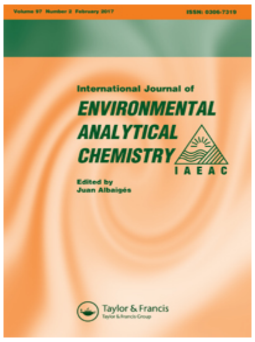

International Journal of Environmental Analytical Chemistry

ISSN: 0306-7319 (Print) 1029-0397 (Online) Journal homepage: https://www.tandfonline.com/loi/geac20

Modified carbon paste electrode for the electrochemical sensing of 3,5,6-trichloro-2-pyridinol

André Luiz dos Santos, Elisete A. Batista, Luís Moreira Gonçalves & Maria del Pilar T. Sotomayor

To cite this article: André Luiz dos Santos, Elisete A. Batista, Luís Moreira Gonçalves & Maria del Pilar T. Sotomayor (2017) Modified carbon paste electrode for the electrochemical sensing of 3,5,6-trichloro-2-pyridinol, International Journal of Environmental Analytical Chemistry, 97:2, 159-167, DOI: 10.1080/03067319.2017.1291809

To link to this article: https://doi.org/10.1080/03067319.2017.1291809

INTERNATIONAL JOURNAL OF ENVIRONMENTAL ANALYTICAL CHEMISTRY, 2017
VOL. 97, NO. 2, 159–167
http://dx.doi.org/10.1080/03067319.2017.1291809

ARTICLE

Modified carbon paste electrode for the electrochemical sensing of 3,5,6-trichloro-2-pyridinol

André Luiz dos Santos[a], Elisete A. Batista[a], Luís Moreira Gonçalves[b] and Maria del Pilar T. Sotomayor[a]

[a]Departamento de Química Analítica, Instituto de Química, UNESP-Univ Estadual Paulista, Araraquara, Brazil; [b]REQUIMTE/LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto (FCUP), Porto, Portugal

ABSTRACT

An electrochemical sensor for the determination of 3,5,6-trichloro-2-pyridinol (TCP), the main metabolite of the pesticide chlorpyrifos, was herein developed. TCP has greater solubility than the source pesticide, and its occurrence in ground and surface water is more frequent and more dangerous. The sensor was fabricated using carbon paste modified with the inorganic complex chloro-5,10,15,20-tetrakis-(pentafluorophenyl)-21 *H*,23 *H*-porphyrin iron(III) (FeTPPCl); this metallic complex has a chemical core structure similar to the heme cofactor of the cytochrome P450 (CYPs). Measurements were performed with square-wave voltammetry. Using the optimised voltammetric parameters and without any sample preparation, the sensor showed a limit of detection of 2.8 mg L^{-1} (14 µmol L^{-1}), recoveries ca. 102%, suitable selectivity and long durability (over 1 month).

ARTICLE HISTORY
Received 12 September 2016
Accepted 31 January 2017

KEYWORDS
Biomimetic sensor; electroanalysis; insecticide; Iron porphyrin complex; modified carbon paste electrode

1. Introduction

Chlorpyrifos is a pesticide used to control mosquitos and flies. Though its residential use has been progressively eliminated, it is still one of the most sold pesticides in the world, including in countries with more developed economies [1–3]. Chlorpyrifos has been reported as the second most commonly detected pesticide in food and water [2]. It has limited solubility and a high soil sorption coefficient. It acts by direct contact and by ingestion. Organophosphorus insecticides connect to the stearic centre of acetylcholinesterase (AChE) inhibiting its function to hydrolyse the neurotransmitter acetylcholine (ACh). Thus, it interferes with the transmission of nervous impulses in insects leading to their death [4,5]. This AChE binding can also occur in humans causing nausea, convulsions, and, with large exposition, respiratory problems and even death. It may be particularly dangerous to pregnant women due to its possible teratogenicity [1].

