Tuning the antioxidant activity of graphene quantum dots: protective nanomaterials against dye decoloration

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Abstract

The antioxidant activity of graphene quantum dots (GQD) with different chemical composition and sp^2-hybridized carbon content has been evaluated and compared to that of a standard antioxidant, ascorbic acid. GQD were prepared by top down and bottom up synthetic approaches from three different precursors (carbon black, glucose and pyrene) in order to vary significantly the chemical composition, electron density and sp^2-hybridized carbon content. For a given radical, the three types of GQD exhibited very different radical scavenging activity (RSA). Moreover, the RSA varied with the type of free radical and reactive oxygen species (ROS) tested, indicating different radical inhibition mechanisms. Overall, GQD with strong hydrogen donor behavior and large content of sp^2–hybridized carbon domains were the most effective radical scavengers. Thus, highly graphitic GQD with abundant edge functional groups produced from pyrene exhibited an extraordinary high antioxidant activity, with inhibition effective concentrations much lower than those of ascorbic acid. The great potential of highly antioxidant GQD as protective films against organic dye decoloration by ROS was demonstrated with two model target molecules, methylene blue and rhodamine B.

1. Introduction

Reactive oxygen species (ROS) play an essential role in damage of biological structures, degradation of chemical products and polymers, food spoilage, etc [1-3]. Among ROS, the hydroxyl radical is the most reactive one and the major inducer of oxidative stress in pathological processes. Therefore, antioxidants and free radical scavengers are crucial for applications in sectors as diverse as health, food, packaging, cosmetics, corrosion protection, etc. In general, compounds with long conjugated C=C chains such as carotenoids are excellent free radical scavengers [4]. By analogy, different types of carbon nanomaterials have been explored as ROS and free-radical scavengers such as fullerene derivatives, whose high superoxide quenching activity was ascribed to a combined electron transfer and adduct formation mechanism [5]. Later on, 1D carbon nanotubes (CNT) with different structure and composition (diameter, length, helicity and functionalization) were explored as free radical scavengers by several groups [6-8], who demonstrated both experimentally and theoretically a remarkable scavenging activity of CNT for hydroxyl and superoxide radicals. As in the case of fullerenes, reactions between CNT and free radicals were interpreted in terms of two different mechanisms: electron transfer and adduct formation by radical addition to sp^2–hybridized CNT network as a result of the high CNT electron donor capability. More recently, different 2D graphene derivatives have also been investigated as antioxidants and their oxidation protection activity was reported to be a combination of antioxidant (UV absorption) activity and radical scavenging [9].

Carbon dots (C-dots) are the latest type of carbon nanostructure that has received attention with regard to their antioxidant activity. Interestingly 0D C-dots are known to be both electron donors and acceptors and hence, together with an antioxidant activity, they can also generate ROS under UV and visible light irradiation [10,11]. This dual activity of C-dots as antioxidants and pro-oxidants was first proposed and studied by Christensen et al. [12], who

established that C-dots can be used for photocatalytic, photodynamic and antioxidant applications. Since then, most research studies have focused on their potential use as pro-oxidants or photosensitizers rather than as antioxidants. Nevertheless, few recent reports have shown that C-dots synthesized by hydrothermal methods from different precursors like date molasses [13], garlic [14] and citric acid [15] exhibit in vitro ROS scavenging activity, in some cases comparable with standard antioxidant molecules. Doping C-dots with heteroatoms such as Nitrogen or Sulfur increases their electron density and consequently their antioxidant activity. This high antioxidant property of some C-dots has proven efficient enough to protect cells against oxidative stress [13,14]. The radical annihilation mechanism proposed in C-dots was a combination of hydrogen donation from functional groups and electron transfer.

