CHAPTER III

DNA Analysis in Natural and Biological Samples and Mutational Studies
The last fifty years of molecular genetics have produced an abundance of new discoveries and data that make it useful to revisit some basic concepts and assumptions in our thinking about genomes and evolution. Chief among these observations are the complex modularity of genome organization, the biological ubiquity of mobile and repetition DNA sequences and the fundamental importance of DNA rearrangements in the evolution of sequenced genomes.

**Genetic Engineering**<sup>(1)</sup>

The branch of biology by which important and permanent changes are created in plants and animals by increasing, decreasing, manipulation and transplantation of genetic units is termed as Genetic Engineering. Altering the behaviour of an organism according to will by way of adding, removing or repairing of a part of genetic material is the main aim of genetic engineering. It is also called as biotechnology of gene therapy, gene manipulation, gene transplantation and algeny.

The genetic information required for the function and multiplication of the biological organism is stored, duplicated and transmitted by means of Nucleic Acids.
All living organisms have a genetic code generally represented by the sequence of nucleotides in their DNA. Since there are four possible bases used in construction of the code denoted by A, G, C and T, each of the four letters is the code carrier to lot of information. Human beings have a genetic code of 3.3 billion base pairs.

Nucleic Acids

The genetic information required for the function and multiplication of the biological organism is stored, duplicated and transmitted by means of nucleic acids. In most organisms genes are segments of Deoxyribonucleic acid (DNA) molecules and function as the basic carrier of genetic information, but in a few phages and many animal and plant viruses, Ribonucleic acid (RNA) is the genetic material. A great deal of what we know about the nature of genes and genes and gene expression has been obtained from the knowledge of the structure of DNA and RNA. From experiment in which DNA is used to carry genetic information from one organism to another or from the studies in which DNA and RNA is altered.

The nucleic acids are informational molecules because their primary structure contains a code or set of directions by which they can duplicate themselves and guide the synthesis of proteins. There are two types of nucleic acids which are polymers found in all living cells they are Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).[2]
DNA is found mainly in the nucleus of the cell of all biological system, while RNA is found mainly in the cytoplasm of the cell, although it is mainly synthesized in the nucleus. DNA contains the genetic code to make RNA and the RNA in turn then contains the codes for the primary sequence of amino acids to make proteins.

**Structure of DNA**

DNA is a very long threadlike molecule, comprised of a large number of fundamental monomeric units—deoxyribonucleotides. The genetic information contained in the sequence of bases, two of them are pyrimidines (Cytosine & Thiamine) and the other two are purines (Adenine & Guanine). Whereas, the sugar and phosphate group-backbone of DNA, perform a structural role. The synthesis of proteins most of which are enzymes ultimately governs the metabolic activities of the cell. DNA plays a very important role in virtually all biological process in which DNA takes part.

In 1953, an American biologist, Watson and Crick\(^6\) an English biologist proofed the double helix structure for DNA

This development set the stage for a new and continuing area of clinical and biological investigations. The two main events in the life of a cell dividing to make exact copies of themselves, and manufacturing proteins, both rely on blue prints, both rely on blue prints coded in our genes.
Primary structure of DNA

It was found by E. Chargaff that the base composition in DNA varies from one species to another. However, in all cases, the amount of adenine was equal to thymine (A=T) and cytosine equal to guanine (C=G). In other words the total amount of purines was equal to pyrimidins i.e. A+G = C+T, but the ratio of AT/GC varied considerably between species.
Secondary Structure of DNA

The two strands in DNA are held together by hydrogen bonds. This hydrogen bonding is very specific because the structure of bases permits only one mode of pairing e.g. guanine is hydrogen bonded to cytosine and adenine to thymine. Thymine and adenine can be joined by two hydrogen bonds while cytosine and guanine can be joined by three hydrogen bonds. No other combination of four bases is possible because
these do not lead to strong hydrogen bonds. The two strands of DNA are said to be complementary to each other. DNA lattices can be right handed as well as left handed. The $\beta$-conformation of DNA having right handed lattices is most stable.

**Denaturation of DNA**\(^{(7-9)}\)

Stability of DNA double helix under physiological conditions is secured mainly by:

- Stacking force between the purine and pyrimidine rings.
- Hydrogen bonds between bases.

