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Microfaradaic Electrochemical Biosensors for the Study of Anticancer Action of DNA Intercalating Drug: Epirubicin

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1. Introduction

In the past two decades chemically modified electrodes (CME) have attracted considerable interest of researchers to exert a direct control over the chemical nature of electrodes. CMEs have found a large number of useful applications in different fields viz. selective electro-organic synthesis, biomedical analysis, electro analyses etc. The ability to manipulate the molecular architecture of bulk matrix of the electrode, particularly its surface has led to a wide range of analytical applications of CMEs and created opportunities for electroanalysts to fabricate useful biosensors

The CMEs have shown remarkable specificity for biological recognition processes which has led to the development of highly selective biosensing devices. The electrochemical biosensors hold a leading position among the bioprobes currently available and hold great promise for the tasks of study of in-vivo mechanism of action of large number of drugs. These electrochemical biosensors consist of two components (1) a biological entity that recognises the target analyte and (2) the electrode transducer that translates the biorecognition event in to a useful electrical signal.

Modern methods of analysis specially designed for drug discovery are mostly high-through put systems. The target samples are either obtained by natural origin generated by combinational chemistry or produced by biochemical methods. Hundreds and thousands of synthetic compounds are available in modern substance libraries, which have to be tested individually for their use as useful drugs. In addition to this, some natural resources including tropical rain forests and marine environments are of great interest for the development of potential new drugs. In the past, a large number of plants and samples obtained from biological sources of these habitats have been used as traditional medicine of native population. Scientific knowledge about these traditional medicines has attracted the attention of scientists working in the field of development of nature derived drugs.

1.1 Biosensors

In the present times most of the screening systems used are enzyme or whole cell based and these biological substances have also been used as biological recognition elements of biosensors. We may consider a biosensor as a device consisting of a biological part and a physical transducer (Figure-1).

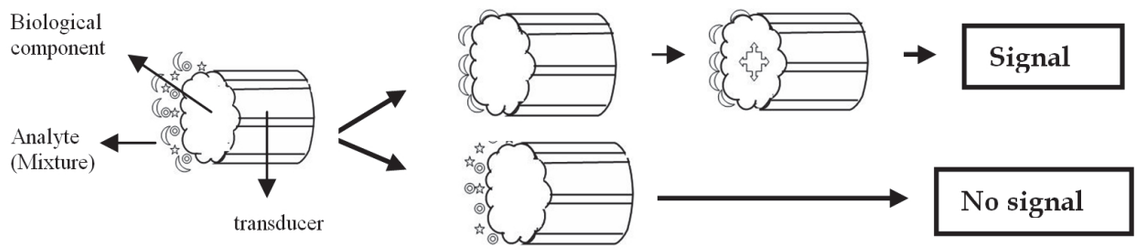


Fig. 1. Typical function of a biosensor. One of the compounds of a mixture of substances interacts with biological part of the biosensor. The biological signal so produced is converted to some physical signal (optical, electric) by the transducer. Compounds which do not interact with biological part do not produce any signal.

According to a recent IUPAC document, a biosensor is defined as a specific type of chemical sensor comprising a biological recognition element and physiochemical transducer. The biological element is capable of recognizing the presence of activity or concentration of specific analyte in solution. The recognition may be either a binding process i.e. affinity ligand based biosensor when the recognition element is an antibody /DNA segment /cell receptor or biocatalytic reaction i.e. enzyme based biosensors.
Both the components of the biosensor are in direct contact and may be used for several measurements. The selectivity of the biosensor depends on integrated biological component. The substances which have a specific tendency interact with the biological part of the biosensor produce optical or electrical signals of the transducers. In addition to cell and enzyme, some other compounds such as DNA, receptor and antibodies have also frequently been used as component of biosensors.
The increasing need to develop specific biosensors for fast routine measurement in several fields of analysis has generated interest of a large number of scientists in the field of biosensor research. As a result, a fairly high number of recently developed specific biosensors are in use for the detection and analysis of hundreds of compounds in analytes of different origin e.g. sugars, amino acids and enzyme co-factor which have utmost biological relevance **(Paddle,1996, Meadows,1996, Bousse,1996, Ziegler et al., 1998).**
Some electrical devices such as semiconductors and electrodes, some optical components such as fiber optics, quartz microbalances have also been used as transducer part of biosensor. These transducers have been miniaturized in order to obtain chip based sensors.
Though, initially biosensors were developed with an aim of clinical diagnosis e.g. in the determination of blood sugar level but in recent years biosensors have been fabricated for their possible application in food industry for quality control purpose, environmental analysis and biomedical analysis etc.

1.2 Electrochemical biosensor

The first scientifically proposed as well as successfully commercialized biosensors were those based on electrochemical sensors for multiple analysis. More than fifty percent sensors reported in the literature are electrochemical and can be classified as amperometric, potentiometric or conductometric sensors **(Meadows,1996).**
Electrochemical biosensors have been studied for a long time. They have been the subject of basic as well as applied research for nearly fifty years. **Leland C Clark** introduced the principle of the first enzyme electrode with immobilized glucose oxidase **(Clark et al., 1962).**

The first commercially produced biosensor was introduced in the market in 1975. This biosensor was used for the fast glucose assay in blood samples from diabetics. Today there is a large number of proposed and already commercialized devises based on the principle of biosensor including those for the analysis of pathogens and toxins.

1.2.1 Amperometric biosensor

In amperometric sensors, an enzyme is typically immobilized at the surface of an amperometric electrode; this immobilized enzyme reacts with the substrate (e.g. phenolic compounds/sugar) and produces current that depends on the concentration of the analyte (Figure-2).

