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# Second-Order Nonlinearity of Graphene Quantum Dots Measured by Hyper-Rayleigh Scattering

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Manoel Leonardo Neto , Renato Silva , [Georges Boudebs](#) \* , [Cid B. De Araújo](#)

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Keywords: Graphene quantum dots; hyper-Rayleigh scattering; hyperpolarizability; biological microscopy imaging



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Article

# Second-Order Nonlinearity of Graphene Quantum Dots Measured by Hyper-Rayleigh Scattering

Manoel L. Silva-Neto <sup>1,†</sup>, Renato Barbosa-Silva <sup>2</sup>, Georges Boudebs <sup>3,\*</sup> and Cid B. de Araújo <sup>2</sup>

<sup>1</sup> Programa de Pós-Graduação em Ciência de Materiais, Universidade Federal de Pernambuco, Recife 50670-901, PE, Brazil

<sup>2</sup> Departamento de Física, Universidade Federal de Pernambuco, Recife 50670-901, PE, Brazil

<sup>3</sup> Univ Angers, LPHIA, SFR MATRIX, F-49000 Angers, France

\* Correspondence: georges.boudebs@univ-angers.fr

† Current address: Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, ON M5S3H6, Canada.

**Abstract:** The first hyperpolarizability of graphene quantum dots (GQDs) suspended in water was determined using the hyper-Rayleigh scattering (HRS) technique. To the best of our knowledge, this is the first application of the HRS technique to characterize GQDs. Two commercial GQDs (Acqua-Cyan and Acqua-Green) with different compositions were studied. The HRS experiments were performed with an excitation laser at 1064 nm. The measured hyperpolarizabilities were  $(1.0 \pm 0.1) \times 10^{-27}$  esu and  $(0.9 \pm 0.1) \times 10^{-27}$  esu for Acqua-Cyan and Acqua-Green, respectively. The results were used to estimate the hyperpolarizability per nanosheet obtained by assuming that each GQDs has five nanosheets with 0.3 nm thickness. The two-level model, used to calculate the static hyperpolarizability per nanosheet, provides the values of  $(2.4 \pm 0.1) \times 10^{-28}$  esu (Acqua-Cyan) and  $(0.5 \pm 0.1) \times 10^{-28}$  esu (Acqua-Green). The origin of the nonlinearity is discussed on the basis of polarized resolved HRS experiments, and an electric quadrupolar behavior with a strong dependence on surface effects is found. The nontoxic characteristics and order of magnitude indicate that these GQDs may be useful for biological microscopy imaging.

**Keywords:** keyword1; keyword2; keyword3

## 1. Introduction

The Hyper-Rayleigh scattering (HRS) technique have been employed for several years to investigate the nonlinear optical properties of molecules in liquid suspensions as well as colloidal metal particles in aqueous solutions, nanocrystals in liquid suspensions and various other materials. It entails directing a laser beam onto a sample and detecting the scattered light, which has double the frequency of the incident light. The intensity of the scattered light is contingent on the first hyperpolarizability of the sample, a measure of its ability to alter the polarization of light. In principle, HRS technique provides a direct assessment of the magnitude of the first hyperpolarizability of molecules and nanoparticles that describes the second-order nonlinear optical (NLO) response. Substantial NLO activity is a crucial prerequisite for emerging technological photonic applications such as photonics and all-optical switching and optical parametric oscillators, that have intensified the quest for NLO-active materials. For example, previous research has underscored the remarkable NLO response in solutions of metallic nanometer-sized particles in solutions. The origin of this response lies in the partial one- or two-photon resonance of the incident laser light used in the experiments, coupled with the particles' strong surface plasmon absorption. Graphene quantum dots (GQDs) are nanometer-size graphene segments that are small enough to exhibit exciton confinement and quantum size effects. Unlike graphene, they present non-zero energy bandgap that is responsible for their characteristic electrical and optical properties.

Generally, the optical absorption spectra of GQDs present a strong peak in the ultraviolet ( $\approx 230$  nm) due to the  $\pi \rightarrow \pi^*$  excitation of the  $\pi$  bonds of aromatic C=C and a weaker peak at  $\approx 300$  nm due to  $n \rightarrow \pi^*$  transitions of C=O bonds [1,2]. Introduction of functional groups in the

GQDs may lead to new absorption features and may change the GQDs optical properties [3,4]. Moreover, since they present molecule-like structure its bandgap can be tuned by changing the GQD size and by surface chemistry. Therefore, the optical properties of GQDs are dependent on the preparation method as well as the functional groups added to the graphene segments [3–8].

When compared to heavy metal-based semiconductor quantum dots that causes concern for in-vivo bioimaging, GQDs due to their low toxicity [9,10] and strong two-photon induced fluorescence have been exploited for biological applications by many authors [3,11,12]. Likewise, there are reported applications of GQDs for energy conversion, environmental monitoring, and electronic sensors for humidity detection, among other applications [3,4,7,13,14]. Recently, Qi *et al.* reported second harmonic generation (SHG) in boron doped-graphene quantum dots (B-GQD) and their application in stem cell imaging and tracking wound healing. These applications were achieved because the doping promoted the break of symmetry in the B-GQD allowing the SHG signal [15].

