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Innovative Configurations of Electrochemical DNA Biosensors (A Review)

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Abstract: In the field of electrochemical biosensing, transition metal complexes achieved a significant importance as hybridization indicators or electroactive markers of DNA. Their incorporation in electro-chemical DNA biosensors enables to offer a promising perspective in understanding of the biological activity of some chemical compounds. In this context, the development of innovative configurations of electrochemical DNA biosensors applied to life sciences during the last years were reviewed in the present article. In the first part, a brief introduction of nanomaterial based electrochemical DNA biosensors is given. In the second part, the complexes of transition metals with biological interest and their applications in electrochemical DNA biosensors are being described.

Keywords: Electrochemical biosensors; Electroanalysis; Nanomaterials; Transition metals; DNA interactions; Review.

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Introduction

Since the discovery of carbon nanotubes (CNTs) by Iijima in 1991 ([1-3]; see Fig. 1), this new form of carbon has become soon the subject of very intensive investigation. Among others, CNTs have attracted considerable attention in electroanalysis due to their special chemical, electrical, and mechanical properties, as well as a wide range of potentially interesting applications [4]. Considering the advantages of both single-wall (SW)- and multi-wall (MW)CNTs, such as high surface area, good conductance, favorable electronic properties and electrocatalytic effect, they represent, indeed, an impressive platform for constructing electrochemical (bio)sensors [4-6].

Helical microtubules of graphitic carbon

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THE synthesis of molecular carbon structures in the form of C_{60} and other fullerenes[1] has stimulated intense interest in the structures accessible to graphitic carbon sheets. Here I report the preparation of a new type of finite carbon structure consisting of needle-like tubes. Produced using an arc-discharge evaporation method similar to that used for fullerene synthesis, the needles grow at the negative end of the electrode used for the arc discharge. Electron microscopy reveals that each needle comprises coaxial tubes of graphitic sheets, ranging in number from 2 up to about 50. On each tube the carbon-atom hexagons are arranged in a helical fashion about the needle axis. The helical pitch varies from needle to needle and from tube to tube within a single needle. It appears that this helical structure may aid the growth process. The formation of these needles, ranging from a few to a few tens of nanometres in diameter, suggests that engineering of carbon structures should be possible on scales considerably greater than those relevant to the fullerenes.

Solids of elemental carbon in the sp^2 bonding state can form a variety of graphitic structures. Graphite filaments can be produced, for instance, when amorphous carbon filaments formed by thermal decomposition of hydrocarbon species are subsequently graphitized by heat treatment[2,3]. Graphite filaments can also grow directly from the vapour-phase deposition of carbon[4,5], which also produces soot and other novel structures such as the C_{60} molecule[6–8].

Graphitic carbon needles, ranging from 4 to 30 nm in diameter and up to 1 μm in length, were grown on the negative end of the carbon electrode used in the d.c. arc-discharge evaporation of carbon in an argon-filled vessel (100 torr). The gas pressure was much lower than that reported for the production of thicker graphite filaments[5]. The apparatus is very similar to that used for mass production of C_{60} (ref. 9). The needles seem to grow plentifully on only certain regions of the electrode. The electrode on which carbon was deposited also contained polyhedral particles with spherical shell structures, which were 5–20 nm in diameter. The needle structures were examined by transmission electron microscopy (electron energies of 200 keV).

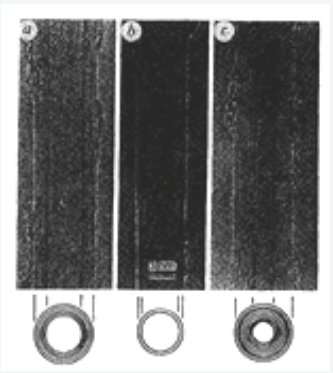

FIG. 1 Electron micrographs of microtubules of graphitic carbon. Parallel dark lines correspond to the (002) lattice images of graphite. A cross-section of each tubule is illustrated. *a*, Tube consisting of five graphitic sheets, diameter 6.7 nm. *b*, Two-sheet tube, diameter 5.5 nm. *c*, Seven-sheet tube, diameter 6.5 nm, which has the smallest hollow diameter (2.2 nm).

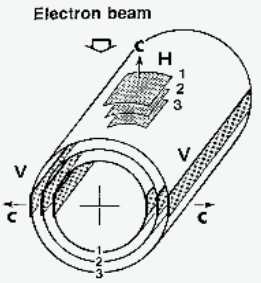

FIG. 2 Clinographic view of a possible structural model for a graphitic tubule. Each cylinder represents a coaxial closed layer of carbon hexagons. The meaning of the labels V and H is explained in the text.

High-resolution electron micrographs of typical needles show (002) lattice images of the graphite structure along the needle axes (Fig. 1). The appearance of the same number of lattice fringes from both sides of a needle suggests that it has a seamless and tubular structure. The thinnest needle, consisting of only two carbon-hexagon sheets (Fig. 1*b*), has an outer and inner tube, separated by a distance of 0.34 nm, which are 5.5 nm and 4.8 nm in diameter. The separation matches that in bulk graphite. Wall thicknesses of the tubules range from 2 to 50 sheets, but thicker tubules tend to be polygonized. This low dimensionality and cylindrical structure are extremely uncommon features in inorganic crystals, although cylindrical crystals such as serpentine[10] do exist naturally.

The smallest tube observed was 2.2 nm in diameter and was the innermost tube in one of the needles (Fig. 1*c*). The diameter corresponds roughly to a ring of 30 carbon hexagons; this small diameter imposes strain on the planar bonds of the hexagons and this causes two neighbouring hexagons on the ring to meet at an angle of ~6°. For the C_{60} molecule, the bending angle is 42°, which is much larger than for these tubes. The C–C bond energy calculated for the C_{60} molecule is smaller than that of graphite[11], suggesting that bending the hexagons in C_{60} lowers the bond energy. A similar effect of the bending on bonding energies might apply here. One of the key questions about the tubular structure is how the ABAB hexagonal stacking sequence found in graphite is relaxed, as it is impossible to retain this ideal graphite structure for coaxial tubes. There should be a shortage of 8–9 hexagons in going from one circumference of a tube to that inside it. Disordered graphitic stacking is known as turbostratic stacking, but no detailed accounts of stacking patterns in such structures have been reported. The argument here is also applicable to the spherical graphitic particles mentioned earlier[6].

