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Using Electrochemical Method to Study the Interaction Between DNA and a compound Known to Have Anticancer Property.

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Using Electrochemical Method to Study the Interaction Between DNA and a Compound Known

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In partial fulfillment

of the requirements for the degree

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by

Alexander Kamasah

December 2011

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Dr. Peng Sun

Dr. Chu Ngi Ho

Dr. Yu-Lin Jiang

Keywords: cyclic voltammogram, DNA modified gold electrode, intercalation

ABSTRACT

Using Electrochemical Method to Study the Interaction Between DNA and a compound Known

to Have Anticancer Property

by

Alexander Kamasah

The interaction of 4-(1'-[8'-(1''-pyrenyl)naphthyl])-2,6-diaminopyridine bis-glycamide

bishydrocholride known to have anticancer properties with deoxyribonucleic acid (DNA) had

been studied on a modified gold electrode by self-assembled monolayer using cyclic

voltammetry. $K_4Fe(CN)_6$ was used as a probe to obtain electrochemical information on the

electrode surface.

A bare gold (Au) electrode was modified with cysteamine and our results showed no change in

the limiting steady state current as compared to the bare Au electrode. There was a reduction in

the steady state current after the modification of the gold electrode with DNA. Immobilization

of the DNA modified gold electrode with the anticancer compound also revealed a further

reduction in the steady state current. The reduction in the steady state current is attributed to the

receptor and DNA forming a nonelectrochemical complex due to the intercalation of the receptor

and DNA on the gold electrode surface.

DEDICATION

To Marian Osei-Mensah, my dearest wife, Gladys Doku, my Mother, and Samuel Boi-Kamasah, my Father.

ACKNOWLEDGEMENTS

I am most grateful to the Almighty God for His protection and guidance through my graduate studies.

I would also want to express my sincere gratitude to Dr. Peng Sun for his excellent mentorship and constructive criticism of this research. I am most grateful to him. I am also most grateful to Dr. Chu-Ngi Ho and Dr. Yu-Lin Jiang for their willingness to serve on my committee.

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CHAPTER 1

INTRODUCTION

Cellular processes such as recombination, replication, transcription, and repair involve the interaction of DNA and protein. Quiet a large number of the intracellular processes of living things rely on a distinctive and controlled interaction between nucleic acids and proteins [1]. Also, many of the proteins are involved in specific sequence DNA binding in the gene promoter region that regulates gene expression and maintenance [2]. The preciseness and potency of these interactions are controlled by the exact structure of the interacting molecule. Apart from these specific interactions, there are other many proteins that bind in a non-specific way to nucleic acid and to other proteins [1]. There are certain organic molecules that bind to these DNA with high affinity and end up influencing its gene expression and therefore affect the cell proliferation. Apart from these specific interactions, there are other many proteins that bind in a non-specific way to nucleic acid and to other proteins [3].

The continuous exposure of DNA cells either by chemical or physical methods to chemicals destroy the viability of the cells [4]. If these impaired DNA bases are not repaired, it could cause mutation in the DNA cells [5] and can lead to the death of the cell by obstructing DNA replication [6]. It is of great significance to study damaged DNA surface because by doing so we would be able to identify early diagnostic of genetic deficiencies and detection of infectious diseases. [7, 8]. Quite a number of methods have been used to study the interaction between a DNA surface and a protein molecule some of which includes UV spectroscopy, electrochemical impedance spectroscopy, surface plasmon resonance, colorimetric, and electrochemical methods [9, 10]. Among these methods electrochemical

methods (using cyclic voltammetry) have been found to be the most convenient to use due to its high sensitivity, low cost characteristic, and compatibility with microarray technology.

A very useful and efficient analytical tool is therefore needed to study these interactions between DNA and protein molecules as it would give us the opportunity to evaluate and predict possible anticancer drugs-DNA interactions. In recent years a lot of studies on the properties of anti-carcinogenic drugs and their interactions with DNA are becoming more and more important in developing new cancer therapy treatment. The study of these interactions between DNA and proteins is becoming more important due to the resemblance of electrochemical reactions and biological reactions such as oxidation mechanism taking place at the electrode surface and also those occurring in living organism [11].

Electroanalytical analyst has adopted a better way of studying the surface properties and interactions between protein molecules and DNA. This involves the use of a modified electrode because of its higher sensitivity and specificity as compared to the unmodified electrode. The use of DNA modified electrode overcomes the limitations of other methods in large requirement for DNA samples and they also exhibit more useful electrochemical properties than the unmodified electrode [12].

<div align="center">Electrochemistry</div>

Cyclic Voltammetry

Cyclic voltammetry (CV) is a type of electrochemical technique that involves the potential of a stationary working electrode being changed linearly with time starting from a potential where no electrode reaction occurs and moving to potentials where reduction or oxidation of solute (the material being studied) occurs. Cyclic voltammetry provides a simple correlation between experimental results and redox potential, diffusion coefficients, and rate

constant [13]. This type of technique is generally used to study the electrochemical properties

of an analyte in solution and the analyte needs to be redox active within the experimental

potential framework. The analyte is electrolyzed (oxidized or reduced) by placing the solution in

contact with the electrode surface and making that surface positive or negative in voltage to

enhance electron transfer. The transfer of electrons or the flow of current comes about when the

potential of the electrode which is very positive of the formal potential of the electrode (E^O) and

it is adjusted negatively, then reduction begins once the potential reaches the vicinity of (E^O). A

rapid increase in current occurs, as the surface concentration of the reactive species becomes

smaller. The current will increase as the potential reaches the reduction potential of the analyte

but then falls off as the concentration of the analyte is depleted close to the electrode surface.

A reversal of the potential to a more positive value causes the reduced species to be

oxidized as the current moves towards (E^O). A graph of current (I) against voltage (v) can be

plotted and two measured parameters that is the ratio of the peak currents (I_{pa}/I_{pc}) and the

separation of the peak potential E_{pa}-E_{pc} can be obtained. [5]. An example of cyclic voltamogram

is shown in Figure1.
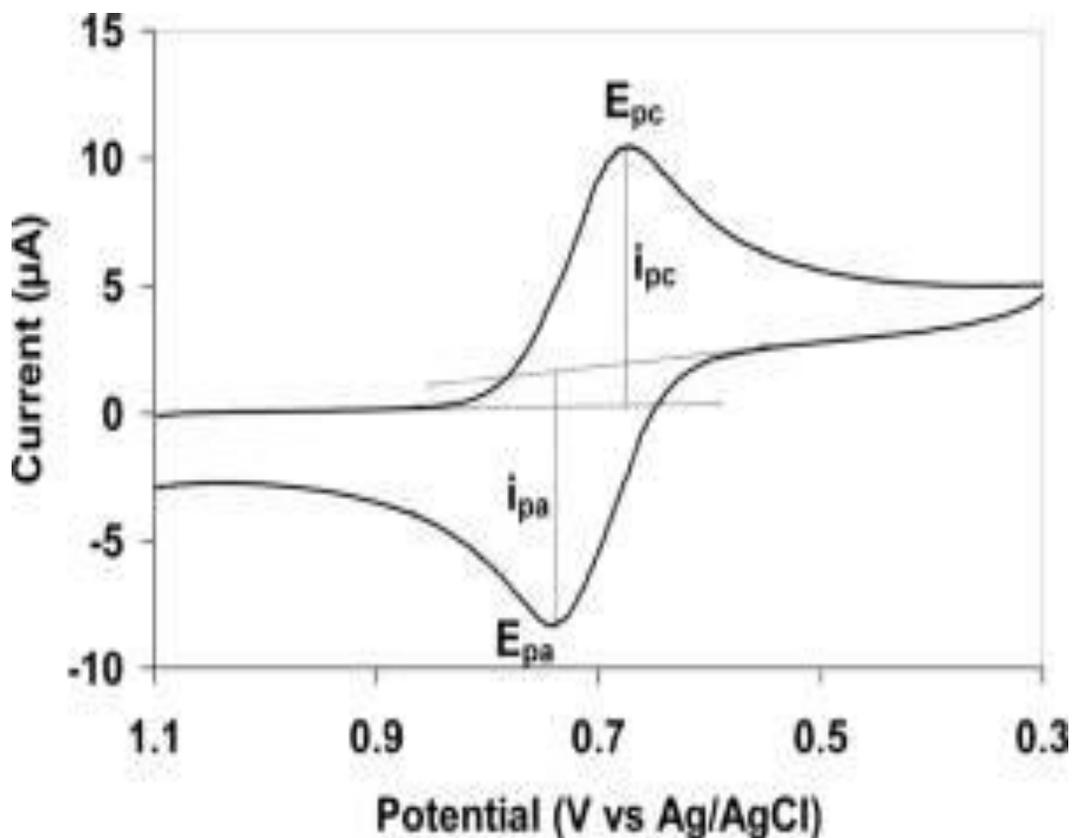
Figure 1: A cyclic voltammogram for a bare gold electrode. The anodic and cathodic peak current has been indicated by I_{pa} and I_{pc} respectively