Although optical absorption, linear luminescence, second harmonic generation and the third-order nonlinearity of GQDs have been studied [5–8,16–19], the second-order nonlinearity, associated to the first hyperpolarizability,  $\beta(2\omega)$ , did not receive much attention. It is true that the experiments to be done are not very easy to set up, such as measuring nonlinear refraction in a 4f setup (see for example [20,21]). Computational results were reported in [22–24] for some GQDs but no experimental result is available. Here we want to estimate the order of magnitude of these coefficients knowing that the manufacturing methods relative to new materials, sometimes different from one lab to another, could influence slightly the result. The absorption spectra of certain DIY items, assumed to be identical, exhibit partial mismatches owing to the presence of significant residues (impurities) within the final solution. Hence, it proves beneficial to examine readily accessible, commercially available samples. Preparations employing hybrid manufacturing methods may yield slightly divergent results but should not alter the core conclusion in this context.

In the present work the hyper-Rayleigh scattering (HRS) technique [25,26] was employed for the first time to determine the effective first hyperpolarizability,  $\langle\beta(2\omega)\rangle_{eff}$ , of GQDs suspended in water. The HRS is an incoherent nonlinear process in which two photons with frequency  $\omega$  are instantaneously combined to generate new photon with frequency  $2\omega$  due to the interaction with a material. Unlike the two-photon induced luminescence process the generation of second harmonic in the HRS process does not require on-resonance excitation of the medium to high energy levels. The HRS technique has been routinely used to study molecules [25–29], metal nanoparticles in liquid suspensions [30–32], and dielectric nanocrystals [33–37]. The first hyperpolarizabilities of molecules and nanoparticles can be determined as well as the origin of the incoherent second harmonic generation (SHG) signal detected.

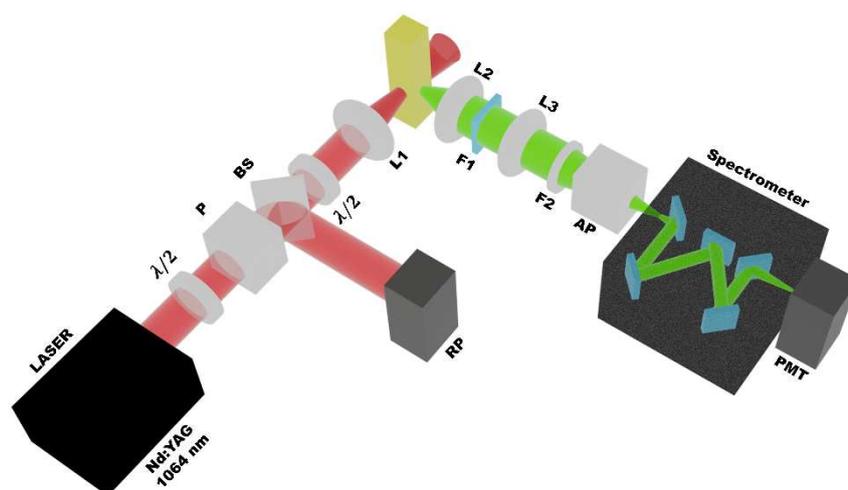
In the present paper we report values for the first hyperpolarizabilities of two different GQDs [38] investigated by measuring the intensity of the incoherent SHG signal upon excitation using a laser off-resonance with absorption transitions of the GQDs. Values of  $\langle\beta(2\omega)\rangle_{eff}$  were determined applying the HRS technique using for calibration para-nitroaniline (*p*-NA) which is the usual reference standard for HRS measurements. To determine the static hyperpolarizability per nanosheet,  $\beta(0)^{NS}$ , the classical two-level model [37,39] was applied in combination with the results for  $\langle\beta(2\omega)\rangle_{eff}$  and considering that each GQD has five nanosheets with thickness of 0.3 nm each. Studying the nonlinear optical properties of these materials can provide insights into their structure and functionality involving the interaction of light with nanoparticles at a nanoscale level.

## 2. Experimental Details

The experiments were performed with commercially available GQDs (Acqua-Green and Acqua-Cyan) purchased from STREM Chemical Incorporation [38,40]. Typically, these quantum dots, composed of graphene nanosheets, have diameters of about  $(5 \pm 1)$  nm and average height of  $(1.5 \pm 0.5)$  nm, corresponding to five graphene sheets. The GQD concentration in the present experiments was  $3.4 \times 10^{16}$  particles/cm<sup>3</sup> for both samples that were suspended in water. Experiments were also performed with more diluted samples to verify their behavior with the laser intensity. The concentration of nanosheets per cm<sup>3</sup> was estimated considering the data sheet provided by the

manufacturer. The absorbance spectra of the samples were measured with a commercial spectrophotometer from 200 to 1500 nm. The photoluminescence (PL) spectra and their temporal evolution were measured using a fast photodetector with time resolution of 1 ns.