Compared to spherical C-dots, the antioxidant activity of planar graphene quantum dots (GQD) has been rarely investigated despite a likely higher activity associated with their sp^2–hybridized carbon network. To the best of our knowledge, there is only a very recent report showing that GQD produced by chemical oxidation of graphite exhibit both pro-oxidant and anti-oxidant activity in the presence or absence of light respectively [16]. As seen in other carbon nanostructures, the antioxidant activity is expected to depend largely on GQD composition and carbon network hybridization. Recent progress in GQD synthetic methods has allowed tuning their size, thickness, chemical composition, carbon hybridization, band gap, which in turn determine their electronic properties and chemical reactivity [17]. Motivated by the scarce knowledge on the antioxidant properties of GQD, in this work we have evaluated the RSA of GQD with different chemical composition synthesized by bottom up and top-down methods from different precursors, specifically: graphene oxide quantum dots prepared by oxidative exfoliation of carbon black nanoparticles (GQDv), N-doped GQD by hydrothermal treatment of glucose in ammonia solution (GQDg) and highly crystalline GQD by hydrothermal condensation of pyrene (GQDp). To the best of our knowledge, there

are no studies comparing the antioxidant activity of GQD with different chemical composition. In this context, the present work has revealed notable differences between the three types of GQD, allowing us to identify materials with higher activity than well-known standard antioxidants like ascorbic acid.

2. Experimental

2.1. Materials

Vulcan® XC-72 carbon black was purchased from Cabot Corporation. Pyrene, hydrochloric acid, ammonia, hydrazine monohydrate, nitric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium permanganate, L-ascorbic acid, methanol and hydrogen peroxide were purchased from Sigma Aldrich. Methylene blue and Rhodamine B were purchased from Scharlau and Acros Organics respectively. All chemicals were of analytical grade and used without further purification. Aqueous solutions were prepared with ultrapure water of Synthesis A10 from Millipore (18Ω.cm) (Massachusetts, USA).

2.2. Synthesis of GQD

GQDp were synthesized by hydrothermal condensation of pyrene, according to a method reported elsewhere [18]. Briefly, in a typical procedure pyrene (1g) was nitrated into 1,3,6-trinitropyrene in 65% wt. HNO_3 (80 mL) under refluxing and stirring for 16h. After cooled to room temperature, the mixture was diluted with deionized (DI) water and filtered through a 0.22 μm nylon membrane. Part of the resultant 1,3,6-trinitropyrene (1g) was dispersed by ultrasonication in a DI water solution (0.1 L) containing 0.4 M ammonia and 1.5 M hydrazine hydrate for 1 h. The suspension was transferred to a Teflon-lined autoclave and heated at 200ºC for 8 h. After cooled to room temperature, the GQDp solution was filtered through a 0.22 μm nylon membrane to remove insoluble carbon product, and further dialysed in a

dialysis bag (retained molecular weight: 3500 Da) for 1 day to remove salt and small molecules.

GQDv were synthesized by oxidative exfoliation of carbon black particles adapting a method reported elsewhere [19]. Typically Vulcan® XC-72 carbon black (1 g) was refluxed in 250 mL of 6M HNO_3 for 24 h. After cooling to room temperature, the suspension was centrifuged (3500 rpm) for 30 min to obtain a supernatant and a sediment. The supernatant was heated to evaporate the water and nitric acid and a reddish-brown solid (GQDv) was obtained.

GQDg were synthesized by hydrothermal pyrolysis of glucose modifying a previously reported protocol for microwave-assisted pyrolysis [20]. Briefly, glucose (1.5 g) was dissolved in a DI water solution (50 mL) containing 16.7 M ammonia. The solution was transferred to a Teflon-lined autoclave and heated at 180ºC for 1 h. After cooled to room temperature, the GQDg solution was filtered through a 0.22 μm nylon membrane and further dialysed in a dialysis bag (retained molecular weight: 3500 Da) for 1 day.

2.3. Material characterization

TEM images of GQD on carbon-coated copper grids were taken using a TECNAI G2 20 TWIN (FEI) transmission electron microscope, operating at an accelerating voltage of 200 KV in a bright-field image mode. AFM images on mica substrates were obtained in a tapping mode at room temperature using a scanning probe microscope (Molecular Imagings PicoScan) equipped with a Nanosensors tips/cantilever, at a resonance frequency of 330 kHz and a spring constant of about 42 N/m with a tip nominal radius lower than 7 nm. FTIR spectra were recorded at room temperature using a Jasco 4100LE spectrophotometer with 4 cm^{-1} resolution in a wavenumber range from 4000 to 400 cm^{-1}. XPS experiments were performed in a SPECS Sage HR 100 spectrometer with a non-monochromatic X-ray source (Magnesium Kα line of 1253.6 eV energy and a power applied of 250 W) and calibrated using

the $3d_{5/2}$ line of Ag with a full width at half maximum (FWHM) of 1.1 eV. Fluorescence spectra were measured in a Varian Cary Eclipse fluorescence spectrophotometer.

