



Immobilization of metallothionein to carbon paste electrode surface via anti-MT antibodies and its use for biosensing of silver

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ABSTRACT

In this paper, heavy metal biosensor based on immobilization of metallothionein (MT) to the surface of carbon paste electrode (CPE) via anti-MT-antibodies is reported. First, the evaluation of MT electroactivity was done. The attention was focused on the capturing of MT to the CPE surface. Antibodies incorporated and mixed into carbon paste were stable; even after two weeks the observed changes in signal height were lower than 5%. Further, the interaction of MT with polyclonal chicken antibodies incorporated in carbon paste electrode was determined by square-wave voltammetry. In the voltammogram, two signals – labelled as cys_{MT} and W_a – were observed. The cys_{MT} corresponded to –SH moieties of MT and W_a corresponded to tryptophan residues of chicken antibodies. Time of interaction (300 s) and MT concentration (125 $\mu\text{g}/\text{ml}$) were optimized to suggest a silver(I) ions biosensor. Biosensor (CPE modified with anti-MT antibody) prepared under the optimized conditions was then used for silver(I) ions detection. The detection limit (3 S/N) for silver(I) ions was estimated as 0.5 nM. The proposed biosensor was tested by detection spiking of silver(I) ions in various water samples (from very pure distilled water to rainwater). Recoveries varied from 74 to 104%.

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1. Introduction

Toxic effect of silver(I) ions on water organisms has been repeatedly reported (Bielsmyer et al., 2008; Gorsuch and Klaine, 1998; Hogstrand et al., 1996; Wood et al., 1996). In water environment, silver(I) ions are stable in a wide range of pH. Under alkaline pH, AgOH and $\text{Ag}(\text{OH})_2^-$ are formed. In addition, in such environment there are many compounds which interact with silver(I) ions. The most important ones are chloride anions, which form insoluble precipitate with silver(I) ions (AgCl). The insoluble silver compounds do not represent any threat to aquatic organisms. Toxicity of silver in its soluble form is probably caused by its high affinity to proteins and also to nucleic acids. The binding of silver ions into the active sites of enzymes leads to their distinctive inhibition.

The use of carbon electrode as working electrode for the determination of silver has been previously reported by several authors (Guo and Khoo, 1999; Schildkraut et al., 1998; Svancara et al., 1996, 2001; Szymanski et al., 2010). Recently, our group introduced a heavy metal biosensor based on interaction of metal ions with a low-molecular mass protein called metallothionein (MT)

(Adam et al., 2010; Eckschlager et al., 2009; Hamer, 1986) which was adsorbed on the surface of hanging mercury drop electrode (HMDE). This biosensor has been successfully used for the detection of cadmium(II) and zinc(II) ions (Adam et al., 2005), cisplatin (Petrova et al., 2006b), cisplatin-DNA adducts (Krizkova et al., 2007), and palladium(II) ions (Adam et al., 2007a). Hanging mercury drop electrode has many advantages to be used for electroanalysis, but due to its physico-chemical properties it cannot be used in flow-automated instruments. Carbon electrodes represent a very promising alternative for the detection of biomolecules and for suggestion of biosensors (Cosnier, 1999; Li et al., 2007; Liu et al., 2008; Sivanesan and John, 2007). On the basis of the convincing results with MT as a biological component, in this paper heavy metal biosensor based on immobilization of metallothionein to the surface of carbon paste electrode (CPE) via anti-MT antibodies is suggested as capable tool for biosensing the silver. Schematic proposal of the suggested silver(I) ions biosensor with metallothionein as a biological component and carbon paste electrode as a transducer is shown in Fig. 1.

2. Materials and methods

2.1. Chemicals and materials

Silver nitrate and all other reagents in ACS purity (chemicals meet the specifications of the American Chemical Society)

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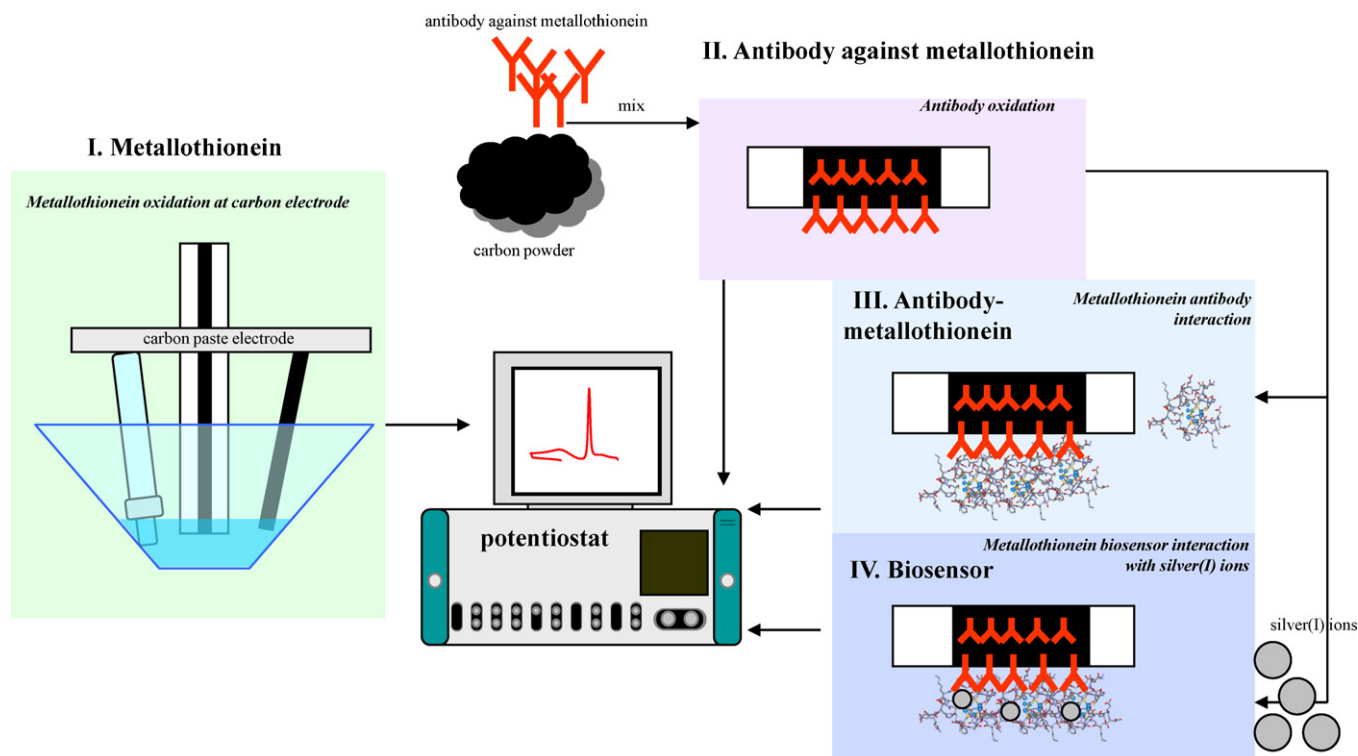


