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Review

Electrochemical approach of anticancer drugs–DNA interaction

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Abstract

The interaction of drugs with DNA is among the most important aspects of biological studies in drug discovery and pharmaceutical development processes. In recent years there has been a growing interest in the electrochemical investigation of interaction between anticancer drugs and DNA. Observing the pre and post electrochemical signals of DNA or drug interaction provides good evidence for the interaction mechanism to be elucidated. Also this interaction could be used for the quantification of these drugs and for the determination of new drugs targeting DNA. Electrochemical approach can provide new insight into rational drug design and would lead to further understanding of the interaction mechanism between anticancer drugs and DNA.

Keywords: Anticancer drugs; DNA; Interaction; Electrochemical DNA biosensor

Contents

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1. Introduction

Deoxyribonucleic acid plays an important role in the life process because it carries heritage information and instructs the biological synthesis of proteins and enzymes through the process of replication and transcription of genetic information in living cells. Studies on the binding mechanism of some small molecules with DNA have been identified as one of the key topics during the past few decades [1,2]. Moreover it is of great help to understand the structural properties of DNA, the mutation of genes, the origin of some diseases, the action mechanism of some antitumour and antivirus drugs and, therefore, to design new and more efficient DNA targeted drugs to deal with genetic diseases.

Anticancer drugs interact with DNA in many different ways. These include intercalation, non-covalent groove binding, covalent binding/cross-linking, DNA cleaving and nucleoside-analog incorporation [3]. As a result of complex formation occurring between DNA and drug, the thermodynamic stability and the functional properties of DNA change [4]. Understanding how complexation affects both the structural and mechanical properties of DNA is an important step towards elucidating the functional mechanism of binding agents and may also provide information towards more rational drug design.

1.1. Types of drug–DNA interaction

The interaction of the anticancer drugs with DNA occurs principally by three different ways [3–5] (Fig. 1). The first one is through control of transcription factors and polymerases; in which drug interacts with proteins that bind to DNA. The second is through RNA binding either to the DNA double helix to form nucleic acid triple helix structures or to exposed DNA single strand forming DNA–RNA hybrids that may interfere with transcriptional activity. The third type of interaction involves the binding of small aromatic ligand molecules to DNA double helical structures.

The binding of small molecules to DNA involves, electrostatic interaction with the negatively charged nucleic acid sugar-phosphate structure (which is generally non-specific), intercalation of planar aromatic ring systems between base pairs (planar organic molecules containing several aromatic condensed rings often bind to DNA in an intercalative mode; (for example daunomycin, epirubicin and actinomycin D) and minor and major DNA grooves binding interaction. Minor groove binding makes intimate contacts with the walls of the groove, and as a result of this interaction numerous hydrogen binding and electrostatic interactions occur between anticancer drugs and DNA (DNA bases and the phosphate backbone); e.g. in case of mithramycin. Major groove binding occurs via the hydrogen bonding to the DNA and can form a DNA triple helix, such as norfloxacin [59]. The chemical structures of some electroactive anticancer drugs have been illustrated in Fig. 2.

This review will focus on the role of electrochemical methods to study the anticancer drugs–DNA interaction and summarizes the use of electrochemical methods to obtain information about the anticancer drug mechanism of action. In addition it will provide a brief overview of recent developments in this direction and will throw light on some future prospects.

2. Techniques used to study drug–DNA interaction

Over the past few years, structure based design strategies exploiting drug–DNA interaction have yielded new DNA-binding agents with clinical promise [6–13]. The interaction of DNA with nucleic acid binding molecules has been extensively studied by a variety of techniques. These techniques are discussed one by one as follows

2.1. DNA-footprinting

DNA-footprinting is a family of techniques collectively known as footprinting. In this technique, the sites to which the drug is bound are protected by a chemical reagent (e.g. DNAase I) and are visualized at single bond resolution as gaps in the autoradiograph of a denaturing polyacrylamide gel, revealing both the position and length of each ligand binding site [14]. Selectivity of binding is of the utmost importance in designing new ligands for the treatment of disease. Footprinting has been the best method for this purpose, but suffers from experimental disadvantages where large number of compounds has to be tested [15].

2.2. Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy involves transition of a nucleus from one spin state to another with the resultant absorption of electromagnetic radiation by spin active nuclei (having nuclear spin not equal to zero)

