Int. J. Electrochem. Sci., 5 **(2010)** 429 - 447

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Review

Automated Electrochemical Analyzer as a New Tool for Detection of Thiols

Vojtech Adam [1], Ivo Fabrik [1], Veronika Kohoutkova [2], Petr Babula [2], Jaromir Hubalek [3], Radimir Vrba [3], Libuse Trnkova [4,5] and Rene Kizek [1,]*

[1] Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic
[2] Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, CZ-612 42 Brno, Czech Republic
[3] Department of Microelectronics, Faculty of Electrical Engineering and Communication, Brno University of Technology, Udolni 53, CZ-602 00 Brno, Czech Republic
[4] Department of Chemistry, and 5 Research Centre for Environmental Chemistry and Ecotoxicology, Faculty of Science, Masaryk University, Kotlarska 2, CZ-611 37 Brno, Czech Republic
[*]E-mail: : kizek@sci.muni.cz

Received: 12 March 2010 / *Accepted:* 15 April 2010 / *Published:* 30 April 2010

Stationary electrochemical analysers are advantageous tools for detection of electroactive species. There advantages are low cost, sensitivity and repeatability. However, non-automated injection of samples belongs to their main disadvantages. We aim our attention to present a new electrochemical tool connected with unique autosampler enabling us to inject units of µl, which is essential in for detection of biological samples. The analyser can be used for detection of peptides, proteins, nucleic acids, drugs. Moreover, the highest sensitivity has for thiols. The recent advances in the field of electrochemistry of mentioned biologically active compounds, mainly metallothionein, are also discussed.

Keywords: Automated Electrochemical Detection; Drugs; Tumour Disease Markers; Proteins; Metallothioneins

1. INTRODUCTION

The modern procedures based on the new knowledge are more and more available to the broad public (in the developed countries and also in the countries with the fast-growing economics) [1]. The techniques, such as the magnetic resonance, the computer tomography, the ultrasound examination, and latterly the positron emission tomography expressively help in the diagnostic as well as in the

treatment of serious diseases. In spite of the increasing availability of these very expensive methods their workload is still enormous. In addition, despite of the considerable progression in these imaging methods they are still insufficient in many cases, above all in the area of the detection of the small tumours. In this area, it is possible to expect the great success of the positron emission tomography focus on the monitoring of the chosen metabolism markers [2-10]. Except of these technologies the investigation of the physiological (chemical) indicators is shifted too. The clinical laboratory medicine advances towards to the fully automated and robotized workplaces as minimizing of the operator intervention (reduction of the errors caused by the staff). The infectious disease contamination risk is also reduced [11-15]. The technological progress and development in the area of the clinical laboratory medicine is directed to i) the emergency laboratories (represents the centres in the proximity of the intensive care units, eventually resuscitation units), these laboratories are limited by the number of the available laboratory examinations; ii) the big centralized laboratory complexes, where are available more exacting analytical techniques, including the mass detection, the proteins and nucleic acids analysis; iii) the laboratories providing community facilities (also the equipped by the exacting instrumentation) [1]. In addition to these significant aims, the tendency of moving towards to patient (to his sickbed on intensive care unit) is well observable [1]. However, this advancement brings the essential necessity to simplify completely the analytical procedure, guarantee the high reproducibility of the obtained results and the arrangement reliability.

In Fig. 1 there are shown the possible ways of laboratory monitoring of patient on intensive care unit. The conventional way A is connected with the periodical sample taking and its transport to the central laboratory, where the request of laboratory examination is identified and subsequently, the laboratory measurement is carried out. The result are verified and subsequently delivered to the information system, where is accessible to the medical staff, which accordingly to results adjust the treatment. B way is directed to the smaller emergency laboratory, where is at disposal the technical equipment for the urgent laboratory data (e.g. pH, pCO_2, O_2, glycaemia). The results are immediately delivered to the medical staff. The very significant marker of these units is the rapidity of the necessary data obtaining in relation to the acute patient condition. In addition, it is guaranteed the device operating by the educated laboratory staff. The way C is based on the monitoring of the required marker directly on the acute sickbed. The indisputable advantage is the possibility of the monitoring of the requested marker practically promptly or even to constantly monitor of this data.

2. THE ELECTROCHEMICAL ANALYZERS IN THE CLINICAL LABORATORY

From the available literature sources it is well evident that the appreciable attention since the end of 20th century is devoted on the techniques of the mass detection, from which we expect the strictly accurate results with the possibility of identification of searched compound. However there is also the question, whether this approach is useful or not (these techniques still require very expensive laboratory arrangement including the very efficient attendance). Let us return our attention on the electrochemical arrangements and their possible utilization in the clinical practice. The increasing of

interest in the electrochemical detection is possible to found out since 90th years of the last century. Since that time the significant increase of the number of the published papers aimed at this theme in the database Web of Science was observed (Fig. 2). The increasing attention in this area of the experimental research is especially focused on the searching of the simple, universal and for the broad public accessible monitoring arrangements [16,17]. The success like this is represented by the electrochemical detectors for glycaemia or for the level of the alcohol in breath [18]. Miniaturization of the detection arrangements advances very quickly and it heads for the broadly accessible instruments that are marked as the personal laboratories or lab on a chip [19].

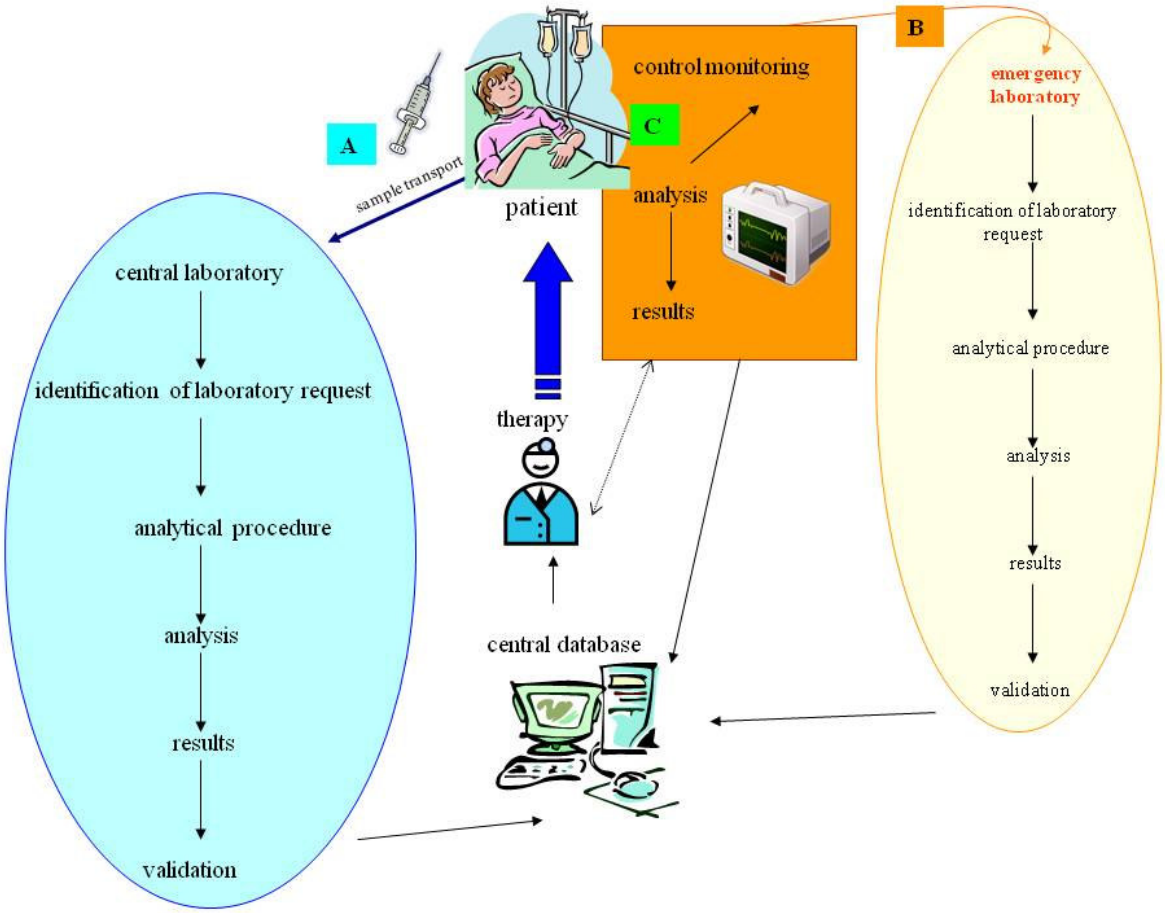

Figure 1. Possible ways of patient monitoring A: central laboratory, B: emergency laboratory, C: individual monitoring directly at patient.

