Hydroxamic acid-functionalized graphene thin films as nanocatalysts towards organophosphate degradation

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Abstract
Herein we describe the covalent functionalization of graphene oxide with hydroxamic acid groups (GOHD) anchored directly on the carboxylate groups. The functionalization was confirmed by several characterization techniques, such as infrared spectroscopy, thermogravimetric analysis, scanning electron microscopy and Raman spectroscopy. The anchored groups have many potential applications, but we focused on its catalytic activity towards organophosphate (OPs) degradation, a serious environmental and health worldwide issue. The material was applied as nanocatalysts towards the degradation of the organophosphate of diethyl 2,4-dinitrophenyl phosphate and the real-life pesticide dimethyl 4-nitrophenyl phosphate (Paraoxon). High catalytic increments were obtained up to $10^8$ fold (Paraoxon). Thus, reactions that would take over millions of years are reduced to a couple of days. Overall, the nanocatalyst shows potential as efficient detoxifying agents to eliminate chemical warfare stocks and pesticides by employing a practical material that can be deposited on any substrate and even over large areas.

1. Introduction
The agricultural growth is intrinsically linked to the high consumption of pesticides, which leads to numerous problems such as people's health, contaminated water, poisoning, among others [1, 2]. The relationship between the two in Brazil is alarming, because the ratio used per planted area (kg ha$^{-1}$) increased significantly in the last years positioning Brazil as the largest consumer of pesticides in the world [3].

One of the toxic agents found in many pesticides, also used in chemical weapons and insecticides are organophosphate compounds (OP), especially phosphate esters, which are highly toxic and can be fatal. The use of OPs is illegal in many countries, but it is still present in many products and is a major threat today [4]. Among the different classes of OP, the triesters stand out, due to its non-natural occurrence, only synthesized for use as pesticides and chemical weapons and their high toxicity and stability [5].

There has been an increasing interest in monitoring the presence of OP as well as their degradation (i.e. eliminating their toxicity) One way to eliminate OPs is by promoting their dephosphorylation (cleaving P-O bond) using $\alpha$-nucleophiles. This class of compound is defined by the presence of a high electronegativity atom with non-shared electrons adjacent to the nucleophilic center, for example hydroxylamine (NH$_2$OH), hydroxamic acid, hydrazine and hydroperoxide. Due to this characteristic these compounds have a higher reactivity as compared to nucleophiles with the same basicity (pKa). Many studies in the literature have shown that hydroxamic acid is the most efficient group to react with OP, but they usually have many drawbacks. For example, when present in simple low-mass molecules they are unstable and rapidly degrade. Also, due to their high reactivity, they sometimes are not selective and can react with phosphorus and carbon center of OPs, leading to multiple reaction pathways and different side products.

Aiming at building catalysts for the degradation of OFs, some studies describe the functionalization of different matrices with various nucleophiles in an attempt to build artificial enzymes, for example, amphiphilic peptides [6], polyethylenimine derivatives [7] and polymers [8, 9]. Given the potential, a new
possibility is the use of graphene as a support for the immobilization of different species, allowing materials with synergistic properties to be obtained and new detection techniques to be developed.

Functionalized carbon materials have a good application as catalysts [10]. We recently showed the high potential of functionalized graphene oxide (GO) for dephosphorylation reactions. The modification occurred in the oxygenated groups (carboxylate groups) of the GO anchoring through amide bonds two highly reactive groups: cysteamine (made in the form of thin-film and powder) [11, 12] and imidazole (made in the form of powder and thin-film) [13]. The formation of the thin films was possible by a self-assembling route [14] at liquid-liquid (L/L) interfaces (water/toluene). This method is of high interest in catalysis due to the easy recovery of the catalyst. We observed a high catalytic increase for the dephosphorylation reaction of 2,4-dinitrophenyl phosphate (DEDNPP—model molecule for dephosphorylation reactions), up to 10^6 times compared to the reaction in its absence. Herein, we explored a novel functional group anchored covalently on GO: hydroxamic acid. Indeed, these groups are very promising towards OPs detoxification, presenting among the highest catalytic activity. To the best of our knowledge there is no report on hydroxamate-derived GO obtained by covalent functionalization and this material showed to be very catalytic. In addition, this material presents many other potential applications due to its known affinity with metals, especially for heavy metal elimination of contaminated media. The nanocatalyst was synthesized through the interfacial covalent functionalization of GO with HD (hydroxylamine hydrochloride), leading to hydroxamic acid groups (is most reactive deprotonated: hydroxamate), namely GOHD, figure 1. The nanocatalyst was employed in the destruction of OPs, included the actual pesticide Paraaxon. We show that when anchored on GO, by the proposed method, the final materials guarantee stability to the hydroxamic acid moieties since they don’t degrade and can be reused several times as catalysts. Besides, it is selective leading only to one reaction pathway (at phosphorus atom). Overall, it is desirable to obtain multifunctional nanocatalysts that may have several applications that will have a significant impact on the neutralization and detection of toxic agents (pesticides and even chemical weapons) to eliminate unwanted stocks and alert abusive usage or attacks.