The magnitude of the peak currents, that is Ipa and Ipc and the potential at which the various peaks occur (Epa and Epc), are the most important parameters of cyclic voltammetry[14]. In cyclic voltammetry, three types of electrodes are used and these are the working electrode, reference electrode, and the auxiliary or counter electrode [15]. All the electrodes are then placed in a glass vial that is filled with a redox probe solution and the whole system is referred to as an electrochemical cell. A typical electrochemical cell has been illustrated in Figure 2.
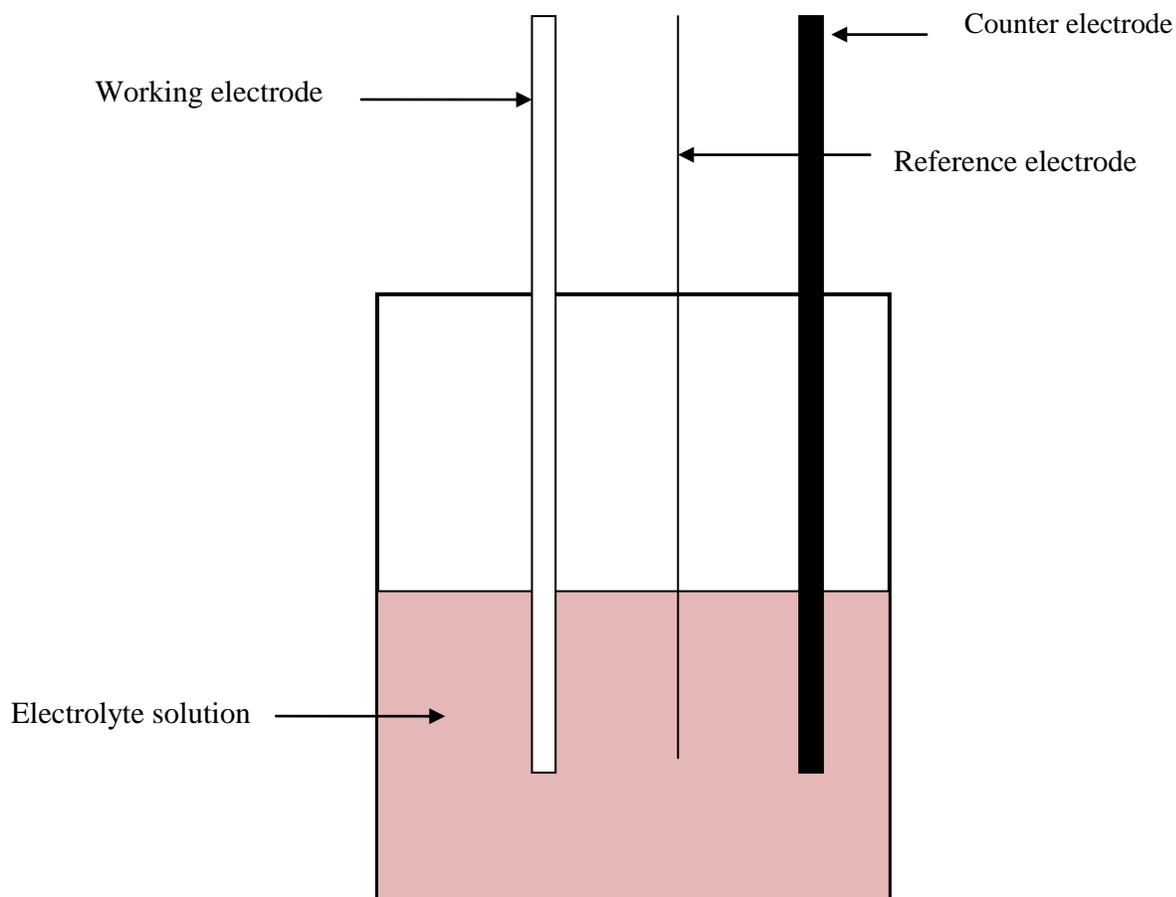
Figure 2: A general diagram of an electrochemical cell, showing the three electrodes system

employed in cyclic voltammetry

The working electrode is the electrode in electrochemical system on which the reaction of interest is occurring. The working electrode can either be cathodic or anodic depending on whether the reaction on the electrode is oxidation or reduction. The counter electrode is usually made of an inert material such as platinum wire that simply serves to conduct electricity from the signal source through the solution to the other electrodes. The counter electrode often has a large surface area that is much larger than that of the working electrode to ensure that the half reaction occurring at the counter electrode can take place faster so as not to limit the process at the working electrode. The

reference electrode consists of a piece of silver wire that is coated with AgCl. The reference electrode is always at a known potential; this constant potential value offers a standard against which to measure the working electrode potential [16].

Immobilization of DNA on Gold Surfaces

Quiet a number of techniques have been postulated to explain the surface-immobilization of DNA on electrodes and among such methods include chemical adsorption [17, 18], covalent binding [19, 20, 21], electrostatic attraction [22, 23, 24], and co-polymerization [25]. As a result of the unprecedented growth in the development of electrochemical DNA biosensors, it would be of great importance to seek for new methods of surface-immobilization on gold electrode surface to improve upon the sensitivity of a DNA biosensor.

Two ideal techniques have been found to be useful for DNA surface modification on electrode and these are Self-assembly monolayer (SAM) and Layer by Layer method.

Self-Assembly Monolayer (SAM)

The formation of self-assembled monolayers involves the presence of a bifunctional linker such as cysteamine that serves as a means to bind the DNA to an electrode surface. It involves a layer of material that binds to a given surface in an ordered form due to chemical or physical forces. The length of alkyl chains present in alkanethiols determines the rate of electron transfer processes. Longer chains show a great resistant in electron transfer as compared to shorter alkane chains [26, 27]. Figure 3 illustrates the formation of self-assembled monolayer (SAM).

S-(CH$_2$)$_2$NH$_3^+$

+ SH-(CH$_2$)$_2$-NH$_2$ $\longrightarrow$ S-(CH$_2$)$_2$NH$_3$+ $\xrightarrow{\text{OLIGONUCLEOTIDE}}$

S-(CH$_2$)$_2$NH$_3^+$

BARE GOLD ELECTRODE

S-(CH$_2$)$_2$-NH$_3^+$

S-(CH$_2$)$_2$-NH$_3^+$

S-(CH$_2$)$_2$-NH$_3^+$
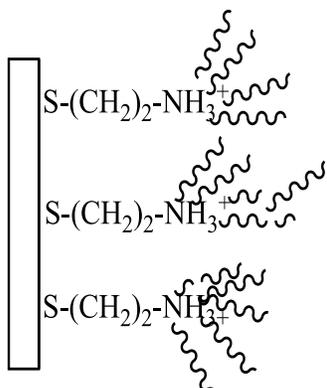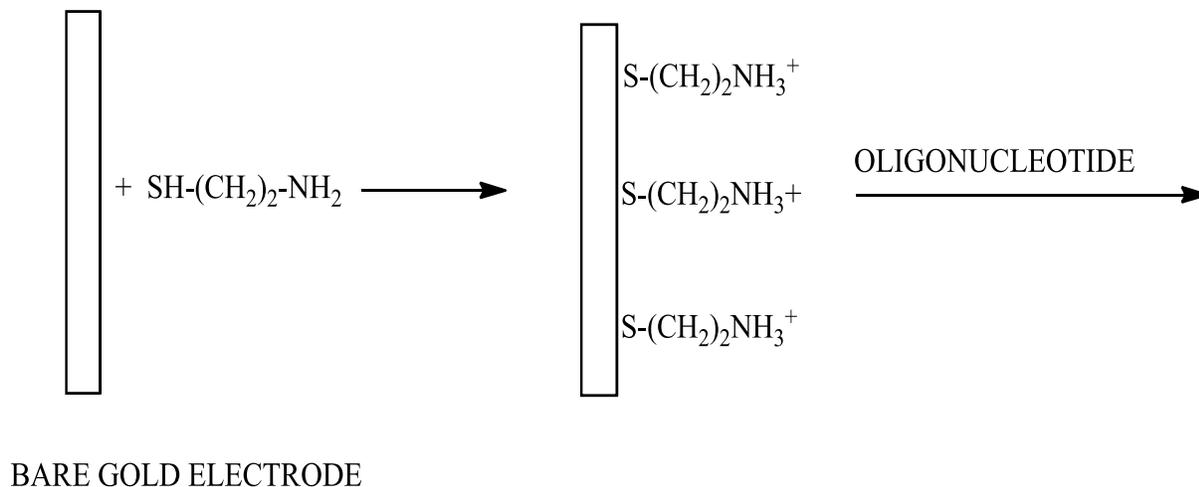
Figure 3: An illustration of a self-assembled DNA monolayer on a gold electrode

