Int. J. Electrochem. Sci., 12 **(2017)** 11465 – 11472, doi: 10.20964/2017.12.76

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Determination of Caffeine in Tea Using a Polydopamine-Gold Nanocomposite

Guolin Zhang[1,3], *Haiping Fu*[2†], *Dongsheng Zou*[1,*], *Runlin Xiao*[3], *Jun Liu*[4] *and Shaojing Li*[4]

[1] College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, 410128, P.R. China
[2] Tea Research Institute of Hunan Academy of Agricultural Science, Changsha, 410125, P.R. China
[3] The Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, 410125, P.R. China
[4] Qingdao Engineering Research Center for Rural Environment, Qingdao Agricultural University, Qingdao, 266109, P.R. China
[†] Co-first author
[*]E-mail: dongshengzouBio@foxmail.com

Received: 17 August 2017 / *Accepted:* 7 October 2017 / *Published:* 12 November 2017

A polydopamine-gold nanocomposite (PDA/AuNPs)-based caffeine sensor with high sensitivity was fabricated. The electrochemical performance of the caffeine sensor was characterized in various electrolyte solutions, including sodium perchlorate, phosphate buffer saline, and choline chloride containing oxalic acid. The influence of interference agents, normally present in caffeine-containing samples, on caffeine detection was also studied. The PDA/AuNPs/GCE-based sensor showed excellent performance in the determination of caffeine in tea samples.

Keywords: Caffeine; Electrochemical sensor; Graphene; Polydopamine; Children asthmatic attack

1. INTRODUCTION

Tea is a healthy drink and the most enjoyed beverage worldwide, with medical benefits that include antimicrobial, anti-inflammatory, antidepressant, anti-obesity, and anticancer activity, among others [1]. Tea is a complex mixture of phytochemicals that include polyphenols, catechins, and caffeine, which have attracted substantial attention for their functional impact on human health. Polyphenolic substances, such as gallic acid, can show cytotoxic activity on cancer cell lines [2]. In addition, flavon-3-ol structured catechins can act as scavenging agents for free radicals and as powerful antioxidants [3]. Additionally, the structure of catechins determines their biological activities.

It has been proved that caffeine that co-exists with polyphenols functions as an ergogenic substance and can enhance the oxidization of fatty acid [4].

Caffeine is not only a constituent of tea and coffee and used as an additive in large numbers of soft drinks but also the most pervasive drug in modern society. Some drugs that contain caffeine and other active substances have been discontinued because the therapeutic utility of its association with other active components has been poorly confirmed, or some associations have been found to result in unwanted effects. However, the use of caffeine is ubiquitous in the pharmacological preparation of analgesics, diet aids [5], and cold/flu remedies. Ingested caffeine normally undergoes extensive biotransformation in the human body, generating at least 17 detectable urinary metabolites, such as theobromine (3,7-dimethylxanthine), paraxanthine (1,7-dimethylxanthine), theophylline (1,3-dimethylxanthine), and 1,3,7-trimethylurate [6]. Caffeine is also a stimulant of the central nervous system and can affect wakefulness and alertness [7]. In addition, caffeine is a vasoconstrictor, which can increase blood pressure [8], stimulate gastric secretion [9-11], and increase respiration cycles, while acting as an effective diuretic causing emesis and dehydration [12, 13]. Furthermore, caffeine is considered a risk factor for cardiovascular diseases, and it may cause bone loss by mobilizing calcium from cells [14-16].