2.4. Antioxidant activity and radical scavenging assays

DPPH· free radical assay. A procedure similar to that reported by Fukumoto et al [21] was used, where the concentration of DPPH· was monitored by UV-Vis absorption spectroscopy at 515 nm in a Biotek microplate reader. In the presence of antioxidants in the darkness DPPH· changes its color from purple to yellow. Freshly prepared DPPH· (100 µM) solution in absolute methanol was mixed with methanol solutions of different GQD (1-500 µg/mL) and incubated in the dark for 1h (total volume 1 mL). The RSA of each GQD at different concentrations was evaluated from the absorbance decrease at 515 nm for GQD-DPPH· mixtures (A_{GQD}) with respect to the absorbance of the control (GQD-free DPPH· solution, A_c) as RSA (%) = $(A_c - A_{GQD})/A_c \times 100$. Ascorbic acid was used as standard. Measurements were replicated three times.

Hydroxyl radical scavenging assay. Rhodamine B (RHB) was used as model colorimetric target molecule for oxidant attack and the protective effect of GQD on RHB decoloration in presence of ·OH was taken as a measure of their antioxidant activity. Hydroxyl radicals were generated by photolysis of H_2O_2. Vials containing mixtures (1 mL) of RHB (0.02 mM), GQD (1-1000 µg/mL) and H_2O_2 (0.03 M) were irradiated with 302 nm UV light in a benchtop UV transilluminator (UVP M-20V) for 1h. After 1h of UV exposure, the absorbance of the different mixtures was monitored by UV-Vis absorption spectroscopy in a Biotek microplate reader. In absence of antioxidant, magenta RHB solutions are totally bleached by reaction with photogenerated ·OH. The RSA of each GQD at different concentrations was assessed from its protective effect against RHB decoloration as RSA (%) = $A_{GQD}/A_c \times 100$, where A_{GQD} and A_c are the absorbance at 554 nm of GQD-RHB-H_2O_2 mixtures and the control

(GQD and H_2O_2-free RHB solution) respectively after 1h of UV exposure. At the highest GQD concentrations, the contribution of GQD absorbance at this wavelength was subtracted. Ascorbic acid was used as standard. Measurements were replicated three times.

$KMnO_4$ reduction assay. The antioxidant activity of GQD was also evaluated with the $KMnO_4$ reduction assay following the protocol by Das et al. [13]. Acidified (pH 3) $KMnO_4$ solutions (100 μM) were mixed with aqueous solutions of different GQD (0.1-750 μg/mL) and incubated in the dark for 1h (total volume 1 mL). After incubation absorbance of the different mixtures was monitored by UV-Vis absorption spectroscopy at 515 nm in a Biotek microplate reader. Upon reduction, $KMnO_4$ solutions change from purple to colourless. The RSA of each GQD at different concentrations was evaluated from the absorbance decrease at 515 nm for GQD-$KMnO_4$ mixtures with respect to the absorbance of the control (GQD-free $KMnO_4$ solution, A_c) as RSA (%) = $(A_c - A_{GQD})/A_c \times 100$. Ascorbic acid was used as standard. Measurements were replicated three times.

2.5. Preparation and characterization of dye and GQD-coated membranes

Nylon (Magna, 0.22 μm pore size) membranes were first coated with GQD by immersion for 30 minutes in aqueous solutions with different GQD concentrations (0.02-0.2 mg/mL). The membranes were thoroughly rinsed with DI water and dried at room temperature. In a second step, GQD-coated membranes were immersed in methylene blue (0.05 mg/mL) or RHB (0.05 mg/mL) aqueous solutions containing 0.01 M H_2O_2 for 90 minutes. Dye decoloration on GQD-coated membranes was monitored with a Konica Minolta CM-2600D colorimeter. Color differences in CIELAB coordinates were estimated as $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$.