<u>Layer by Layer Method</u>

The layer by layer method enables multiple layers to form on a gold electrode. This is achieved by the immersion of gold electrode in a solution containing cysteamine. The cysteamine forms a positive charge monolayer on the gold electrode which then makes it possible for the negatively charged phosphate backbone of the DNA to be attached to it through electrostatic force of attraction. Another positively charged compound can then be immobilized as shown in Figure 4 [28].
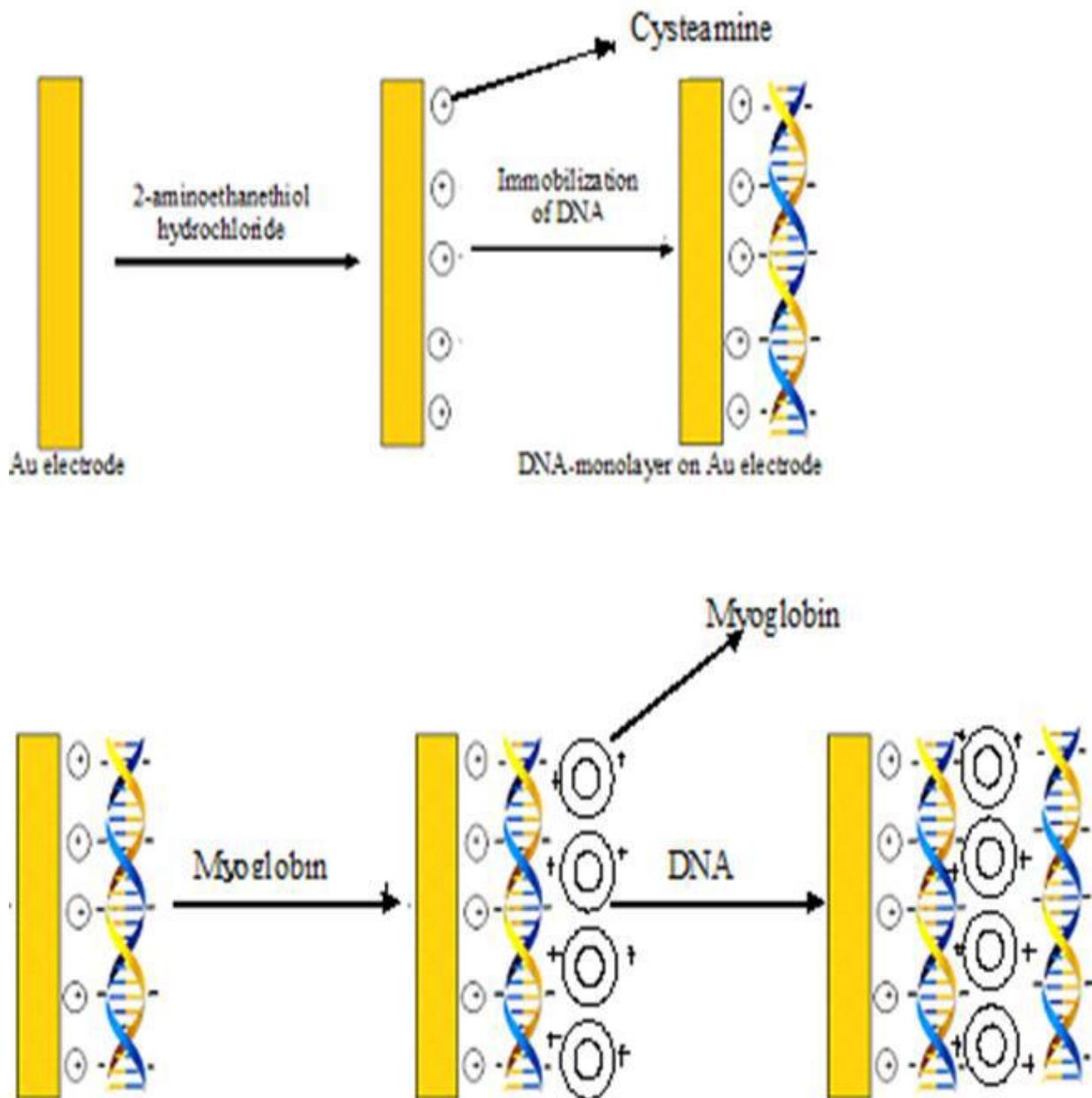
Figure 4: Schematic diagram showing layer by layer assembly method

Structure of DNA

A single strand of DNA is made up of a series of nucleotides. Nucleotides are made up of a nitrogenous base, a phosphate group and a 5- carbon sugar (pentose). The nitrogenous base together with the pentose is called nucleoside. There are four types of nucleotides found DNA, differing only in the nitrogenous base, and these bases are purines: which consists of adenine (A) and guanine (G), or pyrimidines: which also consists of thymine (T) and cytosine (C). In both DNA and RNA one of the pyrimidines is cytosine (C), and the other important pyrimidine is thymine (T) that is basically found in DNA and uracil (U) also found in RNA. The DNA molecule consists of two strands that form a double helix by coiling around its axis. The strands are antiparallel in nature and that demonstrates its 5',3'-phosphodiester bonds moves in opposite directions. On the outside of the double helix are negatively charged phosphate groups and the hydrophilic regions of the pentoses. The hydrophobic regions of the bases are inside the double helical structure of the DNA. The complementary nature of the interactions between the two strands of a DNA helix is determined by the base present on each strand. The conformation of the two strands creates two types of grooves called the major groove and the minor groove in each base pair. The major and minor groove can be of different sizes depending on the conformation of DNA and can be important structural determinants in the interactions between DNA and proteins. The structure of DNA presents a variety of sites where ligands or small molecules may interact to bind to the DNA. The major categories of non-covalent binding to the DNA are ionic interactions with the phosphate backbone, hydrophobic intercalative binding with the bases, and mixed interactions with the major or minor grooves [32].   Examples of the various nitrogenous bases and the interaction between Adenine and Thymine are shown in Figures 5 and 6 respectively.

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Adeninie · Guanine · Thymine
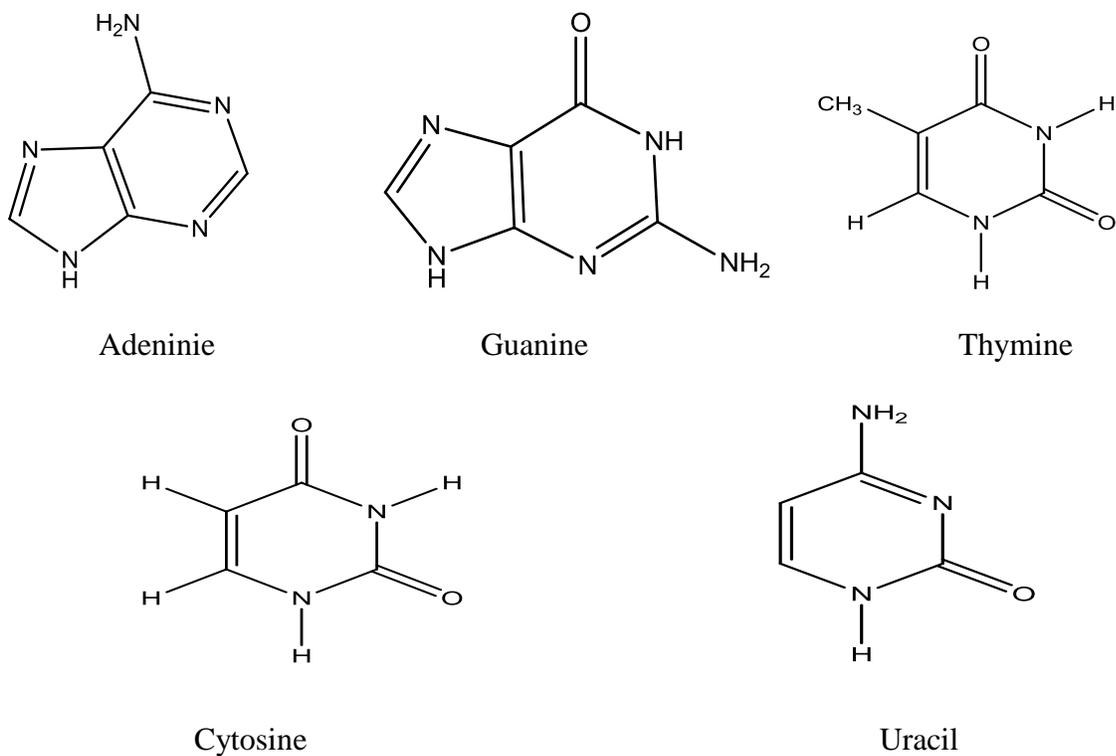
Cytosine · Uracil