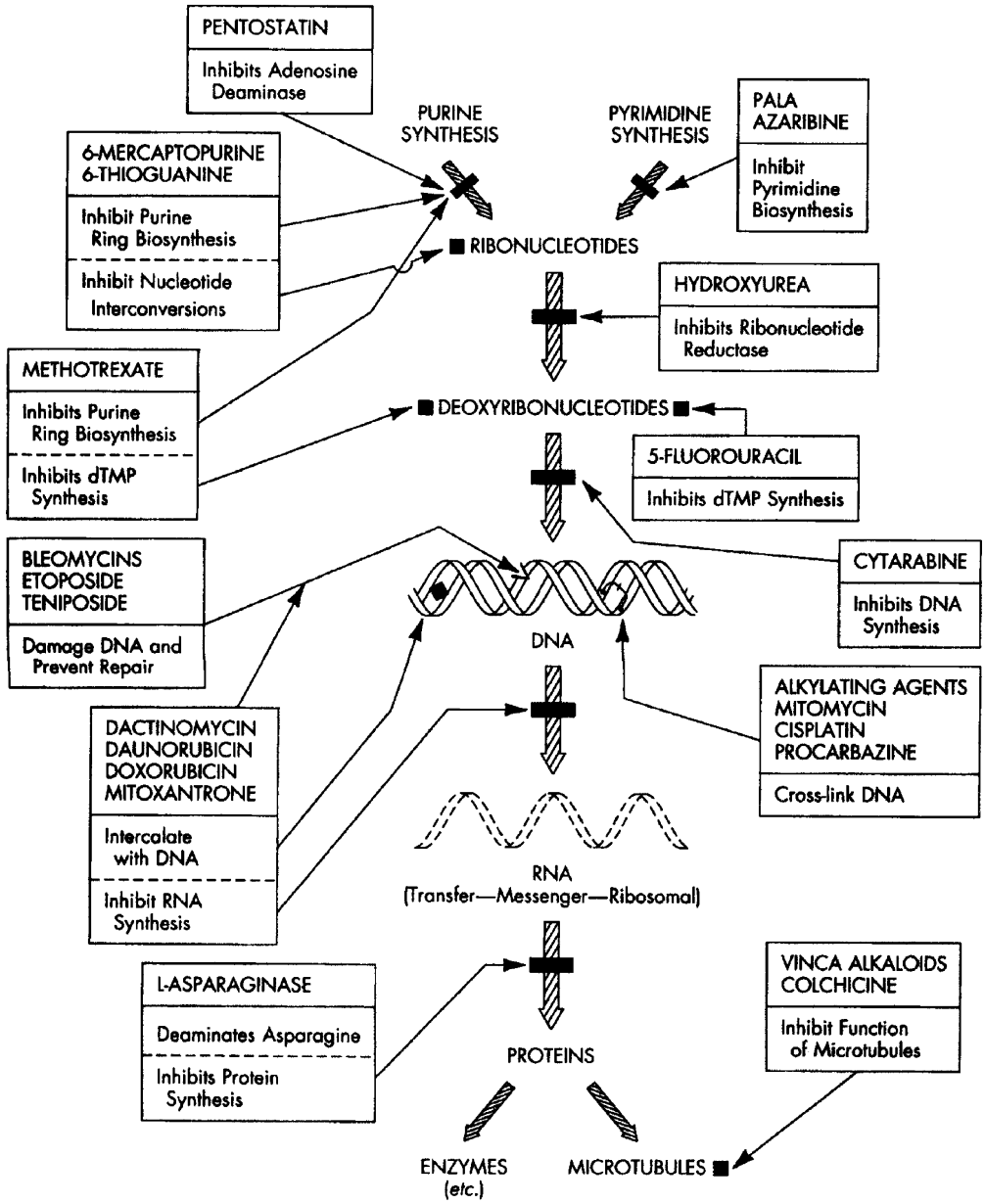

Fig. 1. Summary of mechanism of action of anticancer drugs [5].

when they are placed in a magnetic field. The energy associated with NMR experiments is insufficient to disrupt even the weakest chemical bonds [16]. NMR can therefore provide very detailed, highly localized information on molecules and materials. NMR-based screens may readily identify very weak binders that may be missed in traditional assays [17]. Unfortunately, there are a number of disadvantages associated with purely NMR-based screening approaches. For example, large quantities of proteins ($100\,mg\,g^{-1}$) are required for a typical screen due to the inherent insensitivity of the technique. Additionally, NMR experiments used in a screening effort generally require long acquisition time and isotope enrichment of the substance to be screened.

2.3. Mass spectrometry

Mass spectrometry (MS) deals with the examination of the characteristic fragments (ions) arising from the breakdown of a substance. The ions are produced by using different techniques such as electrospray ionization [18], matrix assisted laser desorption ionization (MALDI) [19], chemical ionization [20] and fast atom bombardment (FAB) [21]. After the ions transferred into the gas phase is accelerated by the voltage at the gateway of the instrument it is then transferred into a mass analyzer and separated by the mass to charge (m/e) ratio. There are many types of mass analyzers, such as double focusing magnetic and electric selectors, quadruple mass filters, quadruple ion traps,

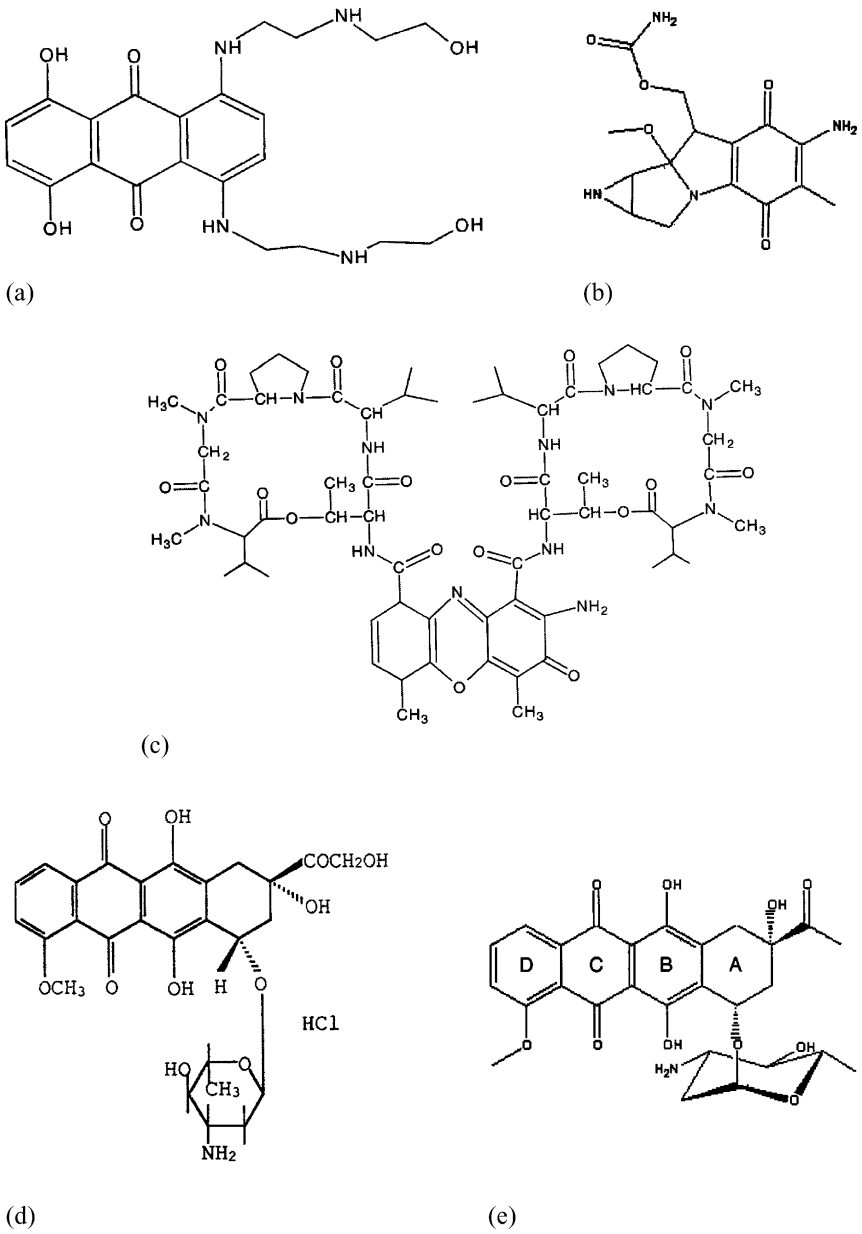

Fig. 2. Chemical structures of some DNA targeted anticancer drugs: (a) mitoxantrone, (b) mitomycin C, (c) actinomycin D, (d) pharmorubicin and (e) daunomycin.

time of flight and Fourier transform ion cyclotron machine [22]. Mass spectrometry has been a powerful tool for studying drug metabolism and disposition [23] and the dynamics of endogenous biologically active substances [24]. The main disadvantage of the MS screens is the inability of the method to discriminate between specific and non-specific binding of the drug to the targeted substance. Also, because of the increased in sensitivity MS screen is more likely to identify weak non-specific binders. Additionally screening by MS does not provide any direct information regarding the binding site of the ligand and structure of the complex formed after the interaction [17].

