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Interactions of Platinum-Based Cytostatics with Metallothionein Revealed by Electrochemistry

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Platinum-based cytostatics play an important role in the chemotherapy of various tumour diseases. Therefore, it is not surprising that there is a great effort for studying of the cytostatics and their fate in an organism. In this study, we aimed our attention at determination of cisplatin, carboplatin and oxaliplatin using stationary and flow electrochemical methods. Primarily, determination of the platinum based cytostatics using differential pulse voltammetry at hanging mercury drop electrode was optimized. Under the optimal conditions (supporting electrolyte: 2 ml of 0.36 M sulphuric acid containing 0.24 ml of hydrazine (10 mM) and 0.01 ml of formaldehyde (37 % aqueous solution, *v*/*v*), pH of the supporting electrolyte: 1.8, potential of accumulation: -0.7 V, time of accumulation: 120 s), limits of detection were estimated (3 S/N) down to tens of pg per ml for the studied drugs. Further, we investigated the interactions of the characterized drugs with peptide fragments of protein metallothionein, because the overexpression of metallothionein in tumour cells belongs to the one of the generally accepted mechanisms of resistance to these cytostatics. For this purpose, flow injection analysis with electrochemical detection was utilized. As it is well evident from the obtained experimental data, interactions between peptide fragments and platinum-based complexes proceeded differently, where oxaliplatin demonstrated the highest ability to form complex.

Keywords: cisplatin; carboplatin, oxaliplatin; metallothionein; metallomics; voltammetry; flow injection analysis; anticancer therapy

1. INTRODUCTION

Malignant tumours are one of the most common causes of death in developed countries [1]. A treatment with cytostatics belongs to the one of the often used therapeutic modalities [2]. Platinum-based cytostatics play an important role in the chemotherapy of various tumour diseases [1,3-5]. Cisplatin, carboplatin and oxaliplatin are the metal-containing anticancer cytostatic drugs that have found application in clinical practice. The effect of these drugs is based on the inhibition the growth of tissues with high proliferative capacity. The effect of cytotoxic agents is not limited to cancer cells but also affects healthy tissue with a high frequency of cell division, producing the undesirable secondary effects. Platinum-based drugs have significant antitumor activity caused particularly by the crosslinking of DNA and formation of DNA adducts with subsequent triggering the apoptosis leading to cell death [6-9]. Cisplatin, carboplatin and oxaliplatin are usually used in combination with other cytostatics for therapy of non–small and small cell lung cancers, lower gastrointestinal malignancies, breast cancer, bladder cancer, gynaecologic malignancies, germ cells malignancies, head and neck cancers, brain tumours, sarcomas including osteosarcoma and hepatoblastoma and some non–Hodgkin's lymphomas [10]. Development new platinum drugs is focused on: a) reduction the toxicity of cisplatin (nausea, ototoxicity, vomiting, and particularly nephrotoxicity); b) overcoming the acquired drug resistance; c) increase the spectrum of anticancer activity [11]. In the light of the above-mentioned facts, it is highly necessary to develop sensitive techniques to analyse the platinum compounds in a wide range of biological matrices [12-16].

There are many analytical methods for the determination of platinum. Atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS) [17-19], high performance liquid chromatography (HPLC) with UV detection [20] and capillary electrophoresis technique [21-27] have been applied for the determination of platinum in a variety of matrices. The above-mentioned methods are mostly limited to determination of the relatively high concentrations of platinum in samples. In addition, they usually require a higher sample volume, however, in many cases, only small amounts of biological samples are available for analysis. This problem may be solved using a pre-concentration or matrix-separation steps, but these steps cause losses in amount of samples. Application of adsorptive stripping voltammetry allows omission of these steps without loss of precision. The voltammetric measurements are based on a potential-activated accumulation step of platinum on the surface of electrode [28]. Hanging mercury drop electrode (HMDE) provides the best working electrode for the determination of electrochemically reducible substances due to the atomically smooth surface potential wide window in the cathodic region. In addition, surface of HMDE may be easily renewed. This characteristic minimizes the most of problems connected with the electrode passivation [29]. Generally, electrochemical methods represent the suitable techniques for determination of trace amounts of elements including platinum [30-34]. Due to high sensitivity to this metal, voltammetry techniques have been developed and have found application potential in the analysis of platinum in various matrices, including biological materials. With respect the trace levels of platinum in plant

material, animal tissues and human samples like blood, urine, hair and excreta, this technique has been successfully used in their analyses [35].

The aim of this study was to determine platinum, cisplatin, carboplatin and oxaliplatin using stationary and flow electrochemical methods. Further, we used flow injection analysis with electrochemical detection for studying of complexes between metallothionein as metal binding protein and platinum based cytostatics.

2. EXPERIMENTAL PART

2.1 Chemicals

Sodium acetate, acetic acid, water and other chemicals were purchased from Sigma Aldrich (USA) in ACS purity unless noted otherwise. $PtCl_2$ was supplied by Pliva-Lachema (Brno, Czech Republic). Cisplatin (0.5 $mg.ml^{-1}$) was supplied by EBEWE Pharma (Austria), carboplatin (10 $mg.ml^{-1}$) by Teva Pharmaceuticals (Czech Republic) and oxaliplatin (5 $mg.ml^{-1}$) by Merck Génériques (France). Fragments of metallothionein (FMTs) were synthesized by Clonestar (Czech Republic). Standard solutions were prepared with ACS water and stock standard solutions of cisplatin (500 $\mu g.ml^{-1}$) were prepared with sodium chloride solution (0.75 M, pH 5.0) and stored in the dark at -20 °C. Pipetting was performed by certified pipettes (Eppendorf, Germany). The pH was measured using an inoLab Level 3 (Wissenschaftlich-Technische Werkstatten GmbH; Weilheim, Germany).

2.2 Electrochemical measurements on mercury electrode

Determination of platinum by differential pulse voltammetry were performed with a 797 VA Computrace instrument connected to 813 Compact Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes. A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was the working electrode. An Ag/AgCl/3M KCl electrode was the reference and a platinum electrode was auxiliary. 797 VA Computrace software (Metrohm) was employed for data processing. Software GPES 4.9 supplied by Metrohm was employed for smoothing and baseline correction. The analysed samples were deoxygenated prior the measurements by purging with argon for 120 s (99.999%) %) [36,37]. Supporting electrolyte used in this study was as follows: 2 ml of 0.36 M sulphuric acid containing 0.24 ml of hydrazine (10 mM) and 0.01 ml of formaldehyde (37 % aqueous solution, *v/v*) [12].

