History of Science has shown that real fundamental progress is always due to those who had ignored the boundaries of science and who had treated science as a whole.\(^1\) Certainly we want science to be used for the betterment of human being and of humanity. Research in the field of pure science is important because the search for truth is always important. Nevertheless, we want to apply it for the betterment of human beings. That is not only justified, but is right.\(^2\)

Over the past quarter of the century along with enrichment of basic science, the science of biology which is no doubt a part of human life and existence, biochemistry, biotechnology and some other branches of science have generated a remarkable symbiosis between scientists, technologist and industrial entrepreneurs, with exciting possibilities. In no other area of human endeavor dose one see such a close connectivity between absolutely basic curiosity driven research at the frontier of human knowledge on one end and application in biofields and production of commercial value on the other.

In the present modern age, a particular field of science dose not purely represents its old traditional form, but it represents an interdisciplinary field increasingly interacting and integrating with chemistry, physics, material sciences, biosciences etc.
However, it was really with the advent of modern science a few hundred years ago and availability of powerful techniques to sciences from chemistry, physics, engineering science and biosciences etc, that the headlong progress towards a basic understanding of living forms and the in-vivo and in-vitro interaction of different physiological, biomedical and other reactions responsible for drug action, became a reality. The invention of microscope and discovery of bacteria by Leeuwenhoek in the 17th century and then the work of Pasteur in the 19th century which laid foundation of microbiology and immunology were landmark events. The last century witnessed revolutionary improvements in the field of analytical chemistry by way of discoveries in the field of techniques of analysis like spectroscopy (UV, IR XRD, Elecromicroscopy, AAS etc),(3-8) and the development in the field of modern electroanalytical chemistry etc. The new tool of recombinant DNA technology and genetic engineering helped the accelerated growth of a clear-cut understanding of mechanism of action of different biomolecules and drugs in different biological [in-vivo/in-vitro] processes.

Such an analysis, would not merely deal with their expression but would unravel the basis of their interrelationship, orchestrated coordination, and strategy for combination, which has led to diverse genotypes against rigors of natural selection, through more than billion years. Possibly that unraveling would be the greatest challenge in the near future.
Similarly other biomolecules like enzymes play a key role in different biochemical reactions. The mechanism of enzyme action has always been elusive and still remains incomprehensive in many aspects. The enzyme substrate (ES) complex formation during the reaction involves modification of enzyme structure, so as to suit catalysis of the substrate. Enzymes are considered to be the catalytic machinery of living systems. They are remarkably effective catalyst, responsible for thousands of coordinated chemical reactions involved in biological process of living systems.\(^9\)

Enzymes are usually intracellular in nature but there are certain enzymes which are extracellular. The major part of the enzymes is produced by GRAS (Generally Recognized AS Safe) status microorganisms given by food and drug administration (FDA) as industrial enzymes. Only a limited number of enzymes are commercially available. Presently the enzymes are commonly being used in many industrial applications. As such there is a great demand for stable, highly active and specific enzymes.

In the recent past, the field of organic electrochemistry has been enriched by analytical scientists by way of improvements made in the field of electro-analytical methods like differential pulse polarography/voltammetry (DPP/DPV) and stripping voltammetry (DPASV/DPCSV) for their possible use in the analysis of different types of organic compounds in sample of different origin in general and in samples of biological relevance in particular.
So much so, that looking at the usefulness of electrochemical methods the scientist working in the field of the analysis of biomolecules has recognized it as a field of *Bioelectrochemistry*.\(^{10}\) The said electrochemical methods have also been found useful for the qualitative as well as quantitative analysis of different organic compounds.

We know that the drug action mechanism of a particular drug plays a key role in understanding and increasing the potency of the drug by way of modifying the drug i.e. molecular designing. Since almost all the drugs are organic compounds, their *in-vivo* or *in-vitro* interaction mechanism can be understood using the said electrochemical methods. The electrochemical analysis of organic compounds has been named broadly as *Organic Electrochemistry*.\(^{11}\)

Looking at the need of the hour, scientist’s working in the field, have made successful attempts to improve upon the detection limits of the electrochemical methods and have developed comprehensive procedures for the analysis in biofluids, with minimum possibility of matrix effect.