2.4. Spectrophotometric methods

A compound, when being united to the DNA, modifies its phantom of absorption, since it undergoes modifications in his electronic structure. In the absorption phantom displacements in the maximums with respect to binding take place free. This one can be by displacement to greater bathochromic effect, wavelengths, along with a diminution of the molar extinction coefficient. These spectral changes allow knowing aspects like the binding constant of the complex and the size of the union site [25]. In case of substances, which are non-absorbing, this technique cannot be employed.

2.5. FT-IR and Raman spectroscopy

Vibrational spectroscopy (Raman and infrared) is often used to characterize the nature of drug–DNA interaction and to monitor the effects of various drugs on DNA structure [26]. Fourier transform infrared (FT-IR) and laser Raman difference spectroscopy are used to determine drug binding sites, sequence preference and DNA secondary structure, as well as the structural variations of drug–DNA complexes in aqueous solution [27]. The disadvantage for both techniques is the sensitivity and quantitative determination of drugs.

2.6. Molecular modeling techniques

The development of modeling tools for the molecular interactions is also essential for rational design of therapeutic drugs and new synthetic proteins that can cure diseases and improve the quality of life for all of us. Molecular mechanics with improved force fields has been used to compute interaction energy between DNA and various drugs including both intercalators and groove binders [28]. Kollman and colleagues employed a free energy perturbation/molecular dynamics approach to compute the free energy difference between ligand-DNA complexes having different base pair sequences [29]. Molecular modeling is widely used in the study of the mechanism of drugs and has contributed to the design of several drugs [30]. New methods are emerging along with other new developments in biomedical science and biotechnology.

2.7. Equilibrium dialysis

The objective of an equilibrium dialysis method is usually to measure the amount of a ligand bound to a macromolecule. This is usually done through an indirect method because in any mixture of the ligand and macromolecule, it is difficult to distinguish between bound and free ligand [31]. The relationship between binding and ligand concentration is used to determine the number of binding sites, ligand affinity, association constant and thermodynamics of the binding reaction can be derived. However, Equilibrium dialysis is an indirect method and requires different other techniques in order to measure the interaction.

2.8. Electric linear dichroism

Linear dichroism ΔA is defined as the difference between the absorbance for light polarized parallel ($A\|$) and perpendicular ($A\perp$) to the applied field at a given wavelength. The reduced dichroism is $\Delta A/A = (A\| - A\perp)/A$, where A is the isotropic absorbance of the sample measured in the absence of field at the same wavelength and under the same path length. Because of axial symmetry around the electric field direction, the changes in absorbance $\Delta A\| = A\| - A$ and $\Delta A\perp = A\perp - A$ are related by $\Delta A\| = -2\Delta A\perp$; thus, measurement of $\Delta A\|$ alone is sufficient for the calculation of the reduced dichroism $\Delta A/A$ [32]. Linear dichroism provides a rapid and sensitive method to study the structure and function of nucleic acids as well as for determining the orientation of drugs upon binding to DNA [33]. Applied to DNA, this technique is probably the most direct method to evaluate intercalating versus groove binding drugs. Linear dichroism is valuable to investigate the sequence-dependent recognition of DNA by drugs [34,35]. Circular dichroism can distinguish between groove binders and intercalators but cannot identify individual binding sites [36].

2.9. Capillary electrophoresis

Capillary electrophoresis (CE) is a relatively new technique, which is based on the separation of analytes in microcapillaries (10–100 μm i.d.) under the influence of a high electric field. Several reports have been described the use of capillary electrophoresis as a method to study ligand–macromolecule interactions, including drug–DNA [37], drug–protein [38] and antigen–antibody interaction [39]. CE can be used to probe the sequence preference of DNA-binding agents, including minor groove binders and intercalators. Cooperativity of binding can be directly probed by observing the change in peak height of the free nucleotide as a function of drug concentration [40]. Perhaps the major limitation in CE is the fact that relatively high concentrations of analyte are required, in other words there is a low concentration limit of detection [41].

2.10. Surface plasmon resonance

Surface plasmon resonance (SPR) based instruments use an optical method to measure the refractive index near (within ~300 nm) a sensor surface. In order to detect an interaction one molecule (ligand) is immobilized onto the sensor surface. Its binding partner (the analyte) is injected in aqueous solution (sample buffer) through the flow cell, also under continuous flow. As the analyte binds to the ligand the accumulation of the protein on the surface results in an increase in the refractive index. This change in refractive index is measured in real time and the result plotted as response or resonance units (RU's) versus time (a sensogram) [42]. The advantages of SPR are mainly that no labeling is required, a large number of actuated sensor chips are commercially available allowing the immobilization of either proteins or target DNA or RNA, the amount of both ligand and analyte needed to obtain informative results is low, the assay is rapid and sensor chips could be re-used many times. However, the limitation of this technique is that it cannot verify the stability of the complex formed during drug binding to DNA and does not even provide the information [43]. This limitation is a consequence of the adsorption of any species at the interface cause a change in SPE signal regardless of whether there is a specific interface with DNA or not. It is also not suitable for concentration measurements, because these require the analysis of many

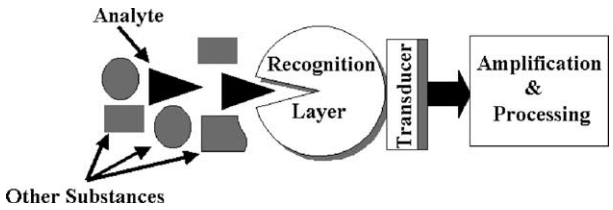

Fig. 3. Schematic representation of a biosensor.

samples in parallel, including the standard curve [44]. A high surface concentration of active immobilized ligand (∼1 mM) is needed, and this is difficult to achieve. Furthermore at such high ligand densities accurate kinetic analysis is not possible because of mass transport limitations and re-binding [45].

