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Pharmacogenomic study using bio- and nanobioelectrochemistry: Drug–DNA interaction

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ABSTRACT

Small molecules that bind genomic DNA have proven that they can be effective anticancer, antibiotic and antiviral therapeutic agents that affect the well-being of millions of people worldwide. Drug–DNA interaction affects DNA replication and division; causes strand breaks, and mutations. Therefore, the investigation of drug–DNA interaction is needed to understand the mechanism of drug action as well as in designing DNA-targeted drugs. On the other hand, the interaction between DNA and drugs can cause chemical and conformational modifications and, thus, variation of the electrochemical properties of nucleobases. For this purpose, electrochemical methods/biosensors can be used toward detection of drug–DNA interactions. The present paper reviews the drug–DNA interactions, their types and applications of electrochemical techniques used to study interactions between DNA and drugs or small ligand molecules that are potentially of pharmaceutical interest. The results are used to determine drug binding sites and sequence preference, as well as conformational changes due to drug–DNA interactions. Also, the intention of this review is to give an overview of the present state of the drug–DNA interaction cognition. The applications of electrochemical techniques for investigation of drug–DNA interaction were reviewed and we have discussed the type of qualitative or quantitative information that can be obtained from the use of each technique.

Contents

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1. Introduction

DNA is the pharmacological target of many of the drugs that are currently in clinical use or in advanced clinical trials [1]. The study of the interaction of drug and DNA plays a key role in pharmacology and it is of great significance for designing and synthesizing the new drugs targeted to DNA and their effectiveness depends on the mode and affinity of the binding [2,3]. The interaction of drugs with DNA is among the most important aspects of biological studies in drug discovery and pharmaceutical development processes. The recognition of DNA binders involves a complex relationship of different interactive forces. It includes the intercalation between adjacent base pairs, intrusion into the minor groove and major groove, and electrostatic interaction [4,5]. The resulting drug–DNA complex is stabilized by a number of non-covalent (such as van der Waals interactions, hydrophobic forces and hydrogen bonds) and covalent interactions.

1.1. Types of drug–DNA interactions

Small molecules such as drugs can interact with or bind with DNA and these interactions can be classified by their mechanism of action. The main classes of DNA binding molecules are:

1) Groove binders that sit in the minor groove;
2) Intercalators that are sandwiched between base pairs;
3) Alkylators that can chemically react with DNA, resulting in DNA alkylation; and
4) DNA cleavage agents that have the ability to break DNA chains.

Each of these classes of molecules has a different structure and interacts with DNA in a different way.

1.1.1. Groove binders

Minor groove binding molecules are usually constructed of a series of heterocyclic or aromatic hydrocarbon rings that possess rotational freedom. This allows the molecule to fit into the minor groove, with displacement of water. The most famous minor groove binding drugs are netropsin, berenil, distamycin and mithramycin. They usually have crescent shape, which complements the shape of the groove [6,7] and facilitates binding by promoting van der Waals interactions. Distamycin and netropsin (Fig. 1) are natural products possessing amido groups and, respectively, three and two N-methylpyrrole rings (distamycin can be denoted PyPyPy, and netropsin PyPy).

Distamycin and netropsin interact with AT-rich regions of DNA in the minor groove by forming hydrogen bonding and hydrophobic interactions (Fig. 2). The terminal amidine group of the small molecule is basic, and serves to attract the drug molecule to the negatively charged DNA phosphodiester backbone. The 2-amino group of guanine prevents distamycin from binding to the minor groove of G·C base pairs by steric hindrance, thus conferring AT-selectivity on the drug molecule.

Also, a series of dimers and trimers of distamycin and netropsin have been synthesized and studied in an attempt to increase the DNA binding region from 3 base pairs (monomeric drug) to 10 base pairs or more. These semi-synthetic compounds have been named lexitropsins. As well as pyrrole rings (Py), some lexitropsins also incorporate imidazole (Im) rings. The ability to recognize specific DNA sequences of more than 10 base pairs would give rise to a powerful tool in molecular biology and antisense/antigene therapeutics (see Oligonucleotides as drugs), as this length of sequence starts to become meaningful in the context of the human genome.

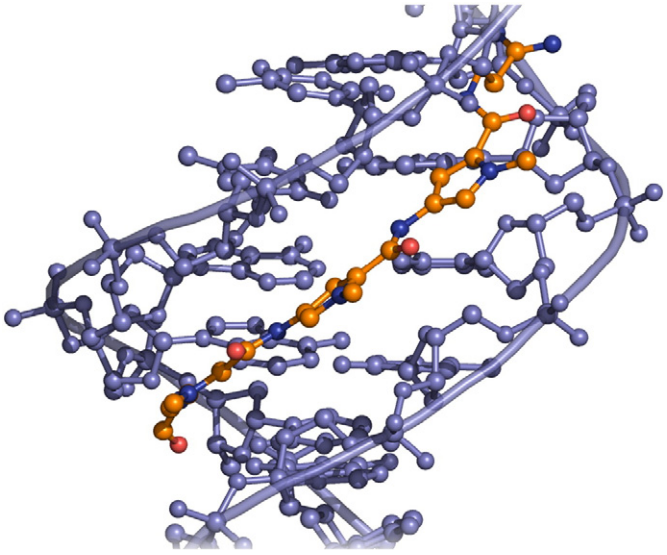

Fig. 2. Distamycin DNA binding view from the three-dimensional structure of a complex between distamycin (orange) and a DNA duplex, showing the binding of distamycin in the minor groove.

Unfortunately, simple oligomeric compounds like distamycin and netropsin do not have the ideal crescent shape to wrap around the minor groove of DNA, and they fail to recognize longer stretches of DNA. Dervan took this approach further in synthesizing a series of oligomeric "hairpin" polyamide molecules containing pyrrole and imidazole ring systems that are able to bind side by side in the minor groove of DNA with high affinity and in a sequence-specific manner. These molecules can be prepared by solid-phase methods.

1.1.2. Intercalators

Certain flat aromatic or heteroaromatic molecules can slide between the base pairs of DNA (intercalate) and stabilize the duplex without disrupting base pairing. Intercalation has the effect of lengthening the duplex by around 3 Å per bound drug molecule, causes unwinding of DNA, and prevents replication and transcription by interfering with the action of topoisomerases. The degree of unwinding depends on the structure of the intercalating molecule and the site of intercalation. The tight ternary complex formed between the intercalated drug,

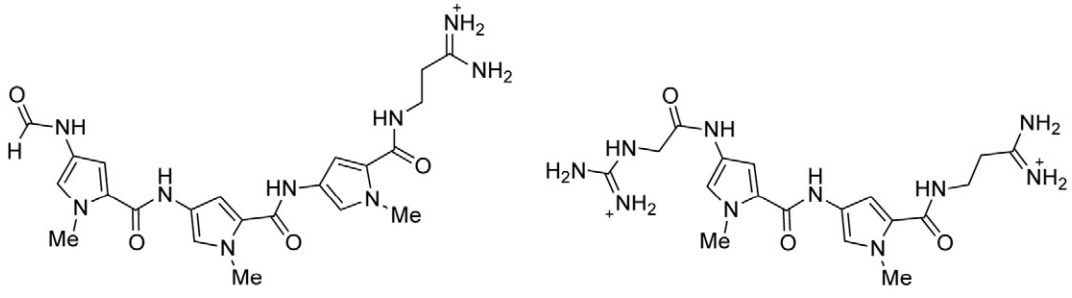

Fig. 1. Distamycin and netropsin structures of the naturally-occurring minor groove binders distamycin and netropsin.

the DNA and the topoisomerase is lethal to proliferating cells, so intercalators are often more toxic to cancer cells than to normal cells. Intercalators are molecules that stack perpendicular to the DNA backbone without forming covalent bonds and without breaking up the hydrogen bonds between the DNA bases. The only known forces that sustain the stability of the DNA–intercalator complex, even more than DNA alone, are van der Waals, hydrogen bonding, hydrophobic, and/or charge transfer forces [8–12]. Intercalation stabilizes, lengthens, stiffens, and unwinds the DNA double helix [13]. Intercalators contain planar heterocyclic groups which stack between adjacent DNA base pairs, forming a complex which is stabilized by stacking interactions between the drug and DNA. Intercalators can be mono- or bi-functional, depending on the number of aromatic moieties. There are simple mono-intercalators like acridine derivatives: proflavine, acriflavine, acriflavine neutral (euflavine), aminacrine, ethacridine, and more complex like daunomycin and Adriamycin, with four fused six membered rings with substituents in positions 7 and 9. These intercalators are often called the threading intercalators since they thread one of the substituents on opposite sides of the intercalating aromatic ring between the base pairs at the intercalation site. In bi-functional intercalators, two planar aromatic moieties are present and separated by some voluminous system like a fairly rigid cyclic peptide system stabilized through disulfide bridge as in the case of triostin A and echinomycin. Below are some of examples for these drugs' function and their interaction mechanism with DNA.

a) *Acridines*

Acridines originated from the aniline dye industry and have been used as anti-malarial and antibacterial drugs. Amsacrine (Fig. 3) is used in the treatment of leukemia and proflavine (Fig. 3) was used in the Second World War to treat wounds. Proflavine contains amino groups that interact ionically with the negatively charged phosphates groups on DNA, whilst the aromatic ring system intercalates.

b) *Polypeptide*

The Actinomycins (Fig. 4) are polypeptide antibiotics isolated from Streptomyces strains.