**ENZYMES**\(^{12}\)

The living cell is the site of tremendous biochemical activity called metabolism. This is the process of chemical and physical change which goes on continuously in the living organism i.e. the build-up of new tissue, replacement of old tissue, conversion of
food to energy, disposal of waste materials, reproduction - all the activities that we characterize as "life".\(^{13}\)

This building up and tearing down takes place in the face of an apparent paradox. The greatest majority of these biochemical reactions do not take place spontaneously. The phenomenon of catalysis makes possible biochemical reactions necessary for all life processes. Catalysis is defined "as the acceleration of a chemical reaction by some substance which itself undergoes no permanent chemical change".\(^{14}\) The catalysts of biochemical reactions are enzymes and are responsible for bringing about almost all of the chemical reactions in living organisms. Without enzymes, these reactions take place at a rate far too slow for the pace of metabolism.

Enzymes are biological catalysts responsible for supporting almost all of the chemical reactions that maintain animal homeostasis. Because of their role in maintaining life processes, the assay and pharmacological regulation of enzymes have become key elements in clinical diagnosis and therapeutics. Enzymes are found in all tissues and fluids of the body. Intracellular enzymes catalyze the reactions of metabolic pathways. Plasma membrane enzymes regulate catalysis within cells in response to extracellular signals, and enzymes of the circulatory system are responsible for regulating the clotting of blood. Almost every significant life process is dependent on enzyme.
In cells and organisms most reactions are catalyzed by enzymes\(^{15}\), which are regenerated during the course of a reaction. These biological catalysts are physiologically important because they speed up the rates of reactions that would otherwise be too slow to support life. Enzymes increase reaction rates sometimes by as much as one million fold, but more typically by about one thousand fold. Catalysts speed up the forward and reverse reactions proportionately so that, although the magnitude of the rate constants of the forward and reverse reactions are increased, the ratio of the rate constants remains the same in the presence or absence of enzyme. Since the equilibrium constant is equal to a ratio of rate constants, it is apparent that enzymes and other catalysts have no effect on the equilibrium constant of the reactions they catalyze.

Enzymes increase reaction rates by decreasing the amount of energy required to form a complex of reactants that is competent to produce reaction products. This complex is known as the activated state or transition state complex for the reaction. Enzymes and other catalysts accelerate reactions by lowering the energy of the transition state. The free energy required to form an activated complex is much lower in the catalyzed reaction. The amount of energy required to achieve the transition state is lowered; consequently, at any instant a greater proportion of the molecules in the population can achieve the transition state. The result is that the reaction rate is increased.
The use of enzymes in the diagnosis of disease is one of the important benefits derived from the intensive research in biochemistry since 1940's.\textsuperscript{16} Enzymes have provided the basis for the field of clinical chemistry. It is, however, only within the recent past few decades that interest in diagnostic enzymology has multiplied. Many methods currently on record in the literature are not in wide use, and there are still large areas of medical research in which the diagnostic potential of enzyme reactions has not been explored at all.\textsuperscript{17}

**GENETIC MATERIALS\textsuperscript{18}**

An important milestone in the history of life is genetics. **Genetics is the science of inheritance or hereditary**, which is the process by which all living things produce offspring like themselves, and it is concerned with the physical and chemical properties of the hereditary material. It is a wide ranging science that explores that transmission of biological properties from parent to offspring, the expression and variation of these biological properties, the structure and function of the genetic material i.e. DNA.

**Deoxyribonucleic acid (DNA) is the master computer of cells**, which contains a special coded genetic program with detailed instructions for biological function. DNA is also the hereditary material passed on to future generation and it ultimately accounts for the great diversity in the biological world.\textsuperscript{19-20}
The genetic information required for the function and multiplication of the biological organism is stored, duplicated and transmitted by means of nucleic acids. There are two types of nucleic acids occurring in nature: Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA). DNA is found in all biological systems, with the exception of certain viruses, it is genetically active component of chromosomes and function as the basic carrier of genetic information. RNA usually function as a secondary carrier of genetic information, serving as an intermediate in the process where the information (originally contained in DNA) is translated into a specific amino acids sequence of a polypeptide chain. DNA is a very long, thread like macromolecule comprised of a large number of fundamental monomeric units- deoxyribonucleotides. DNA structure plays a very important role in virtually all biologically processes in which DNA takes part.

BIOSENSOR

In the last quarter of the past century a great deal of attention has been given to the modification of the electrodes.