Fig. 1. Suggestion of heavy metal biosensor based on immobilization of metallothionein on the surface of carbon paste electrode (CPE) via MT-antibodies. (I) Oxidation signals of MT were measured at the surface of CPE; (II) carbon paste was mixed with polyclonal antibodies to MT; (III) CPE with the antibodies binds with MT; (IV) CPE with selectively bounded MT interacts with heavy metals.

were purchased from Sigma–Aldrich (USA), unless noted otherwise. Chicken antibody against metallothionein was obtained from Hena (Prague, Czech Republic) according to procedure published by Hodek et al. (2004). Stock standard solutions were prepared with ACS water and stored in the dark at -20°C . Working standard solutions were freshly prepared on the day of experiment by dilution of the stock solutions. All solutions were filtered through a $0.45\text{ }\mu\text{m}$ nylon filter discs (MetaChem, Torrance, USA) prior to analysis.

2.2. Dot immunobinding assay

For immunobinding assay the polyvinylidene fluoride (PVDF) membrane (Bio-Rad, USA) was used. Antigen (metallothionein from rabbit liver, $1\text{ }\mu\text{l}$) was applied with a micropipette and air-dried. Further the membrane was blocked in 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS: 137 mM NaCl , 2.7 mM KCl , $1.4\text{ mM NaH}_2\text{PO}_4$, $4.3\text{ mM Na}_2\text{HPO}_4$, pH 7.4) for 30 min with constant shaking. Then, the membrane was rinsed in 0.05% (v/v) Tween-20 in PBS (PBS-T). The incubation with chicken primary antibody (dilution 1:500 in 0.1% (w/v) BSA in PBS) was carried out for 1 h at 37°C under shaking. After the three times repeated washing in 0.05% PBS-T for 5 min the membrane was incubated in the presence of anti-chicken antibody labelled with horseradish peroxidase (dilution 1:1500 in 0.1% (w/v) BSA in PBS) for 1 h at 37°C . Then the membrane was washed three times in 0.05% PBS-T for 5 min and incubated in chromogenic substrate (0.4 mg/ml AEC (3-aminoethyl-9-carbazole) in 0.01 M acetate buffer with 0.1% H_2O_2 , pH 5.5). After the sufficient colouring the reaction was stopped by rinsing in water. The dot intensity was evaluated densitometrically by Biolight software (Vilber-Lourmat, France).

2.3. Electrochemical measurement

Square wave voltammetric (SWV) measurements were performed using an AUTOLAB analyser (EcoChemie, The Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), using a standard cell with three electrodes. Carbon paste electrode was employed as the working electrode. An Ag/AgCl/3 M KCl electrode served as the reference electrode. Glassy carbon electrode was used as the auxiliary electrode. SWV parameters were as follows: initial potential 0.0 V, end potential 1.6 V, modulation amplitude 25 mV, and step potential 0.5 mV. All experiments were carried out at room temperature. Borate buffer (0.2 M, pH 9.6) was used as the supporting electrolyte. Savitzky and Golay filter included in the software GPES 4.9 supplied by EcoChemie was employed for background correction (with following parameters: smoothing – level 2, baseline correction – peak width 0.03).

The carbon paste was made of 70% graphite powder and 30% mineral oil (free of DNase, RNase, and protease). Primary chicken antibody was diluted 1:500 with PBS buffer. Diluted solution with chicken antibody ($100\text{ }\mu\text{l}$) was mixed with 500 mg of carbon paste. The carbon paste was housed in a Teflon body of a 2.5 mm diameter of active disk surface. The electrode surface was polished before each determination with a soft filter paper prior to measurement (Kizek et al., 2005; Masarik et al., 2003; Petrlova et al., 2007a,c).

2.4. Descriptive statistics

Results are expressed as mean $\pm$ S.D. unless noted otherwise. The detection limit (3 S/N) and quantification limit (10 S/N) were calculated according to Long and Winefordner (1983), whereas N was expressed as standard deviation of noise determined in the signal domain.