All the electron diffraction patterns (Fig. 3) taken from individual carbon needles are indexed by the $\{h0l\}$ and $\{hk0\}$ spots for hexagonal symmetry. The patterns always show strong $(00l)$ spots when the needle axes are perpendicular to the [001] axis, supporting the idea of a coaxial arrangement of graphitic tubes. As shown in Fig. 2, two side portions of each tube (indicated by shading and labelled 'V') will be oriented so that the

Fig. 1A: *Authentic appearance of the title page of the first paper on carbon nanotubes by Sumio Iijima* (reproduced from free-access website [2])

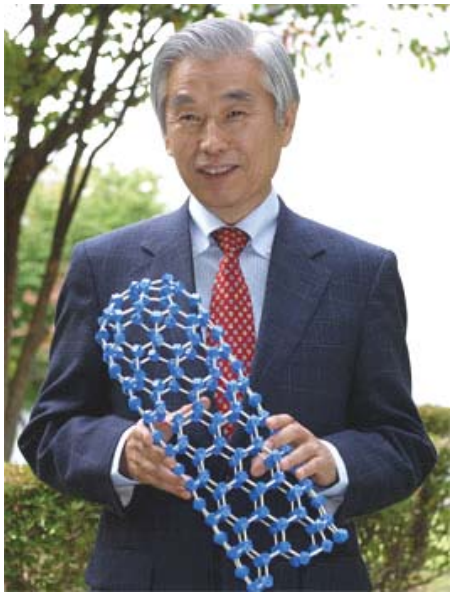

Fig. 1B:
Professor ***Sumio Iijima*** *with the model of "his" carbon nanotube*. An illustrative photo from the late-2000s (from free-access website [3])

Note: Iiijima's contribution in *NATURE* is now generally considered as the very first report on the carbon nanotubes despite the fact that the author himself had named this alternate form of carbon as "helical microtubules"; *i.e.*, even without the prefix "nano". Last but not least, it is worth of mentioning that the already classic contribution by Iijima is apparently the most quoted scientific report in modern chemistry and physics. Up to the date of writing this note*, it has been cited in 11,542 publications (!)

* November 18, (2011).

Electrochemical DNA Biosensors Based on Carbon Nanotubes

In general, carbon nanotubes can be incorporated into the electrode configuration in the form of a paste (composite), in similar fashion to the traditional carbon paste electrode, CPE [4]. This approach leads to a very attractive combination of the advantages of both materials and provides the feasibility to incorporate different substances, low background currents, easy renewal, and composite nature [2]. A variety of binders such as mineral oils (e.g., Nujol® oil) or related silicone oils / greases can be used to produce the desired composites [4-6].

The integration of polyaniline nanofibers (PANI) in a chitosan (CHIT) film in fabricating electrochemical biosensor with improved sensitivity for the detection of DNA probes was presented by Yang et al [7]. The PANI-MWNT/CHIT composites were modified on a carbon paste electrode (CPE) surface. The biosensor was applied to the sensitive detection of the DNA specific sequences of the transgenic genes in the genetically modified crops, such as the phosphinothricin acetyltransferase gene (PAT) and the terminator of nopaline synthase gene (NOS). The DNA hybridization events were monitored with an electrochemical impedance spectroscopy (EIS) label-free detection strategy.

Under the optimal conditions, the detection limit for the PAT gene fragment was an amazing value of 2.7×10^{-14} mol L^{-1}. The NOS gene was also detected satisfactorily.

Utilizing the excellent properties of cobalt phthalocyanine (CoPc) and carbon nanotubes (CNTs), Balan et al [8] fabricated a new paste electrode for the determination of guanine. The modification of a CNTPE with this compound results in amplification of the guanine oxidation response in contrast to that on the unmodified CNTPE. 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 also applied to determine the single-stranded DNA by differential pulse voltammetry with a detection limit of 9.86 $x10^{-8}$ mol L^{-1}.

Using cyclic voltammetry (CV) and square-wave anodic stripping voltammetry (SWASV) in combination with a new type of DNA and carbon nanotube (CNT) mixed paste electrode Ly et al [9] presents an assay of riboflavin (RF). Since the proposed method has a detection limit of 0.2 ng L^{-1} (5.31 $x10^{-3}$ mol L^{-1} RF) was successfully applied to an actual human urine and drug sample, and can be applied to assays of other biological samples.

A paste electrode assembled by MWCNTs and bismuth (BCNE) has been developed again by Ly et al [10] for the voltammetric assay of *Helicobacter pylori* DNA. The analytical cyclic voltammetry (CV) peak potential was obtained at a 0.4 V reduction scan, where the diagnostic optimum square-wave (SW) stripping working range was achieved at 0.72–7.92 μg mL^{-1} H. pylori DNA (11 points). Under optimum conditions, detection limit was 0.06 μg mL^{-1}. Since the sensing time was only two minutes, the process could be simpler compared to common PCR amplification and electrophoresis photometric detection systems. The diagnostic biosensor was successfully applied in detecting amounts of trace labels of HP DNA in gastritis and peptic-ulcer-disease patients.

Carboxylic group-functionalized carbon nanotubes (c-CNT) were used to modify the surface of a carbon paste electrode (CPE) in order to form a conducting precursor film [11]. Positively charged poly-L-lysine (pLys) and negatively charged double-stranded DNA (dsDNA) were alternately adsorbed on the c-CNT-modified electrode, forming (pLys/dsDNA)n layer-by-layer (LBL) films. The nature of the multilayer films growth is characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) of the electroactive probe $[Fe(CN)6]^{3-/4-}$. Differential pulse voltammetry (DPV), with methylene violet (MV) as the intercalation redox probe, was used to study the oxidative DNA damage induced by Cd^{2+} ion.

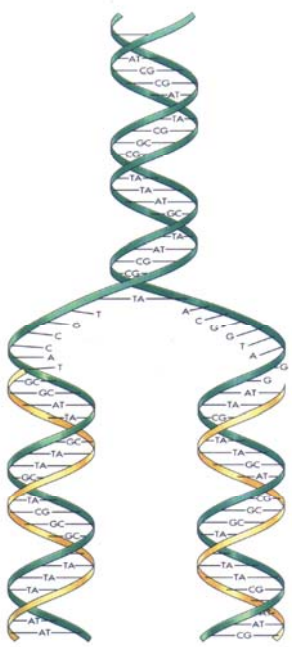

Fig. 2: *The molecule of DNA* (An illustrative image from the authors' archive)

The modification of a DNA –linked carbon nanotubes electrode (BIDCE) with bovine IgG has been reported by Ly and Chob [12] .The bovine IgG was immobilized with cyclic voltammetry, in which the BIDCE sensor was set at optimum parameters via square wave (SW) stripping and cyclic voltammetry (CV) scan. The sensor was used for the development of an effective bioassay for human hepatitis B virus (HBV). Moreover, diagnostic application was performed through direct assay of HBV in non-treated human blood.

