Synergistic exploitation of the superoxide scavenger properties of reduced graphene oxide and a trityl organic radical for the impedimetric sensing of xanthine

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This work is based on synergetically exploiting the activity of graphene-based materials and trityl free radicals to sense xanthine (X) by their combined scavenging properties for superoxide anion radical ($O_2^{\cdot-}$). For this, reduced graphene oxide (rGO) and rGO covalently functionalized with a perchlorotriphenylmethyl (PTM) radical derivative (rGO@PTM) have been synthesized, characterized and casted on an electrode surface to achieve a highly sensitive electrochemical recognition platform for xanthine determination. The electrochemical analysis is based on impedimetrically monitoring a radical-involved reaction on the graphene-based electrode surface after reacting with such $O_2^{\cdot-}$ derived from the xanthine/xanthine oxidase (X/XO) enzymatic system. The presented strategy yields to determine X at nM levels, decreasing the detection limit 100 times with respect to previously reported (bio)sensors.

1. Introduction

Xanthine (3,7-dihydro-purine-2,6-dione, X) is a purine base derived from hypoxanthine by xanthine oxidase (XO).^[1] X, which is the metabolic precursor of uric acid, it is the first indicator of an abnormal purine profile, which can serve as a marker of acute hypoxia stress.^[2] Since excess of X in serum/urine can provoke serious pathologies like a rare genetic disorder called xanthinuria, renal failure and gout, fast diagnosis methodologies for X determination are crucial.^[2] Several analytical (such as HPLC, capillary column gas chromatography, electrophoresis and flow injection), colorimetric and fluorimetric methods have been employed for determination of X in biological materials.^[3] These methods are usually expensive, complicated, time consuming and they entail costly machinery to operate. Electrochemical sensors may overcome these limitations thanks to their simplicity, rapidness, sensitivity, specificity, low cost, and automated methodologies.^[4–6]

The enzyme XO is a type of liver enzyme that catalyses the univalent and divalent reduction of ground state oxygen to generate both superoxide anion radical $O_2^{\cdot-}$ and hydrogen peroxide (H_2O_2) via a one-electron and a two-electron reduction respectively, resulting in the oxidation of hypoxanthine to X and then X to uric acid.^[7] To date, the determination of X through an electrochemical device is mainly focused on immobilizing XO onto different films and membranes, where the H_2O_2 resulting from the Xanthine/Xanthine Oxidase (X/XO) enzymatic system is amperometrically biosensed.^[3] However, these XO-based amperometric biosensors have few drawbacks, such as poor stability and slow electron transfer, yielding to determine X at μM levels. More recently, biosensors based on metal or metal oxide nanoparticles mixed with carbon-based nanomaterials, including graphene related nanomaterials, have shown some electrocatalytic properties towards X determination but the limit of detection was not improved significantly.^[1,3]

In this regard, graphene and graphene-based materials, such as graphene oxide (GO) and reduced graphene oxide (rGO), have shown enhanced electrochemical and electrocatalytic activity over conventional carbon materials (*e.g.*, graphite or carbon nanotubes) for (bio)sensing purposes.^[8–12] While graphene is an electrical conductor, GO can be electrically insulating, being its reduction to rGO a simple strategy to partially restore the π electron network of graphene and hence its electrical conductivity. Among several novel applications,^[13] graphene-based materials have shown to possess antioxidant activity, *i.e.* they can act as radical scavengers against various reactive oxygen species (ROS), especially for hydroxyl radicals or superoxides.^[14,15] These studies showed that the primary active sites are associated with the pristine sp^2 -carbon network, rather than oxygen-containing functional groups.