Many interesting and important phenomena such as chemical transformation, energy conversion, catalysis and molecular recognition occur at the electrode surface or electrolyte interfaces, but these phenomena have a small area in the field of electrochemistry. Hence the design of the electrochemical interfaces constitutes the central theme in electrochemical...
science and technology and is a fascinating area of research, by way of modification of the electrode in the form of Sensor.

There are many electrodes like DME, platinum electrode, gold electrode, graphite electrode, glassy carbon electrode etc, which are used for the direct or indirect determination of various active components in the different natural origin and biological samples, but they are not specific for a particular electroactive species and they work as an ordinary tool for the analysis of electroactive components. But, when a film of electroactive species is deposited at the electrode surface or electrode surface is coated with the electroactive species, then it may become specific for that particular electroactive species and this type of electrode is called as a modified electrode. These electrodes are more sensitive for the particular electroactive component when other materials are presents in the sample.

These modified electrodes or sensors offer sensitivity, selectivity, fast response time and allow ease of preparation. However these modified electrodes are very useful in lowering the over potential, increasing the rate of electro-chemical reactions. These modified electrodes also offer the possibility of adjustable physical and chemical properties of the electrode surface. In the present work modified GCPE (Glassy Carbon Fiber Electrodes) were used as a biosensor. Biosensor is a device that can sense to biospecies and can also study the biochemical reactions.\textsuperscript{21-25}
In the present study GCFE was used because of its high electrical conductivity, impermeability to gases, high chemical resistance and reasonable mechanism. Biosensors have many potential advantages for the study of bioactive, genetic and other samples which are involved in the *in-vivo* biochemical reactions and possessing unique capabilities for species investigation and measurement of the chemically labile free fraction.

This carbon based material has received considerable attention in electro-analytical chemistry. It is generally inert and offers a large aerating potential window in both aqueous and nonaqueous media. The electrode is also useful in the determination of electro inactive molecules by the way of pre-concentration of the component, promotion of electron transfer across the electrode and electrolyte inheritance.

Almost all the people living on this planet have heard of cancer, yet only a few understand its causes, symptoms and varied forms. Cancer occurs in all climates, places and races. Though its' distribution is not uniform but the difference in environmental factors, food habits and life styles explains the difference in its manifestation in different places.

**CANCER**

Cancer is a disorder of cells growth.\(^{26}\) Like normal body cells, cancer cells also grow, divide and group together. However,
normal cells get organized to form organs and tissues, which are useful for the body system. Whereas, cancer cells are abnormal cells which do not cause until they have multiplied enough to form a sizable mass. An aggregate of these abnormal cells is called Neoplasm (new growth of abnormal tissue or tumor).

Two types of tumors are found in our body, they are benign and malignant. The benign tumors are collection of healthy cells which multiply faster then normal cells to give enlarged masses of tissues which may become harmful if they grow too large. Benign tumors remain localized and do not spread to neighboring tissues or other parts of the body. On the other hand malignant tumors are the aggregation of abnormal cells, whose growth is faster as compared to cell in benign tumors. These tumors do not remains localised cells.

Anticancer drugs either kill cancer or modify their growth, as such disturbing the DNA cross linking reaction. Therefore the study of drug-DNA interaction becomes very important.\textsuperscript{27-30}

Whereas, Antibiotic drugs are one of a group of organic compounds, varying in structure that are produced by microorganism and can kill or inhibit activities of other microorganisms. One of the best-known examples is PENICILLINE, which was discovered by Sir Alexander Fleming. Another example is STREPTOMYCIN. Antibiotics are widely used in medicine to combat bacterial infections. Over use can lead to
microorganisms becoming resistant to a particular antibiotic. Antibiotic can also be produced synthetically.\textsuperscript{[31-33]}

**ELECTROCHEMICAL METHODS**

Analytical chemistry serves as a stepping stone for other branches of chemistry and has been playing a key role in the field of research and development. The significance of analytical chemistry in connection of scientific, technological and industrial areas can be easily understood when one considers its impact on biochemical and pharmaceutical research, clinical analysis research in the field of solid state, electronics, environmental science, space and in the analysis of the samples of natural origin. As such, analytical chemistry is an important player in the advancement of scientific and industrial community.

Analysts have paid special attention on electroanalytic techniques because of their ease in application, cost factor, simplicity, high accuracy, selectivity, sensitivity and reproducibility of results.

