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# SPECTROSCOPIC AND ELECTROCHEMICAL STUDIES ON THE DNA BINDING STUDIES OF SOME ASYMMETRIC COMPOUNDS

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## ABSTRACT

DNA binding behavior of four asymmetric compounds has been investigated by UV-Visible spectroscopy and cyclic voltammetry. Calf Thymus DNA was used for interaction at blood $p^H$ of 7.4. Four compounds **A-D** [4-Hydroxy-3- (3-oxo-1-phenylbutyl) -2H-chromen-2-one], [4-Hydroxy-3- (3-oxo-1,3-diphenylpropyl) -2H-chromen-2-one], [4-Hydroxy 3-[1-(4 Bromophenyl) -3-oxo-3-phenylpropyl] -2H-chromen-2-one] and [4-Hydroxy-3- [1-(4-bromophenyl) -3-oxo-3-phenylpropyl] -2H-chromen-2-one] are asymmetric compounds and are the derivatives of 4-Hydroxycoumarin. From UV and CV studies, the binding constants $K_b$ and free energy $\Delta G$ were calculated. All the studied compounds showed weak to moderate interactions with DNA. UV results showed that compound **A** interacted with DNA through electrostatic interactions, **B** and **C** through groove bindings while compound **D** showed typical intercalation mode. Same response was observed after CV results.

**KEYWORDS:** Coumarins, interactions, groove bindings, intercalation.

## INTRODUCTION

Over the last few decades structure of DNA and its interaction with different molecular moieties has gained a great interest in the field of organic synthesis and pharmacology. DNA is a nucleic acid that contains all the information necessary for specifying the biological development of all living bodies. It is a molecule that controls hereditary information transferred to the offspring.[1-3] During reproduction DNA is replicated and transmitted to the new trait. In this process the sequence of DNA base pairs defines the characters of individuals ranging from physical traits to disease susceptibility.

It is necessary to understand at molecular level gene expression and their mechanism of transfer to offspring. This could be helpful to understand the transfer of diseases. It is also a key step towards the development of new chemotherapeutic strategies.[4]

DNA is antiparallel double helix held together by hydrogen bonding interactions between DNA base pairs. It could be further divided into major and minor grooves.

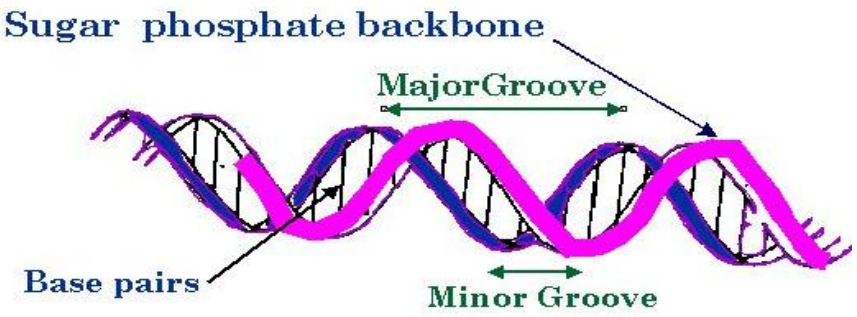



**Figure (1): Structure of DNA.**

Watson and Crick published above structure of DNA along with the hypothesis that accurate chemical properties of the base pairs suggest the exact copying of genetic material.[5] If the copying is disturbed then the characters of the traits could be changed. In recent years the study of DNA and its interaction with newly synthesized drugs has gained an importance in investigating anticancer activities of the drugs. The drugs could interact with DNA in different ways. The drugs could interact at DNA base pairs by the breakdown of hydrogen bonding (intercalaters) while some moieties could interact at groove sites (groove binders).

The first evidence of interaction was published in 1961 when Lerman demonstrated that acridine dye could intercalate between DNA base pairs.[6] It was concluded after this research that only molecules with flat, aromatic structure can intercalate with DNA and are considered to be good anticancer drugs.[7-9] There are certain cases where the cytotoxicity is parallel to anticancer activity. A number of compounds like vitamins, harmones, vitamin antagonists, antidepressants and antihistamines are also good intercalaters.[10]

The non-planer structures mostly interact with DNA through groove bindings, which do not disturb the base pairs but just interact through outside bindings.

Generally those drugs are considered to be best anticancer which are organometallic in nature.[11-12] These drugs are intercalaters as well.

In this paper DNA interaction of four asymmetric compounds with DNA is reported. The interaction is carried out with Calf Thymus DNA and studies carefully via spectroscopic cyclic voltammetric analysis. Their synthesis has been reported earlier.[13] The experimental results showed that some asymmetric drugs showed intercalation while others showed groove bindings.

## EXPERIMENTAL

## MATERIALS AND METHOD

The enantiomeric excess was determined by using chiral columns, Chiralpak AD-H (phenomenix). The instrument used for this technique was HPLC PerkinElmer.

Sodium salt of calf thymus DNA was purchased from (Acros) and was used as received. Calf Thymus DNA (CT-DNA) (50 mg) was dissolved by stirring for overnight in double deionized water (pH = 7.0) and kept at 4°C. Doubly distilled water was used to prepare buffers (20 mM Phosphate buffer ($NaH_2PO_4$-$Na_2HPO_4$), pH = 7.2). A solution of (CT-DNA) in the buffer gave a ratio of UV absorbance at 260 and 280 nm ($A_{260}/A_{280}$) of 1.8, indicating that the DNA was sufficiently free of protein.[14]

The DNA concentration was determined via absorption spectroscopy using the molar absorption coefficient of 6,600 $M^{-1}$ $cm^{-1}$ (260 nm) for CT-DNA and was found to be 2.0 $\times 10^{-4}$ M. The compound was dissolved in 70% ethanol to form stock solution of 2mM strength. The UV absorption titrations were performed by keeping the concentration of the complex fixed while varying the CT-DNA concentration. Equivalent solutions of CT-DNA were added to the complex and reference solutions to eliminate the absorbance of DNA itself. Complex-DNA solutions were allowed to incubate for about 10 min at room temperature before measurements were made. Absorption spectra were recorded using cuvettes of 1 cm path length at room temperature (25 ± 1 °C).

