Synthesis of graphene quantum dots from *Moringa oleifera* seed biomass doped with phosphate

Síntese de pontos quânticos de grafeno de biomassa de semente de *Moringa oleifera* dopados com fosfato

Alexandre Rodrigues Simões  
ORCID: https://orcid.org/0009-0008-8912-5614  
Centro Universitário de Adamantina (FAI), Brasil  
E-mail: simoes@fai.com.br

Alexandre Teixeira de Souza  
ORCID: https://orcid.org/0000-0003-0357-0925  
Centro Universitário de Adamantina (FAI), Brasil  
E-mail: alteiso@fai.com.br

Eduardo César Meurer  
ORCID: https://orcid.org/0000-0003-4835-7773  
Universidade Federal do Paraná (UFPR), Brasil  
E-mail: eduardo.meurer@ufpr.br

Évelin Lemos de Oliveira  
ORCID: https://orcid.org/0000-0002-8208-0950  
Universidade Estadual de Maringá (UEM), Brasil  
E-mail: elemosoliveira01@gmail.com

Helton José Alves  
ORCID: https://orcid.org/0000-0001-6942-1020  
Universidade Federal do Paraná (UFPR), Brasil  
E-mail: helquim@gmail.com

Lázaro José Gasparrini  
ORCID: https://orcid.org/0000-0002-0015-5489  
Universidade Federal do Paraná (UFPR), Brasil  
E-mail: lazarogasparrini@gmail.com

Mara Heloisa Neves Olsen Scaliante  
ORCID: https://orcid.org/0000-0001-9090-9274  
Universidade Estadual de Maringá (UEM), Brasil  
E-mail: msnoscaliante2@uem.br

Wilker Caetano  
ORCID: https://orcid.org/0000-0002-9402-8324  
Universidade Estadual de Maringá (UEM), Brasil  
E-mail: wcaetano@uem.br
ABSTRACT

In this work, graphene quantum dots (GQDs) were synthesized from Moringa oleifera seed biomass doped with phosphorus, using a low-cost, renewable hydrothermal method. The biomass was ground and sieved (30 mesh), and the classified powder was transferred to a Teflon capsule, placed in a stainless-steel reactor (210 °C, 24 h). Afterwards, 20 mL of distilled water were added to the synthesized GQDs, stirred for 30 min, and filtered through Whatman filter paper. Then they were centrifuged (12000 rpm, 10 min) and filtered through a 0.22 μm membrane. The filtrate was dialyzed in a cellulose dialysis bag, with a cut-off size of 3.5 kDa, for 7 days in absolute ethanol (99.5%), with gentle stirring, and the dialysate was exchanged every 24 h. The dialysate was dried at 105 °C for 24 h. The GQDs were characterized by X-ray energy dispersive spectroscopy (EDX), Fourier-transform infrared spectroscopy (FTIR), UV-vis electronic absorption, fluorescence emission, X-ray Diffraction and high-resolution transmission electron microscopy (HRTEM). The results show the GQDs with an average size of 18.63 ± 4.24 nm and high fluorescence (bright blue) under ultraviolet light (365 nm). Our GQDs are promising for application in bioimaging and bioanalysis.

Keywords: Nanotechnology; Light emission; Biomass; Biomedicine; Zero waste.

