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# 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*

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**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).

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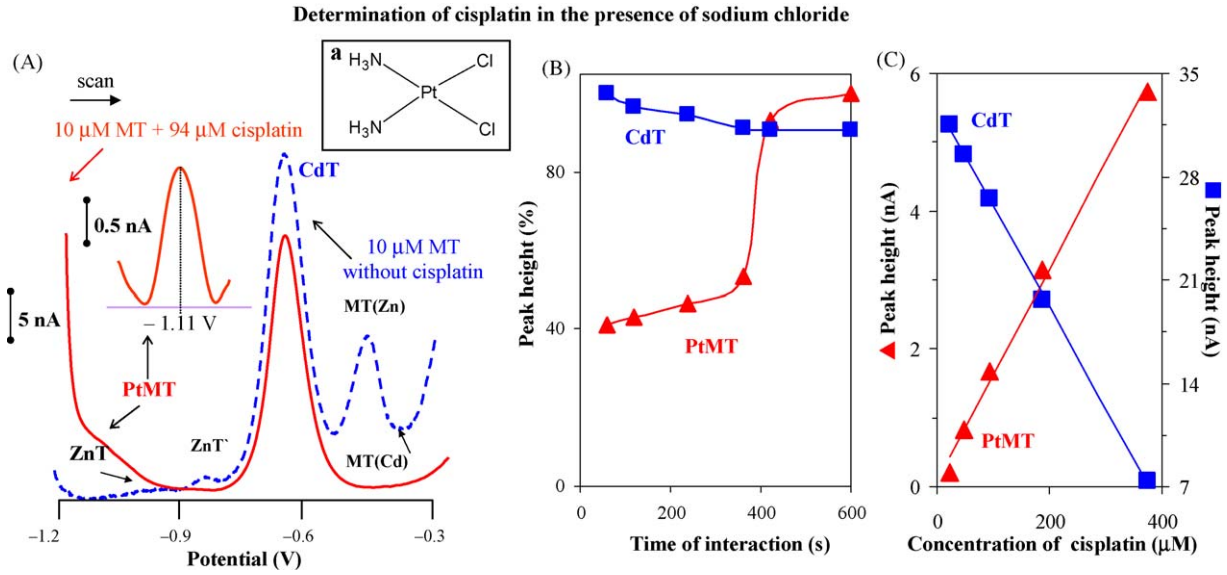


Fig. 1. Cisplatin – $[Pt^{II}(NH_3)_2Cl_2]^0$; anti-cancer drug – detection in 0.5 M NaCl. Typical DPV voltammograms of 10 μM MT without addition of cisplatin and 10 μM MT + 94 μM of cisplatin (A); inset: peak of PtMT after the baseline correction. AdTS DPV parameters were as follows: the initial potential 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