This stacking force/attractive farce that produce the three-dimensional structure of molecules, such as the hydrogen bonding between DNA base pairs are fairly weak and easily disrupted by heat. When the hydrogen bond are disrupted, the DNA is said to be denatured; whereas the hydrogen bonds are intact, as in double stranded DNA in nature the DNA is said to be Native DNA. The transition from the native state to denatured state is called **Denaturation**, and the formed DNA is called **Denatured DNA**.

**Factors of Denaturation of DNA are:**

- Temperature.
- Change of the pH.
- Absorption of UV-Visible light etc.
- Renaturation of DNA.
A solution of denatured DNA can be treated in such a way that native DNA reforms. The process is called Renaturation or reannealing and the reformed DNA is called renaturated DNA.

**Molecular Weight**\(^{(10,11)}\)

DNA molecules are very long. The bacterial chromosome of E. Coli is formed by a single molecule of double helical DNA consisting of 3.4 million base pairs. The molecular weight of this DNA is \(2.3 \times 10^9\). Even the smallest DNA molecules isolated from viruses are highly elongated. Thus DNA from Polyoma Virus consists of 46,000 base pairs and has a contour length of 1.6 \(\mu\text{m}\). The molecular weight of DNA sample so far used for polarographic measurements varied mostly around \(10^7\).

**Ionization of DNA**\(^{(12,13)}\)

Above pH 3 each phosphate group in DNA carries a single negative charge. Ionizable group present on the nucleotide base including ring nitrogen, which accept proton below pH 4-5 and enolic hydroxyl group, which ionize above pH 9. Thus in the range of pH 5-9 DNA is an anionic electrolyte.

Screening of negative charge of phosphate group play an important role in the stabilization of DNA conformation. Single strand DNA is sensitive to charge in ionic strength below 1.0, while with double stranded DNA charge repulsion become significant at ionic strength below 0.1-0.2.
CHAPTER 3  DNA ANALYSIS IN NATURAL AND BIOLOGICAL SAMPLES AND MUTATIONAL STUDIES

MUTATION

The entire genetic message that controls the chemistry of every cell of the body instructing it to act in a specific way is actually written in the language of just four nitrogenous bases.

This four latter code/nitrogenous bases read in a line along the DNA. However, it is the specific combination of these bases farming a unique sequence that spells out the coded message in the DNA molecule. Just as 26 alphabets of English language can be assembled in millions of ways forming as many meaningful sentences as would make up the entire past, present and even future literature. So are these four latter of the genetic code arranged in almost unlimited number of unique sequence, specifying the genetic information for the entire living world.

It is known that 99% of base sequence is same in the DNA of all human beings. Only the sequence of vary short stretches of DNA, sprinkled over the total DNA of a cell when is about three billion base pairs, differs from person to person.

However, under certain physiological conditions of the environment, when may be chemical or physical, certain sudden and permanent changes do sometime occur in the DNA molecule, when subsequently are carried to the next progeny and obviously become hereditary, such change are known as Mutation.

The terms mutant and mutation are commonly confused. Strictly speaking a mutant is an organism whose genotype (or
more precisely, its DNA base sequence) differs from the wild type. Whereas mutation are the raw material of evolution, providing the changes on which selection can act. Since the time of Margan, genetics have used radiation or chemicals to increase the rate of production of mutations. This process is called **mutagenesis**.

**Classification of Mutation**

These changes or mutations of DNA can be classified according to the exact nature of the difference between the new (mutant) form of the gene and the old (wild type) form.

**Kinds of Mutation**

(I) **On the basis of the Types of Cell**

- Somatic Mutation
- Germinal Mutation
  - Gamatic (Genetic) Mutation
  - Zygotic Mutation

(II) **On the basis of Size and Quality of Mutant**

- Gene (paint) Mutation
  - Deletion Mutation
  - Substitution Mutation
- Chromosomal Mutation
  - Addition Mutation
  - Change in the No. Set of the Chromosom
  - Change in the Structure of the Chromosome
(III) On the basis of Origin of Mutant

- Spontaneous Mutation (by radiation, Temperature)
- Induced Mutation (by Chemicals, X & Y rays, Mutagenic agent)

