**Nanostructured platform for the sensitive determination of paraoxon by using an electrode modified with a film of graphite-immobilized bismuth**

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**Abstract**

The authors describe a graphite immobilized bismuth film electrode with enhanced sensitivity for the organophosphorus pesticide paraoxon. The film was formed by ex-situ electroplating of bismuth onto the large surface of a glassy carbon electrode modified with graphite nanopowder (Bi/Gr/GCE). The modified GCE was characterized by chronoamperometry using p-nitrophenol as a model nitro compound. The modification of the GCE results in an increase of the electroactive area (to 27.7 mm²) and of the electrocatalytic activity (the catalytic rate constant being 4140 L⋅mol⁻¹⋅s⁻¹). This results in an enhancement of the current for the electroreduction of paraoxon by a factor of 4.3 (compared to a plain GCE). The modified GCE was applied to the sensitive determination of paraoxon by differential pulse voltammetry. At a typical working potential of -0.45 V vs. Ag/AgCl, the LOD is 2 nmol⋅L⁻¹ of paraoxon, which is comparable to the LOD of some cholinesterases-based electrochemical sensors and is lower than the LOD of the organophosphorus hydrolase-based electrochemical sensors for paraoxon. In addition, the new GCE is more stable than enzyme-based sensors, and it can be renewed.

**Keywords** Paraoxon. Cathodic reduction. Bi film electrode. Graphite nanopowder.

**Introduction**

Electrochemical analysis is considered as a low-cost alternative of the currently used chromatographic methods, for the rapid and sensitive, in situ monitoring and assessment of organophosphorus pesticides (OPs) [1]. For this purpose, numerous well-suited cholinesterases- and organophosphorus hydrolase-based electrochemical sensors were developed [1-5]. The OPs act as specific cholinesterases inhibitors and organophosphorus hydrolase substrates. The enzyme-catalyzed reactions give rise of electroactive products. However, despite of their analytical potential and extensive development, the electrochemical biosensors for OPs determination are still not commercialized and their application to real samples analysis is limited. One of their major drawbacks is the lack of a long-term stability, due to enzyme deactivation. In this context, the implementation of non-enzymatic electrochemical sensors for OPs determination is of great interest. Some typical examples include the electrochemical determination of dichlorvos, dicrotophos, chlorfenvinphos, and crotoxyphos on mercury electrodes by reduction of the carbon-carbon double bond in a two-electron process [6], as well as of parathion, methyl parathion, and fenitrothion determination by the nitro group electrochemical reduction [7]. Nevertheless, current efforts are focused on the development of nanomaterials modified solid electrodes with improved analytical performances, which are more appropriate for in field measurements. The ZrO2 nanoparticles modified electrodes, as an example, revealed excellent performances for OPs determination [8], because of the ZrO2 affinity for phosphoric groups, exploited in solid-phase extraction. However, a loss of surface area was observed, when ZrO2 served as high surface area catalyst [9]. Therefore, carbon nanotubes (CNTs) were also used as appropriate sorbent for nitro phenyl-substituted OPs, because of the π-conjugated interactions upon the benzene ring [9]. Moreover, the CNTs modified electrodes, because of the large surface to volume ratio and electrocatalytic properties of the nanomaterial, display an enhanced electron transfer rate, increased sensitivity, and reduced surface fouling. Hence, CNTs- and CNTs composites-modified electrodes were applied, among other, for the sensitive OPs determination [10-13]. However, the price of the CNTs is still too high [14]. Thus, the use of the inexpensive graphite nanopowder can be an option for electrode modification. Graphite nanopowder is a graphitic carbon with high surface area. Stable aqueous suspensions of graphite can be obtained by using clays as dispersing agents. Such mixtures are currently used in pencil leads production at industrial scale. An effective dispersant of the carbonaceous materials, including graphite, graphene, and CNTs is the synthetic clay laponite [15-18].