The most used electrochemical measurement in the clinical laboratory is the pH and inorganic ions detection by using the ion selective electrode. Much less extended are the liquid chromatographs with the electrochemical detector (especially the analysis of catecholamines) [20]. However, voltammetric analysis is practically not supplied in the clinical laboratories. There are several reasons of this phenomenon, but the requirement of the qualified attendance for the analyzer and with it connected costingness of operation and servicing (eventually groundless fear from the mercury while

using working HMDE electrode) belong among the most important. In addition, tendency in the construction of the clinical instruments is the increasing of so-called closeness of such arrangements. Importance of this access is quite clear – for the guarantee of the correct instrument working certificated by producer it is quite necessary hold on the apparatus from all external influences including of the human. Despite of these disadvantages, the electrochemical analyzers have a lot of advantages such as the excellent repeatability of the analysis, unpretentious of the sample adjustment and manipulation and above all cheap costs of operation of apparatus (one analysis costs below 1 Euro). Possibilities of the electrochemical analysis are especially in the detection of the heavy metals ions, but now over again we can observe its possibilities in the area of the nucleic acids and proteins detection.

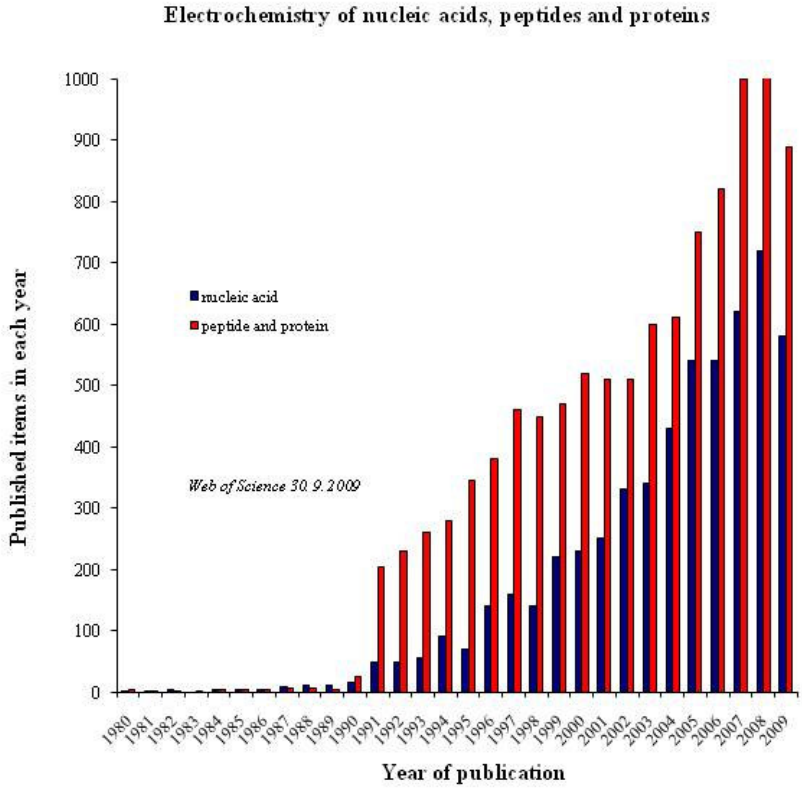

Figure 2. The simple profile of the published records on the Web of Science by using the key words nucleic acid, DNA, RNA, peptide*, protein* and electrochem*, voltamm*. Data has been evaluated to the day 12. 3. 2009. During the analysis, it was not carried out next cross database browsing. The data only predicate the tendency in the number of records.

2.1 Electrochemical determination of heavy metals ions

Polarographic, later voltammetric methods were since their description by Professor Heyrovsky used at first for the heavy metals ions analysis and at the beginning of 21st century electrochemistry itself has its use in this analysis [21-26]. Later it was described the adsorptive stripping technique,

which results from the accumulation of the compound of analysed metal ion on the surface of working electrode (mercury). This compound is in the following electrochemical cycle stripped. Obtained electrochemical response enables decrease of the detection limits to the subnanomolar concentrations of the determined analyte. In addition, by using of these techniques it was possible to determine many metal ions in which other analytical techniques failed [27-35]. Next advantage of the electrochemical analysis is the contemporary determination of the several heavy metals ions in one analysis with the aim of the gradually automation of the whole measurement like this [27-32,36-41].

2.1.1 Automated determination of heavy metals ions

It is possible to use for this purpose subsequent arrangement, which is shown in Fig. 3. The polarograph (757 VA Computrace from Metrohm, Herisau, Switzerland) employs a conventional three-electrode configuration with a hanging mercury drop electrode (HMDE) working electrode, Ag/AgCl/3MKCl as reference electrode, and a platinum auxiliary electrode. The following setup assembled of automated voltammetric analysis is supplied by Metrohm. A sample changer (Metrohm 813 Compact Autosampler) performs the sequential analysis of up to 18 samples contained in plastic test tubes. Samples are placed in the odd positions while the wash solution in the even ones. For the addition of standard solutions and reagents, two automatic dispensers (Metrohm 765 Dosimat) are used, while two peristaltic pumps (Metrohm 772 Pump Unit, controlled by Metrohm 731 Relay Box) are employed for transferring the rinsing solution in the cell and for removing solutions from the voltammetric cell. In this regard, it is important noting that the mercury drops formed during analyses are also aspirated and removed from the cell. All the units are connected to the polarograph via plastic tubings and controlled by the software. The validation on this arrangement was recently published in the journal Analytica Chimica Acta [42].

2.1.2 The Automated determination of the electroactive compounds

It is possible to use the introduced technical arrangement for the analysis of the large spectrum of the electroactive compounds including the detection of the pharmaceutical preparations. An example the record of the automated detection of the samples of the antitumour pharmaceutical doxorubicin is shown in Fig. 4. The obtained electrochemical response was sensitive and it was possible to analyse doxorubicin in the concentrations about 100 nM, which is comparable with stationary electrochemical instrument used for the detection of this drug [43]. The advantage of this analysis is the rapidity, when the result is available in the several minutes at maintenance of the negligible expenses to this measurement. Therefore, this determination would bring the advantage to the patients of the periodic monitoring of the actual concentration of the pharmaceuticals and possible dosage adjustment.

Figure 3. The real view of automated electrochemical system for the voltammetric/amperometric measurement with potentiostat/galvanostat. The sample is transported into the electrochemical cell by the help of the peristaltic pump. The electrolyte is dosed by the dosing unit. The rinsing cycle is done by two independent peristaltic pumps.

2.2 Electrochemical determination of nucleic acids

The structure of the nucleic acids is known over 50 years. The oscilopolarographic DNA records at the end of the fifties of last century brought information about electroactivity of the nucleic acids [44,45]. It was shown that the mercury electrode was very useful for this purpose; later the dropping electrode has been replaced by the hanging mercury drop (HMDE) [46-51]. In the electrochemical records obtained by using of the mercury electrode the electrochemical reduction signals of adenine and cytosine were measured at potentials about -1.4 V. However, signal of guanine has been also obtained, but after its previous electrochemical reduction in the negative potentials [52-54]. At the beginning of the eighties of last century carbon electrodes were used for the nucleic acids analysis by Brabec [55-57]. Recently it was possible to obtain the records of all nucleic acids bases presented in monitored sequence by using of the mathematically analysed data [58-66]. The maximum attention in the area of the nucleic acids sensors is devoted to the monitoring of hybridization (determination of the specific sequence), namely both directly on genomic DNA and on mRNA at its

transcription by the help of reverse-transcription polymerase chain reaction [61,67-77]. The electrochemical sensors for DNA damage monitoring represent next important group [78,79]. Newly the magnetizable particles for these technologies are developed.

