



The use of gamma radiation on graphene oxide and graphene oxide Amino-PEG sterilization for biomedical applications

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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, must be sterilized prior to use in medical devices, and the gamma irradiation shows a promising option for this purpose. 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 com Amino-PEG (GO-PEG-NH₂) irradiated with doses (15, 25, 35 and 50 kGy) that have been used to sterilize medical devices and at rate dose 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.

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 several areas in nanoscience and nanotechnology fields, especially as a component of new conductive nanocomposites and biomaterials [2].

The GO has a high dispersion capacity, large surface area and present functional oxygen groups capable of promoting biological interactions [3]. Despite advances in its applications in the medicine area, there are some concerns about the potential biocompatibility and toxicity of graphene-based nanomaterials [4].

However, when the GO surface is chemically modified, for example, functionalized with polyethylene glycol, its compatibility with the physiological environment is increased [5]. Polyethylene glycol, PEG, is widely used in biomedical applications due to its excellent physicochemical and biological properties such as minimal toxicity, good solubility in water and organic solvents. In addition, the terminal hydroxyl groups of PEG can be converted into reactive primary amino groups (PEG-NH₂) increasing their biocompatibility [6].

The GO functionalized with amino-PEG (GO-PEG-NH₂) exhibits excellent stability in the presence of high salt and protein concentrations and appears to be less toxic than its raw form *in vitro* and *in vivo* [7]. For medical use, materials must be sterilized, and gamma radiation is an effective method. Sterilization by ionizing energy through gamma rays, generated by Cobalt-60 self-disintegration, consists in exposing the materials to short electromagnetic waves [8].

In this study the effects of gamma radiation GO and GO-PEGNH₂ surface was evaluated. The samples were irradiated with four different doses that have being used to sterilized materials for medical devices.

2. MATERIALS AND METHODS

2.1 Materials

Graphite powder with a purity of 99.99%, potassium permanganate (KMnO₄), sodium nitrate (NaNO₃), hydrogen peroxide (H₂O₂), ethyl ether were purchased from Merck, N-(3-dimethylaminopropyl-N'-ethylcarbodiimide) hydrochloride (EDC), potassium phthalimide, pyridine, tosyl chloride, hydrazine sulfate from Aldrich Chemistry, concentrated sulfuric acid (H₂SO₄) and N,N-dimethylformamide from Vetec Química Fina, hydrochloric acid, sodium hydroxide (NaOH) and dichloromethane from CAAL, polyethylene glycol (PEG 4.000), ethanol, anhydrous potassium carbonate (K₂CO₃) and anhydrous sodium sulfate (Na₂SO₄) from Labsynth, Amicon centrifugal filter device, 15 mL, 10 kDa MWCO (Millipore).

2.2 Equipment

Structural analysis of materials was performed in infrared absorption spectrometry (FT-IR) Alpha model from Bruker, and Raman spectroscopy was performed using a Renishaw inVia 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-NH₂ 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 [9]. In details, 3 g of graphite and 3 g of NaNO₃ were mixed with 140 mL of H₂SO₄ and stirred in an ice bath for 1 h. 18 g of KMnO₄ 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 H₂O₂ (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 12.000 rpm. The final product was dried and exfoliated by the ultrasonic treatment.

2.5 Synthesis of Amino-PEG (PEG-NH₂)

Amino-PEG (PEG-NH₂) was synthesized according to the method described by Mutter with modifications [10]. Briefly, 0.5 g of tosyl chloride and 1 g of polyethylene glycol (PEG 4.000) were mixture using pyridine and dichloromethane as solvents. The solution was kept under stirring for 12 h at room temperature and HCl [1M] was added. The organic phase was washed with distilled water and dried over Na₂SO₄. The solution was filtered and to the remaining solid an aprotic solvent (N,N-dimethylformamide) and 1.5 g of potassium phthalimide were added. The mixture was stirred under N₂ at 120 °C for 4 h. The solution was cooled down and after adding ethyl ether the mixture was filtered and to the white solid compound, 0.5 g of hydrazine sulfate and 0.53 g of K₂CO₃ were added. The mixture was kept in reflux for 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-NH₂)

Graphene oxide functionalization was performed by amidation employing the amino-PEG (PEG-NH₂) as described by Yang et al [11]. Briefly, 100 mL of dispersed GO (1mg/mL), 100 mg of PEG-NH₂ and 20 mg of EDC were kept stirring vigorously at room temperature overnight. The product obtained was centrifuged at 12.000 rpm and the supernatant solution was washed using DI water and centrifuged on a 15 mL Amicon filter (MWCO=10kDa) and the final product GO-PEG-NH₂ in water solution was obtained.

