Electrochemical sensor based on polyamide 6/polypyrrole electrospun nanofibers coated with reduced graphene oxide for malathion pesticide detection

Fernanda I. Migliorini¹², Rafaela C Sanfelice³, Luiza A Mercante¹³, Murilo H M Facure¹⁴ and Daniel S Correa¹⁴

¹ Nanotechnology National Laboratory for Agriculture (LNNA), Embrapa Instrumentação, São Carlos, SP, Brazil
² Department of Chemical Engineering, Federal University of the Triângulo Mineiro, Uberaba, MG, Brazil
³ PPG-CEM, Department of Materials Engineering, Federal University of São Carlos (UFSCar), São Carlos, SP, Brazil
⁴PPQG, Department of Chemistry, Center for Exact Sciences and Technology, Federal University of São Carlos (UFSCar), São Carlos, SP, Brazil
⁵ Authors to whom any correspondence should be addressed.

E-mail: fernandamigliorini@yahoo.com.br and daniel.correa@embrapa.br

Keywords: electrochemical sensor, graphene, electrospun nanofibers, pesticide detection, malathion

Supplementary material for this article is available [online](https://doi.org/10.1088/2053-1591/ab5744)

Abstract

An electrochemical sensor based on polymeric electrospun nanofibers of polyamide 6 (PA6)/polypyrrole (PPy) surface-modified with two forms of graphene was developed for the detection of malathion, an organophosphorus pesticide. The materials were chosen considering the electrospun nanofibers possess large surface area and porosity, while reduced graphene oxide has remarkable electrical conductivity, which is a good strategy to increase sensor sensitivity and improve the limit of detection towards the pesticide. The surface modification of nanofibers with graphene was carried out using chemically (CRGO) and electrochemically reduced graphene oxide (ERGO), in order to demonstrate how the degree of reduction of graphene oxide can influence its electrical conductivity. The influence of the reduction on the conductivity properties of graphene-based films was studied through electrochemical techniques: cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The platform modified with CRGO, which presented higher electrical conductivity compared to ERGO, was used in the electrochemical detection of the pesticide malathion and exhibited a low detection limit of 0.8 ng ml⁻¹ ($S/N = 3$). The results indicate that chemically reduced graphene oxide is a potential alternative for modifying electrodes surfaces designed for sensing distinct pollutants of environmental or agricultural interest.

1. Introduction

The modern world has witnessed intense technological, scientific and population growth, which is accompanied by the increase of pollution arising from all types of chemicals released in the environment [1–3]. As a consequence, humans and the environment can be affected directly and indirectly, once soil, water, air, living beings and food are the targets of contamination by chemical pollutants. Among the pollutants, pesticides used in agriculture are considered hazardous chemicals if not properly used or managed [4–6]. Specifically, organophosphorus pesticides (OPs) are a group of organic pesticides that present high toxicity, and contribute with about 38% of the total pesticide used worldwide [7, 8]. Such pesticides released in the natural environment can generate severe pollution problems, once they are not chemically specific and as a consequence, can affect several non-target species [9]. In addition, the OPs can be absorbed by the body in several ways and very rapidly, for example, through the skin, mucous membranes, lungs and the gastrointestinal tract [7, 10, 11]. The symptoms caused by contamination by malathion, one of the typicals OPs, can be headache, nausea, chest
tightness, even convulsions, unconsciousness and can also act to suppress the immune system at relatively high doses [7].