On the basis of above discussion it is clear that no single method can contribute to the whole understanding of drug–DNA interaction. There is an urgent need for rapid, high throughput, continuous and low cost techniques for analysis of the interaction between DNA, proteins and drugs in order to speed up drug discovery and drug approval processes. Electrochemical approach could contribute a lot in order to speed up the drug screening process because these methods can overcome most of the problems as discussed above.

3. Electrochemical approach

One of the practical applications of electrochemistry is the determination of electrode redox processes. Due to the existing resemblance between electrochemical and biological reactions it can be assumed that the oxidation mechanisms taking place at the electrode and in the body share similar principles [46,47]. Electrochemical investigations of nucleic acid binding molecules–DNA interactions can provide a useful complement to the spectroscopic methods, e.g. spectroscopically inactive species, and yield information about the mechanism of intercalation and the conformation of anticancer drug–DNA adduct [94].

In recent years, there has been a growing interest in the electrochemical investigation of interaction between anticancer drugs and DNA. The recent developments of DNA biosensors have attracted substantial research efforts directed toward clinical diagnostics as well as forensic and biomedical applications. Electrochemical DNA biosensors enable us to evaluate and predict anticancer drugs–DNA interaction.

Biosensors are small devices, which utilize biological reactions for detecting target analytes [48]. A typical biosensor construct has three features a recognition element, a signal transducing structure and an amplification/processing element (Fig. 3). Various transduction mechanisms such as electrochemical, optical, thermal and piezoelectric have been employed [49]. There are two types of biosensors depending on the nature of recognition element. Bioaffinity devices rely on the selective binding of the target analyte to a surface confined ligand partner (e.g. antibody and oligonucleotide). In contrast, in biocatalytic devices, an immobilized enzyme is used for recognizing the target substrate. For example, sensor strips with immobilized glucose oxidase have been widely used for personal monitoring of diabetes [50].

Electrochemical DNA biosensors comprise a nucleic acid recognition layer, which is immobilized over an electrochemical transducer. The role of the nucleic acid recognition layer is to detect the changes occurred in the DNA structure during interaction with DNA-binding molecules or to selectively detect a specific sequence of DNA. The signal transducer must determine the change that has occurred at the recognition layer due to the binding molecules or due to the hybridization; converting this into an electronic signal which then be relayed to the end user [51].

Observing the electrochemical signal related to DNA–DNA interactions or DNA–drug interactions can provide evidence for the interaction mechanism, the nature of the complex formed, binding constant, binding site size and the role of free radicals generated during interaction in the drug action.

3.1. How electrochemical methods work

The explanation of the mechanism of interaction between anticancer drugs and DNA by electrochemical methods is mainly based on the electrochemical behavior of the anticancer drug in the absence or presence of DNA. Different types of electrode materials are used for the investigation of the interaction between anticancer drugs and DNA such as carbon paste electrode (CPE) [52,70,71], hanging mercury drop electrode (HMDE) [52,67,80,91], gold electrode [53], pencil graphite electrode (PGE) [74], glassy carbon electrode (GCE) [66,86] and screen-printed electrodes (SPEs) [92]. During all electrochemical procedures, a three-electrode system consisting of working electrode, reference electrode (an Ag/AgCl or saturated **c**alomel electrode) and an auxiliary electrode (Pt wire) were used. Drug–DNA interactions are investigated by using different electrochemical techniques. These include cyclic voltammetry [54], square wave voltammetry [55,56], differential pulse voltammetry [57] and chronopotentiometry [58]. The interaction mechanism can be investigated in three different ways, i.e. DNA modified electrode, drug-modified electrode and interaction in solution [59]. These are described below.

3.1.1. DNA modified electrode

The immobilization of DNA onto an electrode surface is in many ways the crucial aspect of the development of DNA biosensors for monitoring drug interactions because it will dictate the accessibility of the DNA to drugs in solution and hence can influence the affinity of drug binding. DNA is typically immobilized on the surface of the electrode by

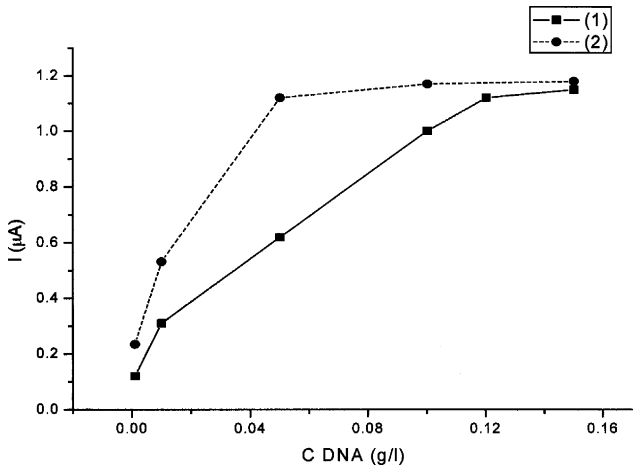

Fig. 4. (1) Dependence of peak current of the characteristic guanine peak of dsDNA in relation with increasing concentration of dsDNA. (2) Dependence of peak current of the characteristic guanine peak of ssDNA in relation with increasing concentration of ssDNA [52].

covalent attachment, by electrostatic attraction or by entrapment within a polymer layer [60–63]. The key criteria for DNA immobilization is that the DNA is maintained at the electrode interface, that the DNA is accessible to binding of the target molecule in solution and that the method of immobilization is compatible with the method of transduction. End-point covalent attachment of DNA to an electrode surface ensures the DNA is easily accessible to drug compounds in solution [64]. In contrast, DNA immobilized throughout a polymer layer [65] is far less accessible to molecules in solution to bind with this DNA particularly if the drug compounds are large macromolecules. With regards to the method of immobilization being compatible with the method of transduction, an example is the change in the ability of oxidation of guanine and adenine bases upon drug binding. The oxidation of guanine and adenine bases requires the bases to be close to the electrode surface so that electron transfer can occur. As a consequence, end-point immobilization of the DNA would be inappropriate as the bases located at the distal end to the link to the electrode will be too remote from the electrode to allow electron transfer to occur. As a consequence Gherghi et al. [52] who used to approach to study the interaction of actinomycin D and DNA used electrostatic binding of the DNA to a carbon paste electrode (CPE). They modified the CPE with DNA as follows. After the pretreatment of CPE at +1.7 V for 1 min in phosphate buffer pH 7.4, DNA modified electrode was prepared by immersing the electrode in DNA solution at +0.5 V for 5 min. Different concentrations of DNA were used and immobilized on the CPE in order to detect the optimal concentration for full coverage of CPE surface. Fig. 4 represents the different peak heights obtained by varying DNA concentrations. A concentration of 0.1 g/l (in the case of dsDNA) and 0.05 g/l (in the case of ssDNA) were selected as the most suitable, since at these concentrations full electrode surface coverage was observed.

