Nanotechnologies for society. New designs and applications of nanosensors and nanobiosensors in medicine and environmental analysis

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Abstract: One of the areas rapidly gaining popularity in all parts of scientific research is Nanotechnology. The pioneering mechanical and electrical engineering has been overtaken by life sciences including chemistry and biology as well as biophysics and biomedicine. The attractiveness of

nanodimensions increases in combination with possible life saving function. Therefore the opportunity of application of nano-size objects such as nanoparticles of various types and nature as drug carriers and/or tumour detecting agents is of great interest and number of research publications devoted to this field is increasing rapidly in last years. The complexity of this field requires combined effort of multidisciplinary teams joining researches with diverse expertise such as engineers, physicists, biologists, chemists as well as medical doctors and clinicians. In this review, short summary of development in the field on nanoparticles and nano-based electrodes as well as new software for treatment of data obtained from the electrodes is shown.

Keywords: paramagnetic bead; particles; electrode; streptavidin-avidin technology; electrochemical detection; voltammetry; glutathione; quantum dot.

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1 Past, present and future of electrochemistry in medicine

Cancer, 'plague of the world', is a word, which presents a threat to humans as well as a subject of investigation for broaden scientific public (almost 90,000 papers appearing in 2009 in Web of Science included 'cancer*' within article titles, keywords or abstracts, particularly, almost 100 of them has been published in *Nature*). The success of treatment of the disease depends on many factors such as prevention, early and sensitive diagnostics. That means the sooner a cancer is detected, the better the chances to treat it successfully. A very interesting idea how we could diagnose a tumour disease was created 70 years ago in Czechoslovakia of that time.

Rudolf Brdicka, born more than 100 years ago, published in *Nature* in 1937 his discoveries about using polarography to diagnose a tumour disease [1,2]. He found out a sensitive polarographic 'protein effect', conspicuously exhibited by serum, which he explained as due to the catalytic activity of the sulphydryl groups of proteins. The 'protein effect' consisting of the appearance of characteristic wave on the current voltage curve, has been always found larger in normal serum sample than when the same procedure with cancer serum was used [1,2]. One year later, Brdicka's colleague Jaroslav Heyrovsky, holder of Nobel Prize in Chemistry 1959, published a paper in the same journal, where he summarised results obtained in the field of Polarographic Research on Cancer [3]. Heyrovsky believed that this field of study would be of general interest of many scientific groups around the world. But he was mistaken. Since then, electrochemistry has been slowly disappearing from tumour disease diagnostics due to using modern techniques of analytical chemistry and molecular biology.

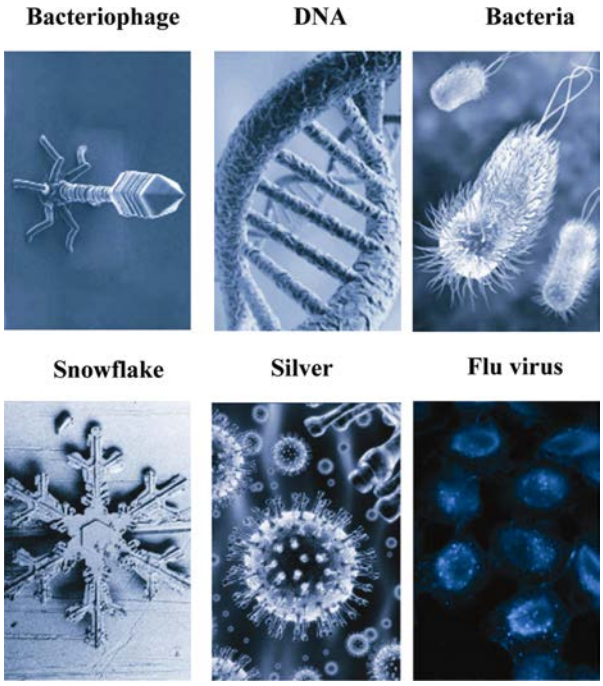
Could electrochemistry be used in 21st century besides robust techniques such as mass spectrometry, nuclear magnetic resonance and many others? You could raise an objection that this 'old technique' cannot compete with these modern methods. But contrary is the case. The obtained results show that electrochemical techniques could be brought back to the investigation of cancer and could enable easy, fast and low cost cancer diagnostics. It is possible that a wish of Jaroslav Heyrovsky and his follower and colleague Rudolf Brdicka will be fulfilled and this field of cancer study will be developed

intensively not only in Czech laboratories but also around the world with great help of nanotechnology. In this paper, we aim our attention at showing various branches of bio-research, in which nanotechnology can be successfully employed. We mainly focus on isolation and detection of peptides, proteins and nucleic acids. Besides bio-research, fabrication of various sensors is discussed. In addition, the advantages of application of elimination voltammetry in nano-research are shown.

2 Nanotechnologies in medicine

Molecular nanotechnology appears from measuring scale, in which is operating [4–7]. This measuring scale represents hundredths to thousandths nanometres (in principle size of molecules). We can imagine that three or four atoms fit into one nanometre. In the case of imagination of objects of nanotechnologies, we can say that size of virus is about 100 nm (Figure 1) and diameter of human hair is about 200 µm. Basic structural element of molecular nanotechnology is atom. By various organisations of atoms, properties of resulting product, such as elasticity, plasticity, strength, or conductibility can be modified. Typical example is rebuilding of atoms in graphite into very rigid structure of diamante. Modification and reorganising of atoms of quartz sand may lead to formation of silicon plate – basic intermediate product for manufacturing of semiconductor components.

Figure 1 Objects with dimensions from units to thousands of nm (see online version for colours)



Nanomaterials are now being designed to aid the transport of diagnostic or therapeutic agents through biological barriers; to gain access to molecules; to mediate molecular

interactions; and to detect molecular changes in a sensitive, highthroughput manner. In contrast to macroscopic materials, nanomaterials have a high ratio of surface area to volume as well as tuneable optical, electronic, magnetic, and biological properties, and they can be engineered to have different sizes, shapes, chemical compositions, surface chemical characteristics, and hollow or solid structures [8–10]. These properties are being incorporated into new generations of drug-delivery vehicles, contrast agents, and diagnostic devices, some of which are currently undergoing clinical investigation or have been approved by the Food and Drug Administration (FDA) for use in humans. It is not surprise that these great successes have led to forming of new scientific branches called 'Nanomedicine' [11]. Nanomedicine, which one the newest member of branches in molecular nanotechnology, can be defined as monitoring, repairing, building, and control of biological systems on a molecular level, which is carried out by nanocomponents and nanosystems. Dramatic advancement of these technologies can be expected, especially in diagnostics of diseases in their early stages [12–16]. Besides this, there are currently used bio-implants based on nanotechnologies in the field of skin regeneration (special polymers, silver nanoparticles etc.). In the approaches with real future perspectives, we may see therapeutic abilities of nanorobots in microsurgery and in treatment of various types of diseases, such as coronary thrombosis or malignant tumours. We can expect that medicinal nanorobots with size up to 1000 nm can be injected into human body (some milliards of nanorobots correspond to one millilitre). There they can help the immune system to participate in processes of metabolism, perform repairing operations, eventually cluster together into higher structures and form more complicated and effective repairing and protective systems. Small size of nanorobots is accentuated, especially because of the ability to enter all organs in human body. These technological arrangements will be equipped with suitable drive, control unit (computer), and supply of a drug. It is warranted that used arrangements will be programmable (in human body by the help of wire-less communication). Therapeutic profile may be significantly broadened for common bacterial and viral infections (end of colds and similar infections that are trivial but significantly affect productivity). Nanorobots could be used also in cryobiology and cryonics, science branches interested in possibilities of preservation and conservation of tissues in order of future use. Basic components – nanocomponents, constructed enzymes, nucleic acids (small interfering RNA) and other biological products will be soon used in medicinal applications, nevertheless, multiyear research and testing procedures will precede to this usage. Besides direct therapeutic effect, nanotechnologies and nano-based instruments can be also used as tools for sensing and/or biosensing diseases. These are ambitious goals of research and development at the beginning of the 21st century to be achieved. Some of the possibilities of technological progress are discussed in this review. The aim of our work is to establish and develop electrochemistry coupled with nano-based materials and instruments for biological applications.