Figure 5: Structures of adenine, guanine, cytosine, thymine, and Uracil
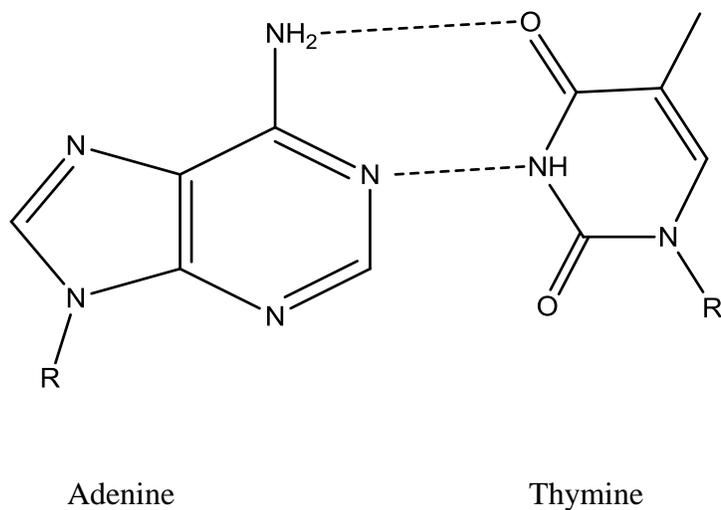
Adenine · Thymine

Figure 6: Base pairing between adenine and thymine

In nucleic acids two types of ribose can be identified, and these are deoxy-D-ribose of the DNA and D-ribose from RNA. The two types of ribose in DNA and RNA are five membered cyclic rings, with the difference being the substituent at carbon 2. The two types of ribose are shown in Figure 7.

D-Ribose (in RNA)   2'Deoxy-D-Ribose (in RNA)
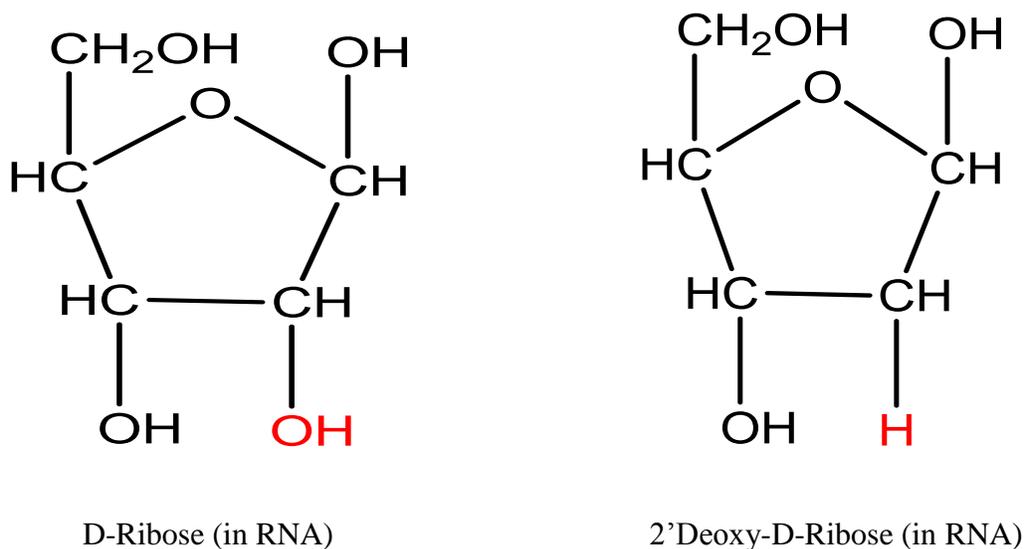
Figure 7: An illustration of the two forms of ribose

DNA exist in living organisms as a pair of molecules held tightly together and they entangle into each other to form a double helix that is stabilized by hydrogen bonding between the nucleotide and base-stacking interactions among the aromatic bases [32]. Long chain of nucleotides of DNA and RNA is due to the presence of covalent bond through phosphate-group bridges that link the nucleotides together. This involves the joining together of a 5'-hydroxyl group of one nucleotide with a 3'-hydroxyl group of the next available nucleotide [33].

Uracil is widely known to occur naturally as a pyrimidine derivative [34]. It is found in ribonucleic acid RNA and it base-pairs through hydrogen bonding with adenine and takes the place of thymine throughout the process of DNA transcription. Uracil can be converted to thymine through methylation that has been found to improve the efficiency of DNA replication. Uracil has the potential to bind with a ribose sugar to produce ribonucleoside uridine [35]. D.J Brown and his group investigated on the tautomeric property of uracil and they came up with amide-imidic acid tautomeric shift. The amide tautomer is known as the lactam structure, while the imidic acid tautomer is also called lactim structure. The tautomeric forms of the uracil are predominant at pH 7 and the lactam structure was found to be the most common form of uracil [36]. The tautomeric form of uracil is shown in Figure 8.

Imide   Lactim
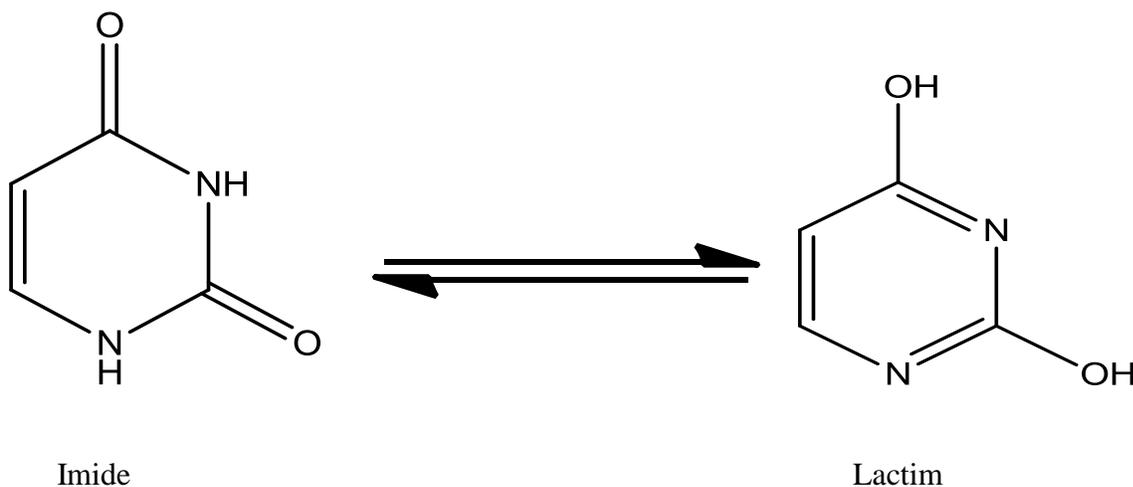
Figure 8: A schematic illustration of tautomers of uracil

Uracil can undergo degradation to produce compounds such as carbon dioxide, ammonia, and aspartate. Uracil can also undergo oxidative degradation to produce urea and maleic acid in the presence of peroxides and Fe^{2+} [34]. Gishan et al. and his group had been reported to synthesize uracil in the laboratory, the simplest of them being addition of water to cytosine to produce uracil and ammonia [35].

$$C_4H_5N_3O + H_2O \longrightarrow C_4H_4N_2O_2 + NH_3 \qquad\qquad \text{Equation 1}$$
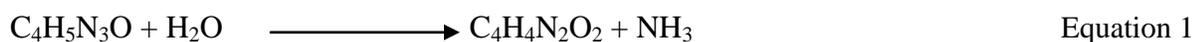
The most commonly used method in synthesizing uracil involves the condensation of maleic acid with urea in fuming sulfuric acid [36]. In recent times uracil and its derivatives had been studied due to the role that they play during molecular recognition, more importantly uracil and thymine had been found to have relation to human diseases and cancers [37]. Figure 9 illustrates the various derivatives of uracil.