Recently, many studies have shown that the biological amines DA [17], L–DOPA [18], and norepinephrine [19] can be adsorbed onto almost any surface and can function as a biocompatible adhesive layer. An adhesive PDA layer can be formed on many different materials through the spontaneous oxidization of biological amines that occurs under an atmospheric oxygen and alkaline pH environment. PDA films show a facile interaction with the surface of many organic and inorganic materials, including ceramics, semiconductors, metal oxides, and conductive polymers. PDA coatings show several distinct properties relevant to the fields of nanoscience and nanotechnology. First, polymerization requires exceptionally mild conditions, occurring at ambient temperature in open air. In addition, a thin and adherent PDA layer can be formed on a few surfaces by self-polymerized biological amines [20]. Furthermore, the active functional entities produced by the secondary reaction at the PDA surface can promote the formation of covalent bonding with molecules that contain primary amine and thiol groups [21]. Moreover, surface multifunctional groups present on the PDA surface can reduce a few noble metal salts to metallic nanoparticles (NPs) [22, 23]. Metal NP coatings on polymeric surfaces can show desirable biological, electrochemical, electrical, and optical properties, which have recently been utilized in the electrical, nanomedicine, photocatalysis, and bio-detection fields. In particular, Au NPs are an exceptionally active material that can link with nearly all kinds of substrates. Au NPs can easily link with other Au NPs via covalent bonding through the amine and dihydroxy functional groups present on the surface of PDA substrates. Furthermore, PDA coatings can be used as the reductive agent for Au deposition.

In this study, detection of caffeine with high sensitivity was achieved through a continuous growth of Au NPs on an active support composed of an adhesive PDA layer. The functional groups present on the PDA surface provide an amenable environment for the continuous growth of Au NPs, which improves the specific surface area, surface negativity, and conductivity of the working electrode. In addition, the electrochemical sensor demonstrated exceptional performance in the determination of caffeine levels in tea samples.

2. EXPERIMENTS

2.1. Chemicals

Hydrogen tetrachloroaurate(III), glutaraldehyde, 3,4-ethylenedioxythiophene, dopamine hydrochloride (DA), potassium ferrocyanide, 4-aminophenazone, adenosine 5′-triphosphate disodium salt hydrate, triolein, glycerol-3-phosphate oxidase, glycerol kinase, sodium perchlorate monohydrate ($NaClO_4$), choline chloride ($C_5H_{14}ClNO$), and caffeine were obtained from Sigma–Aldrich. For the preparation of phosphate buffer solution (PBS), $Na_2H_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}12H_2O$ in the required amounts were dissolved into deionized (DI) water. All reagents were of analytical grade and used without additional purification. Green tea was provided by the Xiangfeng tea factory, Changsha, Hunan Province, P. R. China.

2.2. Characterization

A CHI−660D electrochemical workstation equipped with a three-electrode configuration was used for all electrochemical characterizations, with a Hg/Hg_2Cl_2/3 M KCl standard calomel electrode (SCE) as the reference electrode and a Pt wire as the counter electrode. All electrochemical reactions were conducted under a nitrogen (N_2) atmosphere. A Lambda 750 UV−Vis-NIR spectrophotometer was used to measure the film thickness. An ESCALAB 250Xi X−ray photoelectron spectrometer (XPS) was used to study the elemental composition of PDA and Au.

2.3. Preparation of PDA/Au NPs nanocomposite

Oxidized DA molecules were deposited onto a GCE using 50 cycles of positive cyclic voltammetry (CV) (scanning from –400 mV to 300 mV) in DA (pH 7.5, 3 mM; 10 mM PBS) under a N_2 atmosphere, with a scan rate of 10 mV/s. Then, the PDA product was yielded. The PDA substrates were stored at 4 °C before use. A modified CV measurement was used to characterize the deposition of Au NPs. This reaction was conducted using 2 cycles of negative CV (scanning from 800 mV to –500 mV) in $HAuCl_4$ solution (2 mM), with a scan rate of 5 mV/s. The final product was denoted as PDA/AuNPs.

2.4. Electrochemical measurement

A CHI430A electrochemical workstation in the three-electrode configuration was used for all electrochemical measurements, with working, reference, and auxiliary electrodes of PDA/AuNPs/GCE, Ag/AgCl, and Pt wire, respectively. Caffeine levels in samples were determined by CV measurement in PBS (0.1 M), scanning from 1.0 to 1.5 V (scan rate: 50 mV/s). DPV was calculated in a scan range of 1.0 to 1.5 V (scan rate: 0.6 mV/s), with 0.05 s modulation at an interval of 0.2 s.