2. Materials and methods

All reagents were used untreated: H_2O_2 30% (Vetec), H_2SO_4 98% (Impex), HNO_3 65% (Biotec), K_MnO_4 99% (Synth), N-hydroxysuccinimide NHS (Sigma-Aldrich), N-ethyl-N-(3-(dimethylamino)propyl) carbodiimide EDC (Sigma-Aldrich), NaBH_4 98% (Acros), NaNO_3 99% (Vetec), Graphite (National Graphite, Brazil), HCl 37% (Impex), Ethanol (Dynamic) and Toluene (Neon). All solutions were prepared with deionized water.

2.1. GOHD functionalization

Experimental details for obtaining graphene oxide (GO) are contained in the ESI (Electronic Supplementary Information), (https://stacks.iop.org/JPhysMaterials/3/034003/mmedia) based on the Hummer’s method adapted methodology [15].

GOHD (GO functionalized with HD) thin films were prepared by the GO-liquid interfacial functionalization (L/L) route of GO [12]. The reaction occurs primarily with the formation of the thin film of GO with the addition (20 ml) of an aqueous dispersion (0.02 mg ml) of this material in a toluene solution (10 ml) in a 50 ml round bottom flask under magnetic stirring (1000 rpm) for 12 h. Then the system was kept in an ice bath for 15 min to stabilize the temperature. Then 0.68 g NHS (30.0 mmol) and 1.14 g EDC (30.0 mmol) were added and the system was stirred under magnetic stirring for 1 h at low temperature (0 °C). After the ice bath was removed and the system was kept for an additional 1 h under ambient condition under agitation. Then, HD (0.22 mg) was added and the mixture was allowed to stir for 12 h. When magnetic stirring is interrupted, the dispersed material in the aqueous phase migrates spontaneously...
and provides the following information: (i) initially, the release of the water mass below 18°C was done on a FTIR Bio-Rad spectrophotometer over the range of 4000–400 cm⁻¹ concerning the stretching and deformation modes resulting from the graphite oxidized process:

The catalytic efficiency of the nanocatalyst was analyzed by kinetic studies accompanied by UV–Vis spectroscopy. Reactions were monitored by the formation at 400 nm of 2,4-dinitrophenol (DNP) and carboxylic acid C=O (1737 cm⁻¹), and δH2O (1637 cm⁻¹). Absorbance versus time curves followed a pseudo-first order profile and was adjusted with the Origin program as nonlinear regressions using the Levenberg-Marquardt algorithm to find the least sum of squares, the adjustments resulted in pseudo-first order constants, kobs, with correlation coefficients greater than 0.99.

Reactions were accompanied by at least 3 half-lives. The rate constant for Paraoxon was obtained by the initial ratio method, carried out by analyzing the consumption after 20 d of reaction by UV–Vis spectrum and calculated the constant rate, considering a pseudo-first behavior for Paraoxon. In both procedures it was possible to obtain the following rate constants: kobs, kcat and kactive. The first is the direct rate constant obtained from the kinetic fits, the second is the ratio of the rate constant and the total mass of catalyst and the last was obtained by the ratio of the rate constant by the mass of active site, i.e. hydroxamate groups. This mass was obtained through the thermogravimetric analysis considering the mass of the functionalized group.