 5-Formyluracil (5FoU)   5-Fluorouracil (5FU)   5-Nitrouracil (5FNiU)
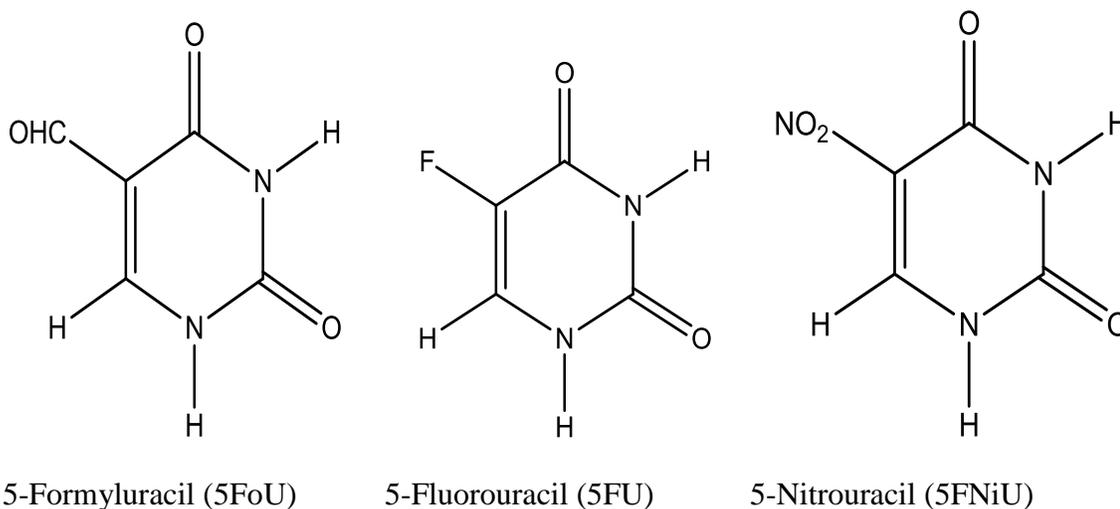
Figure 9: Structures of the derivatives of uracil

5-Fluorouracil (5-FU) had been found to be very useful diagnosing cancers, also uracil and 5-formyluracil are widely known due to their ability to damage DNA bases while 5-Nitrouracil (5-NiU) are also known to exhibit some antiviral properties because they are able to inhibit thymidylate synthetase [38]. 5-Flurouracil (5-FU) has been increasingly used alone or in combination with other various cytoxic drugs and hormones in treating some tumors such as breast, colorectal, and gastric cancers [39]. 5-Flurouracil has been found to exhibit poor tumor selectivity and high incidence of toxicity in the bone marrow, skin, and the central nervous system [40-43].

<u>Drug -DNA Interactions</u>

The interaction of small molecules including drugs with DNA can either be specific or non-specific. For non-specific interactions, the sequence of nucleotide is not all that important, as far as the binding interactions are concerned. However, specific DNA-protein interactions depend upon the sequence of bases in the DNA (orientation of the bases). These protein-DNA interactions are normally controlled by three major forces (a) hydrogen bonding, (b) ionic interactions, (c) other forces such as Van der Waals. The interactions of protein molecules with DNA including drugs have generated lot of interest from biological scientist and pharmaceutical analyst. For some years now there has been an increasing concern to understand the active mechanisms of some anticancer drug and their carcinogenic compounds and to come up with DNA-targeted drug. Interactions of DNA with some anticancer drugs occurs via three different methods (a) the first being under the control of transcription factors and polymerases that drug interacts with proteins that bind to DNA, (b) the second being small aromatic compounds that bind to the DNA double helical structure and (c) lastly through RNA binding to the DNA double helix that brings about suppression of the transcription activity [44].

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A very common interaction that is observed in DNA-protein is between an arginine residue on the protein and a guanine residue as illustrated in Figure 10. A specific combination of several intermolecular interactions between residues of a given protein and a given DNA sequence will ensure a particular protein only binds tightly to a particular DNA sequence to which it is targeted. The higher the number of point's contacts that exist between the DNA sequence and the protein, the more specific the interaction becomes [45]. Certain antibodies or DNA-binding molecules such as Bovine serum albumin (BSA) showed no modification of signal with DNA monolayer. This phenomenon was attributed to the fact that no interaction was observed between BSA and the DNA because of the repulsion between their negative charges [46]. The overall charge of Bovine serum albumin is -18 but after methylation of the compound under acidic conditions produces methylated BSA (me-BSA) [47] that has a positive charge of 8.5 [48]. The DNA with a negative charge is then able to bind with the me-BSA through ionic interactions. Figure 10 illustrate how argentine residue and guanine residue interact through hydrogen bonding.
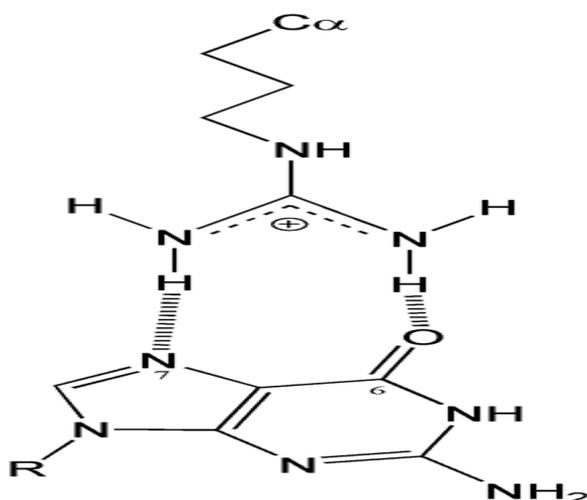
Figure 10: An illustration of the interaction between an arginine residue and guanine residue

Barton et al. [46] used an electrochemical assay of protein binding to a modified DNA electrode based upon detection of disturbances in DNA base stacking. They modified the gold surface with loosely packed DNA stands and covalently crosslinked it to a redox-active intercalator containing the binding sites of the test protein. A considerable reduction in current was observed in the presence of base-flipping enzyme Hhal methylase and uracil DNA glycosylase due to charge transfer between the DNA and the protein that causes some disturbances on the DNA strand.

Drug Used for Immobilization

4-(1'-[8'-(1''-Pyrenyl)naphthyl])-2,6-diaminopyridine Bis-glycamide Bishydrocholride (receptor) has been found to mimic the active site in uracil DNA glycosylase (UDG). UDG is responsible for pulling unwanted uracil into its active site through hydrogen bonding or $\pi - \pi$ during DNA repair processes. The drug has been found to bind selectively to uracil and its derivatives and also some DNA and RNA nucleosides. The 2,6-bis(glycylamino)pyridinyl group in the drug was found to be responsible for mimicking the amino acid residues in UDG that participate in hydrogen bonding and also the presence of a pyrenyl group mimics the phenyl group that undergoes $\pi - \pi$ stacking interactions in the active site of UDG [49]. The drug to be used to for immobilization is shown in Figure 11.
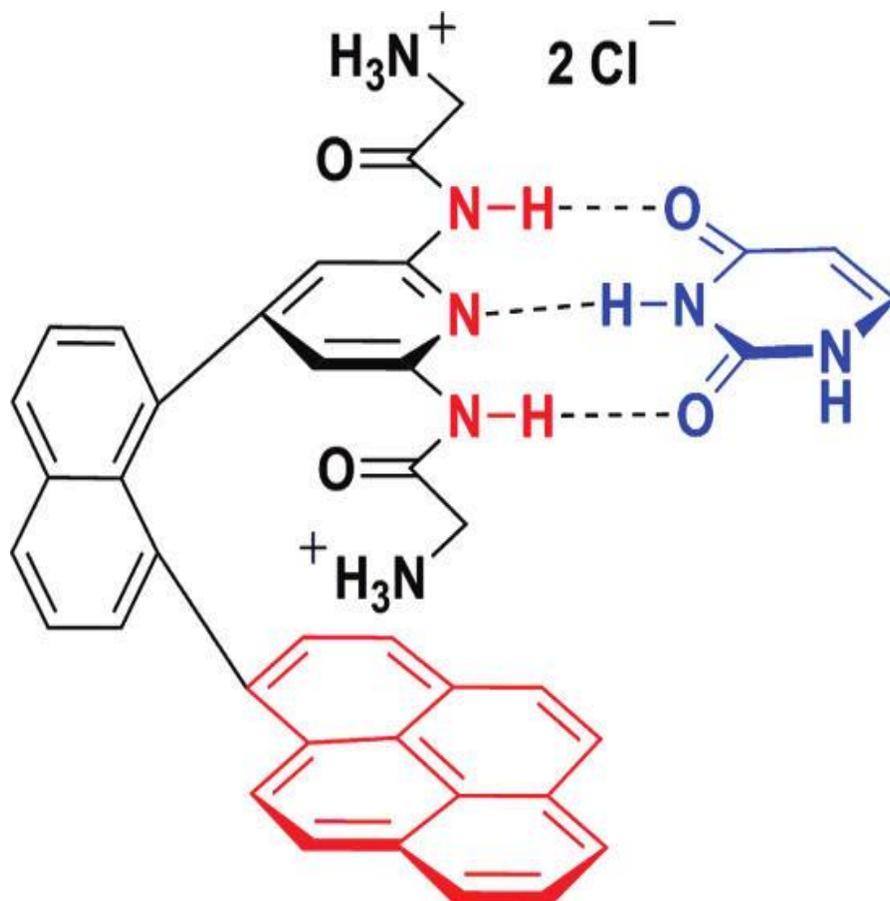
Figure 11: A chemical structure of the receptor binding with uracil used in immobilization 4-(1'-[8'-(1''-pyrenyl)naphthyl])-2,6-diaminopyridine bis-glycamide bishydrocholride

Research Objective

The main aim of this research is to design, construct, and study the interaction between DNA and (4-(1'-[8'-(1''-pyrenyl)naphthyl])-2,6-diaminopyridine bis-glycamide bishydrocholride) known to have anticancer properties using electrochemical technique. A monolayer of double stranded or single stranded DNA of a particular sequence was bound to the surface of gold electrode. Cyclic voltammetry was used to characterize the DNA monolayer on the gold surface. After the characterization of the DNA monolayer, it was then placed in a

solution for about 30 minutes containing the drug that had been dissolved in a buffer solution.