3. RESULTS AND DISCUSSION

UV−Vis (UVS) and XPS characterization was used to confirm the presence of PDA and PDA/AuNPs, as shown in Figure 1. The UVS data (Figure 1A) showed absorption peaks *ca.* 333, 400, 495, and 650 nm for PDA with a melanin−like structure; the peak at 550 nm observed for Au suggested that Au NPs were deposited onto the PDA surface. Moreover, XPS data (Figure 1B) showed Au peaks at 85.3 (Au $4f_{7/2}$) and 89.3 eV (Au $4f_{5/2}$) and PDA peaks at 286.0 (C 1s), 400 (N 1s) and 532.0 eV (O 1s). These data confirmed that PDA and PDA/AuNPs coatings were successfully deposited onto the GCE.

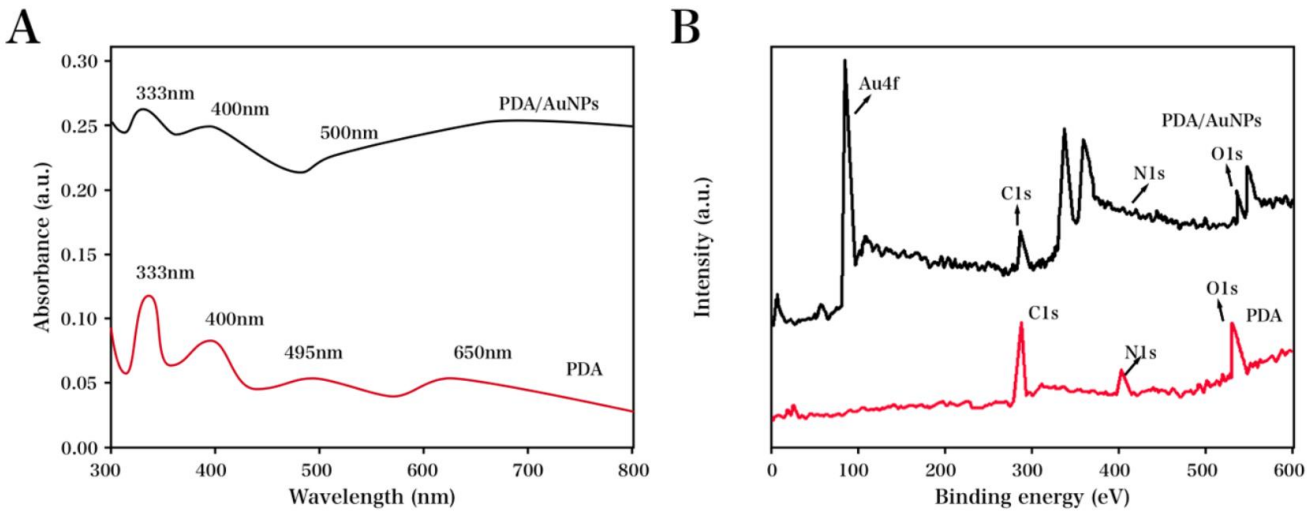

Figure 1. (A) UV − vis. absorption (UVS) and (B) XPS characterization for PDA and PDA/AuNPs.