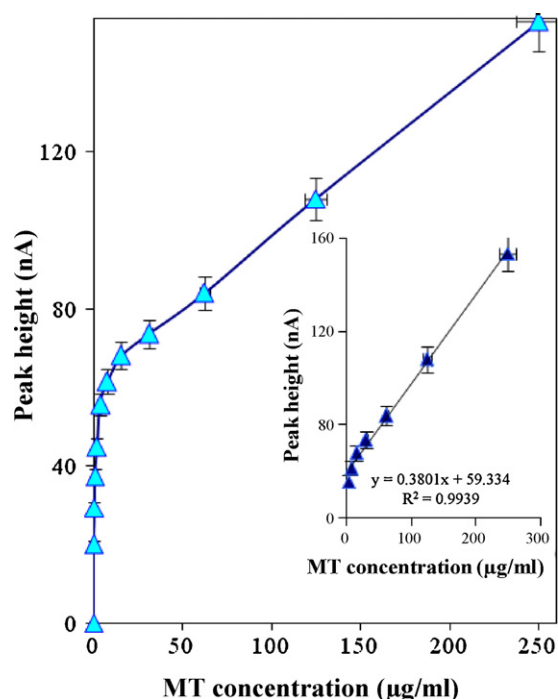


Fig. 2. Dependence of MT oxidation signal on its concentration with the range from 0.2 to 250 $\mu\text{g/ml}$; in inset: within the linear range from 4 to 250 $\mu\text{g/ml}$.

3. Results and discussion

3.1. Electrochemical detection of MT using carbon electrode (oxidative signals of $-\text{SH}$ groups)

This study was focused on basic electrochemical behaviour of MT at the surface of carbon paste electrode (Fig. 1I). It has been found previously that MT gave the highest current response at 750 mV measured at glassy carbon electrode coupled with flow injection analysis (Stejskal et al., 2008). Based on this result, we attempted to detect MT at carbon paste electrode by using SWV. It is known that aromatic aminoacids – especially tryptophan and tyrosine – are responsible for proteins electroactivity (Brabec and Mornstein, 1980; MacDonald and Roscoe, 1996). However, aromatic aminoacids are usually not present in MT structure and therefore the signals of tryptophan and tyrosine residues cannot be detected (Blindauer, 2008). Another aminoacid which exhibits a significant electrochemical activity is cysteine (Adam et al., 2005; Heyrovsky, 2004). This aminoacid is highly abundant in MT structure (more than 30%) (Margoshes and Vallee, 1957) and therefore the observed signal at the potential of 0.6 V was attributed to cysteine residues and was called cys_{MT} . This signal probably corresponds to cysteine $-\text{SH}$ moieties (Stejskal et al., 2008). The calibration curve for the examined concentration range may be splitted into two linear regression lines (Fig. 2): (i) concentration of MT from 15 to 250 $\mu\text{g/ml}$, $y = 0.3801x + 59.334$, $R^2 = 0.9939$; and (ii) concentration of MT from 0.2 to 2 $\mu\text{g/ml}$, $y = 13.378x + 20.652$, $R^2 = 0.9114$. At concentration lower than 0.1 $\mu\text{g/ml}$, the response was not proportional to MT concentration and relative standard deviation (RSD) exceeded 10% ($n = 10$). Detection limit (3 S/N) for MT was determined as 0.1 $\mu\text{g/ml}$. Based on the obtained results, it may be concluded that MT gives a reproducible electrochemical response and it is possible to achieve effective protein capturing to the electrode surface.

3.2. Modification of carbon paste electrode by antibodies against MT

After evaluation of the electroactivity of MT by SWV, our attention was targeted on the way of reliable and reproducible capturing of MT to the surface of CPE. To achieve this goal, anti-MT antibody obtained from chicken yolk was chosen (Fig. 1II) (Krizkova et al., 2009a,b). Firstly, the optimal rate between antibody concentration and antigen (MT) using dot immunobinding assay was determined. Based on the results obtained, primary chicken antibody was diluted 1:500 with PBS. Diluted solution with chicken antibody (100 μl) was mixed with 500 mg of carbon paste. Measurements were carried out in the presence of borate buffer by using of SWV (Masarik et al., 2003). Typical SWV voltammogram of anti-MT antibodies inserted in the body of CPE is presented in the inset of Fig. 3A. Signal observed at 1.4 V was called W_a and probably corresponded to oxidation of tryptophan residues which were contained in polyclonal antibodies used. Masarik et al. (2003) determined that tryptophan and tyrosine give signals at 1.0 V and 0.8 V at surface of CPE, the observed potential shift of tryptophan signal is probably caused by incorporation of antibodies into the body of carbon paste electrode. The incorporated antibodies mixed with carbon paste were stable; even after two-weeks storage at 4 $^{\circ}\text{C}$ the observed changes in peak height were lower than 5%. Moreover, the effect of frequency on voltammetric signal was examined. The highest signal was measured at 50 Hz (Fig. 3A).

3.3. MT capturing via polyclonal antibodies modified carbon paste electrode

The interaction between polyclonal antibodies and MT using dot immunobinding assay was tested (inset in Fig. 3B). It is evident from the results obtained that even 0.78 ng of MT (e.g. 115 nM) can be detected by this assay; it confirms that MT interacts with the antibodies. Further the interaction of MT with polyclonal chicken antibodies incorporated in carbon paste electrode was determined by SWV (Fig. 1III). Two signals were observed in voltammograms (Fig. 3B). Signal measured at 0.6 V is probably associated with oxidation of $-\text{SH}$ moieties of MT and was called cys_{MT} as mentioned above. At 1.4 V, well developed and distinguishable oxidative signal of tryptophan (W_a) was detected. The process of the MT-antibodies interaction can be well characterized by changes in heights of cys_{MT} and W_a peaks. The effect of both time of interaction and MT concentration were studied. The surface of modified CPE interacted with MT (10 $\mu\text{g/ml}$) in time interval from 60 s to 900 s. In Fig. 3C, the decrease of W_a signal related to MT binding to the antibodies is shown. Together with the decrease of W_a signal the gradual increase in cys_{MT} peak was determined; however this signal demonstrated RSD higher than 10%. In addition, the increasing MT concentration produced the same results – as the MT concentration increased, the W_a peak decreased and cys_{MT} increased (Fig. 3D). Coming out from these results, in the following experiments the MT concentration of 125 $\mu\text{g/ml}$ and time of interaction of 300 s were used.

