PROJECT REPORT

On

“EXTRACTION OF DNA FROM BANANA AND STUDY OF ITS INTERACTION WITH METHYLENE BLUE”

Submitted by

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In partial fulfillment for the award of the BSc Degree in Chemistry

POST GRADUATE AND RESEARCH DEPARTMENT OF CHEMISTRY

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Year of Work : 2018-2019

This is to certify that the project "**EXTRACTION OF DNA FROM BANANA AND STUDY OF ITS INTERACTION WITH METHYLENE BLUE**" is the work done by **ANJALI.U.R, SAHALA NAYEEM.A.A, VANDHANA.T.B, SREELAKSHMI.S**

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Submitted to the Examination of Bachelor's Degree in Chemistry
Date:……………
Examiners:……………………………………….

: ……………………………………….

DECLARATION

We hereby declare that the project work entitled "EXTRACTION OF DNA FROM BANANA AND STUDY OF ITS INTERACTION WITH METHYLENE BLUE" submitted to Department of Chemistry, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, is a record of an original work done by us under the guidance of Dr. Elizabeth Kuruvila, Assistant Professor, Department of Chemistry, St. Teresa's College (Autonomous), Ernakulam and this project work is submitted in the partial fulfillment of the requirements for the award of the degree of Bachelor of Science in Chemistry.

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ANJALI.U.R, SAHALA NAYEEM.A.A, VANDHANA.T.B, SREELAKSHMI.S

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Chapter 1

Introduction

1.1. DNA

Nucleic acids are the biopolymers, essential to all known forms of life. The monomers present in the nucleic acids are the nucleotides which consists of three main parts and they are a phosphate molecule, a pentose sugar and nitrogen bases. Based on the pentose sugar and the nitrogen bases present, the nucleic acids are divided into two: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). There are two kinds of bases present; purines and pyrimidines. Adenine and guanine are the purine bases. Cytosine and thymine are the pyrimidine bases present in DNA while in RNA thymine is replaced by uracil. The pentose sugar present in the DNA molecule is the deoxyribose sugar. The backbone of each strand is the repeating pattern of a 5- carbon sugar and a phosphate molecule. Each sugar is attached to one of the four nitrogen bases- Adenine (A), Thymine (T), Guanine (G) and Cytosine (C). Each nitrogen bases are bonded through hydrogen bonding. Adenine and thymine are paired by two hydrogen bonds whereas cytosine and guanine are paired by three hydrogen bonds. The bases are stacked up the ladder and the hydrophobic bonding between the bases gives the DNA molecule stability[1]. The two DNA strands run in opposite directions that

is, they are antiparallel. X-ray studies have shown that each turn of the helix contains 10 nucleotide pairs and the diameter of the helix is 20 $A^{0.}$ The spacing between adjacent pairs is 3.4 A^{0}. It can be seen from this arrangement of the helices that the two DNA chains must be complementary to each other, i.e., a chain with given sequences of bases can pair only with another which has the complementary sequences of bases[4]. Figure 1 shows the structures of nitrogen bases of DNA paired with its complementary bases. Deoxyribonucleic acid is a molecule composed of two chains of nucleotides that coil around each other to form a double helical structure carrying the genetic instructions used in the growth, development, functioning and reproduction of all known living organisms and many viruses. DNA stores the biological information. Since the DNA contains genes that carry hereditary characters, DNA is the genetic material of the cell.

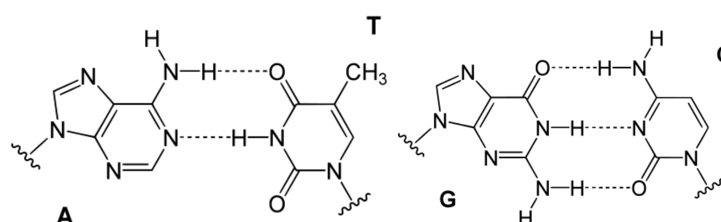

Figure 1. Base pairing in DNA

The history of genetic research began with Gregor Mendel, father of genetics. He had performed an experiment with plants in 1857 that led to increased interest in study of genetics. In 1869, Friedrich Miescher discovered a substance called "Nuclein". It was first isolated by him and in 1889 his pupil named it as nucleic

acid[3]. Thereafter two researchers, Rosalind Franklin and Maurice Wilkins studied the crystal structure of DNA through the X-ray studies and they found that DNA molecule has helical shape[4]. In 1953, James Watson and Francis Crick put forward the double helical model of DNA in which the nitrogen bases on two different strands were connected through the hydrogen bonds[5]. Figure 2 shows the double helical model of DNA.