The traditional techniques for OPs detection are based on chromatography techniques, including gas chromatography (GC) and high-performance liquid chromatography (HPLC). These techniques, however, are time consuming, expensive and require highly skilled labor. In this sense, there is a crucial need for alternative methods that are reliable, rapid and sensitive for the detection of pesticides. In this direction, electrochemical sensors have been developed and widely applied due to their high sensitivity, low cost, fast detection time and direct operation [12, 13]. For instance, differential pulse voltammetry (DPV) technique has been applied with electrochemical sensors for the determination of organophosphorus pesticides, once DPV is sensitive to extremely small amounts of chemicals. Recently, a number of materials have been developed to improve the performance of electrochemical sensors both for greater sensitivity and for the immobilization of bioreceptors. In this sense, the modification of electrode surfaces with distinct nanomaterials, such as nanofibers, metal nanoparticles, carbon materials, among others, stand out for their unique characteristics, making them candidates for a wide variety of applications [14, 15]. Electrospun nanofibers, for instance, present 3D structures with large surface area and porosity [16–18], whose surface can be further modified with varied materials, including carbon materials. Carbon materials display excellent characteristics, such as low cost, wide window of electrical potential and good electrocatalytic activity [19]. Reduced graphene oxide (RGO) falls within this class of materials and present excellent adsorptivity and conductivity, high strength and good flexibility for chemical modification [20–24]. The RGO can be reduced on various substrates and through different processes, including electrochemical and chemical processes [25, 26].

In this work, we report the development of a novel nanostructured electrochemical sensing platform for detecting the organophosphorus pesticide malathion in water. The platform was composed of polymeric electrospun nanofibers of polyamide 6 (PA6) and polypyrrole (PPy) deposited on fluorine tin oxide (FTO) and modified with RGO. For that purpose, chemically reduced graphene oxide (CRGO) and electrochemically reduced graphene oxide (ERGO) were employed in order to evaluate how the degree of reduction of graphene oxide can influence its electrical conductivity properties, and consequently its sensing properties.

2. Experimental

2.1. Materials

Polyamide 6 (PA6, $M_w = 20,000$ g mol$^{-1}$), fluorine doped tin oxide (FTO) substrates, polypyrrole (PPy doped, 5 wt% dispersion in water), formic acid, ascorbic acid (AA) and 2,4-D Pestanal were purchased from Sigma-Aldrich. Graphite flakes, sulfuric acid (H$_2$SO$_4$), potassium permanganate (KMnO$_4$), hydrochloric acid (HCl) and hydrogen peroxide (H$_2$O$_2$) used in the synthesis of graphene oxide were purchased from Dinamica, Brazil. Malathion and cadusafos pesticides (commercial grade samples) were obtained from FMC (Brazil). The stock solutions of pesticides were prepared in acetone (6.25 μmol l$^{-1}$) and stored in amber flasks at 4 °C. All aqueous solutions were prepared with double-distilled water, and the chemicals were used without further purification.

2.2. Electrospinning of PA6/PPy nanofibers

PPy was combined to PA6 (PA6/PPy (7:1 w/w)) in formic acid solution in a concentration of 10% (w/v in respect to the solvent) and submitted to stirring for 4 h (room temperature). The electrospun nanofibers were obtained through an electrospinning apparatus operating with an electric voltage of 20kV and a solution feed rate of 0.02 ml h$^{-1}$. A working distance of 7 cm was maintained between the syringe and the metallic collector, and a 0.8 mm diameter steel needle was also used in the nanofiber production system. The electrospun nanofibers were directly collected onto the FTO substrates to obtain the modified electrodes (FTO/PA6/PPy). The FTO substrates were kept at the same position on the metallic collector, and the nanofibers were collected for approximately 40 min.

2.3. Synthesis and reduction of GO

Synthesis of graphene oxide (GO) and chemically reduced graphene oxide (CRGO) were performed according to the procedure described in previous works of our group [27, 28].

The GO and CRGO were first exfoliated in double-distilled water. A total of 1.0 mg ml$^{-1}$ of GO and CRGO were ultrasonicated for 40 min. The electrospun nanofibers collected on FTO were immersed into the GO and CRGO dispersion for 1 h to provide surface modifications of the nanofibers. Next, they were rinsed with distilled water and dried under ambient conditions.

The electrochemical reduction of the electrodes modified with the GO (in order to obtain ERGO) was carried out using cyclic voltammetry (CV, $−1.5 ~ 1$ V, 50 mV s$^{-1}$, 7 cycles) in 0.1 M Na$_2$SO$_4$ solution in a standard three-electrode system.
2.4. Characterization of nanofibers morphology

The morphology of the nanofibers was evaluated using scanning electron microscopy (SEM) images, which were collected with a JEOL 6510 microscope operating at 10 kV. AFM was carried out using a Dimension V (Veeco) microscope. All images were obtained in the Tapping mode with a scan rate of 0.6 Hz, using silicon nitride tips attached to a cantilever of a spring constant of 2.5 N m$^{-1}$.