3 Nanoparticles

3.1 Magnetisable nanoparticles

Paramagnetic and superparamagnetic nanoparticles (MNPs) are of great interest due to their unique properties. These particles become magnetic under a strong magnetic

field but retain no residual magnetism in the absence of magnetic field. These particles do not interact with each other when removed from the magnetic field. Especially in medicine and pharmaceutics, application of MNPs is promising. The possibilities of their modification by biologically active compounds are very interesting using them in controlled drug delivery systems [17], as agents in magnetic resonance imaging and for magnetic-induced tumour treatment via hyperthermia [18].

Several methods are generally employed for iron oxide NPs preparation including co-precipitation [19,20], which is preferred due to its simplicity and thermal de-composition, which seems to give the best control of NPs size and morphology. The resulting physico-chemical properties of nanosized magnetic product obviously depend strongly on fabricating conditions, especially on material origin, concentration and pH of solution as well as on the way of thermal treatment (annealing temperature, atmosphere and rate of heating/cooling). It was found that ferromagnetic low temperature phase γ-Fe_2O_3 can be easily transformed to antiferromagnetic more stable phase α-Fe_2O_3 when the temperature exceeds 500°C [21]. The particle size plays also a crucial role. Typical particle sizes for the ferro- to superparamagnetic phase transformation are between 10 nm and 20 nm for oxides and 1–3 nm for metals [22]. We utilised process given by Drbohlavova et al. for fabricating un-modified Fe_2O_3 magnetic particles [23]. These particles revealed the magnetic properties in water suspension when external magnetic field was applied (Figure 2(A)). Scanning electron microscopic (SEM) analysis demonstrated that the nanoparticles were generally spherical in the shape and their particle size was about 70 nm (Figure 2(B)). Due to possible application of these particles for biomedical purposes, further characterisation was needed. Therefore, the prepared unmodified Fe_2O_3 powder consisted of 60% iron oxide, 30% of sylvite (KCl) and small portion of amorphous phase was further analysed by Mössbauer spectrum as well as X-Ray diffraction (XRD) according to [23]. Since X-ray powder diffraction cannot distinguish between maghemite and magnetite nanoparticles, Mössbauer spectroscopy (Figure 2(C)) analysis was performed to distinguish these crystallographic modifications. The results showed the maghemite phase being dominant (36%) with a small amount of magnetite Fe_3O_4.

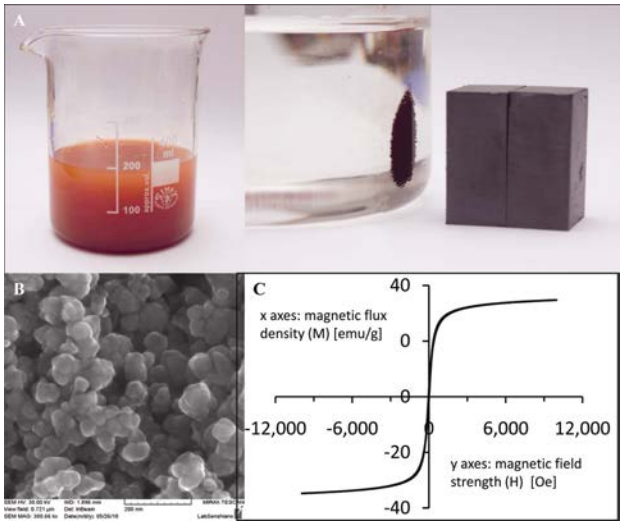
Functionalisation of magnetic NPs with amino group, silica, polymer, various surfactants or other organic compounds is usually provided in order to achieve better physical and chemical properties [24]. Moreover, the core/shell structures of magnetic NPs have the advantages of good dispersion, high stability against oxidation and appreciable amount of drug can be loaded to the polymer shell [25,26]. Furthermore, lots of functional groups from polymers on the surface can be used for further functionalisation to get various properties. It was found the surface functionalisation plays also the key role in nanoparticle toxicity [27,28]. Based on this fact the above prepared particles were conjugated by streptavidin according to procedure described by Liu et al. [29]. Fe_2O_3 magnetic nanoparticles were successfully modified with streptavidin due to its extremely high affinity interaction with biotin. Because biotin can be easily conjugated to many different diagnostic reagents, a variety of assays for different analytes in several formats can be developed [30].

3.2 Light emitting nanoparticles

Quantum dots (QDs) from semiconductor materials are very promising candidates for medicinal purposes, mainly as biosensors and labels in biological imaging [31–33].

QDs exhibit exceptional physical and optical properties and moreover, there is a possibility of various biomolecules attaching to their surface, which allows the detection of DNA and proteins [34–36]. Majority of sensing techniques employing colloidal form of QDs in biological systems are applied in solution [37–39]. To date, two original approaches have been reported for the synthesis of colloidal QDs. The organometallic way produces QDs, which are generally capped with hydrophobic ligands (e.g., trioctylphosphine oxide – TOPO) and hence cannot be directly employed in bioapplications. The second way is the aqueous synthesis route, producing QDs with excellent water solubility, biological compatibility, and stability. Thiol-capped QDs could be prepared directly in aqueous solution with thiols as efficient stabilisers. Cystein [40,41], mercaptopropionic acid [42,43] and reduced glutathione (GSH) [44–46] are the most popular coatings among thiols, however quantum yields only up to 10% were typically obtained without any following treatment [47]. Capillary electrophoresis laser induced fluorescence was used for monitoring of the stability of glutathionylitated-QDs during storage in the dark at 4°C. Moreover, the glutathione was conjugated with biotin (B) due to possibility to use them in avidin-biotin technologies. QDs were sampled per six hours and the signal height of B-GSH-QDs was observed. The height of the signal steadily decreased with increasing time of the storage. After three days, the height decreased for more than 80%. Based on the results obtained it can be concluded that the B-GSH-QDs are less stable in comparison with the QDs coated with unmodified reduced glutathione (GSH-QDs) created according to the same procedure [48,49].

Figure 2 (A) Magnetic properties illustration of Fe_2O_3 nanoparticles dispersed in water. (B) Scanning electron microscope image of Fe_2O_3 (Tescan, Czech Republic). (C) Hysteresis loop of Fe_2O_3 nanoparticles. The structure of the samples was checked by X-ray diffraction (XRD) using X'PERT diffractometer from PANalytical (The Netherlands) and CoKα radiation with qualitative analysis carried out by HighScore software and the JCPDS PDF-2 database. For a quantitative analysis of the XRD patterns, we took HighScore plus with Rietveld structural models based on the ICSD database. ^{57}Fe Mössbauer spectra used for phase analysis were measured using ^{57}Co/Rh source in standard transmission at room temperature and in a cryostat down to 28 K. Spectrum calibrations were done using α-Fe standard (see online version for colours)



Nevertheless, there is also a demand for QDs in planar form (so-called lab-on-chip) deposited on various solid surfaces. This new approach seems to be very interesting and promising for *in situ* biosensing, mainly due to the simplicity of detection, for example, as a sensor array for mass screening [18]. However, only few papers concerning this topic can be found in literature [50,51].

The deposited QDs are mostly fabricated through traditional top-down patterning methods like epitaxy or lithographic techniques (mainly photolithography and e-beam lithography), which are expensive and time-consuming. Hence new more sophisticated techniques for QDs synthesis should be studied regarding the nanosensors reproducibility and price availability [52]. Recently, the scientists revealed the template-based methods as a suitable way for nanostructured material synthesis. Concerning the template, the most extensively studied materials are porous alumina, polymer gel, surfactant, activated carbon and carbon fibre [53].

4 Magnetisable particles for isolation of nucleic acids and proteins

4.1 Magnetisable particles for isolation of nucleic acids

Extraction of nucleic acids is the most important method used in molecular biology. It is a starting point for many techniques such as detection, amplification, cloning, sequencing, electrophoresis, hybridisation, cDNA synthesis, gene therapy, which is the default technique for the study of biological patterns in an organism, organ, tissue or individual cells and organelles [54,55]. Four basic steps play important roles in the isolation of nucleic acids as

- effective disruption of cells or tissues,
- the denaturation of complexes,
- inactivation of nucleases – such as RNase for RNA extraction, DNase for DNA extraction,
- getting rid of contaminants.

