Insights in The Chemical Composition of Graphene Oxide via A Simple and Versatile Fluorescent Labelling Method

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Insights in the chemical composition of graphene oxide *via* a simple and versatile fluorescent labelling method

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Abstract: Graphene oxide (GO) is a promising 2D material with a complex chemical structure which characterization remains as a challenge. The widely-accepted chemical structure of GO includes epoxides, hydroxyls and carboxylic acids. Recently, alkene groups of GO have been used for selective GO functionalization. Despite the different efforts that exist to elucidate the exact GO chemical structure, the chemical composition of the main GO functionalities is fairly limited. By knowing the GO chemical composition, chemical strategies can be developed to create improved GO-based materials. Herein, we reported a simple and versatile fluorescent approach based on fluorescamine for the quantification of GO functionalities. This method offers important advantages such as highly selective, reliable, rapid quantification in bulk and can be extended to follow any primary amine containing molecule grafted onto GO.

Introduction

Graphene oxide (GO) has received an important attraction due to the remarkable and exotic properties that can be achieved after its chemical conversion to obtain suitable graphene-based materials for many applications, including energy storage, bioimaging, biosensors, and depollution.^[1-6] Such applications require specific GO chemical modifications in order to have the better performance during the application.^[7] Hence, it becomes fundamental to know the chemistry and composition of the GO before its application. In this context, two aspects are necessary to elucidate: i) the chemical structure of GO and ii) the concentration of each functional group after GO synthesis. The chemical structure of the GO is fairly complex and diverse due to the variety of chemical routes to synthesis it.^[8] The synthesis of GO is conducted by three principal methods which are Hummer's, Staudenmaier's and Brodie's method.^[9] Since exist several chemical routes that produced a non-stoichiometry GO,^[10] the chemical structure of the GO has been extensively studied by X-Ray photoelectron spectroscopy (XPS),^[11] transmission electron microscope (TEM),^[12] infrared spectroscopy (FTIR),^[13] solid NMR,^[13] and computational simulation.^[14] The widely-accepted chemical structure of GO was proposed by Lerf and Klinowsky, in which epoxide and hydroxyl groups are in the basal plane whereas carboxylic acids are inserted at the edges.^[8,14] This chemical structure of the GO strongly depends of some synthesis parameters such as the

nature of the pristine graphite and its particle size, oxidizing reagents, temperature and the oxidation time.^[9,10,15] Slight changes in those conditions can lead to additional oxygen functional groups (lactone, quinone, ketone, etc.),^[16] but also change the concentration of epoxides, alcohols and carboxylic acids throughout the GO surface. These last functional groups are the basis for developing GO-based materials with tuned properties.^[17,18]

On the other hand, the second fundamental aspect is to estimate the concentration of the GO functional groups. By knowing the concentration of functional groups of GO, it can be possible to select the chemical routes to take full advantage of the chemistry of GO. Computational simulations, XPS and voltammetry studies are some strategies that have been used to analyze the chemical composition of GO. Computational simulation has established that hydroxyl and epoxide groups are the most representative functional groups through the GO surface.^[14,19] Taking an O/C ratio of 25%, it has been proposed a 2:1 ratio of hydroxyl groups with respect to epoxides.^[14] XPS is a recognized characterization tool to know the surface elemental composition and the concentration of GO functionalities.^[8,11,20] Despite the whole information that can be obtained from XPS, it is necessary additional approaches to support this characterization tool and face limitations such as the sensitivity to surface contamination,^[21] and the need to perform several measurements to know the surface elemental distribution.^[22] An alternative approach suggests voltammetry as an efficient and absolute technique to quantify functional groups of GO.^[23,24] Nonetheless, this method is based on slight changes in the cyclic voltammogram which can be on the error of the measurement. Thus, new strategies with high sensibility must be considered for the quantification of the functional groups of the GO. In this context, fluorescamine arises as a promising tool for labelling the GO functional groups due to the low limit of detection, high sensibility, high reliability, it can be used for quantification of GO functionalities in bulk, not subjected to contamination and versatile tool for quantification of amino acids, proteins, peptides and primary amines.^[25,26] Fluorescamine reacts rapidly and specifically with -NH₂ moieties to form fluorescent pyrrolinone with an excitation wavelength of 390 nm and exhibits a maximum emission at 475 nm.^[25] In order to extend the application of fluorescamine to GO, it is necessary to label the specific functional group with a primary amine moiety for the subsequent titration with fluorescamine.

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Scheme 1. a) Functionalization of GO with BocDA to produce GO 1, b) reaction to produce rGO 1 and c) production of rGO 2

Since the study of the chemical structure of GO and its chemical composition remains as an open question to be elucidated,^[14,16] here we propose a simple and versatile fluorescent approach based on fluorescamine for the quantification of GO functional groups. We focused in three functional groups which are epoxides, carboxylic acids and alkenes groups. Epoxide and carboxylic acids are the basis of the chemical functionalization of GO.^[27] Recently, we found that the alkene groups of GO represent an important approach for the functionalization of GO using photochemical thiol-ene reactions (TER).^[28]