3.1.2. Drug-modified electrode

In order to prepare the drug-modified electrode, the target drug is immobilized on the electrode surface. The electrochemical signals of the drug are monitored and then the changes in these signals after interaction with DNA are observed. Interaction between adriamycin and DNA was studied by using adriamycin modified glassy carbon electrode (GCE) [68]. Adriamycin modified glassy carbon electrodes were prepared by immersing the electrode in a 5 μM adriamycin solution for 10 min at a deposition potential of +0.4 V. After deposition the electrodes were rinsed with deionized water and then transferred to DNA solution whereupon voltammetric measurements to investigate the interaction between the DNA and the adriamycin were preformed.

3.1.3. Interaction in solution

For detection of interaction in the solution, drug and DNA are placed in the same solution and after some given time of interaction, the changes in the electrochemical signals of anticancer drug–DNA complex are compared with the signals obtained with DNA or drug alone in the solution. Xia et al. [88] determined the interaction between pharmorubicin (epirubicin) in solution using glassy carbon electrode (GCE) by single sweep cyclic voltammetric experiment. First of all voltammetric signal of drug was obtained and then calf thymus DNA was added to the solution of drug. After some given time of interaction, voltammetric measurement was performed to obtain information about the complex formed.

4. Applications of electrochemical approach

4.1. Determination of anticancer drug–DNA interaction

The electrochemical methods enable us to evaluate and predict DNA interactions and damage caused to DNA by DNA-binding compounds. This information is valuable in drug discovery and can speed up the investigation of new drug candidates. How electrochemical method can explain the mechanism of interaction between anticancer drugs and DNA is illustrated briefly in the following examples.

Adriamycin is an antibiotic of the family of anthracyclines with a wide spectrum of chemotherapeutic applications and antineoplasic action. However, it may cause cardiotoxicity that ranges from a delayed and insidious cardiomyopathy to irreversible heart failure [66,67]. Adriamycin is a complex molecule and different groups can be oxidized or reduced. Brett et al. [68] detected the in situ adriamycin oxidative damage to DNA and suggested the result was the formation of 8-oxoguanine. They modified the glassy carbon electrode with a thick layer of DNA and placed it in the solution of adriamycin in order to obtain information regarding the adriamycin–DNA interaction. When a potential of −0.6 V was applied, adriamycin was reduced at the DNA modified electrode. This redox process occurred within the double helix and involved the simultaneous oxidation of

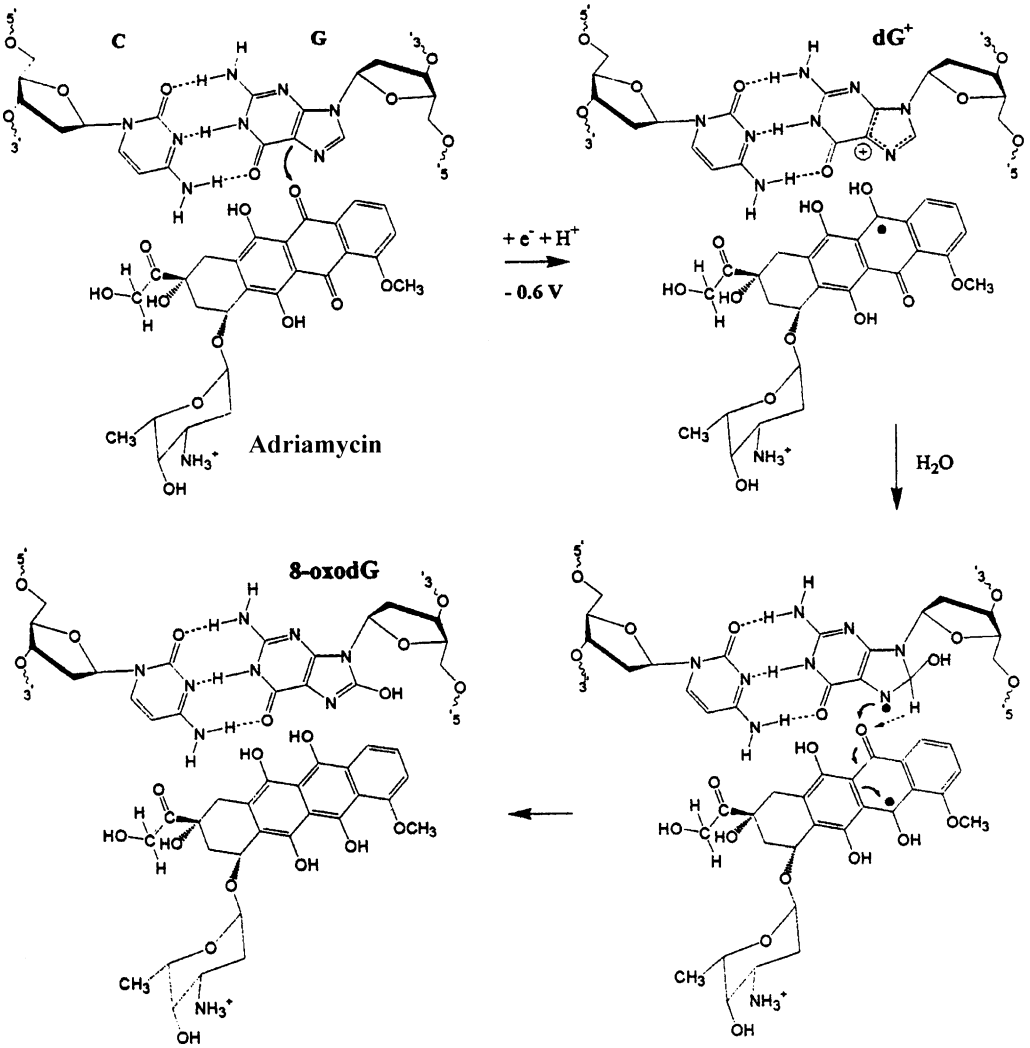

Fig. 5. Mechanism of electrochemical in situ adriamycin oxidative damage to DNA [68].

one neighboring guanine. In this way electron transfer from the guanine moiety to the quinone group of adriamycin without hydrogen abstraction is likely to be the predominant reaction leading to the formation of the guanine cation. Due to the fast hydrolysis of the cation, the semiquinone undergoes further reduction to the fully reduced adriamycin and the formation of the 8-oxoguanine occurred (Fig. 5). The formation of 8-oxoguanine was confirmed by comparing the oxidation peak potential of pure 8-oxoguanine at +0.45 V. The generation of this product of guanine oxidation within DNA is strongly mutagenic and can contribute to cell dysfunction. The electrochemical model proposed by Brett et al. may be used to explain the levels of 8-oxoguanine found when cells are treated with adriamycin.