Automated electrochemical analysis on HMDE

Automated voltammetric system consisted from the measuring analyser 797 VA Computrace connected to a PC via an USB port (Fig. 1A). PC software controls the measurement, records the measuring data and treats it [36,38,39]. The analyzer (797 VA Computrace) employs a conventional three-electrode configuration with a hanging mercury drop electrode (with a drop area of 0.4 mm^2) as working electrode, an Ag/AgCl/3M KCl as reference electrode, and a platinum auxiliary electrode. A sample changer (Metrohm 813 Compact Autosampler) allows performing the analysis of 36 samples in plastic test tubes (Fig. 1B). This autosampler transfers sample from test tube via dosing needle,

peristaltic pump and capillary to the electrochemical cell. For the addition of standard solutions and reagents, automatic dispenser (Metrohm 800 Dosimat) was used, while peristaltic pump stations (Metrohm 772 Pump Unit) were employed for transferring the rinsing solution into the cell and for removing solutions from the voltammetric cell. Metrohm 731 Relay Box ensured connection and control of peristaltic pumps (Fig. 1B).

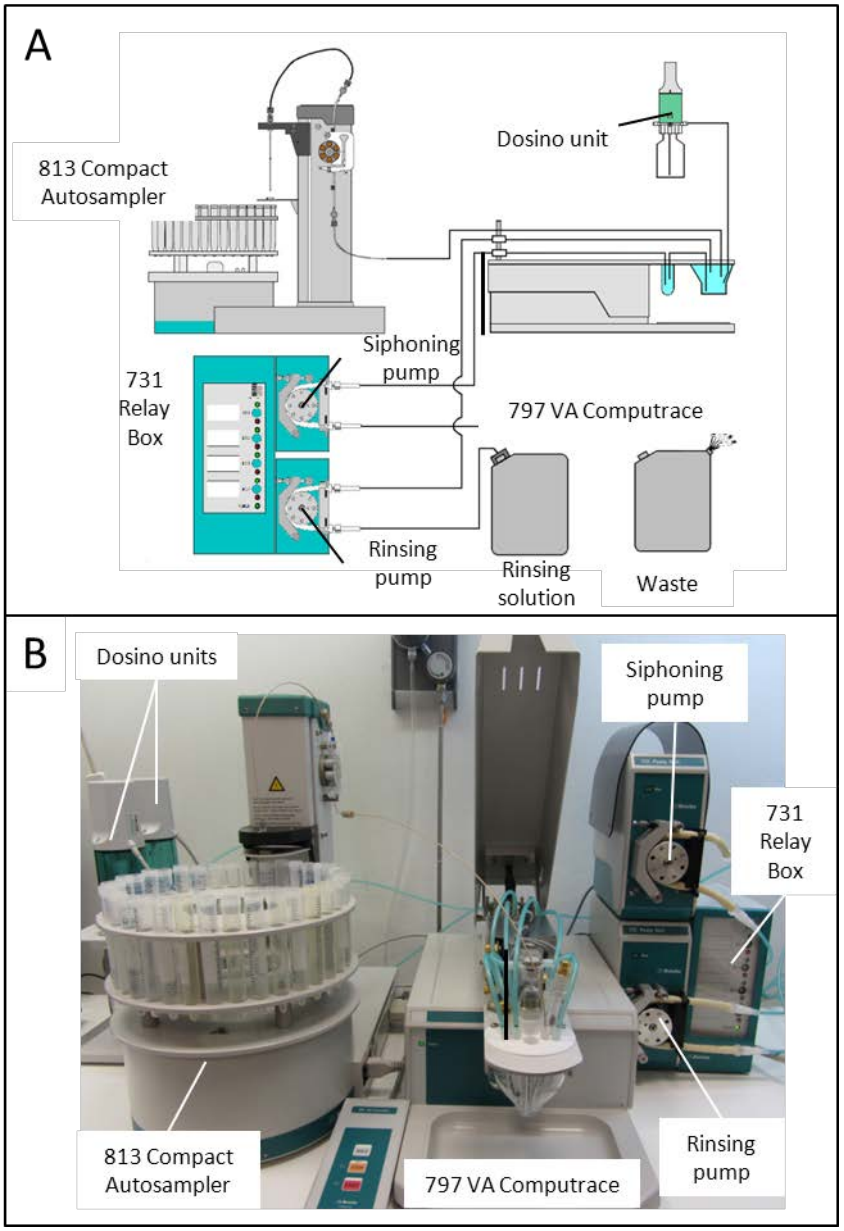

Figure 1. (**A**) Schematic view of measuring system Metrohm. (**B**) Measuring electrochemical system.

2.3 Flow injection analysis with electrochemical detection

The instrument for flow injection analysis with electrochemical detection (FIA-ED) consisted of a solvent delivery pump operating in the range of 0.001-9.999 ml.min^{-1} (Model 582 ESA Inc., Chelmsford, MA, USA), a reaction coil (1 m) and an electrochemical detector. The electrochemical detector includes one low volume flow-through analytical cell (Model 5040, ESA, USA), which consisted of a glassy carbon working electrode, a hydrogen-palladium electrode as a reference

electrode and an auxiliary electrode, and Coulochem III as a control module. Phosphate buffer (pH 7.5, 20 mM) was used as a mobile phase. Flow rate of the mobile phase was 1 ml. min^{-1}. The sample (20 µl) was injected using an autosampler (Model 542, ESA, USA). The data obtained were processed by Clarity software (Version 1.2.4, Data Apex, Czech Republic). The experiments were carried out at a room temperature. A glassy carbon electrode was polished mechanically by 0.1 µm of alumina (ESA Inc., USA) and sonicated at room temperature for 5 min using a Sonorex Digital 10 P Sonicator (Bandelin, Berlin, Germany) at 40 W [40-42].

2.4 Mathematical treatment of data and estimation of detection limits

Mathematical analysis of the data and their graphical interpretation was realized by software Matlab (version 7.11.). Results are expressed as mean ± standard deviation (S.D.) unless noted otherwise (EXCEL®). The detection limits (3 signal/noise, S/N) and the quantification limits (10 signal/noise, S/N) were calculated according to Long and Winefordner [43], whereas N was expressed as a standard deviation of noise determined in the signal domain unless stated otherwise.

