Chapter I

Introduction

This chapter is general introduction and theoretical background of the nucleic acids and methods used. In this chapter the nucleic acids, their structure and their role in origin, evolution and functioning of life has been discussed. The methods of voltammetry, the dropping mercury electrode (dme) and investigation of the kinetics of halogenations at rotating platinum electrode (RPE) by hydrodynamic voltammetry have also been discussed. The scope of our study has been outlined.
1.1 Introduction

“Life” is mysterious and as yet, no scientist has been able to define it precisely. Nonetheless, attempts to unveil the garb of mystery shrouding this term are relentlessly on. Study of life has always drawn the attention of the world in the endeavor of humans to understand “Life”. All living organisms share a master plan related to the blue print of their structure and functioning.

Chemistry has central role in understanding “Life” and all other sciences. It is the widest known science from centuries. In olden times alchemy was famous with various discoveries like drugs, perfumes, coffee, dyes and much more. Sulfuric acid, the king of chemicals was developed by Jabir ibn Hayyan, the famous Persian alchemist and this discovery is thought to be corner stone for transformation of alchemy into modern chemistry [1]. Chemistry today is largest branch of science with a wide service to the solution of problems faced by humans daily. The vastness of the chemistry is unlimited with different fields of study, but focus of this study is mainly towards nucleic acid chemistry.

![Figure 1.1 Chemistry as central science](51]

1.2 Nucleic Acids

Nucleic acids are important biomolecules present in every living being from unicellular bacteria’s to highest living chordates [2]. They are biopolymers present inside the nucleus of cells with a key role in origin, evolution and functioning of life on earth [2-3].
Before the discovery of nucleic acids, it was not known that there are some regulating molecules in life which are linked with transmission of traits, character and diseases from parents to offspring’s [2-4]. Since their discovery, nucleic acid chemistry has revolutionized the understanding of life and genetic diseases. The development of biotechnology as a new and vast field to understand the nucleic acids and obtain engineered products for serving humanity is now possible.

1.2.1 History of Nucleic Acids:

Nucleic acids have posed a mystery from the past 150 years and it was the discovery of Friedrich Miescher a doctor by profession that was trail-blazing. He was interested to study the fundamental particle of life so he left the field of practicing medicine and joined research in Germany [5]. Friedrich Miescher started working on cell chemistry, he specifically selected lymphocytes and started obtaining them from lymph nodes, but it was difficult for him to separate these cells so he later shifted to pus cell from fresh surgical bandage [5].

Miescher later started analyzing the cellular medium of the cell and finally remained concerned towards the nucleus. He observed a new material which was precipitating out with the addition of acid and dissolving with the addition of base. He was sure that this was a novel thing which did not seem to be proteins [5-6].

Later the analysis provided the view that the compound consists of nitrogen, oxygen, carbon and hydrogen. The most important thing was that the substance was lacking sulfur content which is mostly present in proteins. The phosphorous content was high in the sample, later the substance was named as nuclein because their major presence in the nucleus of cell [5,7].

The work of the nucleic acid was published and there was not any appreciation from the scientific community for this work, but Miescher was interested in this study and later he studied sperm cells of salmon which made him get enough nuclear material for the analysis due to the bigger size of sperm nucleus. Till 50 years, the work on nucleic acid remained still and the progress in this work was incited when Albrecht Kossel (1891), Jules Piccard (1874) and Jokab Muller (1874) discovered that nuclein was composed of four bases. Albrecht Kossel was awarded the Nobel Prize in medicine for this work [8].
However, after the death of Miescher, nucleic acids remained out of attention and it was not thought that nucleic acids were the materials for transmission of hereditary traits. In place, proteins were thought to be linked with transmission of hereditary characters because they were composed of twenty different types of amino acids and the nucleic acids were having only four[5,8].

In 1953 Watson and Crick explained the structure of DNA and its working, paving the way for the scientists Hargobind Khurana, Marshel Weilberg, Robert Holley and colleagues to crack the genetic code [6-7] and this was the beginning of the new field of science, molecular biology and genetics and study in this field finally solved the explanation of the trait transmission concept.

Today nucleic acid chemistry is well known as a highly engineered science having wide range of applications in research and development to cure of diseases and increase agricultural productivity to eradicate hunger from undeveloped nations. Today nucleic acids are considered more than a molecule and have become the icon of modern biosciences [9].