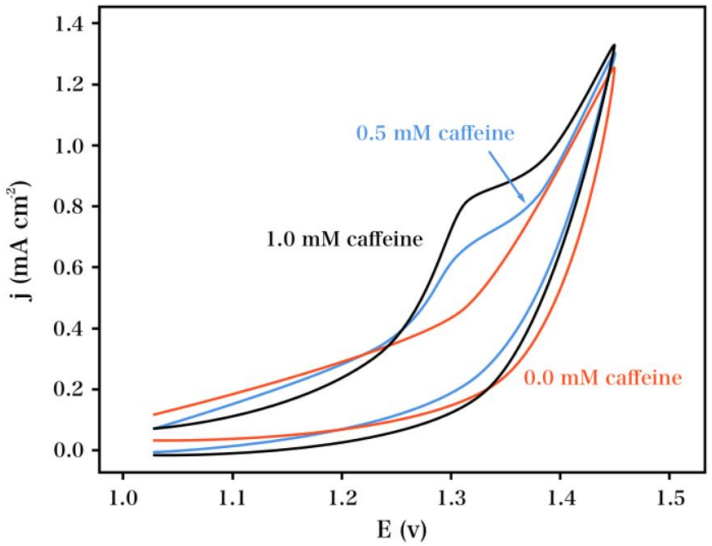

Figure 2. CV for bare PDA/AuNPs/GCE in PBS (0.1 M, pH 7.0) + caffeine (0.0, 0.5 and 1.0 mM).

CV measurements were carried out to study the electrochemical performance of the bare PDA/AuNPs/GCE for caffeine detection. As shown in Figure 2, an anodic peak was observed for the

forward scan at a high potential of *ca.* +1.25 V; however, no cathodic peak was observed during the reverse scan, which suggests irreversible oxidization.

The electrolyte solution is a potential influencing factor for the electrochemical performance of caffeine detection. Therefore, the sensor response was assessed in sodium perchlorate, phosphate buffer saline, and choline chloride solutions. As shown in Figure 3A, DPV scans were used to detect caffeine at increasing concentrations (the inset shows the corresponding calibration curve). The as-prepared sensor exhibited a sensitivity of 146 μA/cm^2/mM, with a limit of detection (LOD) of 44 nM. Sodium perchlorate can show exceptional solubility (even in organic solvents) and oxidization features and was used as the second test solution [24]. The operation of caffeine sensors in perchloric acid media has been studied previously [25]. Figure 3B shows DPV characterization in $NaClO_4$ (0.1 M); the data showed a lower sensitivity for caffeine detection of 104 μA/cm^2/mM than that obtained in PBS (0.1 M) and a higher LOD of 122 nM. In addition, the absence of any complexes in solution is suggested by the peak current linearly increasing with the increase in caffeine concentration.

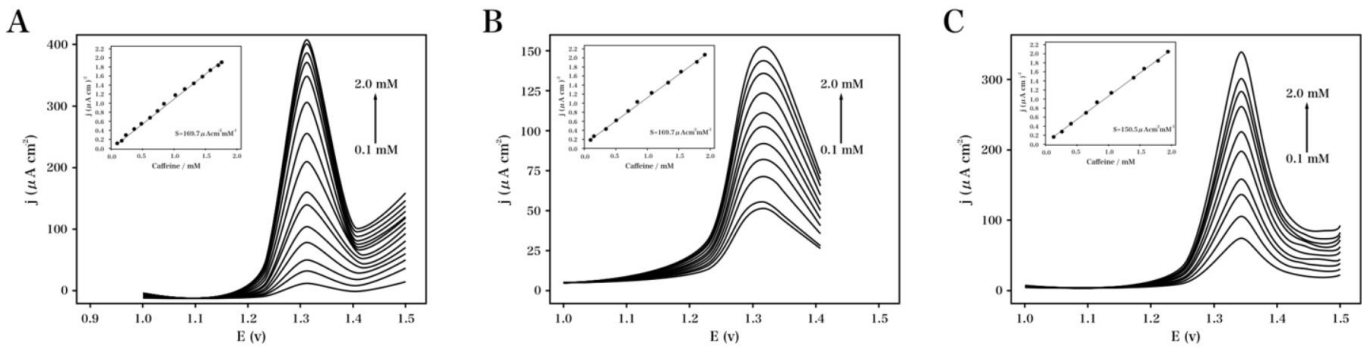

Figure 3. DPV characterization recorded at PDA/AuNPs/GCE for caffeine at various concentrations in (A) PBS (0.1 M), (B) sodium perchlorate (0.1 M), and (C) ChCl (0.05 M) + oxalic acid (0.05 M). Inset: corresponding calibration plots.

