Sensing in Electroanalysis

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Electrochemical Techniques as Promising Analytical Tools in the DNA Electrochemistry (A Review)

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Abstract: In the present article, the research and development of electrochemical techniques, namely voltammetric measurements, applied in the DNA electroanalysis during the past years have been reviewed (in five parts). In the first section, the applications of DNA electrochemistry in the detection of metals are being thoroughly described. The second part gives a critical view in the study of interaction between metals and DNA using electrochemical techniques. Furthermore, attention is paid to the metals and DNA damage detection using selected electrochemical techniques. The following part offers discussion on the detection of DNA sequences / oligonucleotides using metals and electrochemical techniques. Finally, in the last part, the interactions between the antitumor drugs and DNA when using electrochemical techniques are being concerned and critically evaluated.

Keywords: electrochemistry**,** solid electrodes, DNA, metals, DNA interactions, oligonucleotides, DNA sequences, antitumor drugs.

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Introduction

The purine bases, guanine and adenine, and the pyrimidine bases, thymine and cytosine are fundamental compounds in biological systems. They participate in the respective processes as distinct as energy transduction, metabolic cofactors and cell signaling, and are essential building blocks of nucleic acids [1].

Electrochemical techniques such as cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV) and adsorptive stripping analysis are proven to

be significant methods in trace analysis because of its broad scope of applications and relatively simple and low cost instrumentation.

Most applications have used a hanging mercury drop electrode (for reducible species), carbon and platinum disk electrodes (for oxidizable species). A biosensor is an analytical device comprising a biological recognition element (e.g., enzyme, receptor, DNA, antibody, or microorganism) in intimate contact with an electrochemical, optical, thermal, or acoustic signal transducer, that together permit analyses of chemical properties or quantities [2]. Various types of affinity biosensors as "Genosensor" for nucleic acid recognition processes have been rapidly developed incorporating different nanomaterials for the detection of specific nucleic acid sequences related with genetic and infectious diseases.

Immediate applications will include direct quantification of DNA samples for use in sequencing or polymerase chain reactions, or pharmaceutical testing, environmental and quality control. The following parts show a general picture of the different applications of voltammetric techniques in the study of DNA electroanalysis.

Applications of DNA Electrochemistry in the Detection of Metals

Metal ions, could be hazardous pollutants in the living environment, which represent important risk to human health due to their undesirable effects (oxidative stress, changes in activities of many enzymes, interactions with biomolecules including DNA and RNA). Meanwhile, some metal ions, such as Mn(II) ions, were found to be essential elements for a number of DNA binding proteins, which possess structural rather than enzymatic activity or are able to change the enzymatic activity of some nuclear proteins. In order to possess physiological or toxic effects, metal ions have to enter the bacterial cell.

The toxicity of heavy metal ions inside the cell may occur through the displacement of essential metals from their native binding sites or through ligand interactions. Especially, heavy metal cations with high atomic numbers, e.g., Hg(II), Cd(II) and Ag(I), tend to bind SH groups [3-5]. By binding to SH groups, the heavy metal ions may inhibit the activity and/or the functioning of sensitive enzymes. Cations can also be segregated into complex compounds by thiol-containing molecules while on the other hand some heavy metal ions may be reduced to less toxic oxidation states [5]. A metal compound that can be reduced should be able to diffuse out of the cell. Most divalent heavy metal ions are accumulated within the cells by the fast and unspecific CorA (metal transport system) Mg(II) transport

system [5]. Thus, it is of great importance to develop sensitive and selective methods for detection of trace amounts of them. Fig. 1 summarizes the application of electrochemistry of DNA on the detection of metals.

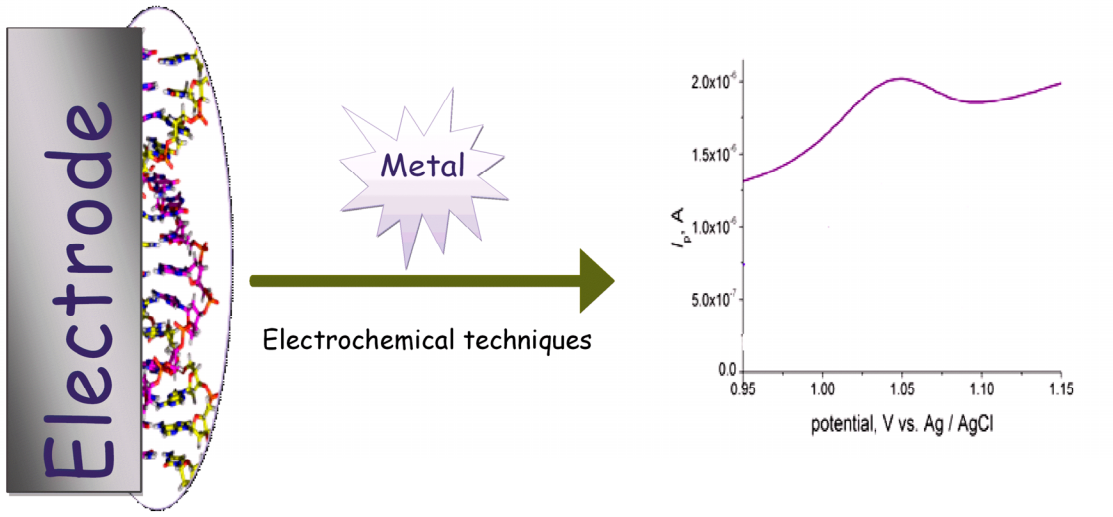

Fig. 1. *Schematic illustration of applications of the electrochemistry of DNA in detection of metals*

For example, Wu et al. [6] had developed an electrochemical sensor based on target-induced structure-switching DNA, that detects sensitively and selectively detection mercury (II) ions. A 33-mer oligonucleotide 1 (5′-SH-$(CH_2)_6$-TCATGTTTGTTTGTTGGCCCCCCTTCT-TTCTTA-3′, S_1) with five self complementary base pairs separated by seven thymine–thymine mismatches was first immobilized on the electrode (gold electrode) via self-assembly of the terminal thiol moiety and then hybridized with a ferrocene-tagged oligonucleotide 2 (5′-ACAAACATGA-$(CH_2)_6$-NH_2-3′, S_2), leading to a high redox current. In the presence of Hg^{2+}, mercury-mediated base pairs (T–Hg^{2+}–T) induced the folding of the oligonucleotide 1 into a hairpin structure, resulting in the release of the ferrocene-tagged oligonucleotide 2 from the electrode surface with a substantially decreased redox current.

The redox-tagged probe is totally dissociated away from the electrode, which imparts a maximized signal-to-background for the new design. The response characteristics of the sensor were investigated using CV, DPV and electrochemical impedance spectroscopy (EIS) at room temperature on a gold electrode. The results reveal that the developed sensor affords a high selectivity and sensitivity for Hg^{2+} detection. The effect of the reaction temperature on

the response of the sensor was also studied. The results revealed that the sensor showed sensitive response to Hg^{2+} in a concentration range from 0.1 nM to 5 μM with a detection limit of 0.06 nM. In addition, this strategy afforded selectivity for Hg^{2+} against other environmentally related metal ions. Furthermore, mercury detection is performed in river water to demonstrate the practical use of this sensor.

Wang et al. [7] illustrated an example of electrically contacted enzyme based on dual hairpin DNA structure and its application for amplified detection of Hg^{2+}. This study was based on a dual hairpin DNA structure, a novel system of electrically contacted enzyme and its signal amplification for ultra sensitive detection of Hg^{2+} was demonstrated. In the presence of Hg^{2+}, with the interaction of thymine–Hg^{2+}–thymine (T–Hg^{2+}–T), DNA sequence dully labeled with ferrocene (Fc) at 5′ end and horseradish peroxidase (HRP) at 3′ end, hybridized to the capture probe and formed the dual hairpin structure on a modified glassy carbon electrode (GCE) with gold nanoparticles (GNP)–polydopamine (PDA)–carbon nanospheres (CNSP). EIS measurements were performed to study the modified electrodes with different interfacial processes. CV was used to monitor the behavior of the dual hairpin DNA system with and without the addition of H_2O_2. Fc unit acted as a relay that electrically contacts HRP with the electrode and activates the bioelectrocatalyzed reduction of H_2O_2. And based on the bioelectrocatalyzed signal amplification of the presented system, Hg^{2+} could be quantitatively detected in the range of 10^{-10}–10^{-6} M with a low detection limit of 52 pM using CV. And it also demonstrated excellent selectivity against other interferential metal ions.

An electrochemical biosensor with high sensitivity and selectivity for mercuric ion detection, based on DNA self-assembly electrode, was designed by Cao et al. [8]. Thiol functionalized poly-T oligonucleotides were used as gold electrode modifier through formation of Au–S bond between DNA and gold electrode. In presence of Hg^{2+} ions, the specific coordination between Hg^{2+} and thymine bases can change parallel ss-DNA from linear to hairpin structures, which can cause the release of partial DNA molecules from the surface of the electrode.

The density of DNA on the surface of electrode correlated with the concentration of mercury in the solution and can be monitored by electrochemical impedance spectroscopy. The limit of detection of this method is pM level of mercuric ions which is far below the upper limit of Hg^{2+} mandated by United States Environmental Protection Agency (EPA), 2 ppb (10 nM). In addition, this method showed excellent selectivity. A series of divalent metal ions, including Ni^{2+}, Co^{2+}, Mg2+, Zn^{2+}, Ba^{2+} and Cd^{2+}, had little interference with the detection of Hg^{2+}.