3. Results and discussion

3.1. GQD characterization

The three types of GQD whose antioxidant properties are investigated in this work were prepared using synthetic approaches known to yield carbon nanomaterials with different composition and carbon hybridization ratios. Specifically, both bottom-up and top-down methods and starting carbon precursors (carbon black, glucose and pyrene) were used with the aim of analyzing the effect of GQD synthesis method and resulting GQD composition on their antioxidant activity. The synthetic protocols were adapted to yield GQD with similar size (as shown below) to avoid changing several structural parameters in the study. Throughout the text we will refer to GQD produced by oxidative exfoliation of Vulcan® XC-72 nanoparticles as GQDv, by hydrothermal pyrolysis of glucose in ammonia solutions as GQDg and by hydrothermal condensation of pyrene as GQDp. First, the lateral size and thickness distribution of the three types of GQD was investigated by TEM and AFM, respectively. As shown in TEM images (Fig. 1), there are no remarkable differences in lateral size distribution between the three GQD samples, with GQDp having the most uniform size distribution: the average lateral size was 2.7 ± 0.4 nm (GQDp), 2.2 ± 0.8 nm (GQDg) and 2.8 ± 0.7 nm (GQDv). The thickness distribution of the three types of GQD determined by AFM (Fig. 1) was: 1.5 ± 0.4 nm (GQDp), 2.2 ± 0.6 (GQDg) and 1.0 ± 0.3 (GQDv), indicating that most of the GQD were composed of 2-6 layers. In general, GQDv produced by a top-down approach comprise less (1-3) layers as a result of their higher functionalization degree (vide infra) that hinders layer stacking.

Next the chemical composition of the GQD was examined by XPS. The general survey spectra of the three GQD samples show the characteristic O 1s, N 1s and C 1s photoelectron peaks from oxygen, nitrogen and carbon and their respective O KLL, N KLL and C KLL Auger peaks (supporting information, Fig. S1). The chemical composition of each GQD estimated from the XPS survey spectrum is summarized in Table 1. As expected, GQDv have the highest oxygen content (37.0 at.%) as a result of the strong oxidative conditions used in

the synthesis. The nitrogen and oxygen content in GQDg and GQDp originate from the precursors and media (glucose, 1,3,6-trinitropyrene, hydrazine and ammonia) used in the hydrothermal pyrolysis and condensation reactions respectively.

Table 1- Chemical composition of GQD determined by XPS

	C (at. %)	O (at. %)	N (at. %)
GQDp	65.7	19.3	15.0
GQDg	63.8	25.5	10.8
GQDv	56.7	37.0	6.3

The high-resolution C 1s, O 1s and N 1s spectra provide further information about the functional groups present in each type of GQD (Fig. 2). The C 1s fitted spectra of GQDg and GQDv (Fig. 2a, 2d) show the peaks associated to C-C bonds at around 284.8 eV while GQDp (Fig. 2g) with a more graphitic nature presents the sp^2 C-C bonds at around 284.4 eV [22]. There are other components between 286.1 eV and 287.6 eV associated to C-N and C-O bonds [22,23] present in different relative content in each GQD. Despite the high oxygen content in GQDp (19.3%), the C-O peak in the C 1s spectrum (Fig. 2g) has small intensity, as noticed also by other authors for similar GQD [18]. GQDv shows additional components at around 288.4 and 291.8 eV associated with C=O and O-C=O bonds [23] while GQDp shows the characteristic π- π^* shake-up of graphitic materials at around 290.0 eV [22]. The O 1s fitted spectra of the three GQD (Fig. 2b, 2e and 2h) show the peaks associated to the C-O bonds at around 532.5 eV. Additionally, the O 1s spectra of GQDg and GQDv (Fig. 2b and 2e) exhibit the presence of N-O bonds at around 531.6 eV [24] from pyridinic N-oxide sites in GQDg and chemisorbed nitric acid residue from the synthesis in GQDv. The O 1s spectra of GQDv also shows the presence of C=O bonds at 531.2 eV [22], which is probably overlapped with the peak from the N-O bonds. The fitted N 1s spectrum of the GQDg sample (Fig. 2c) shows four components: the peaks associated to pyridinic, pyrrolic and graphitic N-C bonds at

around 399.2, 400.1 and 401.4 eV [20] and a minor component at around 405.8 eV from N-O bonds in the pyridinic N-oxide sites [22,23]. The N 1s spectra of GDQv and GQDp (Figs. 2f and 2i) show only three components associated to N-H and C-N bonds at around 399.5 and 401.2 eV, respectively [18,19] and another component at around 405.8 attributed to N-O bonds in chemisorbed nitrogen.