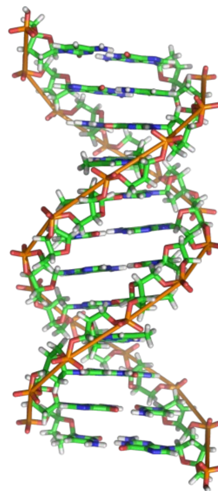

Figure 2. Double helical structure of DNA

The double strand structure of DNA is stabilized when various weak forces comes together[6].

1. Hydrogen bonding (2-3 kcal/mol per base pair)
   They are able to stabilize the helix because a large number of H-bonds is present in DNA.
2. Stacking interactions (4-15 kcal/mol per base pair)
   They are also known as van der Waals interactions. These interactions

are weak, but large amount of these interactions help to stabilize the overall structure of the helix.

Double helix structure is stabilized by hydrophobic effects by keeping the hydrophobic bases clustered in the interior of helix, away from surrounding water.

By van der waals force the stacked base pairs are attracted to each other. Energy associated with a single van der Waals interaction has small significance to the overall DNA structure. But the net effect of numerous atom pairs results in substantial stability.

3. Charge- charge interactions

It refers to the electrostatic (ion-ion) repulsion of the negatively charged phosphate. It is potentially unstable. Double helix is however stabilized by the presence of Mg^{2+} and cationic proteins with abundant arginine and lysine residues. The phosphates on outside interact with H_2O and counter ions (Mg^{2+}, K^+ etc).

1.2. Drug-DNA Interaction

DNA as carrier of genetic information is a major target for drug interaction because of the ability to interfere with transcription and DNA replication, a major step in cell growth and division. There are three principally different ways of drug-binding[7]. First, through control of transcription factors and polymerases. Here, the drugs interact with the proteins that bind to DNA. Second, through RNA binding to DNA double helices to form nucleic acid triple helical structures or RNA hybridization (sequence specific binding) to exposed DNA single strand regions forming DNA-RNA hybrids that may interfere with transcriptional

activity. Third, small aromatic ligand molecules that bind to DNA double helical structures by (see figure 3) (i) intercalating between stacked base pairs thereby distorting the DNA backbone conformation and interfering with DNA-protein interaction or (ii) the minor groove binders. Both work through non covalent interaction.

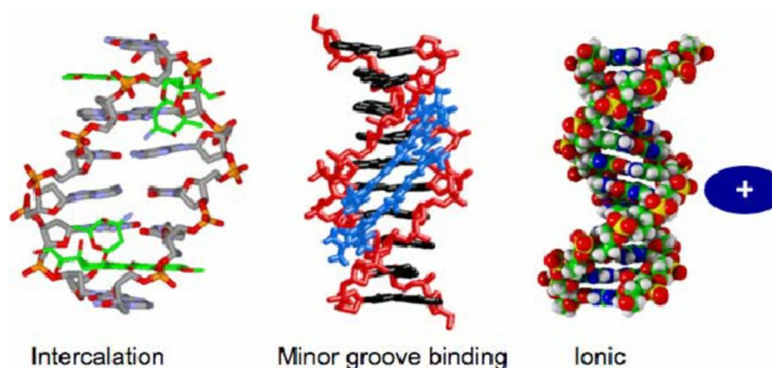

Figure 3. Different non-covalent interactions of small molecules with DNA.

Our objective was to isolate and purify DNA from banana extract and study the interaction of methylene blue dye with the DNA. Methylene blue has a planar aromatic structure with a positive charge. Therefore it can interact with DNA through intercalation as well as electrostatically.