Scheme 1 shows the reactions conducted in this work. To quantify epoxides, GO was reacted with *N*-Boc-ethylenediamine (BocDA) by nucleophilic attack to produce GO 1. Since the epoxide groups are susceptible to be attacked by nucleophiles, a thermally reduced GO (rGO) was employed for the absolute quantification of carboxylic acids and alkenes. To quantify carboxylic acids, rGO was reacted with BocDA using coupling agents to produce rGO 1. For the quantification of alkene groups, rGO was reacted with cysteamine (CA) using photochemical functionalization to produce rGO 2. Finally, the primary amine from the CA and the deprotected BocDA was labelled using fluorescamine for the respective quantification. Moreover, the fluorescent method based on fluorescamine reported here can be extended to follow the kinetics of the molecules containing primary amines grafted onto GO surface.^[29]

Results and Discussion

For the quantification of the epoxides, carboxylic acids and alkene groups of GO, it is necessary to react the specific functional group with molecules containing -NH₂ moieties. BocDA and CA were used to derivatize specific GO functional groups using the chemical routes showed in Scheme 1. ATR-FTIR spectroscopy, Raman spectroscopy and XPS were used to confirm the complete

functionalization on epoxides, carboxylic acids and alkene groups. Figure 1 shows the ATR-FTIR spectroscopy results where GO exhibited the characteristic bands attributed to carboxylic acids (1730 cm⁻¹), skeleton carbon bonds (1624 cm⁻¹), alcohols (1379 and 1046 cm⁻¹) and epoxide groups (1218 and 970 cm⁻¹).^[30-32] After the chemical functionalization on epoxide groups, GO 1 showed new bands at 1592 cm⁻¹, 1355 cm⁻¹, 1244 cm⁻¹ and 1154 cm⁻¹ associated to N-H and N-C bonds for the BocDA bonded to GO,^[33-35] whereas epoxide bands (1218 and 970 cm⁻¹) were suppressed (see Figure 1a). The fact that the band attributed to epoxide groups in GO 1 was no longer observed in Figure 1a was associated to a full reaction on epoxide groups. Indeed, epoxide groups are well-known to react under a wide variety of conditions or by nucleophilic attack with high yields.^[18,36] In addition, carboxylic acid band (1730 cm⁻¹) was maintained in Figure 1a as an overlapped band after the chemical functionalization. This information suggests that the functionalization in GO 1 proceeded on epoxide groups. In the case of the chemical functionalization on carboxylic acid groups and alkene groups, a thermal reduction of GO was carried out as first step. In Figure 1b, rGO material exhibited a decrease in the bands at 1218 and 970 cm⁻¹ associated to epoxides, whereas the rest of the groups presented a similar intensity as compared to GO. The thermal reduction of GO was carried out at 200 °C during 12 h (see Figure S1 in ESI), and previous studies reported that epoxides are almost completely eliminated between 160 °C and 300 °C.^[17] This information is consistent with the results presented in Figure 1b corroborating that in rGO sample practically all the epoxides were removed. After the functionalization on carboxylic acid groups, rGO 1 displayed bands attributed to N-C vibrations (1355 cm⁻¹, 1244 cm⁻¹ and 1154 cm⁻¹) similar to GO 1 due to the introduction of BocDA in the GO.^[33-35] In addition, the carboxylic acid band for rGO 1 sample was completely suppressed due to the formation of amide bonds. The absence of carboxylic acid groups band at 1730 cm⁻¹ in rGO 1 confirms that carboxylic acids

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were completely reacted with BocDA. Indeed, a new band was detected for rGO 1 in Figure 1b at 1526 cm^{-1} , attributed to amide

II mode.^[37,38] This information confirms the successful functionalization of carboxylic acid groups with BocDA.

Figure 1. ATR-FTIR characterization of GO, rGO, GO 1, rGO 1 and rGO 2

On the other hand, Figure 1c presents the ATR-FTIR spectrum of rGO 2 which not revealed new bands. Generally, C-S bonds are less intense in GO and thus it becomes complicated to distinguish those vibrations in ATR-FTIR spectroscopy.^[39] Nonetheless, after the functionalization, rGO 2 conserved the oxygen functional groups of rGO since similar vibration contributions were found as compared to rGO. This information confirms TER as an orthogonal reaction that not produce by-products with the carboxylic acids or alcohols of rGO.

Figure 2. Raman spectroscopy characterization of GO, rGO, GO 1, rGO 1 and rGO 2.

Raman spectroscopy characterization was performed to corroborate the functionalization in the different samples. In the case of GO 1 and rGO 1, G band presented a downshift of 3 cm^{-1} and 7 cm^{-1} , respectively (see Figure 2a and Figure 2b). The downshift observed in GO 1 and rGO 1 is consistent with a surface N-type doping due to N atoms bonded to the graphitic lattice.^[40,41] This information confirmed the functionalization of GO 1 and rGO 1 with BocDA and supports the ATR-FTIR spectroscopy results of Figure 1a and 1b. Notably, the G band in rGO 2 material exhibited an upshift of 8 cm^{-1} as compared to rGO (see Figure 2b). Despite the introduction N and S atoms from CA moieties in rGO structure, the trend of the G band was opposite as compared to GO 1 and rGO 1. In order to explain this behavior, the Raman information was subtracted from Figure 2 and the $I(D)/I(G)$ ratio was calculated.

