Available online at www.sciencedirect.com
SCIENCE DIRECT
Electrochimica Acta 51 (2006) 5169–5173
ELECTROCHIMICA Acta
www.elsevier.com/locate/electacta

Cisplatin electrochemical biosensor

Jitka Petrlova [a], David Potesil [a,d], Josef Zehnalek [a], Bernd Sures [b], Vojtech Adam [a,d], Libuse Trnkova [c], Rene Kizek [a,*]

[a] *Department of Chemistry and Biochemistry, Mendel University of Agriculture and Forestry, Zemedelska 1, 613 00 Brno, Czech Republic*
[b] *Universität Karlsruhe, Zoologisches Institut I, Ökologie-Parasitolie, 76128 Karlsruhe, Germany*
[c] *Department of Theoretical and Physical Chemistry, Masaryk University Faculty of Science, Kotlarska 2, 611 37 Brno, Czech Republic*
[d] *Department of Analytical Chemistry, Masaryk University Faculty of Science, Kotlarska 2, 611 37 Brno, Czech Republic*

Received 1 June 2005
Available online 4 May 2006

Abstract

Platinum complexes play an important role in the chemotherapy of various tumour diseases. The aim of this paper was to investigate if a metallothionein (MT) modified hanging mercury drop electrode can be applied as a cisplatin electrochemical biosensor. The modification of the mercury electrode surface by MT and the determination of cisplatin were performed by adsorptive transfer stripping technique and differential pulse voltammetry. The detection limit (3 S/N) of cisplatin ($[Pt^{II}(NH_3)_2Cl_2]^0$) calculated from the decrease of CdT peak was about 2.5 pmol in 5 μl (0.5 μM) at the interaction time of 400 s. Moreover, we tested the influence of human blood serum as a complex biological matrix on the way of determination of cisplatin. On the basis of the obtained results we estimated that we are able to determine tens of picomoles of cisplatin (5 μl drop) in the presence of human blood serum.

Keywords: Biosensor; Metallothionein; Cisplatin; Platinum based cytostatic; Adsorptive transfer stripping analysis; Differential pulse voltammetry; Human blood serum

1. Introduction

The pollution of the environment with toxic metals is a result of many human activities, such as mining and metallurgy, and the effects of these metals on the ecosystems are of large economic and public-health significance [1,2], because these substances are not biodegradable and retained by the ecological system [3]. Besides "standard" toxic metals such as cadmium, lead and mercury, which have been monitoring for many years, following the introduction of automobile catalytic converters the platinum group metals (platinum and rhodium) gain on increasing interest in environmental research [4–7]. Moreover, platinum complexes play an important role in the chemotherapy of various tumour diseases [8–11]. As a consequence of the increasing employment of platinum for exhaust purification, in industry and tumour diseases treatment, it became necessary to analyse the platinum compounds in a wide range of biological and environmental matrices.

Conventional analytical techniques for platinum environmental determination are atomic absorption spectrometry [5,6,12–15], inductively coupled plasma mass spectrometry [16–21] and stripping voltammetry [21–31]. In addition there are many techniques, which have been used for the determination of platinum based cytostatic drugs such as HPLC coupled to different kinds of detectors [32–34] and/or electrochemical methods [35–43]. On the other hand biosensors have the advantages of specificity, low cost, ease of use, portability and the ability to furnish continuous real time signals [3,44–47]. A number of recently published papers have described determination of platinum using electrochemical biosensors [35,42,43,48–50].

In the present work, we applied the metallothionein (MT) modified electrode (heavy metals biosensor) to determine commonly used platinum cytostatics, cisplatin (for chemical structure see Fig. 1A, inset 'a'). Furthermore we tested the influence of complex biological matrix (human blood serum) on the cisplatin determination.

* Corresponding author. Tel.: +420 5 4513 3350; fax: +420 5 4521 2044.
E-mail address: kizek@sci.muni.cz (R. Kizek).