2.5. Electrochemical measurements

The electrochemical experiments were performed using a potentiostat PGSTAT30 Autolab electrochemical system (Metrohm) controlled by Nova software (1.10). FTO substrate (area = 0.25 cm$^2$) modified by PA6/PPy/CRGO or PA6/PPy/ERGO were used as the working electrodes, while a Platinum (Pt) foil and Ag/AgCl (3 mol l$^{-1}$ KCl) served as the counter (CE) and reference (RE) electrodes, respectively. The electrochemical characterizations were carried out via cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The CV measurements were performed using 0.1 mol l$^{-1}$ KCl containing 0.05 mol l$^{-1}$ [Fe(CN)$_6$]$^{3−/4−}$ and a potential range from −0.1 to 0.7 V at a scan rate of 50 mV s$^{-1}$. Electrochemical impedance spectroscopy (EIS) technique allows to evaluate the electroanalytical performance of the proposed system. The EIS measurements were performed using 0.05 mol l$^{-1}$ [Fe(CN)$_6$]$^{3−/4−}$ containing 0.1 mol l$^{-1}$ KCl as redox probe, at an open circuit potential (OCP) over the frequency range from 0.1 Hz to 100 kHz, using a voltage amplitude of 10 mV. The electrochemical essay for malathion detection was performed using 0.1 mol l$^{-1}$ phosphate buffer solution (pH 7.4) at room temperature. DPV measurements were carried out from −0.6 to 0 V using pulse amplitude of 50 mV, pulse width of 0.4 s and pulse period of 0.5 s.

3. Results and discussion

3.1. Choice of materials

The choice of materials employed was based on improving sensor sensitivity and detection limit. In this way, the nanofiber was fabricated using polyamide 6 as polymeric matrix due to its nanofiber-forming ability and polypyrrole because of its conductivity and ability to dope nanofibers. Reduced graphene oxide was used with the objective of modifying the surface of nanofibers and consequently increasing the electrical conductivity,
improving the detection of the pesticide. For this reason, we also study different ways of reducing graphene oxide.

3.2. Characterization of FTO/PA6/PPy and FTO/PA6/PPy/CRGO and FTO/PA6/PPy/ERGO electrospun nanofibers

Due to the different methods used for the reduction of the GO, the possible morphological alterations on the electrospun nanofibers surface were evaluated by imaging the surfaces of the electrodes using SEM. The two micrographs of figure 1 reveal that the nanofibers modified with CRGO (figure 1(A)) shows a more homogeneous and exfoliated deposition of graphene sheets onto the nanofibers surface when compared to the sample modified with ERGO, where more agglomerates can be observed onto the nanofibers surface (figure 1(B)). These results corroborate with data available in the literature [29, 30], which demonstrate that ERGO presents more defects in its structural organization when compared to CRGO.

AFM images of the electrodes surface modified with CRGO and ERGO are shown in figure 2 A and B, respectively. Through these results it was possible to calculate the surface roughness for both samples and the values found were 152 and 120 nm for CRGO and ERGO, respectively. The greater roughness of the sample modified with CRGO can affect the electrochemical response, which will be further discussed.