Chen et al. [9] fabricated a method to construct Au and Ag electrodes on chip, utilizing the different solubility of gold and silver in different etching solutions. KI-I_2 etching solution and 50% HNO_3 were chosen to dissolve the metal layers alternatively. Planar electrodes with gold and silver could be simultaneously and accurately patterned on chip using photolithographic technique. The asprepared electrode could be directly served as integrated three-electrode system for electrochemical measurement. Based on it, a sensing strategy had been carried out using home-made electrochemical sensing (ECS) chip, which depended on the competition of double strand DNA and Hg(II)-mediated T–T base pairs (T-Hg(II)-T). Actually, a mercury specific oligonucleotide (MSO) was immobilized onto the thus-fabricated gold working electrode and employed as the sensing element. Chronocoulometry (CC) was chosen to monitor the differences of surface charge volume and quantify the concentrations of Hg(II) ions with a low detection limit down to 1 nM. Therefore, a facile method to fabricate Au and Ag electrodes had been demonstrated to simplify the production of ECS chip. The ECS chip was utilized for constructing an effective sensing platform for sensitive Hg(II) determination, which held promising potential for designing ECS chip in lab-on-a-chip device or point-of-care diagnosis.

A sensitive electrochemical sensor for Ag^+ detection based on Ag^+-induced conformational change of cytosine-rich single stranded DNA C-rich ssDNA probe and the controlled assembly of MWCNTs was illustrated by Yan et al. [10]. Measurements carried out using DPV. In this protocol, the gold electrode was first modified with a dense 16-mercaptohexadecanoic acid self-assembled monolayer (MHA/SAM). The hydrophobic MHA/SAM isolated the electrode from the electroactive indicator (ferrocenecarboxylic acid, FcCOOH) in the aqueous solution, which resulted in the electronic transmission blocking. In the presence of Ag^+, C–Ag^+–C coordination induced the conformational change of C-rich ssDNA probe from random-coil structure to fold into a hairpin structure, which could not wrap on the surface of the MWCNTs.

Then the “naked” MWCNTs could be assembled on the MHA/SAM gold electrode, mediating the electron transfer between the electrode and the electroactive indicator. It generated measurable electrochemical signals. The resulting change in electron transfer efficiency was readily measured by DPV at target Ag^+ concentrations as low as 1.3 nM. The linear response range for Ag^+ detection was ranged from 10 to 500 nM. This method did not need of electroactive molecules labeling on the C-rich ssDNA probe. Moreover, it had good selectivity to other environmentally relevant metal ions. Therefore, the developed

electrochemical assay has sufficient advantages including sensitivity, selectivity, simplicity, low-cost, and no requirement for probe label preparation.

Zhao et al. [11] reported an ultra-sensitive method for the selective detection of Ag^+ ions using an electrochemical technique based on Ag^+-assisted isothermal exponential degradation reaction. Cyclic voltammetry (CV) and chronocoulometry (CC) were the elextrochemical techniques which were used in this study.In the presence of Ag^+, mismatched trigger DNA can transiently bind to template DNA immobilized on an electrode (gold electrode) surface through the formation of C–Ag^+–C base pair, which then initiates the isothermal exponential degradation reaction. As a result, the mismatched trigger DNA may melt off the cleaved template DNA to trigger rounds of elongation and cutting. After the cyclic degradation reactions, removal of the template DNA immobilized on the electrode surface could be efficiently monitored by using electrochemical technique to show the status of the electrode surface, which could be the be used to determine the presence of Ag^+. Further studies revealed that the proposed method couldn be ultra-sensitive to detect Ag^+ at a picomolar level. The selectivity of the detection was satisfactory, thus the proposed method for the Ag^+ ions detection maybe potentially useful in the future.

An electrochemical assay for the detection of silver ion was reported by Gong et al. [12], which was based on the interaction of the Y-type, C-rich ds-DNA with Ag^+. Upon addition of Ag^+, Y-type, C-rich ds-DNA could form an intramolecular duplex, in which Ag^+ can selectively bind to cytosine–cytosine (C–C) mismatches forming C–Ag^+–C complex. The binding result was evaluated by EIS on a DNA-gold modified electrode and analyzed with the help of Randles' equivalent circuits. The differences of charge transfer resistance, DRCT, after and before the addition of Ag^+, allows the detection and quantitative analysis of Ag^+ with a detection limit of 10 fM. Moreover, cysteine (Cys) was applied to remove Ag^+ from the C–Ag^+–C complex, which allowed the Ag^+ sensor to be reproduced.

In the same way, DRCT for the C–Ag^+–C system in the absence and presence of Cys allows the detection of Cys at a concentration as low as 100 fM. Finally, the potential application of the Ag^+ sensor was also explored, such as in lake and drinking water.

Wei et al. [13] demonstrated a DNAzyme-based colorimetric sensor for the determination of lead (Pb^{2+}) using unmodified gold nanoparticle probes. Novel functional oligonucleotides, especially DNAzymes with RNA-cleavage activity, have been intensively studied due to their potential applications in therapeutics and sensors. In this work, the authors reported a simple, sensitive and label-free, 5′-CAT CTC TTC TCC GAG CCG GTC GAA ATA GTGAGT -3′ oligonucleotide (17E DNAzyme)-based sensor for Pb^{2+} detection using

unmodified gold nanoparticles (GNPs) based on the fact that unfolded single-stranded DNA could be adsorbed on the citrate protected GNPs while double-stranded DNA could not. The substrate cleavage by the 17E DNAzyme in the presence of Pb^{2+} could be monitored by color change of GNPs, thereby Pb^{2+} detection was realized. The detection of Pb^{2+} could be realized within 20 min, with a detection limit of 500 nM. The selectivity of the sensor was investigated by challenging the sensing system with other divalent metal ions. Since common steps such as modification and separation could be successfully avoided, this sensor could provide a simple, cost-effective yet rapid and sensitive measurement tool for Pb^{2+} detection and may prove useful in the development of sensors for clinical toxicology and environmental monitoring.

An electrochemical DNAzyme sensor for sensitive and selective detection of lead ion (Pb^{2+}) had been developed by Shen et al. [14], taking advantage of catalytic reactions of a DNAzyme upon its binding to Pb^{2+} and the use of DNA-Au bio-bar codes to achieve signal enhancement. A specific DNAzyme for Pb^{2+} was immobilized onto an Au electrode surface via a thiol-Au interaction. The DNAzyme hybridizes to a specially designed complementary substrate strand that had an overhang, which in turn hybridized to the DNA-Au biobar code (short oligonucleotides attached to 13 nm gold nanoparticles). A redox mediator, $Ru(NH_3)_6^{3+}$, which can bind to the anionic phosphate of DNA through electrostatic interactions, served as the electrochemical signal transducer. Upon binding of Pb^{2+} to the DNAzyme, the DNAzyme catalyzed the hydrolytic cleavage of the substrate, resulting in the removal of the substrate strand along with the DNA bio-bar code and the bound $Ru(NH_3)_6^{3+}$ from the Au electrode surface. The release of $Ru(NH_3)_6^{3+}$ resulted in lower electrochemical signal of $Ru(NH_3)_6^{3+}$ confined on the electrode surface.

DPV signals of $Ru(NH_3)_6^{3+}$ provided quantitative measures of the concentrations of Pb^{2+}, with a linear calibration ranging from 5 nM to 0.1 μM. Because each nanoparticle carried a large number of DNA strands that binds to the signal transducer molecule $Ru(NH_3)_6^{3+}$, the use of DNA-Au bio-bar codes enhanced the detection sensitivity by five times, enabling the detection of Pb^{2+} at a very low level (1 nM). The DPV signal response of the DNAzyme sensor was negligible for other divalent metal ions, indicating that the sensor is highly selective for Pb^{2+}. Although this DNAzyme sensor was demonstrated for the detection of Pb^{2+}, it has the potential to serve as a general platform for design sensors for other small molecules and heavy metal ions.

The feasibility of using gold electrodes modified with short-chain ssDNA oligonucleotides for determination of uranyl cation was examined [15]. Valtammetric measurements were made by means of CV and SWV. Interaction between UO_2^{2+} and

proposed recognition layer was studied by means of voltammetric and quartz crystal microbalance measurements. It was postulated that ssDNA recognition layer functions via strong binding of UO_2^{2+} to phosphate DNA backbone. The methylene blue was used as a redox marker for analytical signal generation. Biosensor response was based on the difference in electrochemical signal before and after subjecting it to sample containing uranyl ion. The lower detection limit of 30 nmol L−1 for UO_2^{2+} was observed for a sample incubation time of 60 min. Proposed ssDNA-modified electrodes demonstrated good selectivity towards UO_2^{2+} against common metal cations, with only Pb^{2+} and Ca^{2+} showing considerable interfering effect.

Study of Interaction between Metals and DNA using Electrochemical Techniques

Metal ion–DNA interactions are important in nature, often changing the genetic material's structure and function. The interaction of DNA with heavy metals has been extensively investigated since they are involved in processes leading to DNA damage. The harmful metal ions have been found in different sources, foods, beverages, soil, plants, natural waters, etc. The DNA has four different potential sites for binding of metal ions, the negatively charged phosphate oxygen atoms, the ribose hydroxyls, the base ring nitrogens, and the exocyclic base keto groups [16]. Alkali and alkaline earth cations prefer to bind to the phosphate groups along the backbone of DNA [17-19], which essentially reduces electrostatic repulsion between the strands.

Most transition metal ions interact with more than two different sites and their interactions with DNA are more complicated. They frequently bind indirectly to the phosphate groups and directly to the bases with the N7 atom of purines or N3 of pyrimidines [20]. The interaction of metal ions with ds-DNA films has drawn more attention recently due to its application in the electrochemical detection of single nucleotide mismatches and its application in electrochemical gene chips [21-23]. In this chapter we report some interesting studies, which illustrate in the best manner how metals interact with DNA via electrochemical techniques (see Fig. 2).