Table 1. Information of Raman spectra of Figure 2

Material	G (cm^{-1})	$I(D)/I(G)$	$(D''+D''')$	D'
GO	1590	0.81	0.71	0.52
rGO	1590	0.80	0.71	0.59
GO 1	1587	0.82	0.72	0.55
rGO 1	1583	0.85	0.70	0.60
rGO 2	1598	0.84	0.78	0.58

In Table 1 is possible to observe that GO and rGO exhibited similar $I(D)/I(G)$ ratios confirming the mild thermal reduction that GO was undergone. In addition, the $I(D)/I(G)$ ratio of GO 1, rGO 1 and rGO 2 increased with respect to rGO due to the formation of disordered graphitic lattice, functionalization, vacancies, defects and the creation of bonds in graphene surface. In the case of rGO 2, TER was applied for the functionalization with CA breaking the C=C bonds and increasing the sp^3 hybridization. A deeper analysis was conducted fitting the Raman spectrum of Figure 2 into the Raman components, as reported elsewhere.^[42,43] The bands D' , D'' and D''' were fit and assigned as presented in Figure S2 in ESI, and the information is presented in Table 1. D'' and D''' have been associated to the presence of oxygen species and the degree of crystallinity.^[42] The sum of both bands can be used to follow the functionalization of GO and rGO. In the case of rGO 2, it was observed an increase of the sum of D'' and D''' which could be attributed to the increase of sp^3 domain.^[42,43] This information confirmed the introduction of CA moieties in rGO 2. Finally, D' band is also reported in Table 1 that can be assigned

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to the degree of disorder.^[43] This band tends to increase as GO is reduced. However, the three functionalized materials showed similar values of D' . This information points that the reactions performed does not alter the structure and order of the graphene sheets.

Figure 3. N1s high-resolution XPS deconvolution: a) GO 1 and b) rGO 1. S2p high-resolution XPS deconvolution of rGO 2. XPS characterization was conducted to further confirm the complete functionalization of GO 1, rGO 1 and rGO 2. XPS survey of all the functionalized materials showed a clear Nitrogen (N) atomic contribution, whereas rGO 2 contains additional sulfur (S) atomic contribution (see Figure S3 in ESI). The C1s high-resolution XPS deconvolution of GO and rGO was performed and

the results are presented in Figure S4 in ESI. The peak centered at 286.5 eV was attributed to epoxides and ether groups.^[17] After the thermal reduction, rGO exhibited the peak at 286.5 eV with lower intensity as compared to GO. The peak area ratio of C(O) epoxy/ether bonds to the C=C was estimated, as reported elsewhere (see Table S1 of ESI).^[44] The thermal reduction of GO was enough to released only epoxide groups since ether groups inserted in the aromatic domain and alcohols require higher temperatures (> 600 °C) to release them from the GO surface.^[45] We can observe in Table S1 a difference of 0.47 in the area ratio of GO and rGO that could be associated to the loss of epoxide groups, and this information is consistent with the ATR-FTIR results of Figure 1b. The C1s high-resolution XPS deconvolutions of GO 1, rGO 1 and rGO 2 are presented in Figure S5 of ESI. As compared to GO, in GO 1 material was possible to find an additional peak at 285.4 eV attributed to N-C bonds due to the BocDA bonded to GO by ring opening reactions.^[46,47] In the case of rGO 1, this sample exhibited two additional peaks at 285.4 eV and 287.5 eV attributed to N-C and N-C=O of amide bonds (see Figure S5 in ESI).^[41,46,47] The amine bonds detected in C1s deconvolution are consistent with ATR-FTIR spectroscopy results of Figure 1b.

N1s high-resolution XPS deconvolution of GO 1 is presented in Figure 3a where is possible to observe the peaks at 399.8 eV and 401.8 eV attributed to -NH_2 and -NH_3^+ due to the presence of BocDA once it was deprotected (see Figure 3a).^[46,48] Moreover, rGO 1 was possible to fit into three different peaks at 398.5 eV, 399.5 eV and 401.2 eV associated to -NH_2 , HN-C=O and -NH_3^+ , respectively (see Figure 3b).^[46-48] When amides are formed on GO, the peaks can be shifted due to the existence of resonance structures, as reported elsewhere.^[22] This information confirmed that epoxide and carboxylic acids in GO 1 and rGO 1 respectively, were derivatized with -NH_2 for the subsequent fluorescamine titration.

On the other hand, the C1s deconvolution of rGO 2 presented a new peak at 284.7 eV which could be associated to C-S bonds (see Figure S5 in ESI).^[49] The S2p deconvolution of rGO 2 confirmed the successful functionalization with CA, since the doublet peak at 163.5 eV and 164.7 eV attributed to C-S and C-S-C bonds were identified (see Figure 3c).^[29,49] Thus, despite that ATR-FTIR spectra for rGO 2 presented no significant changes, XPS deconvolution allowed to corroborate the chemical functionalization. TER is a highly efficient reaction for the addition of thiols to a wide variety of unsaturated systems.^[50] This advantage of TER allows to react the alkene groups of GO with an expected high yield. Notably, residual epoxides could be reacted during the functionalization of rGO, when rGO 1 and rGO 2 materials were produced. It is important to highlight that the reaction conditions can activate only residuals epoxides and not alcohols or ether groups of GO. Thus, any change in the area of the peak centered at 286.5 eV can be associated to a reaction in residual epoxides of rGO. However, the results of the peak area ratio of C(O) to C=C presented in Table S1 showed a difference of 0.05 and 0.03 for rGO 1 and rGO 2, respectively as compared to rGO. This information is an indicative of a low density of residual epoxides in the rGO material since similar C(O)/C=C values were found for rGO, rGO 1 and rGO 2. So, ATR-FTIR, Raman spectroscopy and XPS results offer clear evidence of the full derivatization of epoxide, carboxylic acid and alkene groups of GO. The marked functional groups can be quantified in a subsequent step using fluorescamine.