3.3. Electrochemical characterization of FTO/PA6/PPy/CRGO and FTO/PA6/PPy/ERGO electrospun nanofibers

In order to evaluate the electron transfer of the developed sensing platforms, electrochemical impedance measurements were performed and the data were analyzed and adjusted using the Randle’s equivalent circuit
shown in the inset of figure 3(A) [31]. In this equivalent circuit $R_s$ represents the electrolyte resistance, $R_{ct}$ represents the charge (electron) transfer resistance, $C_d$ represents the interface capacitance, and $Z_w$ is the Warburg impedance. Besides, based on the literature [32, 33], the diffusional processes can be ascribed to the linear region at low frequencies and the semicircular region located at high frequencies is associated with an interfacial charge-transfer process and its diameter corresponds to the charge transfer resistance ($R_{ct}$). The Nyquist plots and the CVs measurements for FTO/PA6/PPy, FTO/PA6/PPy modified with CRGO and FTO/PA6/PPy modified with ERGO are shown in figure 3. EIS measurements (figure 3(A)) show clearly that the FTO/PA6/PPy/CRGO presented smaller electron transfer resistance as the semicircle portion of the Nyquist plot was smaller than the ones for the other samples. $R_{ct}$ values were determined as 29 $\Omega$ for FTO/PA6/PPy, 27 $\Omega$ for FTO/PA6/PPy/ERGO and 14 $\Omega$ for FTO/PA6/PPy/CRGO. Figure 3(B) shows the cyclic voltammograms where a higher current value is observed for the electrode modified with CRGO due to their higher electroactive area and roughness in comparison with the electrode modified with ERGO, as previously
Table 1. Comparison of the electrochemical sensing performance of different nanocomposites modified electrodes toward malathion detection.

| Sensing Material                        | Transduction method | Limit of detection (ng ml$^{-1}$) | Detection range (ng ml$^{-1}$) | References |
|------------------------------------------|---------------------|-----------------------------------|-------------------------------|------------|
| Chitosan-iron oxide                      | DPV                 | 0.001                             | 0.001–10                      | [7]        |
| Carbon nanotube-encapsulated polypyrrole and polyaniline | CV                  | 0.9                               | 0.009–500                     | [32]       |
| Carbon paste electrode                   | DPV                 | 69–1300                           | 56                            | [33]       |
| Chitosan/gold nanoparticles-graphene nanosheet composite | DPV                 | 0.5                               | 0.5–500                       | [34]       |
| Carbon nanotubes nanocomposites          | DPV                 | 0.00000003                        | 0.0000003–330                 | [35]       |
| FTO/PA6/PPy/CRGO                        | DPV                 | 0.8                               | 500–2 × 10$^4$                | This work  |

1DPV–Differential Pulse Voltammetry.
2CV–Cyclic Voltammetry.

discussed. Besides, as can be seen in figure 3, the capacitive effect was more pronounced for FTO/PA6/PPy/CRGO electrode providing the lowest $R_{ct}$ and the highest current density.

3.4. Electrochemical detection of malathion

The nanofibers modified with CRGO was employed as a sensing platform for the electrochemical detection of malathion. For that purpose, the electrodes were tested using differential pulse voltammetry (DPV) technique. The DPV method was chosen due to its high sensitivity, once the effects of the charging currents can be reduced. In this way the detection measurements can be performed using extremely small amounts of the analytes [4]. DPV curves, displayed in figure 4(A), show the decrease of the reduction peak (centered at −0.43 V) as the malathion concentration increases. The peak decrease is due to the oxidation process of malathion which produces electrons and consequently enhances the reduction process of FTO.$S_{\text{PPy modi}}$ as indicated in figure 4(C). It is observed that the current decreases linearly with malathion concentration (figure 4(B)) in the range 0.5–20.0 μg ml$^{-1}$. The linear regression equation obtained is $I (\mu A) = 2.2–4.2 × 10^{-2} [\text{malathion}]$ (μg ml$^{-1}$) $R^2 = 0.9$.

For the calculation of the detection limit (DL) we used the standard deviation of the response and the slope of the curve presented in figure 4(B). The DL reached for the electrode modified with CRGO was 0.8 ng ml$^{-1}$ for the concentration range of 0.5–20.0 μg ml$^{-1}$, considering $DL = 3.3 \sigma/S$, where $\sigma$ is the standard deviation of DPV signal (measurements of five replicates), and $S$ is the slope of the calibration curve [34]. The sensitivity of the sensor calculated from the slope of the calibration plot was 174 μA (mg/ml$^{-1}$) cm$^{-2}$.

Repeated experiments were realized using malathion solutions with concentrations of 10 μg ml$^{-1}$ (six successive measurements) in order to evaluate the inter-electrode and intra-electrode reproducibility of the FTO/PA6/PPy modified with CRGO. From these results it was possible to observe that the developed platform presented a good performance for the electrochemical detection of the pesticide in terms of reproducibility, once the relative standard deviation (RSD) for a given electrode was 1.4%, which value increased to 2.0% when three identical electrodes were used. The developed electrochemical sensor presented similar or even better performance for detecting malathion compared to other results reported in the literature, as displayed in table 1 [7, 35–37].

