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Catalytic Electrochemical Analysis of Platinum in Pt-DNA Adducts

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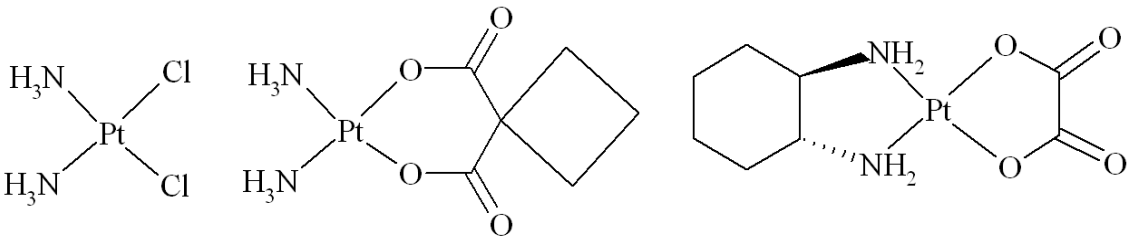
Using of platinum based cytostatic drugs in tumour diseases treatment results in the need to detect these drugs in biological samples and to determine and study the Pt-DNA adducts, which is highly important for investigation of cisplatin-resistance developed during anti-tumour treatment. The aim of this study was not only electrochemical detection of cisplatin with the use of formazone method but also preparation of platinum-DNA complex and platinum content detection in DNA. Primarily, we optimized manual electrochemical detection using differential pulse voltammetry (DPV) for $PtCl_2$ and cisplatin. The detection detection limits (3 S/N) were estimated as 10 pM and 30 nM for $PtCl_2$ and cisplatin, respectively. Further, we automated the optimized method and reached 50 pM detection limit for $PtCl_2$, but the method was proved to be versatile also for detection of cisplatin, which was verified by determination of cisplatin added cell culture medium. The method was also used to monitor the content of cisplatin bound into DNA and was verified by agarose gel electrophoresis. Moreover, we used UV irradiation and ultrasound to study changes in Pt-DNA adducts using above optimized DPV for detection of platinum signals and square wave voltammetry for detection of DNA signals. The suggested method could be applied for measurements of low volumes of isolated DNA due to the low detection limits and versatility.

Keywords: cisplatin; square wave voltammetry; differential pulse voltammetry; DNA; metallomics

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 [1-3], because these substances are not biodegradable and retained by the ecological system [4]. Besides "standard" toxic metals such as cadmium, lead and mercury, which have been monitored for many years, following the introduction of automobile catalytic converters the platinum group metals (platinum and rhodium) gain on increasing interest in environmental research [5-9]. Moreover platinum complexes play an important role in the chemotherapy of various tumour diseases [10-13]. The biological activity of the first platinum based cytostatic drug – cisplatin (*cis*-diamminedichloroplatinum(II)), which is still one of the most frequently used cytotoxic agent, was discovered in 1965 by Rosenberg during his studies on the effects of an electric current on bacterial growth [14]. Since then, hundreds of platinum(II) and platinum(IV) complexes have been synthesized and evaluated as anticancer agents over past 40 years [10,13,15-19]. To be specific, the "second generation" Pt drug carboplatin [*cis*-diammine(1,1-cyclobutane-dicarboxylato)platinum(II)] was developed in the 1980s as a less toxic alternative to cisplatin, providing less severe side effects [20-22]. Oxaliplatin [oxalato-1,2-diaminocyclohexane platinum(II)] was developed in the 1990s as a novel ("third generation") Pt drug to overcome cisplatin and carboplatin resistance [23-25]. Their structures are shown in Fig. 1. Besides these commonly used anticancer drugs, many research groups still develop new ones such as LA-12 [(OC-6-43)-bis-(acetato)(1-adamantylamine)amminedichloroplatinum(IV)] [26-28], analogues of clinically ineffective transplatin [29,30] and new platinum based complexes as nedaplatin, lobaplatin, heptaplatin, satraplatin and picoplatin [31]. 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 [32-34].



Cisplatin Carboplatin Oxaliplatin

Figure 1. Chemical structure of cisplatin, carboplatin and oxaliplatin, as drugs in clinical use for treatment of tumour diseases.

It was of interest of many researchers to find how cisplatin interacts with DNA but the exact mechanism is still unclear [35-37]. Current accepted opinion is that the main biochemical mechanism of cisplatin action involves the binding of the drug to DNA in the cell nucleus with subsequent interference with normal transcription and/or DNA replication [38,39]. In intracellular environment,

chlorine atoms of cisplatin undergo aquation and these are in equilibrium with their hydroxo forms. The cis-$[Pt(NH_3)_2(H_2O)_2]^{+2}$ unit binds bifunctionaly to N7 positions of adjacent guanine or adenosine bases of DNA, which results in the formation of several intrastrand and interstrand cross links, where platinum coordinates to two neighbouring nucleotides [13,19,40-42]. The formation of Pt–DNA adducts causes significant structural distortions of the double helix, which are recognized by specific proteins and eventually lead to tumour cell apoptosis. Although cisplatin can induce apoptosis selectively in cancer cells through binding to DNA, it undergoes many nonselective reactions with a variety of biomolecules [43-46], which can decrease therapeutic concentration of this drug.