1.2.2 Role of nucleic acids in life:

Nucleic acids serve an important role in the life as it was unknown before, but the discovery DNA structure by Watson and Crick revolutionized the field of molecular biology and genetic studies [2-9]. The roles of these molecules are vast in life and are categorized as origin, evolution and functioning of life and each of them is as below.

1.2.2.1 Role in origin of life:

There are many theories regarding the origin of life with some experts suggesting that life originated spontaneously and some suggest that life was generated from pre living organism. Life has been brought to earth from elsewhere is also said by few, but each evidence suggested had merits and demerits [10].

Since, the Millers experiment about the origin of life, the observed results from the experiment were important evidences that life has been originated from water, nucleic acids and few proteins were few early molecules generated in the form of life [6-10].

Nucleic acids specifically RNA is thought as the oldest organic substance which has served as genetic material. The mystery, how the RNA has been the molecule in the
origination of life was explained by the Miller experiment which suggested that the heterocyclic nucleobases like adenine, guanine, uracil, cytosine, and 17 amino acids were also analyzed from liquid broth used in his experiment[8-11]. This seems to provide evidence that the nucleic acid have link in the origin of life.

How do heterocyclic nucleobases get assembled in the form of life? Some theories suggest that after a long enough period, sugar molecules were formed in water and they combined with these heterocyclic molecules and phosphate group to start polymerization. The polymerization initiated was catalyzed by RNA. The small unit of RNA was formed. The replication of RNA is the basis for life to form through the evolution [6, 8] with the generation of viral particles. Even today few of them have RNA as genetic material.

1.2.2.2 Role in evolution of Life:

Evolution has started with the formation of unicellular cells and their nuclear material, the genetic material playing an important role in the modification due to changes in it either by spontaneous mutations or by other means.

The early genetic material has been RNA a single strand. From this strand the double stranded DNA helix in less compact form is still seen in bacteria, with the presence in the central of bacterial cell without having proper nuclear boundary. The genetic material was in a simple form as DNA double strand [16, 10].

The start of replication and transference of genetic information to protein synthesis in these bacteria were simple in organization with few characters and so the nuclear material was less organised.

This unicellular bacterial was transformed in to multi cellular primitive organism with nuclear material as DNA but in more compact form than bacteria [4-14], having increased characters. The organisms were of simple structures both in plant and animal kingdoms and the compactness of the DNA as genetic material was getting increased with increase in characters of the organisms.

The transformation simple cellular mass to organs of body and then in organ systems took huge amount of time and potential but with the basic role of nucleic acids, all the transformation was due to changes genetic material mostly DNA.
The genetic material in bacteria was less compact with few characters. The highest organised form of genetic material was observed in the form of chromosomes. The pairing in the chromosomes has played an important role in the meiosis and mitosis cell divisions which have generated the sexual form of reproduction in multi cellular organisms. The change of genetic material from RNA to DNA took billions of years and the transformation of unicellular bacteria to highest living chordate human beings took another billion of years [16-18].

The mystery of humans that has evolved from apes also took a long time with a clear difference between their genome and our genome being only one chromosome. They have 23 pairs of chromosomes and humans have 24 pairs of chromosomes [9-17]. The evolution process is still running according to evolution experts and will not stop and is all due to changes in the genetic material DNA.

1.2.2.3 Role in the functioning of life:

Functioning of the entire living world is dependent on nucleic acids. The stored information of living characters and its expression is encoded with nucleic acids. The color of eyes, color of skin, height of human beings and much more is regulated by our genetic material, as the old proverb says “like begets like”.

The modern science has proved that, progeny is transferred only through the genetic material. The characters are passed from parents to offspring’s by genetic material only.

The functioning of genetic material is regulated by other cell organelles which cooperated in the cell to make flow of genetic information successful. The information coded on DNA is taken by RNA specially mRNA, tRNA and this information is transferred to protein factory Ribosome’s which synthesize proteins for specific character and serves the functioning of body[18]. The role in the generation of cell from old cells by mitotic division and halving the genetic material of sex cells by meiosis is solely regulated by genetic material.