Polyaniline nanofibers (PANnano) were used by Zhou et al [13] as a kind of dopping material to prepare a modified carbon paste electrode (PANnano/CPE) which served an excellent affinity interface for the subsequent immobilization of the hybrid of carbon nanotubes and gold nanoparticles (Au-nano-CNT).

The immobilization and hybridization of the DNA probe on the nano-Au–CNT/PAN-nano films were investigated with differential pulse voltammetry (DPV) and cyclic voltammetry (CV) using methylene blue (MB) as indicator, and electrochemical impedance spectroscopy (EIS) using $[Fe(CN)_6]^{3-/4-}$ as redox probe. The sequence-specific DNA of the transgenic genes in the genetically modified crops, such as the phosphinothricin acetyltransferase (PAT) gene and the polymerase chain reaction (PCR) amplification of the nopaline synthase gene (NOS) from a transgenic-modified bean sample were satisfactorily detected with the proposed label-free electrochemical impedance spectroscopic (EIS) method. Under the optimal conditions, the detection limit for the PAT gene fragment could be estimated extremely low: 6×10^{-13} mol L^{-1}.

As shown, the glassy carbon electrode (GCE) offer a high efficiency when modified with carbon nanotubes (CNTs) due to the well defined surface; so modified electrodes are then a good platform for immobilizing DNA, giving rise to the enlarged transducer surface area [5].

A composite film (MWCNTs–PNF) which contains multi-walled carbon nanotubes (MWCNTs) along with the incorporation of poly(newfuchsin) (PNF) has been synthesized on glassy carbon electrode (GCE), gold (Au) and indium tin oxide (ITO) by potentiostatic methods for the simultaneous determination of adenine, guanine, and thymine [14]. The MWCNTs composite film enhances surface coverage concentration, increase the electron transfer rate and also exhibits promising enhanced electrocatalytic activity towards the mixture of the biochemical compoundsThe electrocatalytic responses of analytes at MWCNTs and MWCNTs–PNF films were measured using both cyclic voltammetry (CV) and differential pulse voltammetry (DPV). From the results, well separated voltammetric peaks have been obtained at the composite film for AD, GU and THY, with the peak separation of 320.3 and 132.7mV between GU–AD and AD–Thy. The sensitivity of the composite film towards AD, GU and THY in DPV technique is 218.18, 12.62 and 78.22 mA L mol^{-1} cm^{-2} respectively, which are higher than MWCNTs film.

A third-generation H_2O_2 biosensor based on the immobilization of horseradish peroxidase (HRP) on glassy carbon (GC) electrode with DNA functionalized SWCNTs has been developed and characterized by Zeng et al [15]. The resulted HRP-DNA-SWCNTs/GC electrode showed an electrochemical activity to the reduction of H_2O_2 without the aid of any electron mediator. The proposed biosensor exhibited fast amperometric response, wide linear range, high sensitivity and stability, good reproducibility and low detection limit and also a good selectivity.

A novel amperometric immunosensor for the determination of alpha-fetoprotein (AFP) was fabricated by immobilizing multiple layers of multi-wall carbon nanotubes (MWCNTs)-poly(diallydimethlammonium chloride)(PDDA)/DNA/thionine/nano-Au on a nano-Au layer, which was deposited on the GCE [16]. The modification process was characterized by cyclic voltammetry (CV) and scanning electron microscope (SEM). Under optimal conditions, the proposed immunosensor has a very low detection limit of 0.04 ng/mL, when the selectivity, repeatability, as well as the stability of this immunosensor were acceptable, too.

A sensitive electrochemical DNA biosensor for the detection of target DNA was designed by Zhang et al [17]. Firstly, aminobenzoic acid (ABA) was felectropolymerized on the surface of the glassy carbon electrode (GCE) modified with multi-walled carbon nanotubes with carboxyl groups (MWCNTs) by cyclic voltammetry (CV). Secondly, gold nanoparticles (AuNPs) were subsequently introduced to the surface of PABA–MWNTs composite film by electrochemical deposition mode. Thirdly, probe DNA was immobilized

on the surface of AuNPs via Au–S bond. Scanning electron microscopy (SEM), cyclic voltammetry (CV) and electrochemical impedance spectra (EIS) were used to investigate the film assembly process. The hybridization event was monitored by differential pulse voltammetry (DPV) using adriamycin as an electroactive indicator. Under the optimal conditions, the detection limit of the complementary oligonucleotides was 3.5 x 10^{-13} mol L^{-1}. Moreover, this DNA biosensor has had a good stability and reproducibility.

DNA damage induced by in situ generated bisphenol A (BPA) radicals through electro-oxidation was investigated by Qiu et al [18] using the GCE as the modified variant. Namely, the latter were dsDNA and a nanocomposite as a mixture of the multi-walled CNTs and chitosan. The dsDNA/ MWNT-Chit/GCE was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). According the results, MWNT-chit nano-composite represents a viable platform for the immobilization of DNA that effectively promotes electron transfer between DNA and the electrode. The mode of interaction between DNA and BPA was investigated by differential pulse voltammetry (DPV) and UV-vis spectrophotometry.

A GCE modified first with the incorporation of DNA and multi-walled carbonwith multi-walled carbon nanotubes–poly(amidoamine)–chitosan (MWNT–PAMAM– Chit) nanocomposite and subsequently by immobilizing Cytochrome C (Cyt C), was constructed for the determination of nitrite by Chen et al [19]. The nitrite biosensor based on the biocatalytic oxidation of the immobilized Cyt c showed a fast response to nitrite (less than 5 s), a detection limit of 0.03 μ mol L^{-1} and a good reproducibility and stability. Also, it has been successfully demonstrated for detection of nitrite in food material.