Actinomycins inhibit both DNA synthesis and RNA synthesis by blocking chain elongation. They interact with G·C base pairs as they require the 2-amino group of guanine for binding. The phenoxazone ring slides into the double helix and intercalates, while the pentapeptide side chains interact with the DNA minor groove by forming hydrogen bonding and hydrophobic interactions. The result of these two mechanisms of interaction between small molecule and DNA (intercalation and minor-groove binding) is a very stable complex (Fig. 5).

Doxorubicin (Adriamycin) and daunorubicin (daunomycin), isolated from Streptomyces strains, are important examples of anthracycline antitumour antibiotics (Fig. 6). Both possess an amino group on the

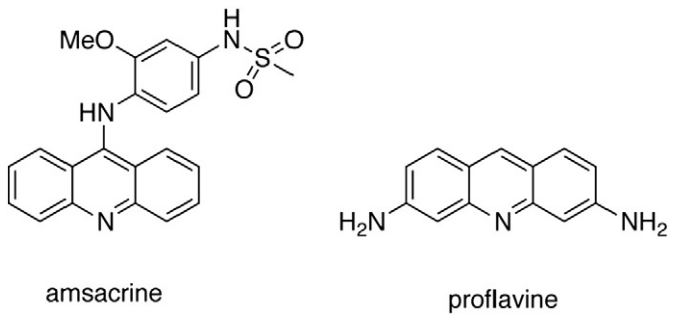

Fig. 3. Amsacrine and proflavine structures of the acridines amsacrine and proflavine, which intercalate between DNA base pairs.

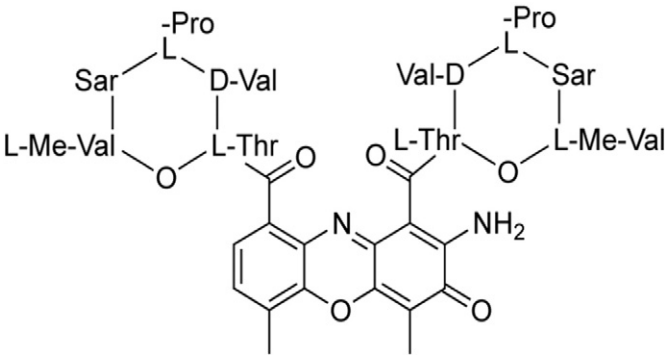

Fig. 4. Actinomycin D structure of actinomyin D (dactinomycin), a polypeptide DNA-binding molecule.

sugar which, when protonated, forms an ionic interaction with the negatively charged DNA phosphate backbone. This bond helps to hold the molecule in place, allowing the planar aromatic ring system to slide into the double helix. Although doxorubicin and daunorubicin differ by only one hydroxyl group, they have different activities: daunorubicin is active only against leukemias , but doxorubicin is active against leukemias and a wide range of solid tumors.

In general, the phenomenon of intercalating involves the aromatic part of a drug molecule positioning itself between base pairs. Intercalating agents inure in hydrophobic interactions with DNA because the hydrophobic, aromatic side chains interact favorably with the aromatic environment of the base pairs. These agents introduce strong structural perturbations in DNA molecule by increasing the distance between the adjacent base pairs. In order for an intercalator to fit between base pairs, the DNA must dynamically open a space between its base pairs. Fortunately, the resultant helix distortion is compensated by adjustments in the sugar-phosphate backbone unwinding of the duplex. Stacking interactions between the bases and the intercalating molecule are the major stabilizing factors for the complex formed. For example, there are direct hydrogen bonds between the functional groups of the drug that are essential for the drug action (like hydroxyl group of the ring A or NH_3^+ group in the 3′ position in daunomycin) and functional groups N_2 and N_3 of guanine, or O_2 of cytosine at the intercalation site. Very often, water-mediated contacts occur rather than a direct hydrogen bonded

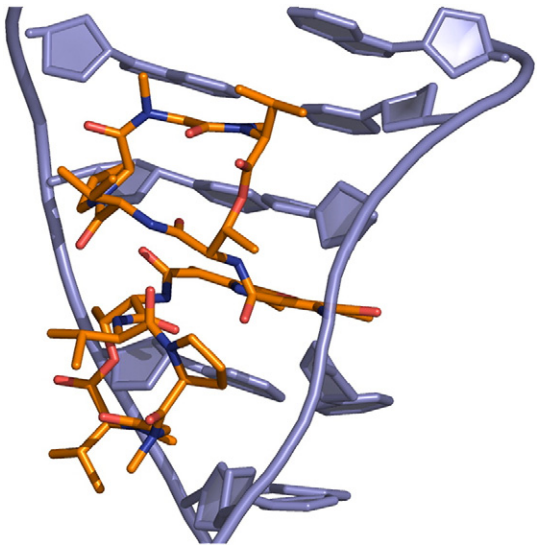

Fig. 5. Actinomycin DNA binding view from the three-dimensional structure of a complex between actinomycin D (orange) and a DNA duplex, showing the intercalation of actinomycin D in double-stranded DNA.

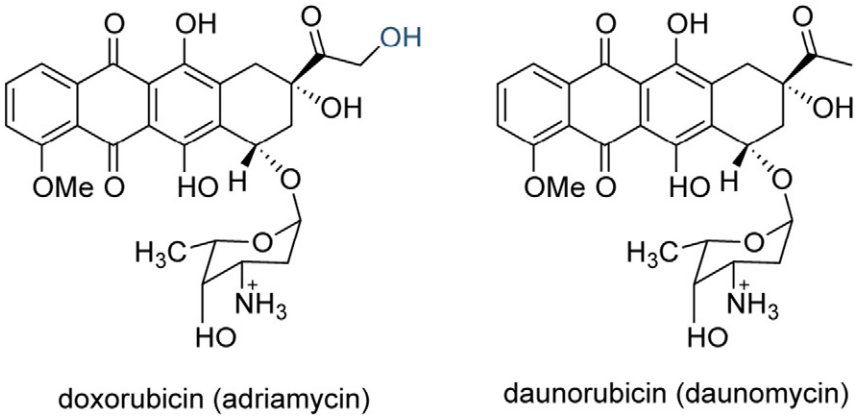

Fig. 6. Doxorubicin and daunorubicin structures of the anthracyclines doxorubicin (Adriamycin) and daunorubicin (daunomycin).

interaction. Anyway, the total amount of surface bound water is reduced after the complex formation. Besides this general scheme of complex formation, the interaction between the DNA and drug also depends on the sequence of the bases adjacent to the intercalation site, and on the chemical modifications of the intercalating drug.

Bifunctional intercalators are able to intercalate in DNA molecule in two different ways: I—intramolecular cross-linking, when both aromatic moieties intercalate with the same DNA molecule, and II—intermolecular cross-linking, when intercalating moieties interact with two separate DNA molecules. There are also multi-intercalators containing three or more intercalation rings, which were synthesized because, as potential drugs, their high DNA binding constants are expected to enhance their therapeutic activity.

1.1.3. Alkylators

Alkylators are strongly electrophilic compounds that react chemically with nucleophilic groups on DNA to form covalent bonds. The resulting DNA adducts are irreversible inhibitors of transcription and translation.

Nucleophilic substitution reactions at the DNA bases occur by both SN_1 and SN_2 mechanisms. The most reactive sites are those that are both nucleophilic and exposed in the grooves of the DNA duplex. The N(7) atom of guanine and the N(3) atom of adenine fulfill both of these criteria. Simple nucleophiles, for example ethyleneimines and methane sulfonates, tend to react via a SN_2 mechanism, whereas the nitrogen mustards can form aziridinium ions that react via an SN_1 mechanism. There are several different classes of DNA alkylators, including nitrogen mustards, ethyleneimines, methanesulfonates, nitrosoureas, triazenes and cis platinum complexes. In this sub-section we discuss platinum complexes for example. Cisplatin and carboplatin (Fig. 7) represent a group of anti-cancer agents used in the treatment of testicular and ovarian tumors. Cisplatin and carboplatin form strong platinum-nitrogen bonds with guanine and adenine bases. The cis configuration leads to intra-strand cross-links, causing unwinding of the helix, preventing transcription and leading to cell death.

The trans isomer, trans-platin, is not an active anti-cancer agent, probably because it cannot readily form intra-strand cross-links. It tends to cross-link separate strands and such lesions are more easily repaired. Fig. 8 show cisplatin DNA binding.