(IV) On the basis of Phenotypic effects

- Dominate visible Mutation
- Intermediate visible Mutation
- Autosomal Recessive Mutality
- Lethal Mutation
- Determental Mutation
- Harmful Mutation
- Reversible Mutation

SURVEY OF LITERATURE

Polarographic and Voltammetric analysis of compounds has been a complex science theoretically as well as experimentally.\(^{(14)}\) However, in the past two- three decades the use of differential pulse polarography/voltammetry has proved to be a strong analytical tool\(^{(15)}\) for the said purpose with great accuracy and precision of determination and that too in samples obtained from different origins.\(^{(16)}\)
It is widely believed that thousands of genes and their products, in a given living organism function in a complicated way that creates the mystery of life.\(^ {17} \) Many other methods\(^ {18-21} \) are used for the study of DNA. However, looking at the ability of voltammetric methods in general and DPP in particular for the analysis of organic compounds, useful and accurate polarographic (DPP) procedures have been developed for the analysis of deoxyribonucleic acid (DNA), the basic genetic material in samples obtained from plant product (cauliflower)\(^ {22} \) and biological origins (human blood).\(^ {23} \) The developed procedure has also been used for mutational studies\(^ {24} \) i.e. genetic engineering. The results of which have been reported in the work.

Many compounds bind and interact with DNA causing changes in the structure of DNA and its base sequences leading to change in its behaviour and also in perturbation in DNA replication.\(^ {25} \) The DNA electrochemical biosensor incorporates immobilised DNA as molecular recognition element in the biological active layer on the electrode surface and measures specific binding processes with DNA, being a complementary tool for the study of biomolecular interaction mechanisms of compounds on their binding to DNA and enabling the screening and evolution of the effect caused to DNA by different compounds and substances.\(^ {26,27} \)
The main roles of DNA in the cells are to maintain and property express inform damage in DNA in the cells, upon interaction with a number of chemical and physical agents occurring in the environment, often results in mutation that may subsequent lead to change in DNA behaviour. It is therefore necessary to have analytical methods capable to fast and sensitive detection of DNA and its damaging agents.\(^{(28)}\)

**EXPERIMENTAL**

This part of chapter describes in details, the experimental conditions, employed in carrying out the various electrochemical measurements, methods of preparing the different stock solutions and test solutions, conditions of temperature, pH and so on.

**Instrumentation:**

An Elico µp-based polarographic analyser model CL-362 was used for all Polarographic/Voltammetric measurements (DCP/DPP). This is very versatile assembly which can be operated to carry out a wide verity of electrochemical measurements with a high degree of accuracy and precision. This instrumental set up for differential pulse polarographic (DPP) analysis was follows:

A dropping mercury electrode was used as a working electrode. Potential were recorded against a saturated calomel
electrode. A platinum wire electrode was used as an auxiliary electrode throughout.

All pH measurements in the present work carried out using a Systronics (India) digital \( \mu \)-pH meter model-361, which measured the pH with an accuracy of \( \pm 0.01 \) pH unit. A combination glass Ag/AgCl reference electrode was used in making the measurements. The cell had an arrangement to bobble nitrogen gas through the solution.

**Chemicals:**

Sample of DNA sodium salt (from Herring sperm) Himedia Lab. Pvt. Ltd. Mumbai, was used. Whereas, other chemicals used in the present work were of Merck/Analytical Reagent/BDH grade. They were all analysed by preliminary DP Polarographic measurements to ensure that they did not contain any impurities which were electroactive in the potential range of interest and were used without further purification.

**Supporting electrolyte:**

1.0M sodium sulphates, and 3.0M ammonium tartrate, were used as supporting electrolyte for all analysis.

**Preparation of stock solutions:**

The stock solutions of DNA (0.1%), was prepared by dissolving their requisites quantity in doubly distilled water. Whereas, the solutions of other regents required in the
experiment were prepared by dissolving requisite amount in doubly distilled water.

**Preparation of analyte and recording of polarogram:**

(i) **For analysis of Native DNA:** For polarographic study of native DNA, varying concentration of 0.1% DNA solution was mixed with 2.5ml of 1.0M sodium sulphate and 2.5ml of 3.0M ammonium tartrate as supporting electrolyte, and final volume was made up to 25ml with doubly distilled water. The pH of the test solution was adjusted to pH 6.7±0.1 with NaOH/HCl solution. The analyte was taken in polarographic cell equipped with an electrode assembly described earlier. The solution was deaerated by bubbling nitrogen gas through the solution for ten minutes and polarogram was recorded.