Again cyclic voltammetry was used to study the interaction between the modified DNA

electrode and the anticancer compound by observing the various changes in the voltammogram

and then correlating it with literature values.

CHAPTER 2

EXPERIMENTAL PROCEDURE

List of Chemicals

All aqueous solutions were made using demineralized water. 2-aminoethanethiol hydrochloride was obtained from Sigma Aldrich (St. Louis, MO) Calf thymus double stranded DNA was also obtained from Sigma Aldrich (St. Louis, MO), Potassium Nitrate (KNO_3) was also obtained from Fischer Scientific (NJ, USA) and was used as supporting electrolyte. All the chemicals, unless otherwise mentioned, were of analytical grade and were used as received.

Preparation of Electrodes

A piece of Au wire was slotted into conical shaped capillary tube that was then placed in a heating coil in order to close one of its ends. A vacuum pump was then connected to the other open end of the capillary tube in order to remove air trapped in the tube. An electrical connection was then made to the gold wire by connecting a copper wire to it, after which a little amount of torr seal epoxy was applied to the open end of the glass capillary in other to seal the tube and also provide an electrical connection. The connection between the gold and the copper was verified using a microscope. The gold electrodes were then polished to a mirror-like surface with aluminum slurry on a polishing pad followed by rinsing with deionized water until the surface was smoothed and unscratched. The surface smoothness was verified again with a microscope.

Instrumentation

Cyclic voltammetry (CV) system consisting of four components was used for the experiment. These components were (a) electrolysis cell: that consists of the working electrode, counter electrode, reference electrode, and the electrolytic solution. The working electrode potential was varied linearly

with time, while the reference electrode was maintained at a constant potential. The counter electrode conducted electricity from the signal source to the working electrode. The electrolytic solution served as a source of ions generation needed at the electrode during the process of oxidation and reduction. (b) An electrical device called potentiostat that uses a dc power to generate a potential that is determined correctly, while allowing little amount of currents to be drawn into system without altering the voltage. (c) A current-voltage converter that measures the resulting current and (d) data acquisition system responsible for generating the voltammogram.

Electrochemical measurements (cyclic voltammetry) were carried out in a three-electrode system that consisted of a counter electrode made of platinum, a reference electrode, and a modified working electrode. The electrochemical measurements were carried out in a 10 mL glass cell at room temperature. The electrodes entered the 10 mL glass vial through holes that were created in the vial lid cover. The real electrode area was estimated from cyclic voltammogram (CV) by integrating the cathodic peak for the reduction of the oxide layer in 0.5 M H_2SO_4. The freshly prepared electrodes were scanned until a constant voltammogram was obtained

Preparation of Cysteamine Solution

The cysteamine solution used for all the experiments were freshly prepared by dissolving 0.2840 g of 2- aminoethanethiol hydrochloride in 25 mL of ethanol solution to obtained a 0.1 M cysteamine solution. Prior to the use of the cysteamine solution it was kept in a refrigerator.

Preparation of Redox Probe Solution

The redox probe solution used for all the experiments was freshly prepared by using an analytical balance to weigh 0.0211 g of potassium ferrocynide and transferring it into a 50 mL volumetric flask. This was followed by weighing again 0.5055 g of potassium nitrate serving as a

supporting electrolyte and transferring it into the 500 mL volumetric flask containing the potassium ferrocynide. Distilled water was used in preparing this redox probe solution.

Preparation of DNA Solution

Stock solution of 0.1 M H_3PO_4/ NaH_2PO_4 buffer solution at pH 7.0 was prepared in a 500 mL volumetric flask and kept in the refrigerator. The prepared buffer solution was then used to prepare the DNA solution to be used for the immobilization and this was achieved by dissolving 1 g/L of the ds DNA in 50 mL of the buffer solution. The solution was stirred for about 2 hours until a uniform mixture was obtained.

Cysteamine- Modified Gold Electrode

Modification of the freshly prepared electrode was done by soaking the bare gold electrode (GE) in a 0.1 M freshly prepared cysteamine solution for 24 hours at room temperature in other to form a monolayer of cysteamine. The cysteamine modified gold electrodes were then thoroughly rinsed with ethanol and distilled water to remove physically adsorbed cysteamine. The modified electrodes were then characterized by scanning it in a 1 mmol of the freshly prepared probe solution ($K_4Fe (CN)_6$) using potassium nitrate (KNO_3) serving as the supporting electrolyte and also in 0.5 M H_2SO_4 solution.

DNA-Modified Gold Electrode

The modified cysteamine gold electrodes were then placed in the DNA solution for 24 hrs containing 0.1 M $NaHPO_4$/H_3PO_4 buffer of pH 7 and then thoroughly rinsed with the phosphate buffer solution to remove physically adsorb DNA. The modified cysteamine/DNA electrodes were then characterized in $K_4Fe (CN)_6$ using Ag/AgCl as reference electrode at scan rate 100 mV/s obtain the voltammogram.

Immobilization of Drug on DNA-Modified Electrode

Finally, the modified DNA gold electrodes were immersed in an aqueous solution containing the drug solution for 30 minutes and cyclic voltammogram were obtained in 1 mmol of $k_4Fe(CN)_6$ and 0.1 M KNO_3 as the supporting electrolyte.

CHAPTER 3

RESULTS AND DISCUSSION

This chapter talks about the results obtained in the form of voltammogram carried out in our redox probe solution, sulphuric acid, cysteamine solution, DNA solution, and our drug solution. This chapter further discusses the various changes recorded in the peak current after the modification of the electrode.

<u>Characterization of the Bare Gold Electrode</u>

The fabricated gold electrodes were characterized by obtaining cyclic voltammogram in 0.5 M H_2SO_4 using Ag/AgCl reference electrode at a scan rate of 100 mV/s as shown in Figure 12. The oxidation of the gold produced a well defined peak at 1.18 V and cathodic peak was also observed at 0.75 V representing the reduction of the gold (Au) from +3 to 0 state. The oxidation reaction occurring at the gold electrode surface is given by the equation

$$Au\ -\ 3e \longrightarrow Au^{3+} \qquad\qquad\qquad Equation\ 2$$

The cyclic voltammogram obtained after the characterization is shown Figure 12.
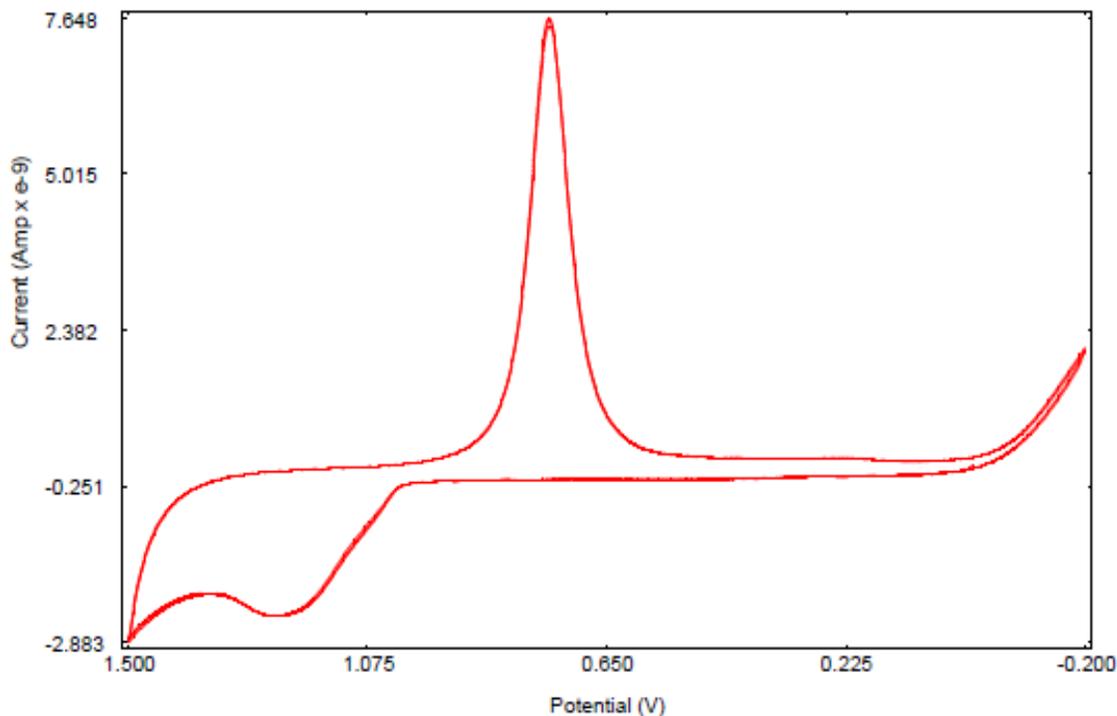
Figure 12: cyclic voltammogram on a bare gold electrode scanned in 0.5 M H_2SO_4 using Ag/AgCl reference electrode at scan rate 100 mV/s