2.7 γ – Irradiation

Samples of GO and GO-PEG-NH₂ were irradiated at dose rate of 7.31 kGy.h⁻¹ in the doses that have been used to sterilize medical devices: 15, 25, 35 and 50 kGy.

3. RESULTS AND DISCUSSION

3.1 The Structures of GO and GO-PEG-NH₂

The synthesis of GO-PEG-NH₂ was carried out using PEG-NH₂ and N-(3-dimethylaminopropyl-N'-ethylcarbodiimide) hydrochloride (EDC) as catalyst. The nitrous [12] acid and ninhydrin tests [13] were positives for primary amine groups in PEG-NH₂ samples.

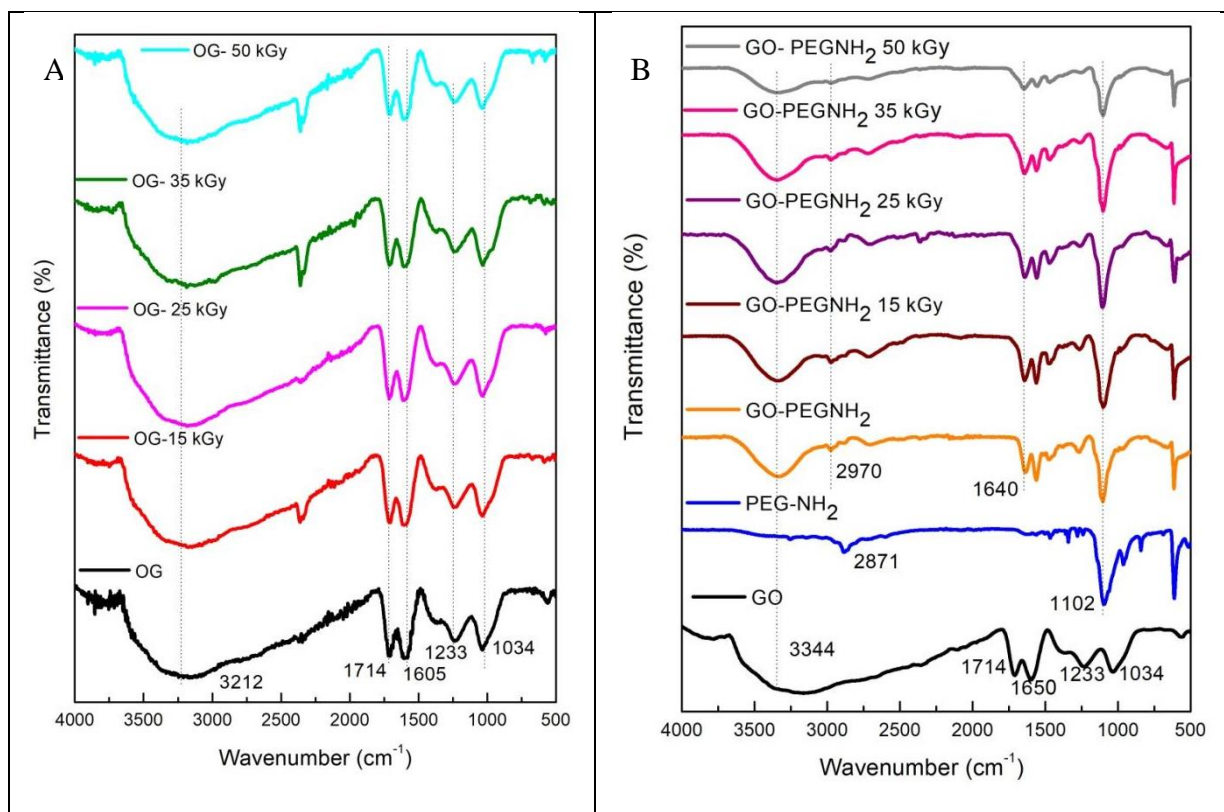
3.1.1 Infrared spectroscopy (FT-IR)

The FT-IR results indicated the presence of oxygenated functional groups on the surface of GO. According to Zhao et al. [14] 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-NH₂, bands at 1640 cm⁻¹ of carbonyls from amides were observed. This result demonstrates that PEG-NH₂ 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-NH₂ spectra showed no changes in disappearance or displacements 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-NH₂, GO-PEG-NH₂, GO-PEG-NH₂ irradiated with 15, 25, 35 and 50 kGy.