3.4. Interaction of silver with MT-antibody-modified CPE

3.4.1. Behaviour of the biosensor

Biosensor proposed according to Fig. 1IV and prepared under the optimized conditions was used for silver(I) ions detection. The biosensor was immersed into 5 μl drop of Ag(I) ions (500 μM) for 300 s. Five oxidative signals (MT(Ag), AgMT, cys_{MT} , W_a and MT(Zn)) were detected in the obtained voltammogram (Fig. 4A). Oxidative signal of silver(I) ions bound to MT molecules probably via non-covalent linkages was measured at 0.25 V and was called MT(Ag). This signal gradually increased with increasing concentration of sil-

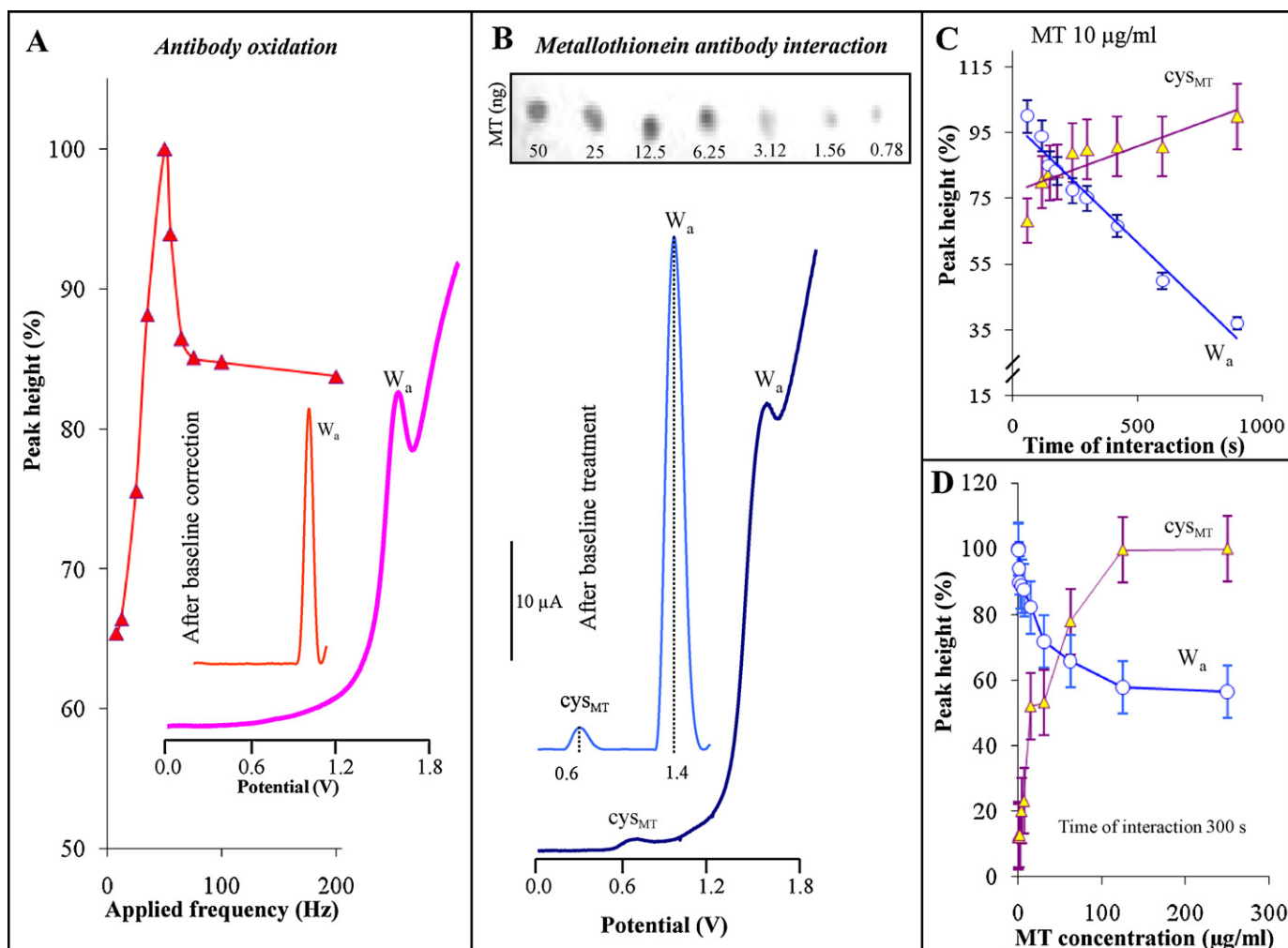


Fig. 3. (A) Dependence of peak height of antibodies on frequency; in inset: SW voltammogram of antibody modified CPE before and after baseline correction. (B) SW voltammogram of MT-antibody modified CPE before and after baseline correction; in inset: dot immunobinding assay of MT using polyclonal antibodies against MT. Dependences of cys_{MT} and W_a peaks heights (C) on time of interaction of MT with antibodies and (D) on MT concentration.

ver(I) ions. When the silver(I) ions concentration exceeded 300 µM, the height of this signal enhanced slowly (Fig. 4B). The highest signal detected in the voltammogram, which was called AgMT, appeared at 0.6 V and probably overlaid negligible cys_{MT} peak (Fig. 4A). AgMT signal may be associated with the oxidation of complex of silver(I) ions with cysteine residues in MT clusters. Height of this signal was proportional to silver(I) ions concentration up to 125 µM according to equation $y = 0.4039x + 5.1877$, $R^2 = 0.9463$. At higher concentration of silver(I) ions the increase was more gradual. This phenomenon is probably associated with binding capacity of MT captured to the surface of antibody modified CPE (Fig. 4C). The presence of polyclonal antibodies was confirmed by W_a oxidative signal at 1.4 V (Fig. 4A). W_a signal moderately declined with increasing silver(I) ions concentration. Potential of this signal shifted to more positive values (for about 0.4 mV per 1 µM of silver(I) ions on average). The decline of W_a signal can be associated with the structural changes and reorganization of protein structures on the electrode surface in the presence of silver(I) ions (Fig. 4C).