Perchlorotriphenylmethyl (PTM) radicals are persistent organic free radicals from the trityl radical family, the chemical and thermal stability of which rely on the full steric blockage of the central carbon atom by the ortho chlorine atoms.^[16] Previous to our work, water soluble PTM derivative was demonstrated to react with high sensitivity and specificity with the superoxide generated from the X/XO enzymatic system. The results pointed to a superoxide-mediated reduction of the PTM radical to PTM anion, which on subsequent protonation yielded to the formation of perchlorotriphenylmethane (α H-PTM, the non-radical form) and molecular oxygen.^[17]

Taking these antecedents into account, in this work, the synergistic effect attained by combining the electrochemical and scavenger properties of both rGO and PTM radicals is exploited for the preparation of a new generation of electrochemical (bio)sensor for xanthine detection. rGO and an rGO-based hybrid-nanomaterial carrying PTM radical molecules (rGO@PTM) have been synthesized, characterized and casted on a carbon-based nanocomposite electrode surface. A highly sensitive recognition platform has been developed, which is based on the indirect impedimetric sensing of X through the interaction of the active

material deposited on an electrode with the *in situ* $O_2^{\cdot -}$ formed from the X/XO enzymatic system (**Figure 1**). The electrochemical performance was evaluated using Electrochemical Impedance Spectroscopy (EIS), since it is a very sensitive tool for surface characterization. Interestingly, by EIS the reactivity of the rGO with the generated $O_2^{\cdot -}$ is clearly observed. Noteworthy, it is demonstrated that upon the grafting of the organic radical, this offers an electrocatalytic activity in terms of enhanced sensitivity if compared to the bare rGO.

2. Results and Discussion

2.1. Synthesis and characterization of the hybrid rGO@PTM material

Reduced graphene oxide (rGO) was prepared by chemical reduction of GO^[18] (synthesized by a modified Hummers' method)^[19] with hydrazine (see Supporting Information). Thermogravimetric analyses (TGA) under flowing air were performed to confirm the reduction of GO. As expected, rGO shows a higher thermal stability than GO (**Figure S1**). In particular, the weight loss at ca. 200 °C observed for the GO sample is no longer visible in the TGA of rGO. This suggests the successful elimination of the majority of the O-based aliphatic functionalities of GO with the hydrazine treatment.

The covalent grafting on sp^2 carbon network via free radical addition has been widely accomplished with aryl diazonium salts.^[20] Here, the rGO@PTM hybrid-nanomaterial was synthesized by the chemical reduction of the diazonium functionalized PTM radical (PTM- $N_2^+BF_4^-$) inducing the formation of the corresponding aryl radicals that reacted with the suspended rGO (see SI, **Scheme S1** and Experimental Section). Very recently we showed the successful electrochemical grafting by means of cyclic voltammetry of the same PTM derivative on highly oriented pyrolytic graphite which was characterized by electrochemical scanning tunneling microscopy.^[21]

The rGO@PTM was characterized using various techniques and compared with the unfunctionalized rGO. UV-Vis spectroscopy of a suspension of rGO@PTM in acetonitrile

showed the distinct bands at 224 nm and 387 nm that corresponded to the PTM radical, in addition to the π - π^* transition at 275 nm that was assigned to the rGO (**Figure 2a**). This band is red-shifted ca. 5 nm with respect to pristine rGO, which is attributed to the chemical modification of the rGO structure (a dotted line has been included in the spectra for ease of comparison). The infrared attenuated total reflection (IR-ATR) spectrum of the rGO@PTM also showed new peaks around 1543, 1387, 1151 and 1070 cm^{-1} that corresponded to the presence of chlorinated phenyl rings of the PTM moiety (grey area in **Figure 2b**). These peaks are less intense and broader if compared with the IR spectrum of the $\text{PTM-N}_2^+\text{BF}_4^-$ in powder, which is a characteristic feature of covalently anchored molecules. In addition, electron paramagnetic resonance spectra (EPR) of both rGO and rGO@PTM nanomaterials were registered, giving a featureless spectrum for the rGO and a broad low intense EPR signal with a g value of 2.0024 for the rGO@PTM, which is a typical value for an organic radical (see SI, **Figure S2**).