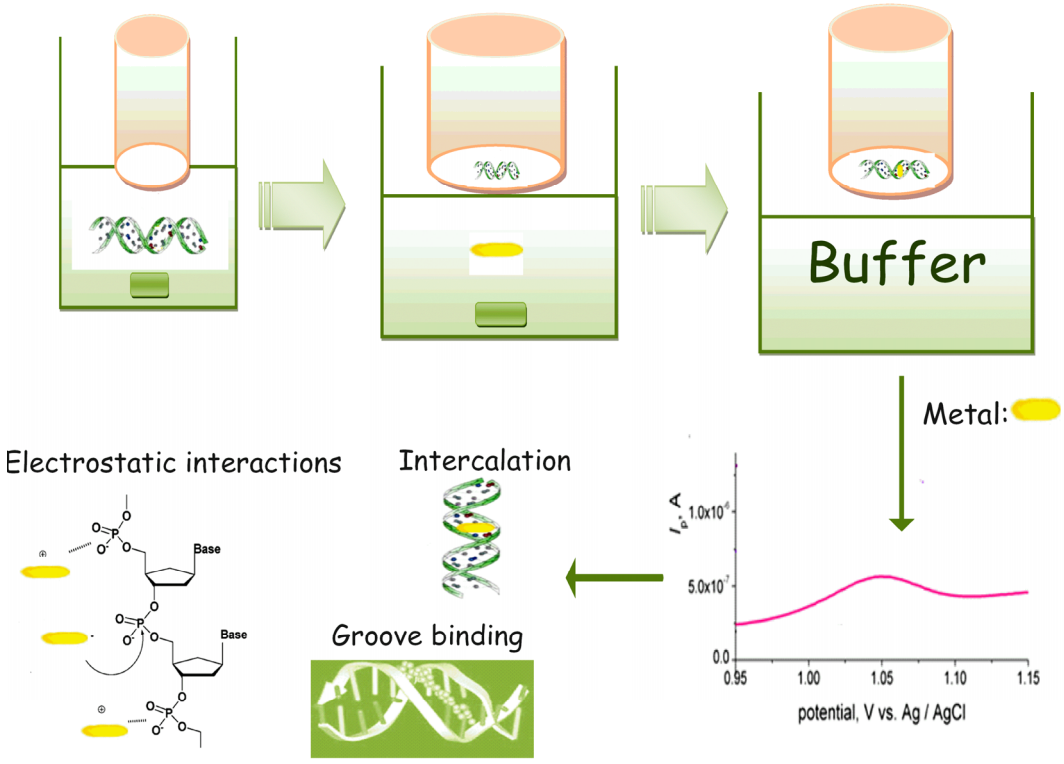

Fig. 2. *Schematic illustration of the applications of the electrochemistry on the determination of the interaction mode between metals and DNA*

For instance Lin et al. [24] studied the conformational switch from hairpin DNA to G-quadruplex induced by Pb^{2+} with EIS in the presence of $[Fe(CN)_6]^{3-/4-}$ as the redox probe. In the presence of Pb^{2+}, the G-rich hairpin DNA opened the stem-loop and forms G-quadruplex structure, which gave rise to a sharp increase in the charge-transfer resistance (RCT) of the film reflected by the EIS.

This structural change was also confirmed by circular dichroism (CD) measurements and UV-Vis spectroscopic analysis and calculated by density functional theory (DFT). On the basis of this, the authors developed a label-free electrochemical DNA biosensor for Pb^{2+} detection. With increasing concentrations of Pb^{2+}, the differences in the charge-transfer resistance RCT before and after the Pb^{2+} incubation was linearly dependent on the logarithm of Pb^{2+} concentration within a range from 50 mM to 0.5 nM. The biosensor also exhibited good selectivity for Pb^{2+} over other metal ions.

A dsDNA-electrochemical biosensor, employing DPV, was used for the in situ evaluation of Pb^{2+}, Cd^{2+} and Ni^{2+} interaction with dsDNA by Oliveira et al. [25] on a glassy carbon electrode (GCE). The results confirm that Pb^{2+}, Cd^{2+} and Ni^{2+} bind to dsDNA, and that this interaction leads to different modifications in the dsDNA structure. These modifications

were electrochemically recognized as changes in the oxidation peaks of guanosine and adenosine bases. Using homopolynucleotides of guanine and adenine it has been proved that the interaction between Pb^{2+} and DNA causes oxidative damage and preferentially takes place at adenine-containing segments, with the formation of 2,8-dihydroxyadenine, the oxidation product of adenine residues and a biomarker of DNA oxidative damage. The Pb^{2+} bound to dsDNA can still undergo oxidation. The interaction of Cd^{2+} and Ni^{2+} causes conformational changes, destabilizing the double helix, which can enable the action of other oxidative agents on DNA.

Bin et al. [26] used EIS to investigate the effects of a number of metal ions with DNA films on gold surfaces exploiting $[Fe(CN)_6]^{3-/4-}$ as a solution-based redox probe. Alkaline earth metal ions Mg^{2+}, Ca^{2+}, trivalent Al^{3+}, La^{3+} and divalent transition metal ions Ni^{2+}, Cu^{2+}, Cd^{2+} and Hg^{2+} have been selected in this study and the results are compared with previous studies on the effects of Zn^{2+} on the EIS of DNA films. In addition, the effect of a single base pair mismatch in the absence and presence of several selected divalent metal cations was investigated. All experimental results were evaluated with the help of equivalent circuits, which allowed the extraction of resistive and capacitive components. For all the metal ions studied, their addition a decrease in the charge transfer resistance. The difference of charge transfer resistance (ΔRct) of ds-DNA films in the presence and absence of the various metal ions was different and particular to any metal ion given. In addition, the EIS of ds-DNA films containing a single A–C mismatch in the presence and absence of Ca^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+} was studied. ΔRct values for ds-DNA films with a single A–C mismatch were smaller than those of fully matched ds-DNA films.

The five transition metal ions which was tested exhibited ΔRct values that followed the order Hg^{2+}> Cd^{2+}>Ni^{2+}> Cu^{2+} > Zn^{2+}, which was approximately inversely proportional to the free energies of hydration of these metal ions. Interestingly, the interaction with Hg^{2+} appeared to be reversible and washing reduced the value of ΔRct close to the value of unmetallated ds-DNA films. Differences between the different transition metals were enhanced in the presence of a single A–C nucleotide mismatch. The difference of ΔRct for the fully matched DNA and one single A–C mismatch DNA (ΔRct) followed the order: Hg^{2+} > Ca^{2+} > Cd^{2+} > Zn^{2+}, suggesting the Hg^{2+} or even Ca^{2+} might be a better choice for mismatch detection and might lead the way to optimizing the procedure for mismatch detection by EIS.

Li et al. [27] investigated the interaction of the metal ions Mg^{2+}, Zn^{2+}, Ni^{2+} and Co^{2+} with DNA-peptide nucleic acid (PNA) films on a gold surface using EIS in the presence of $[Fe(CN)_6]^{3-/4-}$ as the redox probe. Impedance data were analyzed with the help of a modified

Randles' equivalent circuit. Changes in the charge-transfer resistance, R_{CT}, decreases in the order of $Ni^{2+} > Co^{2+} > Zn^{2+} > Mg^{2+}$.We interpret these results in terms of stronger interactions for Ni^{2+} with the DNA-PNA film compared to the other metal ions, potentially involving interactions with the nucleobases, presumably with the N7 of purines or the N3 of pyrimidines. On the basis of these observations, Ni^{2+} was chosen to probe the detection of a C-T mismatch in 15-mer PNA-DNA films. Using Ni^{2+}, it is possible to detect a single C-T mismatch. The resulting ΔR_{CT} is larger for the PNA-DNA hybrid compared to that for the identical 15-mer DNA-DNA hybrid.

Guo et al. [28] found that the presence of Ni^{2+} enables to distinguish the presence of single-nucleotide mismatches in PNA (peptide nucleic acids)–DNA films on gold electrodes by EIS. With the help of a modified Randles' equivalent circuit, differences in the charge transfer resistance (ΔR_{CT}) before and after the addition of Ni^{2+} were a diagnostic measure for the presence of single-nucleotide mismatch. The approach worked under real-life conditions with concentrations of the DNA target strand down to 10 fM, and a PNA capture probe is used to genotype the single-nucleotide mismatch in apoE 4 related to Alzheimer's disease (AD).

Metals and DNA Damage Detection with Electrochemical Techniques

Serious damage to DNA is caused by chemical systems generating free radicals such as reactive oxygen species (ROS) [29]. These radicals oxidize the DNA bases and deoxyribose and lead to a release of the bases and an interruption of the phosphodiester bonds.

The interaction of many chemical compounds, including water, some metal ions and their complexes, small organic molecules and proteins, with DNA is reversible and stabilizes DNA conformations. However, hazard compounds such as drugs and carcinogens interact with DNA causing irreversible damage and these interactions have to be carefully studied. DNA-electrochemical biosensors are a very good model for simulating nucleic acid interactions with cell membranes, potential environmental carcinogenic compounds and clarifying the mechanisms of interaction with drugs and chemotherapeutic agents. In this chapter, we report the most outstanding examples of the way that metals are used in DNA damage determination with elactroanalytical methodologies (see Fig. 3).