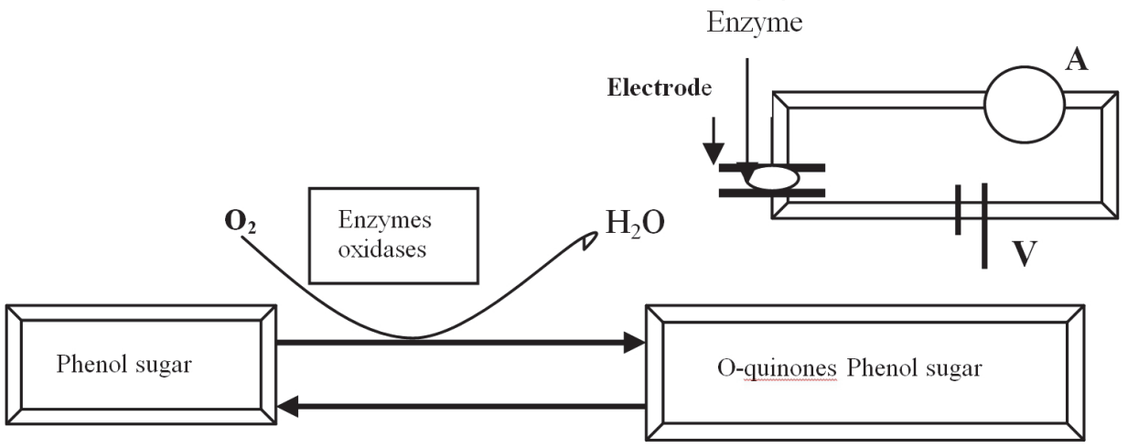


Fig. 2. A typical amperometric sensor for the assay of phenols and sugars: The enzyme is immobilized at the top of electrode, the current between electrodes gives information about the analyte.

In a simple biosensor of above type an oxygen consuming enzyme (phenolase/glucose oxidase) is immobilized on a platinum electrode and the reduction of oxygen at the electrode results in a current that is inversely proportional to the analyte concentration.

1.2.2 Potentiometric biosensor

This type of biosensor is based on the use of ion selective electrodes and ion-sensitive field effect transistors. Possibly the primary out put signal is due to the ions accumulated at the ion-selective membrane interface. The presence of the monitored ion due to reaction at the electrode surface is indicated by change in some physical parameters like pH etc. For example, the enzyme glucose oxidase can be immobilized at the pH electrode surface. The compound glucose has minimal influence on pH in working medium but the formation of gluconate due to its interaction at the enzyme immobilized pH electrode the solution becomes acidic, which can be easily detected. In general a potentiometric biosensor can be represented as under Figure 3.

Some semiconductor based physico-chemical transducers are commonly used for the construction of biosensors. The ion selective field effect transistors (ISFET) and light addressable potentiometric sensors (LAPS) are convenient biosensor materials. The working principle of ISFET is based on the generation of potential by surface ions in a solution

(Yuging et al., 2003, 2005). The generated potential modulates the current flow across silicon semiconductors. A selective membrane fabricated from compounds viz. Si_3N_4 (silicon nitrite), Al_2O_3 (Alumina), ZrO_2 (Zirconium oxide), Ta_2O_5 (Tantalum oxide) is used to cover the transistor gate surface to enable pH measurement. However, the LAPS working are based on semiconductor activation by light emitting diode (LED). Both types of biosensors have proved their applicability for bioassay.

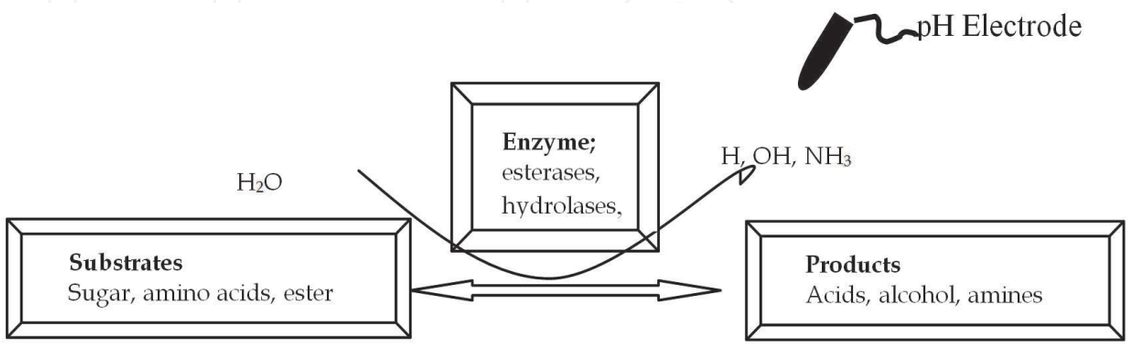


Fig. 3. Principle of working of a potentiometric sensor for the analysis of amino acids, sugars and esters. The enzymes are immobilized at the pH-electrode surface and the change in pH caused due to enzymatic conversion of the substrate is recorded, which is proportional to analyte concentration.

1.2.3 Impedimetric / conductometric biosensors

These types of biosensors measure either impedance or its components resistance/conductance and capacitance. These biosensors have been used for the assay of urea, using urease as biorecognition component. Though, the use of impedimetric biosensor is less frequent as compared to potentiometric and amperometric biosensors but their use in the study of hybridization of DNA fragments previously amplified by a polymerase chain reaction and also in monitoring the microorganism growth due to the production of conductive metabolites **(Silley et al., 1996)** and some other studies has produced promising results.

1.3 Electrochemical biosensors in biomedical analysis

With increasing demand for the development of low cost analytical techniques for selective and accurate analysis of drugs and other analytes and also for suggesting the mechanism of action of drugs, scientists working in the field of electroanalytical chemistry have designed electrochemical biosensors.

The developed biosensors have been successfully used for pharmaceutical analysis **(Gil et al., 2010)** and also for biomedical purpose. Since a large variety of biological systems can be used as recognizing agents, as such, it allows the fabrication of specific biosensors for a large variety of analytes and the electrochemical transducers impart high sensitivity to these devices **(Lojou et al., 2006).** The analyte-bio-recognizing agent interaction is monitored by the transducer and in the case of an electrochemical biosensor the signal detection occurs at the electrode-solution interface, which may be dynamic or static. In case of former methods i.e. voltammetry, amperometric biosensors are used, the interaction involves redox process followed by transfer of electrons. Whereas, in static methods, potentiometric biosensors are

used to monitor the concentration of charged species as a function of electrochemical potential **(Ravishanker, et al 2001)**. The correct choice of a biosensor for the study of a particular analyte depends on the selection of the recognizing agent and transducer, both suited to the target molecule.