0013-4686/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.electacta.2006.03.077

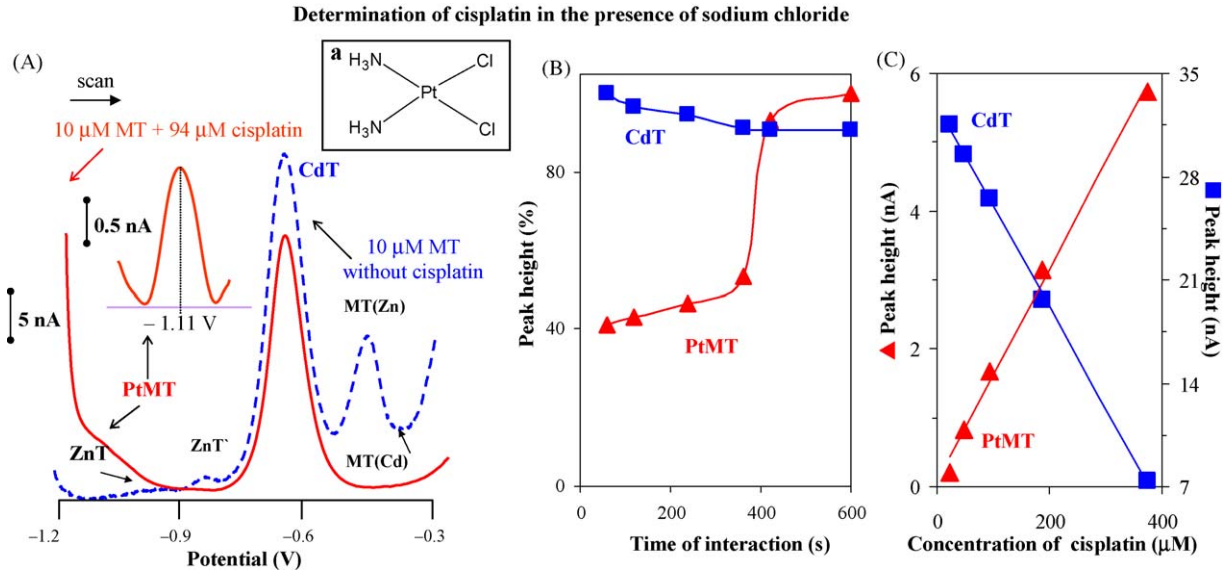

Fig. 1. Cisplatin – $[Pt^{II}(NH_3)_2Cl_2]^0$; anti-cancer drug – detection in 0.5 M NaCl. Typical DPV voltammograms of 10 μM MT without addition of cisplatin and 10 μM MT + 94 μM of cisplatin (A); inset: peak of PtMT after the baseline correction. AdTS DPV parameters were as follows: the initial potential of −1.2 V, the end potential −0.3 V, the modulation time 0.057 s, the interval 0.2 s, the step potential of 1.05 mV/s, the modulation amplitude of 25 mV, E_{ads} = 0 V, time of accumulation 240 s and time of interaction 420 s. Chemical structure of cisplatin (inset 'a'). Effect of interaction time on the peak heights of CdT and PtMT (B). Peak height of 1.72 nA (PtMT signal) and peak height of 29.4 nA (CdT signal) correspond to the 100%. Dependence of PtMT and CdT peak heights on different cisplatin concentrations (C).

2. Materials and methods

2.1. Chemicals

Rabbit liver MT (MW 7143), containing 5.9% Cd and 0.5% Zn, was purchased from Sigma–Aldrich (St. Louis, USA). Tris(2-carboxyethyl)phosphine (TCEP) is produced by Molecular Probes (Evgen, Oregon, USA). Sodium chloride, cadmium nitrate, zinc nitrate and other used chemicals were purchased from Sigma–Aldrich. Stock standard solutions of MT with 10 μg ml^{-1} were prepared by ACS water (Sigma–Aldrich, USA) and stored in the dark at the temperature of −20 °C. Working standard solutions were prepared daily by dilution of the stock solutions and reduced by 1 mM TCEP. The pH value was measured using WTW inoLab Level 3 with terminal Level 3 (Weilheim, Germany), controlled by the personal computer program (MultiLab Pilot; Weilheim, Germany). The pH-electrode (SenTix-H, pH 0–14/3 M KCl) was regularly calibrated by set of WTW buffers (Weilheim, Germany).