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Fluorescamine reacts rapidly and specifically with -NH_2 moieties to form fluorescent pyrrolinone (Scheme 2).^[25] The BocDA molecules grafted onto GO 1 and rGO 1 are associated to the epoxides and carboxylic acid groups concentration respectively, whereas rGO 2 gives information about the concentration of alkene groups, as presented in Figure 4.

Figure 4. Fluorescence titration of GO, rGO, GO 1, rGO 1 and rGO 2 with fluorescamine ($\lambda_{\text{ex}}=390$ nm and $\lambda_{\text{em}}=475$ nm).

The fluorescent titration results are shown in Figure 4 where GO and rGO exhibited a minimum fluorescence signal after being incubated with fluorescamine confirming that no trace of -NH_2 moieties were in the blank materials. The quantification of the functional groups of GO was performed taking the fluorescence intensity at 475 nm from Figure 4 and setting this value to the respective calibration curve of Figure S6 in ESI. The results are

presented in Table 2 and highlight the alkene groups as the functional group with the strongest concentration, followed by carboxylic acids and epoxide groups.

Table 2. Summary of fluorescence and XPS results for the quantification of GO functional groups ($n=3$)

Functional group	Molecules per mg of GO estimated by fluorescent labelling	Molecules per mg of GO estimated by XPS
Carboxylic acids (rGO 1)	4.2×10^{16}	8.7×10^{16}
Alkenes (rGO 2)	6.6×10^{16}	7.1×10^{16}
Epoxides (GO 1)	1.9×10^{16}	8.6×10^{16}

In literature, the study about concentration of functional groups of GO is fairly limited. However, Eng *et al.*^[24] found that the strongest contribution on GO corresponds to the epoxide groups (7.8×10^{17} molecules per mg of GO). Additionally, carboxylic acid groups were reported in a concentration around 1.4×10^{16} by Eng *et al.*,^[24] whereas Barua *et al.*^[51] reported a concentration of 2.8×10^{18} molecules per mg of GO. Both results were obtained from a GO synthesized by modified Hummer's method. Comparing previous reports with the results obtained here, the carboxylic acid groups were found in a concentration of 4.2×10^{16} nearby of the results reported by Eng *et al.*^[24] Regardless of the proximity of this result, the epoxide groups concentration was of 1.9×10^{16} that represents only 2.4 % of the concentration previously reported by Eng *et al.*^[24] As it was mentioned in the introduction, the concentration of the functional groups depends of the pristine graphite and its particle size, oxidizing reagents, temperature and the oxidation time.^[9,10] Therefore, it is expected that slight changes during the GO

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synthesis conduct to different density of functional groups. In this study, natural graphite of size $\sim 1 \mu\text{m}$ was used and given the low dimensions of the initial graphite, the borders are more susceptible to be oxidized and thus, more carboxylic acids groups can be generated.^[52] However, a mixture of $\text{H}_2\text{SO}_4/\text{H}_3\text{PO}_4$ was used in order to have a mild oxidation and thus introduce less defects (such as vacancies) in the graphite lattice, as reported elsewhere.^[53] This could explain the low concentration of carboxylic acids and epoxide groups found. This information highlights the importance of the synthesis parameters during the graphite oxidation to induce a certain quantity of each GO functionality. In addition, alkene groups seem to be an interesting approach to functionalize the GO. Here, the alkene groups concentration was the higher among the functional groups quantified. The origin of this result could be attributed to the wide variety of unsaturated groups that arises during the graphite oxidation.^[52,54] Such unsaturated groups are alkenes, α,β -unsaturated carbonyls, α,β -unsaturated esters and α,β -unsaturated ethers which are susceptible to be activated via TER.^[50,55] Indeed, adding the concentration of the carboxylic acids and epoxides reported in Table 2 (giving a total of 6.1×10^{16}

molecules per mg of GO) and, considering that each functional group break the aromatic domain leading to α,β -unsaturated carbonyls or ethers, the resulted value is nearby to the concentration of alkenes. Therefore, since many defects are introduced throughout the GO layers, it is possible to increase the molecules bonded to GO by TER. This information confirms the alkene groups of GO as a promising tool to create multi-functional GO-based materials via orthogonal reactions.

The fluorescent method was validated estimating the limit of detection (LOD), limit of quantification (LOQ), the coefficient of determination (r^2) and the coefficient of variation (COV). Table 3 contains a summary of the results obtained from the validation method. The r^2 confirmed the linearity of the linear adjust. The molar concentration found in Table 2 were inside of the parameters of the validation method, except for epoxide groups which concentration was slightly lower than LOQ. This can be attribute to the low concentration of epoxide groups in the GO studied here. Since epoxide groups are in lower concentration, the fluorescent method cannot predict with enough accuracy their concentration.