Ibrahim [69] studied the interaction of nogalamycin (NOM), an anthracycline antitumour drug, with calf thymus DNA by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. The experimental results revealed that the reduced form $(H_2NOM)_{red}$, of NOM was bound more strongly to DNA than the oxidized form $(NOM)_{ox}$. The calibration graph for the determination of DNA was obtained by the decrease in the DPV peak current of NOM in the presence of DNA. Furthermore, the results were compared with the similar type of interaction behaviors of daunomycin (DAM), adriamycin (ADM) and 4-deoxyadriamycin (DADM), mainly from the viewpoint of the influence of chemical structure. Binding constants (K) were determined from the voltammetric data i.e. changes in limiting current with addition of DNA. In comparison with DAM, ADM and DADM, nogalamycin displayed high binding affinity to DNA ($K = 4.44 \times 10^5$) and binding affinity increased with the order: DAM < DADM < ADM < NOM. The results also show that a slight change in the chemical structure caused significant changes in the binding affinity to DNA and consequently in clinical properties.

The antimicrobial, anticancer, diuretic, anticonvulsant and anesthesia activities of a variety of acyclic and cyclic α,β-saturated ketones and related Mannich bases such as 4,4′-dihydroxy chalcone (DHC) are well described [70,71]. The activities of these compounds were attributed, in part, to

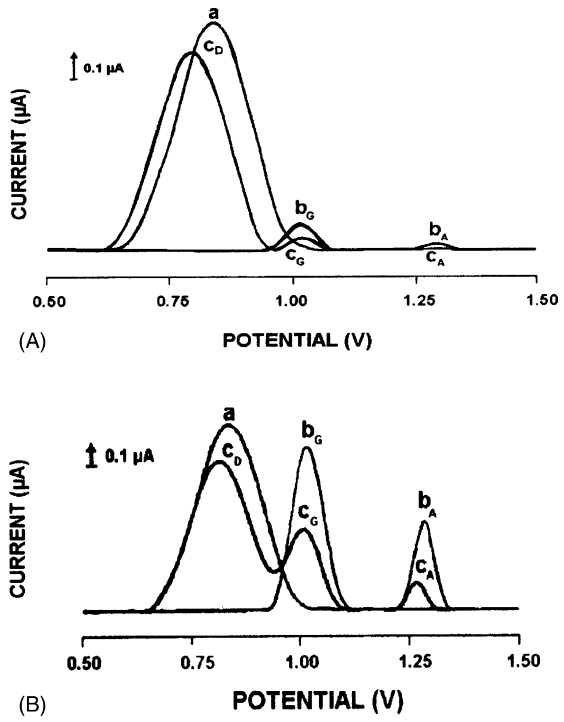

Fig. 6. (A) Differential pulse voltammograms for the interaction of DHC with dsDNA. Oxidation signals of (a) DHC at bare CPE; (b) guanine (b_G), and adenine (b_A) at dsDNA modified CPE; (c) DHC (c_D), guanine (c_G), and adenine (c_A) after the alkylation of 1 μM DHC at dsDNA modified CPE in 0.5 M acetate buffer (pH 4.5). (B) Differential pulse voltammograms for the interaction of DHC with ssDNA. Oxidation signals of (a) DHC at bare CPE; (b) guanine (b_G), and adenine (b_A) at ssDNA modified CPE; (c) DHC (c_D), guanine (c_G) and adenine (c_A) after the alkylation of 1 μM DHC at ssDNA modified CPE in 0.5 M acetate buffer (pH 4.5) (Fig. 6 taken from [72]).

alkylating ability of olefinic groups conjugated with a carbonyl function to guanine bases in DNA. The ability of these alkylating agents to attack guanine bases in DNA results in the cross-linking of DNA. The interaction of 4,4′-dihydroxy chalcone (DHC) with calf thymus double stranded DNA (dsDNA) and calf thymus single stranded DNA (ssDNA) was studied electrochemically based on the oxidation signals of guanine and adenine by using differential pulse voltammetry (DPV) at carbon paste electrode (CPE) [72]. As a result of alkylation of DHC between the base pairs in dsDNA, the voltammetric signals of guanine and adenine were greatly decreased (Fig. 6(A)). After the interaction of DHC with ssDNA modified carbon paste electrode, a higher decrease in the oxidation signals of guanine and adenine were observed under the same conditions indicating a strong alkylation of DHC to the ssDNA (Fig. 6(B)). The reason for this strong alkylation is that in case of ssDNA, guanine and adenine are more exposed to DHC as compared to dsDNA. As a result of this strong alkylation, the detection limit for the determination of DHC at ssDNA-modified electrode was 42 nM and that with dsDNA was 63 nM.

Wang et al. [73] reported the interaction of antitumour drug daunomycin (DM) with dsDNA in solution and at the electrode surface by cyclic voltammetry (CV) and constant current potentiometric stripping analysis (CPSA) with carbon paste electrode. As a result of intercalation of daunomycin between the base pairs in dsDNA, the CPSA daunomycin peak area decreased and a new more positive shoulder (peak) appeared at the potential from +0.79 to +0.81 V. This shoulder was attributed to the oxidation of the drug intercalated in DNA.

Another study about the interaction of daunomycin with DNA was carried out by Chau et al. [74] using rotating disk electrode. They calculated the binding constant ($K = 2.35 \times 10^5\ M^{-1}$) and binding site size of the daunomycin–DNA interaction ($s = 6$) by titration curve and non-linear regression analysis. This means that daunomycin covered six base pairs of DNA after intercalation.

It has been documented in the literature that lycorine inhibited the in vivo growth of a murine ascites tumor and reduced the viability of in vitro grown tumor cells. Moreover, it was reported to inhibit the synthesis of DNA and proteins in murine cells [75]. Karadeniz et al. [76] reported that the voltammetric signals of guanine and adenine were greatly decreased at a pencil graphite DNA modified electrode after the interaction of lycorine with DNA. The decrease both in the signals of guanine and adenine from dsDNA modified pencil graphite electrode was attributed to the binding of lycorine to these bases, this phenomenon could be explained by the shielding of oxidizable groups of electroactive bases such as guanine and adenine while lycorine interacts with DNA at electrode surface.