3.1.2 Raman spectroscopy

Raman spectroscopy is a technique used to analyze the existence of defects and the extent of functionalization [15]. In this sense, it is presented as a complementary analysis to FT-IR in the evaluation of GO and GO-PEG-NH₂ surfaces. For both GO samples the spectra (Figure 2) shows D band at ($\sim 1,348$ cm⁻¹), the G band (~ 1600 cm⁻¹), 2D (~ 2699 cm⁻¹), and D+G call at ($\sim 2,946$ cm⁻¹), in agreement with the studies carried out by Zhao et al. [14]. The D functions are associated with the material disorder, while the G functions contribute to the graphitic organization [16].

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

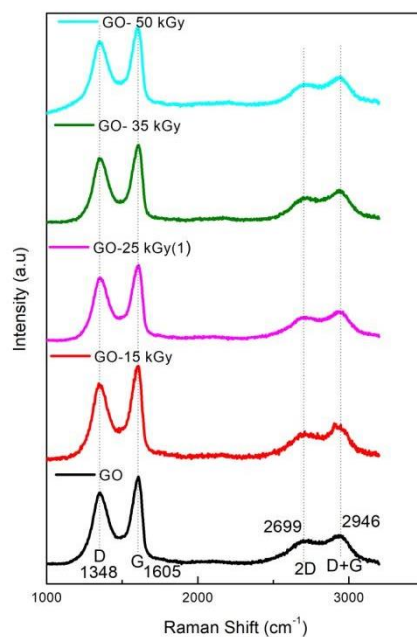
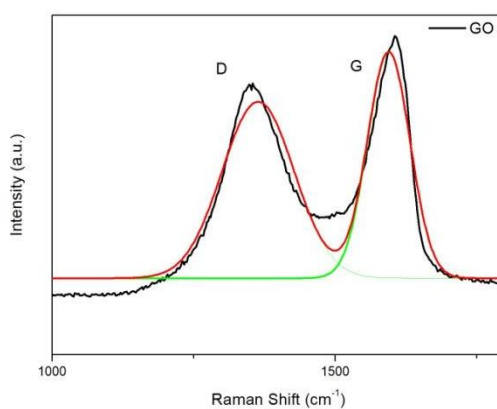