Thus, the selection of the above biosensors depends on the characteristics of the analyte i.e. while using amperometric biosensors the organic/inorganic species should undergo a redox process at working potentials. Whereas, the static methods (using potentiometric biosensors) involve charged species. In pharmaceutical analysis, the commonly used recognizing agents are enzymes, antibodies, DNA, drug receptors etc. For the fabrication of electrochemical biosensors the main transducing elements in use are some noble metals viz. Pt and Au and also some carbon based electrode materials viz. glassy carbon, carbon paste. These electrodes, after proper modification are being widely used in the field of pharmaceutical and biomedical analysis.

In addition to the above materials the advancement made in the field of materials science and nanotechnology, has led to significant development of many electrochemical transducers like conducting polymers with suitable characteristic for electrochemical sensors, carbon nanotubes, nanomaterials with molecular dimensions (porous and monodispersive particles of clay with high superficial area etc).

Nevertheless, the efficient immobilization of recognizing agents for transducer is still a challenging task in electrochemical biosensor technology. Some immobilization techniques have been reported in the literature **(Nakamura et al., 2003).**

The field of research and development of biomedical and pharmaceutical analysis embraces comprehensive procedures in a bid to fulfill the requirements of the analysis i.e. accuracy, selectivity, precision, simplicity and low cost. Since, biosensors fulfill the above requirements, their possible use in the field of biomedical, pharmaceutical, food and environment etc analytes is widely being proposed.

1.4 Electrochemical biosensors in chemotherapy

Chemotherapy is an important weapon for the treatment of cancer. A large number of compounds have been developed as potential candidates for anticancer drugs, but only handful of them have become effective in clinical protocols. As such, the need to develop drugs which can effectively treat various forms of cancer is widely recognized. The development of new antineoplastic drugs (anticancer drugs) requires a clear cut understanding of the mechanism of action of the drug at the cellular and molecular levels. Cancer (neoplasias) are the diseases in which the growth of the cells, exceeds that of healthy tissue, suppressing the organic reserves surrounding the normal tissue. The potential targets for antineoplastic drugs are essentially the four nucleic acids, specific enzymes, microtubules and hormone/growth factor receptors. When the target of the drug are nucleic acids, the DNA damage causes cell death (cytotoxic and genotoxic drugs).

The antineoplastic drugs can bind DNA through several mechanisms. One of the mechanism suggests alkylation of nucleophilic sites with in the double helix. The clinically effective alkylating agents have two moieties capable of producing transient carbocations, which combine covalently to the electron rich sites of DNA like N7 position of guanine. The bifunctional alkylating agents produce cross linking of two strands of DNA which prevents the use of DNA as a template for further DNA and RNA synthesis, causing inhibition of replication and transcription leading to cell death. A large number of alkylating agents are

known which have shown antitumor activity. They include nitrogen mustards (mechlorethamine, cyclophosphamide etc), aziridines and epoxides (thiotepa, mitomycin C etc), alkyl sulphonates (busulfan and its analogues), triazenes, hydrazines and related compound etc.

Another mechanism of drug-DNA binding is intercalation i.e. the insertion of a planar (generally aromatics) ring molecule between two adjacent nucleotides of DNA. Many antitumor antibiotics work through this mechanism. The antibiotic molecule (doxorubicin, daunorubicin etc) is noncovalently but firmly bound to DNA, distorting the shape of the DNA double helix. Thus, inhibiting DNA replication and RNA transcription.

Bleomycins also cause DNA damage through intercalation. These glycopeptides intercalate between guanine, cytosine, DNA base pairs and the end of the peptide binds Fe (II), which is capable to catalyze molecular oxygen reduction to superoxide or hydroxyl radicals, which causes DNA strand scission due to oxidative stress.

Since, the antitumor effect of a drug depends upon its efficiency to interact with DNA. Therefore, the molecular recognition of nucleic acids by low molecular weight compounds is an area of fundamental interest. This very fact has encouraged scientists working in the field of pharmaceutical and biomedical analysis to design experiments using a proper physiochemical technique for the study of interaction of small molecules with DNA.

1.5 Electrochemical methods for biomedical analysis

Among the physiochemical techniques, some modern electrochemical methods have attracted interest of scientists working in the field of biomedical and pharmaceutical analysis. Due to the simplicity and reliability of electrochemical methods, they offer advantages over biological and chemical assays. Since a large number of organic molecules have a tendency to exhibit redox activity, the microelctroanalytical methods have a potential to provide a useful compliment to the previously listed methods of investigation in the field. The use of electrochemical techniques are mainly based on the differences in the redox behavior of organic molecules i.e. nucleic acid binding molecules in the absence and presence of the DNA. The change in the redox behavior of the molecule under study results in the change of formal potential of the redox couple and the decrease of the peak current, which results due to the dramatic change in the diffusion coefficient after the combination of the molecule with DNA. On the other hand, since, DNA is also electrochemically active **(Palacek, 1983)** the drug-DNA interaction can also be described by means of variation of redox behavior of (DNA) nucleobases, such as guanine and adenine in the presence of interacting molecule.

For fabricating an electrochemical biosensor a successful marriage between the transducer, generally a microelectrode (glassy carbon fiber electrode, carbon paste electrode, carbon nanotube electrode etc) and the biological element/chemical (drug) is the primary requirement. The interactions of target analyte with the receptors on transducer (electrode) surface produce characteristic (current/potential) signals. The sensitivity, selectivity, response time and stability of the electrochemical biosensor are the important parameters, which decide the practical utility of the developed biosensor for biomedical analysis and also in the study of in-vivo mechanism of action of the drug. It is a challenging task before the scientists working in the field to design comprehensive analytical procedures setting experiments that are parallel to the expected *in-vivo* interaction conditions.