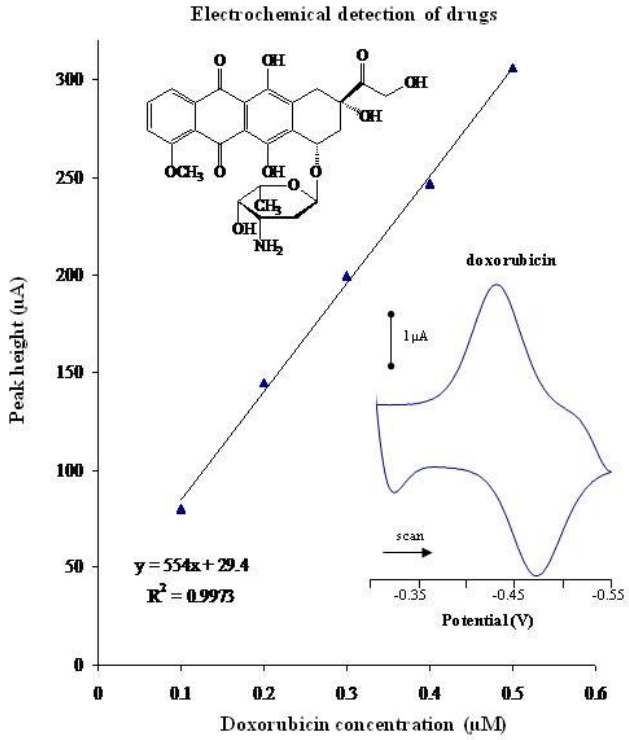

Figure 4. The automated electrochemical analysis of the antitumour pharmaceuticals doxorubicin. Parameters of cyclic voltammetry: scan rate: 100 mV/s, potential step 5 mV, time of accumulation 120 s. The analysed samples were deoxygenated prior to measurements by purging with argon (99.999%), saturated with water for 120 s. All experiments were carried out in the presence of acetate buffer pH 5.0 at room temperature.

2.3 Electrochemical determination of proteins

The early origins of the polarographic studies of the proteins are summarized in the work of Michael Heyrovsky [80]. How it is introduced in this work, the first polarographic analyses of the different body liquids were realized in 1928 [80]. Later Rudolf Brdicka discovered interesting electrochemical behaviour of the proteins in the presence of the cobalt ions [81-84]. It was experimentally detected that the observed changes are of the catalytic character and they are connected with the elimination of the hydrogen out of supporting electrolyte. The observed changes also well corresponds to the concentration of the proteins. In addition it was determined that the intensity of the catalytic signal is related to the presence of free –SH group. The revolutionary fact was subsequently establishment that the polarograms of the patients with the tumour disease are different from the polarograms of the healthy people [85-87].

2.4 Voltammetry in clinical laboratory

The electrochemical detection, in our case voltammetric or amperometric detection, surely should not go away out of the clinical laboratories. The main reasons, why electrochemistry should be kept in the clinical laboratories, are the excellent repeatability and reproducibility, the low detection limit, low costs for the apparatus purchase and for the operation and in some procedures it is also the selectivity of the determination. It is necessary to advise the electrochemical analysis brings one of the most sensitive and selective analytical procedures of the analysis of thiol compounds in real samples of the body liquids and tissues without the previous laborious preparation and the subsequent adjustments (e.g. derivatization). Thanks to this fact, it is possible to carry out the analyses in 60 minutes since the body liquid was sampled and delivered to the laboratory. The advantages of the electrochemical detection can be in addition improved by the connection with the suitable separation technique, e.g. high performance liquid chromatography. This connection brings advantages of the both used methods.

2.5 Automation of electrochemical detection of small sample volume

For our purposes of thiol compounds analysis in the biological samples the electrochemical analyzer from Metrohm Company (the unique arrangement in the whole Central Europe) was tested. The arrangement is conceived as an automat, which is constituted by the electrochemical module (potentiostat/galvanostat, VA Stand 747) and the electrochemical cell is placed on it. Into this electrochemical cell three electrodes (working, reference, auxiliary electrode) are positioned. Next part of the arrangement is the special handler of the samples (Autosampler 695), which fulfils the request on the minimisation of the sample taking. The sample is taken to the Teflon capillary filled by the hydraulic liquid. The capillary is attached to the air pump, which supplies the accurate sample dosage. The amount of the sample that is possible to dose by using of this technology varies between 1 and 100 µl. The capillary is placed on the moving arm with the possibility of the movement in directions *x*, *y* and *z*. The samples and eventually next reagents are placed in three different carousels. Individual carousels are identified by the arrangement according to the placing of magnets on their bottom. In our tested arrangement, it was necessary to place samples into the cooled space. For this purpose the simple water-cooled sample holder, which more than 80 different samples can be placed in, was proposed and subsequently constructed in our laboratory. Remaining carousels serve as the holders for the chemicals, which can be used during the analysis. By this, it is opened the scope for the inexhaustible possibilities of the electrochemical analysis modification, by which we can provide the higher selectivity of determination, eventually lower detection limits. To the provision of the supporting electrolyte and the necessary rinsing steps exchange three pumps (700 Dosino) are available. Two of these pumps supply the perfect rinsing of the working cell (maximum capacity 50 ml) and the third derives the supporting electrolyte (maximum capacity 20 ml). The arrangement is controlled by the microprocessor (746 VA Trace Analyser). The automatic analyzer facilitates of the smaller volume of the supporting electrolyte (to 2 ml), which in the case of the electrochemical analysis is not such common. In addition, setting and customizing is possible almost in case of all

parameters - from the volumes of electrolyte, the sample, or the washing buffer to the automated measurement of calibration curve and real samples (Fig. 5).

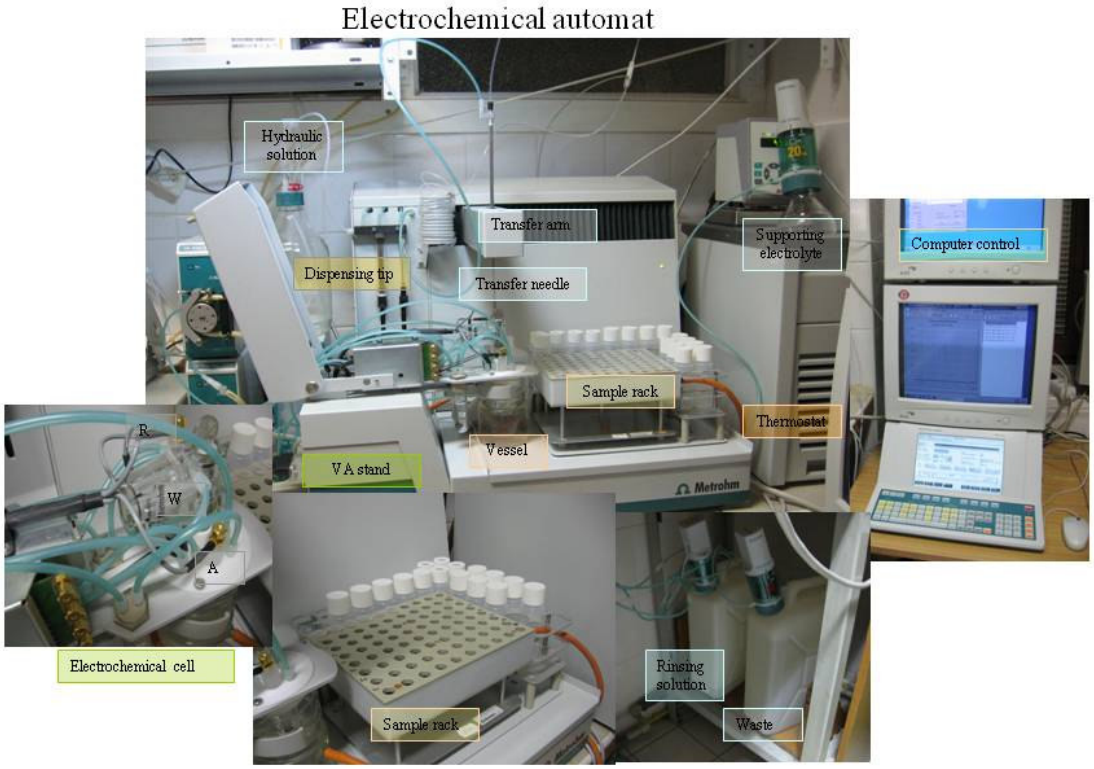

Figure 5. The automated electrochemical analysis of the low-molecular thiol compounds. The electrochemical measurements were performed with 747 VA Stand instrument connected to 746 VA Trace Analyzer and 695 Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes and cooled sample holder (4 °C). 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 auxiliary electrode.