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) [47-49] and high performance liquid chromatography (HPLC) with UV detection [50] have been applied for the determination of platinum in a variety of matrices. The above mentioned methods are mostly limited to determination of relatively high concentrations of platinum in samples as well as to the requirement of higher sample volume. Moreover, there are problems with interfering signals and pre-treatment of samples. Mostly, only small amounts of biological samples are accessible for analysis, which is also a limiting factor, but can be solved using capillary electrophoresis [51-57]. Voltammetry is a powerful technique for the determination of ultra-trace amounts of elements including platinum [58-62]. Due to its high sensitivity for this metal, voltammetry has been widely used for the analysis of Pt in various matrices, including biological materials. With this technique trace levels of Pt in plant material, animal tissues and human samples like blood, urine, hair and excreta were analysed [9]. Recently, voltammetry was used to monitor platinum in blood and urine and it was suggested that this is the only reliable method for the collection of data on blood levels [6,58,63-68]. The formazone-method is often used for the analysis of plasma platinum levels in patients [69,70]. The aim of this study was not only the electrochemical detection of cisplatin with the use of formazone method but also the preparation of platinum-DNA complex and platinum content detection in DNA. Moreover, we used UV irradiation and ultrasound to study changes in Pt-DNA adducts.

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. $PtCl_2$, cisplatin were supplied by Pliva-Lachema (Brno, Czech Republic). Standard solutions were prepared with ACS water unless noted otherwise as in the case of cisplatin. Stock standard solutions of cisplatin (500 μg/ml) 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 inoLab Level 3 (Wissenschaftlich-Technische Werkstatten GmbH; Weilheim, Germany).

2.2 Quantification of DNA

Lyophilised highly polymerized DNA (Reanal, Hungary) was isolated from chicken erythrocytes (Mw = 400 000 g/mol).The DNA amount was quantified using UV spectrometry by measuring the absorbance at three wavelengths: 260, 280, and 320 nm (A260, A280, A320). It was checked that the purity according to the following equations were met: A320<0.010 and 1.7<A*260/A*280 <1.9 with A*260=A260-A320 and A*280=A280-A320. This purity check guarantees the absence of proteins, which can interfere with the quantitation of DNA. DNA concentration in micrograms per millilitre was then given by the equation $c(DNA) = 50 \cdot A*260$. From the total volume of the eluate, the absolute amount of DNA (Mw = 400 000 g/mol) or nucleotide (assuming a mean molecular mass of 330 g/mol) was calculated.

2.3 Electrochemical measurement

Electrochemical manual measurements were performed with AUTOLAB PGS30 Analyzer (EcoChemie, Netherlands) connected to VA-Stand 663 (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 employed as the working electrode. An Ag/AgCl/3M KCl electrode served as the reference electrode. Pt electrode was used as the auxiliary electrode.

Electrochemical automatic measurements were performed with 797 VA Stand instrument 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 glassy carbon electrode was the auxiliary electrode.

2.3.1 Square wave voltammetry with adsorptive transfer technique for DNA detection

Adsorptive transfer technique was used for the electrochemical determination of DNA. The adsorptive transfer technique is based on the sample accumulation (120 s) onto the working electrode surface and consequently on the electrode washing and square wave voltammetric (SWV) measurement. All experiments were carried out at room temperature (25 °C). SWV measurements were carried out in the presence of acetate buffer pH 5.0. SWV parameters: potential step 5 mV, frequency 280 Hz, time of accumulation 120 s [71]. For smoothing and baseline correction the software GPES 4.9 supplied by EcoChemie was employed.

2.3.2 Differential pulse voltammetry for platinum detection

Platinum concentration was measured by differential pulse adsorptive stripping voltammetry in the presence of 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). The analysed samples were deoxygenated prior to measurements by purging with argon (99.999%), saturated with water for 120 s. After that, the Pt(II)-

formazone-complex, which was formed in the electrolyte solution (Fig. 2), was accumulated for 15 s onto the surface of HMDE at -0.7 V with stirring. The concentration of platinum was evaluated from three standard additions. The scan was performed from -0.5 V to -1.2 V. Other parameters of the method were: modulation time 0.057 s, interval time 0.1 s, step potential 1.95 mV, modulation amplitude 49.5 mV. For smoothing and baseline correction the software GPES 4.9 supplied by EcoChemie was employed.

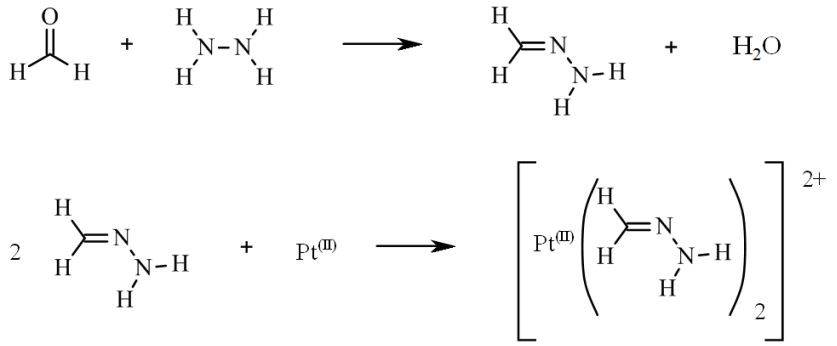


Figure 2. Platinum complex formed in the presence of supporting electrolyte [2 ml of 0.36 M sulphuric acid containing 0.24 ml of hydrazine (10 mM) and 0.01 ml of formaldehyde (37% aqueous solution)].