3. Results and discussions

Functionalization was first confirmed by thermal degradation data (figure 2(A)) of GO and GOHD which is detailed in table 1 and provides the following information: (i) initially, the release of the water mass below 100 °C with GOHD being a discrete loss up to 3.8% and above 13% for GO [17, 18]; (ii) the loss of mass in the range of 135 °C–220 °C due to the oxygenated groups of GO, in this region the functionalized sample has a lower mass loss than that of GO, which means that the various carboxylic groups were functionalized with successful with HD; (iii) functionalized material GOHD has an additional mass loss in the range 135 °C–100 °C with GOHD being a discrete loss up to 30%.

The first is the direct rate constant obtained from the kinetic fits, the second is the ratio of the rate constant and the total mass of catalyst and the last was obtained by the ratio of the rate constant by the mass of active site, i.e. hydroxamate groups. This mass was obtained through the thermogravimetric analysis considering the mass of the functionalized group.

FTIR analysis is shown in figure 2B which shows some discrete bands for GO (less intense due to the thin film) concerning the stretching and deformation modes resulting from the graphite oxidized process: oxygenated groups, OH (3434 cm⁻¹) and carboxylic acid C = O (1737 cm⁻¹), and δH2O (1637 cm⁻¹).
Table 1. Relevant information from TGA analysis for GO and GOHD.

| T (°C)/% Mass Loss | GO Groups | GO       | GOHD     |
|-------------------|-----------|----------|----------|
| Oxygenated        | 120–300/41.0 | 167–268/33.6 |          |
| Hydroxamic acid   | –         | 270–374/30.6 |          |
| Carbon skeleton   | 440–600/47.7 | 375–565/23.2 |          |
| Residue           | –         | 1.9      | 6.0      |

Other bands can also be observed, regarding epoxide $\text{C-O-C (1217 cm}^{-1}$), alkoxy groups $\text{C-O (1065 cm}^{-1}$) and $\delta\text{C-OH at 1388 cm}^{-1}$ [20, 21]. The GOHD spectra shows the disappearance of the band at 1737 cm$^{-1}$ and the appearance at 1640 cm$^{-1}$ of the band for $\text{C}=\text{O}$ amides. Also, other bands related to functionalization emerged: $\text{N-H strain (1542 cm}^{-1}$), $\text{C-N strain (1427 cm}^{-1$) and $\text{N-H amide stretch at 3290 cm}^{-1}$ [22, 23], that confirm obtaining the covalently functionalized GOHD film. Also, some new bands appear in the region of 900 to 1500 cm$^{-1}$ these assigned to 976 cm$^{-1}$ $\delta\text{(N–C = O)}$, 1041 cm$^{-1}$ (N–O), 1150 cm$^{-1}$ $\delta\text{(CNO)}$, 1220 cm$^{-1}$ (C–C), $\delta\text{(N—H)}$, and 1420 cm$^{-1}$ $\delta\text{(C–N–H)}$ and $\delta\text{(OH)}$, confirming the presence of the hydroxamic acid group [24–26].

GOHD was further characterized by Raman spectroscopy. The technique is a powerful tool for the characterization of carbon materials, as it is possible through it to identify defects and structural modifications. Figure 3 shows the typical bands of graphite materials: (i) band D at $\sim$1350 cm$^{-1}$ attributed to defects and edge effects of the graphite region; (ii) G-band at $\sim$1586 cm$^{-1}$ related to the C-C stretch of the graphite structure, and D-band at $\sim$1609 cm$^{-1}$ also correlated to structural defects [27]. Another feature of graphene-based materials can be seen in the region of high frequency with the presence of the 2D ($\sim$2700 cm$^{-1$), 2G ($3130 \text { cm}^{-1$) bands and the $\text{D + D'}$ band at 2936 cm$^{-1$ attributed to the G-band overtone or mode combinations [28].

One of the parameters studied in the Raman spectra of graphene samples is the $\frac{\text{I}_D}{\text{I}_G}$ ratio that provides an estimate of the amount of defects present in the carbonaceous structure allowing the determination of the defect stage of the obtained material that can be directly related to the functionalization [22, 29]. When
comparing GO, and GOHD, the $I_D/I_G$ ratio increased from $1.78 \pm 0.3$ to $2.06 \pm 0.7$, respectively, thus giving a higher level of defects for GOHD that could be attributed to the effective functionalization. The individual response of the hydroxamic acid groups is not visualized due to the overlapping of the functionalized material bands. The high value of the $I_D/I_G$ ratio for the functionalized materials is corroborated with the thermogravimetric analysis since it is possible to correlate the mass loss in the region of the functionalized groups with the increase of the $I_D/I_G$ ratio for GOHD, a fact also observed in other studies [11]. Figure 4 shows the SEM images for GO and GOHD. The homogeneity and transparency of films are verified after functionalization over glass as illustrated in the inset photograph for GOHD. In the SEM image, the material presents crumpled and folded sheets typical of materials derived from graphene [30]. In addition, it can be verified regions with apparently more sheets stacked, present in the edges of the image.