From the other hand, particularly attractive for OPs determination are the bismuth film modified electrodes [19]. Their main advantage over the other solid electrodes resides in the ease of the surface regeneration by electrochemical dissolution and deposition of the Bi film. They are also considered as an alternative to the mercury electrodes, since Bi is less toxic.

The development of a new graphite immobilized Bi-film electrode with enhanced sensitivity for OPs determination is reported in this work. The film was formed by ex situ plating of bismuth onto the large surface of a graphite nanopowder modified glassy carbon electrode. Electrode modification was optimized in terms of graphite load and Bi electrodeposition time. Its electrochemical characteristics were evaluated by chronoamperometry using p-nitrophenol as a model nitro aromatic compound. The graphite immobilized Bi-film electrode was applied for the sensitive paraoxon determination by differential pulse voltammetry (DPV).

**Experimental**

**Reagents and Instrumentation**

Paraoxon-ethyl analytical standard, supplied by Sigma (www.sigmaaldrich.com), was used for the preparation of an aqueous stock solution with a concentration of 10 mmol L-1. Laporte Industry Ltd. (https://beta.companieshouse.gov.uk/company/00031089) provided laponite (monovalent exchange capacity 0.74 mmol g-1). Laponite colloidal suspension (1 mg mL-1) was prepared by ultrasonic dispersion of the laponite powder in deionized water. Graphite nanopowder (99.9% C) with an average particle size <50 nm and a specific surface area in the 30-50 m2 g-1 range was purchased from American Elements, USA (www.americanelements.com). Homogeneous laponite-graphite (LGr) mixtures were obtained by addition of appropriate amounts of graphite nanopowder to the laponite suspension and ultrasonic agitation for 10 min with an UP-800 ultrasonic processor (E-Chrom Tech Co., Ltd., Taiwan, www.taiwantrade.com/company/e-chrom-tech-co-ltd-64863.html). Bi3+ stock solution (1000 mg L-1) was obtained by dissolving Bi(NO3)3 in 5% HNO3. All the experiments were carried out in a Britton-Robinson buffer, which consists of a mixture of H3BO3 0.04 mol L-1, H3PO4 0.04 mol L-1, and CH3COOH 0.04 mol L-1 titrated to the desired pH with NaOH 0.2 mol L-1.

The electrochemical measurements were performed by using a CH Instruments model 440A electrochemical analyzer (CH Instruments Inc., USA, www.chinstruments.com) and an electrolysis cell of a conventional type. The electrolysis cell was equipped with a working glassy carbon electrode (GCE, Tokay GC 20, www.tokaicarbon.co.jp/en/products/fine\_carbon/gc.html, 3 mm diameter, bare or modified), Pt wire as an auxiliary electrode, and Ag, AgCl/KClsat as a reference.

The morphological characterization of the modified electrodes was performed by scanning electron microscopy (SEM) using a JEOL scanning electron microscope JSM-6010PLUS/LV (www.jeolusa.com/PRODUCTS/Scanning-Electron-Microscopes-SEM).

**Glassy carbon electrode modification**

The working GCE was polished using a 0.05 µm alumina polishing abrasive (PACE Technologies, USA, www.metallographic.com), degreased with alcohol, and ultrasonically cleaned. Then, three types of chemically modified electrodes were elaborated, as follows. A Bi-film modified glassy carbon electrode (Bi/GCE) was fabricated by ex situ bismuth electrodeposition from an acetate buffer (pH 4.5) containing 5 mg L-1 Bi3+. The electrodeposition was achieved by constant potential electrolysis (-1 V vs. Ag, AgCl/KClsat) for various time periods under stirring (300 rpm). A graphite nanopowder modified glassy carbon electrode (Gr/GCE) was obtained by spin coating on the surface of the GCE of 5 µL of the LGr mixture and 30 min drying at ambient temperature. Finally, a Bi-film was deposited, as previously described, onto the surface of the graphite composite modified glassy carbon electrode to elaborate the large surface graphite immobilized Bi-film electrode (Bi/Gr/GCE).