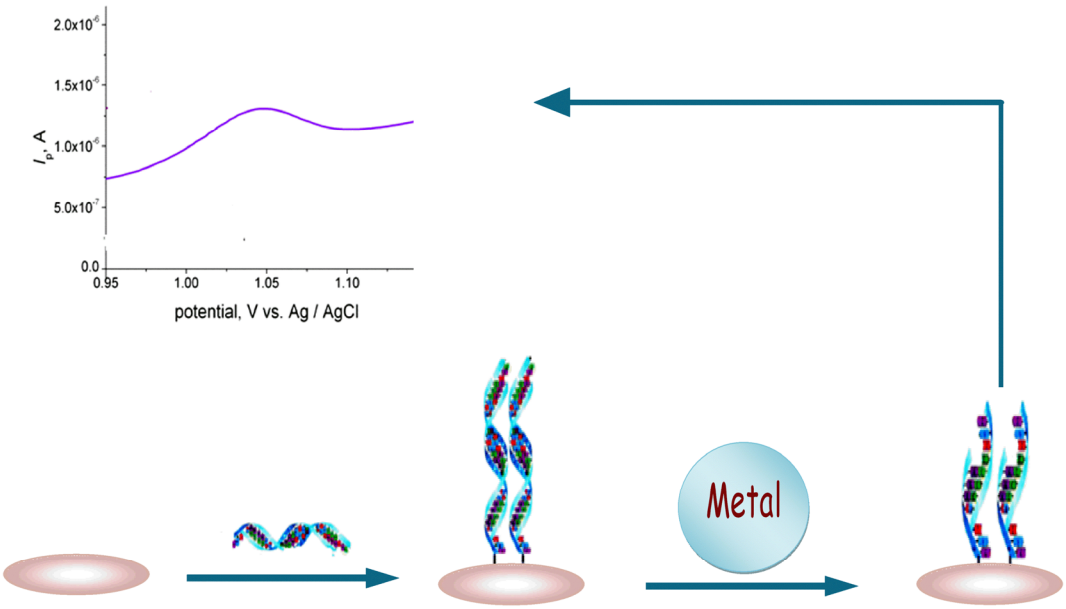

Fig. 3. *Schematic illustration of the applications of the electrochemistry on the determination of DNA damage using metals*

For instance, the cleavage activity of a nicking endonuclease towards metal-ionmediated duplex-like DNA was demonstrated by Li et al. [30]. In this study, the nicking endonuclease (nickase) Nt.AlwI was chosen as a model to probe its cleavage activity towards the Metal-ds DNA. Hg^{2+} was chosen as a model, and the cleavage activity of nickase towards Hg^{2+} ion-mediated duplex-like DNA (Hg^{2+}-dsDNA) was investigated.

A 28-mer ferrocene (Fc) labeled ssDNA, containing the recognition sequence of a nickase, was capable of folding into a hairpin-like stem–loop structured dsDNA in the presence of Hg^{2+}. The nickase cleaved the stem structure of Hg^{2+}-dsDNA at the site where the red arrow indicates, leading to the concomitant Fc tag being released away from the electrode. Electrochemical measurements took place on the gold modified electrode CV was preliminarily employed to follow the binding of Hg^{2+} and cleavage events. In order to define the concentration range of Hg^{2+}, which can modulate the nicking endonuclease activity, the sensitivity of Fc-ssDNA modified electrode for Hg^{2+} was preliminarily determined by DPV. The linear response of concentration range of Fc-ssDNA modified electrode with Hg^{2+} was 0.1 to 3 mM, indicating that the amount of Nt.AlwI was sufficient for dsDNA cleavage, and Hg^{2+} in the concentration range did not inhibit the activity of Nt.AlwI. EIS measurements for the Fc-ssDNA probe modified electrode were performed to follow the DNA conformational change during Hg^{2+} binding and Nt.AlwI digestion Polyacrylamide gel electrophoresis

(PAGE) was also used to confirm the cleavage event of the nickase towards then Hg^{2+}-dsDNA.

Bartosik et al. [31] reported a comparative analysis of the detection of DNA damage modeled by the presence of abasic sites (AP) at defined positions using the chemical modification of the DNA by the complex of osmium tetroxide-2,2-bipyridine (Os, bipy), that selectively binds to unpaired thymine residues in the damaged DNA. AP were detected by electrochemical detection (EC) of the Os, bipy-thymine adducts, by immunoelectrochemical (IE) and by thickness shear mode acoustic methods (TSM). The square-wave voltammetry (SWV) at a pyrolytic graphite electrode (PGE) was used for determination of the concentration of Os, bipy sites at dsDNA. The detection of the interaction of the OsBP7H8 antibody with Os, bipy-modified dsDNA immobilized at the surface of quartz crystal was performed by TSM method on a gold electrode. The acoustics' sensor response was determined by measurement of the electrical impedance, which is related to the complex acoustic impedance. It was found that the electrochemical detection method could discriminate the presence of only one abasic site in 19-mer DNA. Another important advantage of Os, bipy utilized in this study was that the immunogenecity of the DNA-Os, bipy adducts and availability of monoclonal antibodies, which extends possibilities of the analysis to different electrodes and even to non electrochemical methods. The results obtained were rather promising for practical applications in environmental analysis of the compounds that cause DNA damage.

Oliveira et al. [32] studied the in situ evaluation of the direct interaction of chromium species with double-stranded DNA (dsDNA) using DPV at a glassy carbon electrode. The DNA damage was electrochemically detected following the changes in the oxidation peaks of guanosine and adenosine bases. The results obtained revealed the interaction with dsDNA of the Cr(IV) and Cr(V) reactive intermediates of Cr(III) oxidation by O2 dissolved in the solution bound to dsDNA. This interaction resulted to different modifications and caused oxidative damage in the B-DNA structure. Using polyhomonucleotides of guanine and adenine, it was shown that the interaction between reactive intermediates Cr(IV) and Cr(V)–DNA gave oxidative damage and preferentially took place at guanine-rich segments, leading to the formation of 8-oxoguanine, the oxidation product of guanine residues and a biomarker of DNA oxidative damage. The interaction of Cr(VI) with dsDNA caused breaking of hydrogen bonds, conformational changes, and unfolding of the double helix, which enabled easier access of other oxidative agents to interact with DNA, and the occurrence of oxidative damage to DNA.

Serpi et al. [33] studied the DNA damage induced by copper(II) in the presence of curcumin. As a result of the reaction between curcumin (CC) and copper(II) the characteristic peak of curcumin at −1.0V significantly increased, and the peak at −1.6V disappeared. Curcumin probably forms complex with copper(II). The interaction between double stranded dsDNA and curcumin in the presence of Cu(II) was studied in solution, by differential pulse adsorptive transfer voltammetry using carbon paste electrode (CPE) and hanging mercury drop electrode (HMDE). Cu(II)–CC complex generated changes in calf-thymus DNA. The characteristic peak of dsDNA, due to the oxidation of guanine residues, decreased. The increased DNA damage by Cu(II)–CC complex was observed in the presence of various concentrations of copper(II).

Shestivska et al. [34] investigated the electrochemical detection of DNA damage in vitro on a carbon paste electrode using SWV, in order to evaluate the antioxidant properties of transgenic tobacco plant extracts containing metallothionein (MT, Rabbit liver MT containing 5.9 % Cd and 0.5 % Zn was used). This method was based on assessment of efficiency with which the studied plant extracts are able to reduce to the amount of damage of standard chicken DNA by free OH radicals prepared by the Fenton reaction. MT isolated from rabbit liver was tested as a standard reference solution and compared its activity with extracts from wild growing tobacco plants, and from twelve clones of transgenic tobacco plants varying in the carrying of either human or yeast metallothioneins.

The experimental results presented in this report have demonstrated that insertion of a MT gene into the DNA of tobacco plants significantly improves their antioxidant properties in comparison with the non transgenic plants. It seems that the method developed for purpose of this study could be suitable for rapid screening of various plants with respect to their potential in the phytoremediation of heavy metals.

Detection of DNA Sequences / Oligonucleotides Using Metals and Electrochemical Techniques

The detection of DNA has a particular interest in genetics, pathology, criminology, pharmaco-genetics, food safety and many other fields [35]. DNA biosensors are developed for detection and discrimination of either target DNA sequence or single nucleotide polymorphisms. DNA sequence detections have various applications such as detection of target genes, discrimination and classification of various organisms and also detection of genetic based

disorders. Over the last decades, various kinds of biosensor have been developed and miniaturized [36]. Among those, electrochemical biosensor receives an increasing attention due to its high sensitivity and low cost [37]. In addition, the progress made in nanotechnology offers the possibility to employ an ultramicroelectrode array for the multiplex detection [38].

Usually, there are two incidents in a biosensor, bio-recognition and conversion [36]. The former provides the selectivity while the latter the measurable signal. As an electrochemical DNA sensor, its selectivity generally originates from the hybridization with a specific DNA capture. The conversion is then based on the redox reaction, surface modification. In the following examples we demonstrate the use of electrochemical methods upon the detection of DNA and specific sequence oligonucleotide. The following section describes the application of electrochemistry on the determination of nucleic acids general picture in the presence of metals (see Fig. 4, overleaf).

Farias et al. [39] developed a method for the adenine determination in the presence of copper in diluted alkaline electrolyte by adsorptive stripping voltammetry at a mercury film electrode. A nanomolar concentration of adenine was determined in the presence of copper using this method. For an accumulation time of 30 minutes, the detection limit was found to be 0.22 ppb (1.63 x10^{-9} M).

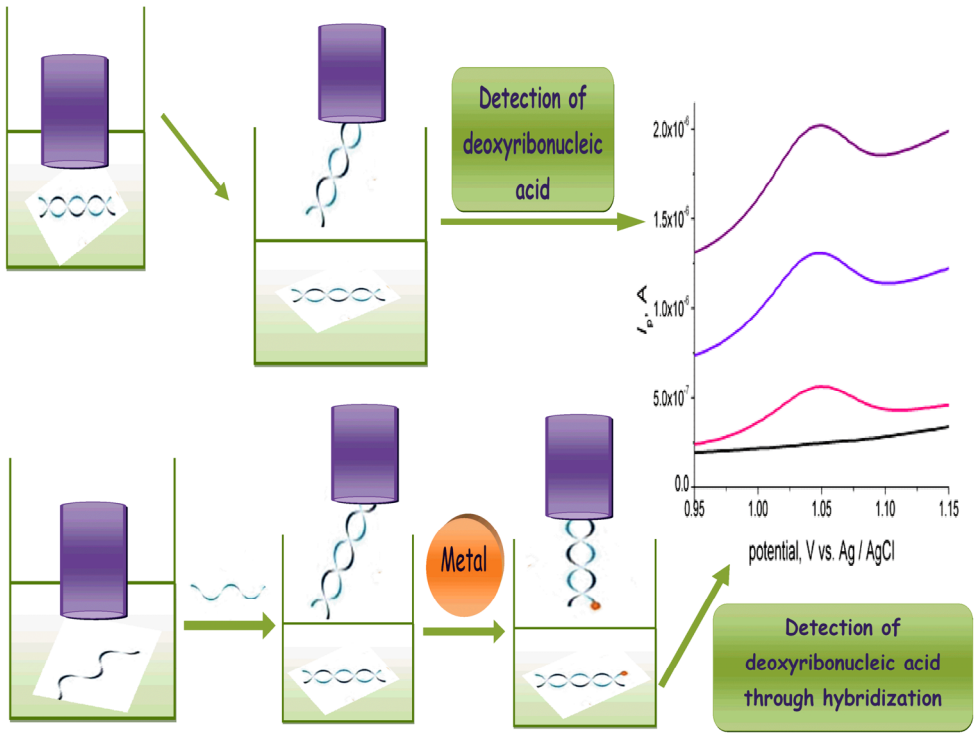

Fig. 4. *Schematic illustration of the applications of the electrochemistry on the determination of nucleic acids using metals.*

The method was based on controlled adsorptive accumulation of adenine-copper at thin-film mercury electrode followed by linear scan voltammetric measurement of the surface species. By applying a condition time of 60 s at -0.9 V, the same thin-film could be used over several measurements. Optimum experimental conditions were found to be the selection of a solution of 5.0 x10^{-3} M NaOH , an accumulation potential in the range of -0.20 to -0.40 V vs. ref., and a scan rate of 100 mV s^{-1}. Then, the response of adenine-copper was found to be linear over the concentration range of 20 – 100 ppb.