2.4 Interaction of platinum with DNA

dsDNA obtained from chicken erythrocytes (100 μg/ml) was modified by cisplatin (100 μg/ml) in the presence of 0.2 M phosphate buffer (pH 7.5). Test tube (1.5 ml, Eppendorf) containing this solution was thermostated at 37 °C in the dark in a thermostatic box (Model TER – 5/1, Chirana, Czech Republic) for 24 hours. After that, dialysis of this solution to separate free cisplatin was carried out. DNA was dialysed against 10 mM Tris-HCl of pH 7.5 using Membrane filters Filter type 0.025 μm (VSWP, Ireland) under mixing at 200 rpm at 6 °C for 24 h. Concentration of dialysed DNA was determined using protocol mentioned in the Section 2.2.

2.5 DNA damage induced by UV light and ultrasound

Pt-DNA adducts (1 μg/ml, as concentration of DNA) were treated with UV light and/or ultrasound. UV light of 312 nm was applied on DNA sample for 10, 20 and/or 30 min. (Vilber Lourmat, France). Ultrasound of 450 Hz was applied on DNA sample for 2, 5 and 7 min. (GM Mini20, Bandelin Sonoplus, Germany). After that, the samples were electrochemically analysed.

2.6 Gel electrophoresis

Agarose gel (0.5%) was prepared by boiling of 1× TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM ethylenediaminetetraacetic acid) for 2 min.in microwave. Then the gel was cooled to 60 °C

and ethidium bromide was added (5 µl per 100 ml of the gel). The gel was transferred into the electrophoretic bath containing TAE buffer. Samples prepared with 5% (*v*/*v*) bromophenol blue and 3% (*v*/*v*) glycerol were loaded into a gel in 5 µl aliquots. The electrophoresis (Biorad, USA) was running at 100 V and 6 °C for 60 min. The bands were visualized using a gel projection system at 312 nm (Vilber-Lourmant, France).

2.7 Microwave digestion

To prepare the samples for quantification of platinum, microwave digestion was used according to recently published papers [72-74]. Briefly, the mineralization of samples took place in a microwave system Multiwave 3000 (Anton-Paar GmbH, Austria). The sample was placed into glass vials MG5 and 700 µl of nitric acid (65%, *w*/*w*) and 300 µl of hydrogen peroxide (30%, *w*/*w*) were added. Prepared samples were sealed and placed into the rotor 64MG5 (Anton-Paar GmbH, Austria). The rotor with the samples was inserted into the microwave system and the microwave digestion was carried out at 100 W and time of mineralization was 15 minutes Maximum temperature was 80 °C. A blank digestion was simultaneously carried out in the same way.

2.8 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 [75], whereas N was expressed as standard deviation of noise determined in the signal domain unless stated otherwise.

3. RESULTS AND DISCUSSION

Electrochemical methods are suitable for detection of metal ions, which has been shown in numerous papers on this topic [60,73,74,76-82]. These methods are sensitive also for platinum group elements, which are still hard to be quantified using spectroscopic techniques [6,9,58]. Some studies on DNA interactions with platinum were carried out using electrochemistry coupled with magnetic separation [83-85]. Based on these facts it is not surprising that electrochemical methods are very useful tools for studying DNA-drug interactions [71,85-88].

3.1 Electrochemical determination of platinum at HMDE

Catalytic hydrogen evolution is likely one of the most sensitive electrochemical methods used for detection of biologically important compounds [89-95]. In this study, we focused on electrochemical detection of platinum ions according to previously published papers using catalytic

signals for this purpose [6,58]. Typical voltammograms of $PtCl_2$ (from 0.391 to 50 ng/ml) measured at HMDE are shown in inset in Fig. 3A. The complex of platinum with the supporting electrolyte provides a well developed and symmetrical catalytic signal. Catalytic signal appeared at -0.9 ± 0.05 V (n = 10). Based on the previously published data, the time of preconcentration of platinum(II) on HMDE within the interval from 60 to 90 s was selected [6,58]. Longer preconcentration led to a decrease in the sensitivity of the method and catalytic reaction of platinum complex did not provide well-developed peaks. It can be assumed that decomposition of platinum complex with hydrazine takes place (Fig. 2) to form molecules that are unable to catalyse hydrogen evolution at HMDE. The potential of the catalytic signal did not change with varying concentrations of platinum(II) (± 5%, n = 3). Moreover, a new signal appeared at -1 V, which is probably related to other type of complex of platinum and the supporting electrolyte. The height of the second signal is 10 times smaller in comparison with the signal measured at -0.9 V and has probably the redox character as we observed in the previously published study [96].