X-Ray photoelectron spectroscopy analysis (see SI, **Figure S3**) of rGO@PTM drop-casted on a gold disc showed similar C1s peaks to that of rGO at 285.1 (C–C and C=C bonds), 286.3 (C–O due to hydroxyl and epoxy groups) and 289.5 eV (C=O bonds). However, the area of the peak at 286.3 eV is around 28% higher than that of the rGO powder, which is attributed to the C–Cl contribution typically observed at this binding energy. In addition, whereas rGO does not show any peak due to the Cl atoms, Cl2p spectrum for the rGO@PTM displayed a peak that was deconvoluted into two peaks at 200.9 and 202.5 eV. These corresponded to the $\text{Cl}2\text{p}^{3/2}$ and $\text{Cl}2\text{p}^{1/2}$ respectively, at a 2:1 intensity ratio and with an energy difference of 1.6 eV as expected, exhibiting the presence of chlorine atoms from the PTM moieties on rGO@PTM. Importantly, the N1s spectra point to the lost of the diazonium group and hence the covalent bonding of the molecule to the substrate (**Figure S3**). **Figure 2c-e** shows the elemental mapping acquired by Energy Filtered Transmission Electron Microscopy (EFTEM) of a functionalized rGO flake. Electron Energy Loss Spectroscopy (EELS) analysis on a

modified flake shows the Cl-L_{2,3} and C-K edges and no peak for N-K (see SI, **Figure S4**). The elemental mapping for carbon (Figure 2c) and chlorine (Figure 2d) were acquired. It is clear that chlorine is only present on the rGO@PTM hybrid-nanomaterial whereas carbon is present on the hybrid and also on the carbon film employed as support for the analysis. The overlap image of the carbon and chlorine (Figure 2e) clearly shows a homogenous distribution of Cl over the entire rGO flake.

Overall, these results show the successful anchoring of the PTM radical onto the rGO sheets, resulting in a suitable graphene-based nanomaterial to be employed for electrochemical sensing purposes.

2.2. Electrochemical characterization

A hand-made nanocomposite carbon paste electrode (NC-CPE) based on an optimum dispersion of multiwalled carbon nanotubes within an insulating epoxy resin (10:90 w/w, respectively) with a geometric area of 28 mm² was prepared and used as a solid electrode support for the electrochemical experiments (see Experimental section).^[22] Bare NC-CPE and NC-CPE modified with either rGO or rGO@PTM were prepared, characterized and compared. The functional electrodes were fabricated by drop-casting 200 µL of dispersion of either rGO or rGO@PTM in acetonitrile (0.25 mg/mL) on the freshly polished NC-CPE surface. Upon evaporation of the solvent, the modified-electrodes were used as the working electrode. The electrochemical studies were carried out by means of Electrochemical Impedance Spectroscopy (EIS). The experiments were performed with a three-electrode configuration electrochemical cell filled with a solution of 10 mM [Fe(CN)₆]^{3-/4-}, 100 µM of diethylene triamine penta acetic acid (DTPA) and 50 mM of tris(hydroxymethyl)aminomethane (Tris)-HCl buffer (pH 7.4) as the electrolyte. DTPA was used as a heavy metal scavenger in order to avoid side reactions with metals in the medium. [Fe(CN)₆]^{3-/4-} was employed as a benchmark redox marker since it is a commonly used redox couple to investigate the electrochemical

performances of modified electrode surfaces. An Ag/AgCl electrode and a Pt wire were used as reference and auxiliary electrodes, respectively.

Nyquist plots are shown in **Figure 3**. While the diameter of the semicircle at higher frequencies corresponds to the charge transfer resistance (R_{CT}) at the electrode surface, the linear part at lower frequencies is related to the diffusion process. Randles circuit was used for fitting the impedance data (Figure 3, inset). The R_{CT} parameter depends on the dielectric and insulating properties of the electrode/electrolyte interface. After modifying the bare NC-PE with either rGO or rGO@PTM nanomaterials, the redox probe exhibited lower permeability, decreasing the Faradaic response and obstructing the penetration of the redox marker significantly. As a result, an increase in the R_{CT} was observed, from 123 Ω for the bare NC-CPE to 240 Ω and 318 Ω for the electrodes containing rGO and rGO@PTM, respectively. The presence of the grafted PTM molecules on the rGO caused a 33% increase on the R_{CT} when compared with the non-functionalized rGO, which is attributed to the steric effect of the bulky PTM on the flakes that hinders the electron transfer process of $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