In short we can say every living being is regulated by genetic machinery which is sole nucleic acids either DNA or RNA. And everything what we do is regulated by them including genetic diseases [3-14].
1.2.3 Structure of nucleic acids:

Nucleic acids are mainly of two types’ deoxyribonucleic acid and ribonucleic acid. There are few differences on the basis of structure and a wide difference on basis of function. The structures of the nucleic acids are similar, but DNA is double stranded and RNA is single stranded. There is also difference in the sugar molecules. The DNA has a 5 carbon sugar known as deoxyribose and RNA has 5 carbon sugar known as ribose sugar [2-19].

The nucleic acids are biopolymers and each monomer is a nucleotide. The nucleotide in turn consists of

1. A pentose sugar
2. Heterocyclic nitrogenous base
3. A phosphate group mainly phosphoric acid.

Regarding the pentose sugars they are of two types that is deoxyribose and ribose sugar the difference only lies former is oxygenated and later lack oxygen atom. The DNA consists of deoxyribose sugar and RNA consists of ribose sugar [12-15]. The structure of these molecules is as.

![Figure 1.2 Structure of pentose sugars][53]

The heterocyclic nitrogenous bases present in nucleic acids are of five different types and are categorized as purines which include two bases adenine and guanine. The structure is given below.
Pyrimidines include three nitrogenous bases; uracil, cytosine and thymine the structure being-

These heterocyclic nitrogenous bases are an important part of a monomer unit. The nucleotidate has three parts which include a pentose sugar group to which is attached a nitrogenous base at position 1 of the pentose sugar and the base may be any of the above.

The thymine nucleobase is only present in DNA and is replaced by uracil in RNA. The phosphate group is also present at the 5 position of sugar group. The whole combination is known as nucleotide and without the phosphate group it is known as nucleoside.

The polymerization of nucleotides leads to the formation of polymer nucleic acid which is a double strand of nucleotides in DNA and a single strand in RNA. The polymerization occurs with bridging of the nucleotides through phosphodiester bond, which is an enzyme catalyzed process for generation of the long strands of nucleic acids DNA/RNA [9-16].

The two strands of DNA are formed collectively and are connected to opposite strands by hydrogen bonds. The pure hydrogen bonds are between nucleobases with C-G as triple bond and A-T as double bond in case of DNA [10-17]. The structure of nucleic acids is made compact by the help of various proteins both histone and non histone proteins and shape them as chromosome in the nucleus of the cell. The length
of DNA strand is much more than that of the cell diameter, even can reach up to few meters but the folding and sub folding by proteins makes it so small and compact in size so, it is embedded in the nucleus of the cell.

1.2.4 Applications of synthetic halogenated nucleobases and derivatives:

The synthetic halogenated pyrimidine and Purine heterocyclic nitrogenous bases of nucleic acids have vast applications as pharmaceuticals [20], and are mostly used as anticancer, antiviral, anti fungal and other various applications [20-21]. The most important halogenated bases studied are fluoro derivatives because 5-fluoro uracil acts as potent anticancer, 5-fluoro cytosine as an important anti fungal and both are practiced as medicines [22]. Vast studies have been done on fluoro derivatives. Other halogenated derivatives also have vast applications specifically bromo and iodo derivatives [20].

The 5-bromo uracil is used as sensitization of bacterial cells by UV irradiations[23], radio labeled 5-bromo cytosine is used in radio diagnosis of body parts[24], 8-bromo adenine is used in vivo enzyme studies because of having an ease of binding with amino acids of proteins[25],

5-Bromouracil is used as mismatch pair study of nucleic acids against thymine [26, 27]. The bromo uracil and bromo cytosine derivatives are used as the substituent in various medicines and as substituent in the synthesis of other important derivatives of nucleobases [28].

5- Iodo uracil has been recently revealed as an important substituent in 8 amino quinoline molecules with increases effectiveness and decreased toxicity against malarial parasites [29].

1.2.5 Damaging effect of in vivo halogenated nucleobases and derivatives:

In vivo halogenation of nucleic acid bases have suggested serious consequences of dangerous disease like cancer and other neurodegenerative diseases [30]. The cause of halogenations in cells is due to the free halide ions in the cellular fluids [31]. During the invasion of infection, the cells secret excess of myeloperoxidase enzyme batteries and hydrogen peroxide convert these halogen species into halo acids like HOCl / HOBr and these acids convert these nucleobases into halogenated form[32]. These
chloro and bromo derivative of nucleobases shows mismatch pairing in the DNA[33] and disturbing the mechanism of genetic coding with effect on protein synthesis and in turn the invasion of numerous diseases.