Quite a good number of compounds have a tendency to interact with DNA, causing changes in its structure and base sequence, which results in disturbing the DNA cross linking

reaction. As such, in the field of medicinal science, the drug-DNA interaction can be highly useful for evaluating the damage caused to DNA by carcinogens and oxidizing substances **(Perry,1996, Blackburn,1996).** In recent years, the use of modified microfaradaic biosensors has proved to be highly significant for the study of interaction mechanism between substances of medicinal relevance **(Brett,1999)**. They also work as electrochemical biosensors **(Niu,2006)** as a simple and inexpensive technology for the diagnosis of genetic diseases and the detection of pathogenic biological species **(Erdem,2005, Rauf,2005)**. We know that some DNA intercalators **(Ozkan,2004, Karadeniz,2003, Pang,2000, Ju,2003, Girousi,2004)** which generally work as anticancer drugs are helpful in detection of the sequence specific hybridization of nucleic acids.

1.6 The anticancer drug-epirubicin

Epirubicin, an antibiotic drug of anthracyclines family, possesses a wide spectrum chemotherapeutic applications and antineoplasic action. Antitumor properties of Epirubicin are known for more than two decades but, the pharmacokinetic and biochemical studies to establish its in-vivo mechanism of action and to improve its administration and anticancer activity are still important goals to achieve. A survey of literature records that Epirubicin and other analogous anthracyclines behave as DNA intercalators and their activity accumulates in nuclear genome **(Ozkan ,2003, Martinez, 2005).**

Since the mid 1980's epirubicin has been extensively used for both early stage and metastatic breast cancer. Epirubicin is the 4'-epimer of the popular anthracyclin antitumor antibiotic, doxorubicin. A number of mechanisms have been suggested to explain the antineoplastic effect of epirubicin; First, it intercalates between DNA nucleotide base pairs, which results in the inhibition of DNA, RNA and protein synthesis. Second, the intercalation leads to topoisomerase II cleavage of DNA, resulting in cytocidal activity and the third mechanism suggests that epirubicin inhibits DNA helicase activity which finally interacts with DNA, RNA replication and transcription.

Due to reorientation of the hydroxyl group in the 4'-position of daunosamine (Figure 4) ring equatorial for epirubicin and axial for doxorubicin, epirubicin possesses several different pharmacological properties as compared to doxorubicin. Its pKa value is lower than that of doxorubicin. Consequently, it is more lipophilic and better able to penetrate cells. Besides, the glucuronidation of epirubicin and epirubicinol to inactivate metabolites results in a shorter terminal half life for epirubicin as compared to doxorubicin.

The below structural difference between epirubicin and doxorubicin is responsible for different safety profiles for the two antineoplastic drugs. Larger doses of epirubicin are required to produce the same degree of toxicity as doxorubicin. The doze ratios for similar toxicities for doxorubicin: epirubicin are 1:1.8 for cardiac, 1:1.5 for nonhematologic and 1:1.2 for hematologic. As such, the superior safety profile of epirubicin compared with that of doxorubicin allows for greater doze escalation that can be achieved safely which clearly means that epirubicin has a greater therapeutic window.

Chemically modified electrodes (CME) as voltammetric biosensors **(Shrivastava,2004)** accumulate analytes selectively and protect them from interference from other ions/electrochemical species, have played a leading role in the analysis of biosynthetic polynucleotides containing adenine/ guanine residues, using species sensitive DPV method. **(Palacek,1983).** As such, looking at the importance of the use of epirubicin, as a anticancer and the usefulness of electrochemical biosensors we have fabricated DNA modified and epirubicin adsorbed GCFE biosensors and studied the interaction of epirubicin in situ with

ds-DNA at its surface. Since, the literature also reports that the in-vivo interaction of the anticancer drug epirubicin with DNA takes place at charged phospholipid membranes and proteins, the studied interaction would be parallel to the in-vivo DNA- epirubicin complex situation, where DNA is in close contact with charged phospholipid membranes and proteins. A suitable mechanism to the above reaction has been proposed.

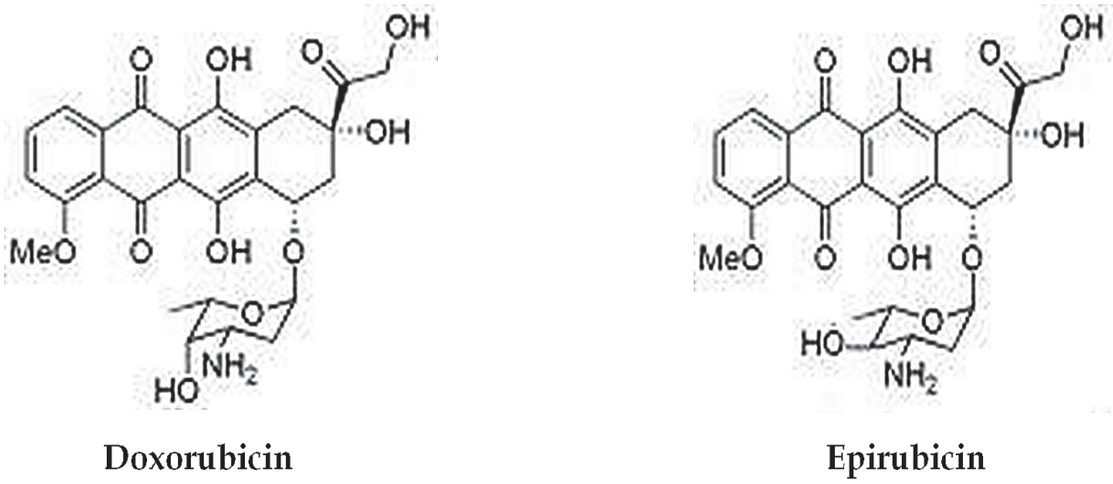


Fig. 4. Chemical structures of doxorubicin and doxorubicin

2. Experimental

2.1 Chemicals and instrumental

Deionised water was used to prepare the solutions of Calf thymus DNA (Himedia ltd. Mumbai) and epirubicin hydrochloride (gift sample supplied by M/S. Dabur Pharma. Ltd. Baddi distt. Solan H.P.).