3. RESULTS AND DISCUSSION

Electrochemical methods are often used for platinum determination [34,35,44-50]. Utilization of these methods for platinum-based cytostatics determination is the main objective of this study. Particularly, we aimed our attention at testing of two types of working electrodes, automation protocol and various conditions for sample preparation.

3.1 Electrochemical determination of platinum based cytostatics

Electrochemical determination of platinum-based cytostatics (cisplatin, carboplatin and oxaliplatin, Fig. 2A) was based on the knowledge of platinum determination in this study. Platinum concentration was determined by differential pulse voltammetry according to our previously published paper [12]. The forming Pt(II)-formazone-complex, which is formed in the supporting electrolyte (Fig. 2B), is accumulated onto the surface of HMDE. The measured scan was started at -0.5 V and ended at -1.2 V. Other parameters of the method were as follows: modulation time 0.057 s, interval time 0.1 s, step potential 1.95 mV, modulation amplitude 49.5 mV. Under these conditions, we focused on the optimizing of time and potential of accumulation, and pH of the supporting electrolyte. For optimizing steps, $PtCl_2$ was used. The first optimized parameter was potential of accumulation. This parameter was optimized within the range from -1.2 V to 0 V. The highest peak was detected at -0.7 V (blue curve, Fig. 2C). The time of accumulation was the second optimized parameter. In this case, time interval was changed from 0 to 240 s. The obtained dependence had growing character with one brake point at 120 s (red curve, Fig. 2C). The signal growth after 120 s was recorded too, however, the time of analysis extended over the obtained signal intensity. For these reasons, we suggested 120 s as the most suitable time of accumulation. Change of electrolyte pH was the final optimized parameter, of which effect was optimized. pH of electrolyte was changed within the range from 1.4 to 1.8 (Fig. 2D). The signal maximum was achieved at pH 1.5. With growing pH value, the signal intensity decreased

linearly up to 71 % of the maximum value at pH 1.8. On the other hand, decrease the pH value to 1.4 caused decreasing of the peak height to 91 %. The optimized conditions (potential of accumulation: -0.7 V, time of accumulation: 120 s, pH of the supporting electrolyte: 1.8) were used for determination of various concentration of platinum as it is shown in Fig. 2E.

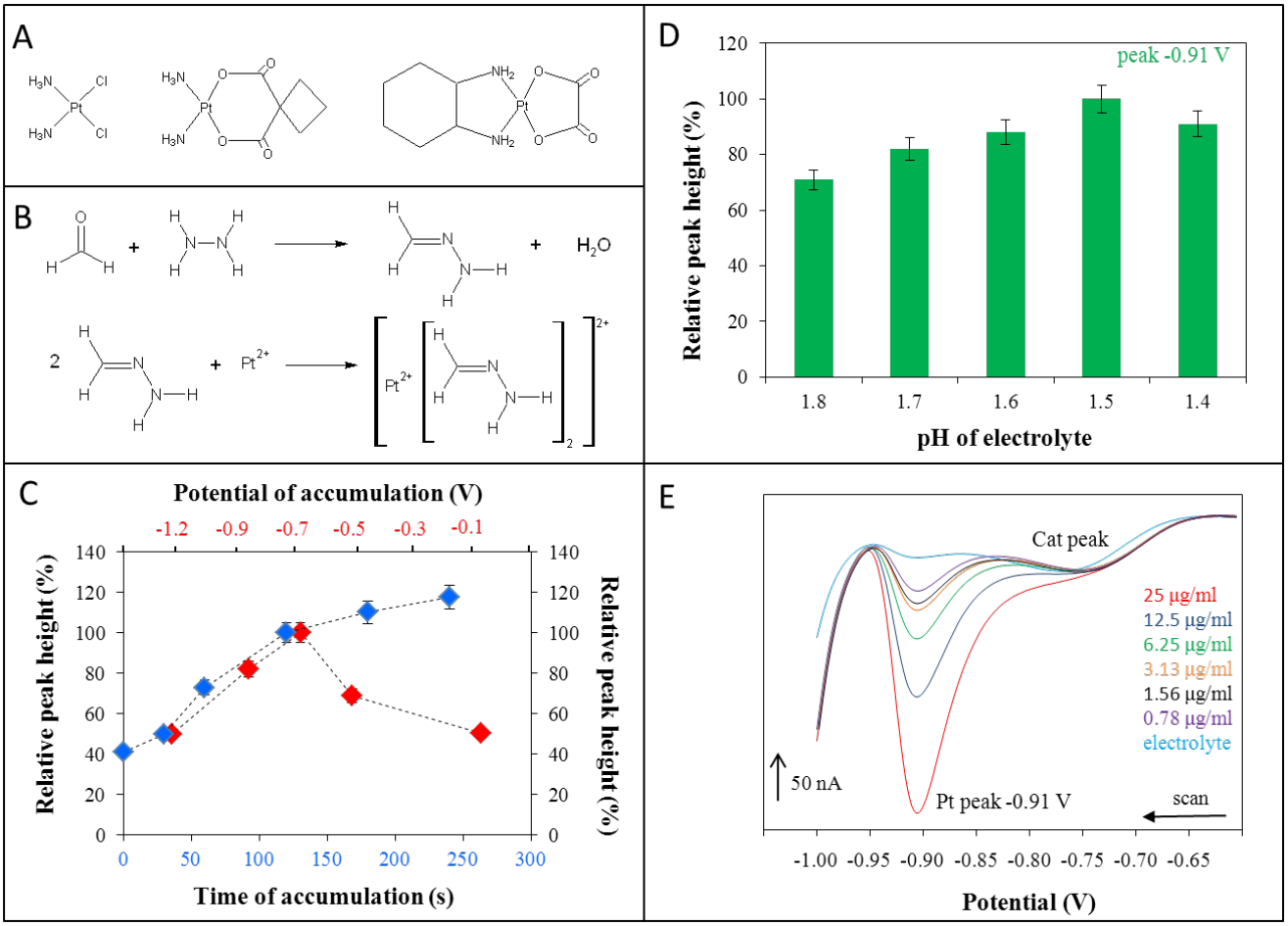

Figure 2. (**A**) Chemical structures of cisplatin, carboplatin and oxaliplatin. (**B**) Platinum complex formed in the presence of the supporting electrolyte [2 ml of 0.36 M sulphuric acid containing 0.24 ml of hydrazine (10 mM) and 0.01 ml of formaldehyde (37 % aqueous solution, *v*/*v*)]. (**C**) The effect of potential of accumulation (blue curve) and of accumulation time (red curve) on the relative platinum peak height. (**D**) The effect of the supporting electrolyte pH on the relative platinum peak height. (**E**) DP voltammograms of detected platinum at various concentrations.