Table 3. Validation of the fluorescent method (n=3)

Functional group	LOD (μM)	LOQ (μM)	r^2	COV	Concentration (μM)
Carboxylic acids (rGO 1)	0.13	0.44	0.992	6.8	0.86
Alkenes (rGO 2)	0.08	0.28	0.996	4.2	0.77
Epoxides (GO 1)	0.16	0.56	0.987	3.5	0.39

In order to corroborate the quantification via fluorescent titration, a theoretical estimation using atomic percentage of XPS survey was conducted. The atomic percentage found of N in the XPS survey was 2.3% and 4.1% for GO 1 and rGO 1 respectively, whereas rGO 2 possessed 1.0% of S atomic contribution. According to the XPS results, the BocDA and CA molecules grafted to GO 1, rGO 1 and rGO 2 were estimated (see experimental section) and presented in Table 2. The concentration found by XPS was slightly higher than the fluorescent titration for two reasons: i) the XPS reveals the N atomic contribution from all the species that could be in the samples, including contamination,^[21] whereas titration test with fluorescamine only detects and quantifies the $-\text{NH}_2$ moieties and ii) XPS results come from a theoretical estimation using the initial molecules added to the reaction. Therefore, the fluorescence titration using fluorescamine can be taken as a reliable method since the functionalization on the respective functional groups was completed and, fluorescamine avoided the quantification of impurities in the samples.

Finally, XPS characterization was used to predict the molar concentration of the molecules bonded to GO and, the information was consistent with fluorescent labelling method. More important, the fluorescent method based on fluorescamine proposed in this work can be extended to monitor the functionalization of GO with any primary amine containing molecules.

Conclusion

Herein we reported a simple and versatile method for the quantification of epoxide, carboxylic acids and alkene groups of GO via fluorescent labelling which is a selective, reliable, easy method and can be used for the quantification of GO functional groups in bulk. The work reported here highlights that the strongest contribution corresponds to alkene groups of GO that arise during the graphite oxidation. Classical methodologies of functionalization take advantage of the oxygen functional group but alkene groups have not been considered with such importance until now. More important, the fluorescent method proposed here is a versatile tool not only to quantify the functional groups of GO, but also it can be extended to monitor primary amine containing molecules grafted onto GO, and quantified them. This opens the opportunity to have a real knowledge about the extent of the reaction after the GO derivatization. Since C=C bonds were found with the stronger concentration, these are a promising tool for creating multi-functionalized graphene oxide materials. This work confirms the importance to study the characteristics of the pristine GO before its application given the structure and chemical composition of the GO strongly depends of the reaction conditions. Further studies consider the analysis of the chemical composition of GO using different methods of synthesis, to have an overview of the chemical structure of GO before its application.

Experimental Section

Materials

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Asbury natural graphite of 1 μm was used for GO synthesis. The reagents used for the graphite oxidation were sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), potassium permanganate (KMnO_4), phosphorus pentoxide (P_2O_5), potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl), all were analytical grade and purchased from Sigma Aldrich. For GO and rGO functionalization, 2-benzyl-2-(dimethylamino)-4'-morpholinobutyrophenone (97%) was used as photoinitiator (PI), cysteamine hydrochloride (CA, $\geq 98\%$), *N*-Boc-ethylenediamine (BocDA, $\geq 98\%$), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), *N,N*-dimethylformamide (DMF, for HPLC, $\geq 99.9\%$), dichloromethane (DCM, $\geq 99.8\%$), fluorescamine ($\geq 98\%$), sodium tetraborate decahydrate (sodium borate, $\geq 99.5\%$) and phosphate buffered saline tablets (PBS) were purchased from Sigma-Aldrich, whereas *N,N*-diisopropylethylamine (DIPEA, 98%) and trifluoroacetic acid (TFA, 99%) was acquired from Alfa Aesar. All the reagents were used as received.

Instruments

For the photochemical functionalization of rGO with CA by TER a UVP CL-1000L Crosslinker was used and operated at 5.2 mW/cm^2 using UV-lamps of 365 nm. Characterization of chemical species was performed using attenuated total reflection-Fourier transform infrared (ATR-FTIR) conducted in a Nicolet Magna-IR 550 FTIR spectrometer, a Micro-Raman spectrometer confocal Renishaw model DM 2500M with a 488 nm laser, a X-Ray photoelectron spectroscopy (XPS) K-ALPHA from Thermo Scientific, with a monochromatic X-ray beam using a k-alpha aluminum line and for the fluorescence characterization a FluoroMax (Jobin-Yvon/Horiba) was used.

GO synthesis

Graphene oxide was synthesized through a modified Hummer's method as reported elsewhere.^[29] In brief, the intercalation of the graphite was carried out in presence of H_2SO_4 , $\text{K}_2\text{S}_2\text{O}_8$ and P_2O_5 during 4.5 h at 80°C . After that, the mixture was diluted with water ($18.2 \text{ M}\Omega\cdot\text{cm}$) and let overnight for the precipitation of the graphite. The intercalated graphite was filtered and dried at 40°C in an oven for 24 h. The intercalated graphite was oxidized using a 7:1 mixture of $\text{H}_2\text{SO}_4/\text{H}_3\text{PO}_4$ and adding KMnO_4 . After 4 h of reaction, H_2O_2 was added to finish the reaction resulting an intense yellow color. The impurities were eliminated adding HCl 10 % v/v and then, the GO was washed by centrifugation using a 1:3 mixture of water/methanol until increase the pH~4.5 approximately. Finally, the methanol was removed by rotavapor and the obtained GO was dispersed only in water.

Thermal reduction of the GO

The GO obtained from the oxidation reaction was freeze-dried during 24 h and then, it was put in an oven at 200°C and 29.5 inHg vacuum pressure during 12 h. The resulting material was dispersed in DMF to obtain the required concentrations for all the reactions using an ultrasonic bath for the exfoliation of the rGO sheets.