RESUMO

Neste trabalho foram sintetizados pontos quânticos de grafeno (PQG) de biomassa da semente de Moringa oleifera dopados com fosfato, através de um método hidrotérmico de baixo custo e fonte renovável. A biomassa foi triturada e peneirada (30 mesh) e o pó classificado transferido para uma cápsula de Teflon, colocada em um reator inox (210 °C, 24h). Após, 20 mL de água destilada foram adicionados aos PQG sintetizados, agitados por 30 min efiltrados em papel de filtro Whatman. Então foram centrifugados (12000 rpm, 10 min) e filtrados em membrana de 0.22 μm. O filtrado foi dialisado em uma bolsa de diálise de celulose, com tamanho de corte de 3.5 kDa, durante 7 dias em etanol absoluto (99.5%), sob leve agitação, sendo que o dialisante foi trocado a cada 24 h. O dialisado foi seco a 105 °C durante 24h. Os PQG foram caracterizados por EDX, FTIR, Absorção eletrônica UV-vis, Emissão de fluorescência, Difração de Raio-X e HRTEM. Os resultados mostram os GQDs com um tamanho médio de 18.63 ± 4.24 nm e alta fluorescência (azul brilhante) sob luz ultravioleta (365 nm). Os nossos GQDs são promissores para aplicação em bioimagem e bioanálise.

Palavras-chave: Nanotecnologia; Emissão de luz; Biomassa; Biomedicina; Resíduo zero.

INTRODUÇÃO

Graphene quantum dots (GQDs) possess diameters below 100 nm. They have garnered significant attention from researchers in recent years due to their robust quantum confinement and edge effects (ABBAS et al., 2021).

GQDs exhibit biologically favorable characteristics such as low toxicity, high biocompatibility, chemical and photostability, good water solubility, adjustable bandgap, stable fluorescence, and excellent photo stability. In addition to their optical and electrical properties, which can be tailored for specific functions (LINGAM et al., 2013; OUYANG et al., 2021). These properties make GQDs suitable for a broad spectrum of applications,
including photocatalysis (SELVAKUMAR et al., 2022), photoelectric conversion (KOUR et al., 2020), bioimaging, detection, and biosensors (CHUNG et al., 2021), medical diagnostics (LATHA et al., 2023), therapeutics and photodynamic (DE OLIVEIRA et al., 2021), optoelectronic devices (TETSUKA et al., 2016), light-emitting diodes (YIN et al., 2019), and agriculture (BAWEJA; JEET, 2019; NXELE; NYOKONG, 2022; RANA et al., 2018)

Many techniques have been employed to synthesize GQDs using different non-renewable precursors such as graphene, carbon fibers, graphite flakes, carbon nanotubes, coal, candle soot, etc. (CHHABRA et al., 2018; LI et al., 2019; RABEYA et al., 2021; TIAN et al., 2018). They are classified into two commonly used classes: bottom-up and top-down (JAHDALY et al., 2021). Bottom-up techniques generate graphene domains from organic molecular precursors, involving the assembly of small molecules into larger nanoparticles, including molecular and polymeric carbonization through chemical reactions (SHEHAB; EBRAHIM; SOLIMAN, 2017). On the other hand, top-down methods break down large carbon structures into smaller ones, such as ultrasound-assisted exfoliation (GAO et al., 2017a), hydrothermal and solvothermal methods (SOHOULI et al., 2023), oxidative cutting of graphene, graphene oxide (GO), or carbon nanotubes (GU et al., 2018), and electrochemically assisted oxidative decomposition (CHEN et al., 2017). These mentioned precursors are expensive and challenging to synthesize, making the search for a renewable, green, and inexpensive alternative indispensable. Biomass residues are green and carbon-rich sources (45-55% by mass), in addition to being renewable, standing out as environmentally friendly precursors for the economical synthesis of GQDs, which can reduce process costs (XIE; GOODELL, 2014).

In recent years, heteroatom doping (largely nitrogen-doped) has emerged as a promising technique to tailor the physicochemical properties such as photoluminescence, quantum yield, including electronic, surface, and local chemical features of GQDs (DWITYA et al., 2023). The heteroatom can be introduced through precursor materials during synthesis or post-synthesis techniques (NGUYEN et al., 2019; WANG et al., 2018; WU et al., 2018). Through surface engineering, including surface oxidation, polymer passivation, and chemical moiety attachment, oxygenated functional groups can be introduced, rendering GQDs hydrophilic for subsequent chemical functionalization. Some plants contain elements such as N, S, and P; thus, these plants can undergo self-doping in the hydrothermal process (SEKIYA et al., 2016).
In this study, graphene quantum dots (GQDs) doped with phosphate were synthesized using the biomass residue from *Moringa oleifera* seeds. The synthesis method employed was the hydrothermal process, known for its low cost, ease of operation, renewable source, and eco-friendly nature. Few studies are reported in the literature concerning the synthesis of phosphate-doped graphene quantum dots.