1.1.4. DNA cleavage agents

DNA cleavage is a reaction that severs one of the covalent sugar-phosphate linkages between nucleotide that compose the sugar phosphate backbone of DNA. It is catalyzed enzymatically, chemically or by radiation. Cleavage may be exonucleolytic-removing the end nucleotide, or endonucleolytic-splitting the strand in two. The classic DNA-cleaving anti-cancer antibiotic is bleomycin, a mixture of related antibiotics isolated from *Streptomyces verticillus*. Bleomycin is a glycopeptide antibiotic, the major component of which is bleomycin A2 (Fig. 9). There are three domains in bleomycin A2: metal-binding domain: the pyrimidine, β-aminoalanine and β-hydroxyimidazole are involved in the formation of a stable complex with Fe(ii). Reaction with O_2 gives a ternary complex believed to be responsible for the DNA cleavage activity.

DNA-binding domain: the bithiazole moiety intercalates into the double helix and the attached side chain containing a sulfonium ion is attracted to the phosphodiester backbone.

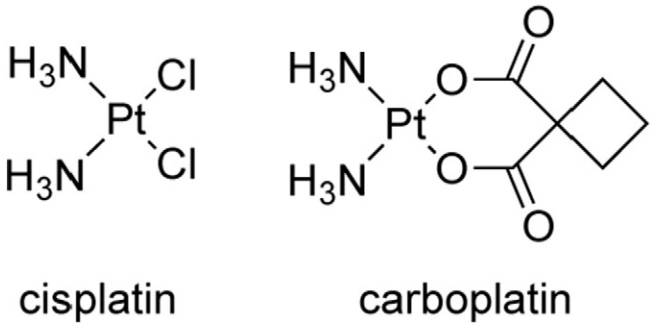

Fig. 7. Cisplatin and carboplatin structures of cisplatin and carboplatin, alkylating agents used in the treatment of testicular and ovarian cancer.

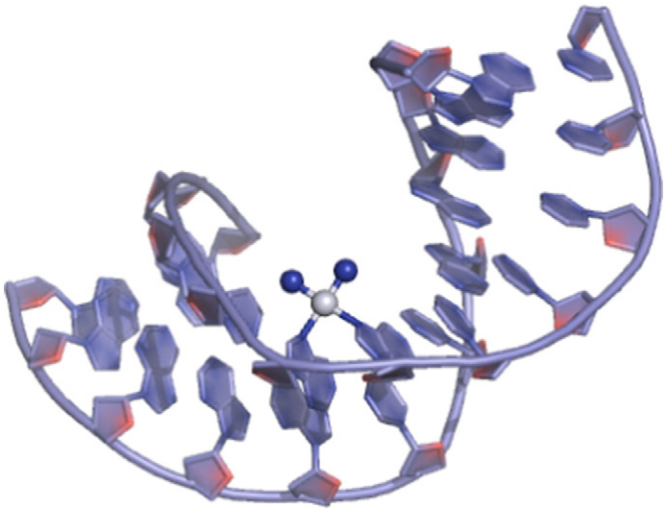

Fig. 8. Cisplatin DNA binding view from the three-dimensional structure of a cisplatin intra-strand adduct. The platinum atom is shown as a white sphere; the NH3 ligands are shown as blue spheres.

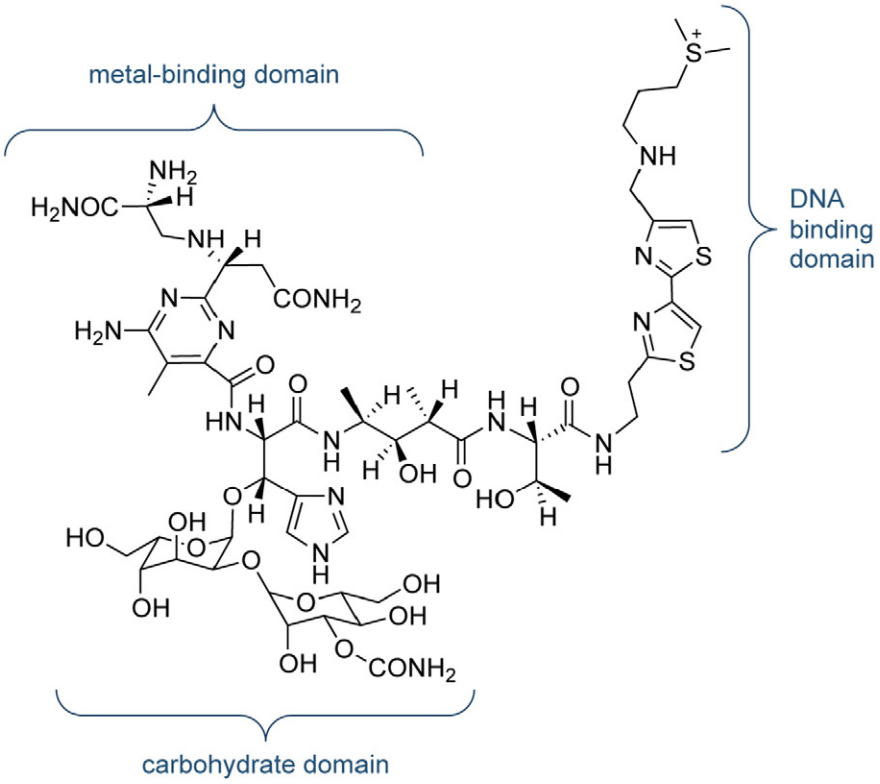

Fig. 9. Bleomycin structure of bleomycin A2, an anti-cancer antibiotic.

Carbohydrate domain: the glucose and carbamoylated mannose disaccharide are thought to be responsible for selected accumulation of bleomycin in some cancer cells. This domain does not appear to be involved directly in DNA cleavage.

Bleomycin binds tightly to guanine bases in DNA, particularly in G-T and G-C-rich sequences. When the ternary complex of Fe(ii), bleomycin and oxygen attacks DNA, it abstracts hydrogen atoms. The resultant radicals react with oxygen to form peroxy species which then fall apart, resulting in chain cleavage.

The enediyne antitumour antibiotics are anti-cancer compounds that cause the oxygen-dependent cleavage of the DNA phosphate backbone. They interact with the minor groove of DNA and then either a thiol or NADPH triggers a reaction that produces radicals. These radicals cleave the DNA chain. The major characteristic of these enediynes is the presence of a macrocyclic ring with at least one double bond and two triple bonds. The first of the enediyne antitumour agents, neocarzinostatin (Fig. 10), was isolated in 1965. Further enediynes, principally esperamicins and calichemicins, were not isolated until many years later. Neocarzinostatin contains an active naphthoate ester which intercalates in DNA, positioning the diyne in the minor groove. Activation with a thiol followed, by a Bergman rearrangement, produces a diradical that is somewhat different from those produced by other enediynes (it is not a 1,4-dehydrobenzene diradical, but it is thought to behave similarly). The reactive diradical effects DNA strand scission by reaction with the C4′- and the C5′-atoms of the deoxyribose sugar, usually at deoxyadenosine and thymidine residues, consuming one equivalent of O_2 per strand break (Fig. 11). As neocarzinostatin is the oldest member of the enediyne class of antitumour agents, it is the most thoroughly studied.

The esperamicins and calichemicins are similar to neocarzinostatin, having a bicyclo [7.3.1] ring and an allylic trisulfide attached to the bridgehead carbon, a 3-ene-1,5-diyne as part of the macrocycle and an α,β-unsaturated ketone in which the double bond is at the bridgehead of the bicyclic system (Fig. 12). The proposed mechanism of activation of the esperamicins and calichemicins begins with reduction of the trisulfide to a thiolate by NADPH or thiols in the cell. This results in an intramolecular Michael addition, which followed by a Bergman rearrangement gives the 1,4-dehydrobenzene diradical, the activated form of the esperamicin or calichemicin (Fig. 13).

Enediynes interact with the DNA minor groove; so that the proradical centers of the enediyne moiety are positioned close to the proton abstraction sites (Fig. 14).

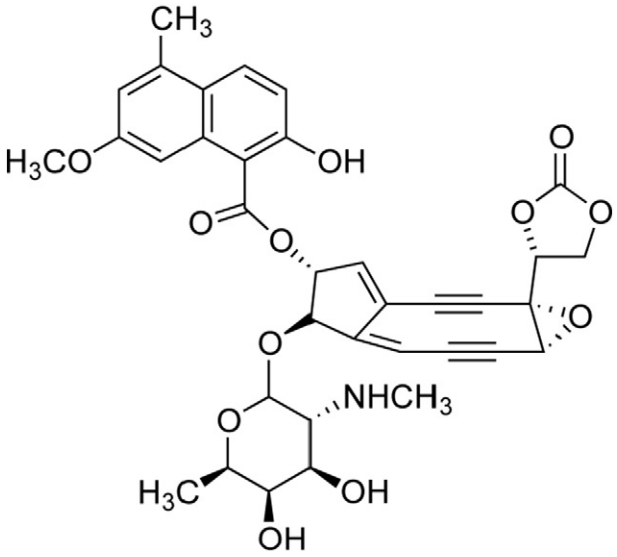

Fig. 10. Neocarzinostatin structure of neocarzinostatin, the first enediyne antitumour agent.