### 3.1. Nanocatalysts for organophosphate destruction

GOHD film was applied as a nanocatalyst in the degradation reaction of DEDNPP and Paraoxon (figure 5). The reaction was followed by the formation of DNP and PNP products of degradation from DEDNPP and Paraoxon, respectively, monitored by UV–Vis by color change. The reaction was performed with the film deposited on glass substrate (four layers) [13] and the material was immersed in the solution with the organophosphate under stirring without any release of the film to the solution. The reaction for DEDNPP was followed at different time intervals by UV–Vis at 400 nm. The spectra and kinetic profiles are illustrated in figures 6(A) and (B) for GOHD. The catalytic evaluation of the film was performed by adjusting the kinetic profile obtained and comparing the $k_{obs}$ value obtained with the spontaneous hydrolysis of the same reaction and other catalysts. The degradation of Paraoxon was also accompanied by UV–Vis at 400 nm, however, the method for monitoring kinetics and obtaining $k_{obs}$, in this case, was performed using the initial velocity method, which is based on the slope of the line at the UV–Vis Paraoxon degradation starting points, figures 6(C) and (D). The value obtained is compared with a degradation reaction of the same pesticide consolidated in the literature.
Table 2 presents the catalytic activity of nanocatalysts evaluated in the destruction of DEDNPP at pH 8.5. For GOHD, it is known that the pKₐ of hydroxamic acids can vary between 6.5–10.0 [31], and also that the hydroxamate specie is the reactive one. Hence, we expect that at pH 8.5, GOHD is in the ionic form: hydroxamate, given its high activity. Second order kinetic constants were obtained from the fit and correlated with the anchored nucleophile mass (k_active) and also the total catalyst mass (k_cat) as described in table 2.

The k_obs obtained for GOHD evidence a high catalytic activity. This is best illustrated considering the amount of catalyst (total mass, k_cat), as the thin film synthesized in this work uses a small mass (~0.0575 mg) compared to typical solid materials (~1.0 mg). Also, if we consider solely the active sites, i.e. hydroxamate groups, obtained from TGA analysis, the rate constant obtained k_active shows a more impressive activity for GOHD. Comparing with the spontaneous reaction (under the same condition with the catalyst in just water), the nanocatalyst herein presents a catalytic increment of 9.8 × 10⁶ fold (k_active/k_H₂O) for GOHD. This is higher than observed with some nanocatalysts already reported (containing imidazole and thiol) [13], and among the most efficient catalyst reported till date towards DEDNPP degradation. This means that a reaction that would normally take over 10 d (DEDNPP hydrolysis) without catalysts, is reduced to 1 h in the presence of GOHD.

In general, the heterogeneous reaction evaluated with nanocatalysts suggests a synergistic effect of the groups anchored with the structure of GO. Importantly, pure GO has no significant catalytic activity, evidencing the potential of the material as a nanocatalyst. GOHD showed the best catalytic increments...
reported and its preparation has not been reported yet. A comparison with the precursor hydroxylamine is not possible since hydroxylamine and hydroxamic acid are known to have different reactivities ($pK_a \sim 6$ for hydroxylamine and $\sim 6.5\text{--}10.0$ for hydroxamic acids)\cite{32}. Moreover, hydroxamic acid when present in simple low-mass molecules are unstable and rapidly degrades, so it is not possible to correlate to the same functional group. On the other hand, results here show that when anchored on GO, the hydroxamic acid groups remain stable and can be very promising for further applications with this specific group.