2.5.1 The basic principle of the measurement

After the choosing of the acceptable method, which is submitted to the control module, it firstly starts the filling of the supporting electrolyte to the electrochemical cell. In our arrangement, the amounts of the used solutions were expressively minimized. The maximal necessary volume for the analysis does not get over 2 ml. The dosing arm is initialized, rinsing of the dosage needle and loading of the sample volume for the taking follow. Then the dosage arm locates the determined position with sample and takes it up. Washing of the dosage needle step and movement of the arm to the electrochemical cell follow. After the dosing of the sample into the electrochemical cell, the electrochemical analysis is initiated. In our case, the time from the cell washing until the sample application is about 5 minutes, but surely the interval could be more cut down, it depends on the character of the analyte and the procedure of the detection.

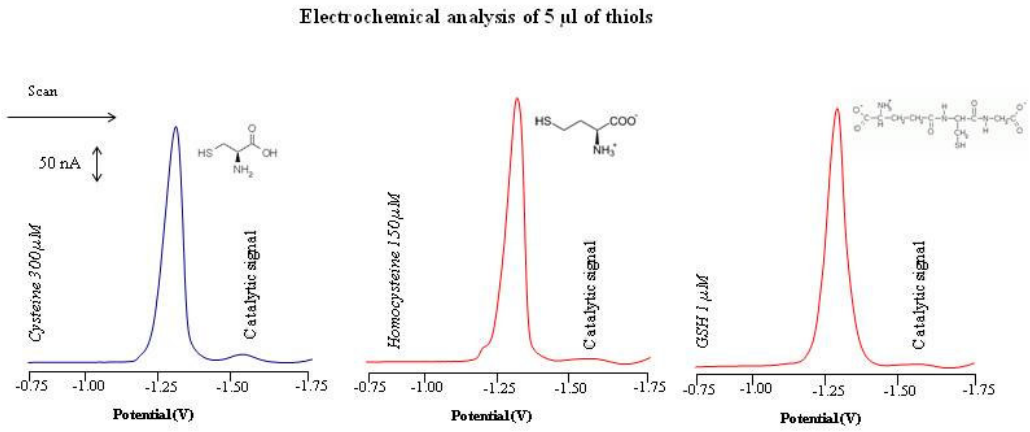

Figure 6. Electrochemical analysis of cystein, homocystein and reduced glutathione (GSH). GPES 4.9 supplied by EcoChemie was employed for data treatment. The Brdicka supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1 M ammonia buffer (NH_3(aq) + NH_4Cl, pH = 9.6) was used and changed per one analysis. Parameters of differential pulse voltammetry were as follows: initial potential of –0.7 V, end potential of –1.75 V, modulation time 0.057 s, time interval 0.2 s, step potential 2 mV, modulation amplitude -250 mV, E_{ads} = 0 V. All experiments were carried out at temperature 4 °C (Julabo F25, Germany). For the other experimental details see in Fig. 5.

2.5.2 The thiol compound detection

Theoretically, it is possible to analyze everything, what can be oxidized or reduced on the surface of the working electrode. It is from the relatively simple analyses of the metals ions samples in the solutions over more peptides detection, after as much as so nowadays discussed proteins analysis. For proteins analysis, we result from the procedure of Professor Rudolf Brdicka who discovered that proteins with –SH groups can create the catalytic responses in the combination with the complexes of the metal ions dissolved in electrolyte (buffer) and mercury drop create catalytic responses [88]. As a complex compound the trivalent complex of the cobalt $Co(NH_3)_6Cl_3$ is used, nevertheless the similar properties in the catalytic electrochemistry, as it seems, have also the another compounds [89]. Our analysis of the thiol compounds is focus on the low molecular mass thiol compounds and proteins. The typical voltammograms of the biologically very important low molecular mass thiol compounds is shown in Fig. 6. The analysis was made by using the above-mentioned electrochemical analyzer, which is able to exactly dose the very small volumes of sample (1 – 10 µl with the error of the sample dosage 1 %). The thiol compounds provide at the Brdicka the reaction catalytic signals, which are proportionate to their concentration. The mentioned progress facilitates the analysis in the submicromolar concentrations. This sensitive analysis can be carried out in the case of all peptides and proteins, which in their molecule contain cysteine. Among this molecules belongs also metallothionein [22,90-112], glutathione [113-119], phytochelatins [106,113,114,120,121] and others [122,123], which have been determined by using of stationary electrochemical instrument without autosampler.

2.6 Biochemical, molecular and biological significance of metallothionein

Metallothionein belongs to the family of the metalloproteins, which are widespread in the animal kingdom. Nevertheless, the similar types of the proteins have been described in the bacteria, plants and fungi. They are low molecular mass (6-10 kDa) on the cysteine moieties rich proteins, which do not contain the aromatic amino acids. About 30 % of the mass is created by the amino acid cysteine, which forms the characteristic properties of metallothionein. Exactly, thanks to the sulfhydryl groups of cysteine, metallothionein is capable to bound seven divalent or twelve monovalent metal ions [124]. Until this time, many physiological functions of metallothionein have been recognized [125-130]. In the first place, it is the important carrier of the metal ions, as was already mentioned in the introduction. Its maximum affinity is to Cu^{+}, but in the most cases metallothionein bounds Zn^{2+}, so it is very intensively connected to the homeostasis of these ions in organism. During the intoxication by the other heavy metals, such as Cd^{2+}, Pb^{2+}, and Hg^{2+}, metallothionein is capable to bond these ions (under the releasing of Zn^{2+}) and this ions are harmless for the cell [129,131,132]. The subsequent detoxification probably proceeds in the kidneys. MT has also the significant antioxidant role. In conjunction with GSH it forms the oxidation-reduction couple, which regulates the occurrence of the free oxygen radicals [125,133]. Together they generate the reduction background, which contributes to the protection nucleic acids, phospholipid membranes and protein structures of the cell against the ionizing effects of the high-energetic radiation and the chemooxidative activity of the toxic reagents [125]. It is recently more and more pointed at the capability of metallothionein to regulate the genome expression. As a reservoir of the zinc, metallothionein is able to transport the essential metals to the transcription factors and activates them. The activated transcription factors subsequently bind onto the regulation DNA sequences and initialize the transcription [126,134,135].

The regulation of metallothionein expression is also associated with the presence of the metal ions [136]. The transcription is controlled by the help of the regulation sequence of DNA called MRE (metal responsive element) and subsequently may be initiated after the binding of the transcription factor onto this sequence. The best-known way of the expression is through MTF- 1 (metal regulatory transcription factor-1). This factor is localised in the cell in the inactivate state with MTI inhibitor bound. After the metal ion enters into the cell this ion binds just to the inhibitor there is activated MTF- 1 and it can after the binding to MRF initiate the transcription of metallothionein after MRE binding. This expression way is used in the therapy of the patients intoxicated by the heavy metals who are given by the zinc to. MTF-1 after initiates the cascade transcription of the gene for metallothionein [137].

2.6.1 The relation of metallothionein to the tumour disease

The relation of metallothionein to the tumour disease has not been satisfactorily clarified until now, however this protein plays the important role in the cancerogenesis [125,138]. Experiments with the genetically modified mice without the gene for metallothionein demonstrated that they are more sensible to the origin of the tumour disease caused by the heavy metal [139,140]. It has been demonstrated too that the proliferating cells contain more metallothionein so the directly connection

with the tumour disease is well evident [125]. The proliferating cells especially need zinc for the newly expressed enzymes and for the regulation of the proteins. The antioxidant activity of metallothionein is used at the deactivation of the oxygen radicals formed during the increased activity of the mitochondria of the proliferating cells [141-143]. There are plenty of studies evaluating metallothionein level at patients [22,98,105,144-155]. The concentration of this protein (MT) is given to the connection with the tumour localization, the tumour stage, the prognostic perspective, but also with the age of the patient, the feeding habits etc. It is necessary to make the intensive investigation in this area for the better understanding of the tumour cell function.