**Renewal of the Bi/Gr/GCE**

The renewal of the Bi/Gr/GCE was achieved by electrochemical dissolution of the Bi film, applying a constant potential of 0.3 V vs. Ag, AgCl/KClsat. for 30 s [19] and subsequent deposition of a new Bi film.

**Paraoxon determination**

Aliquots of the paraoxon stock solution were consecutively added to the Britton-Robinson buffer filled electrolysis cell. Each of the samples was directly quantified by differential pulse voltammetry (DPV) with the following parameters: initial potential -0.25 V, final potential -0.9 V, increment 4 mV, amplitude 50 mV, pulse width 50 ms, sampling width 0.0167 s, and pulse period 0.5 s. The registered DPV curves were analyzed to establish the value of the peak current. A calibration plot (peak current vs. paraoxon concentration) was constructed.

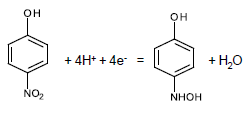
**Results and Discussion**

**Electrode modification optimization**

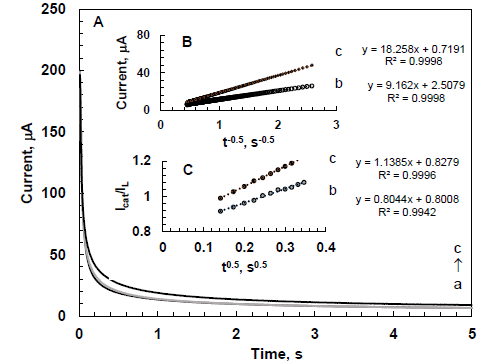
The optimization of the graphite immobilized Bi-film electrode fabrication was performed by analyzing the effect of the graphite load and of the Bi deposition time on the electrode cyclic voltammetric response to paraoxon reduction. Respective data and figures are given in the Electronic Supporting Material (ESM). Best results were obtained by modifying the GCE with a hybrid suspension of laponite 1 mg mL-1 and graphite nanopowder 2 mg L-1, followed by Bi3+ electrodeposition for a period of 5 min.

**Electrochemical characterization of the Bi/Gr/GCE**

PNP (p-nitrophenol) was selected as a model compound for the electrochemical characterization of the Bi/Gr/GCE. As known, PNP reduction, which is irreversible, occurs according to the following reaction [20, 21]:

 (1)

The electrochemical characterization of the Bi/Gr/GCE included the determination of its electroactive surface area, as well as the catalytic rate constant of the PNP reduction at the modified electrode. The evaluation of these parameters was performed using chronoamperometric data (Fig. 1).



**Fig. 1** A. Chronoamperograms obtained at Bi/Gr/GCE in Britton-Robinson buffer solution, pH 6.0. Potential step 400 mV. PNP concentration: (a) 0 mmol L-1; (b) 0.05 mmol L-1; (c) 0.1 mmol L-1. B. Cottrell plot derived from the chronoamperograms; C. *I*C/*I*L vs. *t*0.5 plot derived from the chronoamperograms.

The active surface area value was deduced from the slope of the Cottrell plot, in conformity with the Cottrell equation:

*I=nFAC(D*/π*t*)1/2 (2)

where *I* is the current (A), *n* is the electron transfer number, *F* is the Faraday constant (96 485 C mol-1) *A* is the electrode surface area (cm2), *C* is the PNP concentration (mol cm-3), *D* is the PNP diffusion coefficient (9.19x10-6 cm2 s-1) [22], and *t* is the time elapsed (s).

The catalytic rate constant value was derived from the slope of the *I*C/*I*L vs. *t*1/2 plot, described by the following equation:

*I*C/*I*L = (π*k*h*Ct*)1/2 (3)

where *I*C is the catalytic current of PNP reduction at the modified electrode (A), *I*L is the limiting current in the absence of PNP (A), *k*h is the catalytic rate constant (L mol-1 s-1), *C* is the PNP concentration (mol L-1), and *t* is the time elapsed (s).

For comparison, the same approach was applied for the electrochemical characterization of the bare GCE, as well as of the Bi/GCE and Gr/GCE. The obtained results are presented in Table 1.