Chapter 2

Materials and Methods

2.1. Materials Required

Extraction of DNA from banana

Ripe banana, 150 ml of soap solution, 3.2 g common salt, 2mM sodium chloride, 10 mM disodium hydrogen phosphate, Methylene blue, Coffee filter, Sterilized centrifuge tubes, 500 ml standard flask, 0.2 microns Whatman sterilized syringe filter. Absorption was recorded using ThermoScientific Spectrophotometer.

2.2. Procedure

The procedure for the project can be divided into three steps

1. DNA extraction from fruit
2. Preparation of buffer solution
3. Analysis using UV-Visible spectrophotometry

2.2.1. DNA extraction from fruit

The fruit that we chose in our project was banana. The fleshy portion of banana was smashed and made the pulp of it. This pulp was added to a 250 ml beaker where the 150 ml of soap solution and 2g of salt are mixed.

It was stirred well to attain uniform consistency. This mixture was filtered using coffee filter. It was kept in the freezer for overnight freezing. It was taken out on the next day and 2 ml of the filtered banana pulp was added into a sterilized centrifuge tube and four times 99% of isopropyl alcohol was added into it. The DNA got precipitated out and it was centrifuged. The filtrate was removed and the precipitated DNA was kept in the freezer. The next step was to wash DNA using isopropyl alcohol and water. The tube containing DNA was taken and isopropyl alcohol and water in the ratio 8:2 respectively were added into it. It was shaken well and froze it. It was centrifuged and the filtrate was removed. The process was repeated three times and the DNA was dried.

2.2.2. Preparation of the buffer solution

2mM sodium chloride and 10mM disodium hydrogen phosphate was taken and it was made up in a 500 ml standard flask using water. It was shaken well for uniform concentration. Buffer solution was added into the DNA. The precipitated DNA gets dissolved in it. It was then filtered using 0.2 micron Whatman's sterile syringe filter.

2.2.3. Analysis using Spectrophotometry

The buffer solution was taken in a clean quartz cuvette and analyzed. The blank spectrum was recorded. The spectrum of DNA was recorded and the absorption at 260 nm was taken to calculate the concentration of DNA

using online DNA concentration calculator. Spectrum of methylene blue was recorded and the absorption at 670 nm (molar extinction coefficient 95000 $M^{-1}cm^{-1}$) was taken to calculate the concentration using Beer-Lambert's law. Aliquots of DNA was added from the stock solution and absorbance was measured after each addition.

Chapter 3

Results and discussion

Extraction of DNA: Crushing the fruit physically breaks apart the cell walls. After the cell walls have been disrupted during mechanical mashing of the fruit, the detergent in the shampoo disrupts the cell and nuclear membranes of each cell to release the DNA. It does this by dissolving lipids and proteins that hold the membranes together. The salt neutralizes the negative charges on the DNA and thus enables the DNA strands to stick together. It also causes proteins and carbohydrates to precipitate. DNases or restriction enzymes that destroy DNA are present in the cell's cytoplasm. They are there to protect the cell from invasion by viruses. Once the nuclear membrane is destroyed by the soap, the DNA is now susceptible to the DNases and will quickly be degraded. However, these enzymes are temperature sensitive and cooling the solution slows down the process of degradation. Everything except the DNA will dissolve in ethanol. The ethanol pulls water from the DNA molecule so that it then collapses in on itself and precipitates. The DNA will become visible as white mucous strands that can be spooled with a wooden stick. The precipitated DNA was washed with 80% ethanol water mixture, centrifuged, separated and dried. The dried DNA was dissolved in 2 mM phosphate buffer, filtered and used for further studies. Figure

4 shows the absorption spectrum of the DNA. The absorption maximum occurs at 260 nm, which is characteristic of DNA. Using the absorbance at 260 nm, the concentration of DNA was calculated (1 OD = 50 μg/ml)

Study of interaction of the extracted DNA with Methylene blue: The interaction of methylene blue with the extracted DNA was studied using UV-Visible absorption spectroscopy. Figure 5 shows the change in the absorption spectrum of methylene blue with increasing concentrations of DNA. Methylene blue exhibits absorption from UV to 720 nm with two peaks with maxima at 290 nm and 660 nm.