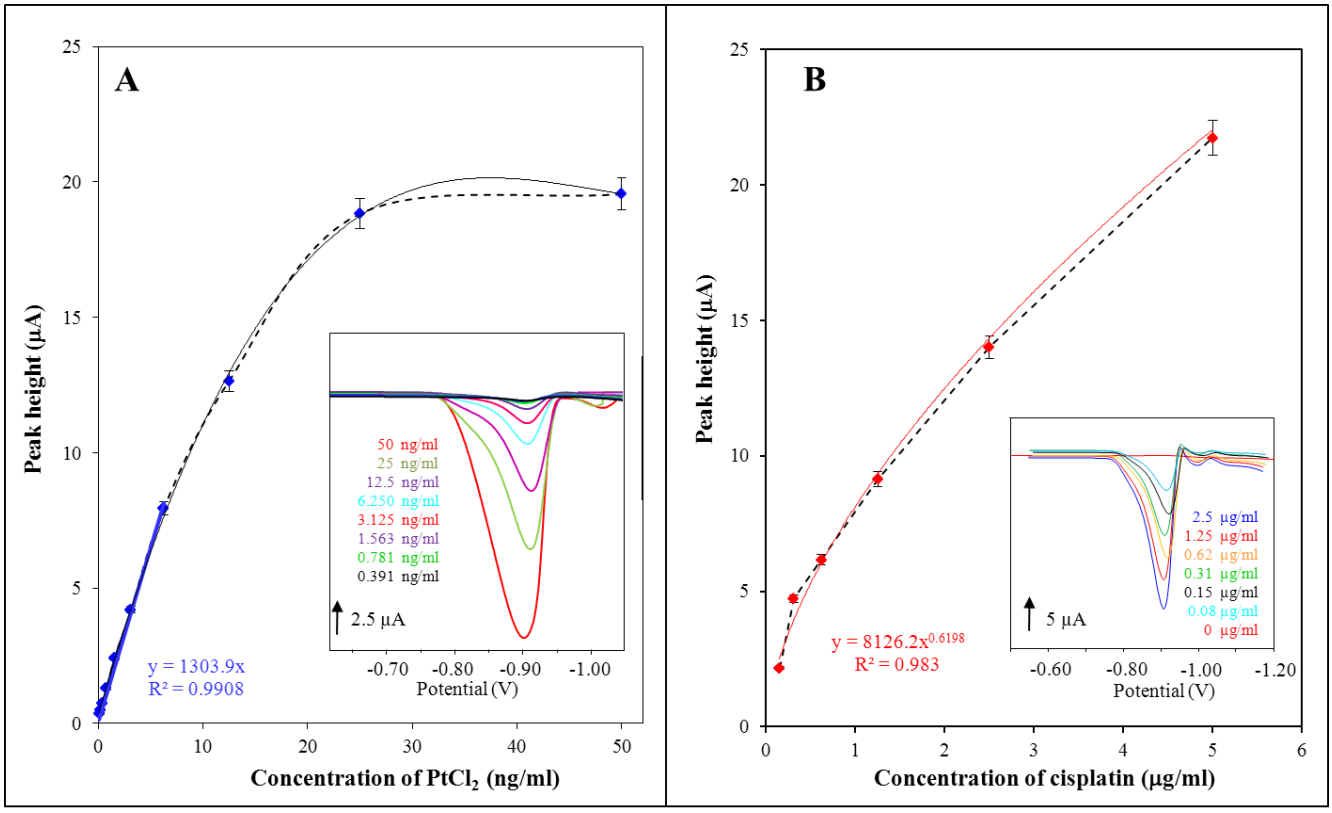


Figure 3. Dependencies of peak height on concentration of (**A**) $PtCl_2$ within concentration range from 0 to 50 ng/ml; in inset: typical DP voltammograms, and of (**B**) cisplatin within concentration range from 0 to 5 µg/ml; in inset: typical DP voltammograms. DPV parameters were as follows: start potential -0.5 V, end potential -1.2 V, modulation time 0.057 s, interval time 0.1 s, step potential 1.95 mV, modulation amplitude 49.5 mV.

It clearly follows from the obtained data that signal of platinum complex increased with the increasing concentration of platinum to 25 ng/ml and the measured dependence was linear ($y = 1303.9x$, $R^2 = 0.9908$, Fig. 3A). At higher concentration of platinum, the height of this signal did not

change. In the case of using a mathematical model, the dependence of platinum complex signal height on concentration of $PtCl_2$ was as follows: $y = 0.2078x^3 - 29.73x^2 + 1352.9x + 269.56$, $R^2 = 0.9996$ (Fig. 3A). The limit of detection (3 S/N) was estimated as 10 pM. The other analytical parameters are shown in Table 1.

In the case of detection of cisplatin, the electrochemical behaviour of the catalytic signal was similar to that of $PtCl_2$. Peak symmetry was very slightly changed (in inset in Fig. 3B). The maximum of the signal shifted from -0.8 to -0.95 V depending on the concentration of cisplatin. It can be assumed that the observed changes are related to the complex formation causing their own catalytic reaction. Formation of cisplatin aqua-complexes plays the important role in the detection of cisplatin. The obtained DP voltammograms document the complexity of these processes (inset in Fig. 3B). Dependence of the signal height on the concentration of cisplatin is shown in Fig. 3B, in which the dependence was linear up to 3 μg/ml. Further increasing concentrations of cisplatin lead to the non-linear increase in the signal. The exponential dependence with the following equation $y = 8126.2x^{0.6198}$, $R^2 = 0.9830$ describes the obtained curve. The limit of detection (3 S/N) was estimated as 30 nM. The results obtained, however, clearly showed a dramatic decrease in the sensitivity of determination of cisplatin compared to the determination of $PtCl_2$. On the other hand, both platinum compounds gave similar signals, which allow us to use both for calibration. The other analytical parameters are shown in Table 2.

