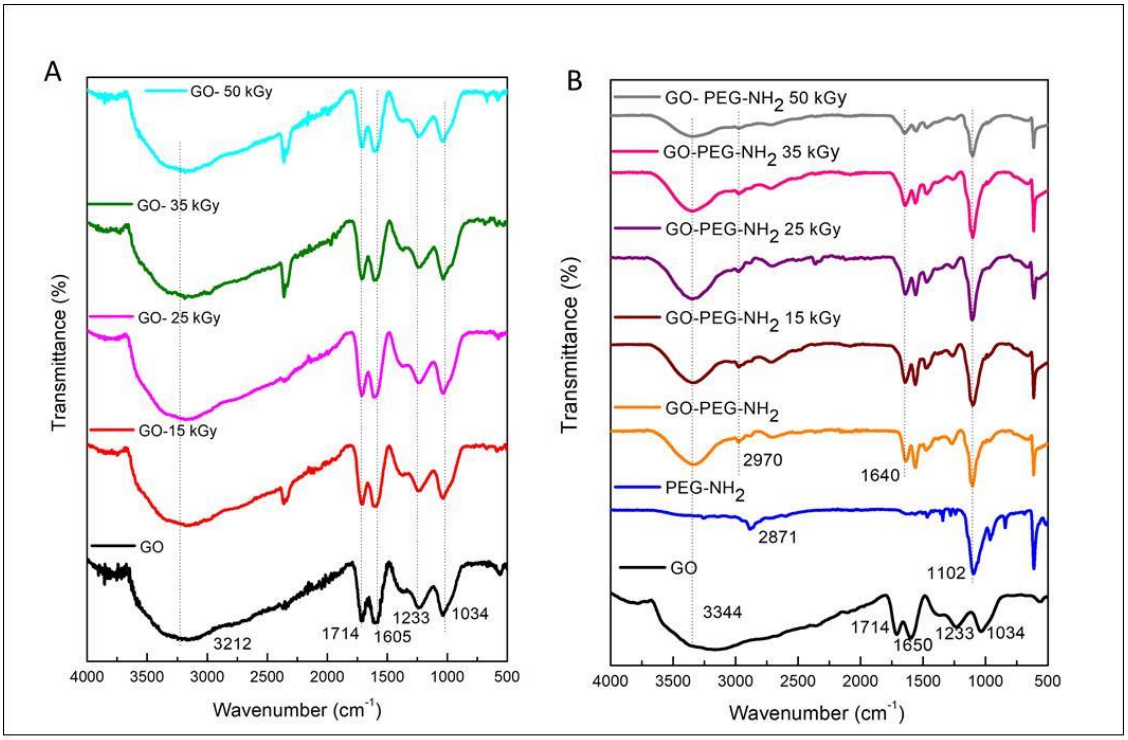
3.1.1 Infrared spectroscopy (FT-IR)

The FT-IR results indicated the presence of oxygenated functional groups on the surface of GO. According to Zhao et al. [15] the main bands corresponding to the hydroxyl groups (~3000 cm^{-1}) carbonyl esters (~1600 cm^{-1}), carboxylic acids (~1700 cm^{-1}), carboxyl C-O (~1300 cm^{-1}) and epoxy (~1080 cm^{-1}).

For GO-PEG-NH_2, bands at 1640 cm^{-1} of carbonyls from amides were observed. This result demonstrates that PEG-NH_2 has been successfully grafted onto the surface of GO by the amidation reaction.

Figure 1 shows that after gamma irradiation, the samples of GO and GO-PEG-NH_2 spectra showed no structural changes of the characteristic bands.

Figure 1: *FT-IR spectra of (A) GO, GO gamma irradiated with 15, 25, 35 and 50 kGy and (B) GO, PEG-NH_2, GO-PEG-NH_2, GO-PEG-NH_2 irradiated with 15, 25, 35 and 50 kGy.*



3.1.2 Raman spectroscopy

Raman spectroscopy is a technique to analyse the existence of defects and the extent of functionalization [16]. In this sense, it is presented as a complementary analysis to FT-IR in the evaluation of GO and GO-PEG-NH_2 surfaces. For both GO samples the spectra (Figure 2) showed D band at (~1348 cm^{-1}), the G band (~1600 cm^{-1}), 2D (~2699 cm^{-1}), and D+G call at (~2946 cm^{-1}), in agreement with the studies carried out by Zhao et al. [15]. The D functions are associated with the nanomaterial disorder, while the G functions contribute to the graphitic organization [17].