2.6.2 The platinum pharmaceuticals

The effects of the best known representative of this group of compounds, cisplatin (cis-diamminedichloridoplatinum complex), were discovered incidentally, when Rosenberg, van Camp and Krigas investigated the influence of the electric current to the colonies of microorganisms (*E. coli*) [156]. They were using the platinum electrodes during the experiments, which generate the cytostatic agent, whose effects are still after almost fifty years intensively used in the oncology [157-159]. It elapsed almost 50 years since the first observation of the cisplatin effect on the cells, but even this event does not change the fact that is used up a lot to the present day. Nevertheless, especially because of the many side effects of therapy is cisplatin nowadays subsequently suppressed by the derivatives of the platinum complexes of the second generation, such as carboplatin [160-162], and more latterly by complexes of the third generation, where oxaliplatin is included [163-165]. What is based therapeutic effect of this compound on? Thanks to the many studies devoted to this question, we nowadays can establish the conception about the probable effect of these pharmaceuticals. Cisplatin must be primarily activated (by elimination of the chlorine ions), which takes the place even in the intracellular space. This activated cisplatin is already toxic for the cell [166-168]. In the nucleus cisplatin is able to interact with purine bases (especially with the guanine), which after the bond creation deviate from the normal structure of the nucleic acids. Thereby cascade of the metabolism processes that optimally result in cell apoptosis is initiated. Unfortunately, this therapy is not successful in the case of all tumours. In prevailing cases the failure of the therapy is caused by resistance formation to cisplatin in the tumour cells [169]. The mechanisms of the cell defence can be divided into the three groups; the cisplatin transport outside of the cell, capture of the cytostatic agent by intracellular thiols and finally the reparation of damaged DNA [168]. MT in conjunction with the glutathione is able to bond cisplatin by it's –SH groups and using this makes it for the cell harmless [168]. The increased level of metallothionein in the patients with the tumour disease consequently can complicate the chemotherapeutical treatment, because its effectiveness is for the tumour cells rapidly reduced [125]. It is unavoidable to approach individually to therapy by cytostatic agents and apply the most optimal therapy.

2.6.3 The automated metallothionein detection

Our automated electrochemical analyzer is very applicable to the routine electrochemical measurement. As it turns out, the serum MT would become the next marker for the monitoring of the cell proliferation and the electrochemical analysis would enable the implementation of this marker to the clinical practice. The characteristic calibration curve of MT obtained by using of the analyzer is shown in Fig. 7. The calibration dependence is strictly linear and the relative standard deviation of the detection is 4.5 %. The repeatability of calibration solutions measurement is very acceptable, how it is demonstrated in the column graph, when after three days there were not the differences in the calibration points major than 2 % since the first measured values. Some next evaluation data are published in the paper of Fabrik et al. [22]. MT detection is very well realisable also in the real samples after their foregoing heat denaturation (this removes most of the thermolabile proteins from the sample). The characteristic analysis of the real samples demonstrates Fig. 8. For MT content the group of patients with the different diagnosed disease was examined. The genetically caused disease leading to the disorders of copper metabolism resulted in the moderate growth of MT concentration in the comparison to the control group of the healthy people. Precancerosis (marked as the others) and the cancer diagnosis indicate the increase of MT level in the serum of these patients. More details will be published elsewhere.

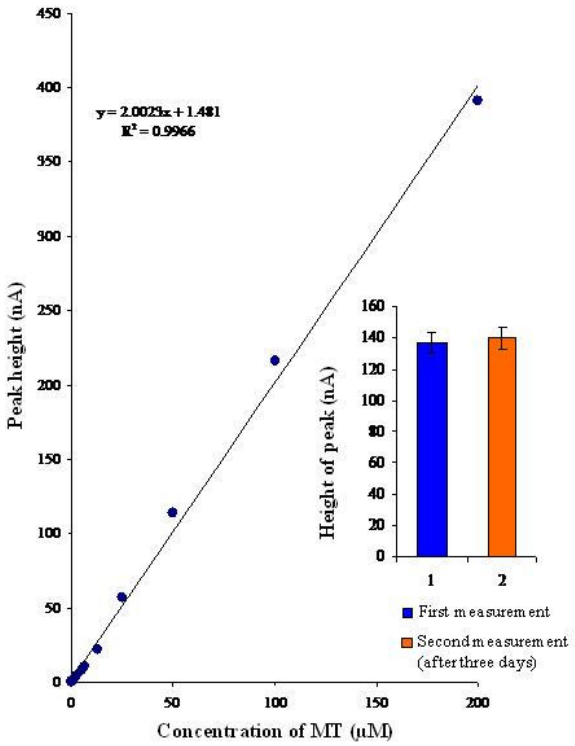

Figure 7. The automated detection of metallothionein. The electrochemical measurements were performed with 747 VA Stand instrument connected to 746 VA Trace Analyzer and 695 Autosampler (Metrohm, Switzerland). For the other experimental details see in Figs. 5 and 6. In inset: the change of MT signal (calibration points) after three days is shown.

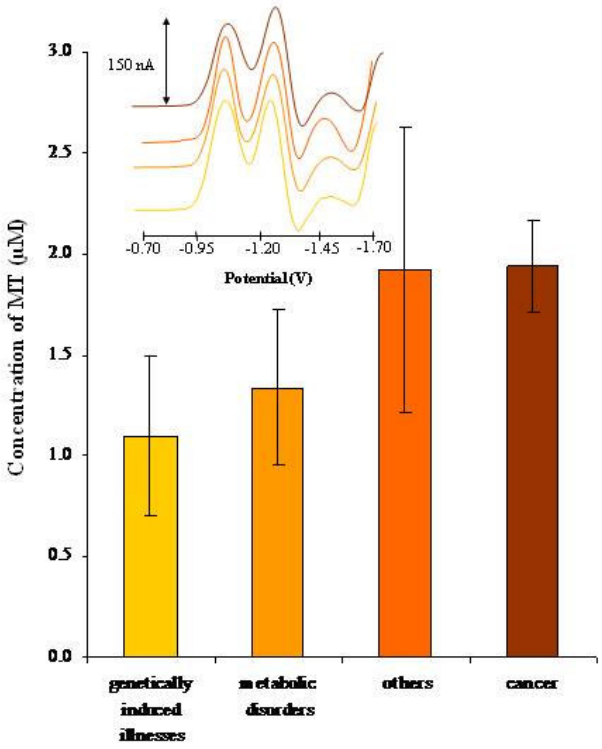

Figure 8. Samples (n = 33) for our studies were obtained from Department of Clinical Biochemistry and Patobiochemistry, 2nd Faculty of Medicine of Charles University in Prague, Czech Republic. All samples passed through the same procedure of the treatment. At first the blood was deep-frozen, subsequently embedded into the thermoblock (Eppendorf 5430, USA) and at the temperature 99 °C incubated for 15 minutes. Then followed the insertion into the centrifuge (Eppendorf 5402, USA) and subsequently samples were centrifuged at temperature 4 °C and 15 000 g. After it supernatant which was automatically dosed into the rinsing cell was detracted. Generally, 28 samples of blood of patients with copper metabolism disorder and four patients treated with cisplatin were analysed. The measurements were carried out on the apparatus 747 VA Stand in the connection with 746 VA Trace Analyzer (Metrohm, Switzerland) and 695 Autosampler. For the other experimental details see in Figs. 5 and 6. The concentration of metallothionein was deducted from the Cat 2 peak height.

3. CONCLUSIONS

The automated and robotized systems can be useful so much for routine analysis and it can be applied here the quite different approaches. The electroanalytical methods are very sensitive, but for their usage in the clinical chemistry, there is some important restrictions, among which belong the increased pretensions to the attendance and the insufficient automation. This gap could be filling up with the introduced apparatus. In addition, the determination of the levels of the selected thiol

compounds can become new markers in the field of the tumour diseases diagnostics, the nutritive therapy, and the evaluation of antioxidant state of the organism.