3.5. Interference studies

A study was also carried out regarding the selectivity of the developed platform (FTO/PA6/PPy/CRGO) in the presence of interfering compounds. This study was also performed using the DPV technique. The interfering pesticides chosen were pestanal and cadusafos because they are common interfering and toxic compounds present in various contaminated environments[38]. The study was carried using a solution containing 15 μg ml$^{-1}$ of malathion and each of the interfering compounds in phosphate buffered (0.1 mol l$^{-1}$ in pH 7.0). Figure 5 shows the DPV studies and observed a maximum interference of 8% in the presence of pestanal, which result reveals the high selectivity of the developed sensing platform toward the detection of malathion.

3.6. Real sample analysis

The novel sensing platform was also used in the analysis of real samples. This test was carried out with the objective of evaluating the performance of the sensing platform for detecting pesticides in tap water and river water using the standard addition method. The river water sample was collected from the Monjolinho River (located in São Carlos–São Paulo/Brazil) and filtered using a paper filter (J Prolab JP42). A concentration of...
Malathion (17 μg ml⁻¹) was added to the water samples and % recovery was calculated, as displayed in Table 2. The obtained recoveries of malathion were in the range of 99%–105% (Table 2), which indicate that the developed platform can be utilized for the detection of malathion in real samples analysis.

4. Conclusion

The developed electrochemical sensing platform composed by electrospun nanofibers of PA6/PPy modified with reduced graphene oxide and deposited onto FTO was successfully used for detecting malathion pesticide. Through the study on the nanofibers modification with chemically and electrochemically reduced graphene oxide, it was possible to observe that the chemical reduction of graphene increased the electrical conductivity of the electrode. The sensor prepared with chemically reduced graphene oxide showed a low detection limit (0.8 ng ml⁻¹) for malathion. Additionally, the platform presented reproducibility values of 1.4% (for a given electrode) and 2.0% (for three identical electrodes). The developed platform also showed a high selectivity for malathion in the presence of interfering compounds and also a good performance for the pesticide detection in real samples. Our results indicate the novel nanoarchitecture as a potential approach to be used in sensors designed for the detection of pesticides or other contaminants.

Acknowledgments

The authors are grateful to CNPq (150208/2016-2), FAPESP (2017/12174-4 and 2017/10582-8), Capes, MCTI-SisNano, and EMBRAPA for the financial support provided for this research.

ORCID iDs

Fernanda L Migliorini https://orcid.org/0000-0002-9605-2790
Luiza A Mercante https://orcid.org/0000-0003-4206-6545
Daniel S Correa https://orcid.org/0000-0002-5592-0627
References

[1] Zhang L and Fang M 2010 Nanomaterials in pollution trace detection and environmental improvement *Nano Today*, 5 128–42

[2] Guillaud D, Ginebreda A, Farr M, Darbra R M, Petrovic M, Gros M and Barceló D 2012 Prioritization of chemicals in the aquatic environment based on risk assessment: Analytical, modeling and regulatory perspective *Sci. Total Environ.* 440 236–52

[3] Wang X, Lu X and Chen J 2014 Development of biosensor technologies for analysis of environmental contaminants *Trends Environ.* 22 25–32

[4] Ali R, Richard K, Hussain Z, Tahira A, Tufail S, Sherazi H and Willander M 2016 Amino acid assisted growth of CoO nanostuctures and their potential application in electrochemical sensing of organophosphate pesticide *Electrochem. Acta* 190 972–9

[5] Song D, Wang Y, Lu X, Gao Y, Li Y and Gao F 2018 Sensors and Actuators B: Chemical MoS2 nanosheet for highly sensitive electrochemical sensing of organophosphorus pesticides *Sensors Actuators B. Chem.* 267 5–13