Further characterization of the bare gold electrodes were carried out in 1mmol $K_4Fe\,(CN)_6$ using Ag/AgCl reference electrode in a potential range from 5 V to -2 V using a scan rate of 100 mV/s. The cyclic voltammogram shown in Figure 13 shows that $K_4Fe\,(CN)_6$ species on the gold electrode surface has been oxidized. The equation below represents the oxidation of the ferrocyanide species on the gold electrode surface.

$$Fe(CN)_6{}^{4-} \;-e \longrightarrow Fe(CN)_6{}^{3-} \qquad\qquad \text{Equation 2}$$

Ideally at any scan rate we expect to have a well shaped voltammogram that has a flat plateau that demonstrates there is no presence of charging current. The shape of the

voltammogram in Figure 13 revealed a rather big charging current that is attributed to the gap

between the glass insulation layer and the gold electrode.
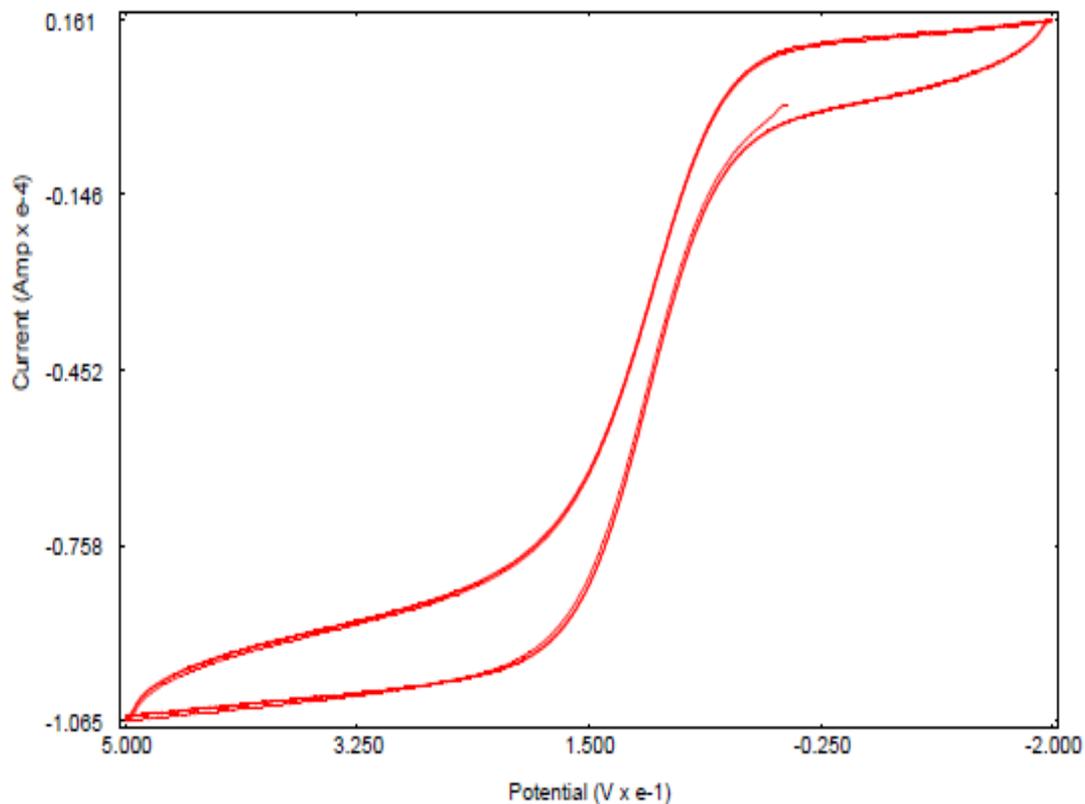
Figure 13: Cyclic voltammogram obtained on a bare gold electrode scanned in 1mmol of

K$_4$Fe (CN)$_6$ using Ag/AgCl reference electrode at scan rate 100 mV/s

Steps were taken to reduce the charging current by placing the electrodes vertically in a

hot liquefied wax for sometime so as to seal the gap upon drying. The electrodes were then

polished so as to remove wax that might have covered the area of interest at the tip of the

electrode. Cyclic voltammetry was carried out on the electrodes but the presence of the

charging current still persisted.

Characterization of Cysteamine Modified Gold Electrode

The characterization of the cysteamine modified gold electrode was done by obtaining cyclic voltammogram in 1 mmol $K_4Fe (CN)_6$ within a potential range of -0.2 V to 0.5 V using a scan rate of 100 mV/s. The voltammogram is shown in Figure 14. Electrochemical reaction that occurred after the immobilization of the bare gold electrode with cysteamine, revealed apparently no change in the magnitude limiting steady state current, compared to the bare gold electrode although the reaction still produced a reduction $Fe (CN)_6^{4+}$ to $Fe (CN)_6^{3+}$. This phenomenon can be attributed to the fact that cysteamine which is made up of short alkanethiol provides an insufficient barrier to block the process of electron transfer between the electrode surface and the probe solution. This then enables most of the redox species in solution to get access to the gold electrode surface.
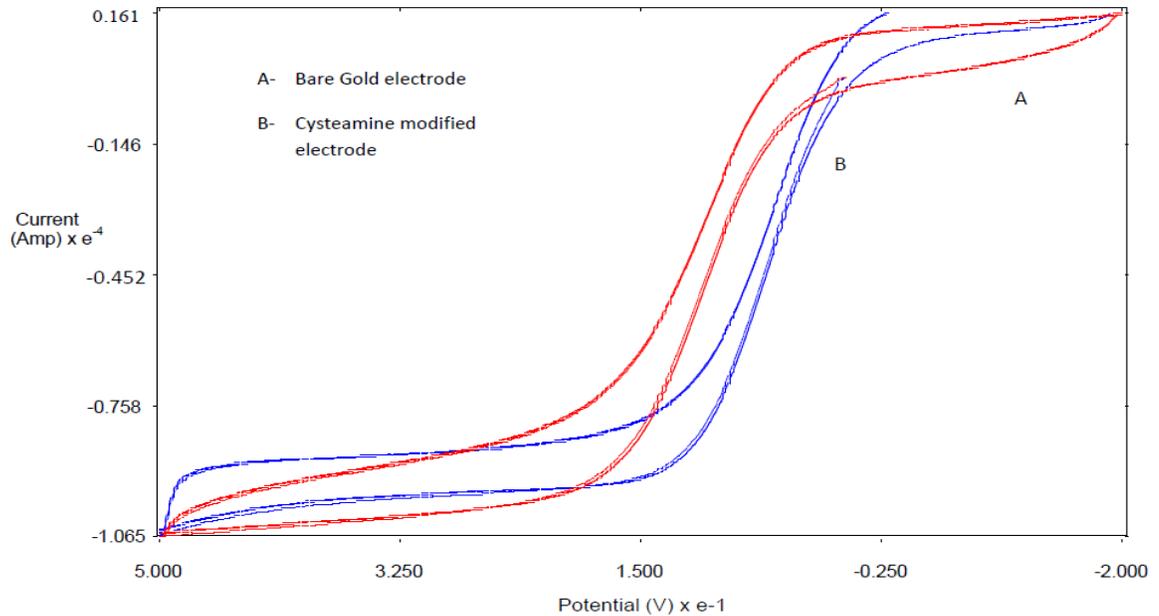
Figure14: Cyclic voltammogram obtained on a cysteamine monolayer modified gold electrode in solution containing 1mmol $K_4Fe (CN)_6$ with Ag/AgCl serving as reference electrode at a scan rate of 100 mV/s