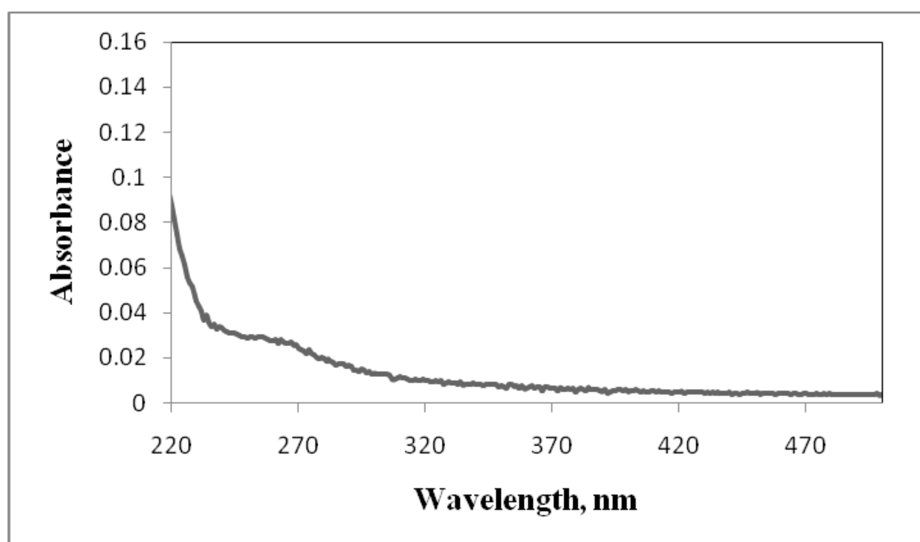

Figure 4. Absorption spectrum of DNA

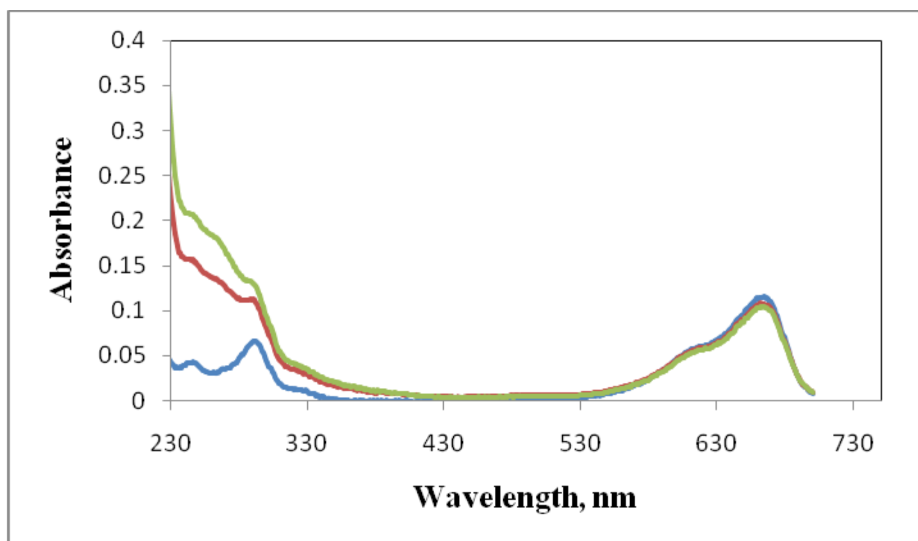

Figure 5. Change in absorption spectra of methylene blue (1.06 µM) with increasing concentrations of DNA. (i) Blue- methylene blue alone (ii) Brown- methylene blue in the presence of 1.38 µg of DNA (iii) Green- methylene blue in the presence of 5.8 µg of DNA.