2.2. Electrochemical measurements

Electrochemical measurements were performed with the AUTOLAB Analyser (EcoChemie, the Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), using a standard cell with three electrodes. The working electrode was a hanging mercury drop electrode (HMDE) with the drop area of 0.4 mm^2. The reference electrode was the Ag/AgCl/3 M KCl electrode and the auxiliary electrode was the graphite electrode. The supporting electrolyte was 0.5 M NaCl (pH 6.4). The analysed samples were deoxygenated prior to measurements by purging with argon (99.999%), saturated with water for 120 s. All experiments were carried out at room temperature. For smoothing and baseline correction, the software GPES 4.4 supplied by EcoChemie was employed.

2.2.1. Suggestion of heavy metals biosensor

A detailed description of the metallothionein modification of the mercury electrode has been previously published [7]. Briefly, scheme of adsorptive transfer stripping technique was used for suggestion of heavy metals biosensor: (1) renewing of the hanging mercury drop electrode surface; (2) adsorbing of MT in a drop solution onto the HMDE surface at open circuit (240 s); (3) washing electrode in sodium chloride (0.5 M, pH 6.4); (4) interaction of cisplatin with the protein modified HMDE surface in a drop solution at open circuit (this parameter was optimised, see Section 3); (5) washing electrode in sodium chloride (0.5 M, pH 6.4); (6) measurement of MT by DPV in 0.5 M sodium chloride, pH 6.4. The samples of the MT were reduced before each measurement by 1 mM tris(2-carboxyethyl)phosphine according to [7,51,52], because reduced metallothionein offers better reproducibility and higher sensitivity of a determination in comparison with non-reduced ones [7]. The supporting electrolyte (sodium chloride: 0.5 M NaCl, pH 6.4) was purchased from Sigma–Aldrich in ACS purity. DPV parameters were as follows: the initial potential of −1.2 V, the end potential −0.3 V, the modulation time 0.057 s, the interval 0.2 s, the step potential of 1.05 mV/s and the modulation amplitude of 25 mV.

2.3. Preparation of cisplatin solutions

The chemotherapeutic drug of cisplatin was synthesized and provided by Pliva-Lachema (Brno, Czech Republic) [53]. Stock standard solutions of cisplatin (10 μg ml^{-1}) were prepared by

sodium chloride solution (0.5 M, pH 6.4) and stored in the dark at the temperature of −20 °C. Working standard solutions were prepared daily by dilution of the stock solutions.

2.4. Preparation of human blood serum

Human blood serum samples were obtained from the Department of Clinical Biochemistry, University Hospital Ponavka in Brno, Czech Republic. Human blood serum was 1000 times diluted with 0.5 M sodium chloride (pH 6.4) before measurements. Moreover, we added cisplatin (10, 20, 40, 80, 160, 350, 450, 530 and 650 μM) to these diluted solutions of human blood. Due to dilution of human blood serum samples before addition of cisplatin and quick analysis of the prepared samples (immediately after addition of cisplatin) we assumed that the amount of the platinum based cytostatic bound to protein containing blood serum was very low and, therefore, did not interfere the analysis.

2.5. Statistical analysis

STATGRAPHICS® (Statistical Graphics Corp®, USA) was used for statistical analyses. Results are expressed as mean ± S.D. unless noted otherwise. Value of $p < 0.05$ was considered significant.

3. Results and discussion

Recently we have published results describing heavy metals biosensor (MT modified surface of HMDE) that we have used for the determination of cadmium and zinc [7]. Here, we were interested in the issue of how the differential pulse voltammetric record of MT modified electrode surface looks like in the presence of cisplatin.