One of the main hydrolysis products of chlorpyrifos is 3,5,6-trichloro-2-pyridinol (TCP). TCP not only might serve as biomarker for chlorpyrifos contamination, since it

CONTACT Maria del Pilar T. Sotomayor ✉ mpilar@iq.unesp.br

is more stable, but it should also be monitored since it is considered to be a more hazardous pollutant than the parent compound [2,6], which is in part explained by the fact that TCP is 20 times more soluble than chlorpyrifos [6].

In a literature search, one can find a large variety of immunoassays for the determination of TCP [7–12] and some chromatographic procedures [13] that can be associated with sample preparation techniques like solid-phase extraction [14] or liquid–liquid [6,15]. However, eletroanalytical alternatives are rather scarce [16,17]. Liu et al. developed a sequential injection analysis system with a thin-layer electrochemical flow cell containing a magnet that fixed TCP antibody-coated magnetic beads; these beads competitively reacted with TCP and horseradish peroxidase (HRP)-labelled TCP, being the HRP activity monitored by square-wave voltammetry achieving a limit of detection (LOD) of 6 ng L^{-1} [16]. Wang et al. presented a portable immunochromatographic electrochemical biosensor working by also measuring HRP activity by square-wave voltammetry, albeit in a different sensor configuration, the obtained LOD was 0.1 µg L^{-1} [17].

In this work, a sensor was developed for the swift detection of TCP by square-wave voltammetry; this sensor made use of a carbon paste modified with a metallic complex mimicking the catalytic action of CYP 450.

2. Experimental

2.1. *Reagents*

All chemicals were of analytical grade and were used as received without further purification. Pesticides (TCP and all other used in the selectivity tests), the metallic complexes, the mineral oil (Nujol) and the graphite powder were all purchased from Sigma-Aldrich, St. Louis, USA. Ultrapure water (resistivity not lower than 18.2 MΩ cm at 298 K) from a Direct-Q 3UV water purification system (Millipore, Billerica, USA) was used in all experiments.

2.2. *Sensor preparation*

Several steps were carried out to prepare the carbon-paste-modified electrodes: (1) 25 mg of the modifying agent, that is, the metallic complex, was mixed with 75 mg of graphite powder; (2) addition of 1 mL of buffer phosphate, 0.1 mol L^{-1}, pH 7.0; (3) the mixture was slowly dried at room environment for about 24 h; (4) over the dried material, 60 µL of the glutting agent, that is, the mineral oil, was added, and after homogenisation, the modified carbon paste was considered ready and kept dry.

The paste was placed in the working electrode surface previously cleaned with isopropyl alcohol. The working electrode consisted in a glass cylinder with a platinum wire in the middle; the paste was deposited on a cavity of 1 mm depth and 3 mm diameter holding a small platinum disc in the base [18].

2.3. *Apparatus*

Electrochemical measurements were performed in a µAutolab controlled with software GPES v.4.9. A three-electrode system was used; it was composed of a platinum wire as the counter-electrode, a Ag|AgCl (in saturated KCl) electrode as the reference electrode and the previously mentioned modified carbon electrode as the working electrode.

All measurements were performed at room temperature and without degasification with nitrogen. Between measurements the working electrode was polished and kept in a dry place.

3. Results and discussion

Biomimetic sensors have been used to obtain a more stable response than conventional biosensors that use a biological element. These devices aim to solve the limitations of enzymatic sensors like cost and stability while maintaining selectivity. Cytochromes P450 (CYPs) are proteins present in the human liver involved in metabolising many different substances, including pharmaceuticals and, notably for this work, pesticides. CYPs are hemoproteins, that is, they contain an heme group as a co-factor. It was precisely the catalytic activity of this group that authors tried to mimic in this work (Figure 1). Several different metallic complexes were tested, namely: 5,10,15,20-tetrakis(pentafluorophenyl)-21 H,23 H-porphyrin iron(III) chloride (FeTPPCl), copper(II) phthalocyanine, cobalt(II) phthalocyanine, iron (II) phthalocyanine bis(pyridine) cobalt(II) 1,2,3,4,8,9,10,11,15,16,17,18,22,23,24,25-hexadecafluoro-29 *H*,31 *H*-phthalocyanine, 5,10,15,20-Tetrakis(4-methoxyphenyl)-21 *H*,23 *H*-porphine cobalt(II) and Iron(III) phthalocyanine chloride. Of all these complexes, FeTPPCl was chosen to carry out the rest of the experiments; this choice was based in the larger signal gain on the TCP