**MATERIALS and METHODS**

**Samples and reagents**

The biomass used was the residue from *M. oleifera* seeds as reported by SIMÕES and colleagues (2023). Absolute ethanol (99.5%) from Dinâmica, Brazil, was used as obtained.

**Synthesis of Graphene Quantum Dots (GQDs)**

The synthesis methodology used in this study involved the bottom-up route through the hydrothermal method to synthesize the GQDs using *M. oleifera* biomass. Figure 1 illustrates the flowchart of the graphene quantum dots (GQDs) synthesis.

**Figure 1** - Synthesis of graphene quantum dots (GQDs) from *M. oleifera* seed biomass.

The biomass was sieved through a 30-mesh sieve and dried at 105 °C for 24 h. 3 g of the classified powder was transferred to a Teflon capsule, adding 10 mL of 0.1 mol
L$^{-1}$ phosphoric acid. The suspension was homogenized and placed in a stainless-steel reactor, heated in a muffle furnace at 210 °C for 24 h. 20 mL of distilled water was added to the synthesized GQDs, stirred for 30 min, and filtered using Whatman filter paper. The solution containing the GQDs was centrifuged at 12,000 rpm for 10 min and filtered through a 0.22 μm membrane. After this process, the filtrate was dialyzed in a cellulose dialysis bag with a cutoff size of 3.5 kDa for 7 days in absolute ethanol (99.5%), under mild agitation, with the dialysate changed every 24 h. The dialysate was transferred to an open glass flask and placed in a muffle furnace at 105 °C for drying.

The GQDs were characterized by EDX, FTIR, UV-Vis electronic absorption, Fluorescence Emission, X-ray Diffraction and HRTEM. Experiments were conducted in triplicate.

Instrumentation

The synthesized graphene quantum dots (GQDs) were characterized by various techniques, such as Energy Dispersive X-ray spectroscopy (EDX, LEO, model 440), High-Resolution Transmission Electron Microscopy (HRTEM, JEOL, model JEM-2100), Dynamic Light Scattering (DLS, BETtersize Nanoptic 90 model), X-ray Diffraction Analysis (XRD, Bruker, model D8 Advance), UV-Visible Absorption Spectrophotometer Cary 60 (Agilent, USA), Fluorescence Spectrum by Cary Eclipse Spectrophotometer (Agilent, USA), and functional groups by Fourier Transform Infrared Spectrophotometer (FTIR, Shimadzu, model IRAffinity 1).

RESULTS AND DISCUSSION

Characterization of graphene quantum dots (GQDs)

Energy Dispersive X-ray spectroscopy (EDX)

The presence of elements in the analyzed structures was confirmed by the EDX technique, indicating the quantity of each element (Figure 2). Oxygen, sulfur, potassium, calcium, magnesium, sodium, phosphorus, and copper were detected on the surface of the nanoparticles, and the presence of oxygen on the surface was significantly notable. The presence of these elements indicates that self-doping of the elements presents in the M. oleifera seed biomass may have occurred in the GQD synthesis process (SEKIYA et al., 2016). M. oleifera seeds contain potassium, sodium, magnesium, calcium, and other
elements (LIANG et al., 2019). EDX analysis detects only the elements present on the surface, not providing a complete elemental analysis. However, the EDX data confirmed the presence of phosphorus on the surface of the GQDs, indicating the formation of phosphate-doped GQDs.

**Figure 2** - Elemental analysis of the structure of GQDs from *M. oleifera* seed biomass using Energy Dispersive X-ray spectroscopy (EDX).