The chemical composition determined by XPS was in agreement with the functional groups identified by FTIR spectroscopy (supporting information, Fig. S2) for the different GQD (chemical structures are depicted in Fig. 2). The main bands in the FTIR spectra of the three GQD can be assigned to the following vibrations: the C=C vibration at 1630 cm^{-1}, the C-OH vibration at 1080 cm^{-1} and the O-H vibration in the 3200-3360 cm^{-1} range. In addition, the spectrum of GQDp exhibits the N-H vibration band from the $NHNH_2$ moiety at 3220 cm^{-1}, the spectrum of GQDg shows the C-N, C=N and N-H vibration bands at 1430, 1660 and 3200 cm^{-1}, respectively and GQDv features the C-O-C, OC-OH and C=O vibration bands at 1213, 1450 and 1720 cm^{-1}.

In order to quantify the sp^2/sp^3 carbon content ratio in the GQD, which is expected to determine their radical scavenging activity, a deeper XPS analysis was conducted by measuring the C KLL spectra as proposed by Turgeon et al. [25] and Mezzi et al. [26]. The method consists of measuring the separation in energy between the most positive maximum and most negative minimum in the first derivative C KLL spectra, that is the so-called D-parameter. The sp^2 content has an approximate linear dependency with the D-parameter and the values can be compared to those of Turgeon et al. [25] and Mezzi et al. [26]. The first derivative of the carbon C KLL spectra for the GQDg, GQDv and GQDp samples are shown in Fig. 3a. The D-parameter (eV) of each sample is plotted against the sp^2 carbon content in Fig. 3b together with the D-parameter values for samples with 0% (sp^3 rich carbon sample) and 100% sp^2 carbon content extracted from references [25] and [26]. The D-parameter

decreased in GQD samples from 17.3 (GQDp) to 13.7 (GQDv) and to 12.9 (GQDg), indicating a gradual transition in the carbon bonding environment from a graphitic to a more amorphous-like structure, with estimated sp^2 carbon contents of 53.0% in GQDp, 16.5% in GQDv and 9.0% in GQDg. That is, the GQDp sample produced from an aromatic precursor as pyrene has comparatively much higher content of sp^2 carbon than GQDv and GQDg, where the highly oxidative conditions and the precursor used respectively, are responsible for the low graphitic carbon content in these GQD.

As a result of their different chemical composition and sp^2 carbon content, the three GQD exhibit different photoluminescence (PL) spectra (supporting information, Fig. S3a). Thus, PL emission wavelength increases in the following order: 395 nm (GQDg) < 490 nm (GQDp) < 530 nm (GQDv) and the corresponding aqueous solutions exhibit bright violet, cyan and green-yellow emission, respectively (photographs in inset of Fig. S3a).

3.2. Radical scavenging activity of GQD

First the free radical scavenging activity of the three GQD was investigated using $DPPH^{\cdot}$ as model radical, a relatively stable free radical commonly used to assess the antioxidant activity of compounds [27]. As indicated in the experimental section, the reaction between $DPPH^{\cdot}$ and a radical scavenger to yield the reduced and stable DPPH-H complex is accompanied by a color change from deep purple to pale yellow/colorless. Fig. 4a shows the absorbance of $DPPH^{\cdot}$ (100 μM) methanol solutions incubated in darkness for 1h with increasing GQDp concentrations. The characteristic $DPPH^{\cdot}$ absorbance band centered at 515 nm gradually disappeared as the GQDp dose was increased. In contrast with the high scavenging activity of GQDp, the other two GQD showed negligible activity in the same concentration range (up to 100 μg/mL). For comparative purposes, Fig. 4b shows the absorbance of $DPPH^{\cdot}$ solutions incubated with a same concentration (10 μg/mL) of the three GQD and a standard antioxidant, ascorbic acid (AA). At this low concentration the RSA decreases in the following order: AA >

GQDp > GQDg ≈ GQDv. The RSA or percent radical inhibition of each scavenger at different concentrations, quantified as the concentration ratio between the residual DPPH· and the control (scavenger-free DPPH· solution), is plotted in Fig. 4c, together with the relative standard deviation (RSD) of the three replicates.