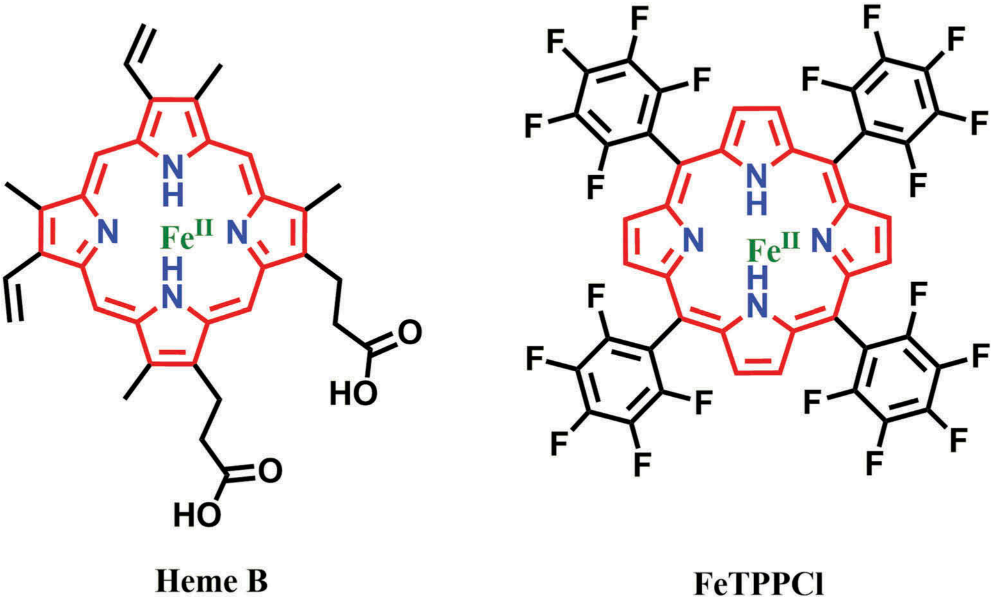

Figure 1. Heme B and FeTPPCl structures.

analysis when compared to the non-modified carbon paste electrodes. Although this signal gain was quite modest (ca. 30%), the greatest advantage seemed to be a greater linearity between signal and concentration. The quantity of the metallic complex within the paste was also subsequently optimised (the following percentages were tested with a TCP calibration curve with concentration from 0.11 to 0.61 g L^{-1}: 0%, 5%, 10%, 15%, 20%, 25% and 30%), there was a large increase in signal starting at 15% that remained rather constant up to 30% (data not shown), a percentage of 25% was chosen to perform the following experiments.

To better understand the process, scan-rate experiments were performed, results are shown in Figure 2. It is known that for a fully diffusional process, in cyclic voltammetry, peak current is proportional to the square root of the scan rate and for a fully adoptional process, peak current is proportional to the scan rate [18,19]. This means that by plotting logarithm of peak current versus logarithm of scan rate, for a diffusional process the slope is 0.5 and for an adsorptive process the slope is 1.0, and one could expect a value between for a mixed process [20,21]. As can be observed in the inlays of Figure 2, in this case we have a mostly diffusional process, thus, due to the irregular surface some *quasi* adsorptive processes might be found to be occurring in simultaneous. The peak potential corresponding to TCP was around 0.0 V versus Ag|AgCl, and the peak present at −0.5 V versus Ag|AgCl corresponds to the oxidation of the metallic complex as can be found in literature [22].