The more convenient ways to measuring adenine in the presence of metals and other nitrogenated bases were also investigated. The adenosine triphosphate (ATP) or deoxyribonucleic acid (DNA) were first treated with acid (e.g., 0.1 M perchloric acid), and the acid-released adenine (without separation from others products of the degradation) was directly determined by adsorptive stripping voltammetry.

Stripping voltammetric determination of purine bases in the presence of copper ions at mercury, amalgam, or carbon-based electrodes has recently been utilized in analysis of DNA or synthetic oligodeoxynucleotides (ODNs). Hason et al. [40] reported on copper-enhanced label-free anodic stripping detection of guanine and adenine bases in acid-hydrolyzed DNA at anodically oxidized borondoped diamond electrode (AO-BDDE). The AO-BDDE was successfully applied in a three-electrode microcell in which a ~50 μL drop of the analyte solution could be efficiently stirred during the accumulation step by streaming of an inert gas. Accelerated mass transport due to the solution motion in the presence of copper resulted in enhancement of the guanine oxidation signal by about 2 orders of magnitude (compared to accumulation of the analyte from still solution not containing copper), allowing an easy detection of ~25 fmol of the ODNs. The proposed technique was shown to be suitable for a determination of purine (particularly guanine) content in DNA samples. Applications of the technique in magnetic bead-based DNA assays (hybridization with DNA sequences exhibiting asymmetrical distribution of purine/pyrimidine nucleotides between the complementary strands or monitoring of amplification of specific DNA fragments in a duplex polymerase chain reaction) were demonstrated.

An electrochemical biosensor for the detection of a synthetic single stranded DNA (ss-DNA) with a detection limit down to femtomolar levels demonstrated by Chen et al [41]. The synthetic ss-DNA with sequence associated with the influenza A virus RNA was used as a model target DNA. The detection scheme was based on a sandwich structure involving a capture probe, the target DNA and a detection probe. An avidin-labeled glucose oxidase (GOx) was attached to a biotinylated detection probe via biotin–avidin interaction.

Subsequently, the DNA hybridization event was transduced and amplified by an in-situ enzyme-catalyzed deposition of cupric hexacyanoferrate (CuHCF) nanoparticles in the presence of glucose, cupric ions and ferricyanide. The peak current in DPV of the deposited CuHCF nanoparticles directly correlated to the concentration of the target DNA. DNA was therefore quantified in the concentration range from 1.0 fM to 10 pM with a detection limit of 1.0 fM and a relative standard derivation of ≤ 11% at 100 fM. Excellent mismatch discrimination was demonstrated in the proposed biosensor.

An assay for the electrochemical detection of guanine based on carbon nanotubes paste electrodes (CNTPEs) modified with cobalt phthalocyanine (CoPc) was investigated by Balan et al [42]. The results indicated that the modification of a CNTPE with this compound results in amplification of the guanine oxidation response in contrast to that on the unmodified CNTPE.

The electrochemical behavior of the modified electrode and the mechanism of the oxidation of guanine were investigated using CV and DPV. The methods parameters were optimized. A detection limit of 1.3 $x10^{-7}$ mol L^{-1} was obtained for guanine using the electrocatalytic oxidation signal corresponding to the Co(II)/Co(III) redox process. This modified electrode was further applied for determination of single-stranded DNA by DPV with a detection limit of 9.86 $x10^{-8}$ mol L^{-1}. The advantages of convenient fabrication, low-cost detection, short analysis time and combination with nanotechnology for increasing the sensitivity made the modified electrodes worthy of special emphasis in the nonlabeled detection of DNA hybridization reaction and in the development of DNA based biosensors for toxic chemicals, toxins and pathogens determination.

A simple strategy of transgenic sequence-specific detection without a special amplification procedure was developed on the basis of aluminum(III)/poly(L-glutamic acid) (PLGA) film by Zhou et al. [43]. An aluminum ion (Al(III)) thin film was assembled on the surface of PLGA via the electrostatic binding of Al(III) with carboxyl, namely Al(III)/PLGA. The immobilization of deoxyribonucleic acid (DNA) was carried out on this carbon paste modified with Al(III)/PLGA film by Al(III)-single strand DNA (ssDNA) interaction. Surface hybridization between the immobilized ssDNA and its complementary ssDNA was monitored by EIS using $[Fe(CN)_6]^{3-/4-}$ as a redox probe. Under the optimal conditions, this DNA electrochemical sensor was applied to determine the specific gene sequence related to phosphinothricin acetyltransferase transgene (PAT) in the transgenic plants by label-free EIS.

Sun et al. [44] had described an electrochemical DNA sensor for the simple, sensitive and specific detection of nucleic acids based on proximity-dependent DNA ligation assays

with the DNAzyme amplification of hairpin substrate signal. A long DNA strand contains the catalytic motif of Mg^{2+}-dependent 10-23 DNAzyme, acting as the recognition probe. When the target DNA was introduced into the system, part of it was complementary to 5′-end of the recognition probe, resulting in the ligation of a stable duplex, the unbinded part of the target DNA was acted as one binding arm for the DNAzyme. This duplex containing a complete 10-23 DNAzyme structure could cleave the purine–pyrimidine cleavage site of the hairpin substrate, which resulted in the fragmentation of the hairpin structure and the release of two single-stranded nucleic acids, one of which was biotinylated and acted as the signal probe. An immobilized thiolated capture probe could bind with the signal probe, using biotin as a tracer in the signal probe, and streptavidin–alkaline phosphatase (SA–ALP) as reporter molecule.

The activity of the immobilized enzyme was voltammetrically determined with DPV on the modified gold electrode by measuring the amount of 1-naphthol generated after 5 min of enzymatic dephosphorylation of 1-naphthyl phosphate. The results revealed that the sensor showed a sensitive response to complementary target sequences of *H. pylori* in a concentration range from 100 fM to 1 nM, with a detection limit of 50 fM. In addition, the sensing system could discriminate the complementary sequence from mismatched sequences, with high sensitivity and reusability.

Mehrgardi et al. [45] designed a strategy for the amplification of the responses of an electrochemical DNA hybridization biosensor using silver nanoparticles (Ag-NPs) as redox reporters and its capability for the detection of a single base mismatches (SBM) including thermodynamically stable ones. In this assay, Ag- NPs were immobilized on the top of recognition layer and their oxidation signals are followed. DPV and EIS were used to monitoring the electrode response. Only for complementary target sequence, electron could transfer between electrode surface and nanoparticles via DNA and Ag-NPs could be oxidized. Therefore, this DNA biosensor could differentiate between complementary target and one containing either SBM or thermodynamically stable G–A and G–T targets through oxidation signal of Ag-NPs. This biosensor is able to detect SBM by overcome the direct electron transfer of redox reporter with electrode surface and positioning of it before the mismatch position.

A new Mn(II) complex of MnL_2Cl_2 (L = azino-di(5,6-azafluorene)-κ^2-*N,N′*) was synthesized and utilized as an electrochemical indicator for the determination of hepatitis B virus (HBV) based on its interaction with MnL_2Cl_2 by Liu et al. [46]. The electrochemical behavior of interaction of MnL_2Cl_2 with salmon sperm DNA was investigated on glassy carbon electrode (GCE). In the presence of salmon sperm DNA, the peak current of $[MnL_2]^{2+}$

was decreased and the peak potential was shifted positively without appearance of new peaks. The binding ratio between $[MnL_2]^{2+}$ and salmon sperm DNA was calculated to be 2:1 and the binding constant was 3.72×10^8 mol^2 L^{-2}. The extent of hybridization was evaluated on the basis of the difference between signals of $[MnL_2]^{2+}$ with probe DNA before and after hybridization with complementary sequence. Control experiments performed with non-complementary and mismatch sequence demonstrated the good selectivity of the biosensor. With this approach, a sequence of the HBV could be quantified over the range from 1.76×10^{-8} to 1.07×10^{-6} mol L^{-1}, with a linear correlation of $r = 0.9904$ and a detection limit of 6.80×10^{-9} mol L^{-1}.

Additionally, the binding mechanism was preliminarily discussed. The mode of interaction between MnL_2Cl_2 and DNA was found to be primary intercalation binding. Peptide nucleic acid (PNA), the DNA mimic with electrically neutral pseudopeptide backbone, is intensively used in biotechnologies and particularly in single-base mismatch detection in the configuration of DNA hybridization sensors.