Figure 2: *Raman spectra of GO, GO gamma irradiated with 15, 25, 35 and 50 kGy.*

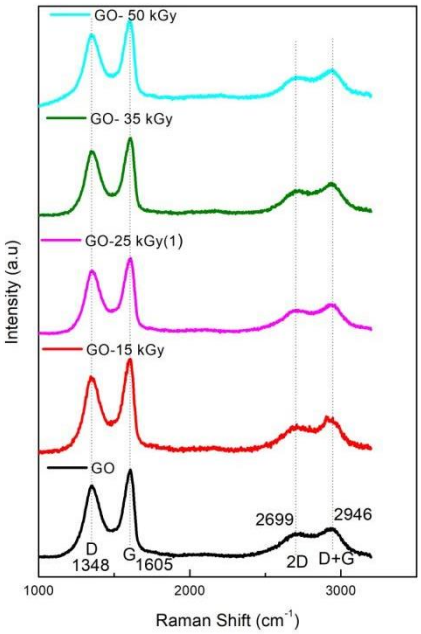
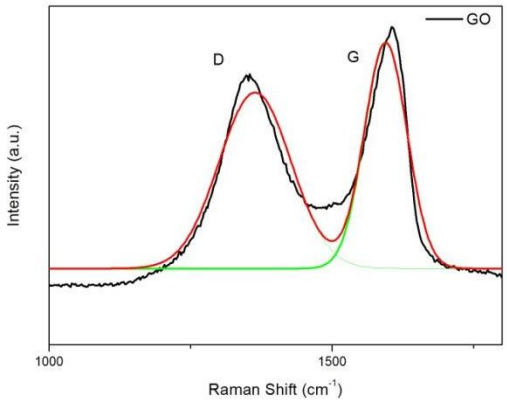


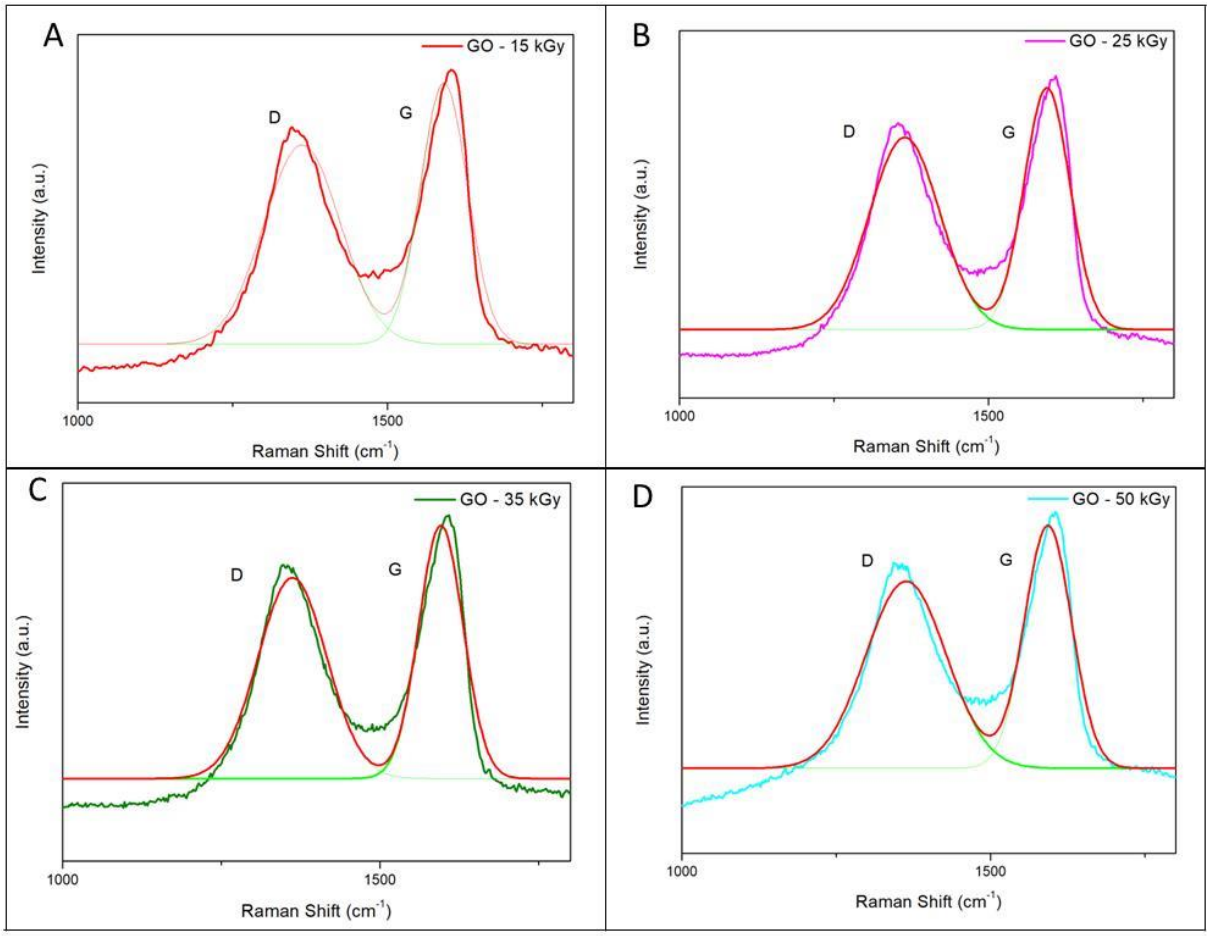
Figure 3 shows D and G bands of the GO, with their respective bands deconvoluted by Gaussian function.