(ii) **For analysis of Denatured DNA:** In the conversion of native DNA into denatured DNA, all hydrogen bonds between the double helix are broken. The stability of double helix of DNA is secured mainly due to stacking force and hydrogen bonding between nitrogenous bases,\(^{29}\) but a large increase in temperature (≈950C) and/or great change in pH (≈11.3)\(^{30}\) may destabilize the double helix of DNA, and native DNA is converted into denatured form.

For its polarographic analysis, varying concentration of 0.1% DNA solution was mixed with 2.5ml of 1.0M sodium sulphate and 2.5ml of 3.0M ammonium tartrate as supporting
electrolyte, and final volume was made up to 25ml with doubly distilled water. The pH of the test solution was adjusted to pH 11.3±0.1 for denaturation of DNA with NaOH solution. The analyte was taken in polarographic cell equipped with an electrode assembly described earlier. The solution was deaerated by bubbling nitrogen gas through the solution for ten minutes and polarogram was recorded.

RESULTS AND DISCUSSION

(I) Polarographic Analysis of Native DNA

The direct current polarogram and differential pulse polarogram of native DNA in 0.1M sodium sulphate and 0.3M ammonium tartrate as supporting electrolyte at pH 6.7±0.1 produces two well defined peaks with half wave/peak potential $E_{1/2}/E_p = -1.09V, 1.53V/-1.07V$ and -1.50V vs SCE for 40μg/ml concentration of DNA. [Fig 3.3(a) & 3.3(b)]

The height of each peak is directly proportional to the DNA concentration.[Fig. 3.4] The first peak with $E_p$ value -1.07V is dependent on DNA conformation and this peak is of nonfaradic nature, whereas, the second peak with $E_p$ value -1.50V vs SCE is due to the reduction of a particular base pair site i.e. Cytosine, involving 2-electrons. Since, under the given experimental conditions cytosine is polarographically reducible whereas, other sites like guanine are polarographically inactive. The half wave potential and height of the second peak is useful for its qualitative and quantitative analysis of DNA.
Fig. 3.2: Direct Current Polarogram for native DNA (blank) in 0.3M ammonium tartrate and 0.1 M sodium sulphate at pH 6.7 ± 0.1

Fig. 3.3 (a): Direct Current Polarogram for 40μg/ml native DNA in 0.3M ammonium tartrate and 0.1 M sodium sulphate at pH 6.7 ± 0.1
Fig. 3.3 (b): Differential Pulse Polarogram for 40μg/ml native DNA in 3M ammonium tartrate and 0.1M sodium sulphate at pH 6.7±0.1

Fig. 3.4: Differential Pulse Polarogram for 80μg/ml native DNA in 3M ammonium tartrate and 0.1M sodium sulphate at pH 6.7±0.1
(II) Polarographic Analysis of Denatured DNA

The direct current polarogram and differential pulse polarogram of denatured DNA in 0.1M sodium sulphate and 0.3M ammonium tartrate as supporting electrolyte at pH 11.3±0.1 produces a well defined peak with peak potential $E_{1/2}/Ep$ value = -1.36V/-1.34V vs SCE. [Fig. 3.5 (a) & 3.5 (b)]

This peak is due to reduction of single strand polynucleotide site. Its peak height was found to be proportional to the concentration of DNA. [Fig. 3.6]

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Fig. 3.5 (a) : Differential Current Polarogram for 40 $\mu$g/ml denatured DNA in 0.3 M ammonium tartrate and 0.1 M sodium sulphate at pH 11.3±0.1
Fig. 3.5 (b): Differential Pulse Polarogram for 40 μg/ml denatured DNA in 0.3 M ammonium tartrate and 0.1 M sodium sulphate at pH 11.3± 0.1

Fig. 3.6: Differential Pulse Polarogram for 80 μg/ml denatured DNA in 0.3 M ammonium tartrate and 0.1 M sodium sulphate at pH 11.3± 0.1
ISOLATION OF DNA FROM PLANT PRODUCT [CAULIFLOWER]