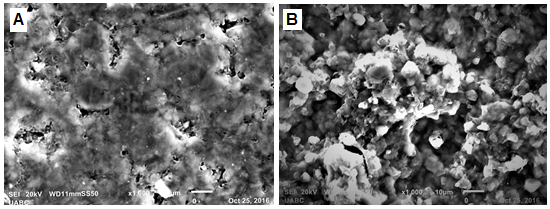
**Table 1** Values of the electroactive surface areas of the bare and modified GCEs and of the catalytic rate constants of the PNP reduction at bare and modified GCEs.

|  |  |  |
| --- | --- | --- |
| Electrode | Active area, mm2 | Catalytic rate constant, L mol-1 s-1 |
| Bare GCE | 21.72 | 2.94x103 |
| Bi/GCE | 21.83 | 3.29x103 |
| Gr/GCE | 27.93 | 3.51x103 |
| Bi/Gr/GCE | 27.71 | 4.14x103 |

As shown in Table 1, the active surface areas of the GCE and the Bi/GCE do not differ considerably, as well as the active surface areas of the Gr/GCE and the Bi/Gr/GCE. These data reveal that the increase of the active surface area is mostly due to the graphite nanopowder modification of the GCE. From the other hand, the comparison of the values of the catalytic rate constants demonstrates that the electrodes modification with a Bi film contributes to the enhancement of their catalytic activity. Hence, the subsequent paraoxon determination was performed by using Bi/Gr/GCE only, because of its increased surface area and enhanced catalytic activity.

**Morphological characterization of the Bi/Gr/GCE**

The morphology of the Bi/Gr/GCE was examined by SEM and was compared with the morphology of the Bi/GCE, as shown in Fig. 2.

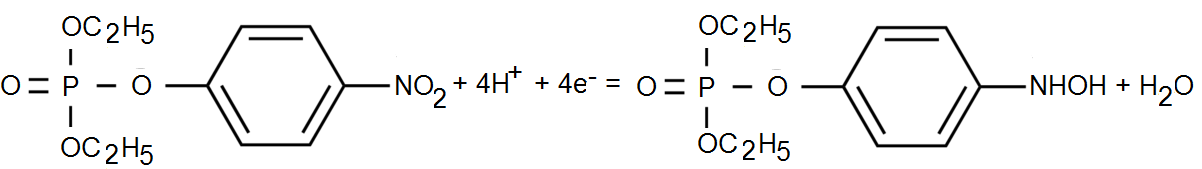


**Fig. 2** SEM images of the surface of the Bi/GCE (A) and Bi/Gr/GCE (B). 1000X magnification, acceleration voltage 20.0 kV, and resolution 10 µm.

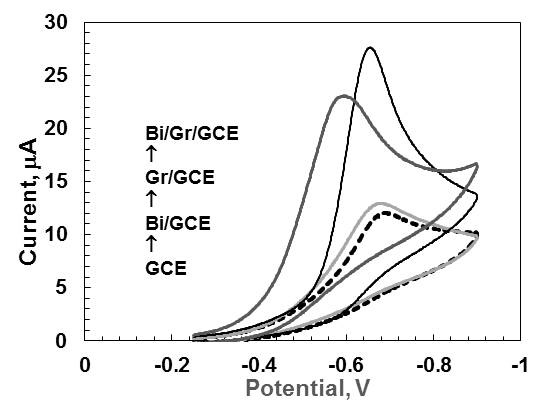
The Bi film deposited onto the GCE displays a flat, smooth, and non-uniform surface. The GCE modification with graphite nanopowder resulted in the formation of a homogeneous rough structure, with regular distribution onto the electrode surface. As the SEM images evidence, the graphite nanoparticles immobilization causes morphological changes, which contribute to the increase of the active electrode surface area.