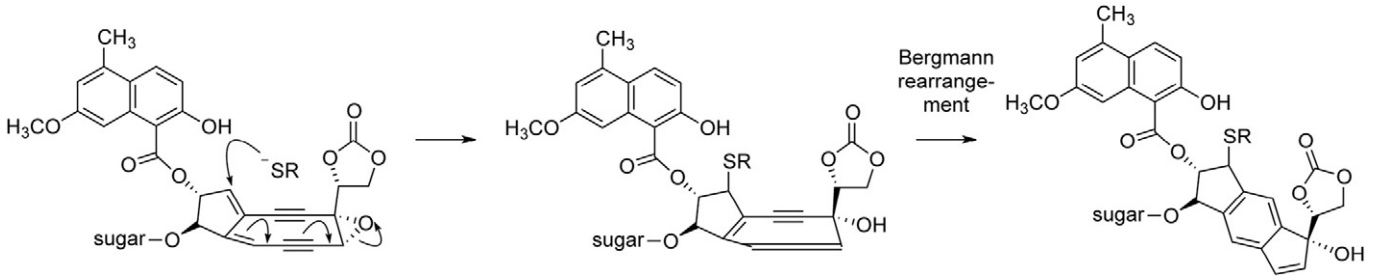

Fig. 11. Neocarzinostatin mechanism; mechanism of activation of neocarzinostatin by thiols.

2. Electroanalytical methods toward detection of drug–DNA interactions

2.1. Why electroanalytical methods/biosensors

Characterization of DNA–drug interaction generally requires a combination of chemical and biological techniques to obtain structural information and to assess the extent and the nature of specific type of binding to DNA, in terms of dissociation constant, stoichiometry, and kinetic constant. Methods able to evaluate the presence of any interaction and, in some cases, to calculate the binding parameter can be classified as mixture- and separation-based methodology. The mixture based type includes UV absorption and fluorescence [14], nuclear magnetic resonance (NMR, [15]), and Raman spectroscopy [16], mass spectrometry (MS, [17]), calorimetry [18], and surface plasma resonance [19]. The separation based methods include dialysis, ultrafiltration, ultracentrifugation, chromatography (liquid chromatography and thin layer chromatography), and electrophoresis (planar and capillary electrophoresis [20,21]). The last two separation methods are generally combined with sensitive detection techniques (hyphenated techniques), such as MS.

Among the physicochemical techniques, there has been a growing interest in electrochemical investigations. Compared to other methods, electrochemical techniques are characterized by simplicity and reliability and require small amounts of sample, thus offering advantages over biological and chemical assays. Since many small molecules exhibit redox activity, electrochemical method can provide a useful complement to the previously listed methods of investigation. The electrochemical methods are mainly based on the differences in the redox behavior of the nucleic acid-binding molecules in the absence and presence of DNA-including the shifts of the formal potential of the redox couple and the decrease of the peak current resulting from the dramatic decrease in the diffusion coefficient after association with DNA. On the other hand, since the discovery of the electrochemical activity of nucleic acids by Paleˇcek at the end of the 1950s [22], also DNA has been on the focus of the electrochemical techniques. The binding of drugs to DNA has been described by means of the variation of the oxidation peak current of the electroactive nucleobases, such as guanine and adenine, in the presence of the interacting species.

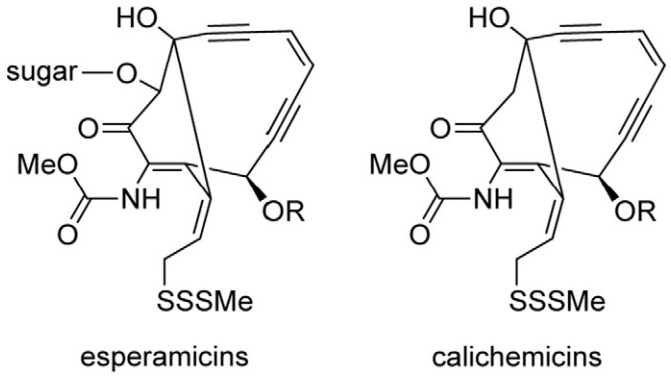

Fig. 12. Esperamicins and calichemicins; structures of esperamicins and calichemicins, enediyne antitumour agents.

The use of electrochemical techniques to increase the knowledge of drug molecules and their mechanism of action is one of the most important phases of drug discovery [23]. On the other hand, the electrochemical DNA-biosensors form a useful model for simulating nucleic acid interactions with cell membranes, potential environmental carcinogenic compounds and clarifying the mechanism of interaction with drugs and chemotherapeutics. However electrochemistry coupled with spectroscopy techniques could provide a relatively easy way to obtain useful insights into the molecular mechanism of drug–DNA interaction, which could be an important step in the development of a new anticancer drug.

According to a recent IUPAC document [24], a biosensor is defined as a specific type of chemical sensor comprising a biological recognition element and a physicochemical transducer. The biological element is capable of recognizing the presence, activity, or concentration of a specific analyte in solution. The recognition may be either a binding process (affinity ligand-based biosensor, when the recognition element is, e.g., an antibody, a DNA segment, or a cell receptor) or a biocatalytic reaction (enzyme-based biosensor). The interaction of the recognition element with a target analyte results in a measurable change in a given property. The transducer converts the change in solution property into a quantifiable signal. The mode of transduction may employ several techniques, including electrochemical, optical, and mass or heat measurements. In our case, the electrochemical DNA biosensor consists of a nucleic acid recognition layer, which is, immobilized over an electrochemical transducer [25]. The signal transducer monitors the change that has occurred as a consequence of the binding, converting this into an electronic signal [26]. Observing the pre- and postelectrochemical signals of drug–DNA interaction provides good evidence for the event. The reproducibility of the experiment is strictly related to the history of the electrode surface. In particular, the preparation of the electrode surface influences the final response. For this reason, the use of disposable, low-cost electrode characterized by high reproducibility overcomes the problem, as far as a new, fresh surface is used in each experiment. Various planar technologies are employed for developing solid-state sensors having the above-said characteristics [27]. Screen printing is especially suitable due to its simplicity, low-cost, high reproducibility, and efficiency in largescale production. This technology enables the deposition of a thick layer of conductive ink on inexpensive substrates and allows precise pattern control. Although systematic research in this field started recently, several seminal review articles have already been focused on this topic [28–41].

The use of DNA-based biosensors is not limited to the study of interaction between drugs and DNA, but many other applications have been reported. On one hand, DNA-ion sensors have been used to test water, food, soil, fish bile [42], and plant samples for the presence of mutagenic pollutants with binding affinities for the structure of DNA [43–51]; on the other hand, DNA-based affinity biosensors have been used to detect specific oligonucleotide sequences in order to find the presence of genes (or mutant genes) associated with particular human diseases [52]. Both

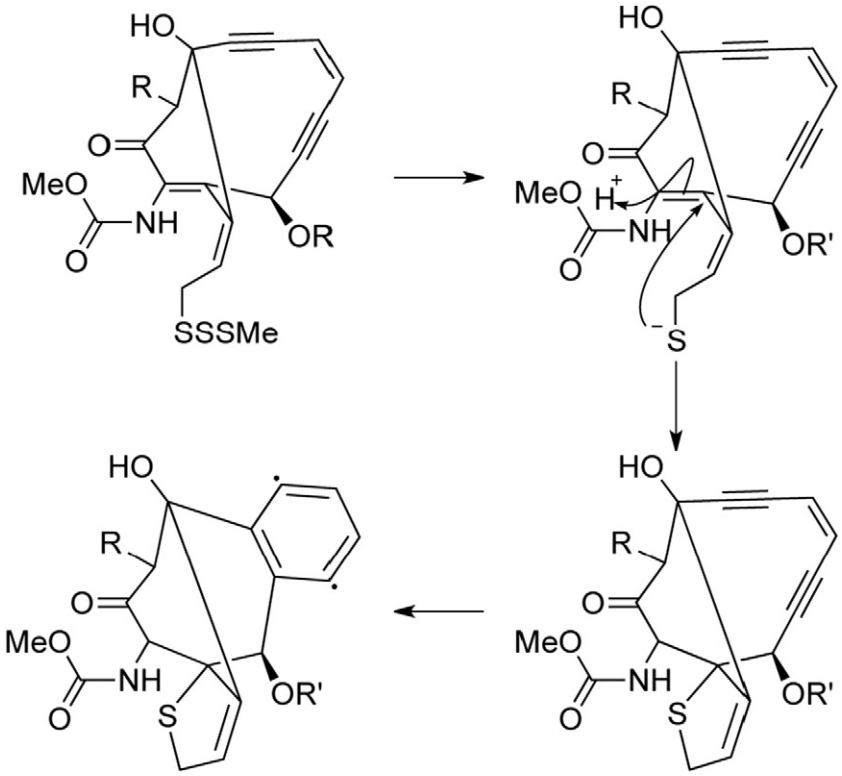

Fig. 13. Esperamicin mechanism; mechanism of activation of esperamicins, enediyne antitumour agents.

aspects are beyond the focus of this paper and will not be discussed further.