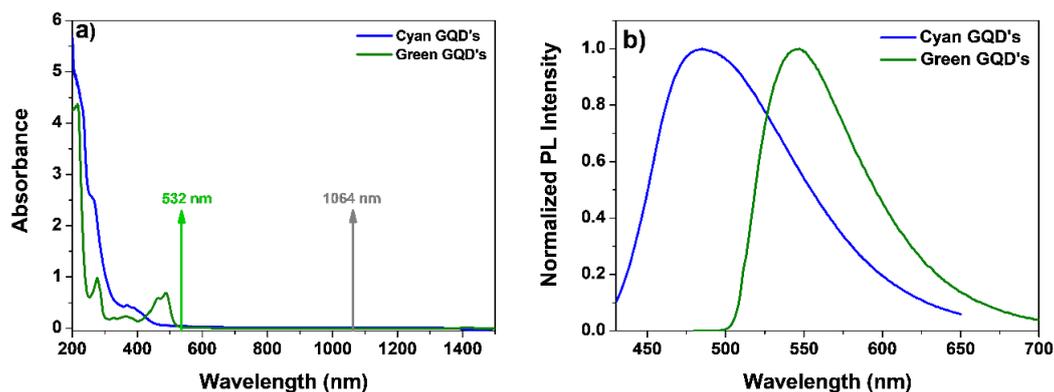
Figure 1 shows the setup used for the HRS experiments. The excitation source was a Q-switched Nd:YAG laser (1064 nm, 6 ns, 10 Hz). A bandpass filter (Filter 1) was used to avoid scattered light from the fundamental beam. A half-wave plate ( $\lambda/2$ ), a polarizer (P), a beam splitter (BS), and a reference photodetector (RP) were employed to control the incident laser intensity on the sample. A 5 cm focal length lens was used to focus the laser beam on the sample that was contained in a 1 cm long quartz cuvette. The HRS signal was collected perpendicularly to the laser beam direction by using two lenses (focal length: 5 cm) to focus the HRS signal on the photomultiplier (PMT) that was coupled to a spectrometer. This set up allowed to analyze the spectral content of the scattered light. Experiments were also performed using an interference filter (F2) centered at 532 nm with full width at half maximum (FWHM) of 8 nm. The photomultiplier was connected to an oscilloscope and a computer for data collection. For temporal analysis of the scattered light the SHG signal was focused on a fast photodetector (response time: 1 ns). Para-nitroaniline (*p*-NA) dissolved in methanol was used as reference standard [25]. For the polarization dependence measurements, a half-wave plate and a polarizer were inserted in the experimental setup and the measurements were performed following the procedure of [30–32]. The second half-wave plate is coupled to a stepper motor controlled by a Labview based program. For the measurements, the stepper motor is rotated in increments of 0-180 degrees. During the measurement, the second polarizer is held in the vertical or horizontal polarization.



**Figure 1.** Experimental setup used for the hyper-Rayleigh scattering experiments. P is a Polarizer, BS is a Beam Splitter, RP is the Reference Photodetector, L1 is a focusing lens, L2 and L3 are collecting lenses, F2 is an interference filter, AP is an Analyzer Polarizer and PMT is a Photomultiplier Tube.

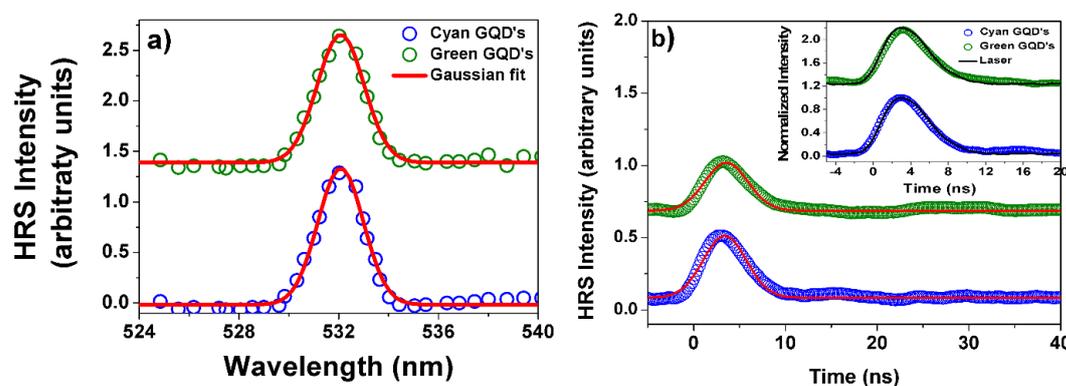
### 3. Results and Discussion

Figure 2a shows the absorbance spectra of both GQDs samples. The absorption bands in the ultraviolet are associated to the  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions. The arrows in Figure 2a indicate the laser and the HRS wavelengths. Notice that for both samples the laser and its second harmonic are off resonance with the absorption bands; therefore, we do not expect a PL signal superimposed on the HRS signal. Figure 2b shows the normalized PL spectra obtained by excitation at 386 nm for the Acqua-Cyan GQDs and 486 nm for the Acqua-Green GQDs using a commercial spectrofluorometer (Fluoromax HORIBA). The temporal decay of the PL signal with maximum at 550 nm (Acqua-Green) and 480 nm (Acqua-Cyan), excited at 850 nm (via two-photon absorption), was fit by a single exponential with decay time of 26 ns (Acqua-Green) and 19 ns (Acqua-Cyan).



**Figure 2.** (a) Optical absorbance spectra; (b) One-photon induced photoluminescence spectra (excitation at 386 nm and 485 nm for Acqua-Cyan and Acqua-Green GQDs, respectively). The normalization factors for luminescence are  $9.5 \times 10^5$  for Acqua-Cyan GQD's and  $1.7 \times 10^6$  Acqua-Green GQD's. Samples concentrations:  $3.4 \times 10^{16}$  particles/cm<sup>3</sup>.