Table 1. Analytical parameters of electrochemical determination of $PtCl_2$.

Substance	Regression equation	Linear dynamic range	Linear dynamic range	R2[1]	LOD[2]	LOD	LOQ[3]	LOQ	RSD[4]
		(nM)	(ng/ml)		(nM)	(ng/ml)	(nM)	(ng/ml)	(%)
Pt	y = 1212.1 + 319.01	0.50 – 32.04	0.098 – 6.25	0.998	0.08	0.02	0.25	0.06	2.31

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

Table 2. Analytical parameters of electrochemical determination of cisplatin.

Substance	Regression equation	Linear dynamic range	Linear dynamic range	R2[1]	LOD[2]	LOD	LOQ[3]	LOQ	RSD[4]
		(μM)	(μg/ml)		(μM)	(ug/ml)	(μM)	(μg/ml)	(%)
Pt	y = 4232.8 + 3651.9	1.03 – 8.33	0.31 – 2.5	0.994	0.1	0.03	0.4	0.1	0.898

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

3.2 Automatized electrochemical determination of cisplatin

The above mentioned method was optimized to be applied on fully automated determination of $PtCl_2$ and cisplatin. For this purpose, electrochemical analyser 797 VA Computrace employing a

conventional three-electrode configuration with a HMDE working electrode, Ag/AgCl/3M KCl as reference electrode, and a platinum auxiliary electrode is used. A sample changer 813 Compact Autosampler performs the sequential analysis of up to 18 samples in plastic test tubes (volume 1 ml). For the addition of standard solutions and reagents, two automatic dispensers (765 Dosimat) are used, while two peristaltic pumps (772 Pump Unit, controlled by 731 Relay Box) are employed for transferring the rinsing solution in the cell and for removing solutions from the voltammetric cell. The sample was mixed with the supporting electrolyte to the final volume of 1 ml. This volume was transferred into the electrochemical cell using a peristaltic pump and the supporting electrolyte was added to 2 ml using automatic dispensers. In DP voltammograms, well developed signals at -0.85 ± 0.02 V (n = 10) were obtained (inset in Fig. 4). We observed signal shift to more positive potentials with the increasing concentration of platinum(II). The calibration dependence obtained is shown in Fig. 4. The dependence was linear within the range from 0.025 to 25 ng/ml of $PtCl_2$. The limit of detection (3 S/N) was estimated as 50 pM, which is well comparable with the manual measurements. The other analytical parameters are shown in Table 3.

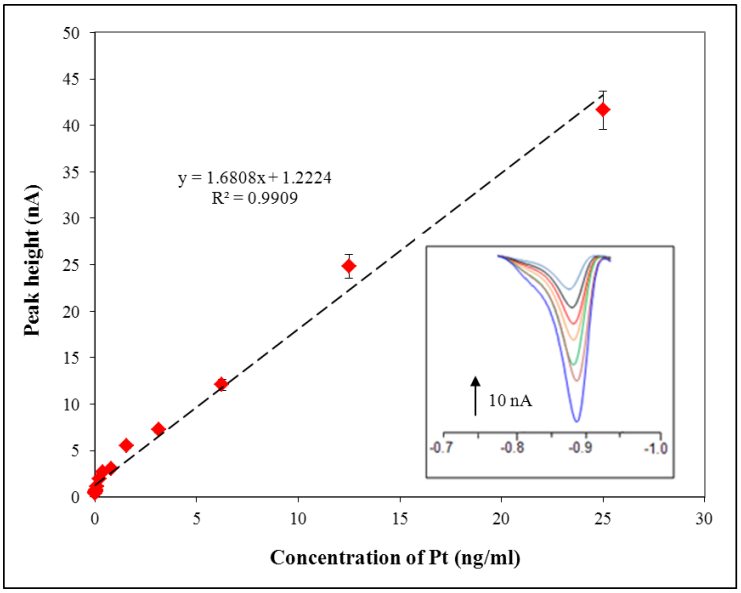


Figure 4. Typical dependence of catalytic signal height of $PtCl_2$ on its concentration. In the inset: DP voltammograms of various concentration of PtCl2. Samples were analysed using automatic determination system Metrohm 797 VA Computrace with 813 Compact Autosampler. DPV parameters were as follows: start potential -0.5 V, end potential -1.2 V, modulation time 0.057 s, interval time 0.1 s, step potential 1.95 mV, modulation amplitude 49.5 mV.

The method was further verified by determination of cisplatin in cell culture medium. Cell cultivation medium is a mixture of high and low molecular mass compounds that can react with each other and above all are able to interact with the platinum(II). The effect of cultivation medium, into which cisplatin (0.625, 1.25 and/or 2.5 μg/ml) was added, on the catalytic signal of Pt(II) was investigated. After mixing, the samples were immediately analysed. We found a relatively strong interaction of cisplatin with the components of the media, which resulted in the decrease of cisplatin

signal to 23.1% (0.625 μg/ml), 32.9% (1.25 μg/ml) and 39.7% (2.5 μg/ml) related to the height of the cisplatin signal without the presence of cultivation medium (Fig. 5). In case of measurement of the same media after mineralization signals corresponded to the amount of cisplatin and were similar to those determined without cultivation medium. It can be assumed that the dramatic reduction of cisplatin free signal in cultivation medium is likely to affect biological effects of this drug on cell lines.