Among GQD, only GQDp have a remarkable dose-dependent scavenging activity very close to that of AA. Thus, the half maximal inhibition effective concentration (EC_{50}) for GQDp was 12 μg/mL, slightly higher than that of AA (5 μg/mL), in agreement with the EC_{50} value previously reported for this standard antioxidant [14]. On the other hand, GQDg and GQDv did not show any radical scavenging activity at concentrations lower than 100 μg/mL, and only a moderate activity in the case of GQDg above this dose threshold. The RSA toward DPPH· of GQDp, in terms of EC_{50} values (12 μg/mL), is considerably higher than that reported for undoped C-dots (EC_{50} = 40 μg/mL) [13], for nitrogen and sulfur co-doped C-dots (EC_{50} = 80 μg/mL) [14] or GQD (EC_{50} ≈ 150 μg/mL) [16] toward the same free radical. This comparatively higher DPPH· scavenging activity of GQDp indicates a strong hydrogen donor behavior [28] as a result of their large amount of functional groups (mostly OH and $NHNH_2$) at edge sites together with a extense sp^2 –hybridized carbon network for the free electron delocalization and stabilization (Fig. 5).

Next, the antioxidant activity of the three GQD toward ·OH was assessed, a radical scavenging assay of great relevance since ·OH are the main radicals producing oxidative stress in biological systems. As indicated in the experimental section, the protective effect of GQD avoiding decoloration of RHB in the presence of photogenerated ·OH was taken as a measure of their antioxidant activity [9]. Thus, the absorbance of vials containing mixtures of RHB (0.02 mM), H_2O_2 (0.03 M) and different GQD concentrations (1-1000 μg/mL) was measured after 1h irradiation with UV light (302 nm). As example, Fig. 6a shows the

absorption spectra of UV-irradiated RHB-H_2O_2-GQDp mixtures with different GQDp concentrations together with the control solution, a GQD and H_2O_2-free 0.02 mM RHB solution. The magenta RHB control solution has a characteristic absorption band centered at 554 nm (top spectrum) that is totally bleached by oxidative attack of UV-photogenerated ·OH in absence of GQDp (down spectrum). As GQDp concentration in the mixtures increased, the absorption band was bleached to less extent, indicating a protective effect of GQDp against RHB decoloration by ·OH oxidative attack. The ·OH scavenging activity of the three GQD at different doses was investigated and benchmarked against that of AA as standard antioxidant. For comparative purposes, the absorption spectra of RHB-H_2O_2 mixtures with the same concentration of antioxidant (10 μg/mL) are shown in Fig. 6b together with the spectra of the control and antioxidant-free RHB solutions. At this low concentration there are significant differences in the ·OH scavenging activity of the three GQD, which is interestingly higher than that of AA in all cases and falls in the order: GQDp > GQDg ≥ GQDv > AA.

In order to compare the dose dependence of antioxidant activity, the percent ·OH inhibition at each concentration was quantified as the ratio between the absorbance of the antioxidant-containing RHB solution and the control. The trends of antioxidant activity obtained for each material with the RSD are plotted in Fig. 6c. Remarkably, GQDp and GQDg have higher antioxidant activity than AA at all concentrations while GQDv again have negligible activity. Thus, EC_{50} values were 7.5, 200 and 350 μg/mL for GQDp, GQDg and AA respectively, highlighting the extraordinary antioxidant activity of GQDp compared not only with AA (whose EC_{50} agrees with literature data [13]) but also with EC_{50} values toward ·OH reported for C dots (250 μg/mL) [13] or N,S-co-doped C dots with negligible ·OH scavenging activity [14]. As shown in Fig. 5 for GQDp, the mechanism for ·OH scavenging in carbon nanomaterials may involve radical adduct formation at sp^2 C sites, electron transfer or hydrogen donation from functional groups [5,7,9]. Thus, the high activity of GQDp can be

attributed to their large sp^2 C content, since the sp^2 C domains been identified as the primary ·OH scavenging sites rather than oxygen-containing functional groups in studies comparing the antioxidant chemistry of different graphene derivatives [9]. In the case of GQDg, despite their lower sp^2 C content the scavenging activity may be explained as a combination of radical adduct formation and electron transfer as they are electron-rich structures due to their large N doping level (10.8 at.%) in the basal graphitic network. In contrast, highly oxidized GQDv (37.0 at.% O) are in general poor in ·OH scavenging sites for radical adduct formation and electron transfer, with only the small fraction of aromatic hydroxyl edge sites having H-donor activity.