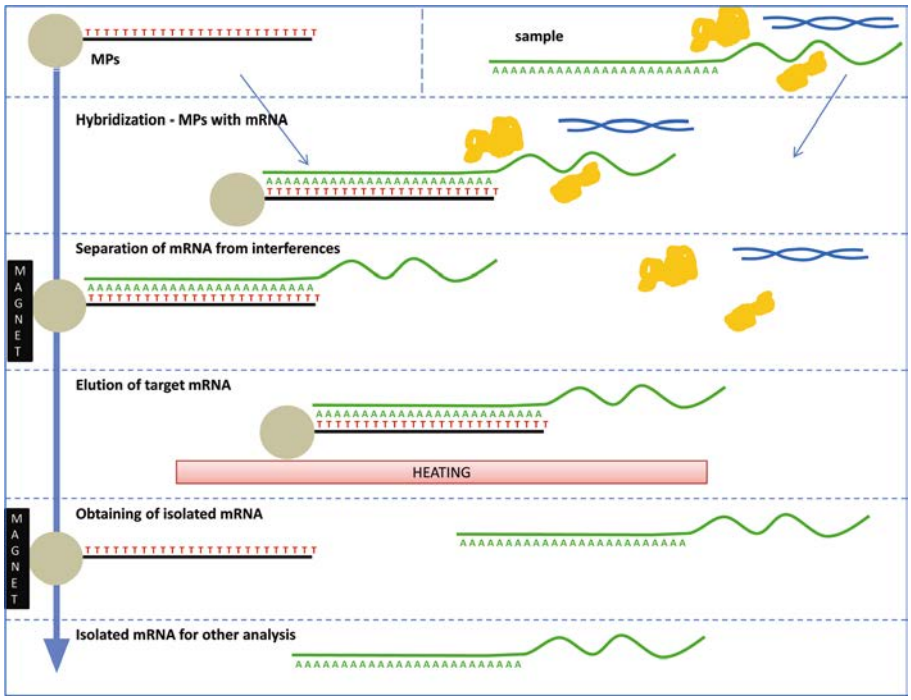
These steps have an impact on the quality and quantity of isolated nucleic acids and consequently directly influence the results of further analysis [54,56]. The standard techniques were time consuming and material-consuming. In addition, the possibility of automation of the standard extraction methods was nearly impossible [55].

In recent years, the conventional methods of extraction of nucleic acids are replacing with an increasingly technology-based paramagnetic or superparamagnetic nano or micro-particles (MPs). MPs react to external magnetic field, thus facilitating the isolation of nucleic acids from biological material and thereby significantly reduce the time of isolation and eliminate the use of organic compounds. Moreover, the whole system can be fully automated [15]. The first magnetic particles to isolate compounds from the solution were used in 1940, when iron oxides were used for the adsorption and removal of dissolved and colloidal substances in biological wastewater treatment [57]. However, the true use of functional magnetic particles for the selective capture of biomolecules has been described in 1974 [57,58]. The current MPs are characterised by several parameters that have a significant role on their effectiveness. In particular, the MPs' uniform size,

surface, their adsorption kinetics, biocompatibility, superparamagnetic properties and magnetic dipole moment. Size of MPs varies in the range from units of nm to units of microns [59].

MPs can be used not only for isolation of total DNA and RNA, but also for isolation specific sequences of nucleic acids [60]. Huska et al. showed the use of commercially available paramagnetic micro-particles for isolation of mRNA as the primary nucleic acid for study of gene expression [15]. The principle of isolation of mRNA using Oligo(deoxythymine)$_{25}$ MPs is based on hybridisation between polyadenylic end (sequence of 50–250 adenines, bearing each mRNA) and sequence of 25 thymine anchored on MPs (Figure 3).

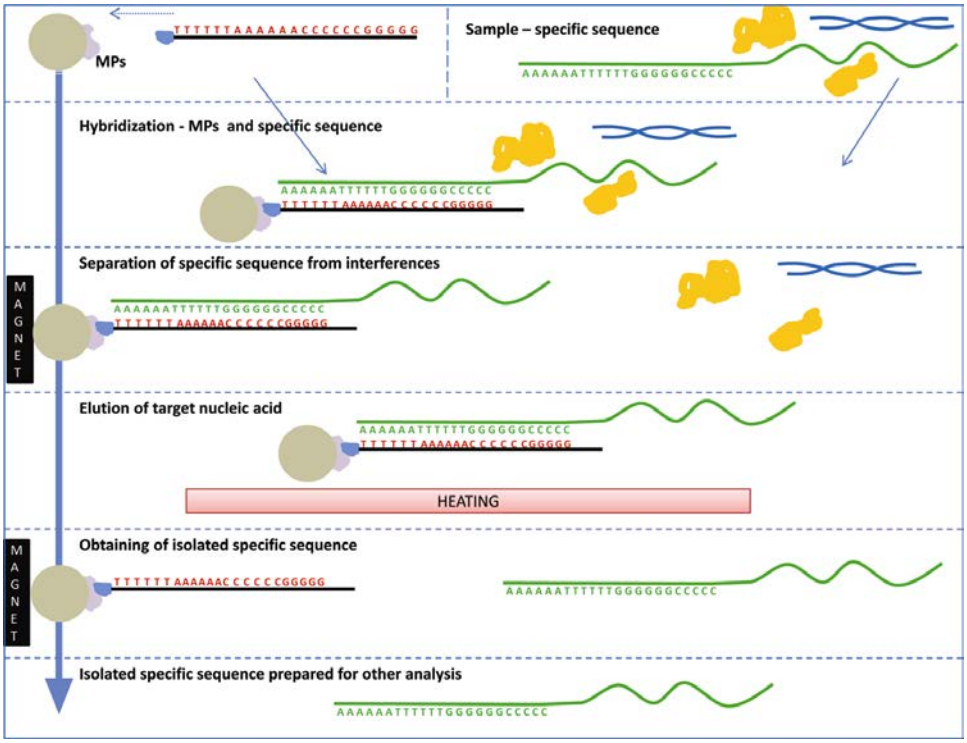
Figure 3 Scheme of mRNA isolation by using of paramagnetic microparticles coated with Oligo(deoxythymine)$_{25}$: (a) washing of the particles, adding of hybridisation solution and a sample; (b) hybridisation of mRNA molecules with strands anchored on the surface of the particles; (c) forcing of the particles by magnetic field and washing the interferences; (d) denaturation of hybridised nucleic acids chains under higher temperature; (e) forcing of the particles by magnetic field and pipetting the target molecules; (f) target nucleic acid is prepared for the following analysis (see online version for colours)



MNPs can be modified by streptavidin, oligonucleotides or using chemical sorption [59]. Streptavidin-coated NPs are suitable for capturing any biotinylated molecules, including nucleic acids [61], aptamers, peptides, proteins, etc. due to high affinity of streptavidin to biotin. The streptavidin/biotin complexes are extremely stable over a wide range of temperature and pH [29]. Amino-functionalised magnetic NPs are also suitable for efficient and direct DNA separation [62]. The particular process consists from several steps. The particles are mixed with a sample. After hybridisation with the targeted

molecule, MPs are anchored to the wall of the test tube by external magnetic field and the remaining solution of interfering substances is removed. By this procedure, we obtain target nucleic acid only, which is ready for further analysis (Figure 4). MPs can standardise procedures for isolation and contribute to an improved study of nucleic acids. MPs may contribute to the construction of entirely new fields of sensors and chips that allow the serial analysis of samples or complex analysis. Great importance is reflected in a rapid and sensitive detection of the presence of viral nucleic acids. Adam et al. showed the use of streptavidin-modified MPs for isolation of viral nucleic acids (Human Immunodeficiency Virus – HIV, influenza virus subtype – H5N1), followed by electrochemical detection [63]. Based on the results from this paper, they synthesised eight specific oligonucleotides for hepatitis B virus (HBV), which were isolated using paramagnetic particles and subsequently detected by cyclic and square wave voltammetry at the hanging mercury drop electrode (HMDE) and solid carbon electrodes modified with nanoparticles. It was necessary to characterise electrochemically the selected oligonucleotides. At HMDE two standard signals as redox signal of adenine and cytosine and oxidative signal of guanine were detected as follows [64].