3.2 Automated determination of platinum cytostatics using HMDE

Analytical instrumentation for trace and ultra-trace analysis is aimed not only at the sensitivity, but also at the possibility of high throughput measurements. The aim of this process is to establish the complex system covering the sample preparation and analysis with minimal operator actions [51,52]. Suggested electroanalytical system was used for the determination of platinum-based cytostatics (Figs. 1A and B). Under the above optimized conditions, the calibration curves for $PtCl_2$, cisplatin, oxaliplatin and carboplatin were measured and are shown in Figs. 3A, B, C and D. The obtained calibration dependence for cisplatin was linear within the range from 0.025 to 25 µg.ml^{-1} with the following equation: $y = 9.501x + 1.919$; $R^2 = 0.998$, n = 5, R.S.D = 3.2. The obtained dependence for

oxaliplatin had linear character within the range from 0.025 to 25 μg.ml^{-1} too. Parameters of this dependence were as follows: y = 10.635x + 8.662; R^2 = 0.993, n = 5, R.S.D = 1.38. Carboplatin had linear dependence at the same concentration range and the parameters were as follows: y = 5.865x + 3.885; R^2 = 0.996, n = 5, R.S.D = 1.62. The lowest detection limit was estimated for oxaliplatin. Other analytical parameters are shown in Table 1.

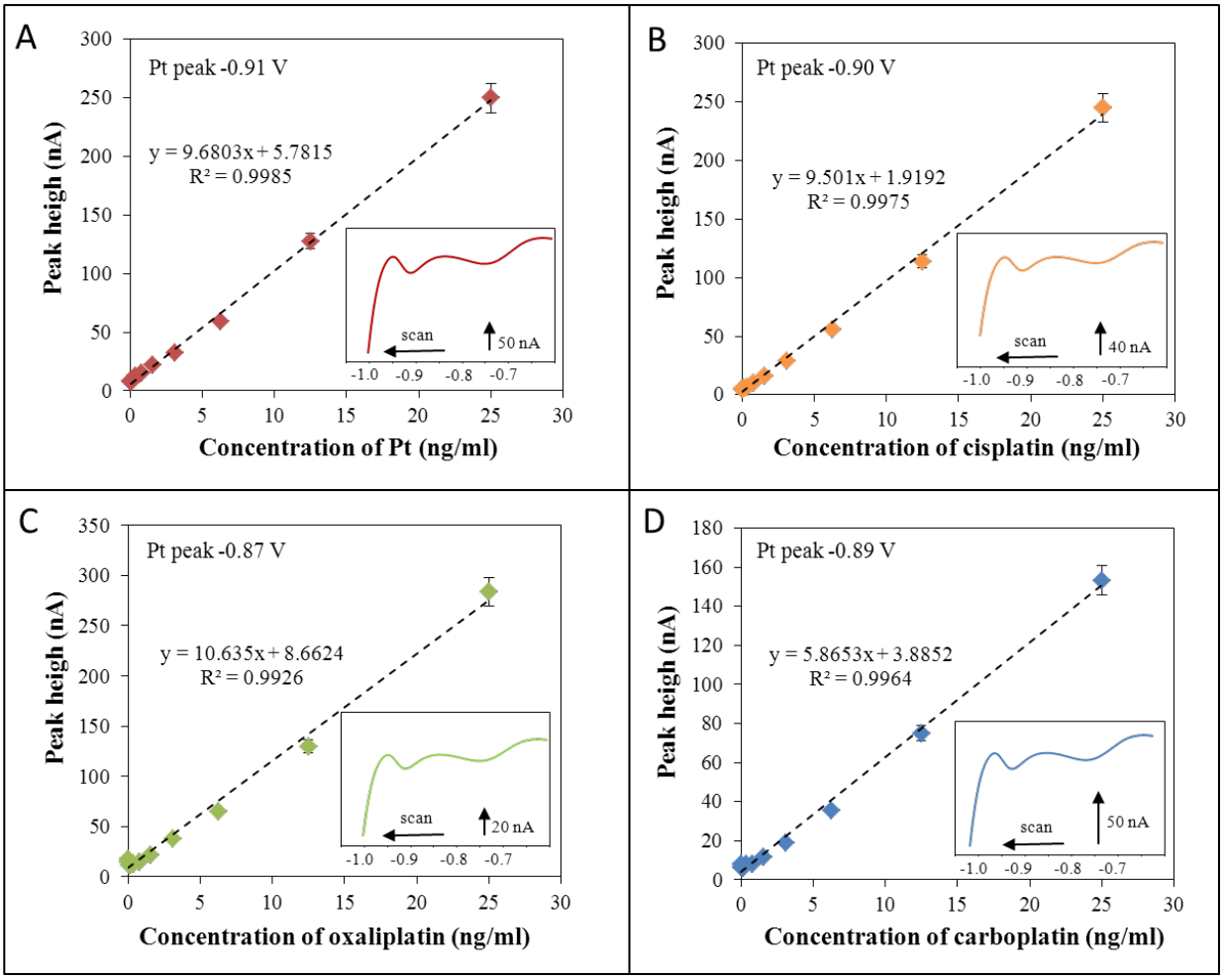

Figure 3. Calibration curves of (**A**) platinum, (**B**) cisplatin, (**C**) oxaliplatin and (**D**) carboplatin measured by automatic electrochemical analyser (813 Compact Autosampler + 797 VA Computrace, both Metrohm, Switzerland). Parameters were as follows: modulation time 0.057 s, interval time 0.1 s, step potential 1.95 mV, modulation amplitude 49.5 mV, supporting electrolyte: 2 ml of 0.36 M sulphuric acid containing 0.24 ml of hydrazine (10 mM) and 0.01 ml of formaldehyde (37 % aqueous solution, *v*/*v*), pH of the supporting electrolyte: 1.8, potential of accumulation: -0.7 V, time of accumulation: 120 s.

Table 1. Analytical parameters of electrochemical determination of platinum and platinum based cytostatics used automatic detection system.