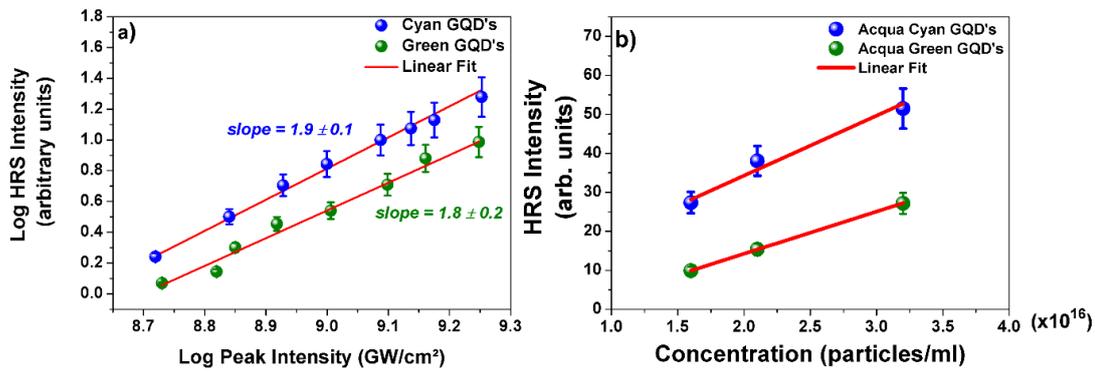
Figure 3 shows the HRS spectra and the temporal behavior of the HRS signal (obtained for a single laser shot). The solid red lines in Figure 3 are the Gaussian fit to the experimental data and the solid black lines on the inset of Figure 3b show the temporal evolution of the excitation laser. The duration for both GQDs was determined to be 4.6 ns from a Gaussian fit. Notice in Figure 3a that the spectra are centered at 532 nm, the second harmonic of the incident laser beam, with the linewidth limited by the spectrometer resolution. The temporal evolution of the HRS signal exhibited in the inset of Figure 3b follows the laser pulse corroborating the above statement that no long-lived luminescence is overlapping with the HRS signal. The HRS is a parametric phenomenon, and the strong correlation between the temporal behavior of the signal and the excitation source supports this assertion. These measurements were performed with laser peak intensity of 12 GW/cm<sup>2</sup>, and the minimum intensity that allowed detection of the HRS signal was 8.0 GW/cm<sup>2</sup>.



**Figure 3.** (a) Spectra of the incoherent second harmonic scattered light for excitation at 1064 nm. The continuous lines are Gaussian fits. (b) The green and blue circles show the temporal HRS data for both samples (single laser shot). The curves are shifted in the vertical direction to allow better visualization. The solid red lines are Gaussian fits to the data. The inset in Figure 3b shows a comparison between the HRS signal and the excitation source. The green and blue circles in the inset shows the temporal data for both samples and the black line illustrates the laser pulse temporal profile. The temporal response of the photodetector is 1ns.

The HRS signal is due to the nonlinear scattering by the randomly oriented GQDs and water molecules. The scattered intensity, at the second harmonic frequency of the incident laser beam, is described by  $I(2\omega) = g \sum_c N_c \langle \beta_c^2(2\omega) \rangle I^2(\omega)$ , where  $I(\omega)$  is the laser intensity,  $\langle \beta_c^2(2\omega) \rangle = \langle \beta_c^2(2\omega; \omega; \omega) \rangle$  is the orientational average of the first hyperpolarizability and the sub-index represents the constituents of the suspension. The symbol  $\langle \rangle$  indicates orientational average. The factor  $g$  depends on the scattering geometry and contains information on the transformation of coordinates from the GQD to the laboratory reference system [33–37].

Figure 4a shows plots of the HRS signal,  $S(2\omega) \propto I(2\omega)$ , versus the incident laser intensity where a quadratic dependence with  $I(\omega)$  was verified. Figure 4b shows linear dependence of  $S(2\omega)$  versus the GQDs concentration,  $N_{GQD}$ , that confirms the contribution of isolated GQDs to the HRS signal. Moreover, we recall that aggregation of GQDs would redshift the linear absorption spectrum, a behavior that was not observed for the various concentration used.



**Figure 4.** (a) HRS intensity signal,  $S(2\omega) \propto I(2\omega)$ , versus the laser intensity for both samples. The slopes of the straight lines were  $1.9 \pm 0.1$  (Acqua-Cyan) and  $1.8 \pm 0.2$  (Acqua-Green). (b) Dependence of  $S(2\omega)$  with the concentration of GQDs per ml. The error bars represent 90% of confidence. The maximum peak intensity of excitation source was  $1.6 \text{ GW/cm}^2$ .