2.3. Xanthine sensing response by EIS

After the satisfactory electrochemical characterization of the functional electrodes, the feasibility of both rGO and rGO@PTM nanomaterials as recognition platforms for the indirect determination of X were impedimetrically evaluated. This indirect determination is based on the reactivity of the components of our material towards the superoxide, which as explained above, is generated from the X/XO enzymatic mixture. The on-surface reaction was followed by the variation of the R_{CT} electrode response to the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ charge transfer. Due to the instability of the XO, a solution of X with a fix concentration was prepared and the amount of XO added to this solution was adjusted. Since the reaction between X and XO is very efficient, the conversion rate is assumed to be 100%. The electrochemical recognition assay is based on two steps: *i*) first, a known amount XO was added to a Tris-HCl buffer

solution (pH 7.4) containing DTPA and a fix concentration of X to generate the $O_2^{\cdot -}$ and *ii*) rapidly, the rGO or rGO@PTM NC-CPE electrode was dipped into this solution for 15 min, promoting the reaction between the active electrode and the $O_2^{\cdot -}$ (see Figure 1). As the concentration of XO is increased, the superoxide radical concentration would increase as a result of more X oxidation. Afterwards, prior to the EIS measurement, the electrodes were rinsed with water.

Electroanalytical analysis represented by means of Nyquist plots showed a consistent increase in the R_{CT} parameter with increasing of [XO], and hence [X] (**Figure 4a**), which can be mainly attributed to the disruption of the sp^2 carbon domains through adduct formation^[23] and the redox reaction with the grafted radicals. To evaluate the detection limit and the linear response range of both graphene-based recognition platforms, a calibration curve was also built (**Figure 4b**), where the analytical signal was expressed as Δ_{RATIO} vs log [X]. The use of this Δ_{RATIO} was necessary in order to obtain independent and reproducible results while comparing the different electrodes used here, and was defined as follows:

$$\Delta_{RATIO} = \Delta_{SIGNAL} / \Delta_{BLANK}$$

$$\Delta_{SIGNAL} = R_{CT \text{ (recognition platform in X/XO)}} - R_{CT \text{ (NC-CPE)}}$$

$$\Delta_{BLANK} = R_{CT \text{ (recognition platform)}} - R_{CT \text{ (NC-CPE)}}$$

where $R_{CT \text{ (recognition platform in X/XO)}}$ is the electron transfer resistance value measured in the buffer with the modified NC-CPE electrode after incubation in the solution containing X/XO, $R_{CT \text{ (recognition platform)}}$ is the electron transfer resistance value measured with the modified NC-CPE surface electrode without incubation with the analyte, and $R_{CT \text{ (NC-CPE)}}$ is the electron transfer resistance value of the bare NC-CPE in the buffer. Each measurement was acquired three times ($n=3$) with three different electrode surfaces ($n=3$) that are regenerated each time by simple polishing ($n_T=9$). The reproducibility of the obtained results is attained to the exhaustive control of several parameters, including: *i*) the experimental setup (*i.e.*, electrode size, controlled area and polishing procedure), *ii*) the deposition methodology (ensuring a

good dispersion of the material by ultrasonication, its concentration and deposited volume), *iii*) the reproducibility of the synthesized active material itself and *iv*) the effectiveness of the reaction.”

Interestingly, the results certainly showed that the rGO exhibits a sensing response for the indirect detection of X, i.e. a clear reactivity towards the $O_2^{\cdot -}$ (**Figure 4b**, line b2). Remarkably, this was substantially enhanced when the rGO@PTM was employed (Figure 4b, line b1). Besides, although both sensing materials showed the same limit of detection (0.52 nM) and linear response in the range (3.7 to 41.5 nM) (see SI, **Figure S5**), a noticeable electrocatalytic effect was observed after the covalently grafting of the organic PTM radical on the rGO surface, yielding to a 60% of enhanced sensitivity from $0.50 \Omega \cdot nM^{-1}$ to $1.31 \Omega \cdot nM^{-1}$ (Figure 4b). As mentioned above, the PTM radicals once exposed to the $O_2^{\cdot -}$ undergoes a redox reaction followed by a protonation step that leads to the formation of the non-radical αH -PTM.^[17] Such extra measured increase in R_{CT} upon the formation of the non-radical species is still an open question but it could be attributed to the contribution of different factors such as: change in the molecular structure, increase of the charge transport resistance across the non-radical species^[24] and a possible modulation of the rGO characteristics due to the different electronic interaction of the radical or the non-radical molecule with the substrate.