Substance	Regression equation	Linear dynamic range (nM)	Linear dynamic range (ng/ml)	R2[1]	LOD[2] (nM)	LOD (ng/ml)	LOQ[3] (nM)	LOQ (ng/ml)	RSD[4] (%)
Pt	y = 9.704x + 35.378	1.03 – 128	0.25 – 25	0.999	0.3	0.05	0.8	0.2	2.08
Cisplatin	y = 9.615x	0.66 – 83	0.25 – 25	0.998	0.2	0.06	0.7	0.2	3.14
Oxaliplatin	y = 11.153x	0.25 – 63	0.10 – 25	0.993	0.08	0.03	0.3	0.1	1.33
Carboplatin	y = 6.099x	0.54– 67	0.25 – 25	0.995	0.1	0.05	0.4	0.2	1.20

1…regression coefficients
2…limits of detection of detector (3 S/N)
3… limits of quantification of detector (10 S/N)
4…relative standard deviations

3.3 Automated electrochemical detection of platinum cytostatics complexes with metallothionein on carbon electrode

It can be concluded that electrochemistry is sensitive enough to determine platinum based cytostatics. Considering the fact that these cytostatics are one of the most frequent drugs used for treatment of tumour diseases, it is obvious that their mechanisms of actions are subjected for numerous studies [53-56]. One of the generally accepted mechanisms of resistance to these cytostatics is the overexpression of metallothionein in tumour cells [12,57-59]. Mammalian metallothioneins (MTs) belonging to the group of intracellular and low molecular mass proteins (app. 6 kDa) are rich in cysteine and have no aromatic amino acids [60-64]. In the following part of our study, we thus utilized electrochemistry, particularly flow injection analysis with amperometric detection, for studying of the interactions between cisplatin, carboplatin and/or oxaliplatin with three synthesized peptides derived from protein metallothionein. The peptides were derived from mouse MT (one isoform) and from human MT (two isoforms, MT I and MT IIA). The evaluation of the results obtained was based on the hydrodynamic voltammogram [65]. For measurements, we used the previously optimized parameters [66]. Phosphate buffer (pH 7.5 and flow rate 1 ml·min^{-1}) was used as an electrolyte. The scheme of the instrument used and its photo are shown in Figs. 4A and B. The detail of electrochemical cell is shown in Fig. 4C.

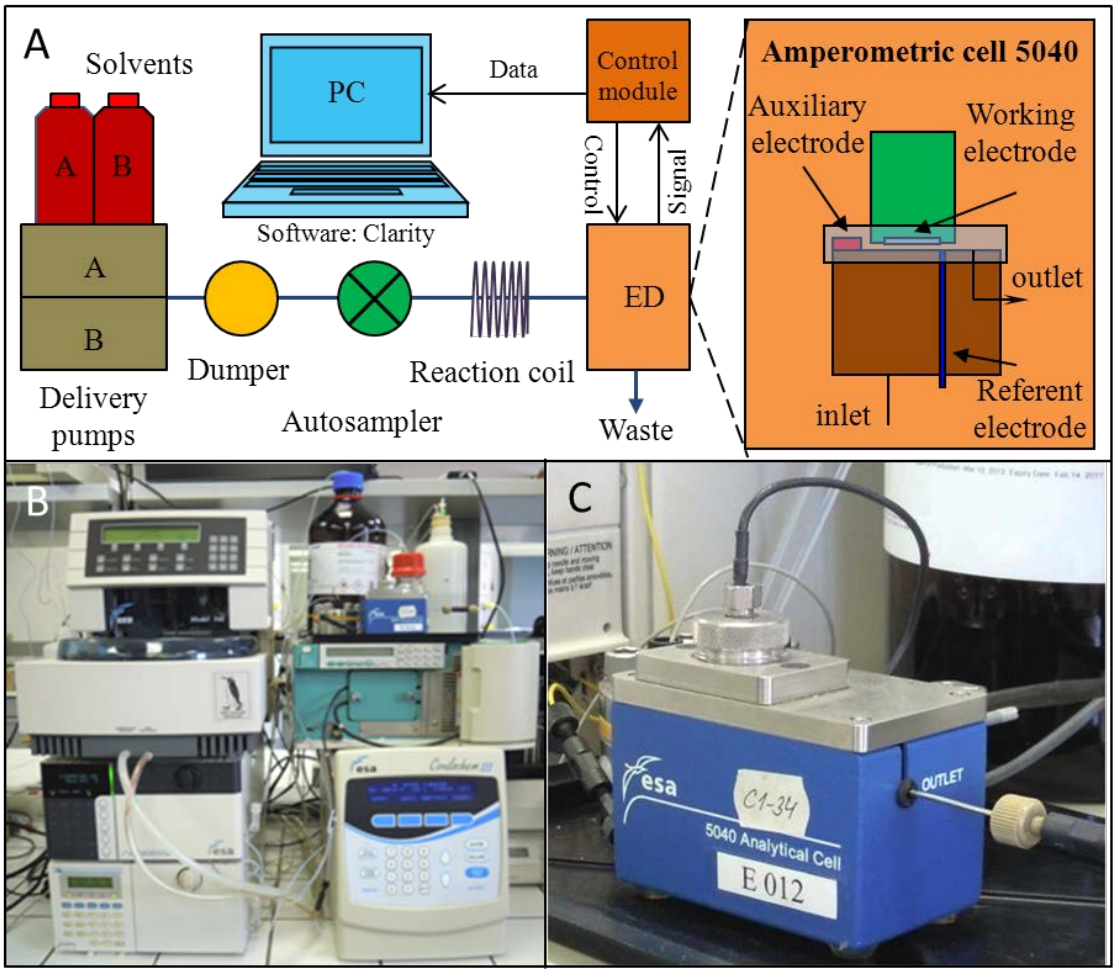

Figure 4. (**A**) Scheme of flow injection analysis system with amperometric cell. (**B**) Photo of FIA-ED instrument. (**C**) Detail of amperometric cell.

Applied working potential was the most important parameter necessary for the achievement of the high sensitivity for platinum-based cytostatics detection. The object of this experiment was to find such conditions that would make the determination of metallothioneins' fragments and platinum based cytostatics. Our procedure was based on the fact that both thiol moieties and some functional groups of platinum-based cytostatics, which may act as interference, will be oxidized on the surface of glassy carbon electrode. Therefore, we determined dependence of the signal height on the applied potential (hydrodynamic voltammograms – HDVs). Our results indicate the suitability of the applied working potential of 500 mV, especially due to the lowest interference of platinum-based derivatives (Fig. 5A). However, in the case of the HDVs courses of platinum-based derivatives demonstrating higher sensitivity compared to the decapeptides themselves, the determination based on the quantification of platinum-based derivatives would be useful. Decapeptides could be considered as interference in this case. However, there are several concerns for this presumption. The HDVs of all platinum-based derivatives should have the same course, but they lack this characteristic compared to chemically similar peptides. This fact is well evident from so-called cumulative hydrodynamic voltammograms (Fig. 5B).