Since the samples consist of GQDs with different dimensions, the experiments provided effective values for the GQD hyperpolarizability,  $\langle \beta^2(2\omega) \rangle_{eff}$ . The external reference method [41], using *p*-NA dissolved in methanol as the reference standard, was applied to measure  $\langle \beta(2\omega) \rangle_{eff}$  and the numerical values were obtained using the equation:

$$\langle \beta^2(2\omega) \rangle_{eff} = \left[ \frac{S_{GQD}(2\omega)}{S_{pNA}(2\omega)} \left\{ \frac{N_{pNA} \langle \beta_{pNA}^2 \rangle + N_{mtOH} \langle \beta_{mtOH}^2 \rangle}{N_{GQD}} \right\} - \frac{N_{water} \langle \beta_{water}^2 \rangle}{N_{GQD}} \right], \quad (1)$$

where *mtOH* refers to methanol,  $\beta_{water} = 0.087 \times 10^{-30} \text{ esu}$  [41],  $N_{water} = 5.0 \times 10^{22} \text{ molecules/cm}^3$ ,  $\beta_{pNA} = 30 \times 10^{-30} \text{ esu}$  [24, 25]

],  $N_{pNA} = 1.0 \times 10^{19} \text{ molecules/cm}^3$ ,  $\beta_{mtOH} = 0.69 \times 10^{-30} \text{ esu}$  [25],  $N_{mtOH} = 1.50 \times 10^{22} \text{ molecules/cm}^3$ . The concentration of GQDs was  $N_{GQD} = 3.4 \times 10^{16} \text{ particles/cm}^3$  for both samples that were suspended in water. Considering the results obtained for  $S_{GQD}(2\omega)/S_{pNA}(2\omega)$  (see Figure 4a), the effective values of  $\langle \beta(2\omega) \rangle_{eff}$  were  $(1.0 \pm 0.1) \times 10^{-27} \text{ esu}$  and  $(0.9 \pm 0.1) \times 10^{-27} \text{ esu}$  for Acqua-Cyan and Acqua-Green, respectively. The quantity  $\langle \beta^2(2\omega) \rangle_{eff}$  is an effective value contributed by GQDs with different number of nanosheets. Consequently, we considered the following approach to estimate the hyperpolarizability per nanosheet,  $\langle \beta^2(2\omega) \rangle_{NS}$ . First we considered the distribution of GQDs sizes shown in the Appendix A; then, assuming the nanosheet thickness 1.5 nm, and a Gaussian distribution of GQDs sizes represented by  $P(n) \propto \exp(-n^2/n_{av}^2)$ , where  $n$  is the number of nanosheets in a GQD, we obtain  $10 \leq n \leq 60$  for Acqua-Cyan and  $15 \leq n \leq 57$  for Acqua Green. The value of  $n_{av}$  is obtained from the AFM measurements given in the SI. The value of  $\langle \beta^2(2\omega) \rangle_{NS}$  is determined by  $\langle \beta^2(2\omega) \rangle_{eff} = \langle \beta^2(2\omega) \rangle_{NS} \{ \sum_1^n n P(n) / \sum_1^n P(n) \}$ . Using

these results, we could estimate the effective first hyperpolarizability per nanosheet,  $\langle\beta(2\omega)\rangle_{eff}^{NS} \sim 10^{-28} \text{ esu}$ , considering that each GQDs have five nanosheets with 0.3 nm of thickness.

The two-level model [39,42] was used to relate  $\langle\beta(2\omega)\rangle_{eff}^{NS}$  with the first static hyperpolarizability per nanosheet,  $\beta(0)^{NS}$ , in each GQD. First, it is necessary to estimate the static hyperpolarizability,  $\beta(0)$ , for the samples. Then, considering the amount of graphene sheets per GQD,  $\beta(0)^{NS}$  is estimated. The following expression was obtained from [39,42]

$$\beta(0) = \left(1 - 4 \frac{\omega^2}{\omega_{eg}^2}\right) \left(1 - \frac{\omega^2}{\omega_{eg}^2}\right) \langle\beta^2(2\omega)\rangle_{NS}, \quad (2)$$

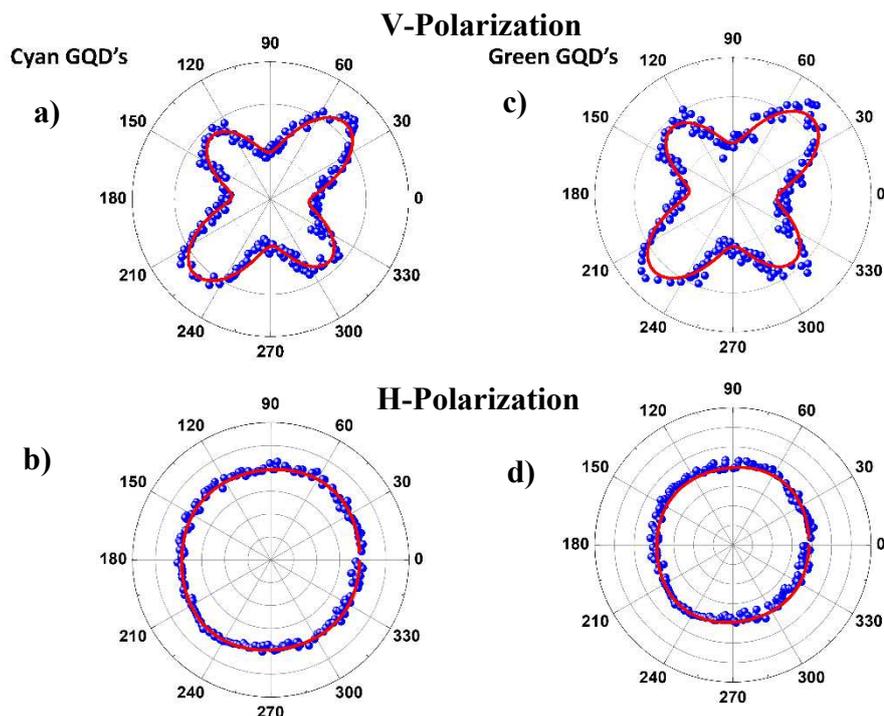
where  $\omega_{eg} = 22222 \text{ cm}^{-1}$  (Acqua-Cyan) and  $\omega_{eg} = 19608 \text{ cm}^{-1}$  (Acqua-Green). The frequency  $\omega_{eg}$  correspond to the smallest band frequency in the absorption spectrum.