Functionalization of epoxide groups with BocDA

For the epoxide groups functionalization, 10 mL of GO (1 mg/mL) dispersed in DMF was bubbled during 30 min with a constant flow of argon (Ar) and under stirring. After that, 10 mL of BocDA (2 mg/mL) was added and the reaction was heated until 50°C keeping the bubbling and the stirring. After 5 min, the system was sealed maintaining the inert

atmosphere and the reaction was let for 24 h producing GO 1 material. Thereafter, the GO 1 was washed first adding ethanol, then centrifuging and removing the supernatant. This wash step was repeated four times. Subsequently, the GO 1 was dispersed in 20 mL of DCM and 1 mL of TFA was added for releasing the Boc group. The mixture was let for 2 h under stirring at room temperature. After this, the GO 1 was washed with ethanol and centrifugation four times. Subsequently, a solution of NaCl (2 M) with pH~2 (the pH was adjusted using HCl) was employed adding 2 mL, and then the material was transferred to a microcentrifuge tube and centrifuged for 2 min at 23900 g. This wash step was repeated six times adding in each time enough NaCl until made it up 2 mL. Finally, the material was washed with water ($18.2 \text{ M}\Omega\cdot\text{cm}$) centrifuging at 23900 g for 10 min and removing the supernatant. This process was repeated five times. At the end, GO 1 was dispersed in 2 mL of water.

Functionalization of carboxylic acid groups with BocDA

In a typical procedure, 10 mL of rGO (1 mg/mL) dispersed in DMF was bubbled during 30 min with a constant flow of argon (Ar) and stirring. After that, 10 mL of EDC (20 mM) dispersed in DMF was added to the system keeping the bubbling and the stirring for another 30 min. Thereafter, 10 mL of BocDA (2 mg/mL) and 100 μL were added and the reaction was heated until 55°C keeping the bubbling and the stirring. After 30 min, the system was sealed maintaining the inert atmosphere and the reaction was let for 48 h producing rGO 1 material. The deprotection of the BocDA and the purification was carried out following the same protocol as described in section Functionalization of epoxide groups with BocDA.

Functionalization of alkene groups with CA

For the photochemical functionalization of GO was performed as reported previously.^[29] GO, CA and PI solutions were prepared in the same mass ratio and mixed in a beaker, then the solution was put inside of the UV chamber under stirring during 60 min to produce rGO 2. The purification was performed using ethanol, a solution of NaCl (2 M) with pH~2 (the pH was adjusted using HCl) and water.

Detection and quantification of BocDA and CA on GO and RGO

For the detection of BocDA and CA in GO 1, rGO 1 and rGO 2, 1 mL of each material (0.05 mg/mL, prepared in PBS buffer) was mixed with 2 mL of sodium borate buffer (pH~9). Then, 1 mL of fluorescamine (0.5 mM dissolved in acetone) was added and the mixture was shaken by hand and then sonicated for 5 s. The fluorescence was measured at 390 nm excitation wavelength ($\lambda_{\text{ex}}=390 \text{ nm}$) and emission of 475 nm ($\lambda_{\text{em}}=475 \text{ nm}$), as reported previously.^[26] For the quantification, three calibration curves were built using: i) BocDA and adding a constant volume of GO (quantification of epoxides), ii) BocDA and adding a constant volume of rGO (quantification of carboxylic acids) and iii) CA and adding a constant volume of rGO (quantification of alkenes). For each calibration curve the following protocol was conducted: 1, 0.8, 0.6, 0.4 and 0.2 mL of BocDA or CA standard (0.02 mM, prepared in PBS) were mixed with 1, 1.2, 1.4, 1.6 and 1.8 mL of sodium borate buffer (pH~9). Then, 1 mL of either GO or rGO solution (0.05 mg/mL, prepared in PBS buffer) was added, followed by the addition of 1 mL of fluorescamine (0.5 mM, dispersed in acetone). The mixture was shaken by hand and then sonicated for 5 s. For the elaboration of each plot, the fluorescence was measured at $\lambda_{\text{ex}}=390 \text{ nm}$ and $\lambda_{\text{em}}=475 \text{ nm}$; each point was obtained from three measurements. The concentration of BocDA or CA in each sample was found setting the

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fluorescence obtained from the detection test at $\lambda_{\text{em}}=475$ nm into the respective calibration curve.

Validation of the method

The fluorescent quantification method was validated using Limit of Detection (LOD), Limit of Quantification (LOQ), coefficient of determination (r^2) and the coefficient of variation (COV). LOD and LOQ were estimated as three and ten times the standard deviation of the y -residuals divided by the slope of the calibration curve, respectively. This validation method can be applied in all cases, and it is more applicable when the analysis method does not involve background noise, since the intensity of signal is too high as compared to the noise.^[66] The COV was calculated as the concentration obtained divided by the standard deviation of the concentration multiplied by 100.

Theoretical estimation of CA and BocDA on GO and RGO using XPS survey information.

For the production of GO 1 and rGO 1 samples, 20 mg of BocDA were used that represents 7.5×10^{19} molecules. The N atomic contribution detected in XPS for GO 1 and rGO 1 were divided by two since each ethylenediamine molecule provides 2 nitrogen atoms per molecule. In the case of rGO 1, the N1s spectrum exhibits more peaks for different functional groups. Therefore, N atomic was multiplied for the fraction of area of the amide bonds and then, this value was multiplied for the number of BocDA molecules. For the GO 1 material, the fraction obtained from the division of the N atomic of XPS was directly multiplied by the initial number of BocDA molecules.