Electroanalytical chemistry encompasses a group of qualitative and quantitative analytical techniques,\textsuperscript{[34-35]} which are based upon the electrical properties of solution of the analyte when it is made part of an electrochemical cell.\textsuperscript{[37-39]} The electroanalytical methods (Polarographic/Voltammetric methods of analysis) find a relatively significant role,\textsuperscript{[40]} due to their good
precision, instrumental simplicity and accuracy in determining the electro-active substance.

Electrochemistry is the relationship between electrical properties and chemical reaction. The electrical properties most commonly measured involve voltage, current or resistance or combination of these.

The rapid advances in electroanalytical techniques has made it possible to use DCP, DPP, NPP, DPASV, & DPCSV for the analysis of trace contents of samples with great accuracy and precision of determination down to nanogram levels in general and picogram level in optimum cases.\(^{41-44}\) The major advantage of these techniques is that several electro active species can be determined in a sample, simultaneously in one run. Therefore, electroanalytical techniques are basically oligo substance detection methods.

**Polarography/Voltammetry**

L. Meites has defined polarography as “A branch of electroanalytical chemistry, which deals with the measurements and interpretation of current-voltage relationship, during the electrolysis of a substance between two electrodes, one of which is very small (micro).\(^{45}\)

Polarography was discovered in 1922 by the Czechoslovakian chemist Jaroslave Heyrovsky, who was awarded the Noble Prize in chemistry in 1959. In polarography the value
of the current, against applied voltage is measured with the help of an instrument, called polarograph and the curves obtained with it as, polarogram.

Recent years have witnessed the development of numerous modification of the original polarographic method as well as development of several methods closely related to polarography. In the polarographic method and its modified modes, dropping mercury electrode (DME) is used as working electrode. If working electrode, other than DME is used, the technique is named as Voltammetry.

A variety of electrodes are used in voltammetry to study different systems in aqueous and non-aqueous media. The voltammograms recorded under varying conditions of temperature, concentration, pH etc, for a given electroactive species, are then interpreted to obtain valuable data for qualitative and quantitative analysis, study of electrochemical behaviour, understanding of reaction mechanism, identification of electroactive groups and investigation of complex formation etc.

Polarographic/Voltammetric experiment consists of three electrode system.\textsuperscript{[46-49]}

\begin{itemize}
  \item Working Electrode or Indicator electrode/Polarizable electrode (DME).
  \item Reference Electrode also called unpolarizable electrode or Saturated calomel electrode.
\end{itemize}
Auxiliary Electrode.

In this technique the electrical current or voltage is varied in a regular manner between two sets of electrodes (indicator and reference) while the current is monitored.

**Working Electrode:**

It consists of liquid mercury (Hg) flowing through a narrow bare capillary tube and is called dropping mercury electrode (DME). This is the electrode where redox reaction or electron transfer of interest is taking place. The oxidation or reduction of substances at the surface of mercury drop working electrode at the appropriate applied potential results in the mass transport of new material to the electrode surface resulting in the generation of current.

The capillary characteristics of (using an open current and 0.1M KCl) were as follows: mercury flow rate (m)-0.6113 mg/sec., mercury column height- 78.5cm, natural drop time- 2 sec.

**Reference Electrode:**

It is an electrode which has stable and well known electrode potential and is crucial for the precise control of the potential of the working electrode.

The reference electrode should provide a reversible half reaction with Nerstian behaviour, be constant over time and be
easy to assemble and maintain. The reference electrode used here is a calomel electrode.

The equilibrium electrode potential is a function of chloride concentration of internal electrolyte (filling solution). The electrolyte in this technique is saturated KCl solution, hence this electrode is called as saturated electrode.

**Counter or Auxiliary Electrode:**

The use of the electrode is to carry the bulk of current and counter the process that occurs at the working electrode, and make it free from any disturbance except to maintain the redox reaction. Hence, this electrode is called as counter electrode. Though this electrode is not important analytically, it is important not to allow the product of the reaction that occurs here to interfere with the process that occurs at the working electrode. For this reason it is often placed in a separate chamber with a fitted glass disc disallowing of the undesirable species to go to the working electrode.

**Theory and Principle:**

The polarographic methods are highly useful for qualitative and quantitative analyses of a substance, if the substance under study is capable of undergoing cathodic reduction or anodic oxidation at the working surface.