The results for  $\beta(0)^{NS}$  are  $(2.4 \pm 0.1) \times 10^{-28} \text{ esu}$  and  $(0.5 \pm 0.1) \times 10^{-28} \text{ esu}$  for Acqua-Cyan and Acqua-Green, respectively. Notice that the value of  $\langle\beta(2\omega)\rangle_{eff}^{NS}$  is approximately equal for both samples but the values of  $\beta(0)^{NS}$  are different because of the different laser frequency detuning with respect to the first excited resonance frequency  $\omega_{eg}$ .

We recall that imaging measurements have been performed using GQDs exploiting their large PL, but SHG microscopy presents an important advantage with respect to PL experiments because lasers operating in a large infrared wavelength range can be used since no resonance with excited states is required. This is a benefit because one may select the laser wavelength according to the transparency window of the biological system of interest. Obviously, care must be taken not to damage living cells and the underlying nanoparticles with too high intensities, as can often happen with thin films irradiated with pulsed lasers in the picosecond regime [43]. To characterize the origin of the HRS signal, polarization-resolved experiments were performed following the usual procedure [30–32]. The laser beam was linearly polarized and the angle  $\gamma$ , between the optical field and the vertical direction, was varied from 0 to  $2\pi$ . The polarization of the HRS signal either in the vertical direction or parallel to the beam propagation axis, was measured. At this point, due to the insertion of half-wave plate and a polarizer (see Figure 1), the laser intensity was  $\approx 0.7 \text{ GW/cm}^2$ . The results are shown in Figure 5 and to fit the data it was used the expression:

$$I_{HRS}^{\Gamma} = a^{\Gamma} \cos^4 \gamma + b^{\Gamma} \cos^2 \gamma \sin^2 \gamma + c^{\Gamma} \sin^4 \gamma + d^{\Gamma} \cos^3 \gamma \sin \gamma + e^{\Gamma} \cos \gamma \sin^3 \gamma, \quad (2)$$

where  $I_{HRS}^{\Gamma}$  is the hyper – Rayleigh scattering intensity and  $\Gamma$  refers to the horizontal (H) or vertical (V) polarization. The coefficients  $a^{\Gamma}$ ,  $b^{\Gamma}$ ,  $c^{\Gamma}$ ,  $d^{\Gamma}$ ,  $e^{\Gamma}$ , were obtained from the fit. The coefficient of multipolarity,  $\varsigma^{\Gamma} = 1 - (a^{\Gamma} + c^{\Gamma} / b^{\Gamma})$ , were also determined and their values are indicated in Table 1.



**Figure 5.** (a) Vertical polarization from Acqua-Cyan GQDs. (b) Horizontal polarization from Acqua-Cyan GQDs. (c) Vertical polarization from Acqua-Green GQDs. (d) Horizontal polarization from Acqua-Green GQDs.

**Table 1.** Depolarization and multipolarity parameters for both GQDs samples.

Sample Parameters	Cyan GQD V polarization	Green GQD V polarization
$a^\Gamma$	0.87	0.79
$b^\Gamma$	0.56	0.59
$c^\Gamma$	0.14	0.19
$\zeta^\Gamma$	0.81	0.66

The origin of the second-order nonlinear effect arising from nanoparticles (NPs) with crystalline structure presenting inversion symmetry was discussed in [44–47]. Two important cases to consider are: NPs with noncentrosymmetrical shape and NPs with centrosymmetric shape [47]. In the former, SHG is due to the induced electric dipole in the NPs volume and in the interfaces. In the latter, the induced electric dipole does not occur because of the inversion symmetry in the crystalline structure of the NPs. Then, besides the contributions from the interfaces an important mechanism of SHG is related to the field's gradients occurring within the NPs bulk [46,47]. However, it is difficult to synthesize NPs with perfect shape and therefore, in real NPs there is a competition between the two main cases above mentioned. Thereby, the coefficient of multipolarity,  $\zeta^V$ , is applied to distinguish both contributions [47]. The case for pure centrosymmetrical shape requires  $a^V = c^V = 0$  and the case for the pure noncentrosymmetrical shapes requires  $b^V = a^V + c^V$ . As one can see in Table 1, the values of  $\zeta^V$  for both samples suggest the dominant mechanism for harmonic generation is the field retardation in the NPs. This fact should be expected in our samples since there is no guarantee that the nanosheets are perfectly compacted and discordances should also occur between the sheets. As a result of such discordances, discontinuities in the electric field should occur in the NPs volume and the quadrupole contributions become dominant.