ACKNOWLEDGEMENTS
The work on this project was supported by the projects GACR 102/06/1624, MSM 0021630503, INCHEMBIOL MSM0021622412 and GACR 102/08/1546. The authors wish to thank Jiri Baloun for the laboratory service and Jiri Kukacka for the providing of the blood samples of the patients.

References

1. E. Schleicher, *Anal. Bioanal. Chem.*, 384 (2006) 124.
2. Y. X. He and Q. Y. Guo, *Postgrad. Med. J.*, 84 (2008) 246.
3. M. Bauer, C. C. Wagner and O. Langer, *Drugs in R&D*, 9 (2008) 73.
4. C. Garcia and P. Flamen, *Curr. Opin. Oncol.*, 20 (2008) 275.
5. K. Bouchelouche and P. Oehr, *Curr. Opin. Oncol.*, 20 (2008) 321.
6. C. C. Wagner, M. Muller, G. Lappin and O. Langer, *Curr. Opin. Drug Discov. Dev.*, 11 (2008) 104.
7. P. Rigo, P. Paulus, B. J. Kaschten, R. Hustinx, T. Bury, G. Jerusalem, T. Benoit and J. FoidartWillems, *Eur. J. Nucl. Med.*, 23 (1996) 1641.
8. S. S. Gambhir, *Nat. Rev. Cancer*, 2 (2002) 683.
9. M. E. Juweid and B. D. Cheson, *N. Engl. J. Med.*, 354 (2006) 496.
10. J. Czernin and M. E. Phelps, *Annu. Rev. Med.*, 53 (2002) 89.
11. M. J. Wheeler, *Ann. Clin. Biochem.*, 44 (2007) 209.
12. D. D. Cunningham, *Anal. Chim. Acta*, 429 (2001) 1.
13. D. Jungkind, *J. Clin. Virol.*, 20 (2001) 1.
14. D. T. Rossi and N. Zhang, *J. Chromatogr. A*, 885 (2000) 97.
15. N. L. Alpert, *Clin. Chem.*, 15 (1969) 1198.
16. A. Economou and A. Nika, *J. Autom. Methods Manag. Chem.* (2006) 9.
17. N. Verma and A. Singh, *J. Autom. Methods Manag. Chem.*, 83427 (2006) 4.
18. R. Nagarajan, A. Gupta, R. Mehrotra and M. M. Bajaj, *J. Autom. Methods Manag. Chem.*, 45102 (2006) 5.
19. L. J. Kricka, *Clin. Chim. Acta*, 307 (2001) 219.
20. O. Gonzalez-Garcia, C. Arino, J. M. Diaz-Cruz and M. Esteban, *Chemometrics Intell. Lab. Syst.*, 93 (2008) 49.
21. R. Y. A. Hassan, I. H. I. Habib and H. N. A. Hassan, *Int. J. Electrochem. Sci.*, 3 (2008) 935.
22. I. Fabrik, S. Krizkova, D. Huska, V. Adam, J. Hubalek, L. Trnkova, T. Eckschlager, J. Kukacka, R. Prusa and R. Kizek, *Electroanalysis*, 20 (2008) 1521.
23. E. Sar, H. Berber, B. Asct and H. Cankitrtaran, *Electroanalysis*, 20 (2008) 1533.
24. P. Abinian, G. G. Wildgoose, L. Xiao and R. G. Compton, *Electroanalysis*, 20 (2008) 1607.
25. Y. H. Li, Y. X. Wang and M. H. Huang, *Electroanalysis*, 20 (2008) 1440.
26. M. Grabarczyk, *Electroanalysis*, 20 (2008) 1495.
27. J. Wang and J. M. Lu, *Anal. Chim. Acta*, 274 (1993) 219.
28. J. Wang and B. Tian, *Anal. Chim. Acta*, 274 (1993) 1.
29. J. Wang, J. M. Lu and Z. Taha, *Electroanalysis*, 4 (1992) 981.
30. J. Wang and B. M. Tian, *Anal. Chim. Acta*, 270 (1992) 137.
31. J. Wang, B. Tian and J. M. Lu, *Talanta*, 39 (1992) 1273.
32. J. Wang, R. Setiadji, L. Chen, J. M. Lu and S. G. Morton, *Electroanalysis*, 4 (1992) 161.
33. F. R. Bento, M. T. Grassi, A. Sales and L. H. Mascaro, *Int. J. Electrochem. Sci.*, 3 (2008) 1523.

34. F. Faridbod, M. R. Ganjali, B. Larijani, M. Hosseini, K. Alizadeh and P. Norouzi, *Int. J. Electrochem. Sci.*, 4 (2009) 1528.
35. M. R. Ganjali, N. Davarkhah, H. Ganjali, B. Larijani, P. Norouzi and M. Hossieni, *Int. J. Electrochem. Sci.*, 4 (2009) 762.
36. R. J. Mascarenhas, A. K. Satpati, S. Yellappa, B. S. Sherigara and A. K. Bopiah, *Anal. Sci.*, 22 (2006) 871.
37. P. C. do Nascimento, M. D. Marques, M. Hilgemann, L. M. de Carvalho, D. Bohrer, S. G. Pomblum and S. Schirmer, *Anal. Lett.*, 39 (2006) 777.
38. N. A. F. Silva, M. I. Lopes, R. A. E. Leitao, H. F. A. Silva and M. J. Matos, *J. Braz. Chem. Soc.*, 16 (2005) 1275.
39. R. M. Palmieri, L. La Pera, G. Di Bella and G. Dugo, *Chemosphere*, 59 (2005) 1161.
40. C. Locatelli, *Electroanalysis*, 16 (2004) 1478.
41. C. Locatelli and G. Torsi, *Microchem J.*, 78 (2004) 175.
42. D. Monticelli, E. Ciceri and C. Dossi, *Anal. Chim. Acta*, 594 (2007) 192.
43. D. Huska, V. Adam, P. Babula, J. Hrabeta, M. Stiborova, T. Eckschlager, L. Trnkova and R. Kizek, *Electroanalysis*, 21 (2009) 487.
44. E. Palecek, *Nature*, 188 (1960) 656.
45. E. Palecek and J. Doskocil, *Anal. Biochem.*, 60 (1974) 518.
46. Y. M. Temmerk, P. Valenta and H. W. Nurnberg, *Bioelectrochem. Bioenerg.*, 7 (1980) 705.
47. P. Valenta, H. W. Nurnberg and D. Krznaric, *Bioelectrochem. Bioenerg.*, 3 (1976) 418.
48. B. Malfoy, J. M. Sequaris, P. Valenta and H. W. Nurnberg, *Bioelectrochem. Bioenerg.*, 3 (1976) 440.
49. J. M. Sequaris, B. Malfoy, P. Valenta and H. W. Nurnberg, *Bioelectrochem. Bioenerg.*, 3 (1976) 461.
50. P. Valenta, H. W. Nurnberg and P. Klahre, *Bioelectrochem. Bioenerg.*, 2 (1975) 204.
51. P. Valenta, H. W. Nurnberg and P. Klahre, *Bioelectrochem. Bioenerg.*, 2 (1975) 245.
52. M. Studnickova, L. Trnkova, J. Zetek and Z. Glatz, *Bioelectrochem. Bioenerg.*, 21 (1989) 83.
53. E. Palecek, F. Jelen and L. Trnkova, *Gen. Physiol. Biophys.*, 5 (1986) 315.
54. L. Trnkova, M. Studnickova and E. Palecek, *Bioelectrochem. Bioenerg.*, 7 (1980) 643.
55. V. Brabec and Z. Balcarova, *Bioelectrochem. Bioenerg.*, 9 (1982) 245.
56. V. Brabec and J. Koudelka, *Stud. Biophys.*, 81 (1980) 99.
57. V. Brabec, *Biopolymers*, 18 (1979) 2397.
58. A. M. Oliveira-Brett, J. A. P. Piedade, L. A. Silva and V. C. Diculescu, *Anal. Biochem.*, 332 (2004) 321.
59. A. M. Oliveira-Brett, V. Diculescu and J. A. P. Piedade, *Bioelectrochemistry*, 55 (2002) 61.
60. A. M. Oliveira-Brett, L. A. da Silva and C. M. A. Brett, *Langmuir*, 18 (2002) 2326.
61. M. Tichoniuk, M. Ligaj and M. Filipiak, *Sensors*, 8 (2008) 2118.
62. I. Stempkowska, M. Liga, J. Jasnowska, J. Langer and M. Filipiak, *Bioelectrochemistry*, 70 (2007) 488.
63. P. Hodek, P. Hanustiak, J. Krizkova, R. Mikelova, S. Krizkova, M. Stiborova, L. Trnkova, A. Horna, M. Beklova and R. Kizek, *Neuroendocrinol. Lett.*, 27 (2006) 14.
64. V. C. Diculescu, M. Vivan and A. M. O. Brett, *Electroanalysis*, 18 (2006) 1963.
65. V. C. Diculescu, M. Vivan and A. M. O. Brett, *Electroanalysis*, 18 (2006) 1800.
66. V. C. Diculescu, M. Vivan and A. M. O. Brett, *Electroanalysis*, 18 (2006) 1808.
67. G. Chatelain, C. Chaix, H. Brisset, C. Moustrou, F. Fages and B. Mandrand, *Sens. Actuator B-Chem.*, 132 (2007) 439.
68. X. Z. Zhang, K. Jiao and X. L. Wang, *Electroanalysis*, 20 (2008) 1361.
69. F. Wei, J. H. Wang, W. Liao, B. G. Zimmermann, D. T. Wong and C. M. Ho, *Nucleic Acids Res.*, 36 (2008) 7.
70. F. Valentini and G. Palleschi, *Anal. Lett.*, 41 (2008) 479.