Palecek et al. [47] propose a simple method of covalent end-labeling of PNA with osmium tetroxide,2,20-bipyridine (Os,bipy). Os,bipy-modified PNA (PNA–Os,bipy) produced voltammetric stripping peaks at carbon and mercury electrodes with adsorptive transfer stripping (AdTS, ex situ) technique and conventional adsorptive stripping (AdS, in situ) DPV. Peak potential (Ep) of one of the anodic peaks of PNA–Os,bipy at the pyrolytic graphite electrode (PGE) differed from Ep of the reagent, allowing PNA–Os,bipy analysis directly in the reaction mixture. At the hanging mercury drop electrode (HMDE) the PNA–Os,bipy yielded a catalytic peak Catp, in addition to the redox couples. Using Catp was possible to detect purified PNA–Os,bipy down to 1 pM concentration at accumulation time 60 s.

A complex OsO_4, 2,2′-bipyridine (Os,bipy), had also been used for electroactive labeling of biopolymers as well as for probing of nucleic acids and protein structure and interactions [48]. Adsorptive transfer stripping square-wave voltammetry (AdTS SWV) and SWV were the voltammetric techniques that were used in this study. In DNA, Os,bipy forms electrochemically active adducts with pyrimidine nucleobases, exhibiting highly selective modification of thymine residues in single-stranded DNA. In this paper, the modification of rare thymine residues (one thymine among several tens of unreactive purine bases) could easily be detected by means of a simple ex situ voltammetric analysis using carbon electrodes. Thus, Os,bipy was utilized as an electroactive probe for unpaired and/or mismatched thymine residues within DNA heteroduplexes. Site-specific chemical modification of the DNA with

the Os,bipy allowed a clear distinction between perfectly base-paired DNA homoduplexes and mismatched heteroduplexes, as well as discrimination among heteroduplexes containing one or two mispaired thymines, a single thymine insertion, or combination of a mispair and an insertion.

Shen et al. [49] developed an electrochemiluminescence (ECL) method for DNA determination using magnetic submicrobeads (SMBs) as the carrier of $Ru(bpy)_3^{2+}$ (bpy=2,2′-bipyridy) and carbon nanotubes (CNTs) as accessorial electrode material. The SMBs with $Ru(bpy)_3^{2+}$ were wrapped with CNTs and then immobilized on an Au electrode.

In the presence of tri-n-propylamine, ECL of the $Ru(bpy)_3^{2+}$ on the SMBs was detected. Since one target DNA (t-DNA) molecule corresponded to one SMB with a large number of $Ru(bpy)_3^{2+}$, the ECL signal was amplified. In addition, the $Ru(bpy)_3^{2+}$-loaded SMBs were wrapped with CNTs that contacted the electrode. The ECL of $Ru(bpy)_3^{2+}$ was greatly increased. Using this method, t-DNA of 3×10^{-16} mol L^{-1} could be detected. The method could be successfully used to quantify mRNA in cells.

Interactions between Antitumor Drugs and DNA using Electrochemical Techniques

Deoxyribonucleic acid (DNA) belongs among the most extensively studied biomolecules. [50]. DNA binding molecules regulate mechanisms closely related to cellular function, including DNA replication and gene expression. The integrity of the structure of DNA and RNA can be varied by interaction with certain classes of drugs, carcinogens, mutagens and dyes, which may have potentially toxic or therapeutic effects. There are two major classes of binding mode: non covalent binding and covalent crosslinking [51]. Typically, three binding modes are recognized at non-covalent DNA interactions: electrostatic interactions of positively charged guest molecules with the negatively charged DNA sugar-phosphate backbone, binding to major or minor grooves of the DNA double helix and an intercalation of or heteroaromatic system to become inserted between adjacent base pairs of DNA without disturbing the overall stacking pattern [52].

Many effective antitumor drugs in clinical use interact with DNA and some of them do so through intercalation. Most of the antitumor drugs employed clinically exert their antitumor effect by inhibiting nucleic acid (DNA or RNA) or protein synthesis [53]. In general antitumor drugs are classified into three major categories (see Fig. 5, overleaf):

- those which directly damage the DNA in the nucleus of the cell.
- those which stop the synthesis of the pre DNA molecule building blocks.
- those which affecting the synthesis or breakdown of the mitotic spindles.

Moreover, study on the properties of anti-carcinogenic medicines and their interaction with DNA is also significantly important in developing new cancer therapy treatments or anticarcinogens. The recognition of DNA binders involves a complex interplay of different interactive forces [54].

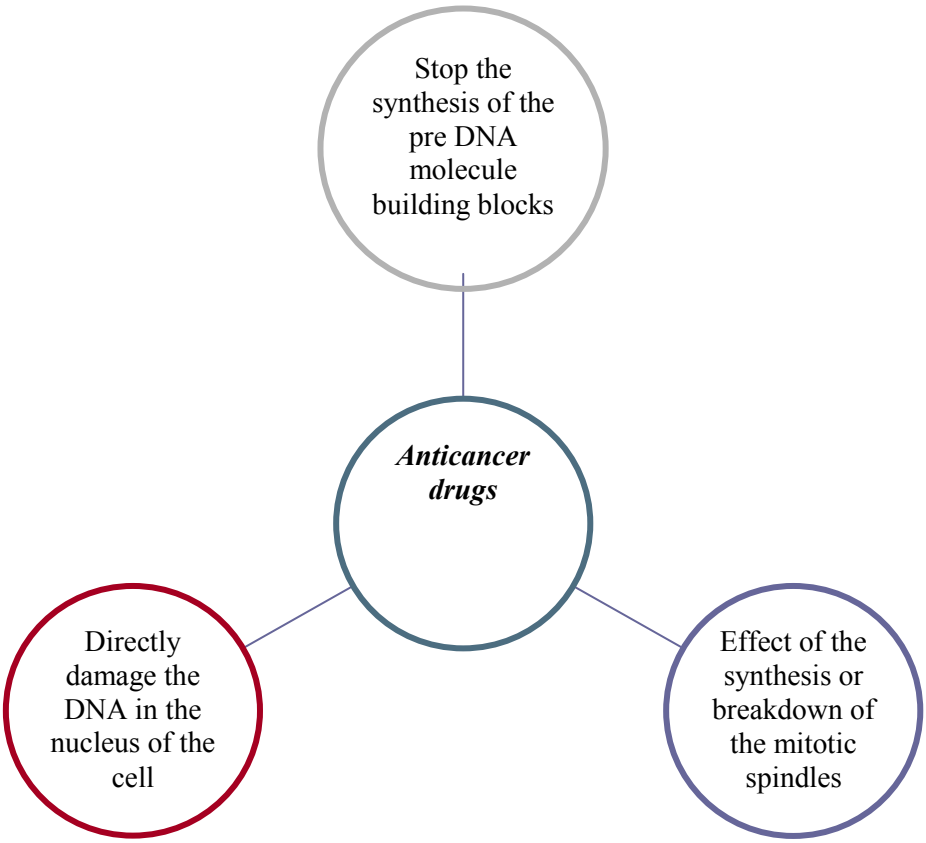

Fig. 5. *Schematic presentation of the categories of antitumor drugs.*

In other words, many efficient antitumor drugs are based on the modification of DNA in cancer cells. The interactions between DNA and drugs molecules have so far been studied by various analytical techniques, such as gel or capillary electrophoresis, high-performance liquid chromatography, X-ray crystallography and surface plasmon resonance [55]. However, most of these methods suffer from high cost, low sensitivity and procedural complication.

Up to now, electrochemical methodologies have attracted appreciable attention due to the inherent specificity and high sensitivity [54].The electroactivity of nucleic acids has allowed the development of new more rapid and more sensitive electrochemical techniques, based on the differences in the electrochemical behavior of DNA-targeted molecules and DNA [55].

DNA damage is, however, also employed for cancer treatment. There is a variety of species that could result in damaging DNA. Figure 6 summarizes the effect of harmful species such as reactive oxygen species (ROS) in DNA and cell membrane. These can damage cell membranes and biomolecules, which can, in turn, induce apoptosis. Damage of the membranes and biomolecules can interfere in numerous crucial biochemical pathways.

Although there is a growing interest for the design of electrochemical DNA biosensors exploiting the interactions between DNA and target drugs, only a few studies concerning evaluation was reported for electrochemical sensing of the interaction between novel potential chemotherapeutic agent or antitumor compounds and DNA [56]. These studies are reviewed here. Figure 6 also illustrates the use of electrochemical techniques in the investigation of the interaction mode of antitumor drugs with DNA.

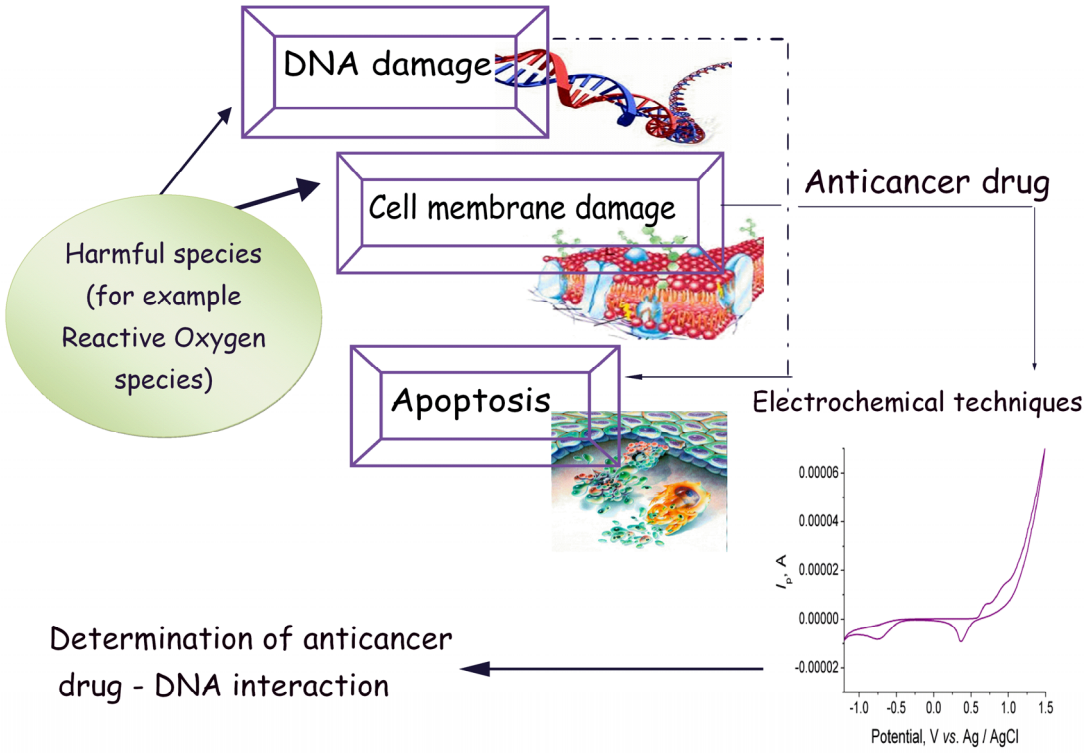

Fig. 6. *Schematic illustration of the action of harmful species and applications of electrochemical techniques for determination of the interaction mode between antitumor drugs and DNA.*