FTIR spectroscopy was used to identify functional groups in the GQDs. The spectrum in Figure 3 illustrates a variety of functions of the groups forming the molecular complex of GQDs.

**Figure 3** - FTIR spectrum of GQDs synthesized from the biomass of *M. oleifera* seeds.

The samples were scanned in a range from 4000 cm$^{-1}$ to 600 cm$^{-1}$. The absorption
band at 3348 cm\(^{-1}\) is attributed to the hydroxyl group stretching vibration (\(\nu_O-H\)), the band at 1641 cm\(^{-1}\) from the amide I group is associated with the \(\text{NH}_2\) group bending, the band at 2981 cm\(^{-1}\) is due to the stretching vibration \(\nu_C-H\) assigned to alkyl groups, and the band at 1085 cm\(^{-1}\) corresponds to the C-O stretching. The absorption band at 1045 cm\(^{-1}\) exhibits stretching of the \(\nu_P-O-C\) bond, possibly related to the phosphate group doped on the surface of the GQDs.

**UV-Vis electronic absorption**

UV-Vis electronic absorption spectra were obtained by adding 2 mL of the sample to a quartz cuvette with a 1 cm optical path length, and measurements were performed on the Cary 60 spectrophotometer (Agilent, USA) with a wavelength scan range of 200 to 800 nm.

**Figure 4 -** UV-Vis electronic absorption spectrum of graphene quantum dots (GQDs).

![Graphene quantum dots (GQD)](image)

Source: authors (2024)

The most common feature of GQDs is the emitted photoluminescence. Figure 4 shows that the GQDs solution appears dark brown under visible light and bright blue under ultraviolet light (365 nm). The UV-vis spectrum of the aqueous GQDs solution exhibits absorption peaks at 207 nm and 220 nm, attributed to the \(\pi-\pi^*\) transition of sp\(^2\) graphitic domains, and another \(n-\pi^*\) absorption band at 360 nm, possibly related to the size and surface effects of GQDs (AHMED et al., 2018). GQDs have a graphene core
with attached "uncertain" chemical groups, where photoluminescence is controlled by both the graphene core and surface chemical groups. The graphene core determines intrinsic emission, while the attached chemical groups control the surface state (TSU et al., 2016).

**Fluorescence emission**

Fluorescence emission spectra were obtained by adding 2 mL of the sample to a quartz cuvette with a 1 cm optical path length, and measurements were performed on the Cary Eclipse fluorescence spectrophotometer (Agilent, USA), with an excitation wavelength range of 250 to 500 nm, slit width 5-5, and a scan wavelength range of (excitation wavelength + 20 nm) to 800 nm. In this study, a cellulose membrane with a cutoff size of 3.5 kDa was employed for GQD purification through the dialysis process.

**Figure 5** - Fluorescence emission spectrum of graphene quantum dots (GQDs).

Many byproducts of the carbon dot formation process remain in the retentate when dialyzed for 24 hours. The required dialysis time is much longer than usual, potentially exceeding 120 hours. Additionally, the cutoff size of the membrane affects the efficiency
of dialysis. Membranes with cutoff sizes ranging from 0.5 kDa to 1.0 kDa exhibit carbon dot byproducts, altering fluorescence emission (CHEN; TSAI; CHANG, 2019).

Figure 5 presents the fluorescence emission spectrum of GQDs. It is observed that an increase in fluorescence emission occurred between excitation wavelengths of 250 to 340 nm, while a decrease in fluorescence emission was noted from 340 to 500 nm. At the excitation wavelength of 340 nm, the photoluminescence spectrum shows a maximum peak.

**High-Resolution Transmission Electron Microscopy (HRTEM) Analysis**

The size, shape, size distribution, and structure of GQDs dialyzed in a cellulose dialysis bag with a cutoff size of 3.5 kDa are shown in Figures 6 (a-d), analyzed by HRTEM. The GQDs were uniformly distributed without agglomeration. The shape and size of the GQDs are depicted in Figure 6a, and the size distribution is shown in Figure 6b. The GQDs exhibited an average size of 18.63 ± 4.24 nm, calculated from the image using ImageJ software (https://imagej.net/software/imagej/).