An Elico (Hyderabad, India) μp-polarographic analyser model CL-362 was used for all voltammetric studies. Glassy carbon fibers (NF-12, Sigity Elititigoitit, U.K.) were used for the fabrication of glassy carbon fiber electrode (GCFE) as working electrode, a saturated calomel electrode and a coiled platinum wire electrode was used as reference and counter electrode, respectively. The pH-measurements were made on a systronic (India) μ-pH system-361. All experiments were performed at room temperature and dissolved oxygen was removed by passing pure nitrogen through the solutions.

For all the DPV (differential pulse voltammetry) experiments the instrumental parameters were used as follows: scan rate 12mV/s, sensitivity 10μA, and pulse amplitude 50mV.

2.2 Fabrication of modified electrodes

2.2.1 Epirubicin adsorbed GCFE

Epirubicin adsorbed GCFE was fabricated by dipping the electrode in a voltammetric cell containing solution of the drug (80μg/ml) for ten min., at a deposition potential of +0.40V. The electrode was taken out of solution, thoroughly rinsed with distilled water and then used for voltammetric measurements. To study the drug-DNA interaction the modified electrode was dipped in a voltammetric cell containing 80μg/ml DNA and 0.1M acetate buffer at pH 4.5±0.1, and the DP Voltammogram was recorded.

2.2.2 Thin layer ds-DNA modified GCFE

For the fabrication of thin layer ds-DNA modified GCFE, the electrode was immersed in 80µg/ml ds-DNA solution at +0.40V applied potential for ten min. This ds-DNA modified GCFE was washed with distilled water and then dipped in epirubicin solution for three min. It was then rinsed with water and transferred to a voltammetric cell containing acetate buffer solution at pH 4.5±0.1 and the differential pulse voltammogram was recorded.

2.2.3 Thick layer ds-DNA modified GCFE

For the fabrication of thick layer ds-DNA modified GCFE, the electrode was dipped in 25mg/ml solution of ds-DNA for ten min. It was taken out of solution and allowed to dry. The electrode was then dipped in (20µg/ml) solution of epirubicin for varying time intervals. Each time the electrode was taken out of the solution, washed with distilled water and dried. The dried electrode was dipped in a solution of acetate buffer (0.1M) at pH 4.5±0.1 and the voltammogram was recorded.

3. Results and discussion

3.1 DPV analysis of epirubicin at bare GCFE

Epirubicin produces two reduction peaks (Table 1) at Ep value -0.46V and -0.62V (Figure 5) due to the reduction of its 5,12-diquinone groups to produce a highly reactive semiquinone radical were observed. However, if the solution is electrolyzed performing positive potential scanning of the working electrode, the resulting differential pulse voltammogram produced a well defined peak at +0.54V (Figure 6), due to the oxidation of 6,11-dihydroquinone of epirubicin. On recording the DP Voltammogram (oxidation peak) for varying concentration of epirubicin (from 5µg/mL to 120µg/mL) under above experiment conditions, a linear relationship between the peak height of the voltammogram and epirubicin concentration was observed. Thus, enabling the quantitative determination of epirubicin. The minimum detection limit was found to be 5µg/mL of the analyte, with good accuracy and precision of determination.

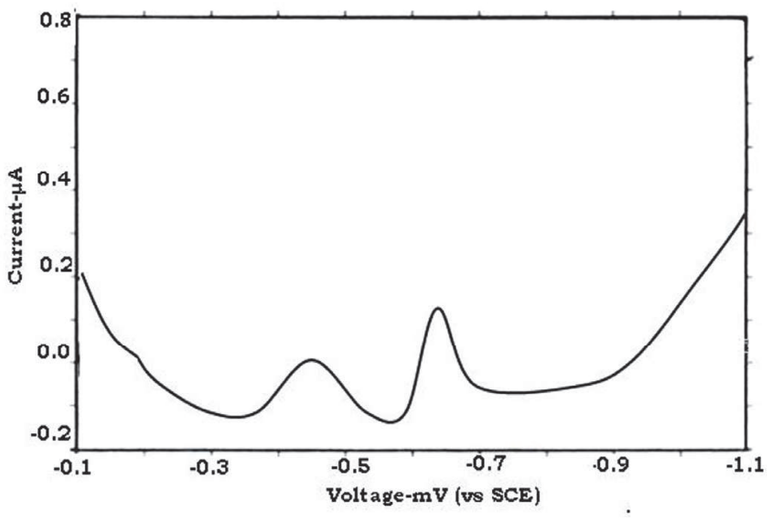


Fig. 5. Differential Pulse Voltammogram for 80µg/ml epirubicin (reduction) in 0.1M acetate buffer at pH 4.5±0.1 at bare GCFE.

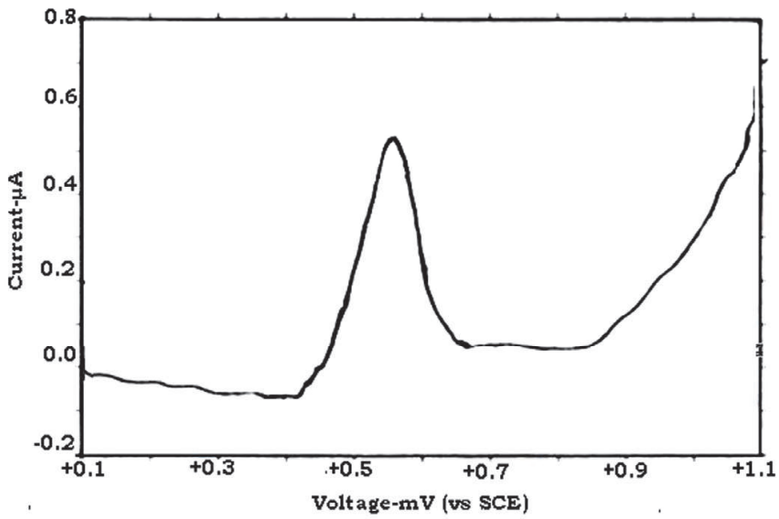


Fig. 6. Differential Pulse Voltammogram for 80µg/ml epirubicin (oxidation) in 0.1M acetate buffer at pH 4.5±0.1 at bare GCFE.