Table 1. Comparison of the main properties of electrochemical sensors for caffeine detection.

Electrode	Linear range upper limit (mM)	Detection limit (μM)	Reference
MWCNT-Nafion/GCE	2.4	0.77	[26]
Nafion/GCE	0.011	—	[27]
Carbon paste electrode	1	200	[28]
Nafion–Gr/GCE	4	0.12	[29]
Edge plane pyrolytic graphite electrode	0.1	0.008	[30]
AHNSA/GCE	0.04	0.137	[31]
4α-Cu^{II}TAPc/GCE	1.4	0.0034	[32]
PDA/AuNPs/GCE	7.5	0.79	This work

The sensitivity of the sensor towards detection of caffeine was investigated using a mixture of oxalic acid (0.05 M) and ChCl (0.05 M). Figure 3C shows the DPV for caffeine at increasing concentration. The data showed a sensitivity of 154 μAcm^2/mM, which was higher than that in

perchlorate solution but lower than that in PBS (0.1 M); the LOD was calculated to be 55 nM. The as-prepared sensor showed the highest sensitivity and lowest LOD in PBS solution (0.1 M, pH 7.0). Therefore, PBS solution was used as the electrolyte in further experiments. A comparison of other modified sensors for the detection of caffeine is shown in Table 1.

The effect of pH value on the caffeine oxidization peak current and peak potential was studied in buffered electrolyte solution (pH: 3.0 to 10) containing 0.5 mM caffeine. The DPV showed only a negligible pH dependence, which was consistent with a previous study using carbon electrodes at an applied potential of *ca.* +1.30 V [28]. The DPV half peak width ($\Delta E_{p/2}$) was obtained in a range of 94 to 114 mV; from CVs measured at various scan rates (Figure 4A), $E_p - E_{p/2}$ was found to be in a range of 60 to 70 mV. These results confirmed that 2 electrons are involved in the first oxidation step. The complete oxidation process requires two oxidation steps, where the second step refers to a $2e^-$, $2H^+$ oxidation and involves $4e^-$ and $4H^+$ overall [33-35]. The peak current showed only a small dependence on the solution pH. In particular, the peak current showed a nominal increase over the pH range of 3.0 to 7.0 but then decreased with a further increase in pH. The highest response to caffeine was obtained in pH 7.0 PBS; therefore, solution with this same pH value was used for further experiments.

To investigate the effect of the CV scan rate on the caffeine oxidization peak current, CVs were recorded using varying scan rates ranging from 10 to 200 mV/s in PBS (0.1 M, pH 7.0) containing caffeine (0.5 mM). A linear relationship was found between the anodic peak current and the square root of the scan rate (Figure 4B), which indicated a diffusion-controlled mechanism for the electrochemical oxidation of caffeine using the GCE.