The fundamental principle of the electrochemical techniques/biosensors detection is based on the fact that the electrode detects the change at the DNA molecule. Upon the interaction with the drug, changes in intensity and position of the voltammetric signals of both drug and DNA may occur. Positive shifts of the peak potential were observed in the binding form via hydrophobic interactions (intercalation), while electrostatic interactions led to negative shift.

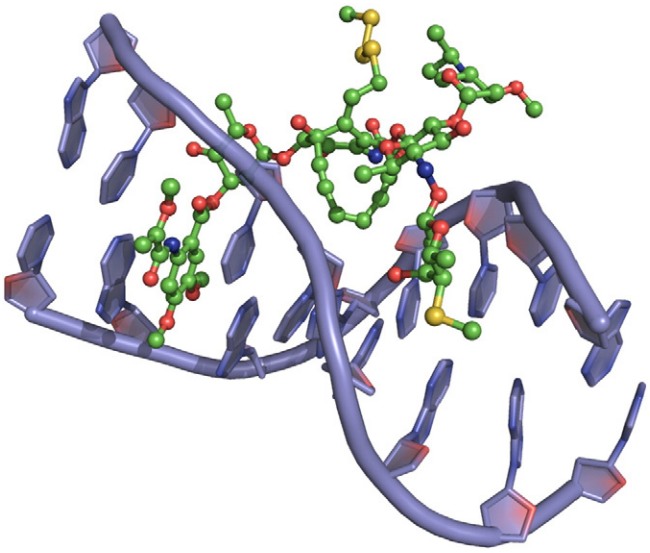

Fig. 14. Esperamicin DNA binding view from the three-dimensional structure of a complex between esperamicin A1 (green) and a DNA duplex, showing the binding of esperamicin A1 in the minor groove of double-stranded DNA.

The application of existing and development of electrochemical biosensor/methods aims to expand research limits regarding to DNA selectivity and bioaffinity towards the drug, as well as for fundamental evaluation of effects of their interaction.

2.2. Types of electroanalytical methods for detection of drug–DNA interaction

2.2.1. Anticancer drugs

Chemotherapy is an effective part of the program for combating cancers. Many anticancer drugs are known to exert their biological activities by interacting with DNA [53]. Therefore, the investigation of drug–DNA interaction is an important aspect of biological studies in drug discovery and pharmaceutical development processes. Many drugs bind to DNA mainly through several types [34]: intercalation, groove-binding, covalent linkage, DNA cleaving, nucleoside analog incorporation and so on. These interactions can result in structural change of both DNA and drug molecule to accommodate complex formation. In the last few years, many technological advances have made us develop new analytical tools to study the interaction of anticancer drugs with DNA. These results have provided useful insights into DNA conformation and drug–DNA interactions. In particular, it has been found that specific atomic sites of DNA bases are often targets for drug binding [54].

In drug discovery and pharmaceutical development studies, the interaction of drugs and DNA is very important. Especially electrochemical investigation of the interaction between anticancer drugs and DNA has been of growing interest because the pre- and post-electrochemical signals of the surface confined interaction between DNA and drugs provide good evidence for the interaction mechanism to be elucidated [55].

After discovery of electroactivity in nucleic acids, there has been a growing interest in the electrochemical investigation of interaction between anticancer drugs and DNA. The electrochemical techniques allow evaluating and predicting interactions and damage caused to DNA. These methods are suitable for investigation of drug–DNA interaction due to their advantages such as high sensitivity, fast response time,

and low cost. In these techniques, DNA electrochemical biosensors are one of the most important groups. Biosensors are small devices, which utilize biological reactions for detecting target analytes. Electrochemical DNA biosensors comprise a nucleic acid recognition layer, which is immobilized over an electrochemical transducer. Different immobilization procedures are used for electrode surface modification such as formation of mono- or multi-layer DNA film, electrostatic adsorption, and evaporation. The investigations of interactions are based on the differences in the electrochemical behavior of DNA such as the decreases/increases of the peak currents and the shifts of the potentials related to guanine and adenine. These DNA probes using differential pulse voltammetry have great sensitivity for detecting small changes of DNA structure and have been successfully used to identify the interaction of anticancer drug with DNA [56].

The interaction of the anticancer drugs with DNA occurs principally by three different ways [57]. 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 [34]. This sub-section 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.

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.

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 [58–68]. Drug–DNA interactions mechanism can be investigated in three different ways, i.e. DNA modified electrode, drug-modified electrode and interaction in solution [34]. These are described below.

2.2.1.1. 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 covalent attachment, by electrostatic attraction or by entrapment within a polymer layer [69–72]. 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 [73]. In contrast, DNA immobilized throughout a polymer layer [74] 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. Recently, Gherghi and coworkers [75] investigated the interaction of actinomycin D and DNA using 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. A concentration of 0.1 g/L (in the case of double-stranded DNA (dsDNA)) and 0.05 g/L (in the case of single-stranded DNA (ssDNA)) were selected as the most suitable, since at these concentrations full electrode surface coverage was observed.

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) [76]. 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.

In this report, 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 and coworkers [77] 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.

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 [60]. Adriamycin is a complex molecule and different groups can be oxidized or reduced. Brett and coworkers [78] 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 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. 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. [78] may be used to explain the levels of 8-oxoguanine found when cells are treated with Adriamycin.

Ibrahim [79] 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 ($K = 4.44 \times 10^5\ M^{-1}$) 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 a cyclic and cyclic α,β- saturated ketones and related Mannich bases such as 4,4_-dihydroxy chalcone (DHC) are well described [80,81]. The activities of these compounds were attributed, in part, to 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 (ds-DNA) 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) [82]. As a result of alkylation of DHC between the base pairs in dsDNA, the voltammetric signals of guanine and adenine were greatly decreased. 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. 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 and coworkers [83] 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. [84] 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 nonlinear 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 [85]. Karadeniz and coworkers [86] 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 [87]. EL-Hady and coworkers [88] 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 NH2 group in guanine moiety. They also calculated the ratio of the equilibrium constant ($K1$) between the reduced free form and the reduced bonded form and the equilibrium constant ($K2$) between the oxidized free form and the oxidized bonded form. It was found that the $K2$ value is 298 times than that of $K1$. 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 [62,89]. 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 [90]. 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 [91]. 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 [92,93]. The interaction of actinomycin D (ACTD) with calf thymus DNA (ctDNA) was studied at an oxidized waxed graphite electrode [94]. Actinomycin D showed a pair of nonsymmetric 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 $K = 7.54 \times 10^9\ cm^3/mol$ and binding site size $s = 8.1$) demonstrated that ACTD binds to ctDNA with a high association constant and covers eight base pairs. Wang and coworkers [95] 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 33,258) 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 [96]. 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 and coworkers [77] 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 [97]. 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 sub-micromolar 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 [98,99]. Kang and coworkers [63] 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–Qu3 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 ds-DNA modified GCE system, the binding number was "1". It was concluded that the ability of complex binding to ds-DNA is stronger than quercetin. Jelen and coworkers [100] 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 ds-DNA by bis-intercalation at the electrode surface.

Flutamide (Flu) is a chemotherapeutic drug, used for the treatment of prostate cancer. It functions by interfering DNA in fast growing cells and preventing them from reproducing. Temerk and Ibrahim [101] investigate the interaction of Flu with single and double stranded DNA at different temperatures and at physiological pH 7.4 (human blood pH).

The voltammetric results of this report indicated that the planar Flu intercalated to the dsDNA. The hyperchromic effect in absorption spectra of Flu–dsDNA complex affirmed the intercalative mode of binding between Flu and dsDNA. The interaction mode of Flu molecules with ssDNA is electrostatic attraction via negative phosphate on the exterior of the ssDNA with Flu. The complete thermodynamic profiles for the binding of Flu to DNA are also constructed and the results show the binding of Flu to DNA is endothermic and a spontaneous process of association. The association between Flu and dsDNA is maximum at 308 K which depicts the most stable complexes are formed at near human body temperature. Thus the human body temperature provides the most favorable conformation of dsDNA which binds to the anticancer Flu, helping this drug to hinder dsDNA replication under the physiological conditions. The obtained results are of potential importance in understanding the mechanism of interaction of the drug with DNA in the living body. Simple, rapid and sensitive voltammetric method is proposed for the determination of dsDNA and ssDNA concentration. The detection limits of dsDNA and ssDNA were found to be 4.27 × 10–7 M and 1.87×10^{-7} M, respectively.