S. No	Drug/DNA Solutions	Working Electrode	Voltammetric Experimental Sequence	Ep V vs SCE I II III
1	80µg/ml Epirubicin	Bare GCFE	Cathodic potential scanning (reduction)	-0.46 -0.62 --
2	80µg/ml Epirubicin	Bare GCFE	Anodic potential scanning (oxidation)	+0.54 -- --
3	80µg/ml ds-DNA	Epirubicin modified GCFE	Applying a potential -0.60V for 60 s, followed by anodic potential scanning	+0.45
4	20µg/ml Epirubicin	Thin layer ds-DNA modified GCFE	Electrode immersed in 20µg/ml Epirubicin for 180 s followed by anodic scanning	+0.54
5	20µg/ml Epirubicin	Thin layer ds-DNA modified GCFE	Applying a potential of -0.60V for 60 s, followed by anodic scanning	+0.40 +0.54 +0.90
6	20µg/ml Epirubicin	Thick layer ds-DNA modified GCFE	Scanning the potential of the working electrode from -0.70V to -0.00V	-0.45 -0.60
7	20µg/ml Epirubicin	Thick layer ds-DNA modified GCFE	Electrode immersed in Epirubicin solution for 300 s, then applied a potential of -0.60V for 60 s, followed by anodic scanning	+0.54 +0.80 +1.1

Table 1. Summary of Voltammetric Experimental Conditions and Observed Data (in 0.1 acetate buffer at pH 4.5, Scan rate 12mV/S, Pulse amplitude- 50mV)

3.2 DPV analysis of epirubicin-DNA interaction at bare GCFE

The first set i.e. without DNA, produced a DPV oxidation peak for epirubicin at +0.54V, which shifted to more electro-positive potential with increasing DNA concentration and the peak current shortened. The shift in Ep value and shortening of peak current may be explained on the basis of change of species that is oxidized at the GCFE surface, i.e. due to the formation of drug-DNA complex.

Although, the above experimental results confirm the formation of Epirubicin-DNA complex, but, to have a clear-cut understanding on the mechanism of the drug-DNA interaction at charged surfaces, the GCFE has been modified in three different ways:

3.3 Epirubicin-DNA interaction at epirubicin adsorbed GCFE

It showed a big peak at +0.54V due to oxidation of adsorbed epirubicin and the other peaks may be due to oxidation of purine bases of DNA. This explanation of the observed voltammogram is based on the presumption that DNA diffuses from bulk of the solution to electrode surface and the chemisorbed epirubicin is intercalated into its double helix. As such, the distortion of double strand takes place, which allows the oxidation of purine bases. However, after the first scan if a potential of -0.60V was applied for 60 s, and then the voltammogram was recorded, it produced a peak at +0.45V (Figure 7). The appearance of this peak is due to the interaction of epirubicin with ds-DNA through guanine rich region.

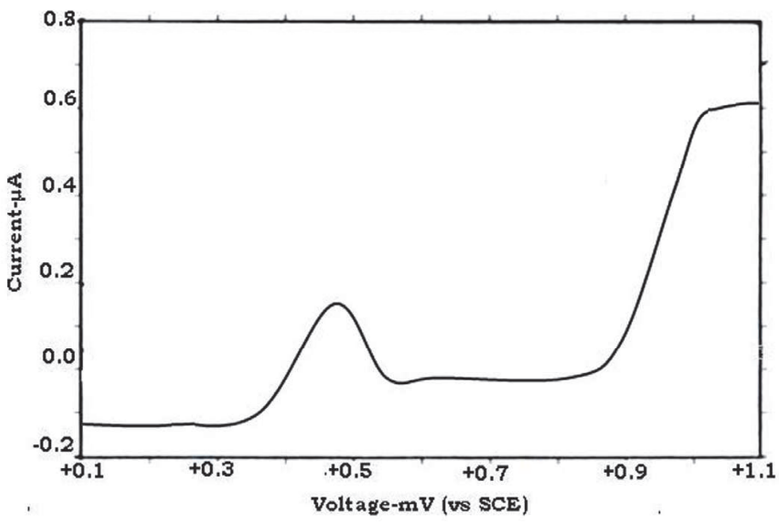


Fig. 7. Differential Pulse Voltammogram for 80µg/ml ds-DNA solution in 0.1M acetate buffer at pH 4.5±0.1, after applying a potential of -0.60V during 60 s, at epirubicin modified GCFE.

3.4 Epirubicin-DNA interaction at thin layer ds-DNA modified GCFE

The DPV for the oxidation of epirubicin, showed a well defined peak with peak potential +0.54V. The peak may be attributed to the oxidation of 6,11-dihydroquinone group of epirubicin molecule.

However, after recording the oxidation peak, a negative potential of -0.60V was applied on the modified electrode for 60 s, followed by recording of DP Voltammogram with positive potential scanning of the working electrode. The resulting voltammogarm showed two new peaks in addition to the epirubicin oxidation peak. The peak at +0.90V (Figure 8) may be

attributed as due to 8-oxo-Guanine (8-oxo-G) oxidation and that at +0.40V may be due to the oxidation of purine bases of DNA. A clear separation of the peak due to 8-oxo-G and epirubicin can be explained on the basis of non-uniform coverage of the GCFE surface by DNA and adsorption of epirubicin at these uncovered surfaces. [The results are in good agreement with those observed using thick layer DNA modified GCFE]. This shift of 8-oxo-G peak to less positive potential informs about the DNA-epirubicin interaction (damage to DNA).