Figure 3: *Raman spectra of GO and curves deconvoluted.*



D and G bands of irradiated samples were deconvoluted using the Gaussian function as shown in Figure 4.

Figure 4: *Raman spectra of samples GO irradiated and curves deconvoluted, (A) GO-15 kGy, (B) GO-25 kGy, (C) GO-35 kGy, (D) GO-50 kGy.*



The intensity (I_{D1} / I_G) and half height (ω_{d1} / ω_g) ratios of all samples were calculated as shown in Table 1. The (I_{D1} / I_G) indicates the degree of the nanomaterial organization, while (ω_{d1} / ω_g) corresponds to amount of structural defects formed due to oxygenated groups incorporated on their surface [18]. With these data, it was possible to relate the contribution of D and G bands.

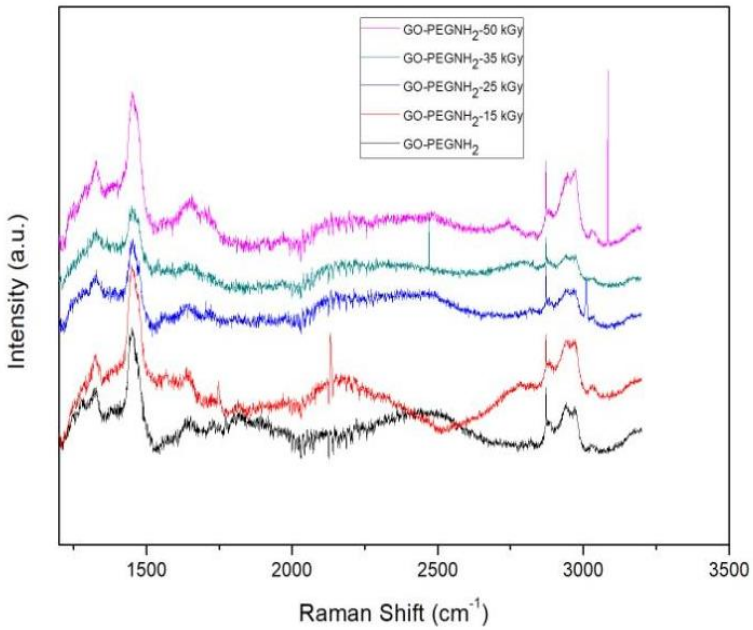
Table 1: Values of (ω_{d1} / ω_g) and (I_{D1} / I_G).

	ωD1/ωG	**ID1/IG**
GO	1.69	1.32
GO – 15 kGy	1.62	1.24
GO – 25 kGy	1.52	1.23
GO – 35 kGy	1.54	1.23
GO – 50 kGy	1.71	1.32

In Table 1, the results indicated that there was no difference in the GO after irradiation even at 50 kGy.

On GO-PEG-NH_2 Raman spectra (Figure 5) the following bands were observed D band at 1351 cm^{-1}, the G band at 1600 cm^{-1}, the 2D at 2688 cm^{-1} and the so-called D + G at 2933 cm^{-1} for all samples [14]. These results corroborated with FT-IR showing that the irradiation did not affect GO and GO-PEG-NH_2 structures.

Figure 5: *Raman spectra of samples GO-PEG-NH_2, GO-PEG-NH_2 irradiated with 15, 25, 35 and 50 kGy.*



4. CONCLUSION

The methods for the synthesis of GO and GO-PEG-NH_2 proved to be effective.

The analyses performed by FT-IR and Raman spectroscopy indicated that the radiation doses used for sterilization of the nanocomposites did not cause modifications structural on their surface, such as disappearance or displacements of the characteristic bands.

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