This method depends on the concentration polarization of the dropping mercury electrode (DME). A metal ion in solution
will not be deposited on the cathode as a metal and no current will flow until the potential between the solution and the cathode reaches a certain critical value. As the potential increases above this value, the rate of reduction and the flow of current increases until a thin layer of solution round the cathode has been depleted of the ionic species is proportional to the difference between the two concentrations and is given by Fick's law.\(^{50-51}\)

\[
\frac{ds}{dt} = \frac{AD}{\delta}[C - C_0]
\]

Where \(A\) is exposed area of a electrode surface, \(D\) is diffusion coefficient of the ions, \(\delta\) is thickness of the hypothetical diffusion layer around the electrode, \(C\) is the ions concentration at the electrode surface and \(C_0\) is the bulk concentration.

The flux of oxidation or reduction at the electrode surface controls the rate of reaction and thus the faradaic current flowing in the cell. The actual value of current is effected by many additional factors, most importantly the concentration of the redox species, the size, shape and material of electrode, the solution resistance, the cell volume and the number of electrons transferred.

Some important terms employed in polarography are as follows:

- Migration current
- Residual Current
- Diffusion Current
Limiting Current
Kinetic and Catalytic Current
Half Wave Potential

**Direct Current Polarography (DCP):**

This classical technique consists of applying a slow linearly increasing potential sweep to dropping mercury electrode (DME) and measuring the corresponding increase in current. The current voltage curve obtained is S-shaped, [Fig.1.1] consisting of a series of oscillations which are due to the continuous growing and dislodging of mercury drops from a fine capillary.

DC polarography can therefore, be exploited to obtain valuable information of an electrode reaction. When DC polarography is applied in quantitative analysis, the parameter of interest is the limiting diffusion current (id) which is related to the concentration of the electroactive species by the Ilkovic equation. In actual practice, when measurements, are carried out all the factors are kept constant in Ilkovic equation except the concentration. Hence the equation gets simplified to :-

\[ i_d = kC \]

Therefore id is directly proportional to the concentration and calibration curve can be utilized to determine an unknown concentration from the measured id. The minimum detection limit of dc polarography, is \(10^{-5}\) M.
Fig. 1.1: A typically current-voltage curve

**Diffusion Current:**

The electrical force on the reducible ions is nullified by an excess of supporting electrolyte present in the solution. Because ions of the supporting electrolyte carry practically all the current and the potential gradient is shortened to a region so very close to the electrode surface that it is no longer operative to attract reducible ions. Under such a condition the limiting current is called diffusion current \((i_d)\). Ilkovic\(^{52-53}\) derived an equation for the diffusion current

\[
i_d = 607nD^{1/2} m^{5/6} t^{1/6} C
\]

Where,

\(i_d = \text{average diffusion current (µA)}\)
n = number of electrons consumed in the redox reaction
D = diffusion coefficient of ions (cm$^2$s$^{-1}$)
m = rate of flow of mercury from Capillary (mgs$^{-1}$)
t = drop time (s)
C = bulk concentration of the electroactive species (mM dm$^{-3}$)

**Half Wave Potential:**

Each electroactive material in polarography is characterized by its half wave potential and is denoted by $E_{1/2}$. It is the potential at the mid point of the diffusion current curve. It is also defined as the potential on the polarographic wave where, the current is equal to one half of the total diffusion current. It depends only on the nature of reducible/oxidisable ions and it is independent of its concentration (for a reversible electrode process).

The significance of half wave potential can be best understood by considering the behaviour of redox system:

$$\text{Ox} + n \text{e}^- \rightarrow \text{Red}$$

The half wave potential ($E_{1/2}$) is characteristic of the specific redox system and may be independent of the concentration of the electroactive species present in solution by the following equation.

$$E = E_{1/2} + \frac{0.0591}{n} \log \frac{i_d}{i}$$

This equation shows the potential as a function of current at any point on the polarographic wave, it is sometimes termed
as the equation of polarographic wave. In above equation if log \( \left( \frac{i_a - i}{i} \right) \) is plotted against corresponding potential of electrode a straight line should be observed.