3.4.2. Analytical properties of the proposed biosensor

3.4.2.1. Calibration curve. Based on the description of the signals above, AgMT for quantification of Ag(I) ions was selected. Due to non-linear behaviour of the selected signal to Ag(I) ions concentration, the calibration curves were divided into two sections (higher and lower concentrations, Table 1). In log scale the sensor exhibited

linearity within concentrations ranging from 15.6 µM to 500 µM (higher concentration, I (nA) = $20.35 \ln(c_{Ag}) - 22.05$, $R^2 = 0.9720$). Height of AgMT was proportional to Ag(I) ions concentration within the range from 10 nM to 15 µM (lower concentration, I (pA) = $0.7340(c_{Ag}) - 6.336$, $R^2 = 0.9590$). In general, dynamic concentration range for an analytical method is dependent on many factors. In the case of a biosensor, the range depends mainly on stability and capacity of its biological part. The advantage of metallothionein is its ability to chelate heavy metal ions independently on environmental factors (temperature, solution components, and pH), which enhances its capacity and thus the dynamic range. The detection limit (3 S/N) for silver(I) ions calculated from AgMT signal were estimated as 0.5 nM and quantification limit (10 S/N) as 1.7 nM.

3.4.2.2. Repeatability and reproducibility. Repeatability and reproducibility of a biosensor is always of great interest. It depends mainly on stability and purity of biological part of the biosensor. The advantage of the proposed biosensor is high stability of metallothionein as a biological part. Reproducibility of intraday and inter day measurements was also investigated. Relative standard deviations (RSD) of inter day measurement with the same biosensor was 2.5% ($n = 5$), for intraday measurement RSD was 5.8% ($n = 5$) and for measurement within one month RSD was lower than 10%. Moreover, the influence of various batches of the biological standard was