The knowledge of gene isolation was developed after gaining concept of physical or chemical characteristics of described DNA fragments; their shape, size and conformation can aid in selection of methods used to isolate and purify those segments.\(^{(31)}\)

Extraction

A standard method has been used for the isolation and precipitation of nuclear DNA from plant sample i.e. cauliflower. In this method;

Using a razor blade, remove 25 gm of the outer 2-3 mm of the cauliflower surface. Place the tissue in a mortar and add 25ml of sodium citrate solution. Grind the mixture until it becomes smooth slurry. Add 150ml of cauliflower homogenization solution to the mortar. Continue grinding the mixture an additional 30 min. Filter the homogenate through a funnel lined with two layers of cheesecloth. Squeeze the cloth to recover any additional liquid. Transfer to liquid to two centrifuge bottles. Add 2 volumes of absolute ethanol to each the bottle while sturring continuously. Balance the bottles and centrifuge them for 5 min at 200 × g at 4OC. Pour off the supernatant, saving the pellet containing nuclei at the bottom of the bottles. Add 1.5 volumes of sodium chloride solution and stir the mixture. Transfer the mixture to a pair of clean centrifuge tubes. Centrifuge them at 10,000 × g for 25 min at 25OC.Save the
supernatant in a clear beaker. Resuspend the pellet in 15ml of sodium chloride solution. Centrifuge the mixture at 10,000 × g for 25 min at 250C. Add the supernatant to the beaker containing supernatant from the previous centrifugation. Slowly add an equal volume of absolute ethanol while slowly stirring with a glass rod. Fibrous DNA strands will collect on the rod; continue stirring until DNA longer adheres to the rod.

Methodology

The extracted sample solution-containing DNA was converted into analyte by mixing 1ml of extracted DNA sample solution with 2.5ml of 1.0M sodium sulphate and 2.5ml of 3.0M ammonium tartrate as supporting electrolyte and total volume of analyte was made upto 25ml with doubly distilled water and pH of the test solution was adjusted to 6.7±0.1. The polarogram was recorded.

Results and Discussion

The differential pulse polarogram of extracted DNA from cauliflower, in 0.1M sodium sulphate and 0.3M ammonium tartrate at pH 6.7±0.1 produces two well defined peaks with peak potential Ep= -1.15V and -1.48V vs SCE. [Fig.3.7]

The method of external spiking [Table 3.1] was used for the qualitative and quantitative analysis of the DNA in the extracted sample and also to counter the matrix effect. The results were confirmed by spiking the sample. [Fig. 3.8]
Fig. 3.7: Differential Pulse Polarogram for Isolated DNA in cauliflower sample in 0.3M ammonium tartrate and 0.1M sodium sulphate at pH 6.7±0.1

Fig. 3.8: Differential Pulse Polarogram for Isolated DNA in cauliflower sample (after spiking) in 0.3M ammonium tartrate and 0.1 M sodium sulphate at pH 6.7±0.1
The resulting polarogram showed an increase in peak height without any change in Ep value thus confirming the presence of DNA in plant product (cauliflower). The percentage recovery and standard deviation of data was 99.2% and 0.98% respectively, which speaks the usefulness of the developed polorographic procedure for the analysis in natural origin sample for its DNA content.

**Table 3.1 : DPP Data for DNA Content in Plant Product (Cauliflower) Sample**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>DNA in cauliflower sample (µg/ml)</th>
<th>Percentage Recovery</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found 520.0</td>
<td>Added —</td>
<td>Recovered —</td>
</tr>
<tr>
<td>1.</td>
<td>520.0 490.0</td>
<td>1002</td>
<td>99.2</td>
</tr>
</tbody>
</table>

Average of four analyses

**Final Results and Discussion:** The amount of DNA content present in cauliflower sample was found to be 5.2%.

**ISOLATION OF DNA FROM HUMAN BLOOD SAMPLE**

In human blood, DNA is found mostly in white blood cells that contain nucleus in which chromosomes, the carrier of heredity are present.\(^{32}\) The concentration of DNA in blood is very low. As such, an analytical procedure has been developed for an accurate analysis of DNA in human blood sample with high accuracy and precision of determination.
Human Blood Sampling

Human blood was sampled by vein puncture of anticubital vein using sterilized polystyrene syringe with stainless steel tips to be used once only. 1.0 ml of 3% sodium citrate solution was taken in the syringe as anticoagulant before blood sampling. Standard method\(^{33}\) using for isolation of DNA from blood sample. In the extraction method blood sample is collected into EDTA blood tubes (e.g. 27.326 tubes) and kept at room temperature or at 4°C. The blood may be frozen is not recommended.