Figure 4 Scheme of isolation of specific nucleic acids sequence by using of paramagnetic microparticles coated with streptavidin: (a) washing of the particles, adding of anti-sense biotinylated oligonucleotide, adding of hybridisation solution and a sample; (b) hybridisation of target nucleic acids sequence with strands anchored on the surface of the particles; (c) forcing of the particles by magnetic field and washing the interferences; (d) denaturation of hybridised nucleic acids chains under higher temperature; (e) forcing of the particles by magnetic field and pipetting the target molecules; (f) target nucleic acid is prepared for the following analysis (see online version for colours)



However, oligonucleotides gave four signals corresponding to cytosine, adenine, thymine and guanine at carbon electrode. After optimising conditions for the electrochemical detection, calibration curves were measured at HMDE due to higher sensitivity. All studied oligonucleotides gave reduction signals dependent on the concentration of oligonucleotide in solution. The curves were linear with the coefficient of determination higher than $R^2 = 0.99$. The single regression equations are shown in Table 1.

Table 1 Analytical parameters of electrochemical detection of HVB oligonucleotides within concentration interval from1 to 100 ng/ml

VHB	*Sequence VHB (5´-3´)*	*Coefficient of determination (R^2)*	*Calibration curve*
VHB 1	TAGGCAGAGGTGAAAAAGTTGC	0.9903	$y = 1.2100x + 0.1377$
VHB 2	AATTTGGAGCTACTGTGGAGTTACT	0.9908	$y = 0.7403x + 0.0626$
VHB 3	GCAGATGAGAAGGCACAGAC	0.9930	$y = 1.1324x + 0.1445$
VHB 4	GCCACCCAAGGAACAGCTTGGAGG CTTGAA	0.9956	$y = 1.8147x + 0.0640$
VHB 5	GGGAGGAGTTGGGGGAGGAGATTA GGTTAA	0.9905	$y = 0.6946x + 0.0902$
VHB 6	ATGATTAGGCAGAGGT	0.9911	$y = 0.8410x + 0.2126$
VHB 7	GACTGTTTGTTTAAAGACTGGGAGG AGTTGG	0.9974	$y = 0.4425x + 0.0680$
VHB 8	CTTGAACAGTAGGACATGAACATG AGATGATT	0.9943	$y = 0.6734x + 0.0801$

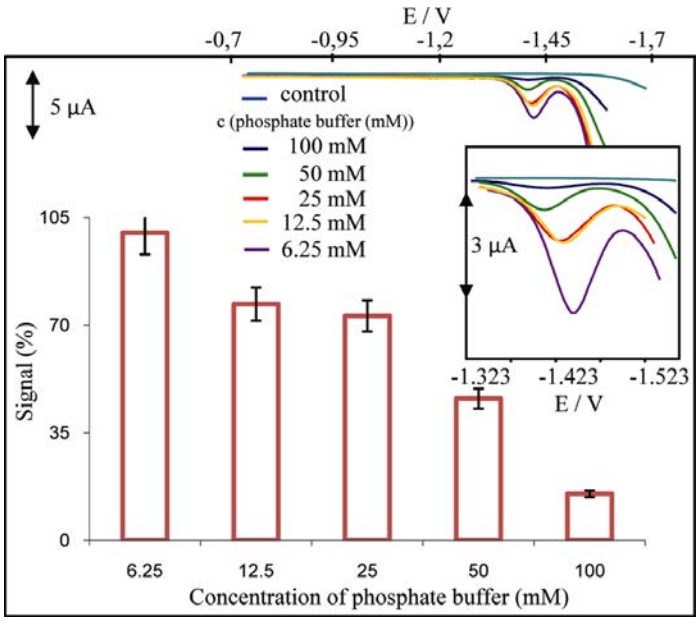
It was also necessary to optimise the conditions for hybridisation of target sequences to ensure the most favourable conditions to establish any real sequence of virus. The most important parameters affecting the bond between the oligonucleotide probe and target sequences of the virus are the hybridisation time and temperature of hybridisation and composition of the hybridisation solution. It follows from the results obtained that the optimal hybridisation temperature is within the range from 10°C to 15°C (under less-stringent conditions) and from 25°C to 30°C (stringent conditions). The optimal hybridisation time was 5 min for less-stringent conditions and 30 min for the stringent conditions. Furthermore, the influence of the concentration of elution solution (6.25, 12.5, 25, 50 and 100 mM of phosphate buffer, $Na_2HPO_4 + NaH_2PO_4$) on the amount of viral target sequences. We found that with the amount of isolated viral nucleic acids increased with increasing concentration of phosphate buffer (Figure 5). Cyclic voltammograms of individual samples are shown in inset in Figure 5.

4.2 Magnetisable particles for isolation of proteins

Magnetisable particles represent new tool in proteomics due to their advanatages including versatility and possibility to be used *in situ*. One of the possible applications includes isolation of metallothioneins (MTs). These proteins belong to group of low molecular mass proteins rich in cysteine. They can be found in cytoplasm, lysosomes, mitochondria and nuclei of cells. MTs participate in intracellular metal

metabolism and detoxification, and also protection against reactive oxygen species [16,65,66]. In addition to these functions, MT play other important biological roles as a reservoir of metals, regulator of transcription, cofactor of enzymes and modulator of cell cycle [67].

Figure 5 The influence of the concentration of elution solution (6.25, 12.5, 25, 50 and 100 mM of phosphate buffer, Na_2HPO_4 + NaH_2PO_4) on the amount of viral target sequences; inset: cyclic voltammograms of individual samples. Electrochemical measurements were performed with AUTOLAB PGS30 Analyser (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. Glassy carbon electrode was used as the auxiliary electrode. For smoothing and baseline correction the software GPES 4.9 supplied by EcoChemie was employed. Cyclic voltammetric parameters were as follows: potential step 5 mV, scan rate 10, 20, 40, 80, 160 and 320 mV/s. The samples measured by square wave voltammetry were deoxygenated prior to measurements by purging with argon (99.999%) saturated with water for 120 s. The temperature of supporting electrolyte was maintained by the flow electrochemical cell coupled with thermostat JULABO F12/ED (Labortechnik GmbH, Germany) (see online version for colours)



4.2.1 Methods for metallothioneins detection

We can divide the techniques for MTs detection into several groups. The first group of methods is based on determination of heavy metal ions in MT molecule. The second group including UV spectrometry, fluorimetry and electrochemistry determines content of –SH groups in MT molecule [16,66,68–86]. The other group includes separation techniques as chromatography and electrophoresis [87,88]. The indirect group of methods is based on detection of gene expression at mRNA level using the specific Taq Man probes for MT genes [89]. The last group of methods for MT detection is based on antigen-antibody interaction. We can use western blotting, immunohistochemistry,

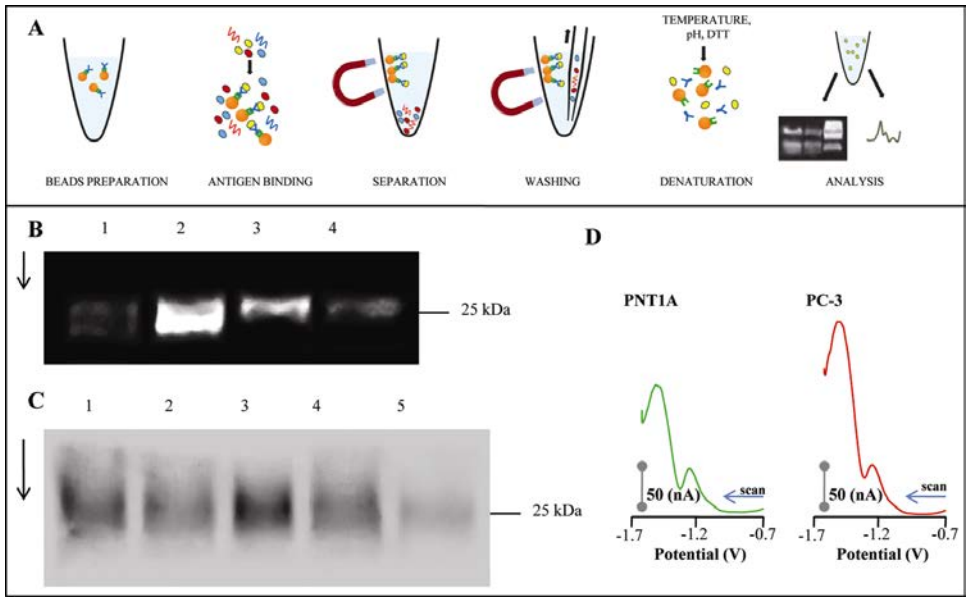
immunofluorescence, radio-immunoanalysis or enzyme-linked immunosorbent assay [66,90]. Immunoprecipitation also belongs to the last group using the ability of antibody (attached at the magnetic beads surface) to bind an antigen (analyte) [91,92]. Magnetic beads-based immunoassay is a relatively new assay configuration where the magnetic particles are the carries of the antibody and can be used for analysis of cell lysates, tissue homogenates, body liquids etc. Paramagnetic particles as immobilising materials are of great interest in the pursuit of increased stability of the surface-bound antibody, improved orientation of the immobilised antibody as well as increase in the protein capturing [92–94]. Recently the particles' size was reduced from micro- to nano-scale, improving the possibility of handling the particles in the suspension [95]. In connection with paramagnetic beads, another very sensitive method, bio-barcode assay, was developed [96]. This assay utilises antibody-coated magnetic beads to capture and concentrate the analytes. The captured analytes are labelled with gold nanoparticles and then with single stranded DNA. Further, we detect the specific DNA sequence which is unique for each protein [97]. A more recent report describes an alternative approach of labelling the analyte after capture onto antibody coated magnetic beads. This approach utilises a biotinylated secondary antibody which is labelled with streptavidin-coated gold nanoparticles and biotinylated barcode DNA [97,98].