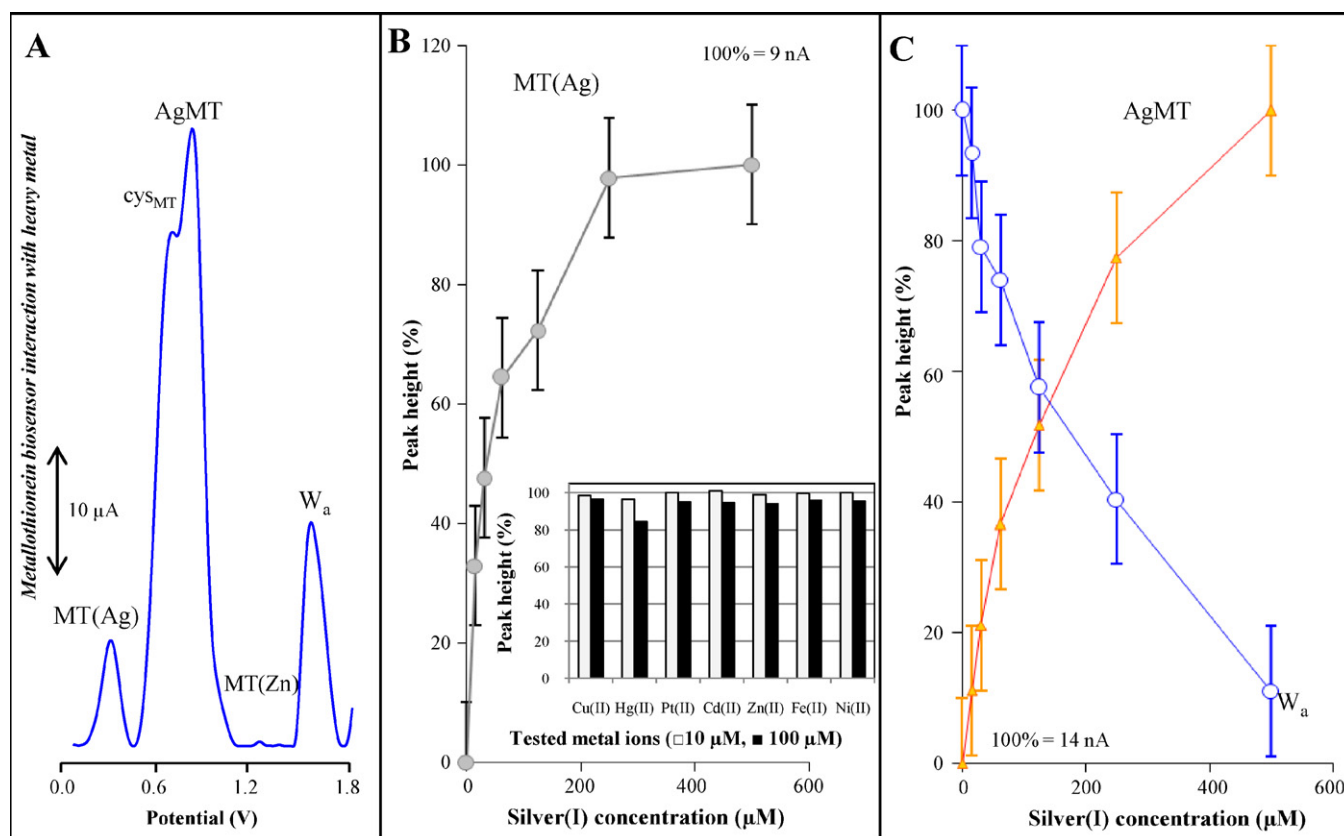


Fig. 4. (A) Typical SW voltammogram of MT-antibody biosensor after interaction with silver(I) ions. Dependences of (B) MT(Ag) and (C) AgMT and W_a peak height on silver(I) ions concentration. MT concentration: 125 $\mu\text{g/ml}$ and time of interaction: 300 s. In inset in (B): the effect of 10 μM (white column) and 100 μM (black column) Cu(II), Hg(II), Pt(II), Cd(II), Zn(II), Fe(II) and Ni(II) ions on height of AgMT signal.

also tested. Data measured by biosensor prepared with standard obtained from different batches have relatively low RSD (less than 10%). In addition, RSD between newly prepared biosensors was examined. RSD of inter day measurement was 7.5% ($n = 5$, number of tested biosensors = 5). Besides repeatability and reproducibility, storage capacity was also investigated and was estimated 10 days.

3.4.2.3. Recovery. The proposed biosensor was tested by detection of silver(I) ions spiked in various water samples (from very pure distilled water to rainwater) according to methodology published previously (Bugianesi et al., 2000; Causon, 1997). Changes in AgMT signals were determined in raw sample. In tested water samples silver(I) ions were not detected directly. The sample of water with spiked silver(I) ions interacted with biosensor for 300 s. The signal recovery in water samples without impurities (Milli Q and distilled water) was very good and varied between 101 and 104% (Table 1). In the case of tap water, rainwater and water Ponávka stream the signal was influenced by sample matrix, which resulted in higher C.V. (from 7.1 to 14%) and lower recovery (from 74 to 93%).

3.4.2.4. Interferences. Due to the fact that biological part of biosensor was protein with the ability to bind almost all metal ions, the other metal ions (Cu(II), Hg(II), Pt(II), Cd(II), Zn(II), Fe(II), and Ni(II)) were tested as interferences. The biosensor was immersed into 5 μl drop of Ag(I) ions (10 μM) for 300 s and voltammogram was measured. Then, the biosensor was immersed into 5 μl drop of particular metal ion (10 μM or 100 μM) for 300 s and voltammogram was measured again. The changes in height of AgMT signal were measured. The effect of by the abovementioned metals on the height of AgMT signal is shown in inset of Fig. 4B. Not only the same, but even 10 times higher concentration of other metal ions (but not the Hg(II) ones) did not have considerable effect on AgMT signal. Ten times higher concentration of these ions caused more than 15% decrease in AgMT signal. This phenomenon may be associated with the fact that Hg(II) ions have slightly higher affinity to MT compared to Ag(I) ions accordingly to $\text{Hg(II)} > \text{Ag(I)} > \text{Cu(I)} > \text{Cd(II)} > \text{Zn(II)}$. Nevertheless, the signals of single heavy metals present in MT structure can be distinguished according to the peaks position which corresponds to the formation of particular MT-heavy metal complex

Table 1
Recovery of silver ions (AgNO_3) measured in the presence of different types of waters ($n = 5$).

Compound of interest	Sample matrix	Filtrate (nA) ^a	Spiking (nA) ^{a,c}	Filtrate + spiking (nA) ^a	Recovery (%)
Silver ions	Milli Q water	nd ^b	7.5 $\pm$ 0.2 (2.7)	7.8 $\pm$ 0.2 (2.6)	104
	Distilled water			7.6 $\pm$ 0.2 (2.6)	101
	Tap water			5.6 $\pm$ 0.8 (14)	74
	Ponávka stream			6.9 $\pm$ 0.5 (7.2)	92
	Rainwater			7.0 $\pm$ 0.5 (7.1)	93

^a Silver ions current response; expressed as mean $\pm$ S.D. (C.V.%).

^b Not detected.

^c Silver ions current response (100 $\mu\text{mol/dm}^3$); expressed as mean $\pm$ S.D. (C.V.%).

(Adam et al., 2007b, 2005; Fabrik et al., 2009; Supalkova et al., 2008; Wu and Lin, 2004).

3.4.3. Comparison of the proposed biosensor with other techniques

Electrochemistry approach seems to be very suitable for the determination of silver(I) ions. Various electrochemical methods and electrode types can be used; the procedures differ in arrangement, electrodes construction, detection limits achieved, and influence by sample matrix (Table 1). The most often used are ion-selective electrodes, with sensitivity comparable to biosensor proposed in this paper, but the interference with sample matrix is often problematic. Nevertheless, Gupta et al. (2009) used an ion-selective electrode for the determination of silver in blood samples. Using of carbon electrodes allows detection of a very low analyte concentration and their application for real samples is more common. Since the subnanomolar detection limits can be achieved, it can be used for monitoring of trace amounts of silver in the environment (Javanbakht et al., 2009; Svancara et al., 1996). Enzyme biosensors exhibit a comparable detection limit in orders of tenth of micromoles per one litre, however the specificity of enzymes inhibition by heavy metals is of great concern (Verma and Singh, 2005; Vopalensky et al., 2007).