Reza et al [57] studied the interaction of irinotecan ((*S*)-4,11-diethyl-3,4,12,14 tetra-hydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3',4':6,7]-indolizino[1,2-*b*] quinolin-9-yl-[1,4'-bi-piperidine]-1'-carboxylate, CPT-11) with double-stranded calf thymus DNA (dsDNA) at the hanging mercury drop electrode (HMDE) employing an irreversible electrochemical equation using cyclic voltammetry (CV). As a result of the reaction with dsDNA, the reduction peaks related to CPT-11 were shifted in a negative direction and the peak currents were decreased. The results demonstrated that the main interaction mode of CPT-11 with dsDNA is electrostatic.

Topkaya et al [58] investigated the interaction of the same antitumor drug irinotecan (CPT-11), which is the inhibitor of the Topoisomerase I enzyme, with fish sperm double stranded deoxyribonucleic acid (dsDNA) and synthetic short oligonucleotides electrochemically based on the oxidation signals of guanine and CPT-11 by using differential pulse voltammetry (DPV) and cyclic voltammetry (CV) at pencil graphite electrode (PGE). The electrochemical signal of guanine and CPT-11 greatly decreased proving that the CPT-11 interacts with the DNA.

Shah et al [59] investigated the interaction of protonated ferrocene (PF) with chicken blood DNA (CB-DNA) in vitro by cyclic voltammetry (CV) at a glassy carbon electrode (GCE) and UV-Vis spectroscopy as well as viscosity measurements under stomach pH and body temperature. The change in the voltammetric response of the drug the addition of DNA observed in CV, hyperchromism in UV absorption titration, an increase in the viscosity of DNA and the results of the effect of ionic strength on the binding constant strongly support the intercalation of the PF into the DNA double helix.

The interactions of C-1305 (5-dimethylaminopropylamino-8-hydroxy-6H-v-triazolo [4,5,1-de]acridin-6-one) with DNA were studied by Nowicka et al [60] using differential pulse voltammetry and UV-vis spectroscopy. All the performed experiments indicated that C-1305 interacts with dsDNA in two ways: by intercalation and by binding to the minor-groove.

A voltammetric DNA biosensor was used by Tiwari et al [61] for the study of the interaction of antitumor drug adriamycin with DNA. The binding mechanism of adriamycin was elucidated by using differential pulse voltammetry (DPV) at DNA modified glassy carbon fiber electrodes (GCFE). The results indicated that the adriamycin intercalated in double helix of DNA can undergo oxidation or reduction and react specifically with the guanine moiety and thus the form mutagenic 8-oxo-G residue.

Adsorptive transfer stripping technique coupled with square wave voltammetry was used by Hynek et al [62] to investigate the interaction of doxorubicin with single stranded

oligonucleotides (ssODN) in various ratios and interaction times. The results showed the assumption that peak height of doxorubicin increased with increasing concentration of the drug intercalated into ssODN, but the signals of redox peaks of adenine and cytocine changes during the interaction suggest the changes in the structure of ssODN indicating a clear interaction between doxorubicin and ssODN.

Continuing the previous work and using the same technique, Hynek et al [50] showed that the interaction between doxorubicin and dsODN is very complex physico-chemically process. The changes, which can be observed electrochemically, seem to be related to the change in the secondary or other types of structures of studied dsODNs and also probably to the ways of interacting with doxorubicin itself with dsODNs.

Interactions of three hydrazides; isonicotinic acid hydrazide (INH), pyrazine carboxylic acid hydrazide (PCH) and 2,4-dihydroxy benzoic acid hydrazide (2,4-DHBAH) with chicken blood ds.DNA were investigated through by UVeVis spectroscopy (UV) and cyclic voltammetry (CV) at physiological conditions of pH and temperature [63]. Experimental results from both techniques were in good agreement and indicated stronger binding and formation of hydrazideseDNA complexes via intercalation. Among the three hydrazides, 2,4-DHBAH has shown promising broad range anti-bacterial activity while all the three compounds have anti-oxidant and cytotoxic potentials.

Cyclic voltammetry coupled with UV/Vis spectroscopic techniques were used by Hajian et al [64] to study the interaction of methotrexate (MTX), an antitumor drug, with double stranded fish sperm DNA (ds-DNA). A considerable decrease in the MTX peak current observed as the result of the interaction of MTX with DNA. The variations in the spectroscopy and electrochemical characteristics of MTX indicated MTX bind to DNA by a groove binding mode.

Few transition metal complexes of tetradentate N_2O_2 donor Schiff base ligands containing 2-hydroxybenzylidene-4-aminoantipyrine and amino acids (alanine/valine) abbreviated to KHL1/KHL2 have been synthesized by Raman et al [65]. The binding behaviors of the complexes to calf thymus DNA have been investigated by absorption spectra, viscosity measurements and cyclic voltammetry. The DNA binding constants reveal that all these complexes interact with DNA through minor groove binding mode.

Organoantimony(V) ferrocenyl benzoates (OFBs) $[p(C_5H_5FeC_5H_4)C_6H_4COO]_2SbR_3$ ($R=C_6H_5$,p-$CH_3C_6H_4$) (**3**, **4**) and $[m\text{-}(C_5H_5FeC_5H_4)C_6H_4COO]_2SbR_3$ (R=p-CH3C6H4) (**5**) were synthesized and characterized by FT-IR, ^1H and ^{13}C NMR, single crystal XRD and elemental analysis [66]. The DNA interaction of the complexes (**3**-**5**) was investigated by

UV/Vis spectroscopy and cyclic voltammetry (CV). Hyperchromism, hypochromism, cathodic and anodic peak potential shifts suggested OFBs to interact with DNA by different modes due to variation in configuration.

Two chlorodiorganotin(IV) complexes with general formula R_2SnClL (R = n-C_4H_9 (**1**) and C_2H_5 (**2**) and a diphenyltin(IV) derivative with general formula Ph_2SnL_2 (**3**), where L = 4-(4-nitrophenyl)piperazine-1-carbodithioate ligand, were prepared and characterized by elemental analysis, Raman, FT-IR, multinuclear NMR (^1H, ^{13}C and ^{119}Sn) and mass spectrometry by Rehman et al [67]. Electrochemical, kinetic and thermodynamic parameters of complexes **1–3**, interacting with DNA were evaluated by cyclic voltammetry (CV). The decrease in peak currents of complexes **1**–**3**, by the addition of DNA evidenced their interaction with DNA. The positive shift of peak potential in case of 2 and 3 by the addition of DNA signified the intercalation mode.

A systematic comparative study of the binding of antitumor Morin and its complexes with DNA has been reported by Temerk et al [68] using voltammetric and spectroscopic methods. Through comparing the changes of voltammetric and spectroscopic characteristics of Morin complexes upon the addition of DNA, it can be drawn that Morin–Cu and Morin–β-CD complexes interacting with DNA via intercalation forms an electrochemically inactive adduct which should be called a supramolecular complex.

The interaction of native calf thymus DNA (CT-DNA) with two anthraquinones including quinizarin (1,4-dihydroxy anthraquinone) and danthron (1,8-dihydroxy anthraquinone) were studied by Gholivand et al by spectrofluorometric and cyclic voltammetry techniques [69]. In the presence of CT-DNA, the cyclic voltammograms (CVs) of the quinones exhibited small shifts in the anodic and cathodic peak potentials followed by decrease in both peak currents, indicating the interaction existing between the quinones and CT-DNA. Also, the data revealed from that the binding modes of quinizarin and danthron with DNA were evaluated to be groove binding.

Cyclic voltammetry (CV) coupled with UV–Vis and fluorescence spectroscopy were used to probe the interaction of a potential antitumor drug, 4-nitrophenylferrocene (NFC), with DNA by Shah et al [70].

The nature of NFC–DNA interaction was examined by CV based upon the difference in the redox behavior of the drug in the absence and presence of DNA, including the shifts in formal potential of the redox couple and the decrease of the peak current due to the DNA. The results of CV and ionic strength effect indicated electrostatic interaction of NFC withDNA as the dominant mode.

Binding properties of ruthenium(II) complexes, $[RuCl_2(dmso)_2(bfmh)]$ (**1**; where "dmso" – dimethyl sulfoxide, bfmh – benzoic acid furan-2-ylmethylene-hydrazide), $[RuCl_2(dmso)_2(btmh)]$ (**2**; btmh – benzoic acid thiophen-2-ylmethylene-hydrazide), $[RuCl_2(dmso)_2(bfeh)]$ (**3**; bfeh – benzoic acid (1-furan-2-yl-ethylidene)-hydrazide) and [RuCl2(dmso)2(bpeh)] (**4**; bpeh – benzoic acid (1-pyridin-2-ylethylidene)-hydrazide) to Herring sperm DNA examined by Mahalingam et al [71] using absorption titration and cyclic voltammetry (CV). All these complexes have been shown to bind DNA through different modes to different extents.