71. S. Reisberg, L. A. Dang, Q. A. Nguyen, B. Piro, V. Noel, P. E. Nielsen, L. A. Le and M. C. Pham, *Talanta*, 76 (2008) 206.
72. M. Seidel and R. Niessner, *Anal. Bioanal. Chem.*, 391 (2008) 1521.
73. K. F. Chow, F. Mavre and R. M. Crooks, *J. Am. Chem. Soc.*, 130 (2008) 7544.
74. D. Kwon, K. Kim and J. Kwak, *Electroanalysis*, 20 (2008) 1204.
75. G. Xu, J. Fan and K. Jiao, *Electroanalysis*, 20 (2008) 1209.
76. H. Y. Ma, L. P. Zhang, Y. Pan, K. Y. Zhang and Y. Z. Zhang, *Electroanalysis*, 20 (2008) 1220.
77. A. Bonanni, D. Calvo and M. del Valle, *Electroanalysis*, 20 (2008) 941.
78. T. R. R. Naik and H. S. B. Naik, *Int. J. Electrochem. Sci.*, 3 (2008) 409.
79. J. B. Raoof, M. S. Hejazi, R. Ojani and E. H. Asl, *Int. J. Electrochem. Sci.*, 4 (2009) 1436.
80. M. Heyrovsky, *Electroanalysis*, 16 (2004) 1067.
81. R. Brdicka, *Coll. Czech. Chem. Commun.*, 2 (1930) 489.
82. R. Brdicka, *Coll. Czech. Chem. Commun.*, 5 (1933) 148.
83. R. Brdicka, *Coll. Czech. Chem. Commun.*, 5 (1933) 112.
84. R. Brdicka, *Coll. Czech. Chem. Commun.*, 5 (1933) 238.
85. R. Brdicka, *Nature*, 139 (1937) 1020.
86. R. Brdicka, *Nature*, 139 (1937) 330.
87. J. Heyrovsky, *Nature*, 142 (1938) 317.
88. R. Brdicka, *Nature*, 139 (1937) 1020.
89. R. Kizek, J. Vacek, L. Trnková, B. Klejdus and L. Havel, *Chem. Listy*, 98 (2004) 160.
90. R. W. Olafson and R. G. Sim, *Anal. Biochem.*, 100 (1979) 343.
91. J. A. J. Thompson and R. P. Cosson, *Marine Environ. Res.*, 11 (1984) 137.
92. R. W. Olafson, *Bioelectrochem. Bioenerg.*, 19 (1988) 111.
93. R. W. Olafson and P. E. Olsson, *Methods Enzymol.*, 205 (1991) 205.
94. M. Dabrio, A. R. Rodríguez, G. Bordin, M. J. Bebianno, M. De Ley, I. Šestáková, M. Vašák and M. Nordberg, *J. Inorg. Biochem.*, 88 (2002) 123.
95. M. Erk, D. Ivanković, B. Raspor and J. Pavičić, *Talanta*, 57 (2002) 1211.
96. L. Trnkova, R. Kizek and J. Vacek, *Bioelectrochemistry*, 56 (2002) 57.
97. M. Strouhal, R. Kizek, J. Vacek, L. Trnkova and M. Nemec, *Bioelectrochemistry*, 60 (2003) 29.
98. R. Prusa, O. Blastik, D. Potesil, L. Trnkova, J. Zehnalek, V. Adam, J. Petrlova, F. Jelen and R. Kizek, *Clin. Chem.*, 51 (2005) A.
99. I. Sestakova and T. Navratil, *Bioinorg. Chem. Appl.*, 3 (2005) 43.
100. J. Szpunar, *Analyst*, 130 (2005) 442.
101. J. Petrlova, D. Potesil, R. Mikelova, O. Blastik, V. Adam, L. Trnkova, F. Jelen, R. Prusa, J. Kukacka and R. Kizek, *Electrochim. Acta*, 51 (2006) 5112.
102. V. Adam, S. Krizkova, O. Zitka, L. Trnkova, J. Petrlova, M. Beklova and R. Kizek, *Electroanalysis*, 19 (2007) 339.
103. V. Adam, J. Baloun, I. Fabrik, L. Trnkova and R. Kizek, *Sensors*, 8 (2008) 2293.
104. V. Adam, O. Blastik, S. Krizkova, P. Lubal, J. Kukacka, R. Prusa and R. Kizek, *Chem. Listy*, 102 (2008) 51.
105. S. Krizkova, I. Fabrik, V. Adam, J. Kukacka, R. Prusa, G. J. Chavis, L. Trnkova, J. Strnadel, V. Horak and R. Kizek, *Sensors*, 8 (2008) 3106.
106. V. Diopan, V. Shestivska, V. Adam, T. Macek, M. Mackova, L. Havel and R. Kizek, *Plant. Cell. Tiss. Org.*, 94 (2008) 291.
107. I. Fabrik, Z. Ruferova, K. Hilscherova, V. Adam, L. Trnkova and R. Kizek, *Sensors*, 8 (2008) 4081.
108. I. Fabrik, Z. Svobodova, V. Adam, S. Krizkova, L. Trnkova, M. Beklova, M. Rodina and R. Kizek, *J. Appl. Ichthyol.*, 24 (2008) 522.