GOHD was also evaluated as a catalyst towards the degradation of a real-life pesticide, Paraoxon, that presents higher stability. Also, the pesticide presents high toxicity and its use is prohibited in Brazil\cite{33–35}. Thus, the formation of the reaction product phenolate (PNP) was accompanied by UV–Vis at 400 nm as described in figure 5 in which it exemplifies the reaction of the nanocatalyst with the pesticide. The method for monitoring kinetics and obtaining $k_{\text{obs}}$, in this case, was performed using the initial velocity method. Thus, the Paraoxon reaction presented a $k_{\text{active}}$ rate constant of $2.0 \text{ g}^{-1} \text{ min}^{-1}$ for GOHD, extremely high, leading to catalytic increment (compared to the spontaneous reaction) of $3 \times 10^8$ fold ($k_{\text{active}}/k_{\text{H}_2\text{O}}$). Thus, a reaction that would take over millions of years (Paraoxon hydrolysis) can take solely 5 d in the presence of GOHD. Therefore, comprising an interesting and promising detoxification process.

Figure 7 illustrates the catalytic increments observed for the DEDNPP and Paraoxon with different catalysts reported in the literature and compared with that applied in this work. The GOHD nanocatalyst presented the highest catalytic increment until now, even if comparing with homogeneous catalysts and materials of a complex nature, such as polymers. The catalytic results described here are better than the GOSH and GOIMZ thin film developed in our group by a similar route\cite{12,13}. Moreover, it is worth highlighting the efficiency of graphene as a support, since the results obtained were better than those functionalized with the same groups in the literature as polyhydroxamate, which is formed by the functionalization of polyhydroxyalkanoates with hydroxylamine, as done here.

After the catalytic studies, the nanocatalyst was characterized to verify possible changes in the material structure after the reaction. The FTIR spectra were obtained before and after the use of GOHD in the DEDNPP dephosphorylation reaction (see SI). It is possible to confirm the presence of amide bond bands, characteristics of the functionalized material, before and after use ($1640 \text{ cm}^{-1}; 1542 \text{ cm}^{-1}$ and $3290 \text{ cm}^{-1}$). The FTIR spectra shown in SI confirms the preservation of the films with no modifications in the amide bands.

The recycling of GOHD nanocatalysts was confirmed by the consecutive use (3 times) of the same material (GOHD) in the DEDNPP reaction. The procedure in this case was done replacing the solution with a new aliquot of DEDNPP. The recycling if confirmed by analyzing the values of $k_{\text{obs}}$ ($7.06, 6.65$ and $6.82 \times 10^{-4}$ for 1st, 2nd and 3rd use, respectively), showing no significant change with the consecutive use. Hence, the nanocatalyst can be recycled.

Further, it is possible to propose a mechanism (figure 8) for the reaction of GOHD with Paraoxon/DEDNPP. First, it is believed that OPs are adsorbed on the surface of the nanocatalyst due to the attraction of van der Waals of hydrophobic carbon structure with the aromatic rings of OPs. Upon
attraction, the surface containing the nucleophilic groups reacts rapidly. Based on other’s works with the reaction with hydroxamic acids (DEDNPP [8] and Paraoxon [40]), the reaction occurs by the anionic O atom. Based on other studies by our group where hydroxamate is anchored in rice hulls and gum arabic, the attack is expected to occur exclusively at the phosphorus center of the OP.

Finally, it is possible to affirm after synthesis, characterization and application of GOHD in a thin film form that the material synthesized in this work has high catalytic performance against model molecules such as DEDNPP and also using the real and highly stable pesticide Paraoxon. The synthesis and application of materials open great prospects for the development of a new way to degrade toxic pesticides by employing a practical material that can be deposited on any substrate and even over large areas.

4. Conclusions

We report a new approach for anchoring hydroxamic acid groups in GO through stable amide bonds, as thin-film. The materials were characterized and the functionalization was confirmed by various techniques. The anchored groups have many potential applications, but we focused on their catalytic activity towards OPs degradation, serious environmental and health worldwide issue. The degradation of OPs was evaluated towards DEDNPP and Paraoxon. The material showed high catalytic performance, where the nanocatalyst was consecutively recycled, maintaining the activity, which was confirmed from post-reaction characterizations. Reactions that would take over millions of years are reduced to a couple of days. The proposed material has potential as efficient detoxifying agents to eliminate chemical warfare stocks and pesticides. Thin films can still be easily handled, washed and reused by simply dipping the film into the contaminated medium. Overall, the deposition of material in a thin film form has proved to be a powerful tool for degrading OP and also for designing sensors. Moreover, other applications can be pursued and extended since hydroxamic acids are known to be applied as chelating agents of heavy metals in decontamination areas, among others.

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