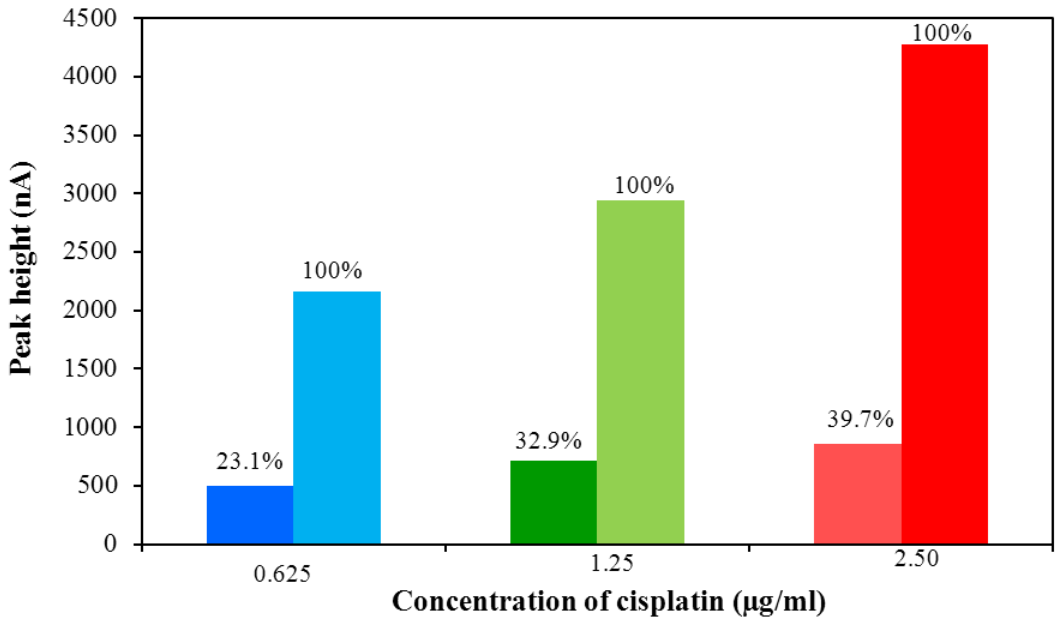


Figure 5. Changes of cisplatin catalytic signals after addition into Dulbecco's Modified Eagle's (DME) medium. Columns with lower values correspond to height of catalytic cisplatin signals measured in the presence of cell cultivation medium; columns with a higher value (100%) correspond to catalytic cisplatin signals measured in supporting electrolyte only. For other details see caption to Figure 4.

Table 3. Analytical parameters of electrochemical determination of $PtCl_2$ using automatic analyser.

Substance	Regression equation	Linear dynamic range	Linear dynamic range	R2[1]	LOD[2]	LOD	LOQ[3]	LOQ	RSD[4]
		(nM)	(ng/ml)		(nM)	(ng/ml)	(nM)	(ng/ml)	(%)
Pt	y = 1.6808 + 1.2224	0.004 – 128.15	0.0008 – 50	0.991	0.3	0.05	1	0.2	2.9

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 Detection of platinum in DNA

The method was also used to monitor the content of cisplatin bound into DNA. For this purpose, erythrocyte genomic DNA modified by cisplatin for 24 and/or 72 h was used. Unbound

cisplatin molecules were subsequently removed by dialysis (24 h, 6 °C). Catalytic signals of cisplatin bound to DNA are shown in inset in Fig. 6A. The character of the signals is changed (Fig. 3B for comparison), which can be explained by formation of the complexes similar to the behaviour of free platinum. The amount of platinum increased with the increasing concentrations of cisplatin used according to the following equation (y = 2.9481x - 0.0281, R = 0.9821, Fig. 6A). The most significant increase in the signal was observed when using low quantities of platinum (up to 200 ng/ml), then the changes were slower.