Ongoing research is focused on assessing the toxicity of graphene-family nanoparticles, with specific attention to GQDs [48]. Factors such as particle size, synthesis methods, and chemical doping

play a role in determining their toxicity, both in vivo and in terms of cytotoxicity [49]. Some researchers argue that GQDs, composed primarily of organic materials, exhibit low toxicity and high biocompatibility, providing an advantage over semiconductor quantum dots. In vitro studies using cell cultures have shown minimal bad effects of GQDs on human cell viability [50]. Fluorescence imaging, a non-radioactive method, allows the visualization of morphological details in various biological specimens, from living cells to animals. Notably, GQDs, unlike many semiconductor quantum dots containing heavy metals, consist of graphene lattices containing light elements. Because they are primarily made of carbon, the most abundant element in biological systems, GQDs are generally considered biocompatible.

#### 4. Summary and Conclusions

In summary, we reported the incoherent second harmonic generation by aqueous suspensions of graphene quantum dots excited at 1064 nm. The hyper-Rayleigh scattering technique was applied for determination of the first hyperpolarizability associated to the GQDs and the results indicate  $\langle\beta(2\omega)\rangle_{eff}^{NS} \sim 10^{-28}$  esu per nanosheet. The spectra of the scattered light (centered at the second harmonic frequency) as well as their fast temporal behavior prove that the signals detected are due to the second harmonic generation and not due to luminescence induced by two-photon absorption. From the hyper-Rayleigh scattering data, using a two-level model, we could determine the static hyperpolarizability per nanosheet,  $\beta(0)^{NS}$ . The values obtained were  $(2.4 \pm 0.1) \times 10^{-28}$  esu for Acqua-Cyan and  $(0.5 \pm 0.1) \times 10^{-28}$  esu and Acqua-Green. In the future, could we develop a straightforward theoretical model for predicting the second-order coefficients of optical nonlinearity, much like we can for third order optical nonlinear coefficients? Theoretical forecasts for the nonlinear refractive index in infrared glasses are achievable [51]. Anyway, the order of magnitude of the GQDs hyperpolarizabilities indicates that the two samples studied have potential for application in microscopy of biological systems. Of course, the possible nontoxicity of the GQDs to living cells is also a relevant characteristic in comparison with many semiconductor quantum dots being used.

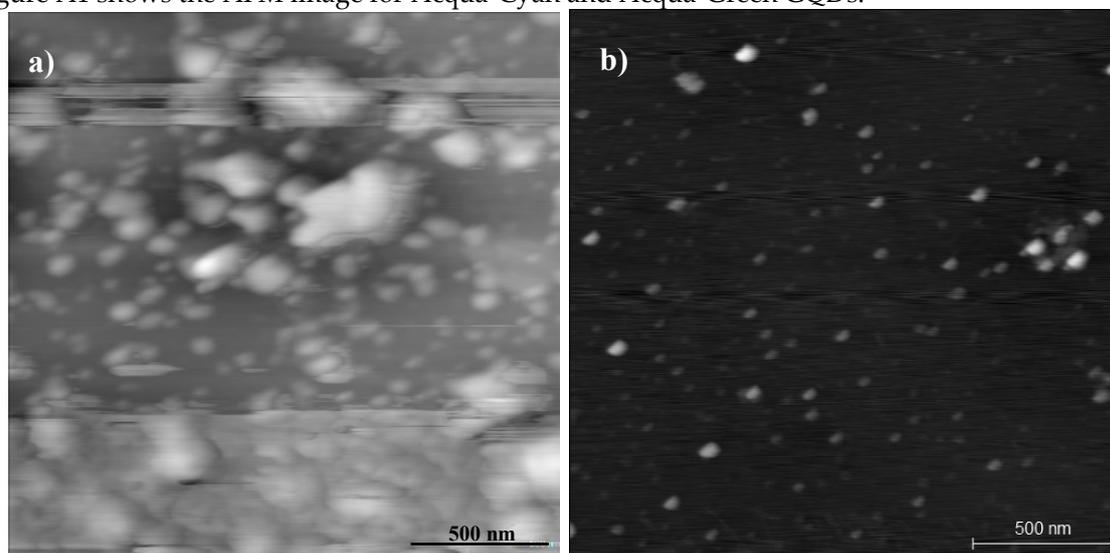
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**Conflicts of Interest:** The authors declare no conflicts of interest.