Square-wave voltammetry was applied to increase sensitivity [23,24]. Optimised square-wave voltammetric parameters were the following: square-wave frequency of 50 Hz, wave amplitude of 150 mV and potential step of 1.5 mV (Figure 3). A calibration curve (n = 28) was constructed to evaluate the sensor's analytical parameters: coefficient of determination (r^2) of 0.994, i_p (A) = (4.0 ± 0.2) × [TCP] (g L^{-1}) − (21 ± 5), linear range up to 0.17 g L^{-1}, LOD and limit of quantification (LOQ) of 2.8 and 9.3 mg L^{-1} (i.e. 14 and 47 μmol L^{-1}), respectively. LOD and LOQ were calculated as 3 and 10 times the standard deviation of the intercept/slope, respectively. When compared to the LODs of other electroanalytical methodologies [16,17], the obtained LOD is indeed higher, however in those cases immunoelectrochemical methodologies with indirect determination were employed. The intra-day repeatability was around 7.5% and inter-day repeatability was around 4.8% (seven calibrations curves, with seven points each, performed in five consecutive days).

To estimate the electrode's durability, the same carbon paste was monitored for around 40 days. This monitoring consisted on making a repeating a calibration curve with seven different concentrations and then compare the obtained slopes, that is, comparing sensitivities. Measurements were performed in the 1st, 2nd, 3rd, 4th, 7th, 14th, 22nd, 31st and 39th day. Only in the 39th a sensitivity below 70% was obtained, in fact in the 31st it was around 94%, this shows that the paste's life time is ca. 1 month. Since this sensor does not make use of any biological element it is not surprisingly that it possesses such durability [25].

To check the sensor selectivity, other five common pesticides were tested: metribuzin, chlorpyrifos, 2,4-dichlorophenoxyacetic acid, carbofuran and profenofos. All these compounds have limited solubility in aqueous solutions, thus we analysed saturated solutions in phosphate buffer, not knowing the precise