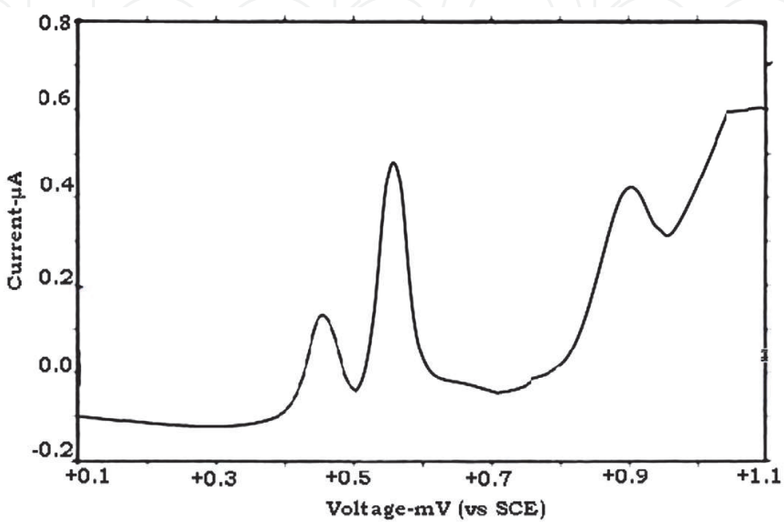


Fig. 8. Differential Pulse Voltammogram in 0.1M acetate buffer at pH 4.5±0.1, obtained with a thin layer ds-DNA modified GCFE after being immersed in 20µg/ml epirubicin solution during 180 s, after applying a potential -0.60V during 60 s.

3.5 Epirubicin-DNA interaction at thick layer ds-DNA modified GCFE

Epirubicin produced a well-defined voltammetric oxidation peak with Ep value +0.54V. The height of the epirubicin oxidation peak with respect to the time of immersion of the thick layer ds-DNA modified GCFE in epirubicin solution was investigated. The results showed a linear relationship between the peak height and time of immersion of the electrode in epirubicin solution i.e.0.00 to 60 min, and then it attained a constant value. Thus, indicating the preconcentration of epirubicin at the thick layer ds-DNA modified electrode surface.

It is important to note that reproducible peak currents were observed for the similar time of immersion of the thick layer ds-DNA modified GCFE in epirubicin solution for the first scan only. However, if the differential pulse voltammogram is recorded using the same modified electrode, an abrupt decrease in the peak current was observed. This suggests a fast consumption of the neoplasic drug at the modified electrode surface.

However, on performing the above voltammetric experiments separately using bare GCFE and thick layer ds-DNA modified GCFE as working electrode and scanning the potential from -0.70V to -0.00V, the resulting DPV curve with bare GCFE produced only one peak at -0.56V. Whereas, using thick layer ds-DNA modified GCFE two peaks were observed at -0.60V and -0.45V, respectively. The observed new peak at -0.45V speaks of a different interaction mechanism of epirubicin-DNA, at the modified GCFE surface.

Since, epirubicin is irreversibly adsorbed at the bare GCFE surface, it becomes necessary to clean the electrode each time before use. Whereas, the thick layer ds-DNA modified GCFE

did not require cleaning. This clearly reveals that the epirubicin is intercalated inside ds-DNA film and could not reach the electrode surface. On the basis of above observations it could be concluded that the voltammetric peaks are observed due to epirubicin which is intercalated into thick layer of ds-DNA. Since, the voltammograms were recorded in acetate buffer supporting electrolyte solution only, the possibility of any contribution to the voltammetric peaks from epirubicin present in solution is ruled out. As such, the observed new peak at -0.45V may be attributed to the epirubicin-guanine site (in DNA) interaction leading to a charge transfer reaction to from epirubicin semiquinone and guanine radical cation. However, the peak at -0.60V may be attributed to the reduction of the epirubicin. As mentioned earlier, epirubicin at bare GCFE produces a peak at -0.56V, the shift in the peak potential for epirubicin reduction at the two different electrode surfaces may be explained due to the change in the electrode surfaces.

However, if the ds-DNA modified GCFE after being dipped in epirubicin for 300s, rinsed and immersed in a buffer solution at pH 4.5±0.1, was subjected to a potential of -0.60V for about 60s and then the voltammogram was recorded by positive potential scanning of the modified electrode, the resulting voltammogram produced two new peaks, one at +0.80V and other at +1.1V (Figure 9). The former peak may be attributed to guanine oxidation and the later due to adenine oxidation.