**Paraoxon electrochemical behavior**

Cyclic voltammetry was applied to investigate the electrochemical behavior of paraoxon on Bi/Gr/GCE in comparison to its behavior on Gr/GCE, Bi/GCE, and bare GCE in the potential range from -0.25 V to -0.90 V vs. Ag.AgCl/KClsat. The voltammograms presented in Fig. 3 allow considering, that it is identical and they can be described by the following equation:

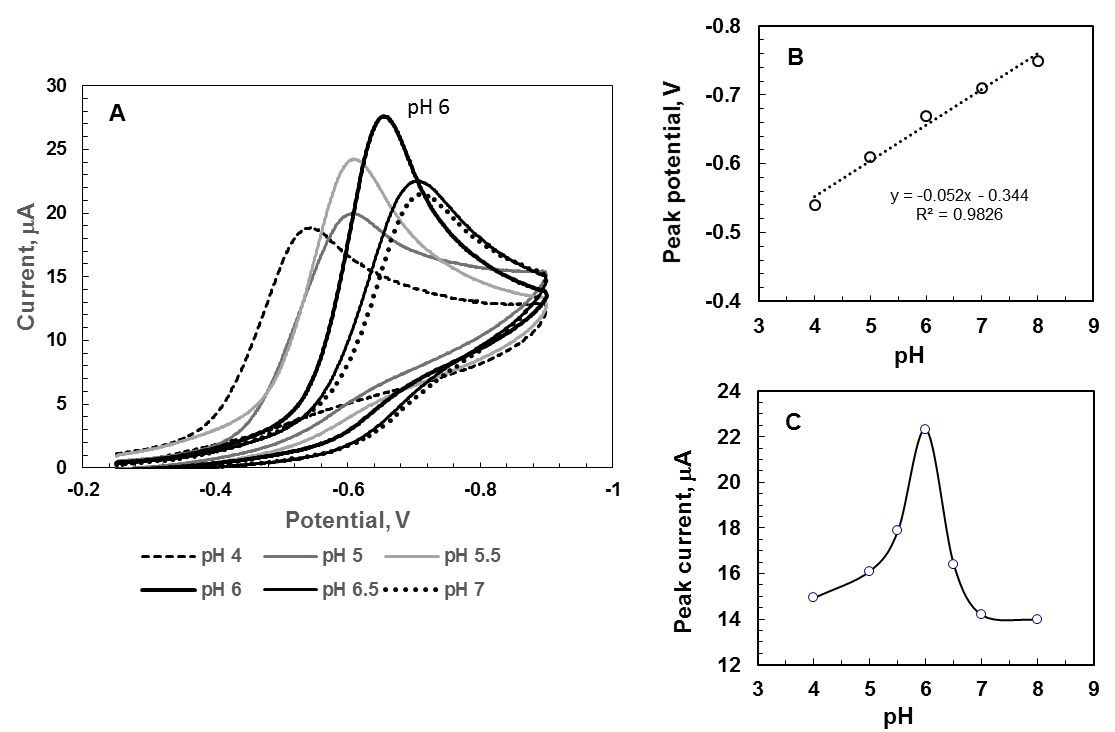
 (4)

The arising peak was ascribed to the irreversible reduction of the nitro-group, like the reduction of the PNP, the other p-nitro phenyl substituted organophosphorus pesticides, and the aromatic and heterocyclic nitro compounds in general, in accordance with data reported in the literature [19-21, 23-26]. Nevertheless, the overpotential of paraoxon reduction, as well as the reduction peak current value changed depending on the electrode material. The positive peak potential shift of 10 mV and of 100 mV on Bi/GCE and on Gr/GCE correspondingly with respect to the peak potential registered on bare GCE demonstrated that electrode modification favored the paraoxon reduction. Although the peak potential of the paraoxon reduction on Bi/Gr/GCE (-0.65 V vs. Ag.AgCl/KClsat) was only with 40 mV more positive in comparison to the recorded peak potential on GCE (-0.69 V vs. Ag.AgCl/KClsat), the increase of the peak current was the most important (4.3-fold vs. GCE, 3.9-fold vs. Bi/GCE, and 1.4-fold vs. Gr/GCE). These results were attributed to the great active surface area and increased catalytic activity of the Bi/Gr/GCE, as commented in the previous section.