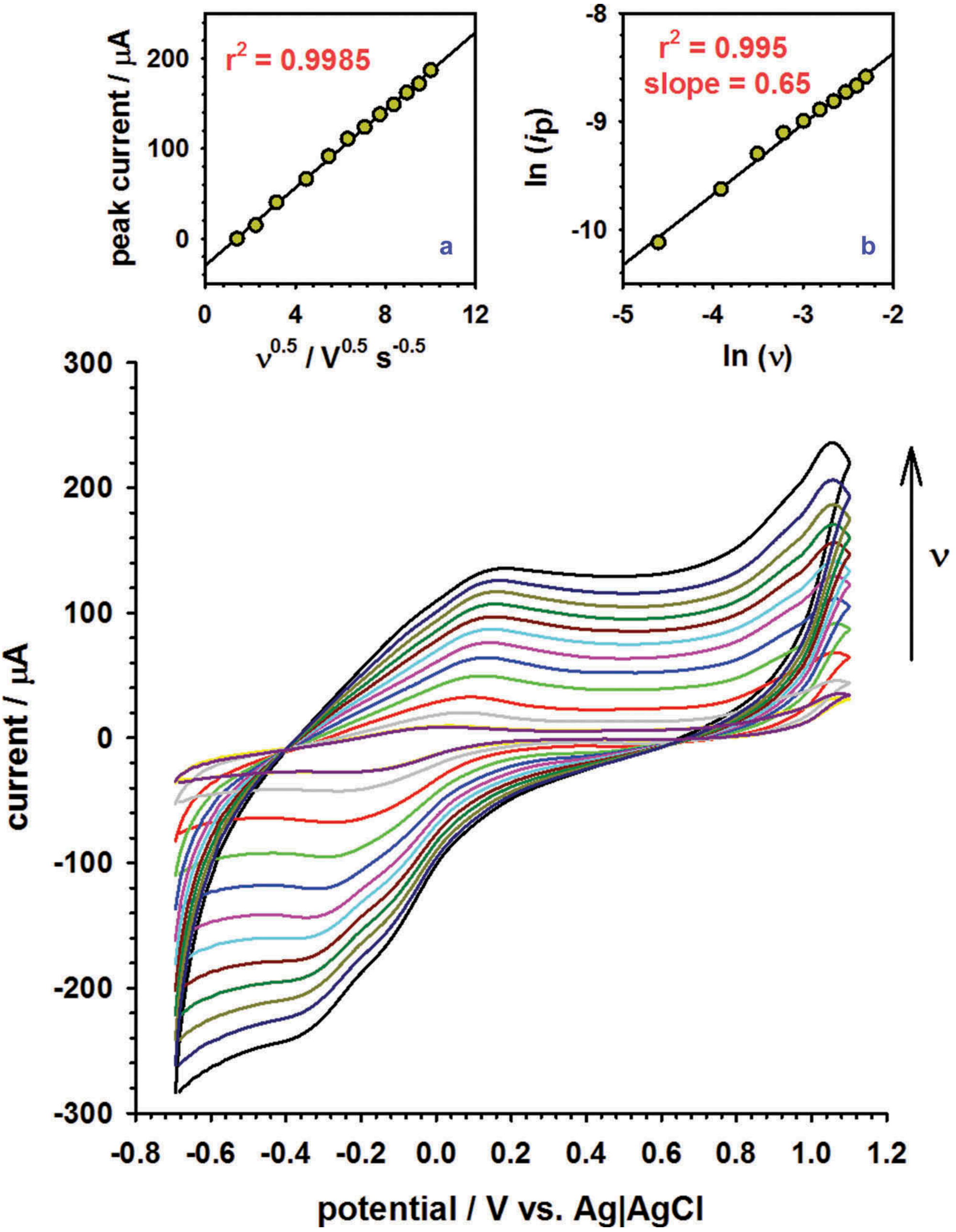

Figure 2. Cyclic voltammetry measurements obtained in the analysis of TCP, 2.08×10^{-2} g L^{-1}, in phosphate buffer 0.02 mol L^{-1}, pH 6.0, at different scan rates (from 2 to 100 mV s^{-1}). Inlay A: Linear variation of peak current as a function of the square root of the scan rate. Inlay B: Linear variation of the logarithm of peak current as a function of the logarithm of the scan rate.