**Figure 6** – a) HRTEM image of GQD size and shape. b) GQD Size Distribution. c) HRTEM image of GQD lattice spacing. d) Lattice spacing profile.
Figure 6c displays the HRTEM image of the GQDs. From high-resolution HRTEM images, it is observed that the lattice fringes located in the synthesized GQDs revealed a lattice spacing of 0.34 nm corresponding to the lattice plane (002) and were calculated using Gatan software (https://www.gatan.com). Figure 6d shows the lattice spacing profile.

Figure 7 – a) HRTEM image of the lattice spacing of GQDs. b) Selected Area Electron Diffraction (SAED). c) and d) Lattice spacing profile.
Figure 7a shows a lattice spacing of 0.24 nm corresponding to the (100) plane and also a crystalline structure with lattice fringes of 0.21 nm attributed to the (100) plane of graphene. Figure 7b displays the Selected Area Electron Diffraction (SAED) image indicating a simple crystalline structure. These results are in accordance with GQDs prepared by other methods (GAO et al., 2017b)

An agglomeration of nanoparticles was also analyzed (Figures 8a and 8b) through the HRTEM image.

**Figure 8** – a) and b) HRTEM image of the nanoparticle cluster. c) Lattice fringe distance. d) Lattice spacing profile.
Figure 8c indicates a crystalline structure with lattice fringes of 0.21 nm attributed to the (100) plane of graphene (MELO; OSTERLOH, 2018), calculated from the lattice spacing profile (Figure 8d). According to the HRTEM images and the lattice spacing profile, the synthesized GQDs exhibited lattice spacings of 0.21 and 0.34 nm, respectively, corresponding to the graphite lattice planes (100) and (002), confirming results found in the literature (AL GHAMDI; AL-GHAMDI, 2023; WANG et al., 2014).

**X-ray Diffraction (XRD) Analysis**

The structures of GQDs were investigated by X-ray diffraction (XRD). A large number of peaks are observed. The XRD analysis (Figure 9) showed peaks of higher intensity at 2θ of 20.7°, 22.9° and 30.4°; peaks of lower intensity at 2θ of 36°, 39.3°, and 47°. The position and intensity of the diffraction peaks are related to the arrangement of atoms in GQDs. The peak near 2θ of 22.9° indicates the (200) plane of GQDs interacting with the GO-COOH (graphene oxide and carboxylic acid) bond (MAO, 2018).
CONCLUSION

Biomass residues are rich in carbon, serving as a natural and environmentally friendly precursor in the synthesis of graphene quantum dots (GQDs). Moreover, they are cost-effective and abundant, resulting in a sustainable, simple, and economical process. In this study, phosphorus-doped graphene quantum dots (GQDs) were synthesized from the biomass of Moringa oleifera seeds using the hydrothermal method at 210 °C for 24 h. The GQDs exhibited high fluorescence, making them promising for development and applications in bioimaging, biosensors, and drug delivery. Other elements were detected on the surface of GQDs, in addition to phosphorus, indicating self-doping, possibly from elements present in the seed biomass. These self-doped elements may contribute to the optical properties of GQDs. The results showed GQDs with an average size of 18.63 ± 4.24 nm, a lattice spacing of 0.34 nm corresponding to the (002) plane, and high fluorescence (bright blue) under ultraviolet light (365 nm) and dark brown in visible light. Furthermore, the proposed biomass synthesis is cost-effective, uses abundant and renewable raw materials, is environmentally friendly by reducing waste generation through biomass reuse, generates a product with added value, and has technological applications. Thus, we can say that the produced GQDs can be considered self-sustainable.

Figure 9 – X-ray Diffraction (XRD) of GQDs.
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