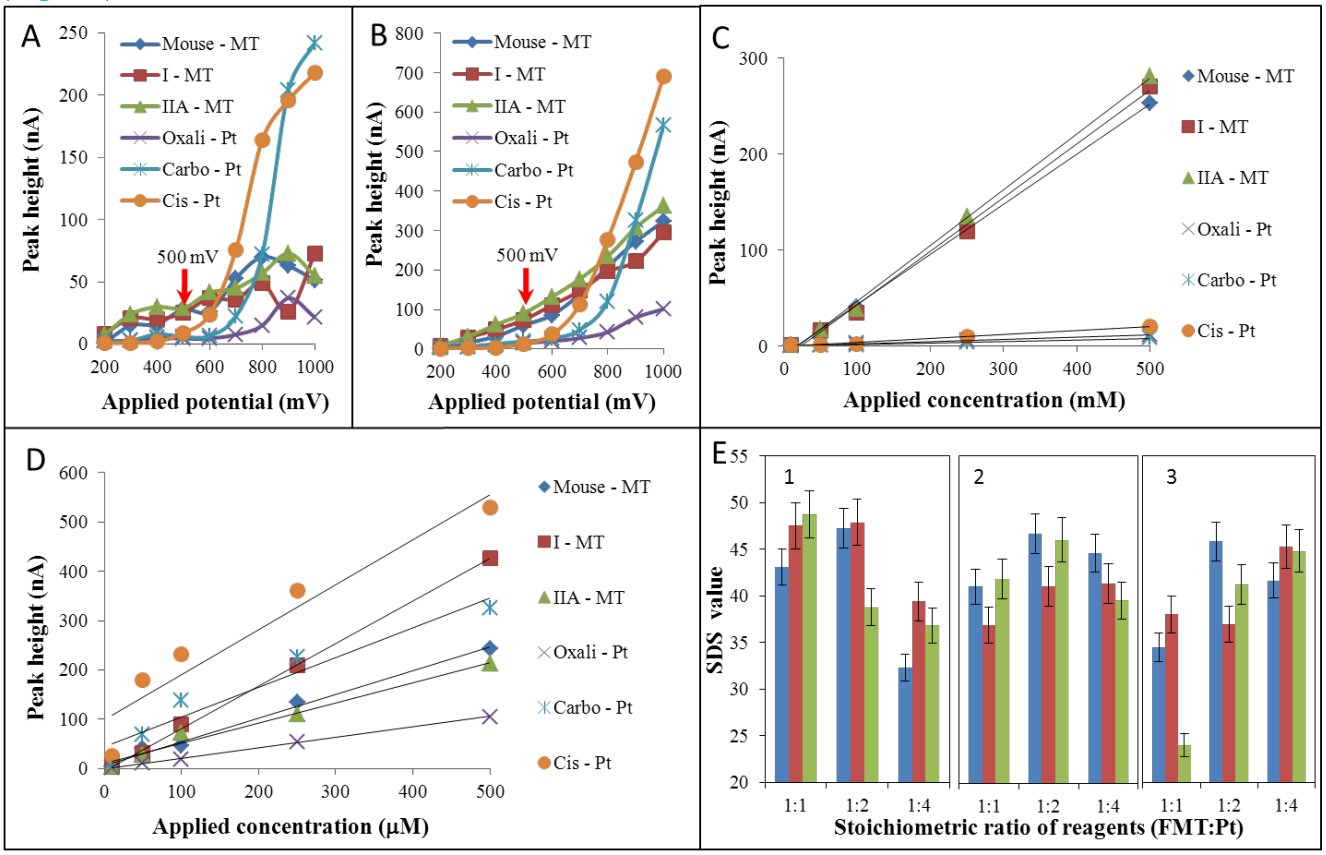

Figure 5. (**A**) The obtained hydrodynamic voltammograms (HDVs) and (**B**) cumulative HDVs for three tested peptides and oxaliplatin, carboplatin and cisplatin. Calibration curve of all studied components for complex were studied at the applied potentials of (**C**) 500 mV and (**D**) 900 mV. (**E**) Different Slope of Decreasing Signal (SDS) values obtained for each platinum-based drug 1) oxaliplatin, 2) carboplatin, and 3) cisplatin for each of peptides tested (Mouse MT-blue, Human MT I – red and Human MT IIA – green column) and three tested stoichiometric ratios (1:1, 1:2, 1:4).

For the evaluation of the rate of interference, we obtained calibration curves at the most suitable potential of 500 mV (Fig. 6C) and at potential that is commonly applied for the oxidation of analytes, which contain functional groups easily oxidized (Fig. 6D). This optimized method was applied for the time-dependent measurement of complexes of three peptides and three platinum-based derivatives, i.e. 9 combinations of two-component mixtures were prepared. In this mixture, one platinum-based derivative and one peptide fragment occurred. In addition, this set was prepared in three different variants that differ in the stoichiometric rate of platinum-based derivative to peptide fragment. Finally, nine mixtures in different stoichiometric rates (peptide fragment:platinum-based derivative) 1:1, 1:2 and 1:4 were prepared. For the variant 1:1, 50 µM concentration was used for both components. For the variant 1:2, we used 50 µM concentration of peptide fragment and 100 µM concentration of platinum-based derivative and for the variant 1:4, 50 µM concentration of fragment and 200 µM concentration of platinum-based derivative were used. Interaction took place in the environment of the phosphate buffer (66 mM, pH 7.5) and the final volume of mixture was 600 µl. All 27 samples were placed in micro test tubes in thermostat and incubated at 37 °C. For the analysis itself, aliquot volume of 100 µl was collected in the strictly defined time intervals (time of interaction: 0, 12 and 24 h) and analysed. The time of interaction was calculated from the insertion of samples into thermostat, which was performed immediately after preparation. Collected aliquots were analysed immediately by the use of the optimized FIA-ED with amperometric detection. Analyses revealed the decreasing signal of free sulfhydryl groups in all samples in the dependence on sequential binding of the platinum-based derivative.