## Appendix A

### Characterisation of the QDs

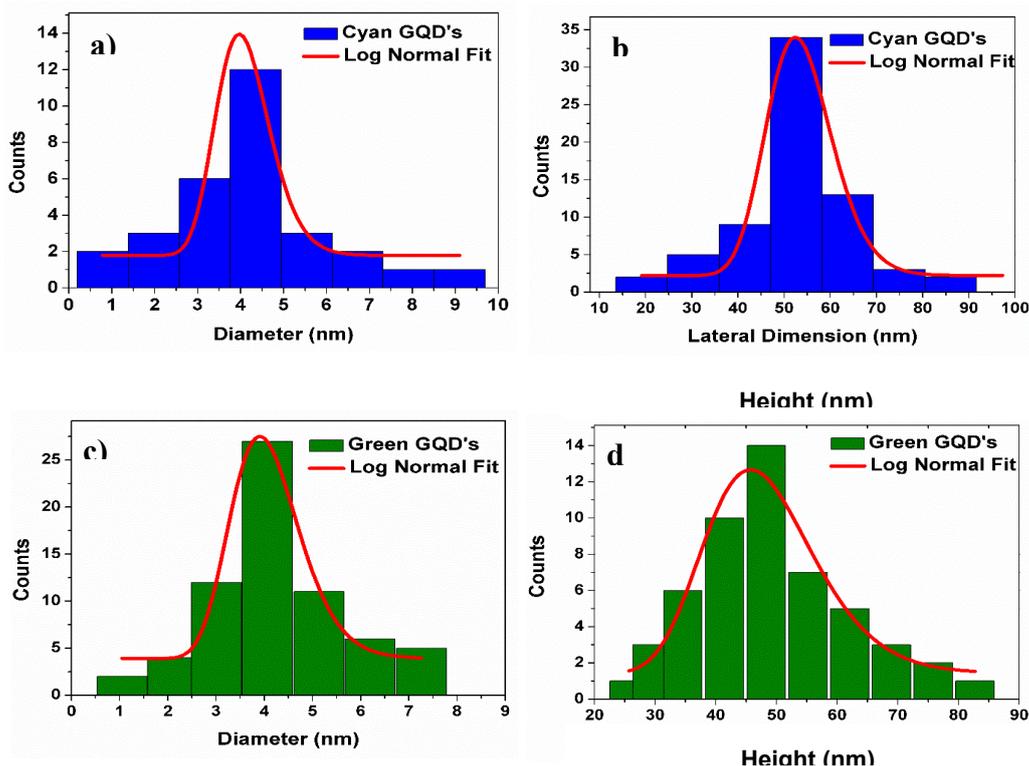
The QDs dimensions were performed by Atomic Force Microscope (AFM) measurements. Figure A1 shows the AFM image for Acqua-Cyan and Acqua-Green QDs.



**Figure A1.** AFM images: (a) Acqua-Cyan QDs; (b) Acqua-Green QDs. The scale is 500 nm for both images.

From the AFM images it was possible to make a histogram of diameter and height of the QDs, as shown below.

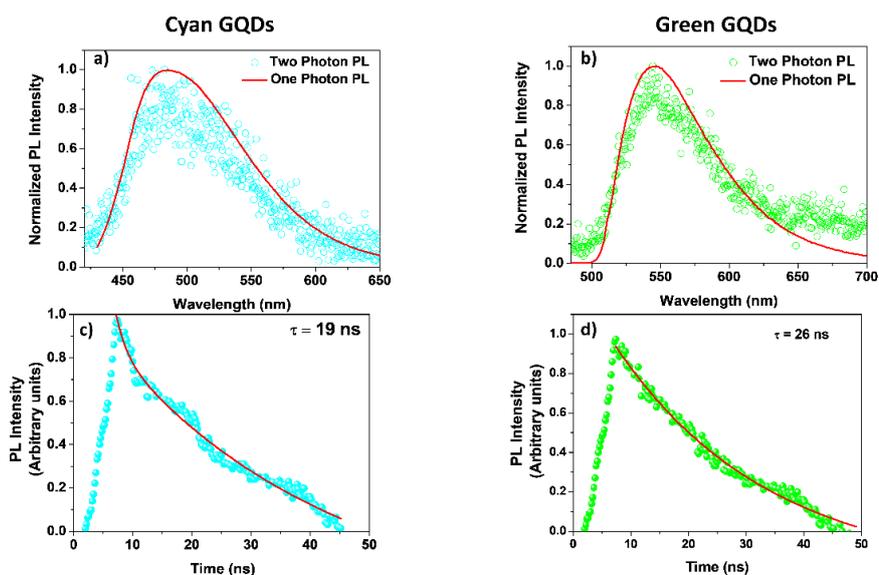
Figure A2 shows the histogram for diameter and height of Acqua-Cyan and Acqua-Green QDs. The log normal fit indicates a maximum of the distribution for diameter of 4 nm and a height of 52 nm for Acqua-Cyan QDs. For the Acqua-Green QDs, the size distribution is peaked for diameter of 3.9 nm and the height of 46 nm.



**Figure A2.** (a) Diameter and (b) lateral size for Acqua-Cyan QDs; (c) Diameter and (d) height for Acqua-Green QDs.

Using the size distribution, we could calculate the concentration of QDs per  $\text{cm}^3$ . The value obtained was  $0.9 \times 10^{15} \text{ QDs}/\text{cm}^3$  for Acqua-Cyan and  $1 \times 10^{15} \text{ QDs}/\text{cm}^3$  for Acqua-Green.

Two photon absorption induced luminescence (2PL) with excitation in 850 nm was performed for both QD's. The Figure 3a) and 3b) show the 1PL (solid lines) and 2PL (dots) spectra for both samples.



**Figure A3.** Normalized 1PL and 2PL Intensity (a) Acqua-Cyan QD's and (b) Acqua Green QD's. 2PL lifetime for (c) Acqua-Cyan QD's and Acqua - Green QD's.

In the Figures A3c and A3d we show the temporal behavior of 2PL, with fluorescence lifetime of 19 ns for Acqua-Cyan QD's and 26 ns for Green QD's.

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