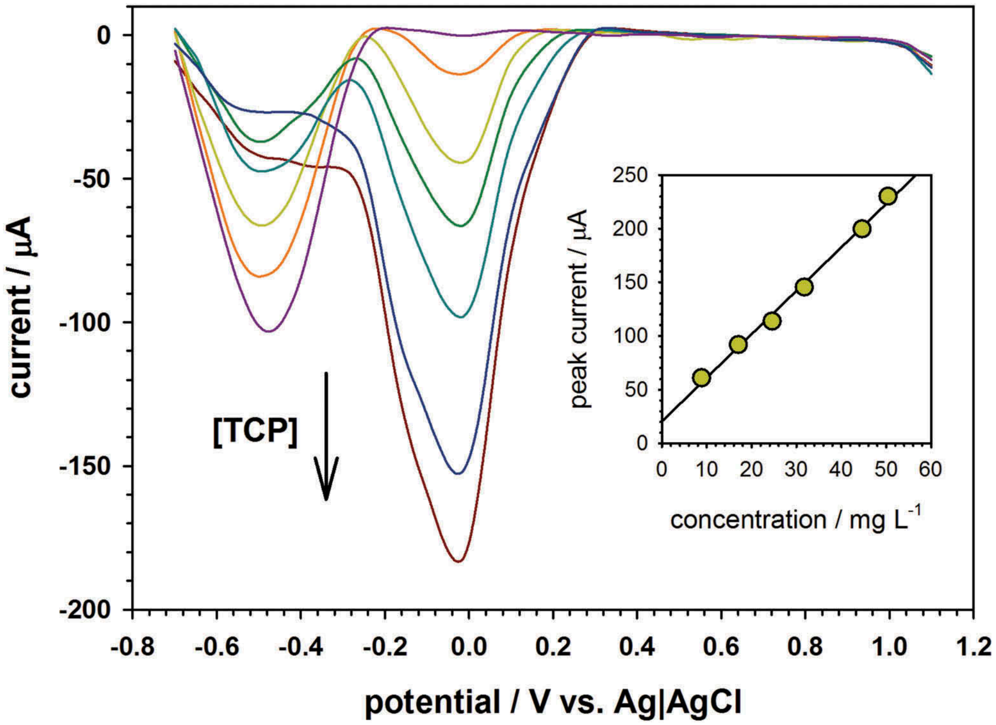

Figure 3. Square-wave voltammograms of TCP, concentrations from 8.73×10^{-3} to 4.96×10^{-2} g L^{-1}, sweep from +1.1 to −0.7 V. Inlay: calibration curve of concentration vs. peak potential.

concentration. None of them produced an electrochemical signal differing from the blank, which indicates very good selectivity of the developed sensor.

Recovery studies were performed over water samples obtained in the Tietê river nearby the Araraquara region. These samples were filtered to remove any solid residues. Then the samples were mixed with phosphate buffer 0.20 mol L^{-1}, pH 7.0 in a one to one proportion. This mixture was spiked with TCP concentrations from 12 to 22 mg L^{-1}; average recoveries obtained were of 102%.

In order to obtain the number of electrons in the electrochemical reaction, the variation of peak potential with the square-wave frequency was studied using the following equation [26]:

$$E = -\frac{2.3RT}{2nF}\log(f)$$

where E is the potential, R is the ideal gas constant, f is the frequency, T is the temperature, F is the Faraday constant and n is the number of electrons. Using this formula, it was calculated that it is a two-electron reaction, thus authors suggest the reaction shown in Figure 4 [18].