**Fig. 3** Cyclic voltammetric response of the bare GCE, Bi/GCE, Gr/GCE, and Bi/Gr/GCE to paraoxon (40 µmol L-1) reduction. Britton-Robinson buffer pH 6. Scan rate 0.1 V s-1. Ambient temperature.

Further studies were performed to investigate the pH dependence of the process of paraoxon reduction on Bi/Gr/GCE, using Britton-Robinson buffers with pH values varying in the range 4-8. The recorded cyclic voltammetric curves and the resulting peak potential vs. pH and peak current vs. pH plots are presented in Fig. 4.

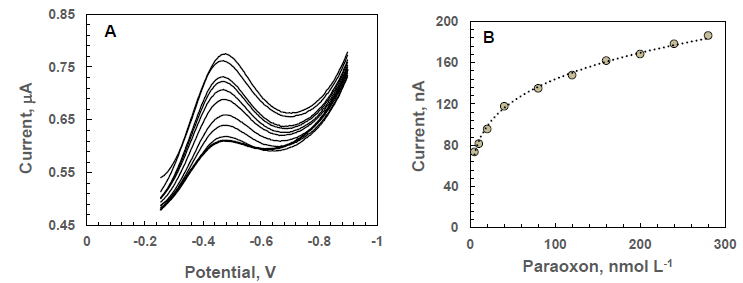


**Fig. 4** A. Cyclic voltammetric response of the Bi/Gr/GCE to paraoxon (40 µmol L-1) reduction. Britton-Robinson buffer with varying pH. Scan rate 0.1 V s-1. Ambient temperature. B. Paraoxon reduction peak potential vs. pH plot. C. Paraoxon reduction peak current vs. pH plot.

The slope of the peak potential vs. pH plot with a value of 0.052 V/pH, close to the predicted Nernstian value of 0.059 V/pH demonstrates that the paraoxon reduction involves the exchange of the same number of protons and electrons, as Eq. 4 shows. The current response of the Bi/Gr/GCE to paraoxon reduction increased with pH up to pH 6 and decreased for higher pH values. Hence, pH 6 was chosen as optimum for the paraoxon determination.

**Analytical characterization of the paraoxon determination**

The analytical performances of the Bi/Gr/GCE for OPs determination were evaluated using paraoxon. The analysis was performed by differential pulse voltammetry, as previously specified. The registered under the optimum conditions for paraoxon quantification DPV-curves and the constructed calibration plot are shown in Fig. 5.



**Fig. 5** A. DPV curves for increasing paraoxon concentrations: (a) 5 nmol L-1; (b) 10 nmol L-1; (c) 20 nmol L-1; (d) 40 nmol L-1; (d) 80 nmol L-1; (e) 120 nmol L-1; (f) 160 nmol L-1; (g) 200 nmol L-1; (h) 240 nmol L-1; (i) 280 nmol L-1. Britton-Robinson buffer pH 6. Ambient temperature. B. Calibration curve for paraoxon determination. Peak potential -0.45 V vs. Ag, AgCl/KClsat.

The peak current, recorded at a potential of -0.45 V vs. Ag, AgCl/KClsat was proportional to the paraoxon concentration in the linear range of 5 nmol L-1 to 40 nmol L-1 with a correlation coefficient of 0.9887. The sensitivity of the determination, defined as the slope of the linear part of the calibration plot was found to be 1.19 nA L nmol-1. The limit of detection (LOD), determined using the criterion S/N=3 was 2 nmol L-1. This LOD is comparable with the LOD attained by some of the inhibition-based cholinesterases electrochemical sensors for paraoxon determination [27-29] and lower than the LOD reached by the OPH-modified sensors [30-35], as shown in Table 2. Thus, the suggested graphite immobilized Bi-film electrode can be considered as suitable for paraoxon determination. In addition, it is stable and renewable, in contrast to the electrochemical biosensors. The stability of the Bi/Gr/GCE was confirmed by performing 25 consecutive measurements of paraoxon 40 nmol L-1 with a R.S.D. of 4.26%. The repeatability and the reproducibility of the paraoxon determination (40 nmol L-1, n=5) were found to be very satisfactory (R.S.D. 1.56% and 4.42%, respectively). The Bi/Gr/GCE was also successfully applied for paraoxon determination in spiked tap water samples with acceptable recovery of 99.2%., thus confirming the accuracy of the determination. The recovery was calculated as the ratio of the mean value of the paraoxon concentration found and the paraoxon concentration added midway the working concentration range (20 nmol L-1, n=3). The spiked sample was prepared by adding, to the tap water, of a known amount of diluted paraoxon analytical standard.