**Advanced Polarographic/ Voltammetric Methods**

Several major modifications of classical polarographic/voltammetric techniques were developed in the advanced polarographic/voltammetric methods, whose theory and instrumentation have undergone significant advancement that enhanced significantly the sensitivity and selectivity of the method. These techniques have better detection limit and have resulted in the form of Normal pulse polarography,\(^{54}\) Differential pulse polarography,\(^{55}\) Differential pulse anodic stripping voltammetry,\(^{56}\) Differential pulse cathodic stripping voltammetry,\(^{57}\) Oscillographic polarography,\(^{58}\) Square-wave polarography,\(^{59}\) and Cyclic Voltammetry.\(^{60-61}\) A brief theoretical description of some of these techniques which have been used in the present work are given below:-

**Pulse Polarography:**

These techniques were developed in order to increase the efficiency and sensitivity of polarography. Since the capacitive current is a major source of error in dc polarography, pulse wave forms were designed to leave it aside while measuring only the faradaic current. To achieve this, a potential pulse of short duration is applied, towards the end of the mercury drop life, at
exactly the same time interval in the life of each drop. It is a known fact that capacitive currents decrease more quickly to zero than faradaic current, hence, it is possible to ignore the former and measure only the latter at the end of a constant pulse period.

Hence, in pulse polarography, a single pulse is applied to each mercury drop, late in the life of the drop as it grows in the test solution. It may be assumed that about 20 to 40 ms. after the application of the pulse, the charging current will have decayed to almost zero. The faradaic current produced by the pulse is measured after this brief time interval and is plotted versus the applied potential. The potential programming is done in two different ways, to give birth to the following techniques:

(i) NPP (Normal Pulse Polarography)
(ii) DPP (Differential Pulse Polarography) of the above two techniques, the author has used DPP for the present work which has been discussed below.

**Differential Pulse Polarography:**

The technique, DPP was developed to improve minimum detection limit and sensitivity by applying voltage pulse at certain time intervals. Pulse polarography introduced by Barker in 1960 has better ability to discriminate against capacitive current because it measures a difference of two measured currents.

DPP involves, firstly, the application of a normal dc voltage ramp to the system under analysis and then towards the end of
the drop lifetime, the application of these pulses, it is possible to separate the unwanted capacitive current from the required faradaic current. The current is measured, just before and soon after, application of the pulse, and the current difference is plotted versus the applied voltage. A peak shaped curve is obtained, with the peak maximum almost coinciding with the $E_{1/2}$.

The discrimination against the charging current that is inherent in all the pulse techniques leads to lower detection limits, which makes this techniques suitable for quantitative analysis.

**Working Process:**

In the DPP process small pulse amplitude (of approximately -50mV) is superimposed on the linearly increasing voltage. The current is sampled twice during the life of each drop, one just before the application of pulse, i.e. at time $t^0$ and again near the end of pulse, i.e. at time $t$. The difference of current ($\Delta i$) is plotted against the applied base potential ($E$). The result of this process is a peak [Fig. 1.2] at a voltage, corresponding to $E_{1/2}$ with a height proportional to the concentration of the analyte/electroactive species.

![Voltammogram for a Differential Pulse](image)

**Fig. 1.2 : Voltammogram for a Differential Pulse**

**Polarography Experiment.** Here $\Delta i = i(t) - i(t')$
Total Current \( (t) \) = Residual Capacitive Current + Faradic Current

Current at Point \( (t') \) = Residual Capacitive Current

\[ \Delta i = t - t' \]

Usually, pulse amplitudes (Ep) of 5-100mV are employed together with drop times \( (t) \) of 0.5 - 3ms., the pulse width \( (tp) \) is usually kept constant at about 50 sec. and scan rates are normally 1-5mV sec\(^{-1}\). In DPP the peak current is given by the following expression:

\[
i_p = \frac{nFAD^\nu C}{\pi^{1/2}(\tau - \tau^0)^{1/2}} = \frac{1 - \sigma}{1 - \sigma}
\]

where

\[
\sigma = \exp \frac{nF \Delta E}{RT \cdot 2}
\]

\[\Delta E = \text{Pulse amplitude}\]

The high resolution and enhanced sensitivity of DPP is dependent on increased faradic current and a very slow capacitance current and therefore, the lower limit of detection can go as low as \(10^{-8} \text{ M}^{(63)}\)

**Stripping Voltammetry:**

Stripping analysis is the advanced from of polarography which has gained wide popularity having general acceptance for trace and ultra-trace analysis of several cation, anions\(^{64,65}\) and organic materials\(^{66}\)

**Working process:**

The stripping voltammetry involves the stripping of the accumulated substrate on the electrode. A stripping voltammetry measurement involves three important steps:
(i) **Preconcentration**: In this step the electroactive species is first deposited on a stationary microelectrode at a predetermined potential usually from a stirred solution.