109. K. Stejskal, S. Krizkova, V. Adam, B. Sures, L. Trnkova, J. Zehnalek, J. Hubalek, M. Beklova, P. Hanustiak, Z. Svobodova, A. Horna and R. Kizek, *IEEE Sens. J.*, 8 (2008) 1578.
110. D. Huska, S. Krizkova, M. Beklova, L. Havel, J. Zehnalek, V. Diopan, V. Adam, L. Zeman, P. Babula and R. Kizek, *Sensors*, 8 (2008) 1039.
111. V. Adam, M. Beklova, J. Pikula, J. Hubalek, L. Trnkova and R. Kizek, *Sensors*, 7 (2007) 2419.
112. J. Kukacka, D. Vajtr, D. Huska, R. Prusa, L. Houstava, F. Samal, V. Diopan, K. Kotaska and R. Kizek, *Neuroendocrinol. Lett.*, 27 (2006) 116.
113. P. Ryant, E. Dolezelova, I. Fabrik, J. Baloun, V. Adam, P. Babula and R. Kizek, *Sensors*, 8 (2008) 3165.
114. V. Supalkova, M. Beklova, J. Baloun, C. Singer, B. Sures, V. Adam, D. Huska, J. Pikula, L. Rauscherova, L. Havel, J. Zehnalek and R. Kizek, *Bioelectrochemistry*, 72 (2008) 59.
115. J. Vacek, J. Petrek, R. Kizek, L. Havel, B. Klejdus, L. Trnkova and F. Jelen, *Bioelectrochemistry*, 63 (2004) 347.
116. M. El Hourch, A. Dudoit and J. C. Amiard, *Anal. Bioanal. Chem.*, 378 (2004) 776.
117. B. Raspor, *J. Electroanal. Chem.*, 503 (2001) 159.
118. B. Raspor, M. Paic and M. Erk, *Talanta*, 55 (2001) 109.
119. B. Raspor and J. Pavicic, *Fres. J. Anal. Chem.*, 354 (1995) 529.
120. V. Dorcak and I. Sestakova, *Bioelectrochemistry*, 68 (2006) 14.
121. M. Fojta, M. Fojtova, L. Havran, H. Pivonkova, V. Dorcak and I. Sestakova, *Anal. Chim. Acta*, 558 (2006) 171.
122. V. Ostatna, B. Uslu, B. Dogan, S. Ozkan and E. Palecek, *J. Electroanal. Chem.*, 593 (2006) 172.
123. D. Huska, V. Adam, O. Zitka, J. Kukacka, R. Prusa and R. Kizek, *Electroanalysis*, 21 (2009) 536.
124. R. Kizek, L. Trnkova and E. Palecek, *Anal. Chem.*, 73 (2001) 4801.
125. T. Eckschlager, V. Adam, J. Hrabeta, K. Figova and R. Kizek, *Curr. Protein Pept. Sci.*, 10 (2009) in press.
126. T. Kimura and N. Itoh, *J. Health Sci.*, 54 (2008) 251.
127. M. Dutsch-Wicherek, J. Sikora and R. Tomaszewska, *Front. Biosci.*, 13 (2008) 4029.
128. M. Penkowa, *Febs J.*, 273 (2006) 1857.
129. J. C. Amiard, C. Amiard-Triquet, S. Barka, J. Pellerin and P. S. Rainbow, *Aquat. Toxicol*, 76 (2006) 160.
130. P. Coyle, J. C. Philcox, L. C. Carey and A. M. Rofe, *Cell. Mol. Life Sci.*, 59 (2002) 627.
131. G. Roesijadi, *Cell. Mol. Biol.*, 46 (2000) 393.
132. C. D. Klaassen, J. Liu and S. Choudhuri, *Annu. Rev. Pharmacol. Toxicol.*, 39 (1999) 267.
133. S. G. Bell and B. L. Vallee, *ChemBioChem*, 10 (2009) 55.
134. C. H. Kim, J. H. Kim, J. Lee and Y. S. Ahn, *Toxicol. Appl. Pharmacol.*, 190 (2003) 189.
135. C. Cobbett and P. Goldsbrough, *Annu. Rev. Plant Biol.*, 53 (2002) 159.
136. F. Otsuka, *J. Health Sci.*, 50 (2004) 332.
137. D. H. Hamer, *Annu. Rev. Biochem.*, 55 (1986) 913.
138. S. Krizkova, I. Fabrik, V. Adam, J. Hrabeta, T. Eckschlager and R. Kizek, *Bratisl. Med. J.-Bratisl. Lek. Listy*, 110 (2009) 93.
139. W. Qu, R. Fuquay, T. Sakurai and M. P. Waalkes, *Mol. Carcinog.*, 45 (2006) 561.
140. J. Liu, Y. P. Liu, R. A. Goyer, W. Achanzar and M. P. Waalkes, *Toxicol. Sci.*, 55 (2000) 460.
141. N. Futakawa, M. Kondoh, S. Ueda, M. Higashimoto, M. Takiguchi, S. Suzuki and M. Sato, *Biol. Pharm. Bull.*, 29 (2006) 2016.
142. M. Sato and M. Kondoh, *Tohoku J. Exp. Med.*, 196 (2001) 9.
143. M. Kondoh, Y. Inoue, S. Atagi, N. Futakawa, M. Higashimoto and M. Sato, *Life Sci.*, 69 (2001) 2137.

144. S. E. Theocharis, A. P. Margeli, J. T. Klijanienko and G. P. Kouraklis, *Histopathology*, 45 (2004) 103.
145. T. Goldmann, D. Ribbert, L. Suter, M. Brode and F. Otto, *J. Exp. Clin. Cancer Res.*, 17 (1998) 483.
146. R. X. Jin, J. X. Huang, P. H. Tan and B. H. Bay, *Pathol. Oncol. Res.*, 10 (2004) 74.
147. D. Mitropoulos, A. Kyroudi-Voulgari, S. Theocharis, E. Serafetinides, E. Moraitis, A. Zervas and C. Kittas, *World J. Gastroenterol.*, 3 (2005) 5.
148. G. Ohshio, T. Imamura, N. Okada, Z. H. Wang, K. Yamaki, T. Kyogoku, H. Suwa, H. Yamabe and M. Imamura, *J. Cancer Res. Clin. Oncol.*, 122 (1996) 351.
149. K. Rossen, T. Haerslev, K. HouJensen and G. K. Jacobsen, *Apmis*, 105 (1997) 35.
150. A. Sauerbrey, F. Zintl and M. Volm, *Ann. Hematol.*, 69 (1994) 111.
151. V. K. Shukla, N. C. Aryya, A. Pitale, M. Pandey, V. K. Dixit, C. D. Reddy and A. Gautam, *Histopathology*, 33 (1998) 154.
152. K. Sugita, O. Yamamoto and M. Asahi, *Am. J. Dermatopathol.*, 23 (2001) 29.
153. P. Surowiak, V. Materna, I. Kaplenko, M. Spaczynski, M. Dietel, H. Lage and M. Zabel, *Virchows Arch.*, 447 (2005) 626.
154. B. Zelger, A. Hittmair, M. Schir, C. Ofner, D. Ofner, P. O. Fritsch, W. Bocker, B. Jasani and K. W. Schmid, *Histopathology*, 23 (1993) 257.
155. V. Adam, I. Fabrik, T. Eckschlager, M. Stiborova, L. Trnkova and R. Kizek, *TRAC-Trends Anal. Chem.*, 29 (2010) 409.
156. B. Rosenberg, L. Van Camp and T. Krigas, *Nature*, 205 (1965) 698.
157. M. A. Fuertes, J. Castilla, C. Alonso and J. M. Perez, *Curr. Med. Chem.*, 10 (2003) 257.
158. S. T. Ong and N. J. Vogelzang, *J. Clin. Oncol.*, 14 (1996) 1007.
159. R. B. Weiss and M. C. Christian, *Drugs*, 46 (1993) 360.
160. G. K. Dy, L. M. Bruzek, G. A. Croghan, S. Mandrekar, C. Erlichman, P. Peethambaram, H. C. Pilot, L. J. Hanson, J. M. Reid, A. Furth, S. Cheng, R. E. Martell, S. H. Kaufmann and A. A. Adjei, *Clin. Cancer Res.*, 11 (2005) 1877.
161. R. S. Go and A. A. Adjei, *J. Clin. Oncol.*, 17 (1999) 409.
162. H. T. See, R. S. Freedman, A. P. Kudelka, T. W. Burke, D. M. Gershenson, S. Tangjitgamol and J. J. Kavanagh, *Int. J. Gynecol. Cancer*, 15 (2005) 209.
163. D. Arango, A. J. Wilson, Q. Shi, G. A. Corner, M. J. Aranes, C. Nicholas, M. Lesser, J. M. Mariadason and L. H. Augenlicht, *Brit. J. Cancer*, 91 (2004) 1931.
164. A. Grothey and R. M. Goldberg, *Expert Opin. Pharmaco.*, 5 (2004) 2159.
165. E. Raymond, S. Faivre, S. Chaney, J. Woynarowski and E. Cvitkovic, *Mol. Cancer. Ther.*, 1 (2002) 227.
166. S. G. Chaney, S. L. Campbell, B. Temple, E. Bassett, Y. B. Wu and M. Faldu, *J. Inorg. Biochem.*, 98 (2004) 1551.
167. M. Kartalou and J. M. Essigmann, *Mutat. Res-Fund. Mol. M.*, 478 (2001) 1.
168. M. Kartalou and J. M. Essigmann, *Mutat. Res-Fund. Mol. M.*, 478 (2001) 23.
169. P. Borst, R. Evers, M. Kool and J. Wijnholds, *J. Natl. Cancer Inst.*, 92 (2000) 1295.

© 2010 by ESG (www.electrochemsci.org)