A new method for mimicking the metal mediated DNA damage pathway in vivo was presented by X. Wang and Jiao [20]. The biosensor was developed by incorporating the mixture of multi-walled carbon nanotubes (MCNTs) and Fe@Fe2O3 nano-necklace into a membrane containing poly(dimethyldiallylammonium chloride) (PDDA) and intact DNA using a glassy carbon electrode (GC) as working electrode. Using $Co(phen)_3^{3+}$ and $Ru(NH_3)_6^{3+}$ as electrochemical indicators, the DNA damage course was monitored by the differential pulse voltammetric (DPV) measurement. This biosensor has had not only good stability and high sensitivity, but also the potentiality to help in understanding of the mechanism of DNA damage induced by oxidative pathways *in vivo*.

The fabrication of a sensitive electrochemical DNA biosensor for the detection of sequence-specific target DNA was reported by J. Wang et al [21]. Firstly, zinc oxide

nanowires (ZnONWs) were immobilized on the surface of a glassy carbon electrode (GC). Then, multi-walled carbon nanotubes (MWCNTs) with carboxyl groups were dropped onto the surface of the ZnONWs. And finally, gold nanoparticles (AuNPs) were subsequently introduced to the surface of the MWNTs/ZnONWs by electrochemical deposition. Scanning electron microscopy (SEM) and cyclic voltammetry (CV) were used to investigate the film assembly process where as differential pulse voltammetry (DPV) was used to monitor DNA hybridization by measuring the electrochemical signals of $[Ru(NH_3)_6]$ bounding to double-stranded DNA (dsDNA). The results indicated that this sensor can detect the target DNA with a detection limit of $3.5x10^{-14}$M and also exhibits fast response, good selectivity and a broad linear range.

Gutiérrez et al [22] presented the selectively determination of 8-OHdG on glassy carbon electrodes modified with carbon nanotubes (CNs) dispersed in polyethylenimine (PEI) in the presence of ascorbic acid and uric acid using the technique of differential pulse anodic stripping voltammetry (DPASV). Since the detection limit was $1.0x10^{-7}$ M the proposed method can be compared with the ones reported using other analytical methods (HPLC-ECD and ELISA).

Weber et al [23] reported the detection of *Salmonella enterica* serovar *Typhimurium* using chemically modified single walled carbon nanotubes (SWNTs) with single stranded DNA (ssDNA) on a polished GCE. Target DNA sensing was accomplished by measuring the change in the overall impedance of the system, as well as measuring the charge transfer resistance. This method of the so-called genosensing is a quick and facile approach to detect DNA without the use of additional labels.

The fabrication of poly (2,6-pyridinedicarboxylic acid)/MWNTs modified glass electrode (PPDA/MWNTs/GCE) was proposed by Deng et al [24] and used for individual or simultaneous determination of guanine and adenine. The performances of the PPDA/MWNTs/GCE were characterized with cyclic voltammetry (CV) while the concentration of guanine and adenine was determinated by differential pulse voltammetry (DPV). The detection limit for guanine and adenine was 0.045 mmol L^{-1} and 0.05 mmol L^{-1}, respectively.

An electrochemical sensor based on gold nanoparticles (GNPs)/multiwalled carbon nanotubes (MWCNTs)/ poly (1,5-naphthalenediamine) films modified glassy carbon electrode (GCE) was fabricated by Zeng et al [25].

Their modified electrode was applied to the determination of cellobiose dehydrogenase gene extracted from *Phanerochaete chrysosporium*. The film assembly and DNA hybridization

processes were investigated by scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The electrochemical sensor was convenient and extremely sensitive, offering an extraordinary LOD down to $1.2x10^{-16}$ mol L^{-1}, representing one of the lowest values ever achieved with an electrochemical sensor [26].

For the study of the electrochemical behavior of rutin (RU) and its interaction with DNA, multi-walled carbon nanotubes functionalized with a carboxylic acid group (MWNTs–COOH) in association with iron oxide (Fe_3O_4) (MWNTs–COOH/Fe_3O_4/GCE) were used by Bian et al [27] as the modifying materials on a glassy carbon electrode (GCE). DNA was covalently immobilized to the MWNTs–COOH/Fe_3O_4/GCE surface with the aid of coupling activator. The direct electrochemistry of RU and its interaction with DNA were studied by cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

A simple approach to fabricate an electrochemical DNA biosensor by introducing the single-walled carbon nanotubes (SWNTs) has been reported by Yang et al [28]. Using high square wave voltammetry (SWV) as the stripping mode and methyl blue (MB) as the electrochemical label, the DNA biosensor has significant advantages of improved sensitivity, sequence-specificity, and large linear dynamic range with low detection limit.

Carbon nanotubes can be used in association with graphite electrodes [6]. Electrochemical monitoring of direct DNA hybridization related to specific sequences of Hepatitis B virus (HBV) was successfully performed by Caliskan et al [29] using disposable graphite sensors (PGEs) modified with the commercial single-walled carboxylic acid functionalized carbon nanotubes (SWCNTs). The performance characteristics of hybridization on the disposable SWCNT-PGEs were studied by measuring the changes at guanine signal in terms of optimum analytical conditions; such as, probe and target concentration, hybridization time, and selectivity. Electrochemical impedance spectroscopy (EIS) was also used to characterize the successful construction of carbon nanotubes modification onto the surface of PGEs complementing the ones obtained via the voltammetric detection.

Differential pulse voltammetry (DPV) in combination with unmodified and modified pencil graphite electrodes (PGE) with single walled carbon nanotubes (SWCNT) was used by Karadeniz al [30] in order to explore the interaction of 4-nonylphenol (NP) with DNA. Before and after interaction of NP with DNA, the changes at the two oxidation signals coming from NP and DNA base, guanine were studied. Also, the effect of NP concentration was investigated in order to determine the optimum experimental conditions. The detection limit and the reproducibility were determined by using CNT-modified electrodes.

A sensitive voltammetric method has been developed by Goyal et al [31] using single-walled carbon nanotubes modified edge plane pyrolytic graphite electrode (SWNT/EPPGE). The proposed sensor has been used for the simultaneous determination of guanine and 8-hydroxyguanine in acid hydrolyzed DNA with satisfactory results. The main advantage of the modified electrode is its selectivity, as it can detect very low concentration of 8-hydroxyguanine without affecting the other products of DNA damage. The detection limits of guanine and 8-hydroxyguanine were calculated to be 0.05 $x10^{-9}$ and 0.01 $x10^{-9}$ mol L^{-1}, respectively; the limits of quantification being then found to be 0.17 $x10^{-9}$ and 0.34 $x10^{-10}$ mol L^{-1} for guanine and 8-hydroxyguanine, respectively.