Primarily we accumulated 10 μM reduced metallothionein, which naturally contains Cd(II) and Zn(II) in its clusters, on the surface of the HMDE for 240 s and analysed the adsorbed MT by differential pulse voltammetry (Fig. 1A, dashed line). The highest observed signal called CdT (−0.65 V) corresponds to adduct of the MT with mercury on the surface of the HMDE (HS-peptide + Hg = HgS-peptide) [7,54]. In addition the MT(Cd) (−0.42 V) and MT(Zn) (−0.49 V) signals relates to reduction of bounded metal [55] were observed. Signals ZnT′ (−0.87 V) and ZnT (−0.99 V) probably results from electrochemical reactions proceeding between MT, heavy metals and mercury electrode. Other details will be published elsewhere. Moreover, we applied the MT modified HMDE to determination of cisplatin (interaction time was 420 s). The resulting voltammogram is shown in Fig. 1A (continuous line). We observed three signals: CdT at the potential of −0.66 V, ZnT′ at −0.87 V and peak at −1.11 V called PtMT that could correspond to reduction of platinum bounded to metallothionein (Fig. 1A). On the other hand MT(Cd), MT(Zn) and ZnT signals disappeared which probably relates with the replacing of cadmium and/or zinc by platinum in the structure of MT adsorbed on the HMDE surface, as it is already described that Pt binds approximately 30 and 10^7 times more firmly to MT than Cd and Zn, respectively [56].

It clearly follows from the results obtained that the voltammogram of adsorbed MT changed in the presence of cisplatin in comparison with the non-cisplatin ones (Fig. 1A). Therefore, we studied time dependence of interaction between MT modified electrode surface and cisplatin on the height of the PtMT and CdT signals. We observed sharp increase of peak PtMT at interaction time of 400 s and gradual decrease of CdT signal during the increase of interaction time (Fig. 1B). The sharp increase of PtMT signal observed relates with releasing of cadmium(II) and zinc(II) atoms from the MT structure, which confirms the decrease of height of MT(Cd), MT(Zn), ZnT′ and ZnT signals (Fig. 1A). We selected interaction time of 420 s as optimal for the following measurements of cisplatin because we did not observe signals of other metals and the height of PtMT was sufficient for sensitive cisplatin determination.

For analytical purposes we studied the dependence of the PtMT peak and/or CdT signal heights on cisplatin concentration (Fig. 1C). The dependences were linear in the studied concentration range of 25–375 μM of cisplatin (Fig. 1C and Table 1). The detection limit (3 S/N) of cisplatin ($[Pt^{II}(NH_3)_2Cl_2]^0$) calculated from the decrease of CdT peak was about 2.5 pmol in 5 μl (0.5 μM) at the interaction time of 400 s.

3.1. Determination of cisplatin in the presence of the human blood serum

For the study of the anti-cancer drugs effectiveness it is necessary to detect their therapeutic level. That is why we were interested in using the MT modified HMDE for determination of cisplatin in the presence of human blood serum. Hence, we added the different cisplatin concentrations (10, 20, 40, 80, 160, 350, 450, 530 and 650 μM) to the human blood serum sam-

Table 1
Influence of biological matrix (human blood serum) on determination of cisplatin by MT modified electrode surface

Sample	Detected compound	Concentration of the cisplatin [μM]	MT signals[a]	Equation	R^2
Supporting electrolyte[b]	Cisplatin	25–375	PtMT	$y = 0.0154x + 0.0833$	0.9939
			CdT[c]	$y = -0.0683x + 32.8$	0.9993
Human blood serum	Cisplatin	10–160	PtMT	$y = 0.0098x - 0.096$	0.9953
		350–650	PtMT	$y = 0.1046x - 34.42$	0.9960
		10–650	CdT[c]	$y = -0.00670x + 25.8$	0.9906

[a] For detailed description of the MT signals see Section 3 and/or [7,55,59–62].
[b] Supporting electrolyte was 0.5 M NaCl.
[c] The CdT signal was used for quantification of the detected compound.