[6] Liu Y, Li B, Liu A, Liang G, Yin L, Pu Y and Wei W 2018 Sensors and Actuators B: Chemical Multicolor sensor for organophosphorus pesticides determination based on the bi-enzyme catalytic etching of gold nanorods *Sensors Actuators B. Chem.* 265 675–81

[7] Prabhakar N, Thakur H, Bharti A and Kaur N 2016 Analytica Chimica Acta Chitosan-iron oxide nanocomposite based electrochemical aptasensor for determination of malathion *Anal. Chem.* 939 105–16

[8] Singh R, Prasad R, Sumana G, Arora K, Sood S, Gupta R K and Malhotra B D 2009 STD sensor based on Biosensors and Bioelectronics nucleic acid functionalized nanostructured polyaniline *Biosens. Bioelectron.* 24 2233–8

[9] Bonner M R, Coble J, Blair A, Freeman L E B, Hoppin J A, Sandler D P and Alavanja M C R 2018 Malathion exposure and the incidence of cancer in the agricultural health study *Orig. Contrib.* 166 1023–34

[10] He L, Cui B, Liu J, Song Y, Wang M and Peng D 2018 Chemical novel electrochemical biosensor based on core–shell nanostructured composite of hollow carbon spheres and polyaniline for selectively detecting malathion *Sensors Actuators B. Chem.* 258 813–21

[11] Xie Y, Yu Y, Liu L, Ma X, Gong L, Huang X, Liu G and Yu Y 2018 CuO nanoparticles decorated 3D graphene nanocomposite as non-enzymatic electrochemical sensing platform for malathion detection *J. Electroanal. Chem.* 812 82–9

[12] Esen E, Kokturk G, Ozer H, Muhammad T, Okter Z, Otkay H, Simsek S, Barut S and Gok M Y 2016 Lab-on-a-chip based biosensor for the real-time detection of a flavonoid *Talanta* 160 381–8

[13] Tan Y, Wei X, Zhang Y, Wang P, Guo L, Lin Z and Yang H 2015 Exonuclease-catalyzed target recycling amplification and immobilization free electrochemical aptasensor exonuclease-catalyzed target recycling amplification and immobilization free electrochemical aptasensor *Anal. Chem.* 87 11826–31

[14] Mercante L A, Scagion V P, Migliorini F L, Mattoso L H C and Correa D S 2017 Electrospinning-based (bio) sensors for food and agricultural applications: a review *Trends Anal. Chem.* 91 91–103

[15] Mei Liu F, Qiu Du Y, Mei Cheng Y, Yin W, Jun Hou C, Qin Hao D, Chen G and Bao Fa H 2016 A selective and sensitive sensor based on highly dispersed cobalt porphyrin-Co3O4-graphene oxide nanocomposites for the detection of methyl parathion *J. Solid State Electrochem.* 20 599–607

[16] Reneker D H and Yarin A L 2008 Electrospinning jets and polymer nanofibers *Polymer (Guildf).* 49 2387–425

[17] Mercante L A, Pavinatto A, Iwaki L E O, Scagion V P, Zucolotto V, Oliveira O N, Mattoso L H C and Correa D S 2015 Electrospun polyamide 6/Poly (allylamine hydrochloride) nanofibers functionalized with carbon nanotubes for electrochemical detection of dopamine *ACS Appl. Mater. Interfaces* 7 4784–90

[18] Andre R S, Pavinatto A, Mercante L A, Paris E C, Mattoso L H C and Correa D S 2015 Improving the electrochemical properties of polyamide 6/polyvinylidene fluoride nanocomposites by surface modification with ZnO nanorods *RSC Adv.* 5 73875–81

[19] McGreevy R L 2008 Advanced carbon electrode materials for molecular electrochemistry *Chem. Rev.* 108 2646–87

[20] Li W and Yang Y J 2014 The reduction of graphene oxide by elemental copper and its application in the fabrication of graphene supercapacitor *18 1621–6

[21] Feng X, Chen W and Yan L 2016 materials - *RSC Adv.* 6 80106–10

[22] Emran M Y, Shenashen M A, Morita H and El-Saffy S A 2018 One-step selective screening of bioactive molecules in living cells using sulfur-doped microporous carbon *Biosens. Bioelectron.* 109 237–45