Metallothionein (MT) can be used as a protein component of biosensors for heavy metals (Adam et al., 2007b; Bin et al., 2009; Fu et al., 2008; Varriale et al., 2007). Gonzalez-Bellavista et al. (2009) proposed a metallothionein-based silver biosensor where MT was a part of ion-selective electrode with detection limit in orders of 10^{-5} M in model solutions. Very good adsorption of MT to the surface of gold and hanging mercury drop (HMDE) electrodes has been reported in numerous papers (Adam et al., 2005; Ju and Leech, 2000; Petrlova et al., 2006a, 2007b; Trnkova et al., 2002). Therefore adsorption of MT was used to introduce a metallothionein-based silver biosensor based on HMDE with 500 nM detection limit of Ag(I) ions (Krizkova et al., 2009c). In comparison to the bare carbon paste electrode the detection limit of the sensor was ten times lowered making it comparable to modified carbon paste electrodes (Krizkova et al., 2009d; Labuda and Vanickova, 1993; Mikelova et al., 2007; Wang et al., 2009; Ye and Khoo, 1997). In this study, 1000-fold lowering of the detection limit compared to HMDE was reached. This phenomenon may be associated with more effective immune-based-capturing of MT onto the electrode surface as compared to simple adsorption. Besides detection limit, the other advantage of the proposed biosensor is possibility of the miniaturization of carbon electrodes for *in situ* analysis.

4. Conclusions

Metallothioneins, low molecular mass proteins rich in cysteine, play an important role in the processes of heavy metals ions metabolism. Due to their unique physicochemical properties they are able to bind heavy metals with high affinity (Zhang et al., 1997). This feature was used to suggest a simple biosensor based on immobilization of MT to the surface of carbon paste electrode via chicken anti-MT antibodies. The outlined biosensor was further successfully employed in detection of silver(I) ions. The main advantage of this biosensor is its easy miniaturization; carbon nanostructures with immobilized MT might be used as working electrodes.