Mitoxantrone intercalates with DNA and produces a MTX-DNA adduct, resulting in the blockage of protein synthesis and excessive production of free radicals in the myocardium which eventually leads to cardiac toxicity. Interestingly, Lad and Agrawal [102] prepared a DNA nanosensor to investigate the interaction of MTX with DNA. Multiwall carbon nanotubes (MWCNTs) were functionalized with carboxyl group and were used for immobilization of DNA to construct the DNA nanosensor. The DNA nanosensor was immersed in MTX solution to monitor MTX–DNA interaction with respect to time and alter the resistance of the nanosensor. It was observed that MTX–DNA interaction is fast initially and as time elapses, the change in interaction gets slow due to formation of MTX-DNA adduct. The limit of detection (LOD) and limit of quantification (LOQ) were found as 150 (ng/mL) and 456 (ng/mL), respectively, for DNA nanosensor. This study suggests that the potentiometric nanosensor allows real-time monitoring of the drug-DNA interaction changes by measuring the potential at DNA-sensor interface which can prove to be an important tool in drug discovery pipelines and molecular toxicology.

Also, Turkey researchers [103] prepared a chitosan/ionic liquid modified pencil graphite electrode (CHIT-IL-PGEs) and applied for enhanced electrochemical monitoring the interaction of the anticancer drug Mitomycin C (MC) and calf thymus double stranded DNA (dsDNA) by measuring the oxidation signals of MC and guanine in the same voltammetric scale.

Advanced breast cancer is an incurable disease and, even if many active antineoplastic agents are available, the aim of the treatment, at present, can be only palliation. Nevertheless, availability of these agents allows to control the disease for long periods of time and to improve the quality of life in the majority of patients. A prolongation of survival, on the contrary, can be hypothesized only in a minority of cases achieving a complete response with currently available first-line treatments, expected response rates range from 50 to 70% [104–109].

Anthracyclines are widely used in combination schedules and are considered the most effective drugs in the treatment of metastatic breast cancer. Epirubicin is an anthracycline drug used for chemotherapy. Epirubicin is favored over doxorubicin, the most popular anthracycline, in some chemotherapy regimens as it appears to cause fewer side-effects. Epirubicin has a different spatial orientation of the hydroxyl group at the 4′ carbon of the sugar, which may account for its faster elimination and reduced toxicity. Epirubicin is primarily used against breast and ovarian cancer, gastric cancer, lung cancer, and lymphomas. The investigation of DNA–EPR binding can provide information of the structural features which determine the therapeutic affectivity of drugs. Interactions with DNA could also be critical for understanding the drug toxicity and its distribution in the organism. It has been an interesting research field in life science, chemistry and clinical medicine [100,110]. For this purpose Hajia and coworkers [111] studied the EPR–DNA interaction in combination with cyclic voltammetry (CV), spectrofluorometry, viscometery and specially UV–Vis spectrophotometry. The apparent binding constant of epirubicin with DNA was found to be 3.8×10^5 mol^{-1}/L and was studied at different temperatures. The experimental results revealed that EPR and ds-DNA had strong interactions. Also, the diffusion coefficients of EP in the absence and presence of ds-DNA were calculated as 5.04×10^{-6} and 1.13×10^{-6} cm^2/s respectively.

Idarubicin (IDA), 4-demethoxydaunorubicin, is an anthracycline derivative and widely used treatment of leukemia. The electrochemical

behavior of IDA was examined at a glassy carbon electrode (GCE) in different aqueous supporting electrolyte by Kara [112]. These researchers found that, the oxidation process of IDA was pH dependent and with irreversible nature. The electroactive center is the hydroxyl group on the aromatic ring which produces a final quinonic product. The diffusion coefficient of IDA was calculated to be DIDA $= 7.47 \times 10^{-6}$ cm^2/s^{-1} in pH = 4.3 0.1 M acetate buffer. The interaction of IDA and double stranded deoxyribonucleic acid (ds-DNA) was investigated using electrochemical ds-DNA biosensor and incubation solution by means of DPV. The DNA damage was detected following the changes in the oxidation peaks of guanosine and adenosine residues. The results obtained showed that IDA interacts with DNA which causes the change in the DNA morphological structure. In addition to these polynucleotides, PolyG and PolyA, biosensors were also used to confirm the interaction between ds-DNA and IDA.

IN 2005, Chinese researchers investigate interaction of mitoxantrone with DNA analyzed by electrochemical and spectroscopic methods [113]. Cyclic voltammetry coupled with different spectroscopic (UV/Vis, fluorescence and Raman) techniques were used to study the interaction of mitoxantrone (MTX), an antitumor drug, with calf thymus DNA in acetate buffer solutions (pH 4.5). The interaction of MTX with DNA could result a considerable decrease in the MTX peak currents and a hypochromic and bathochromic shift in the maximum adsorption bands of MTX as well as the emission quenching in the MTX fluorescence spectra. The variations in the electrochemical and spectral characteristics of MTX indicated that MTX bind to DNA by an intercalative mode. This conclusion was reinforced by Raman data. The merely particular vibrations were affected in Raman, suggesting that only a portion of the chromophore of MTX was involved in the intercalation into DNA duplex. These studies are valuable for a better understanding the detailed mode of MTX–DNA interaction, which should be important in deeper insight into the therapeutic efficacy of MTX and design of new DNA targeted drug.

Interestingly, Li, and coworkers [114] employs functional electrodes immobilized by synthesized thiol-linked DNA as probes to detect DNA-specific binding drugs differing in binding mode and sequence specificity. The alteration of the interfacial properties of electrodes upon Drug-DNA interaction is traced by electrochemical impedance spectroscopy. Results of this report show that, the gold nanoparticle- modified sensing device resulted in detection limits of 5 nM for nogalamycin, 10 nM for mythramycin, and 40 nM for netropsin, respectively, which are 15–40-fold lower compared to flat gold substrates. Researchers of this report, hypothesis that the enhanced sensitivity might be simply explained in terms of changes in the geometry of the electrode surface affecting the binding efficiency of the Drug-DNA interaction and hence direct the improvement of impedance signal changes. The specificity of the interactions of two classical minor groove binders, mythramycin, a G-C specific-DNA binding anticancer drug, netropsin, an A-T specific-DNA binding drug and an intercalator, nogalamycin on AT-rich DNA modified substrate and GC-rich DNA-modified substrate are compared. This strategy might be useful for the sensing of other interfacial interaction such as enzyme-DNA, antibody-antigen, and cell-antibody.

Also, the interaction of mitomycin C (MC) with fish sperm or calf thymus DNA immobilized onto carbon screen-printed electrodes (CSPE) and carbon paste electrode (CPE) have been studied by Turkish researchers [115]. Results of this work show that, after the interaction between DNA and MC on electrode surface, the guanine signal was higher with bare electrode than on DNA-modified one. These results showed that these two different DNA biosensors could be used for the sensitive, rapid and cost effective detection of MC–DNA interaction.

The electrochemical detection of the interaction between MC and DNA by using solid electrodes such as, carbon screen printed electrode (CSPE) and carbon paste electrode (CPE) based on the changes of guanine signal. This report show that the detection of MC–DNA interaction could be done faster, more sensitive and less laborious techniques by using these kinds of solid electrochemical genosensors.

Marin et al. [91] showed the acid activated MC binds to DNA at pH 3.9 by using HMDE surface as detected by the decrease of guanine signal. Additionally they reported that the quinone group in the occurred DNA–MC adduct was reversibly reduced at HMDE resulting in a 10 fold higher and 50 mV more negative cathodic peak. In another work, Marin et al. [116] showed direct determination of submicromolar concentrations of MC with an excess of 5-flurouracil or cisplatin in urine without any cleaning up step by using DNA biosensor.

In another study related to MC–DNA interaction, the cyclic voltammetry in connection with HMDE used for detecting the interaction between MC and DNA was studied based on the cathodic responses of MC both in single-sweep and repeated cycle modes [117].

Perez and coworkers [118] showed the electrochemical detection of DNA–MC adducts at HMDE by using different procedure such as the potential controlled interaction for MC with DNA at electrode surface.

Also, a novel microemulsion-based drug-delivery system was prepared by loading MC into an oil/water system with the aim of investigation of the interaction between MC and dsDNA in the microemulsion phase using a disposable pencil graphite electrode (PGE) and differential pulse voltammetry(DPV) and monitoring the guanine oxidation signal before and after interaction between MC and dsDNA [119]. The effect of different experimental parameters, such as MC concentration, its interaction time with dsDNA, and dsDNA concentration were also studied to find the optimum analytical performance.