It is worth noting that the PL intensity of GQD and RHB was unaffected in solutions containing both fluorophores (supporting information, Fig. S3b) and no PL quenching was noted, ruling out a possible electron transfer between GQD and RHB. UV Exposure of 100 µg/mL GQD solutions to UV light for 1h did not lead to changes in pH values (7.6 for GQDg, 7.5 for GQDp and 3.9 for GQDv). Likewise, the chemical composition of the GQD was not altered after 1h of UV illumination, as revealed by XPS (supporting information, Fig. S4).

Further evaluation of the antioxidant activity of GQD was done with the $KMnO_4$ reduction assay, where the oxide radical scavenging activity of the materials is tested. Reduction of acidified $KMnO_4$ solutions in presence of antioxidants leads to a color change from purple to colorless. Fig. 7a shows the absorption spectra of 100 µM $KMnO_4$ aqueous solutions with different GQDp concentrations (0.1 - 1 µg/mL) after 1h reaction in the dark together with the control, a GQDp-free $KMnO_4$ solution. As GQDp concentration increases, the characteristic bands in the $KMnO_4$ absorption spectrum are gradually bleached while absorbance at shorter wavelengths increase, with an isosbestic point at 503 nm. These spectral changes confirm $KMnO_4$ reduction to Mn^{2+} (λ_{max} at 440 nm) by GQDp, as noted for other reducing compounds

[29,30]. The assay was also conducted with the other two GQD and AA as standard antioxidant. As representative of the trends observed, Fig. 7b compares the absorption spectra at a low antioxidant concentration (10 μg/mL) together with the spectra of the control $KMnO_4$ solution. This assay also reveals that GQDp have the highest antioxidant activity and it decreases in the order: GQDp > AA> GQDg > GQDv, with the latter ones having no activity. This trend is observed at different concentrations, as shown in Fig. 7c, where the antioxidant activity (calculated as the concentration ratio between the residual $KMnO_4$ and the control solution) is plotted vs concentration for all the materials. The EC_{50} values were 0.5, 15 and 70 μg/mL for GQDp, AA and GQDg respectively. Again this assay highlights the extraordinary antioxidant activity of sp^2 C-rich GQDp by electron transfer to $KMnO_4$ (Fig. 5), which exceeds by far the inhibition capacity of AA and that reported for C-dots by several orders of magnitude (EC_{50} = 900 μg/mL) [13]. The electron-rich N-doped GQDg exhibit moderate oxide radical scavenging activity as they can undergo electron transfer to $KMnO_4$ whereas the sp^3 C-rich GQDv with a high content of oxygen-containing groups (37.0 at.% O) do not show activity. This result is in agreement with the poor antioxidant activity encountered for graphene oxide, the 2D counterpart with comparable chemical structure [9]. In summary, these results prove the remarkable influence of GQD composition on their antioxidant chemistry. Designing GQD with higher radical scavenging activity would require more systematic studies to address individually the effect of each GQD parameter in their activity.

3.3. GQD-based protective films against dye decoloration

Given the remarkable free radical and ROS scavenging activity of GQDp, they were selected to investigate their possible use in protective coatings to prevent degradation of organic dyes by in situ photo-generated ROS. Thus, we compared the photocatalytic degradation of two organic dyes, RHB and methylene blue (MB) on bare and GQDp-coated Nylon membranes exposed to UV (302 nm) light. As indicated in the experimental section, a first layer of GQDp

was applied by immersion of the membrane in a GQDp solution following a protocol reported before [31]. In a second step, the dye was stably adsorbed on both bare and GQD-coated membranes by immersion in MB or RHB solutions containing also 0.01 M H_2O_2 as a source of UV-generated ROS. The dry membranes were exposed to UV light for different time. The presence of a uniform GQDp coating was corroborated by the high blue fluorescence across the whole membrane area visible under UV illumination (supporting information, Fig. S5). As can be seen in Fig. 8, both RHB (Fig. 8a) and MB (Fig. 8c) undergo fast decoloration (90 and 30 min. respectively) upon UV irradiation on uncoated membranes. In contrast, visual decoloration of the dyes on GQDp-coated membranes was imperceptible after the same irradiation time, evidencing the protective effect of the GQDp antioxidant coating against oxidative degradation by ROS.