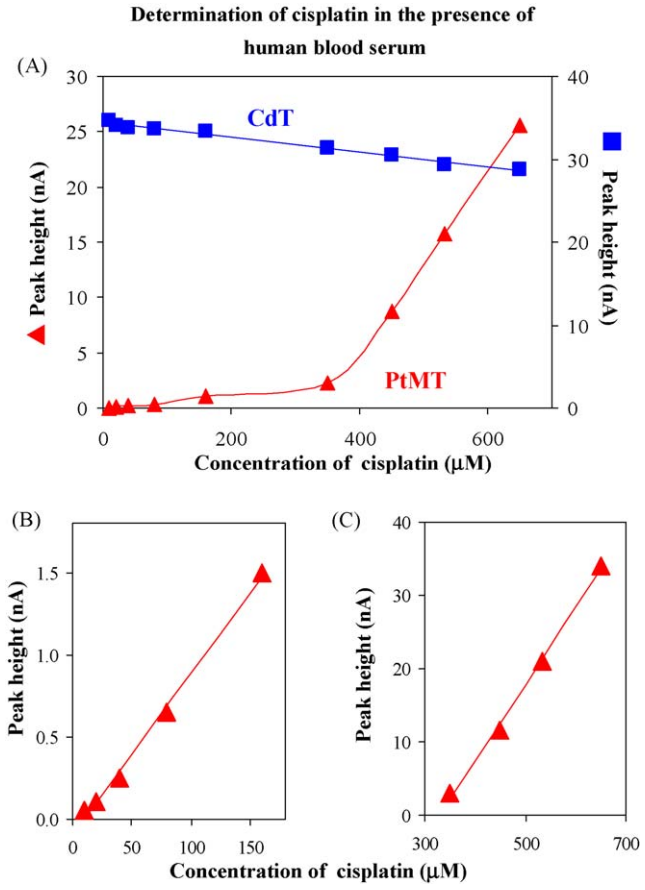

Fig. 2. Detection in human blood serum. Dependences of PtMT and CdT peak heights (A), and PtMT peak heights (B and C) on different cisplatin concentrations. MT concentration: 10 μM. Experimental conditions: time of accumulation 240 s, time of interaction 420 s. DPV parameters are shown in Fig. 1.

ples. The observed changes of CdT and PtMT in peak heights with changing cisplatin concentrations are shown in Fig. 2A. CdT signal specifically decreased with increasing cisplatin concentration. We observed the same phenomenon in the case of determination of cadmium and zinc by MT modified HMDE [7]. Moreover, PtMT signal slowly increased up to cisplatin concentration 350 μM and then increased markedly (Fig. 2A). For analytical purposes we attempted to split the mentioned PtMT curve into two parts: Fig. 2B (10–160 μM) and Fig. 2C (350–650 μM). The detection limit (3 S/N) of cisplatin ($[Pt^{II}(NH_3)_2Cl_2]^0$) calculated from the decrease of CdT peak was about 2.5 μM (R.S.D. was 7.8%, $n = 5$) at the interaction time of 400 s. The suggested approach shows the possible way for simple, sensitive and rapid detection of the anti-cancer drug in the human body fluids at the picomole level (10 pmol in 5 μl drop).

4. Conclusion

Electrochemical biosensors have superior properties over the other existing measurement systems because they can provide quick, simple and low-cost on-field determination of many biologically active species and a number of dangerous pollutants [44,57,58]. It is clear that biosensor technology is a powerful alternative to conventional analytical techniques, combining the specificity and sensitivity of biological systems in small devices. Here we suggested a new biosensor for the determination of platinum based cytostatic, cisplatin. On the basis of the obtained results we assumed that the suggested technique could offer simple, quick and low-cost detection of cisplatin in biological and medical samples.

Acknowledgements

This work was supported by grants: GA CR No. 525/04/P132 and RASO 8/2005.