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References

- Adam, V., Fabrik, I., Eckschlager, T., Stiborova, M., Trnkova, L., Kizek, R., 2010. TRAC-Trends Anal. Chem. 29 (5), 409–418.
- Adam, V., Hanustiak, P., Krizkova, S., Beklova, M., Zehnalek, J., Trnkova, L., Horna, A., Sures, B., Kizek, R., 2007a. Electroanalysis 19 (18), 1909–1914.
- Adam, V., Krizkova, S., Zitka, O., Trnkova, L., Petrlova, J., Beklova, M., Kizek, R., 2007b. Electroanalysis 19 (2–3), 339–347.
- Adam, V., Petrlova, J., Potesil, D., Zehnalek, J., Sures, B., Trnkova, L., Jelen, F., Kizek, R., 2005. Electroanalysis 17 (18), 1649–1657.
- Bielmyer, G.K., Brix, K.V., Grosell, A., 2008. Aquat. Toxicol. 87 (2), 81–87.
- Bin, Y.N., Gao, Y., Xiang, J., Ren, B., 2009. Surf. Interface Anal. 41 (10), 834–838.
- Blindauer, C.A., 2008. J. Inorg. Biochem. 102 (3), 507–521.
- Brabec, V., Mornstein, V., 1980. Biophys. Chem. 12 (2), 159–165.
- Bugianesi, R., Serafini, M., Simone, F., Wu, D.Y., Meydani, S., Ferro-Luzzi, A., Azzini, E., Maiani, G., 2000. Anal. Biochem. 284 (2), 296–300.
- Causon, R., 1997. J. Chromatogr. B 689 (1), 175–180.
- Cosnier, S., 1999. Biosens. Bioelectron. 14 (5), 443–456.
- Eckschlager, T., Adam, V., Hrabeta, J., Figova, K., Kizek, R., 2009. Curr. Protein Pept. Sci. 10 (4), 360–375.
- Fabrik, I., Kukacka, J., Baloun, J., Sotornik, I., Adam, V., Prusa, R., Vajtr, D., Babula, P., Kizek, R., 2009. Electroanalysis 21 (3–5), 650–656.
- Fu, Y., Xu, M.T., Li, X., Du, M., Wang, J.X., Zhou, F.M., 2008. Electroanalysis 20 (8), 888–893.
- Gonzalez-Bellavista, A., Atrian, S., Munoz, M., Capdevila, M., Fabregas, E., 2009. Talanta 77 (4), 1528–1533.
- Gorsuch, J.W., Klaine, S.J., 1998. Environ. Toxicol. Chem. 17 (4), 537–538.
- Guo, S.X., Khoo, S.B., 1999. Electroanalysis 11 (12), 891–898.
- Gupta, V.K., Pal, M.K., Singh, A.K., 2009. Anal. Chim. Acta 631 (2), 161–169.
- Hamer, D.H., 1986. Annu. Rev. Biochem. 55, 913–951.
- Heyrovsky, M., 2004. Electroanalysis 16 (13–14), 1067–1073.
- Hodek, P., Koblas, T., Rydlova, H., Kubickova, B., Sulc, M., Hudecek, J., Stiborova, M., 2004. Collect. Czech. Chem. Commun. 69 (3), 659–673.
- Hogstrand, C., Galvez, F., Wood, C.M., 1996. Environ. Toxicol. Chem. 15 (7), 1102–1108.
- Javanbakht, M., Divsar, F., Badiei, A., Fatollahi, F., Khaniani, Y., Ganjali, M.R., Norouzi, P., Chaloosi, M., Ziarani, G.M., 2009. Electrochim. Acta 54 (23), 5381–5386.
- Ju, H.X., Leech, D., 2000. J. Electroanal. Chem. 484 (2), 150–156.
- Kizek, R., Masarik, M., Kramer, K.J., Potesil, D., Bailey, M., Howard, J.A., Klejdus, B., Mikelova, R., Adam, V., Trnkova, L., Jelen, F., 2005. Anal. Bioanal. Chem. 381 (6), 1167–1178.
- Krizkova, S., Adam, V., Eckschlager, T., Kizek, R., 2009a. Electrophoresis 30 (21), 3726–3735.
- Krizkova, S., Adam, V., Petrlova, J., Zitka, O., Stejskal, K., Zehnalek, J., Sures, B., Trnkova, L., Beklova, M., Kizek, R., 2007. Electroanalysis 19 (2–3), 331–338.
- Krizkova, S., Blahova, P., Nakielna, J., Fabrik, I., Adam, V., Eckschlager, T., Beklova, M., Svobodova, Z., Horak, V., Kizek, R., 2009b. Electroanalysis 21 (23), 2575–2583.
- Krizkova, S., Huska, D., Beklova, M., Hubalek, J., Adam, V., Trnkova, L., Kizek, R., 2009c. Environ. Toxicol. Chem. 29 (3), 492–496.
- Krizkova, S., Krystofova, O., Trnkova, L., Hubalek, J., Adam, V., Beklova, M., Horna, A., Havel, L., Kizek, R., 2009d. Sensors 9 (9), 6934–6950.
- Labuda, J., Vanickova, M., 1993. Electroanalysis 5 (2), 141–144.
- Li, Y.X., Wang, P., Wang, L., Lin, X.Q., 2007. Biosens. Bioelectron. 22 (12), 3120–3125.
- Liu, A.L., Zhang, S.B., Chen, W., Lin, X.H., Xia, X.H., 2008. Biosens. Bioelectron. 23 (10), 1488–1495.
- Long, G.L., Winefordner, J.D., 1983. Anal. Chem. 55 (7), A712–A724.
- MacDonald, S.M., Roscoe, S.G., 1996. J. Colloid Interface Sci. 184 (2), 449–455.
- Margoshes, M., Vallee, B.L., 1957. J. Am. Chem. Soc. 79 (17), 4813–4814.
- Masarik, M., Kizek, R., Kramer, K.J., Billova, S., Brazdova, M., Vacek, J., Bailey, M., Jelen, F., Howard, J.A., 2003. Anal. Chem. 75 (11), 2663–2669.
- Mikelova, R., Baloun, J., Petrlova, J., Adam, V., Havel, L., Petrek, H., Horna, A., Kizek, R., 2007. Bioelectrochemistry 70 (2), 508–518.
- Petrlova, J., Krizkova, S., Supalkova, V., Masarik, M., Adam, V., Havel, L., Kramer, K.J., Kizek, R., 2007a. Plant Soil Environ. 53 (8), 345–349.
- Petrlova, J., Krizkova, S., Zitka, O., Hubalek, J., Prusa, R., Adam, V., Wang, J., Beklova, M., Sures, B., Kizek, R., 2007b. Sens. Actuator B-Chem. 127 (1), 112–119.
- Petrlova, J., Masarik, M., Potesil, D., Adam, V., Trnkova, L., Kizek, R., 2007c. Electroanalysis 19 (11), 1177–1182.
- Petrlova, J., Potesil, D., Mikelova, R., Blastik, O., Adam, V., Trnkova, L., Jelen, F., Prusa, R., Kukacka, J., Kizek, R., 2006a. Electrochim. Acta 51 (24), 5112–5119.
- Petrlova, J., Potesil, D., Zehnalek, J., Sures, B., Adam, V., Trnkova, L., Kizek, R., 2006b. Electrochim. Acta 51 (24), 5169–5173.
- Schildkraut, D.E., Dao, P.T., Twist, J.P., Davis, A.T., Robillard, K.A., 1998. Environ. Toxicol. Chem. 17 (4), 642–649.
- Sivanesan, A., John, S.A., 2007. Biosens. Bioelectron. 23 (5), 708–713.
- Stejskal, K., Krizkova, S., Adam, V., Sures, B., Trnkova, L., Zehnalek, J., Hubalek, J., Beklova, M., Hanustiak, P., Svobodova, Z., Horna, A., Kizek, R., 2008. IEEE Sens. J. 8 (9), 1578–1585.
- Supalkova, V., Beklova, M., Baloun, J., Singer, C., Sures, B., Adam, V., Huska, D., Pikula, J., Rauscherova, L., Havel, L., Zehnalek, J., Kizek, R., 2008. Bioelectrochemistry 72 (1), 59–65.
- Svancara, I., Kalcher, K., Diewald, W., Vytras, K., 1996. Electroanalysis 8 (4), 336–342.

- Svancara, I., Vytras, K., Barek, J., Zima, J., 2001. *Crit. Rev. Anal. Chem.* 31 (4), 311–345.
- Szymanski, M., Turner, A.P.F., Porter, R., 2010. *Electroanalysis* 22 (2), 191–198.
- Trnkova, L., Kizek, R., Vacek, J., 2002. *Bioelectrochemistry* 56 (1–2), 57–61.
- Varriale, A., Staiano, M., Rossi, M., D'Auria, S., 2007. *Anal. Chem.* 79 (15), 5760–5762.
- Verma, N., Singh, M., 2005. *Biometals* 18 (2), 121–129.
- Vopalensky, P., Ruml, T., Kotrba, P., 2007. *Chem. Listy* 101 (6), 468–479.
- Wang, F., Liu, Q.Y., Wu, Y.J., Ye, B.X., 2009. *J. Electroanal. Chem.* 630 (1–2), 49–54.
- Wood, C.M., Hogstrand, C., Galvez, F., Munger, R.S., 1996. *Aquat. Toxicol.* 35 (2), 93–109.
- Wu, C.M., Lin, L.Y., 2004. *Biosens. Bioelectron.* 20 (4), 864–871.
- Ye, R.D., Khoo, S.B., 1997. *Electroanalysis* 9 (6), 481–489.
- Zhang, B.L., Sun, W.Y., Tang, W.X., 1997. *J. Inorg. Biochem.* 65 (4), 295–298.