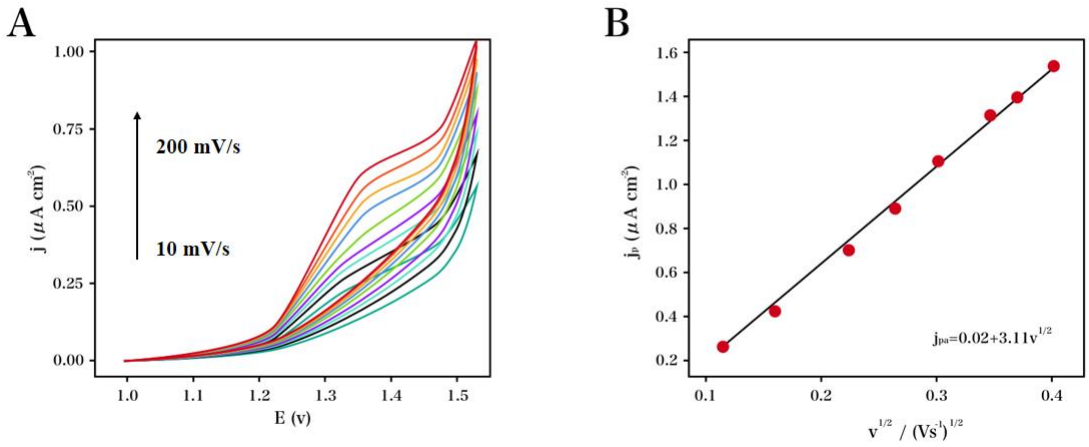

Figure 4. (A) CV characterizations recorded at PDA/AuNPs/GCE in 0.1 M PBS + 0.5 mM caffeine (scan rates: 10 to 200 mV/s) and (B) the linear dependence of peak current versus the square root of scan rate.

The effect of possible interference agents (e.g., sucrose, glucose, fructose, citric acid, and ascorbic acid, normally co-present in drugs and beverages containing caffeine) on the sensor performance was also investigated by using two different interference compounds with caffeine to

interference agent concentration ratios of 1:1 and 1:2. DPV profiles were obtained in PBS (0.1 M, pH 7.0) containing caffeine (0.5 mM) before and after addition of the interference compounds. An interference effect was found for both 1:1 and 1:2 compounds, with an increase in the overall oxidation peak current of 50% and 100%, respectively.

The concentration of caffeine in six green tea samples was measured at the PDA/AuNPs modified GCE using the standard addition method. In particular, an aliquot of the samples was injected into the buffer electrolyte followed by the addition of known amounts of caffeine. As shown in the results in Table 2, the caffeine sensor showed an acceptable level of recovery behaviour.

Table 2. Detection of the concentration of caffeine in tea samples.

Sample	Found (μM)	Added (μM)	Found (μM)	Revovery (%)	RSD (%)
1	0.74	0.5	1.16	93.55	4.51
2	0.55	1	1.51	97.42	2.22
3	1.01	1.25	2.28	100.88	2.36
4	1.02	1.5	2.47	98.02	2.01
5	0.85	1.75	2.53	97.30	4.05
6	0.77	2	2.69	97.11	5.90

4. CONCLUSIONS

Adhesive PDA coatings on a GCE were synthesized under optimal parameters using CV. The continuous growth of Au NPs on the active support provided by the adhesive PDA layer enabled the efficient electrochemical detection of caffeine. Compared with other electrochemical caffeine sensors, the PDA/AuNPs/GCE sensor showed high sensitivity, a low LOD, and a large linear range. In addition, the sensor demonstrated excellent performance in the detection of caffeine in real tea samples.

References

1. C. Fernando and P. Soysa, *Nutrition Journal*, 14 (2015) 74.
2. F. Zhao, H. Lin, S. Zhang, Y. Lin, J. Yang and N. Ye, *Journal of Agricultural and Food Chemistry*, 62 (2014) 2772.
3. Q. He, Y. Lv, L. Zhou and B. Shi, *Journal of Liquid Chromatography & Related Technologies*, 33 (2010) 491.
4. D. Pesta, S. Angadi, M. Burtscher and C. Roberts, *Nutrition & Metabolism*, 10 (2013) 71.
5. M. Westerterp-Plantenga, M. Lejeune and E. Kovacs, *Obesity*, 13 (2005) 1195.
6. M. Nakajima, T. Yokoi, M. Mizutani, S. Shin, F. Kadlubar and T. Kamataki, *Cancer Epidemiology and Prevention Biomarkers*, 3 (1994) 413.
7. D. Krieger, D. Kalman, S. Feldman, L. Arnillas, D. Goldberg, O. Gisbert and S. Nader, *Clinical and Translational Science*, 9 (2016) 246.
8. P. Pereira, Y. Motoyama, G. Esteves, J. Oliveira, R. Pereira, D. Pandeló and P. Azevedo, *Sport Sciences for Health*, 12 (2016) 239.