1.3 Polarography

Polarography is an important voltammetric method of analysis which primarily uses the dropping mercury electrode as cathode and saturated calomel electrode as the reference electrode. The various applications of this technique have been discussed in the instrumentation part of chapter II. Here various types of polarography as an analytical tool to study the different molecules with focus on organic molecules have been discussed.

1.3.1 History of polarography:

History of polarography can be directly traced to Heyrovsky who was interested in chemistry however this subject was not available in the Austro-Hungarian empire so he went to London to study in University College where he worked in the Professor Fredrick George Donnan laboratory for his Ph.D and was supervised by Dr. Roland Edgar Slade, who was working on electrochemistry of aluminum. This task was also given to Heyrovsky for determination of standard reduction potential of aluminum [34].

In 1914 Heyrovsky returned for holiday to his native place and this was the time when the Second World War broke out, Heyrovsky was forced to join medical corps of his country. Heyrovsky interests did not lay him back so he still experimented in the hospital laboratory with electrochemistry of aluminum and collected his results [34, 35].

Heyrovsky submitted his results to Charles University in Prague. During examination of his work one of his examiners was the founder of dropping mercury electrode who had left this work, now regained interests in the past work [36].

Heyrovsky was interested to develop a new method for the determination of potentials and he connected a galvanometer in the circuit to measure the current flow he was not succeeded because of less sensitivity of galvanometer and in 1922 he returned for the experimentation to observe the decomposition of salts like NaCl and other aluminum
salts and in same year he connected a mirror galvanometer with high sensitivity in the circuit in place of old one and he succeeded in obtaining the first ever Polarogram[37].

1.3.2 Organic Polarography:

After prominence of polarography in different field of chemistry the organic molecules were also tried to be observed by polarography and many successes were obtained in the observation of organic molecules at the dropping mercury electrode (dme).

Organic compounds having functional groups have their reducibility dependable on the functional group attached, molecular frame work of functional groups, positioning of the functional group and various other parameters like multiplicity of bonds and electro negativity of the functional groups [34, 36].

It is seen that the compounds having conjugation of double and triple bonds and having C-X bonds are mostly reducible. The reducibility of C-X bonds depends on the polarizability of halogen atom attached the ease of reduction increase with increase in the polarizability of halogen atom as C-I is more reducible than C-F.

The aromatic rings present in the compound increase the reduction process of organic compound as the number of aromatic rings increase in the compound the reduction process becomes easy for the compound.

The organic molecules which are reducible at dropping mercury electrode can be studied directly and irreducible molecules can be studied indirectly at the electrode, the indirect methods include reacting the substance with other group and generating the electroreducible group in the compound so as to make it reducible at dme. The methods are as below.

1.3.2.1 Nitration:

This is the most frequent used method for obtaining the nitro products which are electroreducible, the method mainly fit for hydrocarbons mostly aromatic like benzene and others the reaction is performed with the nitric acid alone or with KNO₃ or H₂SO₄ which are mainly used as nitration mixture. The temperature and other
parameters are regulated so as to obtain the single nitration product so which the polarograms can be obtained.

1.3.2.2 Nitrosation:

The nitroso compound can be easily reduced at the dme. This method is used as an indirect method in which sodium nitrite is used in acidic medium for generation of nitroso group this method can be used to determine the secondary amines in presence of primary and tertiary amines after transformation. The reaction is mainly performed with nitrous acid in acidic condition to transform it into nitroso compound and mostly flavones are transformed.

1.3.2.3 Addition:

This method is mainly used for molecules containing unsaturated bonds and bromine is used to add to these bonds mostly olefins and alpha beta dibromides formed from these compounds which show polarographic reduction curves.

1.3.2.4 Oxidation:

Periodate is mainly used as oxidation agent for organic polarography because selected oxidation can be performed and is mainly used for α-diols, α-aminoalcohols. The oxidation of polyalcohol is mainly performed between pH 4-7 and after oxidation the polarographic oxidation/reduction curves can be observed by indirect method for these species.