References

[1] J. Zehnálek, V. Adam, R. Kizek, Listy Cukrov 120 (2004) 222.
[2] M. Gavrilescu, Eng. Life Sci. 4 (2004) 219.
[3] N. Verma, M. Singh, Biometals 18 (2005) 121.
[4] B. Sures, S. Zimmermann, J. Messerschmidt, A. von Bohlen, F. Alt, Environ. Pollut. 113 (2001) 341.
[5] S. Zimmermann, J. Messerschmidt, A. von Bohlen, B. Sures, Anal. Chim. Acta 498 (2003) 93.
[6] S. Zimmermann, B. Sures, Environ. Sci. Pollut. Res. 11 (2004) 194.
[7] V. Adam, J. Petrlova, D. Potesil, J. Zehnalek, B. Sures, L. Trnkova, F. Jelen, R. Kizek, Electroanalysis 17 (2005) 1649.
[8] M.A. Fuertes, J. Castilla, C. Alonso, J.M. Perez, Curr. Med. Chem. 10 (2003) 257.
[9] T. Boulikas, M. Vougiouka, Oncol. Rep. 10 (2003) 1663.
[10] Z.H. Siddik, Oncogene 22 (2003) 7265.
[11] D. Wang, S.J. Lippard, Nat. Rev. Drug Discov. 4 (2005) 307.
[12] L.N. Moskvin, N.M. Yakimova, I.A. Alekseeva, J. Anal. Chem. 60 (2005) 34.
[13] A. Limbeck, E. Rudolph, S. Hann, G. Koellensperger, G. Stingeder, J. Rendl, J. Anal. At. Spectrom. 19 (2004) 1474.
[14] J. Chwastowska, W. Skwara, E. Sterlinska, L. Pszonicki, Talanta 64 (2004) 224.
[15] M.M.G. Garcia, F.S. Rojas, C.B. Ojeda, A.G. de Torres, J.M.C. Pavon, Anal. Bioanal. Chem. 375 (2003) 1229.
[16] L. Qi, M.F. Zhou, C.Y. Wang, J. Anal. At. Spectrom. 19 (2004) 1335.
[17] S.V.M. Maharaj, Anal. Bioanal. Chem. 380 (2004) 84.
[18] C.A. Heinrich, T. Pettke, W.E. Halter, M. Aigner-Torres, A. Audetat, D. Gunther, B. Hattendorf, D. Bleiner, M. Guillong, I. Horn, Geochim. Cosmochim. Acta 67 (2003) 3473.
[19] R.R. Barefoot, J.C. Van Loon, Talanta 49 (1999) 1.
[20] A.J. Campbell, M. Humayun, Anal. Chem. 71 (1999) 939.
[21] S. Zimmermann, C.M. Menzel, Z. Berner, J.D. Eckhardt, D. Stuben, F. Alt, J. Messerschmidt, H. Taraschewski, B. Sures, Anal. Chim. Acta 439 (2001) 203.
[22] K.Z. Brainina, N.Y. Stozhko, G.M. Belysheva, O.V. Inzhevatova, L.I. Kolyadina, C. Cremisini, M. Galletti, Anal. Chim. Acta 514 (2004) 227.
[23] E. Szlyk, A. Szydlowska-Czerniak, J. Agric. Food Chem. 52 (2004) 4064.
[24] J. Vacek, J. Petrek, R. Kizek, L. Havel, B. Klejdus, L. Trnkova, F. Jelen, Bioelectrochemistry 63 (2004) 347.
[25] O. Mikkelsen, K.H. Schroder, Electroanalysis 15 (2003) 679.
[26] J.H. Pei, M.L. Tercier-Waeber, J. Buffle, Anal. Chem. 72 (2000) 161.
[27] M.A. Baldo, S. Daniele, I. Ciani, C. Bragato, J. Wang, Electroanalysis 16 (2004) 360.
[28] S. Huszal, J. Kowalska, M. Krzeminska, J. Golimowski, Electroanalysis 17 (2005) 299.
[29] C. Locatelli, Electroanalysis 17 (2005) 140.
[30] J. Kowalska, S. Huszal, M.G. Sawicki, M. Asztemborska, E. Stryjewska, E. Szalacha, J. Golimowski, S.W. Gawronski, Electroanalysis 16 (2004) 1266.
[31] C. Leon, H. Emons, P. Ostapczuk, K. Hoppstock, Anal. Chim. Acta 356 (1997) 99.