(ii) **Quiet time**: After an accurately measured period, the electrolysis is discontinued, the stirring is stopped and the deposited analyte is determined by one of the voltammetric procedures.

(iii) **Stripping Step**: During the last step in the analysis, the deposited film is stripped off or out of the working electrode, during a potential scan that reverses the deposition reaction, hence the technique is named as stripping voltammetry.

The stripping peak current is proportional to the concentration of the electroactive species in the bulk of solution. As a result of the preconcentration step, stripping methods yield the lowest detection limits of all voltammetric procedures.

The characterization of the peak is done by peak potential $E_p$, peak current $i_p$ and peak width $b$, i.e. voltage span. The peak current is proportional to the concentration of electroactive species.

A particular feature of this technique is that owing to the much better resolution and greater sensitivity which is achieved. The technique can be used to measures the concentration in the
range of $10^{-6}$ to $10^{-9}$ M. The sensitivity of the stripping technique can be magnified significantly by employing differential pulse voltammetry during stripping process.

**Classification:**

The stripping voltammetry may be divided into the following types:\(^{(67)}\)

**Differential Pulse Anodic Stripping Voltammetry (DPASV)**

In this technique the cathodic deposition is followed by anodic oxidation (stripping), it means that in this technique the microelectrode behaves as a cathode during the deposition step and as an anode during the stripping off step with the analyte being oxidized back to its original form and peaks obtained are oxidation peaks.

**Differential Pulse Cathodic Stripping Voltammetry (DPCSV)**

This technique allows anodic deposition followed by cathodic reduction. The microelectrode/working electrode behaves as an anode during the deposition step and as a cathode during the stripping off step and the cathodic reduction peak is obtained out of the analysis.

**Adsorptive Stripping Voltammetry (ASV)**

At present there is increasing interest in using stripping voltammetry for the analysis of organic compounds which can not be concentrated by the electrolytic process. Alternative
deposition schemes based primarily on adsorption accumulation have been employed for this purpose.

The term adsorptive electroanalytical technique in which the species is deposited/preconcentrated first by adsorption on the working electrode followed by voltammetric measurements of surface species.\(^{(68)}\) The last few year have witnessed the application of ASV to the trace and ultra trace analysis of several compounds of biological importance, such as nucleic acid, hormones, tranquilizers, antibiotic and dopamine.

**AIMS AND SCOPE OF THE WORK**

Looking at the importance of the use of electrochemical methods in the analysis of bioactive and genetic materials and also the importance of replacing the traditional electrodes by modifying them with the bioactive/genetic/antibioactive material, the present work has been planned with an objective of developing the DC Polarographic/Voltammetric methods for the analysis of some bioactive materials viz. enzymes (amylase), coenzyme (Thiamine pyrophosphate), genetic material (DNA). Attempts have been made to standardize electrochemical procedures for the qualitative and quantitative analysis of some bioactive materials/ DNA in natural origin and biological fluid (blood). DCP and DPP methods have been used for the study of mutational changes in DNA.
The analysis of some anticancer drugs viz. Adriamycin and Mitomycin-C has also been done, with an intention to develop voltammetric procedure for their individual analysis and also to study their interaction with DNA for suggesting the mechanism of interaction of the anticancer drug with DNA which will prove to be highly useful in chemotherapy (cancer cure). Besides, DNA modified GCFE, Adriamycin/Mitomycin-C modified GCFE have been prepared for their possible use as Biosensors.

In the field of bioelectro-chemistry, Biosensors (modified electrodes) are the strong analytical tools for the study of different organic compounds and the interaction mechanism with other substances. Attempts have therefore been made to modify the GCFE with some antibiotic viz. Ciprofloxacin and Ofloxacin. Which have proved highly useful for the analysis of the antibiotic drugs understudy and in assigning their interaction mechanism with DNA.

The work undertaken, finds its wide applicability not only in the analysis of bioactive and genetic (DNA) materials individually but also in natural and biological samples. Besides, the prepared bioelectrodes have proved to be of utmost use in the study of the mechanism of action of some anticancer and antibiotic drugs with DNA which in turn may prove to be highly useful in cancer chemotherapy.
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