For rGO 2 sample, 10 mg of CA was used which represents 7.80×10^{19} molecules. After that, the S atomic contribution detected in XPS was multiplied for the number of CA molecules added to the reaction. However, the S atomic percentage of XPS can also contain residual SO_3 moieties inserted to GO graphitic lattice after graphite oxidation.^[31] Therefore, in the S2p high resolution XPS, the area percentage of C-S and C-S-C bonds were only considered, and the fraction of both areas was multiplied for the total S atomic percentage. This final atomic percentage was multiplied for the number of CA molecules. Finally, the number of molecules estimated by XPS and fluorescent labelling was reported per GO or RGO mass.

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Keywords: Fluorescent labelling method • primary amines • quantification • selective labelling

- [1] A. T. Smith, A. M. LaChance, S. Zeng, B. Liu, L. Sun, *Nano Mater. Sci.* **2019**, 1, 31–47.
- [2] H. Ahmad, M. Fan, D. Hui, *Compos. Part B Eng.* **2018**, 145, 270–280.
- [3] Y. Zhang, M. Chen, T. Hu, C. Meng, *ACS Appl. Nano Mater.* **2019**, 2, 2934–2945.
- [4] Z. Zang, X. Zeng, M. Wang, W. Hu, C. Liu, X. Tang, *Sensors Actuators, B Chem.* **2017**, 252, 1179–1186.
- [5] S. Joshi, P. Sharma, R. Siddiqui, K. Kaushal, S. Sharma, G. Verma, A. Saini, *Microchim. Acta* **2020**, 187, 1–15.
- [6] K. Thakur, B. Kandasubramanian, *J. Chem. Eng. Data* **2019**, 64, 833–867.
- [7] Y. I. Avila-Vega, C. C. Leyva-Porras, M. Mireles, M. Quevedo-López, J. Macossay, J. Bonilla-Cruz, *Carbon N. Y.* **2013**, 63, 376–389.
- [8] Y. C. G. Kwan, G. M. Ng, C. H. A. Huan, *Thin Solid Films* **2015**, 590, 40–48.
- [9] J. Sturala, J. Luxa, M. Pumera, Z. Sofer, *Chem. - A Eur. J.* **2018**, 24, 5992–6006.
- [10] M. Acik, G. Lee, C. Mattevi, A. Pirkle, R. M. Wallace, M. Chhowalla, K. Cho, Y. Chabal, *J. Phys. Chem. C* **2011**, 115, 19761–19781.
- [11] K. Dave, K. H. Park, M. Dhayal, *RSC Adv.* **2015**, 5, 95657–95665.
- [12] K. Erickson, R. Ermi, Z. Lee, N. Alem, W. Gannett, A. Zettl, *Adv. Mater.* **2010**, 22, 4467–4472.
- [13] H. R. Thomas, S. P. Day, W. E. Woodruff, C. Vallés, R. J. Young, I. A. Kinloch, G. W. Morley, J. V. Hanna, N. R. Wilson, J. P. Rourke, *Chem. Mater.* **2013**, 25, 3580–3588.
- [14] F. Mouhat, F. X. Coudert, M. L. Bocquet, *Nat. Commun.* **2020**, 11, 1–9.
- [15] Q. Qiao, C. Liu, W. Gao, L. Huang, *Carbon N. Y.* **2019**, 143, 566–577.
- [16] E. Aliyev, V. Filiz, M. M. Khan, Y. J. Lee, C. Abetz, V. Abetz, *Nanomaterials* **2019**, 9, 1180.
- [17] S. Eigler, A. M. Dimiev, Graphene Oxide *Fundam. Appl.* **2016**, 175–229.
- [18] W. Gao, in Graphene Oxide (Ed.: W. Gao), *Springer International Publishing, Cham*, **2015**, pp. 61–95.
- [19] D. W. Boukhvalov, M. I. Katsnelson, *J. Am. Chem. Soc.* **2008**, 130, 10697–10701.
- [20] J. Nimita Jebaranjitham, C. Mageshwari, R. Saravanan, N. Mu, *Compos. Part B Eng.* **2019**, 171, 302–309.
- [21] M. Salim, J. Hurst, M. Montgomery, N. Tolman, H. Liu, *J. Electron Spectros. Relat. Phenomena* **2019**, 235, 8–15.
- [22] M. C. Hsiao, S. H. Liao, M. Y. Yen, P. I. Liu, N. W. Pu, C. A. Wang, C. M. Ma, *ACS Appl. Mater. Interfaces* **2010**, 2, 3092–3099.
- [23] E. L. K. Chng, M. Pumera, *Chem. - An Asian J.* **2011**, 6, 2899–2901.
- [24] A. Y. S. Eng, C. K. Chua, M. Pumera, *Nanoscale* **2015**, 7, 20256–20266.
- [25] S. Udenfriend, S. Stein, P. Böhlen, W. Dairman, W. Leimgruber, M. Weigle, *Science (80-)*, **1972**, 178, 871–872.
- [26] T. Bantan-Polak, M. Kassai, K. B. Grant, *Anal. Biochem.* **2001**, 297, 128–136.
- [27] S. Eigler, A. Hirsch, *Angew. Chemie - Int. Ed.* **2014**, 53, 7720–7738.
- [28] A. Piñeiro-García, S. M. Vega-Díaz, F. Tristán, D. Meneses-Rodríguez, G. J. Labrada-Delgado, V. Semetey, *Ind. Eng. Chem. Res.* **2020**, 59, 13033–13041.
- [29] A. Piñeiro-García, S. M. Vega-Díaz, F. Tristán, D. Meneses-Rodríguez, G. J. Labrada-Delgado, V. Semetey, *FlatChem* **2021**, 26, 100230.
- [30] A. Dimiev, D. V. Kosynkin, L. B. Alemany, P. Chaguine, J. M. Tour, *J. Am. Chem. Soc.* **2012**, 134, 2815–2822.
- [31] L. Zhang, F. Dai, R. Yi, Z. He, Z. Wang, J. Chen, W. Liu, J. Xu, L. Chen, *Appl. Surf. Sci.* **2020**, 520, 146308.
- [32] M. J. Yoo, H. B. Park, *Carbon N. Y.* **2019**, 141, 515–522.
- [33] Y. Ouyang, X. Cai, Q. S. Shi, L. Liu, D. Wan, S. Tan, Y. Ouyang, *Colloids Surfaces B Biointerfaces* **2013**, 107, 107–114.
- [34] H. P. Mungse, O. P. Khatri, *J. Phys. Chem. C* **2014**, 118, 14394–14402.
- [35] Q. Xin, Z. Li, C. Li, S. Wang, Z. Jiang, H. Wu, Y. Zhang, J. Yang, X. Cao, *J. Mater. Chem. A* **2015**, 3, 6629–6641.
- [36] D. R. Dreyer, S. Park, C. W. Bielawski, R. S. Ruoff, *Chem. Soc. Rev.* **2010**, 39, 228–240.
- [37] A. S. K. Kumar, S. J. Jiang, *J. Environ. Chem. Eng.* **2016**, 4, 1698–1713.
- [38] W. Zhou, W. Zhuang, L. Ge, Z. Wang, J. Wu, H. Niu, D. Liu, C. Zhu, Y. Chen, H. Ying, *J. Colloid Interface Sci.* **2019**, 546, 211–220.
- [39] J. Li, Y. Cheng, S. Zhang, Y. Li, J. Sun, C. Qin, J. Wang, L. Dai, *Compos. Part A Appl. Sci. Manuf.* **2017**, 101, 115–122.
- [40] A. Piñeiro-García, F. Tristán, D. Meneses-Rodríguez, V. Semetey, S. M. Vega-Díaz, *Mater. Today Chem.* **2021**, 19, 100386.
- [41] F. Liu, L. Wu, Y. Song, W. Xia, K. Guo, *RSC Adv.* **2015**, 5, 45987–45995.
- [42] P. Vecera, J. C. Chacón-Torres, T. Pichler, S. Reich, H. R. Soni, A. Görling, K. Edlthammer, H. Peterlik, F. Hauke, A. Hirsch, *Nat. Commun.* **2017**, 8.
- [43] W. Liu, G. Speranza, *ACS Omega* **2021**, 6, 6195–6205.
- [44] D. Luo, G. Zhang, J. Liu, X. Sun, *J. Phys. Chem. C* **2011**, 115, 11327–11335.
- [45] S. Hun, *Phys. Appl. Graphene - Exp.* **2011**.
- [46] F. Zhou, H. N. Tien, Q. Dong, W. L. Xu, H. Li, S. Li, M. Yu, *J. Memb. Sci.* **2019**, 573, 184–191.