[32] Z. Stefanka, S. Hann, G. Koellensperger, G. Stingeder, J. Anal. At. Spectrom. 19 (2004) 894.
[33] S. Hann, Z. Stefanka, K. Lenz, G. Stingeder, Anal. Bioanal. Chem. 381 (2005) 405.
[34] V. Nischwitz, B. Michalke, A. Kettrup, J. Chromatogr. A 1016 (2003) 223.
[35] V. Brabec, O. Vrana, V. Kleinwachter, Collect. Czech. Chem. Commun. 48 (1983) 2903.
[36] V. Brabec, O. Vrana, O. Novakova, V. Kleinwachter, F.P. Intini, M. Coluccia, G. Natile, Nucl. Acids Res. 24 (1996) 336.
[37] S.D. Kim, O. Vrana, V. Kleinwachter, K. Niki, V. Brabec, Anal. Lett. 23 (1990) 1505.
[38] O. Vrana, V. Brabec, Bioelectrochem. Bioenerg. 19 (1988) 145.
[39] O. Vrana, V. Brabec, Anal. Biochem. 142 (1984) 16.
[40] O. Vrana, V. Brabec, V. Kleinwachter, Anti-Cancer Drug Des. 1 (1986) 95.
[41] G. Weber, J. Messerschmidt, A.C. Pieck, A.M. Junker, A. Wehmeier, U. Jaehde, Anal. Bioanal. Chem. 380 (2004) 54.
[42] D. Hernandez-Santos, M.B. Gonzalez-Garcia, A. Costa-Garcia, Electrochim. Acta 50 (2005) 1895.
[43] V. Brabec, Electrochim. Acta 45 (2000) 2929.
[44] V. Adam, J. Zehnalek, J. Petrlova, D. Potesil, B. Sures, L. Trnkova, F. Jelen, J. Vitecek, R. Kizek, Sensors 5 (2005) 70.
[45] P.A.E. Piunno, U.J. Krull, Anal. Bioanal. Chem. 381 (2005) 1004.
[46] F. Lucarelli, G. Marrazza, A.P.F. Turner, M. Mascini, Biosens. Bioelectron. 19 (2004) 515.
[47] K.H.R. Baronian, Biosens. Bioelectron. 19 (2004) 953.
[48] S. Rodriguez-Mozaz, M.P. Marco, M.J.L. de Alda, D. Barcelo, Pure Appl. Chem. 76 (2004) 723.
[49] F. Yan, O.A. Sadik, Anal. Chem. 73 (2001) 5272.
[50] F. Yan, O.A. Sadik, J. Am. Chem. Soc. 123 (2001) 11335.
[51] R. Kizek, J. Vacek, L. Trnkova, F. Jelen, Bioelectrochemistry 63 (2004) 19.
[52] R. Kizek, J. Vacek, L. Trnkova, B. Klejdus, L. Havel, Chem. Listy 98 (2004) 166.
[53] P. Dolezel, V. Kuban, Chem. Pap. Chem. Zvesti 56 (2002) 236.
[54] M. Heyrovsky, Electroanalysis 16 (2004) 1067.
[55] M. Erk, B. Raspor, Anal. Chim. Acta 442 (2001) 165.
[56] B.L. Zhang, W.Y. Sun, W.X. Tang, J. Inorg. Biochem. 65 (1997) 295.
[57] C.-M. Wu, L.-Y. Lin, Biosens. Bioelectron. 20 (2004) 864.
[58] R. Kizek, J. Vacek, L. Trnkova, B. Klejdus, V. Kuban, Chem. Listy 97 (2003) 1003.
[59] M.S. Díaz-Cruz, J. Mendieta, R. Tauler, M. Esteban, J. Inorg. Biochem. 66 (1997) 29.
[60] M.S. Díaz-Cruz, J. Mendieta, M. Esteban, Electroanalysis 14 (2002) 50.
[61] M. Erk, D. Ivanković, B. Raspor, J. Pavičić, Talanta 57 (2002) 1211.
[62] B. Yosypchuk, I. Sestakova, L. Novotny, Talanta 59 (2003) 1253.