Tarabine PFS (Cytosar-U) is the most important antimetabolic, antineoplastic agent used in the therapy of myelocytic leukemia [77]. EL-Hady et al. [78] studied the interaction of Tarabine PFS with ssDNA using mercury film electrode. They postulated that a hydrogen bond was formed between the −C=O group in the oxidized form of the drug and $-NH_2$ group in guanine moiety. They also calculated the ratio of the equilibrium constant (K_1) between the reduced free form and the reduced bonded form and the equilibrium constant (K_2) between the oxidized free form and the oxidized bonded form. It was found that the K_2 value is 298 times than that of K_1. Therefore ssDNA interacts preferentially with the oxidized form that contains carbonyl group and in comparison almost negligibly with the reduced form that contains an amino group. Therefore, it was concluded that the −C=O group in the oxidized form is the predominant functional group for the interaction of Tarabine PFS. This type of mechanism had been proposed previously using other methods, e.g. UV-hyperchromaticity and ^{1}H NMR spectroscopy or single cell gel electrophoresis [79,80]. This study suggests that electrochemical approach can be used as a complement to these methods.

Mitoxantrone–DNA interaction was investigated by using dsDNA modified glassy carbon electrode [81]. Differential pulse and square wave voltammetry were applied to develop an electroanalytical procedure for the determination of mitoxantrone and to determine its interaction with dsDNA or ssDNA immobilized on the glassy carbon electrode surface.

Mitoxantrone interaction was not specific to either guanine or adenine bases. The kinetics of the mitoxantrone–DNA interaction was slow and caused damage to DNA.

Clinically applied antitumour agent, mitomycin C (MC) interaction with ssDNA detected by changes of guanine residues were investigated [82]. Acid activated MC bound to DNA at hanging mercury drop electrode (HMDE) surface as detected by the decrease of guanine signal and appearance of redox couple at −0.4 V by using transfer stripping cyclic voltammetry (TSCV). It was concluded that acid activated MC covalently bound to the guanine residues in DNA at pH 3.9 with the accumulation time as 5 min by observing a strong decrease of guanine signal.

Actinomycin D is an antitumour antibiotic that contains a 2-aminophenoxazin-3-one chromophore and two cyclic pentapeptide lactones. This drug has been used clinically for the treatment of highly malignant tumors and also in combination with other antitumor agents to treat high-risk tumors [83,84]. The interaction of actinomycin D (ACTD) with calf thymus DNA (ctDNA) was studied at an oxidized waxed graphite electrode [85]. Actinomycin D showed a pair of non-symmetric peaks at −0.51 and 0.19 V in cyclic voltammetry. As a result of reaction with ctDNA, the voltammetric peaks of actinomycin D disappeared. The disappearance of actinomycin D redox peaks could be attributed to the formation of a non-electroactive ACTD–DNA complex. This means that the ACTD phenoxazone ring, the redox active moiety must bind tightly to native DNA and then it loses its electroactivity. The overall results (binding constant $K = 7.54 \times 10^9$ cm^3 mol^{-1} and binding site size $s = 8.1$) demonstrated that ACTD binds to ctDNA with a high association constant and covers eight base pairs.

Wang et al. [86] presented a rapid method for investigation of the interaction between DNA and electroactive ligands based on an electrochemical equation for irreversible processes. A non-intercalation binder (Hoechst 33258) and two DNA intercalators (mitoxantrone and actinomycin D) were used as model for applying the equation. They were successful in determining binding constant (K) and binding site size (s) simultaneously based on the dependence of the current on the amount added DNA in voltammetry. It was found that Hoechst binds to fish sperm DNA ($K = 2.2 \times 10^8$ cm^3/mol) and covers four base pairs (binding site size $s = 3.9$). It could be supposed that Hoechst bind to minor groove region of DNA since these minor grooves is geometrically suitable for it [87]. The binding constant of mitoxantrone with calf thymus DNA ($K = 8.9 \times 10^9$ cm^3/mol) and binding site size ($s = 3.1$) suggested that mitoxantrone bound tightly to three base pairs of calf thymus DNA. Comparing with Hoechst, the high value of binding constant may be caused by the chelation of two aminoethylamino side chains of mitoxantrone. However, actinomycin D binds to DNA with a highest binding constant ($K = 9.1 \times 10^9$ cm^3/mol) and covers eight base pairs ($s = 8.2$). It is most possible that the hydrogen bonds are formed between the two depsipeptides of actinomycin D and the adjacent amide groups of DNA bases, which reinforces the interaction and results in a high binding constant. This study provides a convenient and sensitive approach for estimating and outlining the interaction between DNA and electroactive targeting compounds.

Xia et al. [88] determined the interaction of anticancer drug pharmorubicin or epirubicin with DNA on a glassy carbon electrode by using cyclic voltammetry. They calculated the binding constant ($K = 7.46 \times 10^4$ M^{-1}) and binding site size ($s = 1.85$) of the pharmorubicin–DNA interaction.

Interaction between some platinum complexes, potent anticancer agents and DNA was studied by using differential pulse voltammetry at wax impregnated graphite electrode coated by linearized plasmid DNA [89]. The sensor relied on monitoring changes in the intrinsic electro-oxidation response of the surface confined DNA resulting from its interaction with platinum compounds and required no label or indicator. Short reaction times (2–10 min) were sufficient for monitoring submicromolar levels of platinum complexes. It was suggested that DNA biosensors could be used for quantitating antitumor platinum drugs in various samples including those used when studying mechanisms underlying their antitumour effectiveness.

Quercetin complexate with metal cations to form stable products**,** which have demonstrated antibacterial properties and antitumour activities [90,91]. Kang et al. [92] studied the electrochemical behavior of Quercetin and its Eu^{3+} complex interaction with calf thymus DNA modified glass carbon electrode by using cyclic voltammetry and double potential step chronocoulometry. Their results suggested that quercetin and Eu–Qu_3 complex can both bind to DNA, but quercetin binds to DNA mainly by electrostatic attraction and the Eu–Qu_3 complex bind to DNA by both intercalation and electrostatic attraction. For the Eu–Qu_3 complex and dsDNA modified glass carbon electrode (GCE) system they obtained binding site size or binding numbers (s or $n = 2$) for the Eu–Qu_3 complex per DNA (base pair). However, for the quercetin and dsDNA modified GCE system, the binding number was “1”. It was concluded that the ability of complex binding to dsDNA is stronger than quercetin.