1.3.3 Polarography as an analytical tool:

Polarography is an important voltammetric method for both qualitative and quantitative analysis. This technique can be used to perform analysis in limited time and the results obtained are accurate [37]. By this method we can perform analysis of very small quantities of the samples.

1.3.3.1 Qualitative analysis by polarography:

The Polarographic waves obtained have diffusion current as one of the parameters and this current is generated by the electro reducible species present in the solution. The potential at which diffusion current is half is known as half –wave reduction potential [38]. This potential is specific for each species to be analyzed.
To obtain the qualitative analysis by polarography we can obtain half-wave reduction potential of analyzing species and comparing the values with standard pre-obtained values or the values can be obtained at the same time by running the polarograms for each species present. The half wave reduction potential can be obtained from polarograms by a simple tangent at half of the diffusion current value which meets the potential axis. The point is half wave reduction potential for the particular species.

There are certain limitations, when there are more than one species in solution to be analyzed and when the polarograms are not much separated then, this poses a hard task to obtain the half wave reduction potentials for both the species, but this problem can be overcome by changing the supporting electrolyte of the analyzing solution. The changing supporting electrolyte has been seen to have good effect in separation of overlapping polarograms.

1.3.3.2 Quantitative analysis by polarography:

The Quantitative analysis can be performed by the famous Ilkovic equation which can be used to obtain the concentration of the analyzing solution by knowing the other parameters of the equation, all other parameters are to be known but diffusion coefficient is hard to obtain because of its dependency on viscosity and temperature of the analyzing solution [39] and uniqueness for each species. The height of the polarogram is a measure of concentration of the electroreducible species.

This can be obtained from the pre-obtained standard value or by chronopotentiometry. The diffusion coefficient can be also obtained for known species by knowing the concentration and measuring the diffusion current.

Quantitative analysis is performed by calibration curve methods in which a calibration curve is generated for the known concentrations and the unknown concentration is obtained by comparing the current for specific species.

The limitation for this method is the overlapping of the polarograms for more than one analyzing species in certain cases, but this can be also erased by changing the supporting electrolyte of the analyzing solution.
1.3.4 Types of polarography:

Polarography can be extended in many types to increase the sensitivity and determination of the electroactive species these are explained as below.

1.3.4.1 A.C Polarography:

In this Polarography alternating potential source is applied to the cell containing the electroactive substances. The alternating potential both in positive and negative direction is applied and current is measured. This polarography can be applied to the reversible reactions also. The peak of the reversible reaction is symmetric and for poorly reversible reactions the peak height is small and wide [40]. The peak height of the polarogram is proportional to the electroactive species and is given by

\[ I_p = 2.5 n^2 F^2 A D^{1/2} \Delta E N^{1/2} C / 4RT \]

Where \( \Delta E \) is amplitude potential in (V), \( A \) is electrode area in cm\(^2\), \( N \) is frequency in Hz, \( D \) is diffusion coefficient (cm\(^2\)/s), \( R \) is gas constant, \( T \) temperature in Kelvin and \( C \) is the concentration.

The advantage of this polarography is to increase the S/N ratio of Faradic current and reducing the charging current drastically, this in turn will increase the S/N ratio and the determination limit in place of classical polarography.

1.3.4.2 Pulse Polarography:

The concentration range of determination of polarography is between \(10^{-2}-10^{-5}\)M and below this concentration increases the noise which is due to the captive current. To avoid the captive current the potential is applied in pulses to the mercury drop for small time and current is measured. Mainly the potential is applied for small time about 57ms and the current is measured for the last 17ms, in the first 40 ms the captive current becomes zero and in last 17 ms the electroreducible species are reduced [40-41].

The captive current is avoided which is the source of noise so, increasing the S/N almost by 100% and the efficiency of determination than classical polarography. The current is given by
\[ I = n F A D^{1/2} C /\delta^{1/2} \pi^{1/2} \]

\( \delta \) is the length of time of pulse applied it is mainly 57-17 = 40ms.

The quantitative determination is mainly done by standard addition and working curve method. This method can be also extended to solid electrode where it is known as pulse voltammetry. This polarography includes differential pulse polarography and square wave polarography.