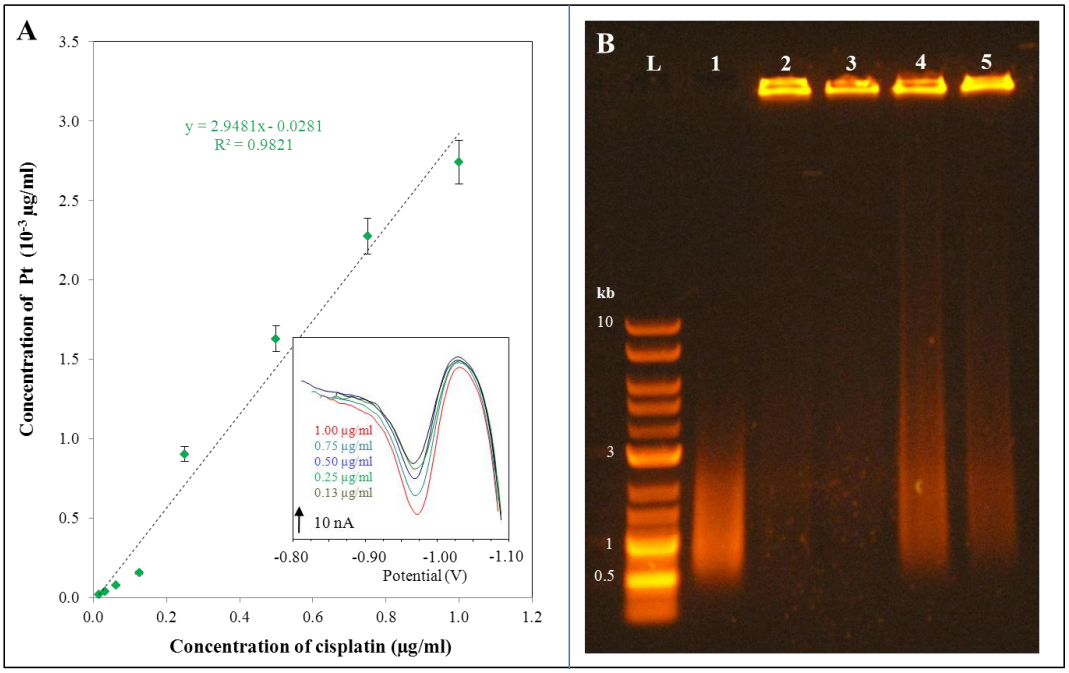


Figure 6. (**A**) Dependence of cisplatin bound into DNA after 24 h dialysis measured by differential pulse voltammetry; in inset: DP voltammograms of cisplatin bound into DNA. (**B**) Gel electrophoresis of chicken DNA in 0.5 % agarose gel, the experimental parameters: 60 min, 100 V. L Ladder, 1 DNA 100 µg/ml, 2 - 5 chicken DNA 100 µg/ml + cisplatin 100 µg/ml (2 after 24 h incubation, 3 after 72 h incubation, 4 after 24 h incubation and 24 h dialysis, 5 after 72 h incubation and 24 h dialysis). For other details see caption to Figure 4.

3.4 Detection of cisplatin bound into DNA, influence of various types of damaging

Further, we aimed our attention to analysis of DNA modified with cisplatin. Primarily, we analysed DNA modified with cisplatin using agarose gel electrophoresis (Fig. 6B). Unmodified DNA (lane 1) was hardly detectable in the gel, only some fragments of low molecular mass were found. If DNA was modified with cisplatin, this molecule was hold in gel and the intensity of the colouring was markedly enhanced (lanes 2, 3, 4 and 5). These changes are probably associated with the fact that preferential DNA cisplatination within the GG doublets resulted in inhibition of the DNA cleavage [37].

After that we characterized DNA modified with cisplatin, DNA was analysed electrochemically. The unmodified DNA yielded two signals due to electrode processes undergone by DNA bases: a cathodic peak CA at -1.4 V (corresponding to irreversible reduction of cytosine and adenine residues) and peak G at -0.2 V (corresponding to reversible oxidation of 7,8-dihydrogen guanine, which is generated at the electrode upon guanine reduction) [86,97-107]. We observed, in well agreement with Vidlakova et al. [83,84], that the shape of cisplatinated DNA voltammograms differed markedly. In the cathodic part, hydrogen evolution is getting sooner (at higher potential) compared to the unmodified DNA. This signal was detected at -1.3 V. This phenomenon subsequently influences CA peak. In addition, there can be found other signals at -1.30 V, -1.45 V and -1.75 V in anodic part according to Vidlakova et al. [83,84]. These authors found that such behaviour suggested a kinetic process coupled to reversible electron-transfer reactions [108,109], most likely catalytic hydrogen evolution accompanying the redox processes of the platinum moieties, which has been observed earlier as a common feature of platinum group metals and their compounds. In this study, peak G produced by the cisplatinated DNA (10 μg/ml) was by 22.5 to 33.8 % (n = 10) lower than the same signal produced by the unmodified DNA and was shifted by 24±8 mV to more negative potentials. It clearly follows from our and previously published results that catalytic currents measured at the HMDE responding proportionally to the DNA cisplatination level. To obtain better developed signal specific for the DNA modification, we used AdTS DPV. The main advantage of AdTS is based on electrode removing from a solution after accumulating of a target molecule on its surface, rinsing of the electrode and transferring to a pure supporting electrolyte, where no interferences are present. Genomic DNA modified with cisplatin was prepared as above and measured by the AdTS DPV under conditions showed in the Experimental section for the measurements of DNA peaks (CA, G and catalytic signal. The latter signal, occurring at -0.28 V, was the only peak observed on the voltammogram corresponding to the unmodified DNA. DNA modified with cisplatin produced, in addition to peak G, another distinct signal at -1.25 V the intensity of which was about proportional to the DNA modification level.

Platinated DNA modified with cisplatin for 24 h and dialysed for the same time was exposed to UV radiation and sonication. UV radiation leads to DNA breaks and formation of dimers. There was increase in CA peak with the increasing time of UV radiation (10 % per 5 minutes of exposure). CA peak increased by about 70 % after 30 min of UV exposure compared with the control. We assume that DNA was damaged and therefore the individual nucleotide bases were more accessible to HMDE surface. Catalytic signal of cisplatin also increased. After 10 min. long UV light exposure, the catalytic signal of platinum increased for more than 300 %, and after 30 min. long UV light exposure for more than 500 % compared to control (Fig. 7A). This effect can be associated with easier access of platinum bound into DNA to HMDE and changing of DNA structure resulting in the formation of the complex catalysing electrochemical reaction. This result therefore suggests that the amount of platinum intercalated into the structure of DNA is higher than the previous experiment revealed. Similar results were obtained using sonication (450 Hz). During this procedure there is a dramatic DNA damage in a number of fragments, breaks, or even single strands formation. The resulting mixture provides significantly higher signal DNA, mainly due to better access the individual fragments to the working electrode surface in comparison to the effect of UV radiation. The increase in CA peak was 200 % of

control. Likewise, there was detected the increase in the content of cisplatin, but the growth of catalytic platinum signal was not as high as in the case of UV radiation and reached a maximum of 300 % of control values (Fig. 7B).