The biomolecular interactions of platinum derivatives widely used as anticancer drugs: cisdiamminedichloroplatinum (II) and oxaliplatin with calf thymus double-stranded DNA were studied by Yapasan et al [32] using differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) in combination with single-walled carbon nanotubes modified graphite electrode (SWCNTs-GE) and unmodified graphite electrode (bare GE). The performance characteristics of these biomolecular interactions were explored at the electrode surface by monitoring the changes at guanine oxidation signal in terms of optimum interaction times by comparing the results of SWCNTs-GE with bare one.

Combining the advantages of nanotechnology and polymer technology, Canavar et al [33] used a single-walled carbon nanotube (SWCNT)/poly(vinylferrocenium) (PVF^+) modified pencil graphite electrode (PGE) for the electrochemical investigation of the interaction between the anticancer drug *Mitomycin C* (MC) and DNA. The changes in the magnitude of guanine oxidation signals were sensitively monitored by using differential pulse voltammetry (DPV) in the absence/presence of MC. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were also used for the characterization of $SWCNT/PVF^+$ modified electrode and PVF^+ modified PGEs. The detection limit corresponded to 625 ng mL^{-1} for MC using calf thymus double-stranded DNA immobilized $SWCNT/PVF^+$ modified PGE.

A single-wall carbon nanotubes (SWCNT) modified edge plane pyrolytic graphite electrode (EPPGE) has been used as a sensor by Goyal et al [34] to monitor the DNA damage in acid hydrolyzed calf thymus DNA. Square wave voltammetry (SWV) was used to detect the DNA damage based on the determination of 2,8-dihydroxyadenine (2,8-DHA). Because of the sensor exhibits a potent and persistent electron- mediating behavior, the oxidation peak of 2,8-DHA was observed with lowering of peak potential and increase in peak current as

compared to bare EPPGE. At optimal experimental conditions, the catalytic oxidative peak current was responsive with the 2,8-DHA concentrations ranging from 0.05 nmol L^{-1} to 100 nmol L^{-1}. The detection limit was 3.8 $x10^{-11}$ mol L^{-1} and limit of quantification was 1.27 x 10^{-10} mol L^{-1}. The modified electrode exhibited high stability and reproducibility.

Among various types of miniaturized electrodes, screen-printed electrodes (SPEs) have several advantages including low cost of preparation, ease use, and mechanical stability. The main advantages of those sensors, which they fabrication technique based to thick-film technology (TFT), are low dimensions, good reproducibility, excellent mechanical and electrical properties of electrodes and well accessible and ecological fabrication process [35].

Hubálek et al [36] prepared two sensor sets with different paste materials. In the first case, a working electrode has been printed from carbon nanotubes based paste on Ag-layer modified with nano-patterned structures and in the second case, vertically aligned carbon nanotubes were grown on the Au working electrode. For the detection of nucleic acids, based on the respective characterization, they used Au-based working electrode. Moreover, this electrochemical sensor was able to estimated melting points of DNA.

The application the MW-CNT-based screen-printed graphite electrodes for the electrochemical monitoring of DNA hybridization related to specific sequences on Hepatitis-B virus (HBV) the macromolecules of DNA were explored by Karadeniz et al [37]. The advantages of MWCNT-SPEs; such as, disposable and portable, providing a higher surface coverage due to modification of CNTs and offering an enhanced response with a high sensitivity in a good reproducibility, leads to a great opportunity for DNA detection using differential pulse voltammetry (DPV) by measuring the guanine oxidation signal observed at +1.0 V vs. ref. in the presence of DNA hybridization between HBV probe and its complementary target.

A disposable electrochemical DNA-based biosensor was developed by Labuda et al [38] using screen-printed carbon electrodes without and with MW-CNTs and applied as a screening device to detect the effect of a synthetically prepared quinazoline derivative on the surface-attached double stranded calf thymus DNA. The quinazoline interaction with DNA was investigated voltammetrically using DNA-bound electrochemical indicators such as $[Co(phen)_3]^{3+}$, $[Ru(bpy)_3]^{2+}$, *Methylene blue*, the $K_3[Fe(CN)_6]$ complex present in the solution phase as well as by electrochemical impedance spectroscopy (EIS).

A nontoxic biosensor with a layer of double-stranded DNA based on a composite of multiwalled carbon nanotubes (MWCNT) in chitosan (CHIT) used as an interface at the screen printed carbon electrode (SPCE) was designed by Galandová et al [39]. The

voltammetric characterization of the modified SPCE with / without DNA has been done using the redox probe $[Fe(CN)_6]^{3-/4-}$ in the CV mode and by linear-scan elimination voltammetry (LSEV).

The modification of a SPCE by using a MW-CNT composite dispersed in polyethylenimine (PEI) followed by using the covering the electrode with the calf thymus dsDNA layer proposed again by Galandová et al [40]. Square wave voltammetry (SWV) with the $[Ru(bpy)_3]^{2+}$ indicator and mediator as well as coupled cyclic voltammetry (CV) and EIS with the $[Fe(CN)_6]^{3-/4-}$ redox pair have been used to characterize the biosensor and to evaluate damage to the surface-attached DNA by quinazolines.

Applications of Transition Metal Complexes in the Study of DNA Electrochemistry

In the previous years, the need to analyse the gene sequences, oxidative damage to DNA, as well as to understand the principles of DNA interactions with molecules or ions, had initiated the rapid development of electrochemical DNA-based methods as a complementary tool to the other biophysical methods, such as UV-visible spectroscopy or some special fluorescence studies. Since numerous metal complexes cannot be studied with such molecular spectra techniques (either due to weak absorption capabilities and forbidden "d-d" transitions or because of overlap of electronic transitions with those in the DNA molecule), it was approached to exploit their redox activity and the respective voltammetric characterisation.

In general, electrochemical measurements of such redox couples of metal-based potential drugs in the presence of DNA are highly sensitive thanks to the resemblance between the electrochemical and biological reactions. In addition, the family of the transition metal complexes offers unique modular systems, holding three dimensional ligand scaffoldings that bear the recognition elements at a site which can be varied by the effective interchange of some ligands. Their high electrochemical activity and generally favourable properties have thus allowed metal complexes to be used as the proper electrochemical probes for DNA binding studies (see e.g. discussion in [41]).

The above topic is of particular interest within our research group (see e.g. [6] and refs. therein), and is thus reviewed in the following sections, covering the studies on the interaction mechanisms between transition metal complexes and DNA, its application in electrochemical DNA biosensors as hybridization indicators or as an electroactive marker of DNA in the configurations of the DNA cleavage electrochemical biosensors.