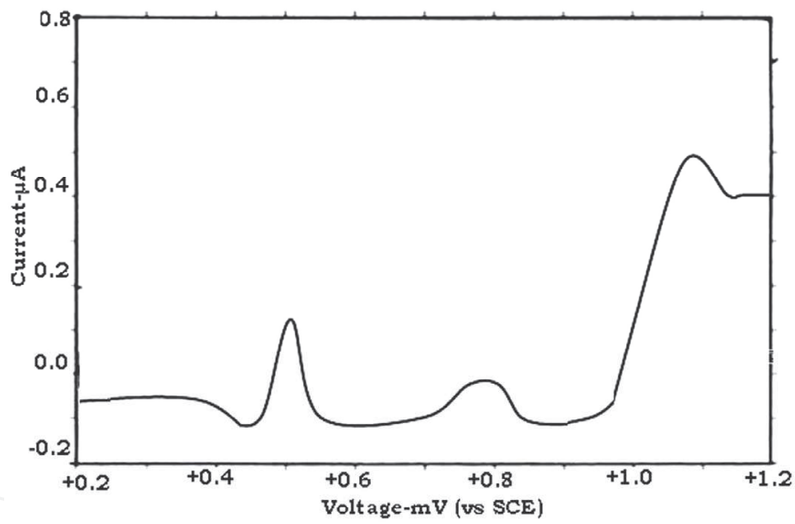
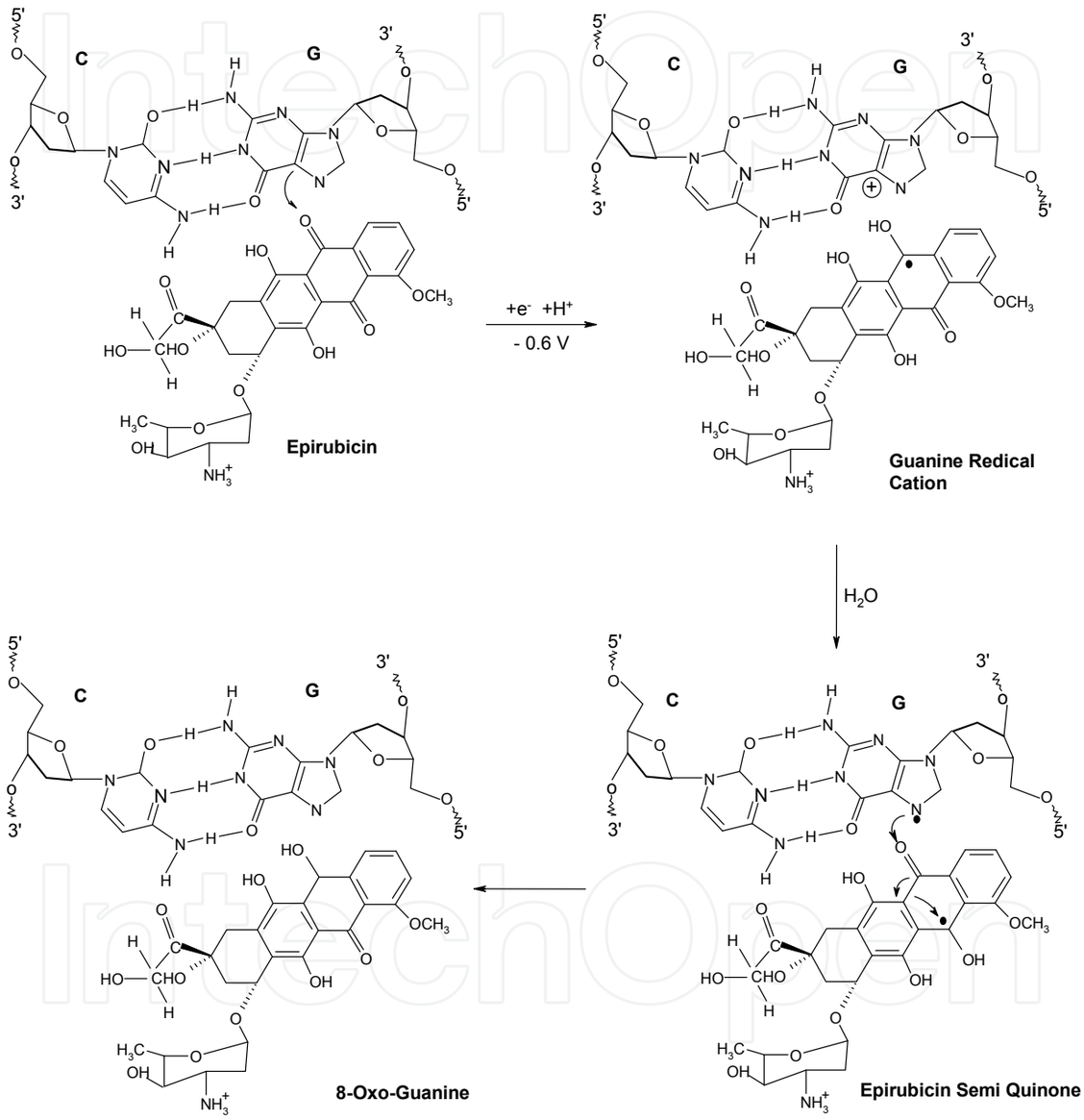


Fig. 9. Differential Pulse Voltammogram in 0.1M acetate buffer at pH4.5±0.1 obtained with a thick later ds-DNA modified GCFE after being immersed in 20µg/ml epirubicin solution for 60 s at potential -0.60V.

4. Mechanism

Epirubicin transfers an electron to its quinone portion **(Perry, 1996)** to generate a free radical. The highly reactive free radical formed at -0.60V may oxidize the guanine site of ds-DNA in which it is intercalated within the double helix, forming drug-DNA complex. Besides, the study on drug-DNA interaction at bare GCFE showed that the peak at +0.54V as observed in case of pure epirubicin oxidation, at bare GCFE shifts to less positive side i.e.+0.45V, on its complexation with ds-DNA, which may be explained as due to interaction between epirubicin and 8-oxo-G which is formed as a result of interaction of epirubicin with

guanine rich region of ds-DNA. As such, one electron transfer from guanine moiety to quinone leading to guanine cation formation appears to be the probable reaction. However, due to the tendency of guanine cation to undergo hydrolysis, finally the semiquinone is further reduced to form epirubicin and 8-oxo-G.
Mechanism model



Mechanism model : Mechanism of electrochemical epirubicin oxidative damage to DNA

5. Conclusion

Voltammetric in-situ sensing of DNA oxidative damage caused by reduced epirubicin intercalated into DNA is possible using ds-DNA modified GCFE microfaradaic biosensor. The results show that epirubicin intercalated in double helix of DNA can undergo oxidation

or reduction and react specifically with the guanine moiety and thus forms mutagenic 8-oxo-G residue. A mechanism model for the reaction may be proposed. The fabricated microfaradaic biosensors are of utmost relevance because the mechanism of interaction of DNA-epirubicin at charged interfaces is parallel to in-vivo DNA-drug complex reaction, where DNA is in close contact with charged phospholipid membranes and proteins rather then when intercalation is in solution. It also promises the use of voltammetric techniques for in situ generation of reaction intermediates. As such, is a complementary tool for the study of biomolecular interaction mechanism of medicinal relevance.

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A biosensor is a detecting device that combines a transducer with a biologically sensitive and selective component. Biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. This book covers a wide range of aspects and issues related to biosensor technology, bringing together researchers from 16 different countries. The book consists of 24 chapters written by 76 authors and divided in three sections: Biosensors Technology and Materials, Biosensors for Health and Biosensors for Environment and Biosecurity.

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