Mix 1 volume of blood with 3 volumes of red cell lysis buffer (155mM ammonium chloride, 10mM potassium bicarbonate, 0.1mM EDTA) in a disposable plastic Universal tube (e.g. Sterilin cat. No. 128A) and place in an ice/water mix for 10 min. Centrifuge at 170 g for 10 min. Discard the supernatant into a dilute solution into dilute solution of sodium hypochlorite (domestic bleach). Resuspend the white cell pellet in 4ml white cell suspension medium (0.3M sodium acetate, 20mM Tris-HCl- pH 7.5, 1mM EDTA) in a 13ml disposable polypropylene centrifuge tube. Add 200μl 10% SDS and mix gently to lyse the cells.

The lysate is extracted gently, but thoroughly, with an equal volume of phenol/chloroform/iso-amyl-alcohol/8-hydroxy-quinoline (50:50:1:0:1) for 5 min. Any shorter time is insufficient for adequate extraction of proteins. Phases are separated by centrifugation at 2000 g.
The upper aqueous layer is removed with a wide bore pipette to a fresh tube and the phenol extraction repeated. The aqueous layer is recovered ones more and extracted with an equal volume of chloroform/iso-amyl-alcohol (24:1) then centrifuge as before. The aqueous layer is transferred to a fresh tube.

DNA is precipitated by addition of 2 volumes of absolute ethanol. The DNA is recovered by centrifugation and rinsed in 2ml of 70% ethanol. The DNA is again recovered by centrifugation and dissolved in an appropriate volume of TE (10mM Tris-HCl, 1mM EDTA-pH 7.6). Shake gently overnight to dissolve.

Methodology

For the diagnosis of DNA in blood sample, the extracted DNA from blood was dissolved in water and it was converted into analyte by mixing 1ml of extracted DNA with 2.5ml solution of 1.0M sodium sulphate and 2.5ml of 3.0M ammonium tartrate as supporting electrolyte and total volume of analyte was made upto 25ml with doubly distilled water and pH of the test solution was adjusted to 6.7±0.1. The polarogram was recorded.

Results and Discussion

The isolated DNA samples from blood produced DPP curve with peak potential $\text{Ep} = -1.5V$ vs SCE. [Fig. 3.9] Thus,
confirming the presence of DNA in the blood sample. The analyte was spiked by addition known amount of DNA solution and the polarogram was recorded again. The resulting polarogram [Fig. 3.10] showed a little shift in Ep value thus confirming peak due to DNA. The shift in Ep value may be due to matrix effect. The amount of DNA in blood sample was calculated and has been reported in [Table 3.2].

The observed percentage recovery of the data is 99.7% and standard deviation 0.8% respectively. Thus, confirming the utility of developed analytical procedure for on accurate analysis of DNA in blood sample.

Fig. 3.9: Differential Pulse Polarogram for Extracted DNA in blood sample in 0.3M ammonium tartrate and 0.1M sodium sulphate at pH 6.7 ± 0.1
Fig. 3.10: Differential Pulse Polarogram for Extracted DNA in blood sample (after spiking) in 0.3M ammonium tartrate and 0.1M sodium sulphate at pH 6.7± 0.1

Table 3.2: DPP Data for DNA Content in Human Blood (White Blood Cells) Sample

<table>
<thead>
<tr>
<th>S.No.</th>
<th>DNA in blood sample (µg/ml)</th>
<th>Percentage Recovery</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found</td>
<td>Added</td>
<td>Recovered</td>
</tr>
<tr>
<td>1.</td>
<td>205.4</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>2.</td>
<td>205.4</td>
<td>205.0</td>
<td>409.4</td>
</tr>
</tbody>
</table>

Average of four analyses

Final Results and Discussion: The amount of DNA present in blood is 205µg/ml.
MUTATIONAL STUDY/GENETIC ENGINEERING

Mutation is the any change in nitrogenous base sequences of DNA molecule and these changes are heritable. Mutation has great importance in elucidating both genetic and biochemical properties of living systems.