Comparing these results with electrochemical (bio)sensors previously reported in the literature for X determination, which are summarized in **Table 1**, the herein proposed impedimetric sensor decreased the limit of detection 10^2 times. This can be ascribed to the ROS scavenging properties of the employed active material offering a high sensitivity when combined with EIS technique in a way that a small surface modification was greatly translated into a detectable output signal. Furthermore, it is important to highlight that the novel proposed strategy for X sensing may avoid possible common interferences since the electrochemical determination is not based on the common amperometric methodologies

where all the electrochemical active species undergoing a redox process at the potential applied are susceptible to interfere at the (bio)sensing platform. In addition, as reported by Kuppusamy and collaborators ^[17] the radical character of the PTM radical was not affected by the presence of other free radicals and biological oxido-reductants highlighting its envisaged specificity. In future work the specificity will be investigated in more complex or real samples.

3. Conclusion

Reduced graphene oxide (rGO) has been successfully chemically modified with a stable organic PTM free radical. It was demonstrated that this hybrid (rGO@PTM) system and the non-modified rGO can be used as active sensing materials for the indirect determination of xanthine by exploiting their reactivity with the superoxide anion radical. The scavenger properties of both, rGO and rGO@PTM nanomaterials, towards these reactive oxygen species have been synergistically exploited to fabricate a novel highly sensitive recognition platform based on the impedimetric determination of xanthine in a buffer media. The grafting of the radical molecules on the rGO led to an electrocatalytic effect enhancing the sensitivity by 60% when compared with the bare rGO. A striking limit of detection as low as 0.52 nM was achieved, decreasing the limit of detection 10^2 times when compared with other electrochemical (bio)sensors found in the literature. To the best of our knowledge, this is first time where a radical-involved reaction was monitored, by means of charge transfer resistance changes, on an electrode surface. We believe that impedimetric sensors based on monitoring radical reactions represents an unexplored field and that this work may open up the exploitation of such graphene-based engineered materials for a new generation of highly sensitive enzyme-based electrochemical (bio)sensors.

4. Experimental Section

Chemicals: $\text{PTM-N}_2^+\text{BF}_4^-$ was synthesized as previously described.^[21] Xanthine, xanthine oxidase (XO from bovine milk, ≥ 0.8 units/mg protein) and diethylenetriaminepentaacetic acid (DTPA) were purchased from Sigma Aldrich.

Reduced graphene oxide preparation: Reduced graphene oxide (rGO) was synthesized by reduction of GO in presence of hydrazine monohydrate (see ESI). PTM radical diazonium salt was synthesized following the literature procedure.^[21] Synthesis of rGO@PTM powder: 5.5 mg rGO powder was dispersed in 60 ml acetonitrile and this suspension was cooled down to 5°C in an ice-water bath. To this mixture 1.7 mg (30 μM) of $\text{PTM-N}_2^+\text{BF}_4^-$ was added. Finally, 0.5 ml of hypophosphorous acid (H_3PO_2 , 50 wt % in H_2O) was added and the reaction mixture was stirred overnight. At the end of this time reaction the mixture was filtered using Nylon filter paper, and the residue was washed with acetonitrile (20 ml x 3) to remove the unreacted species. rGO@PTM powder was characterized using UV-Vis, IR, EPR, XPS, TEM (described in the main text and SI).

Preparation of working electrodes: A hand-made nanocomposite carbon paste electrode (NC-CPE) was prepared by mixing multi-walled carbon nanotubes (SES Research, >95% of carbon purity, 10–30 nm of outer diameter and has about 5–15 μm of length) and epoxy resin (Epotek H77) in a 10:90 (w/w) ratio, which was found to be the best composition for electrochemical analysis. Briefly, a small copper disk was soldered at the end of an electrical female connector and introduced to a PVC tube for the body electrode. Afterwards, the carbon paste was incorporated in the hollow end of the PVC tube and cured during 24h at 80 °C. Finally, the electrode surface was polished with different sandpapers in order to obtain a reproducible electrochemical surface. The resultant geometric area was 28 mm². This type of nanocomposite-based electrode was used because of its several electrochemical benefits in comparison with conventional pure solid carbon electrodes, including robustness from a mechanical point of view, renewable surface, chemical inertness, stable responses, low ohmic

resistances, biocompatibility, printability for miniaturizing and suitability for a variety of biosensing applications, being a nontoxic and environmentally friendly type of electrode. Functionalized NC-CPEs resulted from drop-casting 200 μL of dispersion of either rGO or rGO@PTM nanomaterials in acetonitrile (0.25 mg/mL) on the freshly polished NC-CPE surface. After each analysis, electrodes were reset by a simple polishing step.