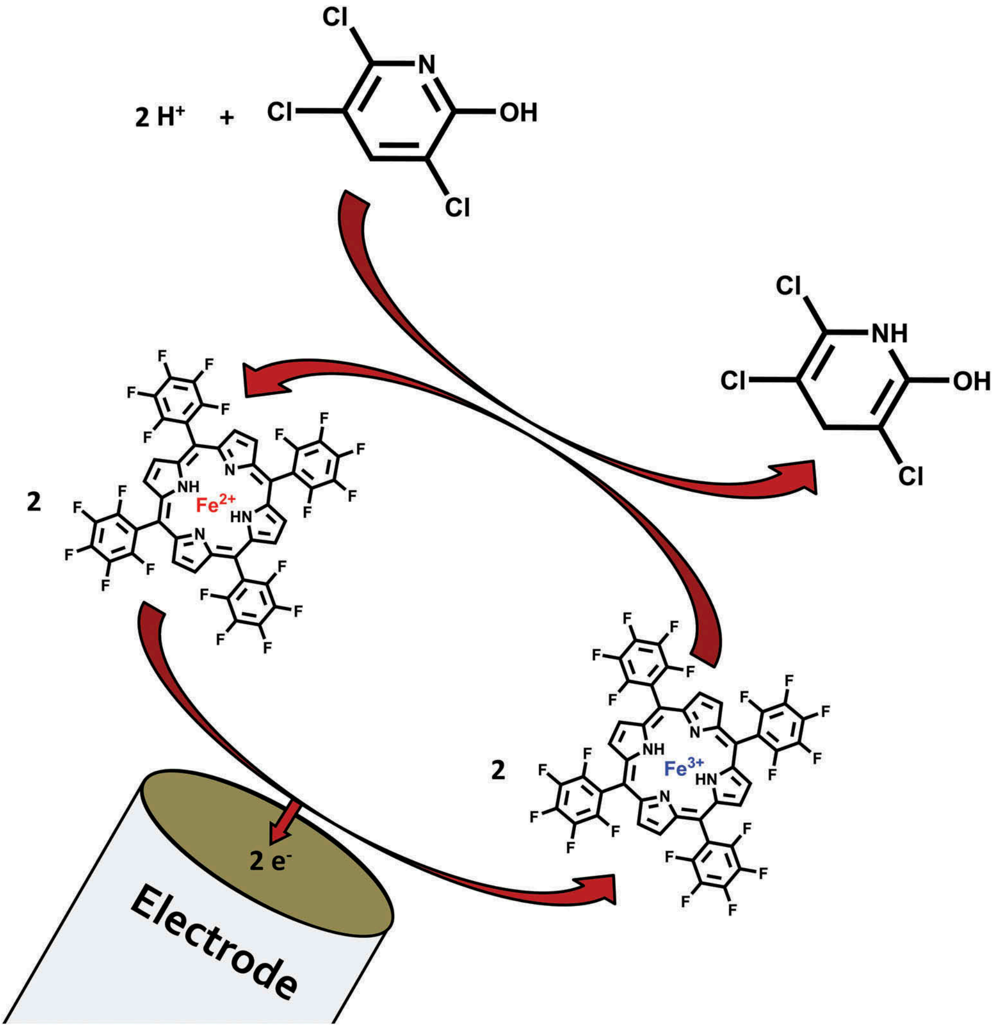

Figure 4. Suggested electrochemical reaction.

4. Conclusions

It is herein presented a carbon paste electrode for the determination of TCP, a dangerous widespread pesticide. To obtain a more suitable response the metallic complex FeTPPCl was included within the paste; this complex mimicked the heme B present in the CYP 450. The analytical sensing was performed by square-wave voltammetry, the sensor had adequate selectivity, LOD and LOQ of 2.8 and 9.3 mg L^{-1}, respectively, recoveries around 102%, 1-month stability and good repeatability.

Acknowledgements

The authors would like to express their sincerest gratitude and indebtedness to the Research Funding Agencies CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico

(400459/2012-4 and 153409/2010-0) and FAPESP (2016/06926-0). LMG (SFRH/BPD/76544/2011) wishes to acknowledge FCT for his post-doctoral grant.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico [153409/2010-0 and 400459/2012-4]; Fundação de Amparo à Pesquisa do Estado de São Paulo [2016/06926-0]; and Fundação para a Ciência e a Tecnologia [SFRH/BPD/76544/2011].

ORCID

Luís Moreira Gonçalves http://orcid.org/0000-0002-4290-656X

References

[1] D.L. Eaton, R.B. Daroff, H. Autrup, J. Bridges, P. Buffler, L.G. Costa, J. Coyle, G. McKhann, W.C. Mobley, L. Nadel, D. Neubert, R. Schulte-Hermann and P.S. Spencer, Crit. Rev. Toxicol **38**, 1 (2008). doi:10.1080/10408440802272158.

[2] E.M. John and J.M. Shaike, Environ. Chem. Lett **13**, 269 (2015). doi:10.1007/s10311-015-0513-7.

[3] J. Fischer, A. Hájková, M. Pereira, M. Křeček, V. Vyskočil and J. Barek, Electrochim. Acta **216**, 510 (2016). doi:10.1016/j.electacta.2016.09.013.

[4] D. Catalina Rodríguez, S. Carvajal and G. Peñuela, Talanta **111**, 1 (2013). doi:10.1016/j.talanta.2013.03.036.

[5] P.R. Bueno, L.M. Gonçalves, F.C.D. Santos, M.L.D. Santos, A.A. Barros and R.C. Faria, Anal. Lett **46**, 258 (2013). doi:10.1080/00032719.2012.713065.

[6] J. Xiong, G. Zhou, Z. Guan, X. Tang, Q. He and L. Wu, J. Liq. Chromatogr. Relat. Technol **37**, 1499 (2014). doi:10.1080/10826076.2012.745146.

[7] L.D. Marestoni, A. Wong, G.T. Feliciano, M.R.R. Marchi, C.R.T. Tarley and M.D.P.T. Sotomayor, J. Braz. Chem. Soc **27**, 109 (2015). doi:10.5935/0103-5053.20150256

[8] A.B. MacKenzie, F.C.A. Striley, E.R. Biagini, E.L. Stettler and J.C. Hines, Bull. Environ. Contam. Toxicol **65**, 1 (2000). doi:10.1007/s0012800086.