The results of this report are of potential use for developing and optimizing drug-delivery systems loaded with DNA-targeted molecules for application in chemotherapy and diagnosis.

The antibiotic mitomycin C (MC) is an antitumor agent used in clinical chemotherapy against a broad spectrum of solid tumors. Early studies indicated that the primary target of the MC action is DNA, as evidenced by its ability to bind covalently to DNA, both mono and bifunctionally [118]. Monitoring the MC-DNA interaction process was reported in earlier studies in literature with different electrochemical detection methods and transducers. Marin and coworkers [91] investigated acid-activated MC and its interaction with single-stranded DNA using hanging mercury drop electrode (HMDE) by cyclic voltammetry(CV) technique. Electrochemical detection of interaction between MC and double-stranded DNA (dsDNA) in microemulsion phase was performed by using differential pulse voltammetry (DPV) in combination with PGE [119]. Erdem et al. [120] developed a graphene oxide (GO) integrated disposable electrochemical sensor for voltammetric monitoring of interaction between MC and DNA by measuring the changes at the oxidation signals of MC and guanine.

The applications of carbon nanotube modified electrochemical biosensor for the purpose of monitoring of drug–DNA interaction have been reported [121–123]. Moreover, the combinations of carbon nanotubes with different polymers have been reported in the literature [124–126]. Recently, carbon nanotubes-chitosan (CNT-CHIT) modified disposable electrochemical biosensor platform was fabricated and applied for monitoring of the surface confined interaction between MC and DNA [127]. The modification of PGEs with CNT-CHIT and the interaction of MC with fsDNA was represented in Fig. 15. In this report, the surfaces of PGEs were modified by CNT-CHIT composite structure. Then, fish sperm double stranded DNA (fsDNA) was immobilized onto the surface of CNT-CHIT/PGEs, and MC interaction with fsDNA was evaluated based on the changes at the oxidation signals of MC and guanine using DPV. Results of this report show that, optimum MC concentration was determined with monitoring the MC oxidation signal by using unmodified and CNT-CHIT/PGE in the concentration range of MC from 10 to 50 mg/mL. The detection limits (DLs) were also calculated based on the MC signals obtained by CNT-CHIT/PGEs corresponded to 1.12 mg/mL (0.33 nmol in 100 mL sample).

Interestingly, CV coupled with UV–vis and fluorescence spectroscopy were used to probe the interaction of potential anticancer drug, 4-nitrophenylferrocene (NFC) with DNA [128]. The electrostatic interaction of the positively charged NFC with the anionic phosphate of DNA

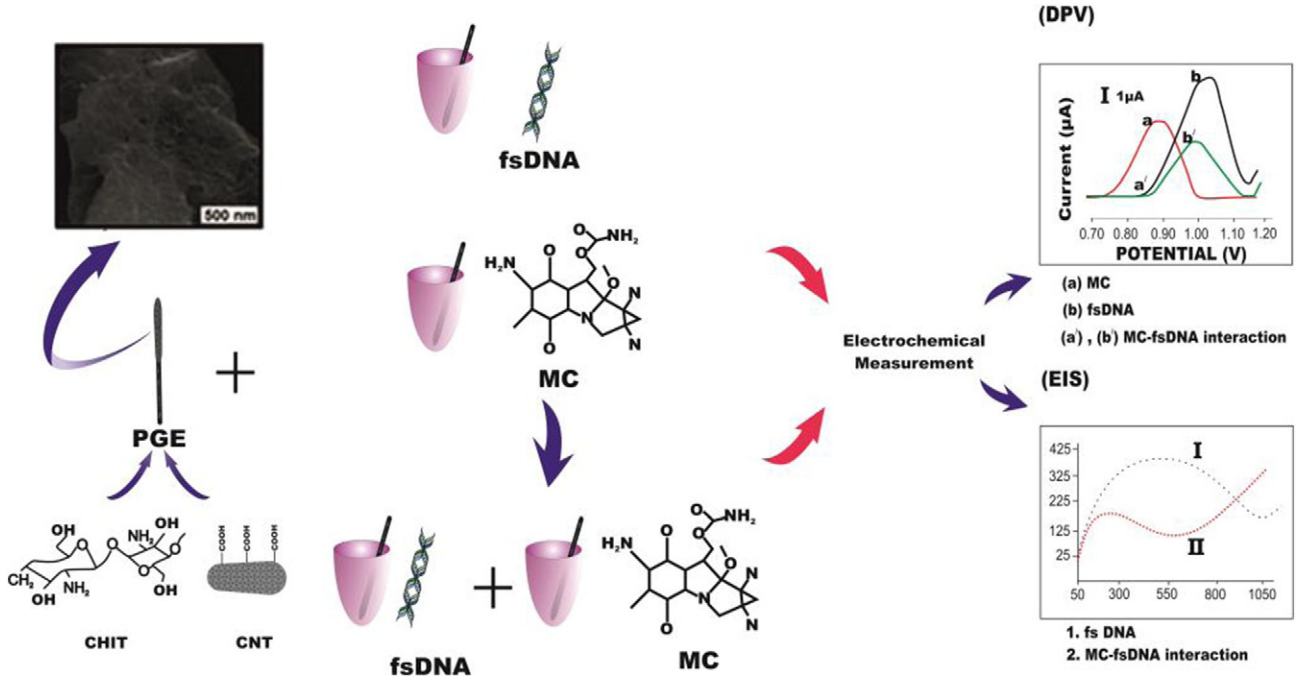

Fig. 15. Development of CNT-CHIT/PGEs and electrochemical investigation of interaction between fsDNA and MC. [127].

was evidenced by the findings like negative formal potential shift in CV, ionic strength effect, smaller bathochromic shift in UV–vis spectroscopy, incomplete quenching in the emission spectra and decrease in viscosity. The nature of NFC–DNA interaction was examined by CV based upon the difference in the redox behavior of the drug in the absence and presence of DNA, including the shifts in the formal potential of the redox couple and the decrease of the peak current due to the remarkable decrease in the diffusion coefficient after binding to DNA. The results of CV and ionic strength effect indicated electrostatic interaction of NFC with DNA as the dominant mode. The binding parameters like binding constant, ratio of binding constants (K_{red}/K_{ox}), binding site size and binding free energy were determined from voltammetric data. $K = 3.85 \times 10^3\ M^{-1}$ and binding site size was 0.9 bp. The small value of s indicates electrostatic interaction of NFC with DNA. Such an interaction may induce perturbation in the normal functioning of DNA which could presumably culminate in the prevention of replication and ultimate cell death.

Concluding this sub-section, it is important to point out that, 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 [129]. 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 [130]. 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 and coworkers [95] 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.

Having in mind that DNA is one of the most important biomacromolecule, the possibility of detection and explanation of the changes resulting upon its interaction with chemotherapeutic drugs deserves a great attention. It is very important to use the most advanced methods in the analysis of drugs and toxic substances that may produce changes in DNA structure. The choice of the appropriate instrumental method allows the complete knowledge of the nature and the type of the mentioned interaction.

Compounds binding with DNA through intercalation usually results in hypochromism and hypsochromism (blue shift) or bathochromism (red shift) of the UV–Vis absorption spectra. In case of electrostatic attraction between the compound and DNA, hyperchromic effect is observed that reflects the changes of DNA conformation and structure. The advantage of molecular fluorescence over other techniques is its high sensitivity, large linear concentration range and selectivity. Interaction with DNA usually causes a significant enhancement of the fluorescence intensity. Fluorescence quenching experiments give information concerning the localization of the drugs and their mode of interaction with DNA. A major advantage that the application of IR spectroscopy offers is that samples can be analyzed in different aggregation states. Fourier transform infrared (FTIR) spectroscopy has been used to determine drug binding sites and sequence preference, as well as conformational changes due to drug–DNA interaction. Chemical shifts in NMR spectra are attributed to the binding between ligand and DNA molecule and are sensitive to conformational changes.

Intercalating drugs cause downfield shift, and the broadening of DNA ^{1}H NMR resonances upon addition of a suitable minor-groove binding compound is usually taken as primary evidence of complex

formation. CD spectra may distinguish between chiral and achiral drug via an induced circular dichroism. The observation of an ICD immediately reveals that the drug actually binds to DNA, while its sign provides qualitative information about the binding mode: a strong, positive ICD is typical of a minor groove binder, while intercalators usually exhibit negative and weaker ICDs. The fundamental principle of the electrochemical biosensor detection is based on the fact that the electrode detects the change at the DNA molecule. Upon the interaction with the drug, changes in intensity and position of the voltammetric signals of both drug and DNA may occur. Positive shifts of the peak potential were observed in the binding form via hydrophobic interactions (intercalation), while electrostatic interactions led to negative shift.

The application of existing and development of new techniques and methods aims to expand research limits regarding to DNA selectivity and bioaffinity towards the drug, as well as for fundamental evaluation of effects of their interaction.