Indeed, in order to corroborate the antioxidant effect of the GQDp coating, dye degradation rate was evaluated for membranes with different GQDp coverage, which can be tuned varying the concentration of the GQDp immersion solutions [31]. The membrane color at different irradiation times was measured with a colorimeter and the corresponding color differences with respect to the non-irradiated membrane (ΔE) for RHB and MB adsorbed on membranes with different GQDp coverage are shown in Fig. 8b and 8d, respectively. For both dyes, decoloration rate decreased with increasing coverage of the protective GQDp coating, suggesting a higher ROS scavenging capacity and hence dye oxidation inhibition. Finally, the protective capacity of GQDp coatings against dye degradation in absence of H_2O_2 and under natural ambient light exposure was examined. Although in general MB decoloration was slower both on bare and GQDp-coated membranes, it was also observed that GQDp lowered substantially the MB degradation rate (supporting information, Fig. S6), suggesting an additional protective effect by UV absorption and not only due to ROS scavenging, as proposed before for graphene materials [9].

4. Conclusions

We have shown the remarkable influence of the chemical composition of a series of GQD on their antioxidant and free radical scavenging activity. By tailoring the synthetic methods to produce GQD with very different C, O and N contents, type and amount of functional groups and sp^2/sp^3 hybridized carbon ratios, markedly different antioxidant activities have been encountered. The RSA was largely GQD, dose and radical-dependent, indicating different radical inhibition mechanisms. In general, GQD with strong hydrogen donor behavior, large content of sp^2–hybridized carbon domains or doped with electron-rich heteroatoms were very effective ROS scavengers. The best-performing GQD exhibited an extraordinary high antioxidant activity, with inhibition effective concentrations for ROS considerably lower than those of ascorbic acid or other carbon nanomaterials. Moreover, they proved very effective to protect organic dyes against decoloration induced by exposure to UV and ambient light. These results will contribute to a rationale design of carbon nanomaterials with high antioxidant activity, which are crucial for the multiple sectors demanding oxidation protection materials.

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Captions

Figure 1. TEM images with corresponding lateral size distributions (top) and AFM images with corresponding thickness distributions (down) for the different GQD.

Figure 2. High resolution C 1s (a, d, g), O 1s (b, e, h) and N 1s (c, f, i) XPS fitted spectra of the different GQD and corresponding chemical structures.

Figure 3. (a) First derivative of the carbon C KLL spectra for the different GQD. (b) D-parameter vs. sp^2 carbon content for the different GQD and reference (0% and 100% sp2 content) samples.

Figure 4. DPPH• scavenging assay: (a) Absorption spectra of 100 μM DPPH· methanol solutions with increasing GQDp concentrations from 0 to 20 μg/mL measured after 1h in dark. (b) Absorption spectra of 100 μM DPPH· solutions with 10 μg/mL of the different GQD, AA and the control measured after 1h in dark. (c) RSA vs. antioxidant concentration.

Figure 5. Mechanism for antioxidant activity of GQD.

Figure 6. Hydroxyl radical scavenging assay: (a) Absorption spectra of 0.02 mM RHB and 0.03 M H_2O_2 solutions with increasing GQDp concentrations from 0 to 20 μg/mL and the control after 1h irradiation with UV light (302 nm). (b) Absorption spectra of 0.02 mM RHB and 0.03 M H_2O_2 solutions with 10 μg/mL of the different antioxidants, no antioxidant and the control after 1h UV irradiation. (c) Antioxidant activity vs. concentration.

Figure 7. $KMnO_4$ reduction assay: (a) Absorption spectra of 100 μM $KMnO_4$ solutions with increasing GQDp concentrations from 0 to 1 μg/mL measured after 1h in dark. (b) Absorption spectra of 100 μM $KMnO_4$ solutions with 10 μg/mL of the different GQD, AA and the control after 1h in dark. (c) Antioxidant activity vs. concentration.

Figure 8. Photocatalytic degradation of RHB (a) and MB (c) on Nylon membranes with and without a protective GQDp antioxidant coating exposed to UV light. Color change (ΔE) vs. UV exposure time for RHB (b) and MB (d) coated membranes pre-coated with different amount of GQDp.

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