### 1.4 Other voltammetric methods

There are various other voltammetric methods used for analytic purposes and few of them are as below

#### 1.4.1 Hydrodynamic voltammetry:

This is a type of voltammetry in which solid electrode is used as working electrode mostly a rotating platinum electrode or stationary disc electrode are used. The platinum electrode is made of a platinum wire at the end of tube the wire is either perpendicular to the tube or at the same angle and rotated at high speed with the solution coming in motion and the electroactive species are reduced/oxidized by convectional flow of solvent and the voltammetry is known as hydrodynamic voltammetry[35,36].

The voltammogram generated are same as that of classical polarography but here diffusion current is represented as limiting current, as the diffusion of electroactive species is not observed. These species come in contact with the electrode by conventional flow and the limiting current is given by

\[ I_{lim} = 1.55nFAD^{2/3} \pi^{1/2} v^{-1/6} N C \]

All other values are same as the above equation except \( v \) which is the kinematic viscosity of the solvent and is the viscosity of the solution divided by density of the solution, having units of cm\(^2\)/s. \( N \) is the rate of rotation of electrode in r/s. More about Hydrodynamic voltammetry is discussed in chapter II.

#### 1.4.2 Cyclic voltammetry:
This is a method of triangular voltammetry in this voltammetry the rapid scanning of voltage is done in small amount of time mainly in 1-2 seconds. The voltage scan applied can be explained in figure below

![Cyclic Voltammogram obtained for Zn (II) ions](image)

**Figure 1.3 Cyclic Voltammogram obtained for Zn (II) ions [46]**

The scanning of voltage starts at point A given in figure above, the increase in current is small from A-B and from B current increases rapidly till C and then starts falling till D. this was cathodic potential applied to this half scan [35-40].

From D scan is reversed now the anodic phase starts and current decrees rapidly from D-E then starts rising slowly from E-G and rapidly increases from H-I and then fall back to J. From J the phase is reversed and cathodic phase starts this completes a cycle and the scanning of potential occurs in cycles so this voltammetry is known as cyclic voltammetry.

The peaks seen in both cathodic and anodic part are of same nature if the reaction under study is reversible and if the reaction is partially reversible the two peaks differ in peak heights and widening of peaks. This voltammetry is an important tool of voltammetric analysis as the rapid scanning in both directions is done and the results obtained are quite fast.
1.5 Reaction Kinetics

Kinetics of a reaction is the study the rates of reactions or the speed of the chemical reactions with which they proceed for the product formation. It is important to monitor the reaction for observing the rate because unless we can’t understand the rate of a reaction we cannot predict the reaction results. There are number of methods developed from time to time to observe reaction kinetics.

One of the old conventional method to study the reactions by titrimetry, but this method cannot be used to study rapid reactions as the rapid reactions take very less time to get completed even less than a second. Most biochemical reactions in nature are rapid and different rapid reaction methods have been developed to study these reactions. The reactions in which one component is a gas can be studied by change in the pressure as the reaction proceeds there will be any change in the volume of gas used and this will show variation in the pressure and monitoring the pressure of the gas can be used to determine the rate of the reactions. Spectrophotometry is also used to observe the kinetics of the reaction but in this case one of the reactant must absorb the radiations from the electromagnetic spectrum and the change in the concentration of the reactant can be predicted by the absorption pattern of the specific reactant or the product.

The reaction which involves the generation of ions can be studied by conductometry methods and the reaction which involves protons can be studied by pH metery. Other methods of determining are emission spectroscopy, mass spectrometry, gas chromatography, EPR, NMR.

1.5.1 Flow Technique:

A fast reaction is one that is completed in less than 1s at measurable reactant concentration. To observe the reaction kinetics of the fast reaction this method is used. In this method the reactants are mixed in the reaction chamber and after mixing they are allowed to flow continuously through an outlet tube and the movable spectrometer is used to observe the reaction kinetics as given in the figure below. There are certain disadvantages of this technique. There is continuous flow of the reactants throughout the tube and a large amount of reactants are used up to make the
continuous flow and the flow must be rapid so as to observe the accurate kinetic results.