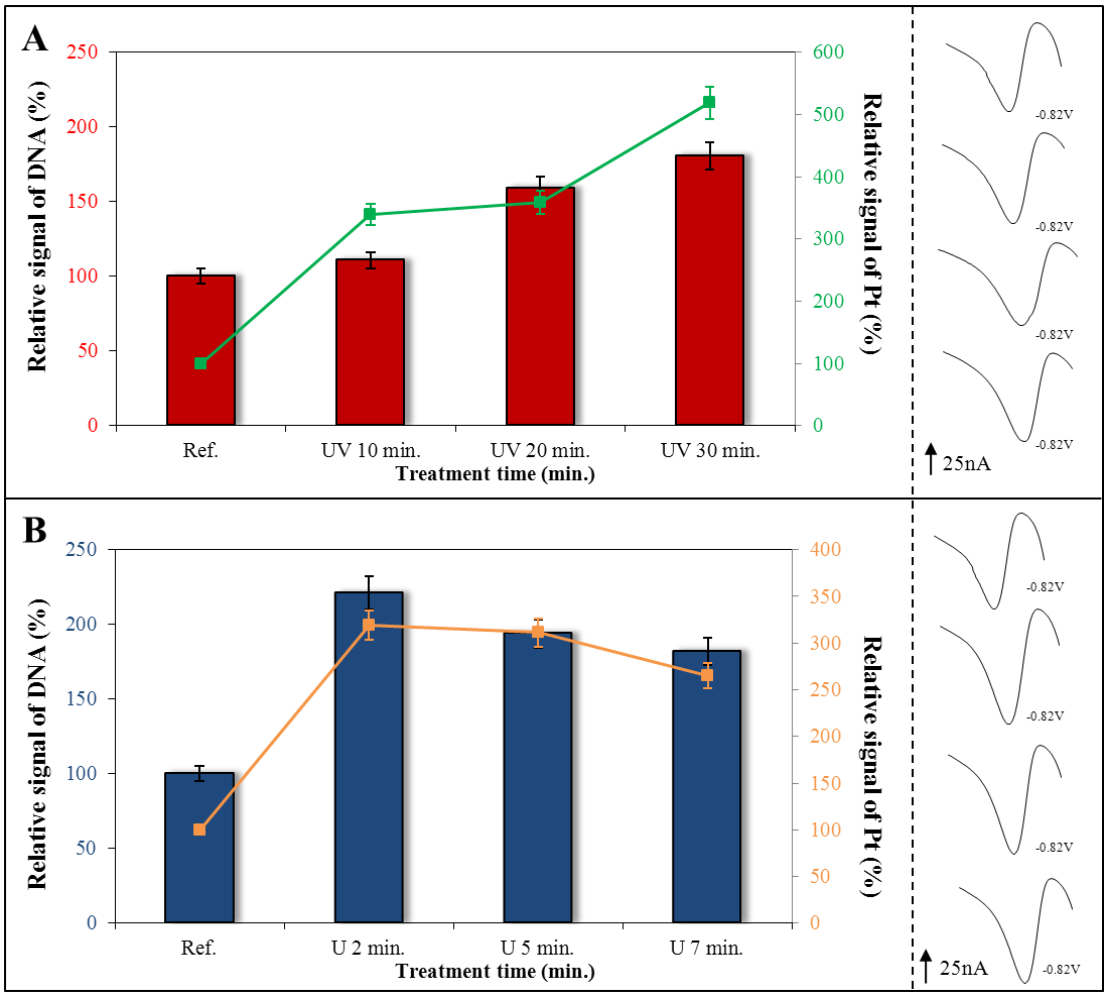


Figure 7. Effect of (**A**) UV radiation and (**B**) ultrasound on peak height of DNA and Pt measured in Pt-DNA adducts after 24h incubation and 24 h dialysis. DNA was measured by square wave voltammetry in the presence of acetate buffer pH 5.0 with these parameters: potential step 5 mV, frequency 280 Hz, time of accumulation 120 s [102]. Pt was measured by AdTS DPV. For other details see caption to Figure 4.

4. CONCLUSIONS

Electrochemical methods proved useful as simple and inexpensive tools for the analysis of natural as well as chemically modified nucleic acids. Voltammetry at mercury electrode was used for the monitoring of DNA modification with platinum complexes, which was associated with the changes in the intensity of nucleic acid signals, particular of guanine. Such approach is rational because N7 of guanine is the primary target of cisplatin and other platinum drugs, and chemical damage within the

guanine imidazole ring results in the decreasing of peaks corresponding to guanine [35,66,110-112]. Moreover, we can also use voltammetry for detection of platinum, which is complementary information to DNA interaction with platinum based drugs.

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