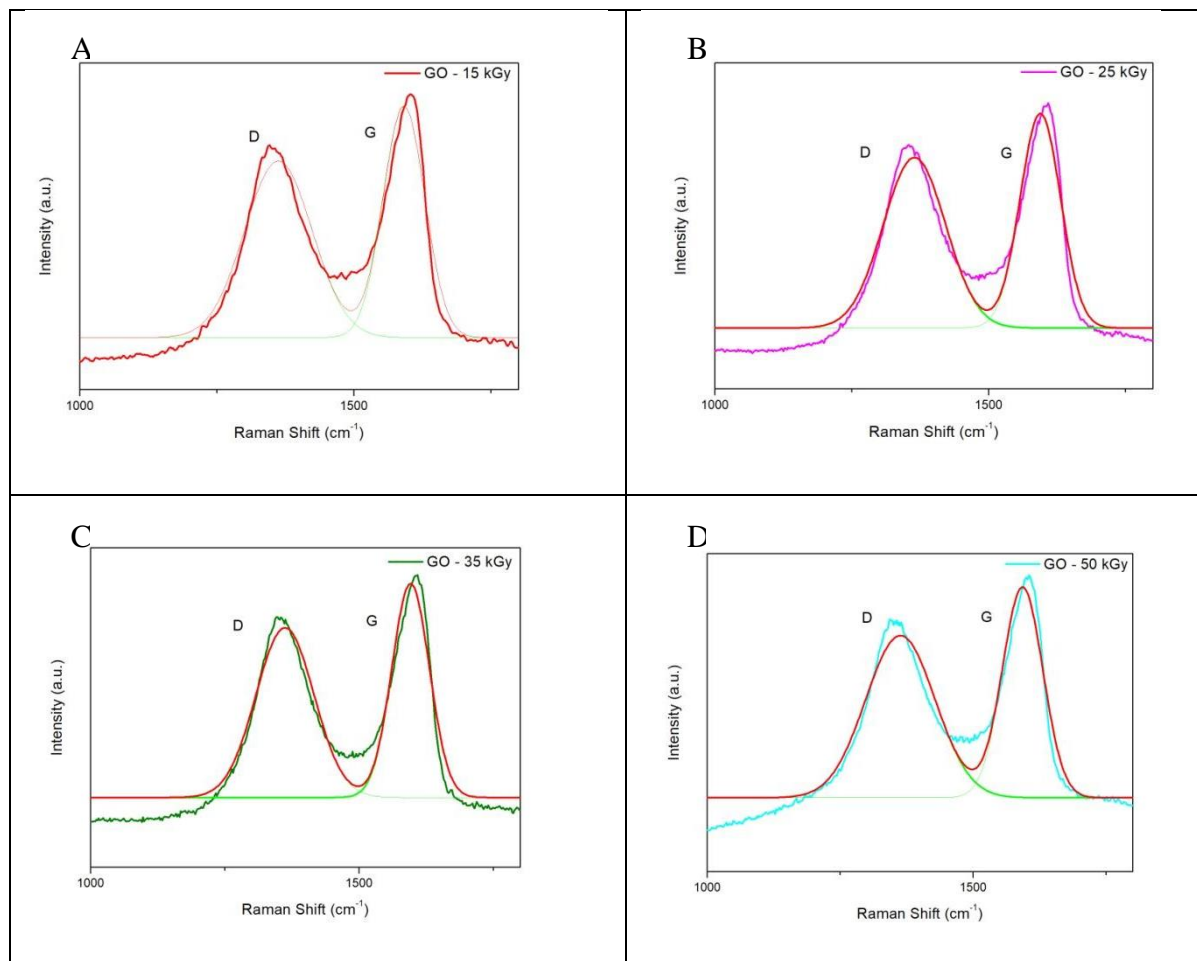
Figure 3 shows D and G bands of the GO, with their respective bands deconvoluted by Gaussian function.

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) ration indicates the degree of the material's organization, while (ω_{d1} / ω_g) ration corresponds to amount of structural defects [17] formed as a consequence of oxygenated groups incorporated on their surface. With these data, it was possible to relate the contribution of D and G bands.

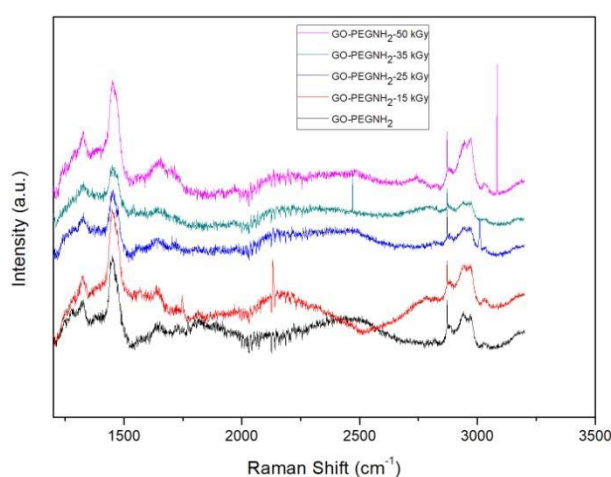
Table 1: Values of (ω_{D1} / ω_G) and (I_{D1} / I_G).

	ω_{D1}/ω_G	I_{D1}/I_G
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 indicate that there is no difference in the GO when irradiated even at 50 kGy.

On GO-PEG-NH₂ Raman spectra (Figure 5) the following bands are 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 does not affect GO and GO-PEG-NH₂ structures.

Figure 5: Raman spectra of samples GO-PEG-NH₂, GO-PEG-NH₂ irradiated with 15, 25, 35 and 50 kGy.



4. CONCLUSION

The methods for the synthesis of GO and GO-PEG-NH₂ proved to be effective since there was confirmation of the surface oxidation of materials and functionalization with the PEG-NH₂ group.

The analyzes performed by FT-IR and Raman spectroscopy indicated that the radiation doses used for sterilization of the nanocomposites did not cause modifications on their surface, neither on the functional groups.

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