To evaluate the rate and effectiveness of the complex formation, we processed obtained data and calculated equation of regression in the form y=Ax+B, where negative value of direction was inverted and expressed in the column graph as Slope of Decreasing Signal (SDS) as it is shown in Fig. 6E. The magnitude of the direction value (it means size of the column) expresses the rate of the complex formation of one of the nine combinations of peptides and platinum-based complexes and for different stoichiometric rates. As it is well evident from the obtained experimental data, interactions between peptide fragments and platinum-based complexes proceeded differently. In addition, trend of intensity of interaction related to the rate of reagents is interesting. Oxaliplatin demonstrated the highest ability to form complex in the presence with MT-IIA in stoichiometric rate 1:1 and in the presence of Mouse and MT-I peptides in the stoichiometric rate 1:2 (Fig. 6E). On the other hand, rate of 1:4 led to the less intensive interaction for all three monitored peptides. Intensity of interaction with carboplatin was the most similar for all peptides in all stoichiometric rates of reagents, however, rate 1:2 seemed to be the most effective (Fig. 6E). For the interaction of MT-I and MT-IIA peptide fragments with cisplatin, the rate of reagents of 1:4 was the most effective. For Mouse peptide fragment, the stoichiometric rate 1:2 was the best, nevertheless, this rate was the worst for the interaction with MT-I. Generally, the lowest intensity of interaction was recorded for MT-IIA and cisplatin in the stoichiometric rate 1:1 (Fig. 6E).

4. CONCLUSIONS

Formation of the resistance to tumour disease treatment by cytostatics is complex process, in which numerous mechanisms are involved. Metallothionein belongs to the proteins with important but not yet

fully understood role in this process. In this study, we showed that electrochemistry can be considered as a powerful tool not only for determining of cytostatics but also for studying of complex formations and their stabilities. These data could be used for suggesting of treatment strategies to overcome the resistance of tumour cells, which could enhance the effectiveness of the treatment.

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References

1. M. A. Fuertes, C. Alonso and J. M. Perez, *Chem. Rev.*, 103 (2003) 645.
2. S. van Zutphen and J. Reedijk, *Coord. Chem. Rev.*, 249 (2005) 2845.
3. T. Boulikas and M. Vougiouka, *Oncol. Rep.*, 10 (2003) 1663.
4. Z. H. Siddik, *Oncogene*, 22 (2003) 7265.
5. D. Wang and S. J. Lippard, *Nat. Rev. Drug Discov.*, 4 (2005) 307.
6. A. Gelasco and S. J. Lippard, *Top. Biol. Inorg. Chem.*, 1 (1999) 1.
7. V. M. Gonzalez, M. A. Fuertes, C. Alonso and J. M. Perez, *Mol. Pharmacol.*, 59 (2001) 657.
8. S. Ahmad, *Chem. Biodivers.*, 7 (2010) 543.
9. R. J. Knox, F. Friedlos, D. A. Lydall and J. J. Roberts, *Cancer Res.*, 46 (1986) 1972.
10. V. T. DeVita, S. Hellman and S. A. Rosenberg, Cancer: Principles and Practice of Oncology, Lippincot Williams & Wilkins, Philadelphia, 2011.
11. A. S. Abu-Surrah and M. Kettunen, *Curr. Med. Chem.*, 13 (2006) 1337.
12. D. Dospivova, K. Smerkova, M. Ryvolova, D. Hynek, V. Adam, P. Kopel, M. Stiborova, T. Eckschlager, J. Hubalek and R. Kizek, *Int. J. Electrochem. Sci.*, 7 (2012) 3072.
13. P. Sobrova, J. Zehnalek, V. Adam, M. Beklova and R. Kizek, *Cent. Eur. J. Chem*, 10 (2012) 1369.
14. F. Alt, J. Messerschmidt and G. Weber, *Anal. Chim. Acta*, 359 (1998) 65.
15. A. M. O. Brett, S. H. P. Serrano and M. A. La-Scalea, Applications of an electrochemical DNA-biosensor to environmental problems, Springer, Dordrecht, 1997.
16. M. E. Farago, P. Kavanagh, R. Blanks, J. Kelly, G. Kazantzis, I. Thornton, P. R. Simpson, J. M. Cook, S. Parry and G. M. Hall, *Fresenius J. Anal. Chem.*, 354 (1996) 660.
17. M. Breda, M. Maffini, A. Mangia, C. Mucchino and M. Musci, *J. Pharm. Biomed. Anal.*, 48 (2008) 435.
18. Z. F. Fan, Z. C. Jiang, F. Yang and B. Hu, *Anal. Chim. Acta*, 510 (2004) 45.
19. M. Krachler, A. Alimonti, F. Petrucci, K. J. Irgolic, F. Forastiere and S. Caroli, *Anal. Chim. Acta*, 363 (1998) 1.
20. S. N. Lanjwani, R. K. Zhu, M. Y. Khuhawar and Z. F. Ding, *J. Pharm. Biomed. Anal.*, 40 (2006) 833.
21. L. S. Foteeva and A. R. Timerbaev, *J. Anal. Chem.*, 64 (2009) 1206.
22. A. R. Timerbaev, *Talanta*, 52 (2000) 573.
23. A. R. Timerbaev, *Electrophoresis*, 23 (2002) 3884.
24. A. R. Timerbaev, *Electrophoresis*, 25 (2004) 4008.
25. A. R. Timerbaev, *Electrophoresis*, 31 (2010) 192.
26. A. R. Timerbaev and B. K. Keppler, *Anal. Biochem.*, 369 (2007) 1.
27. A. R. Timerbaev, K. Pawlak, C. Gabbiani and L. Messori, *TRAC-Trends Anal. Chem.*, 30 (2011) 1120.
28. M. Kolodziej, I. Baranowska and A. Matyja, *Electroanalysis*, 19 (2007) 1585.
29. V. Vyskocil and J. Barek, *Crit. Rev. Anal. Chem.*, 39 (2009) 173.