Although all mentioned techniques show their own specificity, their common aim is to correlate the measurable biophysical parameters with cytotoxicity i.e. anticancer activity of the drug, and to enforce the obtained knowledge for design new DNA ligands for in vitro and in vivo genetic diseases monitoring.

Electrochemical approach has been successfully used to determine the anticancer drug-DNA interaction and can contribute to drug discovery and effective treatment for cancer by providing knowledge regarding the efficacy of drug candidates binding with DNA and also elucidating the mechanism of the drug–DNA interaction.

2.2.2. Antibacterial drugs

In the case of antibacterial drug–DNA interaction, there are two published report. In the first report [131], the interaction between ciprofloxacin and DNA was studied using bare GCE or a nano-SnO_2 modified electrode. In this report, the nano-SnO_2/PVS film was cast onto the surface of GCE and the electrochemical behavior of CFX at the modified electrode was investigated. The results of this report show that, the interaction of CFX and ctDNA was an intercalation mechanism.

At the second report [132], the electrochemical oxidation of gatifloxacin (GTF), at multi-walled carbon nanotube paste electrode was studied by Brahman and coworkers. The results of this report show that, GTF has well-defined oxidation peak at +1.05 V in acetate buffer of pH 5.0 ± 0.01. Also, the peak current is linear over the concentration range 2.13×10^{-5} to 1.7×10–^{4} M with detection limit of 4.5×10–^{9} M. Also, multi-walled carbon nanotube paste electrode was employed to probe the interaction between GTF and calf thymus DNA. The addition of ds-DNA to the analyte containing GTF results in a decrease of the peak current and a positive shift of the peak potential of GTF in differential pulse voltammetry analysis, indicating the dominance of the intercalating interaction. The electrochemical oxidation of GTF under the conditions described in this study is an irreversible adsorption controlled process. Also, the binding constant of this complex was calculated to be 3.620 M^{-1} and the binding number was determined to be 0.438 between GTF and DNA. DNA interacting with GTF forms a complex of 1 mol GTF per 2 mol base pair.

2.2.3. Other compounds

Other than the above discussed drugs, in the literature, other types of drugs–DNA interactions have been reported. In the current subsection, we will briefly discuss some of these interactions. In this subsection of review, we focused our attention on antiandrogenic, anti-inflammatory, antineoplastic, antioxidant, and antiviral drugs. Despite their easy interaction with DNA, few research efforts have been devoted to this electrochemical behavior. Nevertheless, there are few promising works concerning to these drugs–DNA now available in the literature.

Recently, DNA modified carbon paste electrode in combination with differential pulse voltammetry were used to obtain information about the interaction of flutamide with DNA [133]. In this work, flutamide produced a well-defined DPV peak at $E_p = -0.75$ V vs. SCE. The DPV study on the DNA–flutamide interaction clearly demonstrated that flutamide interacts preferentially with adenine and guanine groups in DNA. When a potential of −0.75 V was applied, flutamide was reduced at the DNA-modified electrode. In this way flutamide generates an anion, this reactive anion may oxidize one of the neighboring guanine site of the ds-DNA. During this process, electron transfer from guanine moiety to the nitro group of flutamide without hydrogen abstraction is likely to be the predominant reaction leading to the formation of the guanine cation. The cation will undergo hydrolysis forming semiquinone. This semiquinone may undergo further reduction to the fully reduced flutamide.

Also, Wei and coworkers [134] studied the interaction between diclofenac and DNA by the electrochemical method. The result indicated that the intercalation played a predominant role in the interaction of diclofenac with DNA. Based on the diclofenac–DNA interaction, an electrochemical biosensor for diclofenac was constructed in combination with the promotion of graphene oxide (GO). The prepared biosensor showed a sensitive response to diclofenac in the range of 1 to 130 μM. It was found that diclofenac and DNA formed an electrochemically inactive adduct at a binding ratio of 1:1. The binding constants were calculated to be 6.51×10^{3} M^{-1} in pH 7.0 buffer solution and 4.71×10^{4} M^{-1} in pH 3.0 buffer solution.

Interestingly, cyclic voltammetry coupled with UV/VIS spectroscopic techniques were used to study the interaction of myricetin, a flovonoid compound, with double stranded calf thymus DNA (ds-DNA) in phosphate buffer solution (pH 7.5) [135]. In spectrophotometric studies, maximum absorbance at ca. 330 nm for myricetin does not change significantly by addition of ds-DNA to the solution. The slopes of the calibration curves for methotrexate in the absence and presence of neutral red (NR)-DNA are similar. Because of the inactivity of myricetin on the surface of hanging mercury drop electrode, Hajian and coworkers used neutral red as a probe for electrochemical study of myricetin with ds-DNA. In the electrochemical study of neutral red, the appearance of a pair redox peaks with $\Delta E_p = 3.0$ mV shows the adsorption process. The decreasing of peak currents for neutral red by addition of ds-DNA reveals a high strength binding between neutral red and DNA ($K_b = 2.74 \times 10^{4}$). In the competitive study on the titration of neutral red-DNA with myricetin shows no significant changes on the oxidation or reduction of neutral red-DNA. These studies are valuable addition for a better understanding of the detailed mode of interaction between myricetin with ds-DNA, which should be important in deeper insight into the therapeutic efficacy of myricetin and design of new DNA targeted drugs. All of the electrochemical and spectroscopy studies show that myricetin has a week interaction with ds-DNA.

More recently, interaction of an antipsychotic agent, aripiprazole (ARP), with calf thymus double stranded deoxyribonucleic acid, ct-ds-DNA, was investigated by Sevinc Kurbanogluet al [136]. In this study, the electrochemical ct-ds-DNA biosensor has been successfully performed to characterize the interaction of the drug ARP with dsDNA. The ct-ds-DNA modified GCE was used to obtain the information about the interaction of ARP with ct-dsDNA, based on the changes at ARP and DNA bases signal. The change in DNA bases signal was also confirmed by interaction studies in solution phase. The approach described by these researchers shows the utility of the voltammetric methods and electrochemical DNA based biosensors for simple investigation of DNA–drug-interactions.

It is important to point out that, few papers have reported the electrochemical detection of non anticancer drugs–DNA interaction, so it is promising to develop new electrochemical methods/sensors for this purpose.

3. Conclusions

This review highlighted recent advances in the application of electrochemical techniques/biosensors for the detection of drug–DNA

interactions and summarized their typical applications. Taking into consideration the above examples, we conclude that electrochemical techniques/biosensors are suitable for detecting some binding. We have witnessed explosive growth in work related to the use of electrochemical methods on investigation of drug–DNA interaction especially in the case of anticancer drugs. As we show above, the unique properties of electroanalytical methods led to simple, highly-sensitive procedures that could not be accomplished by other optical methods. However, the preparation of more novel, sensitive electrochemical techniques/biosensors will largely depend on rapid progress in the synthesis of novel modifier with unique shapes and structures for chemical interaction with DNA. So, much effort needs to be devoted to synthesizing novel materials with unique shapes and structures. From the above examples, we conclude that there is a bright opportunity for further advances and developments of DNA modified electrodes and biosensors based on electrochemical methods, especially through further miniaturization and integration into lab on-chip systems. Electrochemists therefore have a great deal to do to address the behavior of electrochemical methods/biosensors for detection of drug–DNA interaction based on novel electrode modifiers in the future.

Despite such advances in this field, there are still challenges to explore new protocols and strategies for improving the sensitivity and the practical applications of electrochemical methods/biosensors for detection of drug–DNA interaction. In short, we believe that electrochemical methods/biosensors hold much promise for the future. In future research, special attention will probably be given to implantable electrochemical biosensors based on nanomaterials in order to improve this research area. In this review, we discuss the promising future of using electrochemical methods/biosensors, specifically their current roles in detecting different types of binding. We present the advantages of these methods, together with their electroanalytical applications in biological systems. But, there are many challenges ahead that must be addressed before DNA based biosensors can be successfully integrated into biomedical devices and technology for investigation drug–DNA interaction. The main advances required include the following: 1 advanced techniques and facile methods are needed to increase the sensitivity of electrochemical methods/biosensors; 2 further research is required to promote selectivity; 3 more specialized coatings are needed to minimize non-specific bonding; 4 protocols and further experiments should be conducted to determine the exact nature of the interaction or binding; and, finally, 5 results raise doubts about the repeatability, reproducibility, and comparability of DNA based biosensors, as, with every emerging technology, standards need to be established to avoid doubts about the lack of reproducibility, repeatability, and compatibility across platforms and laboratories.

In general, monitoring of drug–DNA interaction based on electrochemical methods/biosensors show great promise for future applications in pharmaceutics. Given the impressive progress in electrochemical DNA biosensors, there is no doubt that they will have a major impact on drug discovery and pharmaceutical development processes. We hope that this brief overview has illustrated that electrochemical methods/biosensors have achieved considerable success in academia and the need for pharmacogenomics greater than ever.

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