Paraoxon determination was affected by the presence of PNP and other nitrophenyl-substituted OPs, like methyl parathion and ethyl parathion. The simultaneous determination of the nitro aromatic OPs by electrochemical (bio)sensors remains an analytical challenge. Interferences due to the presence of metal ions such as Zn2+, Pb2+, Cd2+, As3+, Cr3+, and Co2+ (1 ppm of each) were not observed. The recorded DPV curves at the mentioned above optimal conditions for paraoxon determination demonstrated that cathodic peaks for As3+, Cr3+, and Co2+ reduction do not appear in the investigated potential range (-0.25 V to -1.3 V vs. Ag, AgCl/KClsat), although the peak potentials for Cd2+, Pb2+, and Zn2+ reduction were found to be: -0.79 V, -0.98 V, and -1.25 V vs. Ag, AgCl/KClsat, respectively. i.e. too far from the peak potential for paraoxon reduction.

**Table 2** An overview on the analytical performances of some selected biosensors for paraoxon determination.

AChE-acetylcholinesterase; BuChE-butyrylcholinesterase;CB-carbon black; CNTs-carbon nanotubes; CoPC-cobalt phtalocyanine; CP-carbon paste; CS-chitosan; GCE-glassy carbon electrode; MC-mesoporous carbon; OPH organophosphorus hydrolase; PBNPs-Prussian blue nanoparticles; PPy-poly(pyrrole); SPE-screen printed electrodes; SWCNTs-single wall carbon nanotubes.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Electrode | LOD, nmol L-1 | Sensitivity, nA L µmol-1 | Linear range, µmol L-1 | Ref. |
| SPE/AChE | 1.81 | - | - | 27 |
| GCE/PPy-CNTs/AChE | 3.00 | - | - | 28 |
| SPE/PBNPs/BuChE | 4.00 | - | 0.007-0.01 | 29 |
|  |  |  |  |  |
| GCE/SWCNTs/OPH | 10 | 2.40 | 0.5-8.5 | 30 |
| CP/OPH | 20 | 12.00 | 0.02-18 | 31 |
| GCE/CB/MC/OPH | 120 | 198.00 | 0.2-8.0 | 32 |
| GCE/SWCNTs/OPH | 150 | 25.00 | 0.25-4.0 | 33 |
| GCE/MWCNTs/OPH | 310 | 25.95 | 0.5-2.0 | 34 |
| CP/OPH | 900 | 1.45 | 4.6-46.0 | 35 |

Detailed information on the analytical performances of the electrochemical (bio)sensors for OPs determination is provided by Stoytcheva et al. [1-4].

**Conclusion**

A glassy carbon electrode modified by renewable Bi film deposited ex situ was developed using graphite nanopowder incorporated in laponite as intermediate layer to form a sandwich-type structure (Bi/Gr/GCE). The large surface area of the nanomaterial and the high electrocatalytic activity of the Bi layer provoked an enhancement of the current response of the sensor to organophosphorus pesticides (paraoxon) reduction and hence a sensitivity improvement. The attained LOD of 2 nmol L-1 is comparable to the LOD of some cholinesterases-based electrochemical sensors and lower than the LOD of the organophosphorus hydrolase-based electrochemical sensors for paraoxon determination. In addition, the Bi/Gr/GCE is more stable than the enzyme-based sensors and it can be easily renewed.

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