**Figure 1.4 Representation of flow Technique [49]**

1.5.2 Stopped-flow Technique:

The disadvantage in flow technique is overcome here. In this technique the reactants are mixed quickly in the reaction chamber and the flow is stopped by the help of syringes and reaction is allowed to proceed in the reaction chamber. The kinetics determination occurs by UV-Visible absorption, Fluorescence emission and others. The reaction measurements are made as the function of time. The reactions mainly of millisecond and second level are studied by this technique mainly biochemical fast reactions are studied involving protein and enzymes kinetics are studied by this method.

**Figure 1.5 Representation of stopped flow technique [50]**
1.5.3 Flash Photolysis:

In this technique the sample is exposed to a laser beam, the sample absorbs the radiations and shows the process of excitation. During de-excitation they emit spectra detected by different methods. Kinetic analysis is made on the basis of data obtained, the method is quite fast and ultra fast time resolved method are used and the reactions completing in picoseconds or femtosecond are studied by this technique.

Figure 1.6 Representation of flash photolysis technique [52]
1.5.4 Hydrodynamic Voltammetry:

This technique can also be used to study fast reactions as in the method rotating platinum electrode is used to observe the fall in the concentration of the electroactive species and the concentration of these species is observed by current change. In the present study this technique has been used as a sole method to observe fast reactions, the method is detailed explained in chapter II.

1.5.5 Rate and Rate law:

The rate of the reaction is the change in the concentration of reactants or products with time and for a simple reaction can be given as

\[ A + B \rightarrow C \]  

The rate can be given as

\[ \frac{dA}{dt} = \frac{dB}{dt} = \frac{dC}{dt} \]

And the rate law is dependable on the concentration of reactants raised to power and can be given as

\[ V = k [A]^a [B]^b \]

Where k is called as rate constant which is independent of the concentration but depends on the temperature and the above equation represents the rate law. The rate law is an equation which expresses the rate of reaction as the function of concentrations present in the overall reaction [48].

1.5.6 Order of reaction:

The rate law for a simple reaction is given as

\[ V = k [A]^a [B]^b \]

In the above rate law there are components A and B and the powers raised to them are (a) and (b). The order of the reaction is the sum of the powers raised to the
components. The order of reaction can be zero also meaning the rate of reaction is independent of the concentration of the reactants and can be also half.

On the basis of the order reactions are classified as zero, first, second, third order according to dependency of concentration of their reactants.

1.5.7 **Arrhenius equation:**

The Arrhenius equation is given as

\[ k = A e^{-\frac{E_a}{RT}} \]

While \( k \) is the specific reaction rate, \( R \) is gas constant, \( T \) is temperature in Kelvin.

\( E_a \) is the activation energy the activation energy of the reaction is the minimum amount of energy required by the reactants to change into products and is measured in joules.

Where \( A \) is the frequency factor which represents the number of effective collisions as is know that the reactants are constantly colliding with each so as to get transformed into products [48] but all the collisions does not lead to products only the effective collision lead to the products and is unit less quantity.

**1.6 Scope of our study**

This study is based on the determination of nucleobases and nucleotides of DNA/RNA at dropping mercury electrode by polarography, to study these bases is important because of their role in the functioning of life, as these bases are used to study in vitro and in vivo DNA damage also. This study is limited to the determination at the dme and the reactivity study of nucleobases in complementary manner by an allied polarography/voltammetry method. The future of our study may have impact for the determination of halogenated biomarkers of DNA damage by voltammetry.

On the other hand synthetic halogenated nucleobases/nucleotide serves important applications as pharmaceuticals and is widely used as anticancer, anti fungal, antiviral drugs. Here we have observed the kinetics of halogenation of these reactions by hydrodynamic voltammetry as an allied method of polarography/voltammetry. These
halogenations are mostly rapid in aqueous medium and cannot be studied by conventional method. So, this voltammetric method is necessitated as a cheap and easy method to determine the rapid kinetics of these important reactions.

Further these studies are extended to in vitro halogenation study of nucleotide/nucleobases by Hypohalous acids at biological pH. As it is known that in vivo halogenations of nucleobases and nucleotides by Hypohalous acids mostly HOCl/HOBr generated in biological cell by Myeloperoxidase-H2O2 system modify these bases and these modified bases lead to abnormal genetic coding resulting in diseases. So, it remains important to study kinetics of these reactions for the progress in DNA damage studies. Here use of rotating platinum electrode to observe the kinetics of these important reactions has been made.

1.7 References

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