The polarographic analysis is based on the behaviour of electroactive species, but any change in conformation or structure of electroactive species, changes its polarographic nature too. It is due to this reason polarographic method has been used for the study of mutational change in DNA fragment by chemical mutagens.

A chemical mutagen is a substance that can alter a base that is already incorporated in DNA and thereby change its hydrogen-bonding specificity. Two commonly chemical mutagens are:

(I) Hydroxylamine

It (NH₂OH) is a known chemical mutagenic agent. It is often used to mutagenize DNA in vitro, when used in vitro, it react specifically with cytosine site converting it to a modified base (N₄-hydroxy cytosine), that pairs with adenine instead of guanine. This has two consequences:

Hydroxylamine produces only GC→AT transitions

Mutations induced by hydroxylamine cannot be reverted by hydroxylamine (when hydroxylamine is used in-vitro it produces free radical that damage the DNA and this DNA damage include the SOS system resulting in a wide variety of types of mutations.)
Hydroxylamine

Cytosine  →  Intermediate  →  NH₂OH

Sugar  →  Modified Cytosine  ↔  Tautomeric Forms  →  Modified Cytosine

Sugar

Reaction of Hydroxylamine with Cytosine site of DNA

Modified Cytosine  Adenine

Base Pairing of the Proposed Reaction Product
(Hydroxylamine with Cytosine) to Adenine
Methodology and results:

The polargraphic analysis of pure DNA has been mentioned earlier. Its DPP produces two well defined peaks with peak potential $E_p = -1.07V$ and $-1.50V$ vs SCE, [Fig. 2(b)] but on the addition of hydroxylamine as a mutagenic agent, the peak potential of mutant DNA in 0.3M ammonium tartrate and 0.1M sodium sulphate as supporting electrolyte at pH $6.7 \pm 0.1$ shifted to $-1.50V$ to $-1.53V$ vs SCE, [Fig. 3.11] which may be explained on the basis of mutational change. These changes are due to changed DNA conformation.

![Differential Pulse Polarogram for Mutant DNA](image)

**Fig. 3.11**: Differential Pulse Polarogram for Mutant DNA with hydroxylamine in 0.3 M ammonium tartrate and 0.1 M sodium sulphate at pH $6.7 \pm 0.1$

(II) Ethyl Methane Sulfonate

It is an alkylating agent. Many sites in DNA are alkylated by these agents: the major effect of EMS is the addition of an alkyl
group to the hydrogen-bonding oxygen of guanine and thiamine. These alkylations impair the normal hydrogen bonding of the bases and cause mispairing of G and T, leading to the transition AT→GC and GC→AT (the latter markedly predominates). EMS also reacts with adenine and cytosine.

![Ethyl Methane Sulfonate](image)

**Reaction and Base Pairing of Ethyl Methane Sulfonate with Guanine and Cytosine Base Pairs**

**Methodology and Results**:
As has been discussed earlier, DNA produces two well defined peaks with peak potential $E_p = -1.07V$ and -1.50V vs SCE. On adding the mutagenic agent EMS to the DNA Analyte, the
peak potential of the second peak of second peak from -1.50V to -1.48V vs SCE. [Fig. 3.12] Thus indicative the combination of EMS with the cytosine site of DNA.

Resulting in alkylation of the hydrogen bonding oxygen of guanine and thiamine these alkylations impair the normal hydrogen bonding of the bases and cause impairing of G and T bonding to transitions GC→AT.

Fig. 3.12: Differential Pulse Polarogram for Mutant DNA with Ethyl Methane Sulfonate in 0.3 M ammonium tartrate and 0.1 M sodium sulphate at pH 6.7±0.1

CONCLUSION

The significance of the present work using modern electro analytical method in research on nucleic acid properties lies in its ability to ignore thousands of base pairs irregularly arranged in the double helix of DNA and recognize the presence of small
number of bases which form anomalies in the structure. The oligo determination ability of DPP has made it possible to use it for an accurate analysis of DNA in samples of natural origin. Besides, the results have proved the ability of poloragrpnic method for the study of mutational changes i.e. genetic engineering. However, it is only the beginning further possibilities are to be explored in future.
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