Four new organotin(IV) carboxylates, $[Bu_2SnL_2]$ (**1**), $[Et_2SnL_2]$ (**2**), $[Bu_3SnL]_n$ (**3**), $[Me_3SnL]_n$ (**4**), where L = 4-nitrophenylethanoates, were synthesized and characterized by elemental analysis, FT-IR and multinuclear NMR (^1H and ^{13}C) by Muhammad et al [72]. The electrochemical, kinetic and thermodynamic parameters of complexes **1-4**, interacting with DNA evaluated by cyclic voltammetric (CV) technique revealing an intercalative mode.

Mohamed et al [73] synthesized and characterized by elemental analysis, IR, TGA, UV–VIS and magnetic susceptibility measurements two new complexes $[Cu(bpy)(Gly)Cl]\cdot 2H_2O$ (**1**) and $[Cu(dpa)(Gly)Cl]\cdot 2H_2O$ (**2**) (bpy = 2,2'-bipyridine; dpa = 2,2'-dipyridylamine, Gly = glycine). The binding properties of the complexes with CT-DNA were investigated by electronic absorption spectra. The calculated binding strength (Kb) of the two complexes to CT-DNA was found to be of lower magnitude than that of the classical intercalator EB (ethidium bromide) suggesting an electrostatic and/or groove binding mode.

Corduneanu and co-workers [74] reported an atomic force microscopy (AFM) and differential pulse (DP) voltammetric study of the interaction established with double-stranded DNA (dsDNA) with two polynuclear Pd(II) chelates with the biogenic polyamines spermidine (Spd) and spermine (Spm), Pd(II)-Spd and Pd(II)-Spm, as well as with the free ligands Spd and Spm. Due to their polycationic chains, the polyamines Spd and Spm interact specifically with the negatively charged phosphate groups of the DNA double helix by electrostatic forces, thus stabilizing its structure.

Compared with their ligands, the Pd(II)-Spd and Pd(II)-Spm complexes were found to induce greater morphological changes in the dsDNA conformation. The interaction was fairly specific, inducing the effects of distortion and local denaturation of the B-DNA structure with release of some guanine bases.

The study of the interaction of Pd(II) amine complexes with CT-DNA is of special interest for pharmaceutical and biomedical research. Shoukry et al [75] published a study which describes the interaction of two Pd(II) complexes; $[Pd(bpy)(OH_2)_2]^{2+}$ (**1**) and

$[Pd(phenpip)(OH_2)_2]^{2+}$ (**2**) with CT-DNA. All results showed that both complexes presumably intercalate in DNA by electrostatic and/or groove binding mode.

Ibrahim et al [76] described the synthesis of a tridentate ligand, bis(2-benzimidazolyl-methyl-6-sulfonate)amine H_2SBz together with its zinc(II), copper(II), and oxidovanadium(IV) complexes [SBz-M(H2O)2] (M = Zn^{2+} **1**, Cu^{2+} **2**, and VO^{2+} **3**). The ligand and its metal complexes **1-3** were characterized based on elemental analysis, conductivity measurements, spectral, and magnetic studies. The interactions between the oxidovanadium(IV) complex **3** and calf thymus DNA had been investigated using cyclic voltammetry showed that the modified electrode with DNA causes the peak potentials, E_{pc}, and E_{pa} shifted to more positive values. This may be attributed to diffusion of the metal complex bound to the large, slowly diffusing DNA molecule and the resulted peak current due the equilibrium of free and DNA-bound oxidovanadium(IV) complex to the electrode surface.

An electrochemical study of the DNA-spermidine interaction was conducted [77] by differential pulse voltammetry (DVP) at a DNA-modified gold electrode. The guanine oxidation peak in DNA was probed for the monitoring of its interaction with spermidine and the nature of this interaction was found to be mainly the groove-binding mode.

The synthesis and characterization of copper(II) and zinc(II) coordination compounds of 4-(3',4'-dimethoxybenzaldehydene)2-3-dimethyl-1-phenyl-3-pyrazolin-5-semicarbazone (**1a**), 4-(3',4'-dimethoxybenzaldehydene)2-3-dimethyl-1-phenyl-3-pyrazolin-5-thiosemicarba-zone (**1b**), 4-(3'-hydroxy-4'-nitrobenzaldehydene)2-3-dimeth yl-1-phenyl-3-pyrazolin-5-semicarbazone (**1c**) and 4-(3'-hydroxy-4'-nitrobenzaldehydene)2-3-dimethyl-1-phenyl-3-pyrazolin-5-thiosemi-carba-zone (**1d**) was depicted by Raman et al [78]. All the complexes were characterized by elemental analysis, molar conductivity, IR, ^{1}H NMR, UV–vis, ESI-Mass, magnetic susceptibility measurements, cyclic voltammetric measurements, and EPR spectral studies.

The interaction of $CuL_2^{\mathbf{1a–1d}}$ complexes with CT DNA was investigated by spectroscopic, electrochemical and viscosity measurements suggested that the copper complexes bind to DNA via an intercalative mode.

Five new anti-carcinogen 5-Fluorouracil derivatives with DNA were investigated and their DNA binding specificities were compared by electrochemical means with previous reported DNA ligands [54]. Using cyclic voltammetry (CV) to study the elucidation of interactions between DNA and 5-Fluorouracil derivatives, with $Fe(CN)_6^{3-/4-}$ as electroactive indicator of the intercalative interaction dominance, the experimental results indicate that the principal interaction mode of 5-Fluorouracil derivatives with DNA is cooperative interaction.

Horáková et al [79] used voltammetry at the mercury-based electrodes for the monitoring of DNA modification with cisdiamminedichloroplatinum (cisplatin).In square-wave voltammetry, during anodic polarization after prereduction of the cisplatinated DNA, a well-developed, symmetrical signal (peak P) was obtained and a correlation between the peak P intensity and a loss of sequencespecific DNA binding by tumor suppressor protein p53, as well as blockage of DNA digestion by a restriction endonuclease Msp I (both caused by the DNA cisplatination) was demonstrated.

Ravera et al [80] reported the results of electrochemical DNA biosensor assay of the interaction between double-stranded DNA (ds- DNA) and a series of six neutral, anionic or cationic Pt complexes of the general formula $[PtCl_n(NH_3)_{4-n}]^{(2-n)}$ [n = 4, **1**; n = 3, **2**; n = 2, isomers cisplatin (**3**) and transplatin (**4**); n = 1, **5**; n = 0, **6**]. the decrease in the guanine oxidation signal recorded on a screen-printed electrode by using square wave voltammetry (SWV) was measured as fuction of the ability of the electrophilic Pt^{II} agents generated in solution to interact with DNA, and hence to form Pt–DNA adducts The results indicated that the interaction of the PtII complexes of the series with ds-DNA follows the order **2**>**1**> **3** = **4**> **5**>> **6**.

Yapasan et al [81] studied the biomolecular interactions of platinum derivatives widely used as antitumor drugs: cisdiamminedichloroplatinum(II) and oxaliplatin with calf thymus double-strandedDNA using the DPV mode and electrochemical impedance spectroscopy (EIS) in combination with single-walled carbon nanotubes modified graphite electrode (SWCNTs-GE) and unmodified graphite electrode (bare GE). By following the changes at guanine oxidation signal in terms of optimum interaction times and by comparing the results of SWCNTs-GE with bare one, the performance of these biomolecular interactions were carefully explored.

Sönme et al [82] prepared two new mononuclear complexes by reacting symmetric Schiff base, containing pyrimidine rings and the metal chlorides of Pd(II) and Pt(II) in methanol and explored the interaction of these metal complexes with fish sperm double-stranded DNA (dsDNA) electrochemically based on the oxidation signals of guanine and adenine. Differential pulse (DP) voltammetry by using renewable pencil graphite electrode was employed to monitor the DNA interaction at the surface or in solution indicating that Pd(II) and (Pt) complexes strongly interacted with DNA.

The interaction of doxorubicin (DOX) covalently bound via tether molecules to colloidal magnetic nanoparticles (ferrofluid) with calf thymus double stranded DNA (dsDNA) by means of spectroscopic and electrochemical techniques have been examined by Nowika et

al [83]. The experiments clearly indicated that after binding to the magnetic nanoparticles, the DOX molecules are still capable of intercalating the dsDNA helices. Spectroelectrochemical results showed that the intercalation behavior of DOX molecules is only slightly affected by the attachment via the flexible adipoyl tether to the surface of magnetic nanoparticles.

An interaction between the non-toxic agent curcumin and double stranded (ds) calf thymus DNA has beendemonstrated by using voltammetry [53]. The interaction of curcumin (CU) with dsDNA was studied using a carbon paste electrode (CPE) and a hanging mercury drop electrode (HMDE). Significant changes in the characteristic peaks of dsDNA were observed after addition of curcumin to a solution containing dsDNA. All the experimental results have indicated that curcumin was found to be very effective during binding in the minor groove of the DNA double helix. The inducing activity of curcumin appeared in a concentration (of CU)- and time-dependent manner.

Conclusions

Electrochemical research on DNA is of great relevance to explain many biological mechanisms. The DNA-modified electrode is a very good model for simulating the nucleic acid interaction with cell membranes, potential environmental carcinogenic compounds and to clarify the mechanisms of action of drugs used as chemotherapeutic agents.

The application of voltammetric techniques for *in situ* generation of reactive intermediates are a promising analytical tool for the study of biomolecular interaction mechanisms. Voltammetric methods are an inexpensive and involve fast detection procedure.

Additionally, the interpretation of electrochemical data can contribute to elucidation of the mechanism by which DNA is oxidatively damaged by such substances, in an approach to the real action scenario that occurs in the living cell. Moreover, carbon paste electrodes have become a powerful tool applicable to the detection of compounds presenting affinity for DNA, the clarification of their reaction mechanism and the comparison of model compounds with compounds, such as drugs, polymers used in drug delivery and any kind of chemical substance having potent effect into the DNA structure.

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