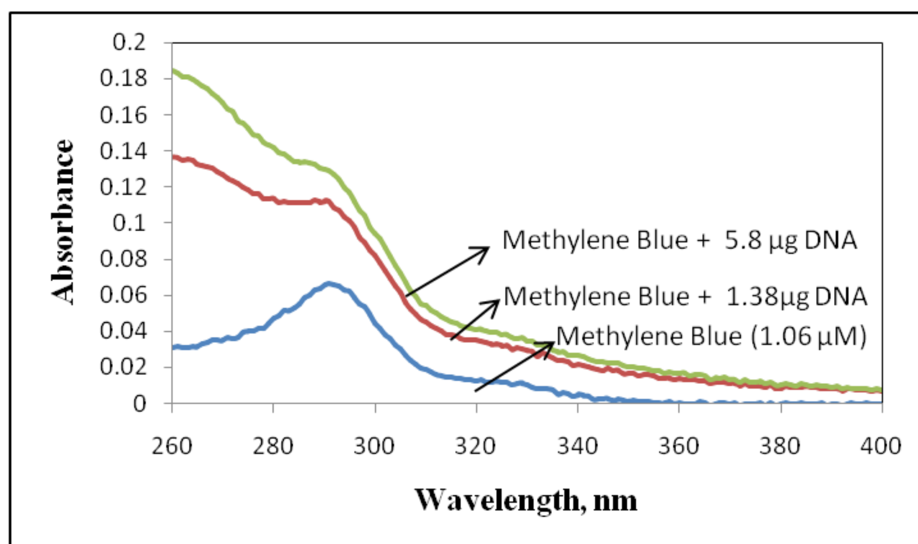

Figure 6. Change in absorption spectra of methylene blue (1.06 µM) with increasing concentrations of DNA.

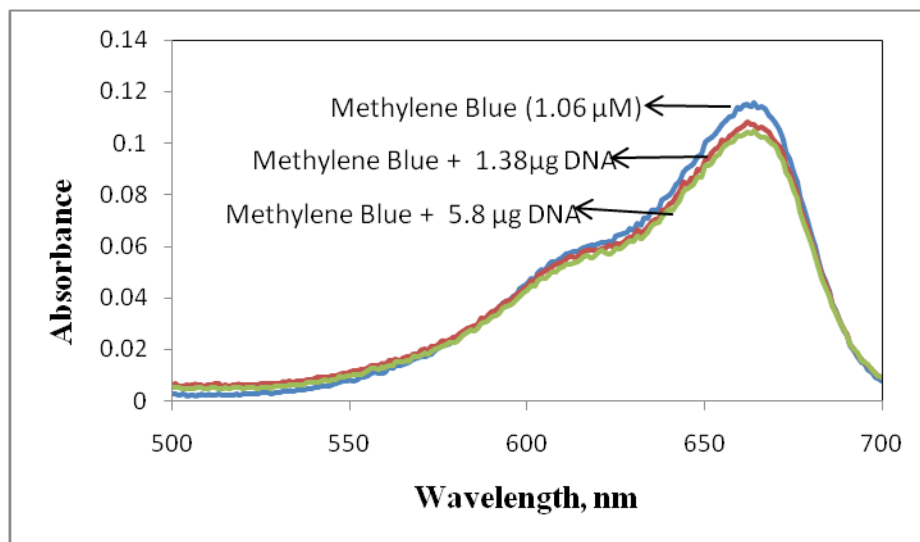

Figure 7. Change in absorption spectra of methylene blue (1.06 µM) with increasing concentrations of DNA.

The band at 660 nm exhibits a shoulder at 607 nm. Figure 6 and 7 shows the enlarged portion of the spectra at 290 nm and 660 nm. The peak at 290 nm shows hyperchromicity with increasing concentration of DNA. The peak at 660 nm shows hypochromicity with a 2 nm red shift. These changes shows that methylene blue undergoes non-covalent interaction with DNA. The hyperchromicity at 290 nm may also be due to the absorption by DNA which has λ_{max} at 260 nm. However the observed hypochromicity with red shift at 660 nm clearly indicate intercalative interactions. Since methylene blue is positively charged, electrostatic interaction will also stablize its complexation with DNA.

Chapter 4

Conclusions

DNA was extracted from banana pulp, purified and obtained in quantitative yields. Interaction of DNA with methylene blue was studied using UV-visible absorption spectroscopy. Our results shows that methylene blue undergoes electrostatic and intercalative interaction with DNA.

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