Drugs, such as transition metal complexes are inhibitors of nucleic acid synthesis. At present biochemists mainly use the coupling of electrophoretic separations and radioisotopic detection are used for DNA sequencing analysis due. Typically, conventional methods are time-consuming and, as usually quite sophisticated, also labor-intensive. On the other hand, electrochemical hybridization biosensors, which are used for the detection of specific DNA sequences significantly reduces the assay time, and simplify its protocol.

In an electrochemical DNA hybridization detector, a short DNA probe, usually 15–20 nucleotides long, is immobilized on the electrode surface to create a DNA recognition element. Immobilization of probe DNA could take place either by covalent or noncovalent binding. The probe-modified electrode is then immersed into a solution of target DNA. A hybrid duplex is formed at the electrode surface, when the target DNA contains a sequence that exactly matches that of the probe DNA. In the absence of a complementarity between the probe and target, no duplex is formed.

For instance, Zhang et al. [42], synthesized $[Mn(Im)_6](teph)\ 4H_2O$ (hexakis (imidazole) manganese(II) terephthalate). In this study, the interaction between $[Mn(Im)_6](tePht)$ x 4 H_2O and DNA was investigated by CV and fluorescence spectroscopy. The experimental results proved that $[Mn(Im)_6](tePht)$ x 4 H_2O could interact with DNA mainly by intercalative mode. Using $[Mn(Im)_6](tePht)$ x 4 H_2O as the electrochemical hybridization indicator, the electrochemical DNA sensor was prepared and tested by covalent interaction. The results demonstrate that $[Mn(Im)_6](teph)\ 4H_2O$ is a promising electrochemical DNA biosensor to determinate the complementary ssDNA.

Niu et al. [43] had chosen Luteolin as a ligand for the synthesis of copper (II) complex (CuL_2). In this study CuL_2 was used as an electroactive indicator for the detection of a synthetic 21-mer sequence of the human hepatitis B virus based on silver nanoparticles and multi-walled carbon nanotubes (Ag/MWCNTs) modified GCE. The interaction between the CuL_2 and dsDNA was investigated by CV and fluorescence spectroscopy methods. The experimental results showed that CuL_2 could bind with dsDNA to form a complex mainly by intercalation. The sensitivity of this electrochemical DNA biosensor for the hybridization detection was improved based on Ag/4-ABA/MWCNTs. The target ssDNA of the human hepatitis B virus (HBV) was quantified in a linear range from 3.2×10^{-12} to 5.3×10^{-9} mol L^{-1} (with $r^2 = 0.998$). A detection limit (3σ) of 6.5×10^{-13} mol L^{-1} (for n = 11) was achieved.

In another study the application of a metallodendrimer-based electrochemical DNA biosensor, a cobalt (II) salicylaldiimine metallodendrimer (SDD–Co^{II}), as a suitable platform to immobilize DNA and a 21-bases oligonucleotide NH_2-5′-GAGGAGTTGGGGGAGCACATT-3′ (pDNA) on gold electrode surface was presented [44]. The complementary oligonucleotide was 5′-AATGTGCTCCCCCAACTCCTC-3′ (tDNA). The electrochemistry of the SDD–Co(II)-modified Au electrode was studied by SWV. Herein, FTIR and UV–vis spectroelectrochemistry were used to study the structural changes associated with the redox processes of the SDD–Co(II) and the hybridization of the probe and target DNA fragments to form dsDNA. EIS experiments confirmed that the hybridization of the biosensor's pDNA with the tDNA to form double-stranded DNA (dsDNA) resulted in an increase of the impedimetric charge transfer resistance, R_{ct}. The limit of detection (LOD), calculated as 3σ of the background noise, and sensitivity of the sensor were 1.29 KΩ /nmol L^{-1} and 0.34 pmol L^{-1}, respectively.

More recently, Ilkhani et al. [45] synthesized an ytterbium complex of $YbCl_3$ (tris(8-hydroxyquinoline-5-sulfonic acid) ytterbium) and utilized it as an electrochemical indicator for the detection of DNA oligonucleotide based on its interaction with $Yb(QS)_3$. The results revealed that $Yb(QS)_3$ presented electrochemical activity on GCE and could intercalate into the double helix of double-stranded DNA (ds-DNA). The binding mechanism of interaction was elucidated on glassy carbon electrode dipped in DNA solution and DNA modified carbon paste electrode by using DPV, CV and a fluorescence method; the binding ratio between this complex and ds-DNA being defined to be 1:1. The extent of hybridization was then evaluated from difference between signals of $Yb(QS)_3$ with a DNA probe before and after hybridization with complementary DNA. With this approach, this DNA could be quantified from 1×10^{-8} to 1.1×10^{-7} mol L^{-1}.

Transition Metal Complexes as DNA Cleavage Electrochemical Biosensors

A number of transition metal ions may cause DNA damage through the production of reactive oxygen species (ROS) (frequently via *Fenton*-type reactions) under aerobic conditions. Therefore, it is necessary to identify free radical scavengers or anti-oxidants that inhibit the oxidative DNA damage [46]. The concern of the biochemists is aroused by the fact drugs are inhibitors of nucleic acid synthesis. At present agarose gel electrophoresis is used in order to study the DNA damage due to physical and chemical environmental agents. Gel

electrophoresis is a time consuming and laborious technique, moreover it is not able to detect small damages to DNA induced by ionizing radiation, enzymatic digestion or treatment with chemicals, damages. In contrast to this, electrochemical methods offer a rapid and relatively inexpensive approach to detect DNA damage.

Wang and coworkers [20] presented an electrochemical biosensor for mimicking the metal-mediated DNA damage pathway in situ. In this investigation, a new DNA damage electrochemical biosensor was developed by incorporating the mixture of MCNTs and $Fe@Fe_2O_3$ nanonecklace into a membrane containing poly(dimethyldiallylammonium chloride) (PDDA) and intact DNA. In the membrane, the DNA damage was caused by the hydroxyl radicals. Under slightly acidic conditions, the hydroxyl radicals were produced during the reaction of iron ions leaking from $Fe@Fe_2O_3$ nanonecklace and H_2O_2 cathodically generated on MCNTs. Both the generation of hydroxyl radicals and the whole course of DNA damage were completed in the membrane, which were just like the course of DNA damage caused by heavy metals in organism. The Fenton reagents (H_2O_2 and iron ion) for the DNA damage were generated in situ with a constant rate from the sensing film. H_2O_2 and iron ion reacted further together to yield hydroxyl radical, which attacked DNA in the film. These courses of DNA damage were just like those happened in organism. The DNA damage was detected by monitoring the differential pulse voltammetric response of an electrochemical indicator, $[Co(phen)_3]^{3+}$.