Jelen et al. [93] investigated the interaction of echinomycin with DNA by cyclic voltammetry with hanging mercury drop electrode (HMDE). Interaction of echinomycin with dsDNA attached to HMDE resulted in high echinomycin signals, suggesting a strong binding of echinomycin to dsDNA by bis-intercalation at the electrode surface.

4.2. Quantification of the anticancer drugs

The development of new methods capable of determining minimal drug concentration is important in pharmaceutical formulations and in biological fluids. Using the anticancer drug–DNA interaction, one can determine the anticancer drug concentration using electrochemical approach.

The analytically useful information can be obtained from the increased DNA (guanine) response, associated with the interaction of drugs with the surface confined

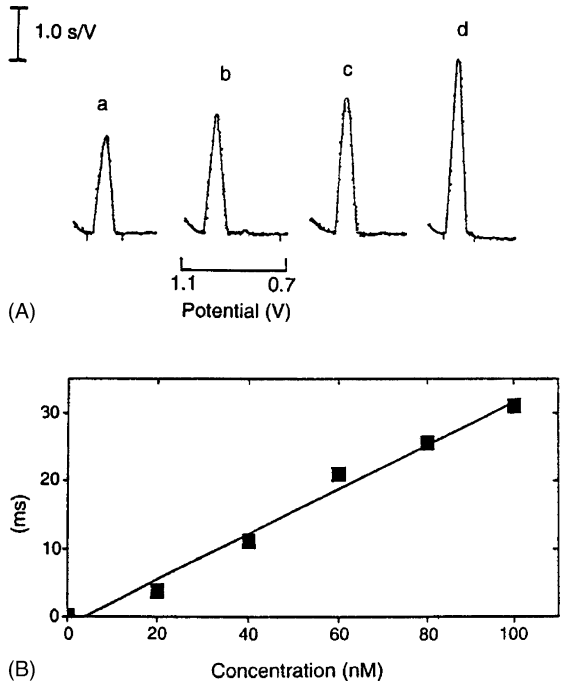

Fig. 7. (A) Chronopotentiograms for daunomycin with increasing concentrations: (a) 0 nM, (b) 20 nM, (c) 40 nM, (d) 60 nM at the dsDNA-modified SPE after interaction (or accumulation) and transfer to the pure electrolyte (0.2 M acetate buffer, pH 5.0). (B) Resulting calibration plot (0–100 nM) (Fig. 7 taken from [94]).

DNA. Daunomycin is commonly used in the treatment of various cancers. Daunomycin is known to intercalate into duplex DNA with preferential binding to the G + C base pairs. Fig. 7(A) displayed the chronopotentiograms at the DNA coated strip for increasing levels of daunomycin in 20 nM steps (b–d), along with the response of the blank solution (a). The guanine peak increases linearly with the drug concentration as indicated from the resulting calibration plot (Fig. 7(B)). A short (2 min) reaction time was sufficient for the detection of nanomolar concentrations of the daunomycin [94]. The increased guanine peak was attributed to the changes in the surface accessibility of the guanine moiety accrued by the unwinding of the DNA duplex during the intercalative binding of the daunomycin.

DNA interactions and quantitative analysis of antineoplasic antibiotic anthramycin was studied by using differential pulse polarography (DPP) in aqueous buffered media at hanging mercury drop electrode (HMDE) [95]. It was reported that anthramycin produced covalent adducts with native DNA. Due to the smaller accessibility of the electroactive groups of guanine in the adduct than that in the DNA, there was a decrease in the guanine signal. By observing decrease in guanine signal analytical determination of anthramycin was possible. The detection limit of 70 nM in 4% methanol buffered media gave the opportunity to develop a useful technique to control doses for this drug from patients.

Additional attention is required for the application of the DNA biosensors to the determination of anticancer drugs in complex biological matrices because of the presence of potential interferences and matrix effects. In a study performed by Brett et al. [96], the analytical quantification of carboplatin in serum samples from women patients with ovarian cancer undergoing treatment with this drug, was described by using a DNA modified glassy carbon electrode. The electrochemical results clearly demonstrated that for low concentrations carboplatin interacted preferentially with adenine rather than guanine groups in the DNA. They reported as its binding to DNA occurred covalently it seemed quite clear that it could be possible to develop an indirect analytical method to determine platinum compounds with antitumour activity by measuring this interaction. As carboplatin was added to the solution containing ssDNA, a decrease was observed in the oxidation current of adenine with increasing concentration of carboplatin in solution while the guanine oxidation currents only decreased slightly. The response range for carboplatin determination in serum samples by the standard addition method using ssDNA solutions was found in the range 6.5×10^{-5} mol/l to 1.5×10^{-3} mol/l and the detection limit in serum samples was calculated as 5.7×10^{-6} mol/l.

5. Conclusions and future perspectives

In electrochemistry considerable progress has recently been made in the development of new and sophisticated techniques to study anticancer drugs–DNA interaction. The field of anticancer drug design will naturally take advantage of this progress. Electrochemical approach has been successively used to determine the anticancer drug–DNA interaction and can contribute to drug discovery and effective treatment for cancer through providing knowledge regarding the efficacy of candidate drug binding with DNA and through providing information of the mechanism of the DNA–drug interaction. The value of electrochemical approaches in studying the anticancer drugs–DNA interaction is that it is a relatively clean chemical system, it is relatively easy to control and can be studied in aprotic and aqueous solutions thus allowing one to evaluate the behavior of free radicals generated in biological systems [97]. The versatility of the electrochemical methodology allows the mimicking of the multitude of biological environments in which the conditions can be widely varied in the attempt to resemble them. Different ranges of pH, oxygen content in the electrochemical cell and solvents of diverse properties can be used. However, standardization is urgently required in terms of methods, electrodes and supporting electrolytes to allow a more general use of the already available data [98].

In addition, new electrochemical methods are currently being developed, which may play an active part in biological and biomedical research in the future. Progress in electrochemical techniques will continue to contribute to the growth of these research fields. For example, the method proposed by Wang et al. [86] for simultaneous determination of binding

constant "*K*" and binding site size "*s*" is a good addition to electrochemical methods.

In view of the developments in electrochemical methods for the determination of anticancer drug–DNA interaction, future is not too far that such methods would be available to measure levels of DNA covalent modifications in target cells in vivo, which is seen as the ultimate form of therapeutic drug monitoring.

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