4.2.2 Immunoprecipitation of MT

The principle of immunoprecipitation technique used for MT determination is shown in Figure 6(A). Firstly it is necessary to make bond between protein G (at the paramagnetic beads surface) and the antibody. After washing procedure, the antibody against MT 1A/2A is added to DB-G (paramagnetic beads with protein G) in appropriate amount and the mixture is incubated for 30 minutes at room temperature on roller to avoid beads sedimentation to form Ab-DB-G complex. During this incubation the antibody is bind to the Dynabeads via their Fc region. After that, tubes are placed on a magnet, so the beads can migrate to the side of the tube facing the magnet and allow easy removing of the supernatant. Unbound antibody is then removed and Ab-DB-G complex is washed with of phosphate buffer, pH 7.4. Then, fixed amount of sample is added and tubes are kept at room temperature on roller for 60 min. Tubes are placed on the magnet, supernatant is removed, samples are washed with phosphate buffer again for three times and the beads-Ab-protein complex is transferred to clean tubes to avoid cross-reactions. According to the detection method (electrochemistry or electrophoresis), acetate buffer or DTT-loading buffer is used, respectively. For each analyte it is important to optimise the conditions of antibody/sample binding as time, buffer and temperature. In case of MT we tested the lowest concentration of MT which we were able to detect after immunoprecipitation. A concentration scale of MT standard (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was prepared and after binding of 5 µg of antibody diluted in PBS various concentrations of MT from subnanograms to micrograms were measured. After denaturating step of immunoprecipitation, samples were loaded to 10% SDS-PAGE gel and stained by Coomassie blue or by silver. We able to detect tens of nanograms of MT in Coomassie staining procedure and units of nanograms of MT by silver staining similarly as immunochemical detection shown in Figure 6(B). In addition, we attempted to detect MT in patient serum with or without prostate carcinoma (Figure 6(B)). It follows from the results obtained that we were able to determine difference between healthy patient and patient with prostate carcinoma and the values varied from 2.3 µM

MT to 2.8 µM MT in patients and from 0.5 µM MT to 0.8 µM MT in healthy individuals. Following optimisation step was to determine optimal binding time of antigen with MT standards. We found the optimal time of incubation as 60 min (Figure 6(C)). This incubation time was used in the further experiments. To disrupt MT-Ab complex, acetate buffer (100 mM, pH 3.5) and high temperature were used. Besides immune-detection and staining, differential pulse voltammetry was used for detection of MT in cell lysates (Figure 6(D)). Using electrochemical methods we determined recovery factor of above described microseparation process, which was approx. 25% for MT. Manual separation procedure can be further adopted for fully robotised preparation of modification of microparticles and nanoparticles by antibodies and isolation of analytes by the use of robotic system EP Motion Blue.

Figure 6 (A) Scheme of immunoprecipitation. Detection of MT after immunoprecipitation. (B) Western blot and subsequent immunodetection of MT in serum samples. 1 – negative control, 2 – MT standard (50 µg/mL), 3 – serum of patient with prostate cancer, 4 – serum of healthy individual. (C) SDS-PAGE and silver staining of MT – time dependence of antigen binding. 1 – 15 min., 2 – 30 min., 3 – 60 min., 4 – 90 min., 5 – control sample (without antibody). (D) Voltammograms of MT after immunoprecipitation procedure from cell lysates of normal prostate (PNT1A) and tumour prostate (PC3) cells (see online version for colours)



5 Nano-based detection instruments

It is necessary to develop precise analytical methods with low limits of detection for the determination of contaminants harmful to our environment and our health. Such techniques may include methods based on electrochemical redox properties of the analyte. The current trend of analytical techniques and procedures aims at the miniaturisation of all components of such equipment [5,99–110]. Electrodes using different types and modes of electrochemical methods can be easily miniaturised and

fulfil the great demands on this property. Currently, solid carbon paste electrodes (CPE) have many applications. These electrodes provide us with many advantages, ease of manufacturing, possibility of miniaturisation, high electrical and mechanical resistance, easy connection to other circuits, low cost and particularly the possible modification of the detection surface [111–118].

5.1 Nucleic acids (DNA, RNA) analysis

More than 40 years ago Palecek discovered using oscillo-polarography that nucleic acids gave two signals:

- redox signal of adenine and cytosine, and
- oxidative signal of guanine [119–122].

Recently elimination voltammetry has been successfully utilised for resolution of reduction signal of adenine and cytosine [123–128] in short oligonucleotides. Moreover it was published that cytosine, adenine, thymine and guanine gave signals at carbon electrodes [129–131]. Based on these promising milestones of electroanalysis of nucleic acids together with the fact that electrochemistry is still one of the most sensitive analytical technique voltammetric methods can be considered as a suitable tool for detection of nucleic acids [132–152].

Three carbon paste electrodes (CPE) with different content of carbon particles with various shapes and sizes were prepared and tested on measuring of nucleic acids. The first carbon paste electrode (called as 'micro CPE') was made of 70% glassy and spherical carbon powder 2–12 μm (*m*/*w*, Sigma-Aldrich) and 30% mineral oil (*m*/*w*, Sigma-Aldrich; free of DNase, RNase, and protease). The second one (called as 'nano CPE I') was made of 60% glassy and spherical carbon powder 2–12 μm, 30% powdered cylinder nanocarbontubes OD = 7–15 nm; ID = 3–6 nm; L = 0.5–200 μm (*m*/*w*, Sigma Aldrich) and 10% mineral oil. The third one (called as 'nano CPE II') was made of 60% glassy and spherical carbon powder, 30 % vertically aligned multiwalled carbon nanotubes (MWCNTs) OD = 30–50 nm; ID = 5–15 nm; L = 0.5–200 μm (*m*/*w*, Sigma Aldrich) and 10% mineral oil [153]. These pastes were housed in a teflon body having a 2.5-mm-diameter disc surface [154–157]. Prior to measurements, the electrode surface was renewed by polishing with a soft filter paper in preparation for measurement of a sample (5 μl). The prepared carbon paste electrodes were used in the following experiments, in which genomic DNA isolated from salmon, DNA isolated from chicken and influenza virus HPI DNA were analysed. For the analysis of samples square wave voltammetry was used. Primarily, it was necessary to find the most appropriate conditions for detection. The influence of accumulation time on the response signal was studied (Figure 7(A)). The results indicate that micro CPE was the most sensitive for the detection of DNA followed by nano CPE II and nano CPE I. It can be concluded that the electrochemical response expressed as the sum of all signals of nucleic bases, increased with increasing accumulation time. Furthermore, the calibration curves were measured at all CPEs. The obtained calibration curves were linear with regression coefficients higher than 0.99 within concentration interval from 1 ng/ml to 100 ng/ml.