Another electrochemical indicator, $[Ru(NH_3)_6]^{3+}$, was also used for monitoring the DNA damage as a complementary means and the minimal detectable amount of DNA damage was 0.16 μg. The result showed that the biosensor had good reproducibility.

Flavonoids are a large group of naturally occurring polyphenolic compounds that are distributed in vascular plants. A wide range of biological activities, including anti-inflammatory, antibacterial, hepatoprotective activities, are attributed to flavonoid antioxidant and chelating abilities (see [47] and reference therein). The respective study was carried out by Zatloukalová et al. who have studied flavonolignans, silybin and its derivatives (2,3-dehydrosilybin, 7-O-methylsilybin, 20-O-methylsilybin) and isosilybin using ex situ (adsorptive transfer, AdT) cyclic and square wave voltammetry (SWV) basal-plane pyrolytic graphite electrode (PGE). The electrochemical results confirmed that 2,3-dehydrosilybin is a relatively strong antioxidant. The oxidation processes and antioxidant parameters of flavonolignans can be affected by transition metal complexation via hydroxyl groups. It was found that silybin and 2,3-dehydrosilybin are able to chelate transition metals, especially Cu^{2+}. In a special study, the formation of silybin/Cu^{II} complexes was confirmed by adsorptive transfer square-wave voltammetry (AdT-SWV) and the same observation was also made by

fluorescence spectroscopy. The electrochemical investigation of DNA interactions and damage caused in the presence of silybin/Cu^{II} complex and hydrogen peroxide was described. It was evidenced that flavonolignans are involved not only in antioxidant abilities but also in the prooxidation effects under *in vitro* conditions.

Transition Metal Complexes and DNA Interactions Studies

Metal complexes interact with DNA in three ways: (i) electrostatic binding along the exterior of the DNA double helix, which is generally nonspecific; (ii) groove binding, in which the bound molecule interacts directly with the edges of base pairs in the minor or major grooves of DNA; and (iii) intercalation of planar aromatic ring systems between base pairs. Electrostatic and groove binding do not usually change DNA conformation, whereas intercalation changes the torsional angles in the sugar–phosphate backbone so as to separate adjacent base pairs enough to allow insertion of the intercalator. Further changes in DNA structure, such as unwinding or bending, can accompany the intercalation process. Using various electrodes, it is possible to study the electron transfer (ET) reactions of the interaction between transition metal complex and DNA by means of fast scan rate cyclic voltammetry and to detect more sensitive electrode processes by differential pulse voltammetry. Electrochemical techniques — best, some pulse techniques — are suitable for studies of biological systems, since they are fast and have high sensitivity. For example, conventional CV can be used for the detailed diagnosis of the interaction between transition metal complexes whith DNA, because it provides a useful complement to the other methods of investigations such as UV-visible spectroscopy and fluorescence studies. On this ground, a diketo-type ligand was synthesized by the so-called *Knoevenagel* condensation reaction of thiophene-2-aldehyde with acetylacetone, subsequently its transition metal complexes with Cu(II), Ni(II), and Co(II) chlorides were also prepared by Tak et al [48]. Interaction of the Cu(II) complex with CT-DNA (calf thymus DNA) was studied by absorption spectral method and CV at a Pt-disc electrode. The k_{obs} values versus [DNA] gave a linear plot suggesting pseudo-first order reaction kinetics. The CV of the Cu(II) complex revealed a quasi-reversible wave attributed to Cu(II)/Cu(I) redox couple. On addition of CT-DNA, there is a shift in the $E_{1/2}$ values (168 mV and 18 mV, respectively), as well as a decrease in E_p values. The shift in $E_{1/2}$ values noticed in the presence of CTDNA suggests one quite strong binding of the Cu(II) complex to CT-DNA.

Arjmand et al [49] synthesized via an in situ one-pot template condensation reaction (IOPTCR) some bis-macrocyclic complexes of Co(III), 1, Ni(II), 2, and Cu(II), 3, containing pyridyl bridges between 13-membered macrocyclic subunits. DNA-binding properties of the complexes 1 and 3 were investigated by absorption and emission titrations, cyclic voltammetry at a Pt micro cylinder electrode, and viscosity measurements. Complexes 1 and 3 are strong DNA binders with formation (binding) constants, K_b = 1.64 x10^5 and 2.05 x10^5 L mol^{-1}, respectively. Hyperchromism, a decrease in emission intensity of DNA-bound ethidium bromide (EB), and changes observed in the viscosity and cyclic voltammograms in the presence of added metal complexes have revealed that the complexes bound to DNA predominantly by electrostatic attraction, substantiated by absorption titration with 5′-GMP.

Another example of the use of CV was made by Phsomas and coworkers [50] who had selected a quintet of novel metal complexes of the quinolone antibacterial agent, *Ciprofloxacin*, with Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+} and MoO_2^{+2} was prepared and characterized with physicochemical, spectroscopic and electrochemical techniques. The CVs of the complexes recorded in dimetrhyl sulphoxide (DMSO) solution and in 1 : 2 DMSO : buffer solution (containing 0.15 M NaCl and 0.015 M trisodium citrate; pH 7.0) and the corresponding redox potentials were estimated.

The biological activity of the complexes was evaluated by examining their ability to bind to CT-DNA with UV and fluorescence spectroscopy and cyclic voltammetry. The UV-studies of the interaction of the complexes with DNA have shown that these compounds can bind to CT-DNA. The binding constants of the complexes with CT-DNA were also calculated. The cyclic voltammograms of the complexes in the presence of CT-DNA showed that the complexes could bind to CT-DNA by both the intercalative and the electrostatic binding mode. Competitive studies with ethidium bromide (EB) revealed also that the complexes exhibit the ability to displace the DNA-bound EB indicating that they coould bind to DNA probably via intercalation in strong competition with EB for the intercalative binding site.