Electrochemical measurements: Impedimetric measurements were carried out using a Novocontrol Alpha-AN impedance analyzer equipped with a potentiostat POT/GAL 30V/2A electrochemical interface. The experiments were performed with a three-electrode configuration electrochemical cell filled with an electrolyte solution of 50 mM TrisHCl buffer (pH 7.4) containing 10 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 100 μM DTPA. $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was used as a benchmark redox marker since it is very sensitive to the electrode surface characteristics. DTPA was used as a heavy metal scavenger in order to avoid side reactions with metals in the medium. An Ag/AgCl electrode and a Pt wire were used as reference and auxiliary electrodes respectively. Finally, a hand-made nanocomposite carbon paste electrode (NC-CPE) was prepared by mixing multi-walled carbon nanotubes and epoxy resin in a 10:90 (w/w) ratio, which was found to be the best composition for electrochemical analysis. Electrodes were dipped into electrolyte solution and the impedance spectra were recorded in the frequency range of 0.1 Hz to 100 kHz (signal amplitude to perturb the system was 5 mV) at the redox equilibrium potential (+ 0.2 V).

Electroanalytical experiments: the working electrode was dipped in the aforementioned buffer solution containing a fixed amount of xanthine (0.5 M) with the desired concentration of xanthine oxidase (from 0.52 to 83 nM). The incubation took place for 15 min under soft stirring, similarly to a common immunoassay. Then, the working electrode was washed with copious amounts of Milli-Q water and dipped into

the electrochemical cell. In order to estimate the reproducibility and repeatability, three different experiments were performed for each surface per triplicate ($n_T=9$).

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Figure 1. Scheme of the reaction between the developed rGO@PTM electrochemical platform and the in situ generated superoxide through the reaction between the xanthine/xanthine oxidase enzymatic system.

Figure 2. UV-Vis (a) and ATR-IR (b) spectra of rGO and rGO@PTM. EFTEM elemental distribution maps for carbon (c) and chlorine (d) of a rGO@PTM flake. Image displayed in (e) corresponds to the overlap of c and d (carbon in red and chlorine in green)

Figure 3. Nyquist plots of bare, rGO and rGO@PTM drop-casted NC-CPEs in 10 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ Tris-HCl *buffer* solution at pH 7.4 and DPTA. Inset: Randles circuit used for fitting the impedance results where R_s is the resistance of the solution, R_{CT} is the electron-transfer resistance, C_{dl} is the double-layer capacitance and W is the Warbug impedance.

Figure 4. a) Nyquist diagrams for different concentrations of XO (*a*) 0.52, *b*) 2.3, *c*) 5.2, *d*) 10.5, *e*) 20.7, *f*) 41.5 and *g*) 83 nM) employing the recognition platform based on rGO@PTM and rGO (inset). b) calibration curve, Δ_{RATIO} vs $\log [\text{xanthine}]$ for the rGO@PTM (b_1) and rGO (b_2).

Table 1. Comparison of the characteristic parameters of different xanthine sensors.

Electrodes	Electrochemical technique	Analyte monitored	LOD [μM]	Ref.
PVC membrane bound XO on a PtE ^{a)}	Amperometry	H ₂ O ₂	0.24	[25]
rGO-carboxymethylcellulose layered with Pt-NPs/PAMAM dendrimer/magnetic nanoparticles on a GCE ^{b)}	Amperometry	H ₂ O ₂	0.013	[26]
GPtNs on a GCE ^{c)}	Amperometry	H ₂ O ₂	0.1	[27]
Au-NPs/GCPE ^{d)}	Chronoamperometry	H ₂ O ₂	0.5	[28]
TiO ₂ -NPs/c-MWCNT carrying XO on a FTOE ^{e)}	Cyclic Voltammetry	H ₂ O ₂	0.05	[1]
rGO on a NC-CPE	Electrochemical Impedance Spectroscopy	O ₂	5.2·10 ⁻⁴	This work
rGO@PTM on a NC-CPE	Electrochemical Impedance Spectroscopy	O ₂	5.2·10 ⁻⁴	This work