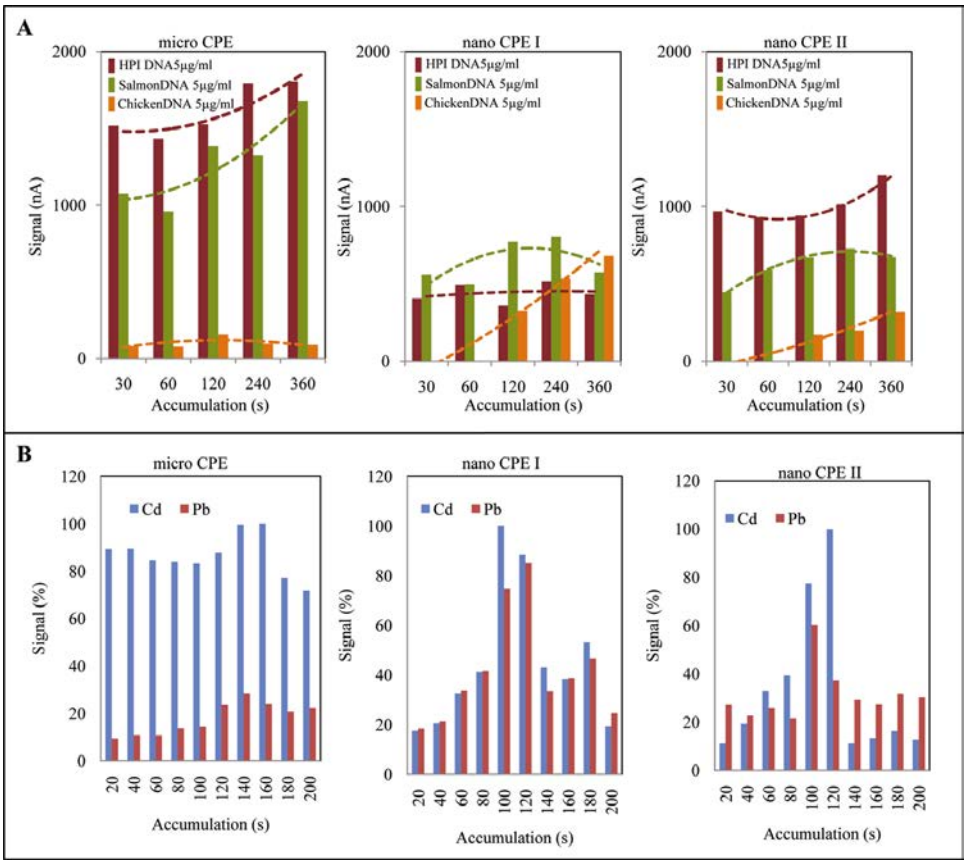
5.2 Heavy metal ions detection

Heavy metal ions are natural components of Earth's crust. The content of them in soil is varying from very low (femtograms) to high (milligrams). However due to anthropogenic activities their content can be elevated in the site of the action. High concentrations of heavy metal ions can injure human health and pollute environment. It is a common knowledge that toxic heavy metal ions (lead, cadmium and mercury) are able to enter to organisms and interfere several important metabolic processes. Due to the above-mentioned facts developing and proposing of simple analytical instruments, methods and procedures with low detection limits are needed [158]. Analytical methods and instruments for detection of cadmium(II) [159–162] and lead(II) [163–167] ions have been several times reviewed. Among very sensitive analytical methods for detection of heavy metal ions belong the electrochemical ones [168–170]. The classic instruments consisted of potentiostat/galvanostat with electrochemical cell including three electrodes (working, reference and auxiliary). However the trend of the analytical techniques is to miniaturise the whole instrument due to many advantages of small devices including portability, low costs and demands on service and operations, sufficient sensitivity and selectivity [171,172]. As the working electrode, hanging mercury drop electrode (HMDE) is commonly used [173,174]. HMDE can be also modified by biologically active substances to improve sensitivity or selectivity of heavy metal ions detection [175–181]. Due to adverse effects of Hg(II) and many restrictions for usage of this metal, carbon electrodes are used as an alternative [182–184]. Moreover, the trend of the analytical techniques is to miniaturise the whole instrument, in which carbon electrodes have much more advantages compared to HMDE [171,172]. Screen-printed carbon electrodes belong to the most convincing carbon electrodes to be used for *in situ* environmental analysis [185–190]. Use of electrochemical techniques coupled with nanostructured materials is the other way to achieve the results of the highest quality and reliability. We focused on the monitoring of heavy metals, especially cadmium(II) and lead(II) ions. Electrochemical techniques using abovementioned CPE, which were enriched with nanotubes, were used for detection of the ions. The influence of time of Cd(II) and Pb(II) accumulation on the surface of the electrodes on their electrochemical response was investigated (Figure 7(B)). The highest response was measured at micro CPE at 140–160 s. In the case of both CPE enriched with nanotubes, the maximum response was reached at 100–120 s. The concentration of both metals in solution was 1 mM.

For automated electrochemical determination of heavy metal ions, it is possible to use the following analyser. 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 to note 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 [191,192].

Figure 7 (A) Dependences of accumulation time of influenza virus HPI DNA, Salmon DNA and chicken DNA (5 µg/ml) on the response (nA) measured at three CPE. (B) Dependences of accumulation time of Cd(II) and Pb(II) (1 mM) on the response (nA) measured at three CPE. Electrochemical measurements were performed with AUTOLAB PGS30 as mentioned in Caption for Figure 4. Carbon paste electrodes were employed as the working electrode. Square wave voltammetric measurements (DNA) were carried out in the presence of acetate buffer pH 5. Square wave voltammetry parameters: potential step 5 mV, frequency 280 Hz. Differential pulse voltammetric (metals) measurements were carried out in the presence of acetate buffer pH 5. Differential pulse voltammetry parameters: initial potential 0 V, end potential –0.8 V, potential step 5 mV. The temperature of supporting electrolyte was maintained by the flow electrochemical cell coupled with thermostat JULABO F12/ED (Labortechnik GmbH, Germany) (see online version for colours)



5.3 Peptides and proteins detection

The early origins of the polarographic studies of the proteins are summarised in the work of Michael Heyrovsky [193]. As introduced in this work, the first polarographic analyses of the different body liquids were realised in 1928. Later Rudolf Brdicka discovered interesting electrochemical behaviour of the proteins in the presence of the cobalt ions.

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 [1,2].

The electrochemical detection, in our case voltammetric or amperometric detection, surely should not disappear from 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 notice that 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., derivatisation). Thanks to this fact, it is possible to carry out the analyses in 60 min 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.

Theoretically, it is possible to analyse everything, what can be oxidised or reduced on the surface of the working electrode [194]. For proteins analysis, we follow 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 [1,2]. 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 [195,196]. Our analysis of the thiol compounds is focus on the low molecular mass thiol compounds and proteins. The thiol compounds provide well developed catalytic signals, which are proportionate to their concentration. The mentioned progress facilitates the determination of subnanomolar concentrations. This sensitive analysis can be carried out in the case of all peptides and proteins, which in their molecule contain cysteine. Among these molecules belongs also MTs [68,72,73,75,82,83,85], glutathione [174,197–201], phytochelatins [199,200,202–204] and others [205,206], which have been determined by using of stationary electrochemical instrument without autosampler.

Besides automated analysis, there was suggested a great help in electrochemical analysis of proteins and other biologically active compounds called adsorptive transfer stripping technique [207]. The principle of the adsorptive transfer stripping technique is based on the strong adsorption of the target molecule on the surface of the working electrode at an open circuit. The hanging mercury drop electrode is periodically renewed. Target molecules are adsorbed on the surface of the renewed working electrode at an open circuit. The electrode is washed with a supporting electrolyte. The electrode with the adsorbed target molecules is measured in the presence of the supporting electrolyte. The adsorptive accumulation procedure can be done under various experimental conditions, different potentiostatic control and in the presence of different types of matrix. The main advantage of this technique is elimination of interfering compounds in bioanalytical investigations, where the matrix of a sample is very complex. In addition, the stripping process can be controlled by various types of not only voltammetric techniques but also chronopotentiometric ones. DPV and SWV offer lower detection limits, but this effect mostly depends on the kind of adsorbed molecules [208].