30. K. Z. Brainina, N. Y. Stozhko, G. M. Belysheva, O. V. Inzhevatova, L. I. Kolyadina, C. Cremisini and M. Galletti, *Anal. Chim. Acta*, 514 (2004) 227.
31. S. Huszal, J. Kowalska, M. Krzeminska and J. Golimowski, *Electroanalysis*, 17 (2005) 299.
32. C. Locatelli, *Electroanalysis*, 17 (2005) 140.
33. J. Vacek, J. Petrek, R. Kizek, L. Havel, B. Klejdus, L. Trnkova and F. Jelen, *Bioelectrochemistry*, 63 (2004) 347.
34. S. Zimmermann, C. M. Menzel, Z. Berner, J. D. Eckhardt, D. Stuben, F. Alt, J. Messerschmidt, H. Taraschewski and B. Sures, *Anal. Chim. Acta*, 439 (2001) 203.
35. N. Haus, T. Eybe, S. Zimmermann and B. Sures, *Anal. Chim. Acta*, 635 (2009) 53.
36. D. Hynek, J. Prasek, J. Pikula, V. Adam, P. Hajkova, L. Krejcova, L. Trnkova, J. Sochor, M. Pohanka, J. Hubalek, M. Beklova, R. Vrba and R. Kizek, *Int. J. Electrochem. Sci.*, 6 (2011) 5980.
37. J. Sochor, O. Zitka, D. Hynek, E. Jilkova, L. Krejcova, L. Trnkova, V. Adam, J. Hubalek, J. Kynicky, R. Vrba and R. Kizek, *Sensors*, 11 (2011) 10638.
38. D. Hynek, L. Krejcova, S. Krizkova, B. Ruttkay-Nedecky, J. Pikula, V. Adam, P. Hajkova, L. Trnkova, J. Sochor, M. Pohanka, J. Hubalek, M. Beklova, R. Vrba and R. Kizek, *Int. J. Electrochem. Sci.*, 7 (2012) 943.
39. P. Majzlik, A. Strasky, V. Adam, M. Nemec, L. Trnkova, J. Zehnalek, J. Hubalek, I. Provaznik and R. Kizek, *Int. J. Electrochem. Sci.*, 6 (2011) 2171.
40. P. Babula, D. Huska, P. Hanustiak, J. Baloun, S. Krizkova, V. Adam, J. Hubalek, L. Havel, M. Zemlicka, A. Horna, M. Beklova and R. Kizek, *Sensors*, 6 (2006) 1466.
41. S. Krizkova, O. Krystofova, L. Trnkova, J. Hubalek, V. Adam, M. Beklova, A. Horna, L. Havel and R. Kizek, *Sensors*, 9 (2009) 6934.
42. R. Mikelova, J. Baloun, J. Petrlova, V. Adam, L. Havel, H. Petrek, A. Horna and R. Kizek, *Bioelectrochemistry*, 70 (2007) 508.
43. G. L. Long and J. D. Winefordner, *Anal. Chem.*, 55 (1983) A712.
44. S. Zimmermann, J. Messerschmidt, A. von Bohlen and B. Sures, *Anal. Chim. Acta*, 498 (2003) 93.
45. V. Brabec, O. Vrana and V. Kleinwachter, *Collect. Czech. Chem. Commun.*, 48 (1983) 2903.
46. S. D. Kim, O. Vrana, V. Kleinwachter, K. Niki and V. Brabec, *Anal. Lett.*, 23 (1990) 1505.
47. O. Vrana and V. Brabec, *Anal. Biochem.*, 142 (1984) 16.
48. O. Vrana and V. Brabec, *Biosens. Bioelectron.*, 19 (1988) 145.
49. O. Vrana, V. Brabec and V. Kleinwachter, *Anti-Cancer Drug Design*, 1 (1986) 95.
50. O. Vrana, V. Kleinwachter and V. Brabec, *Talanta*, 30 (1983) 288.
51. M. J. Wheeler, *Ann. Clin. Biochem.*, 44 (2007) 209.
52. E. Schleicher, *Anal. Bioanal. Chem.*, 384 (2006) 124.
53. L. Galluzzi, L. Senovilla, I. Vitale, J. Michels, I. Martins, O. Kepp, M. Castedo and G. Kroemer, *Oncogene*, 31 (2012) 1869.
54. B. Koberle, M. T. Tomicic, S. Usanova and B. Kaina, *Biochim. Biophys. Acta-Rev. Cancer*, 1806 (2010) 172.
55. E. Jerremalm, I. Wallin and H. Ehrsson, *J. Pharm. Sci.*, 98 (2009) 3879.
56. A. Stein and D. Arnold, *Expert Opin. Pharmacother.*, 13 (2012) 125.
57. T. Eckschlager, V. Adam, J. Hrabeta, K. Figova and R. Kizek, *Curr. Protein Pept. Sci.*, 10 (2009) 360.
58. L. Krejcova, I. Fabrik, D. Hynek, S. Krizkova, J. Gumulec, M. Ryvolova, V. Adam, P. Babula, L. Trnkova, M. Stiborova, J. Hubalek, M. Masarik, H. Binkova, T. Eckschlager and R. Kizek, *Int. J. Electrochem. Sci.*, 7 (2012) 1767.
59. J. Sochor, D. Hynek, L. Krejcova, I. Fabrik, S. Krizkova, J. Gumulec, V. Adam, P. Babula, L. Trnkova, M. Stiborova, J. Hubalek, M. Masarik, H. Binkova, T. Eckschlager and R. Kizek, *Int. J. Electrochem. Sci.*, 7 (2012) 2136.
60. V. Adam, I. Fabrik, T. Eckschlager, M. Stiborova, L. Trnkova and R. Kizek, *TRAC-Trends Anal. Chem.*, 29 (2010) 409.

61. P. Babula, M. Masarik, V. Adam, T. Eckschlager, M. Stiborova, L. Trnkova, H. Skutkova, I. Provaznik, J. Hubalek and R. Kizek, *Metallomics*, 4 (2012) 739.
62. M. Capdevila, R. Bofill, O. Palacios and S. Atrian, *Coord. Chem. Rev.*, 256 (2012) 46.
63. S. Krizkova, I. Fabrik, V. Adam, P. Hrabeta, T. Eckschlager and R. Kizek, *Bratisl. Med. J.*, 110 (2009) 93.
64. M. Vasak and G. Meloni, *J. Biol. Inorg. Chem.*, 16 (2011) 1067.
65. O. Zitka, H. Skutkova, V. Adam, L. Trnkova, P. Babula, J. Hubalek, I. Provaznik and R. Kizek, *Electroanalysis*, 23 (2011) 1556.
66. O. Zitka, M. Kominkova, S. Skalickova, H. Skutkova, I. Provaznik, T. Eckschlager, M. Stiborova, V. Adam, L. Trnkova and R. Kizek, *Int. J. Electrochem. Sci.*, in press (2012).