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- [47] N. Cai, P. Larese-Casanova, *J. Environ. Chem. Eng.* **2016**, 4, 2941–2951.
- [48] O. C. Compton, D. A. Dikin, K. W. Putz, L. C. Brinson, S. T. Nguyen, *Adv. Mater.* **2010**, 22, 892–896.
- [49] P. L. Yap, S. Kabiri, D. N. H. Tran, D. Losic, *ACS Appl. Mater. Interfaces* **2019**, 11, 6350–6362.
- [50] F. Dénès, M. Pichowicz, G. Povie, P. Renaud, *Chem. Rev.* **2014**, 114, 2587–2693.
- [51] M. Barua, M. B. Sreedhara, K. Pramoda, C. N. R. Rao, *Chem. Phys. Lett.* **2017**, 683, 459–466.
- [52] L. Yan, Y. B. Zheng, F. Zhao, S. Li, X. Gao, B. Xu, P. S. Weiss, Y. Zhao, *Chem. Soc. Rev.* **2012**, 41, 97–114.
- [53] D. C. Marcano, D. V. Kosynkin, J. M. Berlin, A. Sinitskii, Z. Sun, A. Slesarev, L. B. Alemany, W. Lu, J. M. Tour, *ACS Nano* **2010**, 4, 4806–4814.
- [54] G. Shao, Y. Lu, F. Wu, C. Yang, F. Zeng, Q. Wu, *J. Mater. Sci.* **2012**, 47, 4400–4409.
- [55] C. E. Hoyle, C. N. Bowman, *Angew. Chemie - Int. Ed.* **2010**, 49, 1540–1573.
- [56] A. Shrivastava, V. Gupta, *Chronicles Young Sci.* 2011, 2, 21.

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Fluorescamine is a non-fluorescent compound that selectively labelled the -NH₂ moieties linked on GO functionalities leading to a fluorescent product