The AdTS technique coupled with the DPV Brdicka reaction can be used to detect MTs in low sample volumes. The technique, however, has its limitations. This technique currently is unable to generate reproducible results when analysing very low sample volumes. We attempted to investigate how changes in drop volume and area of the working electrode influence the repeatability and sensitivity of the measurements. Study of MT (100 μM) drop volumes of 2.5, 5.0, 10 and 15 μL by AdTS DPV Brdicka reaction at HMDE with a drop area of 400 μm^2, resulted in well developed and reproducible Brdicka catalytic signals of 1.5, 3.0, 6.2 and 9.2 ng of MT, respectively. MT measurements (100 nM) with drop volumes of 0.5, 1.0, 1.5, 2.5 and 5.0 μL can be carried out using HMDE with a drop area of 250 μm^2. The Brdicka catalytic peaks were well developed in the measured voltammograms, whereas Cat2 peaks were sufficiently detected even at low MT amounts of 0.3, 0.6, 0.9, 1.5 and 3.0 ng. However, relative standard deviation (R.S.D., %) increased significantly with decreasing drop volume. The R.S.D. measurements of MT in 2.5, 1.5, 1.0 and 0.5 μL were 4%, 8%, 15% and 40%, respectively. This phenomenon was possibly due to the water evaporation from a drop of MT standard solution. Due to this fact, MT concentration increased and a higher peak was observed. Based on the results obtained, this approach cannot be used for quantitative determination of proteins in very low sample volumes at room temperature. This obstacle can be overcome by technically modified current transfer technique. A small square of parafilm (10 × 10 cm, Sigma-Aldrich) is seamed on a microscope slide by a burner. The slide is washed with ethanol and distilled water (Milli Q, 18 MΩ) and transferred to a cooled space, in this case to a beaker filled with distilled water and placed in a tempered water bath (Julabo, Germany) at a temperature of 2°C. Prior to use, the slide is removed from the bath and dried using cellulose. MT low volume drops were pipetted onto the dried slide and then adsorbed on the surface of HMDE at open circuit. The electrode with the adsorbed target molecule is washed and measured. MT measurements (100 μM) with drop volumes of 2.5, 5.0, 10 and 15 μL were carried out using the improved AdTS DPV Brdicka reaction and HMDE with a drop area of 400 μm^2. Compared to results mentioned above the signals were higher and more proportional to MT content with a R^2 value of 1.000. Relative standard deviation of Cat2 (n = 5) peak height did not exceed 4%. The detection limit (3 S/N) of MT was evaluated as 500 zeptomoles per 500 nL (1 pM) and the quantification limit (10 S/N) as 1500 zeptomoles per 500 nl (3 pM) [68].

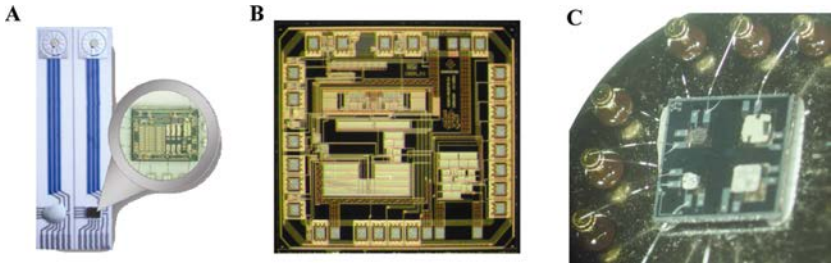
6 Intelligent miniaturised systems for chemical, biological and pharmaceutical applications

6.1 Electronics chips

Two chips for electrochemical applications have been developed. Both chips are designed in sub-micron technology CMOS 0.7 um. The first one is a potentiostat integrated on a silicon chip with 5 current ranges from 100 nA to 1 mA (Figure 8(A)). We have two versions. The first one is without current range 100 nA but it has embedded PROM memory for information about sensor (version, calibration, etc.). The second version has not embedded memory but it has two channels for measurements. Voltage range controlled on auxiliary electrode is ±2.5 V, current of working electrode is transformed to asymmetric current output with resistor 1.5 kΩ which converts output to voltage range

0–5 V with bias at 2.5 V. It means that the current is from 2.5 V to 0 V negative and from 2.5 V to 5 V positive. The chip can be implemented directly within electrodes [209]. The second chip is designed for conductivity measurements using bipolar pulse technique (Figure 8(B)). In this chip the two symmetric current pulses are generated and voltage at the end of the second pulse is read and used for calculation of resistance and finally the conductivity. The chip has 4 ranges for measurement resistance from 10 Ω to 100,000 Ω [210].

Figure 8 (A) Screen printed electrodes with the chip of potentiostat and example of cyclic voltammetry measurement [209]. (B) The conductometric chip [210]. (C) Micro-hotplate gas sensor with screen-printed active layers [23] (see online version for colours)



6.2 GAS sensors

Semiconducting metal-oxides are mostly used for gas sensors based on conductivity dependency on gas concentration. Nanomaterials brought possibilities to increase the sensitivity of this type of sensor. Two approaches were applied. The first was nanopowder used in a paste to print an active layer of nanocrystals. The printed active layers with nanocrystals of metal-oxide semiconductors were also deposited on micro-hotplate sensors (Figure 8(C)). The second way uses mostly the reactive sputtering to deposit nanocrystals over the electrodes. The advantage of screen-printed layer is in a high dynamic range of measured concentration. Thin film deposited by sputtering is able to detect low concentration however the dynamic range is small because of the fast saturation at higher concentration [23,211–214].

7 Elimination voltammetry in nanobiotechnology

Coupling of electrochemistry and sophisticated mathematical approaches is advantageous [215]. Recently, nanobiotechnology has become one of the most exciting forefront fields not only in bioanalytical and biophysical chemistry but also in biomedicine and pharmacy. In all of these areas electrochemistry takes an important position to provide easily workable response of all detectors. The electrochemical contribution consists of a sensitive, selective and cheap biosensing with the fast response characterising a chosen property of biologically important substance. The biological research advancing from microparticles to nanoparticles requires new approaches, in which new instrumentations and methodologies arise and develop. One of the methods expected to contribute significantly is also elimination voltammetry with linear scan.

7.1 Elimination voltammetry as a transformation model

Elimination voltammetry with linear scan (EVLS) [123–125,216–219], named according to its ability to eliminate some chosen current components from the total voltammetric current measured in linear sweep, cyclic or square wave voltammetry (LSV, CV or SWV). In other words, if one current component is eliminated, the other is conserved. The EVLS procedure is based on the different dependence of current component on scan rate, e.g., the diffusion current I_d depends on square root of scan rate, the charging current I_c is the linear function of scan rate, and the kinetic current I_k is independent of scan rate [123,216,220,221]. In a potential-controlled electrochemical experiment, only the total current can be measured. This total voltammetric current is expressed as the sum of these current components. The elimination procedure is achieved by the elimination function *f(I)* expressed as a linear combination of two (the elimination only one current component):

$$f(I) = a_1 I_1 + a_2 I_2 \tag{1}$$

or three (the simultaneous elimination of two current components):

$$f(I) = a_1 I_1 + a_2 I_2 + a_3 I_3 \tag{2}$$

where a_1, a_2, and a_3 are EVLS coefficients and currents I_1, I_2 and I_3 measured at different scan rates. These coefficients were calculated from three equations of the form $a_i I_i = a_i(I_d)_i + a_i(I_k)_i + a_i(I_c)_i$, where index *i* indicates the number of a total current (1, 2, 3). The total current with number 2 is the reference current, to which both currents (1 and 3) are related. These equations have different forms due to different combinations of elimination requirements and scan rate ratios. In square wave voltammetry (SWV) the time-varying value comparable to the scan rate in linear sweep voltammetry (LSV) or cyclic voltammetry (CV) is frequency [222]. For the elimination of I_c and I_k and conservation of I_d using the total currents measured at half ($I_{1/2}$) and double (I_2) reference scan rate (I) we can calculate the EVLS function:

$$f(I) = -11.657\, I_{1/2} + 17.485\, I - 5.8284\, I_2. \tag{3}$$

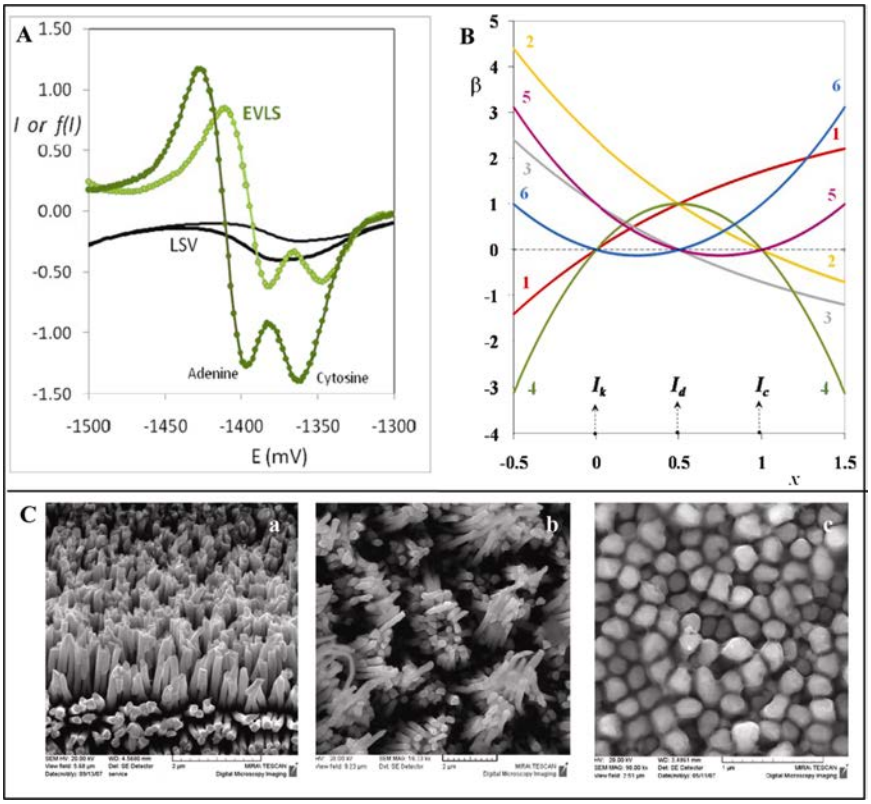
It was found that this elimination function (EVLS E4) is most suitable for the research of biomolecules such as DNA, oligonucleotides, nucleobases, and proteins because it is able to reflect sensitively the processes at polarised interface [64,123,124,126–128,221,223].

7.2 Elimination voltammetry as a sensitive analytical tool in biotechnology

The high sensitivity of E4 consists also in the special signal (peak-counterpeak) which was theoretically verified for a total adsorbed electroactive species undergoing the irreversible electrode process [128]. The reduction overlapped reduction signals of adenine (A) and cytosine (C) for the mixtures of homo-ODNs and hetero-ODNs with different sequence of A and C were analysed on HMDE by EVLS E4 [64,126,127]. The best resolution of AC signals was observed in the case of hetero-ODN 3′- CCCAAACCC-5′, in which three AAA are in the centre of ODN chain [123,127,221,222,224,225]. In contrast to the common voltammetric methods EVLS is capable not only to resolve the overlapping reduction signals of A and C, but also to indicate different sequences of A and C. For the adsorbed electroactive substance, the elimination function E4 actually yields a well-readable peak-counterpeak (Figure 9(A)).

It clearly follows from the figure that the EVLS signals (peak-counterpeaks) are 8–10 times higher than corresponding LSV signals [64,123,127,221,224,225]. Moreover, elimination voltammetry was used in connection with adsorptive stripping techniques (AdS EVLS) for the sensitive detection of purine derivatives on carbon (paste and pencil) electrodes [226–228].

Figure 9 (A) LSV and EVLS voltammograms of hetero-ODN, (3′- CCCAAACCC-5′), in phosphate buffer (pH 6.2), EVLS E4: *f(I)* - elimination function for simultaneous elimination of kinetic and charging currents (I_k, I_c), and conserving the diffusion current. Scan rates for EVLS: 100, 200, 400 mV/s, reference scan 200 mV/s, time of accumulation 90s at –100 mV vs. Ag/AgCl/3MKCl. (B) The dependence of the current elimination coefficient $\beta_{EVLS} = f(Ij)/Ij$ on the rate power coefficient *x* for six elimination functions (integer 2). Arrows with currents I_c, I_k and I_d indicate the points of its elimination ($I_j = 0$). Electrochemical measurements were performed with AUTOLAB PGS30 and under experimental conditions as mentioned in Caption for Figure. 4. (C) Scanning electron microscope images of (a) clustered nickel nanorods, (b) gold nanorods and (c) nickel nanorods (Tescan, Czech Republic) (see online version for colours)



7.3 Elimination voltammetry as a tool for detection of rate determining step in complex processes

The EVLS provides valuable information about nature and kinetics of processes at electrode/electrolyte interfaces [123,125]. It is suitable for the study of the mechanism

of electrode processes and structural and concentration changes of ODNs at charging phase interfaces. According to the different course of various elimination functions, the EVLS is able to identify the current nature changing also through the transition from micro- to nanoparticles. We calculated the dependence of the current elimination coefficient $\beta_{EVLS} = f(I_j)/I_j$ on the rate power coefficient x for various elimination functions using multiple of 2 of the reference scan rate. According to the course of elimination functions in the diagram $\beta_{EVLS} = f(x)$ we are able to determine the nature (diffusion, capacity, kinetic, catalytic, reversible, irreversible currents, etc.) of electrode processes (Figure 9(B)). Using the reduction of DNA components, various kinetic processes at the electrode surface take place. It is well known that the reduction of DNA bases is related to protonisation. Moreover, other kinetic processes can be related to structural changes of DNA on the electrode surface. Regardless of the nature of the actual process, the kinetics can be formally described as a preceding chemical reaction.

7.4 Elimination voltammetry as a pointer of miniaturising electrode systems

The elimination voltammetry was applied to renewed mini- or semimicro-drop mercury electrodes and it was found that EVLS is a sensitive method to the size of an electrode [65,229]. Using miniaturised mercury electrodes EVLS is capable to detect the effect of spherical diffusion regarding to both the scan rates and the size of an electrode drop. It is one of the evidence that the EVLS can count for characterisation of the way to miniaturise the system. In connection with miniaturised mercury electrodes (MME), the EVLS experiment confirmed the assumption that spherical diffusion can be expressed as the kinetics [229].

7.5 Elimination voltammetry as an analyser of nanostructured surfaces

Nanostructural materials have unique properties beside the common material. These especially include a massive increase of the active surface, restriction of the transport processes of the reactive compounds to diffusion, and covering the specific interaction of the measuring compound with the surface of nanoparticles. The aim of research of nanostructured material is the study of different types of not only carbon nanotubes, nanorods, and nanowires, but also of metal and non-metal nanostructures and the effect of experimental conditions (temperature, pH, parameters of the fabricated nanomaterials, concentration and composition of electrolyte) on their formations. We used the metal-coating methods (nickel, zinc, or copper) through the porous mask to create nanostructures (Figure 9(C)). These nanostructures were tested by means of electron microscopy and beside the commonly used electrochemical methods also by means of EVLS which is sensitive to different rate determining steps.

Recently, the electrochemical systems studying the extent of DNA damage, the interaction of DNA with proteins, drugs and mutants were characterised by cyclic voltammeter with redox probe as $[Fe(CN)]^{3-/4-}$. Electrode surface is often modified either by using strong bonds (the biotin-avidin, metal-sulphur), electrostatic interactions (modified carbon nanotubes) or magnetic forces (paramagnetic nanoparticles). Recently, we have reported the DNA biosensor based on multi-walled carbon nanotubes (MWNT) and chitosan (CHIT) [230]. As a signal transducer the screen printed carbon paste electrode (SPCPE) was used. These matrixes were investigated using $[Fe(CN)]^{3-/4-}$ as the redox probe in the mode of CV and EVLS. The EVLS showed that chemical

modification is responsible for the increase of diffusion component of a voltammetric current.

8 Conclusion

At the beginning of 21st century, the field of nanotechnologies and bionanotechnologies comprises especially:

- biological and chemical systems, from molecular to cell level,
- materials and technologies, which are able to affect biological systems as well as technologies constructed by people,
- hybrid bioelectronic and other systems,
- techniques and methods used in nanotechnologies.

We are mainly involved in fabricating and using micro- and nanoparticles and nano-based electrodes. The adsorption of biomolecules directly onto naked surfaces of bulk materials may frequently result in their denaturation and loss of the bioactivity. However, the adsorption of such biomolecules onto the surface of nanoparticles can retain their bioactivity because of the biocompatibility of nanoparticles. Since most of the nanoparticles carry charges, they can electrostatically adsorb biomolecules with different charges. Besides the common electrostatic interaction, some nanoparticles also immobilise biomolecules by other interactions. Gold nanoparticles can immobilise proteins through the covalent bonds formed between the gold atoms and the amine groups and cysteine residues of proteins.

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