Characterization of DNA/ Cysteamine/ Bare Gold Electrode

The characterization of the DNA/ Cysteamine/ Bare gold electrodes was done by obtaining cyclic voltammogram in 1 mmol $K_4Fe(CN)_6$ as shown in the Figure 15.
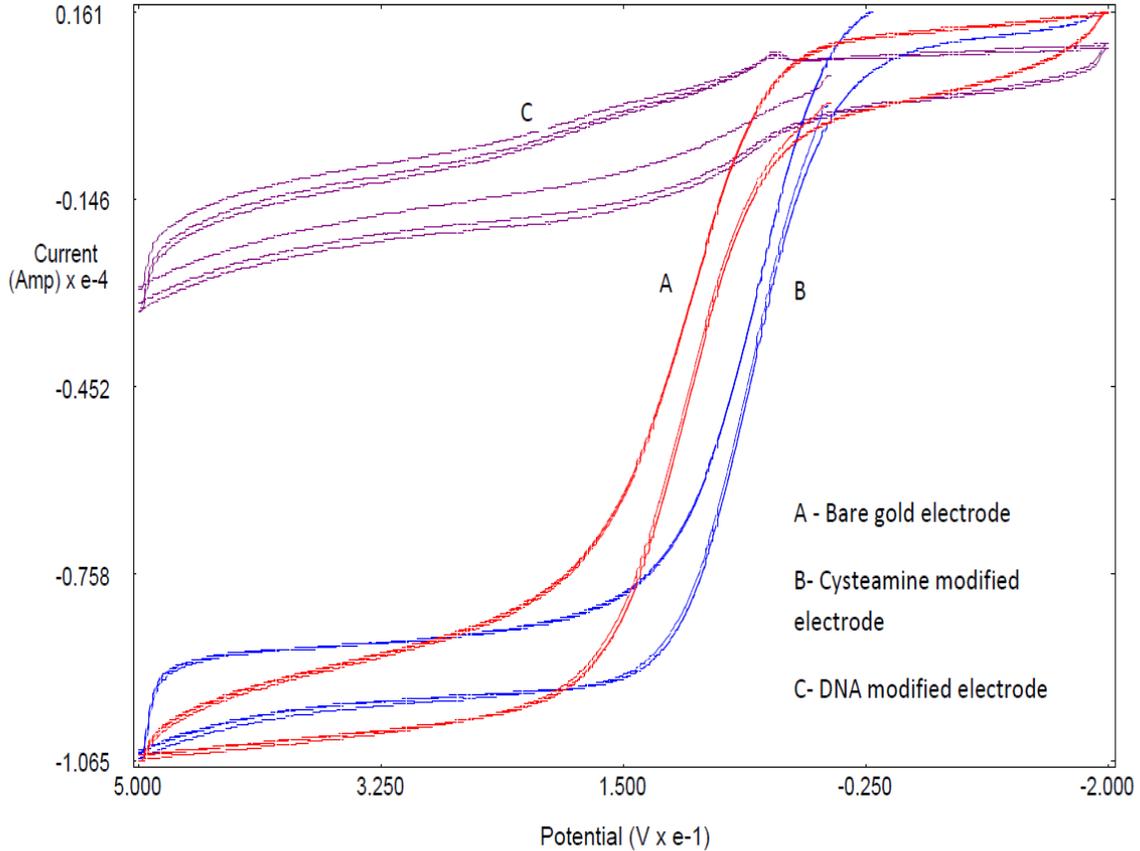
Figure15: Cyclic voltammogram obtained on Bare gold electrode (A), cysteamine modified electrode (B) and DNA modified electrode (C) in 1 mmol of $K_4Fe(CN)_6$ at a scan rate of 100 mV/s

Figure15 clearly shows a drastic reduction in the limiting steady state current observed after the modification of the electrode with DNA compared to those of the cysteamine and the bare gold electrode. The obvious reduction in the limiting steady state current is attributed to the DNA acting as a mass transfer blocking layer and thereby hindering the diffusion of ferricyanide

species towards the electrode surface. This observation shows that the DNA has been successfully assembled on the cysteamine gold electrode surface.

Immobilization of Drug on DNA-Modified Electrode

The modified DNA/ cysteamine/ bare gold electrode was placed into an aqueous solution of the drug solution for about 25 minutes followed by scanning it in 1 mmol M $K_4Fe(CN)_6$ using Ag/AgCl as a reference electrode at scan rate 100 mV/s.

The cyclic voltammogram showed a further reduction in the limiting steady current of the redox probe after the modified DNA electrode was immersed in the receptor solution as shown in Figure16.
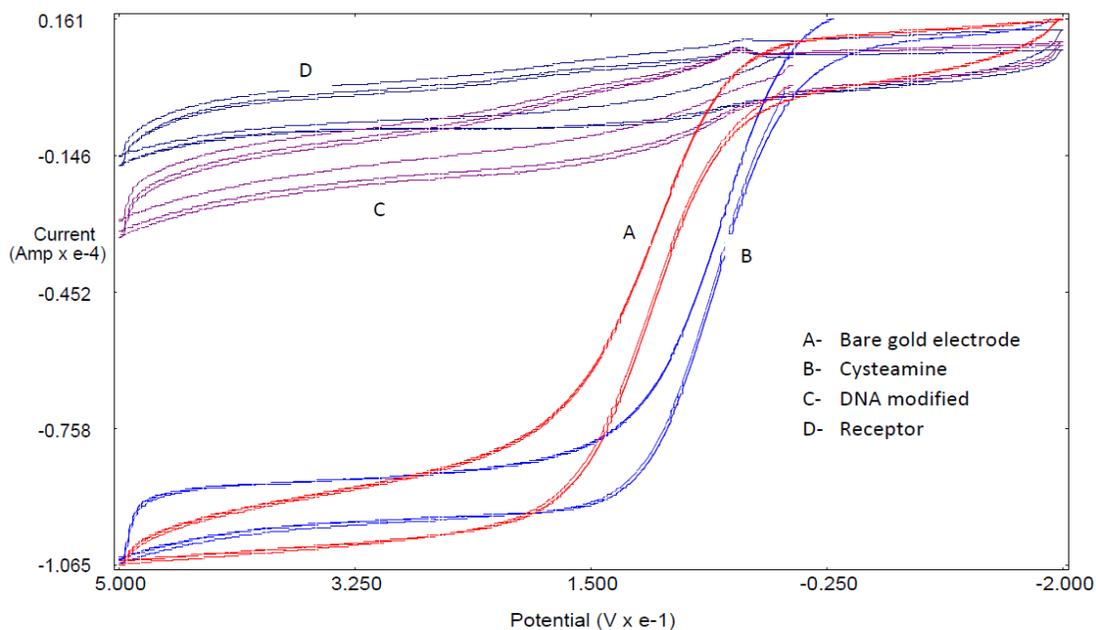
Figure 16: Cyclic voltammogram obtained on Bare Au electrode (A), Cysteamine modified (B), DNA modified electrode, (C) and Drug modified electrode, (D) in 1 mmol $K_4Fe(CN)_6$ at scan rate 100 mV/s

From the voltammogram in Figure 16, electrode (D) showed a further reduction in the limiting steady state current after the immobilization. The reduction in the limiting steady state current of electrode (D) can be attributed to electrostatic interaction between the DNA and the drug (4-(1'-[8'-(1''-Pyrenyl) naphthyl])-2, 6-diaminopyridine Bis-glycamide Bishydrocholride) forming an inert complex that prevent the electroactive species from reaching the electrode surface and that induces the decrease of the electrochemical response due to the receptor binding to the DNA by intercalation. The intercalation process involves the compound finding itself stack in between the adjacent DNA pairs.   Research by Jiang et al.[49] found out that (4-(1'-[8'-(1''-pyrenyl) naphthyl])-2, 6-diaminopyridine bis-glycamide bishydrocholride) has the potential to detect and recognized damaged DNA and bases [49].

CHAPTER 4

CONCLUSION AND RECOMMENDATION

In this research, the electrochemical behavior of the receptor (4-(1'-[8'-(1''-pyrenyl) naphthyl])-2, 6-diaminopyridine bis-glycamide bishydrocholride) and its interaction with a modified DNA was investigated using electrochemical method. All the experimental results agreed with expectations. In summary, the following conclusions were drawn from this research:

a. A modified DNA electrode was fabricated successfully using self-assembled monolayer method

b. Cyclic voltammetry have been used to characterized the modified electrode surface

c. The results obtained indicate that, the principal interaction mode of (4-(1'-[8'-(1''-pyrenyl) naphthyl])-2, 6-diaminopyridine bis-glycamide bishydrocholride) with DNA is by intercalative interaction.

Because the results obtained demonstrate that cyclic voltammetry provides an efficient method to study the mechanism of DNA interaction with a compound, it would be very useful if the interaction can be quantified using spectrophotometry method.

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