^{a)} PVC: Polyvinylchloride, PtE: Platinum Electrode; ^{b)} Pt-NPs: Platinum Nanoparticles, PAMAM: Polyamidoamine, GCE: Glassy Carbon Electrode; ^{c)} GPtNs: Branched Platinum Nanostructures on Reduced Graphene; ^{d)} Au-NPs: Gold Nanoparticles, GCPE: Glassy Carbon Paste Electrode; ^{e)} TiO₂/c-MWCNT: Titanium Dioxide Nanoparticles and Carboxylated Multiwalled Carbon Nanotubes Nanocomposite, FTOE: Fluorine doped Tin Oxide Electrode.

The reactivity of reduced graphene oxide with *in situ* generated superoxide anion radical and the catalytic effect upon its chemical modification with a trityl organic radical are here used to indirectly determine xanthine.

Sensor

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Synergistic exploitation of the superoxide scavenger properties of reduced graphene oxide and a trityl organic radical for the impedimetric sensing of xanthine

Supporting Information

Synergistic exploitation of the superoxide scavenger properties of reduced graphene oxide and a trityl organic radical for the impedimetric sensing of xanthine

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1. Synthesis of graphene oxide (GO).

In a typical synthesis, 2 mg of graphite powder (20 μm , Sigma–Aldrich) were mixed together with 46 mL of H_2SO_4 and 1 g of NaNO_3 , keeping the mixture cooled down to 0 $^\circ\text{C}$ during 30 min. Afterwards, 6 g of KMnO_4 were added slowly and the reaction mixture was subsequently warmed (35 $^\circ\text{C}$) under magnetic stirring. After 30 min, distilled water (100 mL) was added and the mixture was stirred under reflux (98 $^\circ\text{C}$) during 2 h. The dispersion was diluted with 400 mL of water and 2 mL of a 30 % H_2O_2 solution were added. The content was cooled down and the powder was finally centrifuged and washed with distilled water. The washing procedure was repeated until neutral pH was reached.

2. Synthesis of reduced graphene oxide (rGO).

rGO was synthesized by chemical reduction of GO. For this purpose, 100 mg of GO were mixed with 100 mL of distilled water and the dispersion was subsequently sonicated during 2 h. Afterwards, 1 mL of hydrazine monohydrate (98 %, Sigma–Aldrich) was added and the reaction mixture was refluxed (98 $^\circ\text{C}$) during 24 h under continuous stirring. The rGO powder was removed by filtration using a 47 mm hydrophilic PTFE membrane (0.2 μm , Omnipore)

and washed with distilled water until neutral pH was reached. Finally, the sample was dried at 60 °C overnight.

Figure S1. Thermogravimetric analyses of GO and rGO (as prepared by hydrazine reduction). TGA were performed under flowing air at a heating rate of 10 °C.min⁻¹.

Scheme S1. Scheme of the reaction taking place at the electrode surface

Figure S2. EPR spectrum of the rGO@PTM powder.

Figure S3. a) XPS analysis of rGO@PTM and pristine rGO in the region of C1s, Cl2p and N1s. The small signal at 400eV present in both materials can be attributed to the synthesis of graphene oxide by Hummer's method^[1] followed by the hydrazine reduction

Figure S4. EELS spectra acquired for the rGO@PTM. Energy loss peaks with onsets at 200 eV and 284 eV correspond to Cl and C, respectively.

Figure S5. Lineal (A) and logarithmic (B) representation of [Xanthine] vs. Δ_{ratio} at the rGO- (●) and rGO@PTM-based nanocomposite sensors (○). Inset shows the lineal range (from 3.7 to 41.5 nM). Experiments were carried out per triplicate with three different electrodes ($n_T=9$).

References

- [1] D. Kević, S. Sandoval, A. Pérez del Pino, E. György, L. Cabana, B. Ballesteros, G. Tobias *ChemPhysChem* **2017**, *18*, 935