Horakova et al [51] used the CV with the mercury-based electrode for monitoring the modification of DNA with *cis-Platin* as a typical representative of anticancer metallodrug. The cis-Platin-modified DNA yielded the catalytic currents the intensity of which reflected the DNA modification extent. The decrease in G^{ox} peak — due to the chemical change within the imidazole ring upon cis-platination of DNA — has indicated the coordination to its primary target via N7 of guanine. Raman and Selvan [52] used absorption spectroscopy, viscosity measurements, and CV with the GCE to investigate the interactions of Cu^{II}, Zn^{II},

Co^{II} and $V^{IV}O$ complexes with Schiff bases, 4,4'-methylenedianilidene-bis-(3-methoxy-4-hydroxy-benzaldehyde and 4,4'-methylene-dianilidene-bis(3,4-dimethoxybenzaldehyde). Also these results have indicated that the complex can be bound to the DNA molecule by intercalation.

Another interesting study aimed at the investigation of the interaction between DNA and the flavonoid–transition metal complex Cu^{II}–naringin complex [53]. The interaction was evaluated by using electrochemical ssDNA- and dsDNA-based biosensors and the results were supported by UV-VIS, CD, and 1H-NMR data. Electrochemically, the changes in the oxidation peak of guanine and adenine bases are obtainable by SWV evidencing pretty well the interaction. The variations of the spectroscopic characteristics of DNA and Cu^{II}–naringin complex in aqueous medium demonstrated that the predominant interaction mode may be due to the intercalation effect. Cu^{II}–naringin complex interacts to dsDNA probably via N(7) of the guanine site.

Li et al. [54] investigated the interaction of transition metal complexes $Co(phen)_2TATP^{3+}$, $Co(phen)_3^{3+}$ and $Co(bpy)_3^{3+}$ (where TATP = 1,4,8,9-tetra-aza-trisphenylene, phen = 1,10-phenanthroline, and bpy = 2,2′-bipyridine) CT-DNA using the rotating ring-disk electrode technique. The values of the apparent diffusion coefficient and rate constant at the formal potential for reduction of these three poly-pyridyl/Co^{II}-complexes were found to decrease significantly in the presence of DNA as compared with that in the absence of DNA. The formal potentials at which the redox reaction takes place in the absence or presence of DNA were in the order of $Co(phen)_2TATP^{3+/2+}$ > $Co(phen)_3^{3+/2+}$ > $Co(bpy)_3^{3+/2+}$. The interaction between the complexes and DNA varied significantly, depending on the nature of ligands. The binding strength of these complexes to DNA was found in the order of $Co(phen)_2TATP^{3+}$ > $Co(phen)_3^{3+}$ > $Co(bpy)_3^{3+}$. The interaction modes of the polypyridyl cobalt complexes with DNA were discussed in line of electrochemical observations

Yet another couple of related studies [55] can be presented, concerning Pd^{II}- and Pt^{II}-complexes and their specific interaction with DNA. At fisrt, Sönme et al focused thier attention on the interaction of these metal complexes with fish sperm dsDNA in an electrochemical study based on the oxidation signals of guanine and adenine. Differential pulse voltammetry in combination with renewable pencil graphite electrode (PGE) was employed to monitor the DNA interaction at the surface or in solution. The results indicate that Pd^{II} and Pt^{II} complexes with Schiff base ligand having pyrimidine rings strongly

interacted with DNA. The determined DNA binding levels for platinum complex were found to be similar to those of palladium one, showing the strong interaction with DNA. Similarly, Mello et al investigated the interaction of the $[Pt^{II}(dppf)(H_2O)_2]^{2+}$ complex with DNA by DPV at a carbon paste electrode (CPE), combined with special characterisation by 1H-NMR. The results obtained have shown that the interaction process was characterized by changes in the electrochemical parameters of both compounds and the formation of a new anodic current peak close to the anodic current peak of the Pt^{II} complex of interest. In addition, the 1H-NMR spectra revealed that the coordination of Pt(II)(dppf)-complex with dsDNA occurs via N7 of guanine. Within the whole study, others parameters — *e.g.*, pH or ionic strength — that may affect the interaction process were also considered.

Table 1. *Transition metal complexes and electrodes used for their investigation*

Transition metal	**Electrode** and its specification	**Application** / Characterization	**Ref**
Mn	GCE	hybridization indicator	[36]
Cu	Ag/MWCNTs modified GCE	hybridization indicator	[37]
Co	Au	hybridization indicator	[38]
Yb	GCE	hybridization indicator	[39]
Co, Ru	GCE or metallic film-coated GCE	DNA cleavage electrochemical biosensor	[16]
Cu	basal-plane pyrolytic graphite electrode (PyGE)	DNA cleavage electrochemical biosensor	[41]
Cu, Ni, Co	Pt disc	Metal complex - DNA interaction	[42]
Co, Ni, Cu	Pt micro cylinder	Metal complex - DNA interaction	[43]
Fe, Co, Mo, Ni	Pt	Metal complex - DNA interaction	[44]
Pt	Hg	Metal complex - DNA interaction	[45]
Cu, Zn, Co, V	GCE	Metal complex - DNA interaction	[46]
Cu	graphite	Metal complex - DNA interaction	[47]
Co	AFDT06 Au ring-Au electrode	Metal complex - DNA interaction	[48]
Pd, Pt	renewable pencil graphite electrode (PGE)	Metal complex - DNA interaction	[49]
Pt	CPE	Metal complexes - DNA interaction	[50]

The above-given description can be concluded with Table 1, summarizing the application of transition-metal complexes in the DNA electrochemistry along with the corresponding electrodes. These studies show clearly that the individual methods and procedures can advantageously be used in the DNA sequencing analysis. Last but not least, they are useful for understanding of the biological activity of some drugs or the cleavage of DNA strands.

Concluding Remarks

The interaction of small molecules with DNA and electrochemical DNA analysis remains a dynamically developing field in clinical diagnosis, environmental monitoring and drug analysis. Nanomaterials and especially carbon nanotubes gain much interest in the field of DNA sensing as they can improve the effective surface area of the respective electrodes. Moreover, these detection systems are chemically stable and sufficiently conductive so that they may offer a very high performance in modern electrochemical analysis.

The development of new transition metal complexes and nanomaterials are still a matter of significance. Their potential application in biochip devices is promising moreover labelling of DNA using transition metal complexes and the attachment of metal complexes probes to nucleobase, promise potential application in multifunction analysis of DNA by combination of the redox characteristic and photophysical properties of transition metal complexes.

Thus, this arising new class of electrochemical biosensors characterised by the speed of analysis, simplicity of instrumentation, as well as by low cost of the respective experimental procedures, represent very promising analytical tools as shown throughout this text.

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