9. E. de Mejia and M. Ramirez-Mares, *Trends in Endocrinology & Metabolism*, 25 (2014) 489.
10. I. Ulanovsky, N. Haleluya, S. Blazer and A. Weissman, *Journal of Perinatology*, 34 (2014) 620.
11. M. Hensel, M. Pashmakova and B. Porter, *Brazilian Journal of Veterinary Pathology*, 10 (2017) 65.
12. K. Turley, J. Rivas, J. Townsend and A. Morton, *Journal of Caffeine Research*, 7 (2017) 71.
13. R. Rodriguez, R. Haugen, A. Rueber and C. Huang, *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 163 (2014) 47.
14. N. Yamada-Fowler, M. Fredrikson and P. Söderkvist, *PLoS One*, 9 (2014) e99294.
15. J. Petzer and A. Petzer, *Current Medicinal Chemistry*, 22 (2015) 975.
16. X. Cai, C. Fang, S. Hayashi, S. Hao, S. Nishiguchi, H. Tsutsui and J. Sheng, *Journal of Hepatology*, 64 (2016) S670.
17. M. Zhu, Z. Yang, Y. Cheng, Y. Sun, J. Xing, J. Wei, J. Sun, H. Liu and X. Song, *Int. J. Electrochem. Sci*, 12 (2017) 6863.
18. X. Li, Q. Fu, Q. Zhang, X. Jiang, F. Yang, W. Wei and Z. Xia, *Analytical Methods*, 7 (2015) 8227.
19. Y. Zhang, Y. Zhang, S. Yu, Y. Zhang, L. Zhu, P. He and Q. Wang, *Microfluidics and Nanofluidics*, 21 (2017) 97.
20. R. Subair, B. Tripathi, P. Formanek, F. Simon, P. Uhlmann and M. Stamm, *Chemical Engineering Journal*, 295 (2016) 358.
21. A. Ananthi and K. Phani, *Journal of Electroanalytical Chemistry*, 764 (2016) 7.
22. H. Chen, L. Zhao, D. Chen and W. Hu, *Journal of Colloid and Interface Science*, 460 (2015) 258.
23. W. Zhang, Y. Tang, J. Liu, Y. Ma, L. Jiang, W. Huang, F. Huo and D. Tian, *Journal of Materials Chemistry B*, 2 (2014) 8490.
24. E. Urbansky, *Bioremediation Journal*, 2 (1998) 81.
25. T. Alizadeh, M.R. Ganjali, M. Zare and P. Norouzi, *Electrochimica Acta*, 55 (2010) 1568.
26. J. Zhang, L. Wang, W. Guo, X. Peng, M. Li and Z. Yuan, *International Journal of Electrochemical Science*, 6 (2011) 997.
27. B. Brunetti, E. Desimoni and P. Casati, *Electroanalysis*, 19 (2007) 385.
28. G. Mersal, *Food Analytical Methods*, 5 (2012) 520.
29. J. Sun, K. Huang, S. Wei, Z.W. Wu and F. Ren, *Colloids & Surfaces B Biointerfaces*, 84 (2011) 421.
30. R. Goyal, S. Bishnoi and B. Agrawal, *Journal of Electroanalytical Chemistry*, 655 (2011) 97.
31. M. Amare and S. Admassie, *Talanta*, 93 (2012) 122.
32. A. Jeevagan and S.A. John, *Electrochimica Acta*, 77 (2012) 137.
33. R. Nunes and É.T. Cavalheiro, *Journal of the Brazilian Chemical Society*, 23 (2012) 670.
34. N. Spataru, B.V. Sarada, D. Tryk and A. Fujishima, *Electroanalysis*, 14 (2002) 721.
35. J. Sun, K. Huang, S. Wei, Z. Wu and F. Ren, *Colloids and Surfaces B: Biointerfaces*, 84 (2011) 421