[23] Emran M Y, Shenashen M A, Abdelwahab A A, Abdelmotahal M and El-Saffy S A 2018 Facile synthesis of microporous sulfur-doped carbon spheres as electrodes for ultrasensitive detection of ascorbic acid in food and pharmaceutical products *New J. Chem.* 42 5037–44

[24] Emran M Y, El-Saffy S A, Shenashen M A and Minowa T 2019 A well-thought-out sensory protocol for screening of oxygen reactive species released from cancer cells *Sensors Actuators, B Chem.* 284 456–67

[25] Li S J, Xing Y, Deng D H, Shi M M and Guan P P 2015 A comparative study of different types of reduced graphene oxides as electrochemical sensing platforms for hydroquinone and catechol *J. Solid State Electrochem.* 19 861–70

[26] Molina J, Fernández J, Garcia C, Riao A I, Bonastre J and Cases F 2015 Electrochemical characterization of electrochemically reduced graphene coatings on platinum *Electrochem. Acta* 166 54–63

[27] Mercante L A, Fucure M H M, Locilento D A, Sanfelice R C, Migliorini F L, Mattoso L H C and Correa D S 2017 Solution blow spun PMMA nanofibers wrapped with reduced graphene oxide as effective dye adsorbent *New J. Chem.* 41 9087–94

[28] Mercante L A, Facure M H M, Sanfelice R C, Migliorini F L, Mattoso L H C and Correa D S 2017 One-pot preparation of PEDOT:PSS-reduced graphene decorated with Au nanoparticles for enzymatic electrochemical sensing of H2O2, *Appl. Surf. Sci.* 407 162–70

[29] Rocha D P, Dornellas R M, Cardoso R M, Narciso L C D, Silva M N T, Nossel E, Richter E M and Munoz R A 2018 Sensors and actuators b: chemical chemically versus electrochemically reduced graphene oxide: improved amperometric and voltammetric sensors of phenolic compounds on higher roughness surfaces *Sensors Actuators B Chem.* 254 701–8

[30] Guo H, Wang X, Qian Q, Wang F, Xia X and Green A 2009 Approach to the Synthesis of Graphene nanosheets *ACS Nano.* 3 2653–9

[31] Wu Z, Wang B, Cheng Z, Yang X, Dong S and Wang Y 2001 A facile approach to immobilize protein for biosensor: Self-assembled supported bilayer lipid membranes on glassy carbon electrode *Biosens. Bioelectron.* 16 67–72

[32] Jiang J and Du X 2014 Sensitive electrochemical sensors for simultaneous determination of ascorbic acid, dopamine, and uric acid based on Au/Pd-reduced graphene oxide nanocomposites *Nanoscale* 6 11303–9

[33] Wang Q, Moser J E and Gratzel M 2005 Electrochemical impedance spectroscopic analysis of dye-sensitized solar cells *J. Phys. Chem.* 2 109 14945–53

[34] Comité de Dirección NO ICH, Validation of Analytical Procedures: Text and Methodology Q2(R1), Int. Conf. Harmon. 1994 (2005) 17. doi: (http://ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf)

[35] Du D, Ye X, Cai J, Liu J and Zhang A 2010 Biosensors and Bioelectronics Acetylcholinesterase biosensor design based on carbon nanotube-encapsulated polypyrrole and polyaniline copolymer for amperometric detection of organophosphates *Biosens. Bioelectron.* 25 2564–8

8
[36] Bao J, Hou C, Chen M, Li J, Huo D, Yang M, Luo X and Lei Y 2015 Plant-esterase-chitosan/gold nanoparticles—graphene nanosheet composite based biosensor for the ultrasensitive detection of organophosphate pesticides J. Agric. Food Chem. 63 10319–26

[37] Kaur N, Thakur H and Prabhakar N 2016 Conducting polymer and multi-walled carbon nanotubes nanocomposites based amperometric biosensor for detection of organophosphate JEAC. 775 121–8

[38] Facure M H M, Mercante L A, Mattoso L H C and Correa D S 2017 Detection of trace levels of organophosphate pesticides using an electronic tongue based on graphene hybrid nanocomposites Talanta 167 59–66