[9] F. Delgado Reyes, J.M. Fernández Romero and M.D.L.D. Castro, Fresenius. J. Anal. Chem **372**, 366 (2002). doi:10.1007/s00216-001-1184-8

[10] M.A. González-Martínez, R. Puchades, A. Maquieira, J.J. Manclús and A. Montoya, Anal. Chim. Acta **392**, 113 (1999). doi:10.1016/S0003-2670(99)00232-9.

[11] E. Watanabe, R. Hoshino, Y. Kanzaki, H. Tokumoto, H. Kubo and H. Nakazawa, J. Agric. Food Chem **50**, 3637 (2002). doi:10.1021/jf0114684.

[12] W. Zhang, Y. Tang, D. Du, J. Smith, C. Timchalk, D. Liu and Y. Lin, Talanta **114**, 261 (2013). doi:10.1016/j.talanta.2013.06.012.

[13] W.M.J. Chang, Y.C. Lin, W.L. Lo and S.R. Lin, Bull. Environ. Contam. Toxicol **56**, 367 (1996). doi:10.1007/s001289900053.

[14] J. Xiong, X. Tang, G. Zhou, Z. Guan and L. Wu, Anal. Methods **5**, 536 (2013). doi:10.1039/C2AY25972A.

[15] G. Peng, Y. Lu, Q. He, D. Mmereki, G. Zhou, J. Chen and X. Tang, J. Sep. Sci **38**, 4204 (2015). doi:10.1002/jssc.201500736.

[16] G. Liu, S.L. Riechers, C. Timchalk and Y. Lin, Electrochem. Commun **7**, 1463 (2005). doi:10.1016/j.elecom.2005.10.005.

[17] L. Wang, D. Lu, J. Wang, D. Du, Z. Zou, H. Wang, J.N. Smith, C. Timchalk, F. Liu and Y. Lin, Biosens. Bioelectron **26**, 2835 (2011). doi:10.1016/j.bios.2010.11.008.

[18] T.M. Do Prado, M.V. Foguel, L.M. Gonçalves and M.D.P.T. Sotomayor, Sensors Actuators B Chem **210**, 254 (2015). doi:10.1016/j.snb.2014.12.108.

[19] C. Batchelor-McAuley, L.M. Goncalves, L. Xiong, A.A. Barros and R.G. Compton, Chem. Commun **46**, 9037 (2010). doi:10.1039/c0cc03961f.

[20] L.M. Gonçalves, C. Batchelor-McAuley, A.A. Barros and R.G. Compton, J. Phys. Chem. C **114**, 14213 (2010). doi:10.1021/jp1046672.

[21] E.M. Tavares, A.M. Carvalho, L.M. Gonçalves, I.M. Valente, M.M. Moreira, L.F. Guido, J.A. Rodrigues, T. Doneux and A.A. Barros, Electrochim. Acta **90**, 440 (2013). doi:10.1016/j.electacta.2012.12.040.

[22] E.C. Figueira, L.C.S. Neres, M.R.S. Ruy, G.F. Troiano and M.D.P.T. Sotomayor, Anal. Methods **8**, 6353 (2016). doi:10.1039/C6AY01830K.

[23] A.M. Carvalho, L.M. Gonçalves, I.M. Valente, J.A. Rodrigues and A.A. Barros, Phytochem. Anal **23**, 396 (2012). doi:10.1002/pca.1370.

[24] V. Mirceski, R. Gulaboski, M. Lovric, I. Bogeski, R. Kappl and M. Hoth, Electroanalysis **25**, 2411 (2013). doi:10.1002/elan.201300369.

[25] M.D.P.T. Sotomayor, A. Sigoli, M.R.V. Lanza, A.A. Tanaka and L.T. Kubota, J. Braz. Chem. Soc **19**, 734 (2008). doi:10.1590/S0103-50532008000400018.

[26] D. De Souza, S.A.S. Machado and L.A. Avaca, Quím Nova **26**, 81 (2003). doi:10.1590/S0100-40422003000100015