The stock solution of CT-DNA was prepared by using doubly distilled water and stored at 4 °C. The concentration of CT-DNA was determined by UV absorbance at 260 nm (molar coefficient Є of CT-DNA was taken as 6600 $M^{-1}$ $cm^{-1}$).

The absorption spectra were recorded on a Shimadzu 1800 UV-Vis spectrophotometer.

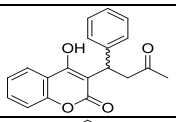
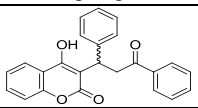
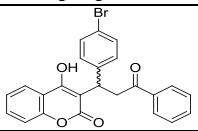
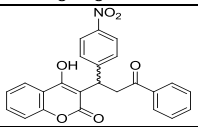
Cyclic Voltammetric (CV) experiments were performed using Eco Chemie Auto lab PGSTAT 12 potentiostat/galvanostat (Utrecht, The Netherlands) equipped with the electrochemical software package GPES 4.9. The measurements were recorded in a single compartment cell with a three electrode configuration i.e, the Ag/AgCl as the reference electrode, a platinum wire of thickness 0.5mm with an exposed end of 10mm as the counter electrode and a bare glassy carbon electrode of 0.071 $cm^2$ surface area as working electrode. Argon gas was purged to flush out oxygen before each measurement. The voltammogram of a known volume of the 0.1M solution of compound was recorded in the absence of Calf Thymus DNA (CT-DNA) at 298K, then CT-DNA of $1.84\times10^{-5}$ M strength was added to solution of compound and voltammogaram was recorded at 298K. The scan rate was 0.2volt/sec. The GCE was cleaned after each reading. 0.1M solutions of each compound were prepared in 20% aqueous ethanol (20% $H_2O$: 80% ethanol).

## RESULTS AND DISCUSSION

### CT-DNA Binding Studies using UV-Visible Spectroscopy

DNA-binding studies of bioactive compounds (**A-D**) were carried out in presence and absence of Calf Thymus DNA. UV-Visible spectra of all the compounds were performed at $p^H$ of 7.4 and normal body temperature ($37^0$C).The effect of varying concentration of DNA ($0.1*10^{-4}$M to $1*10^{-4}$M) on UV absorption spectra of 2.5mM solutions of **A**, **B**, **C** and **D** as is shown in **figures 2-5** respectively. The compound **A** showed three maximum absorptions at 270nm, 280nm and at 310nm. 267nm and 311nm were the typical absorption bands of coumarins. 280nm can be due to the effect of substitutions. The spectra were related to π-π* transitions of these chromophores.

**Table1**: **Compounds for DNA Binding Studies.**

| Sr.No | Code | IUPAC Name | Structure | Enantiomeric Excess (ee) |
|---|---|---|---|---|
| 01 | **A (4a)** | 4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one |  | 50 |
| 02 | **B (4b)** | 4-Hydroxy-3-(3-oxo-1,3-diphenylpropyl)-2*H*-chromen-2-one |  | 50 |
| 03 | **C (4c)** | 4-Hydroxy 3-[1-(4-bromophenyl)-3-oxo-3-phenylpropyl]--2*H*-chromen-2-one |  | 50 |
| 04 | **D (4g)** | 4-Hydroxy-3-[1-(4-nitrophenyl)-3-oxo-3-phenylpropyl]-2*H*-chromen-2-one |  | 50 |

A compound when being united with DNA modifies its UV spectra. These modifications could be the decrease of absorbance (hypochromic shift), increase of wavelength (hyperchromic effect), shift of $\lambda_{max}$ to higher wavelength (bathochromic or red shift) or it could be the shift of $\lambda_{max}$ to lower wavelength (hypsochromic or blue shift). These spectral changes were indicative of different interactions with DNA. The hypochromic effect along with a red shift of less than or equal to 15nm were the indicative of intercalation mode of binding while a red shift of less than or equal to 8nm were the indicative of groove bindings. The hypochromic effect along with blue shifts were the indicative of electrostatic interactions.[15]

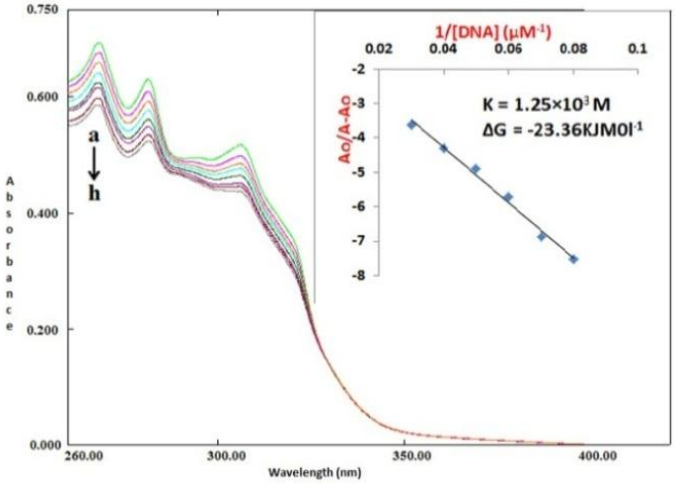



**Figure 2**: **UV Spectrum of A in the presence and absence of DNA.**

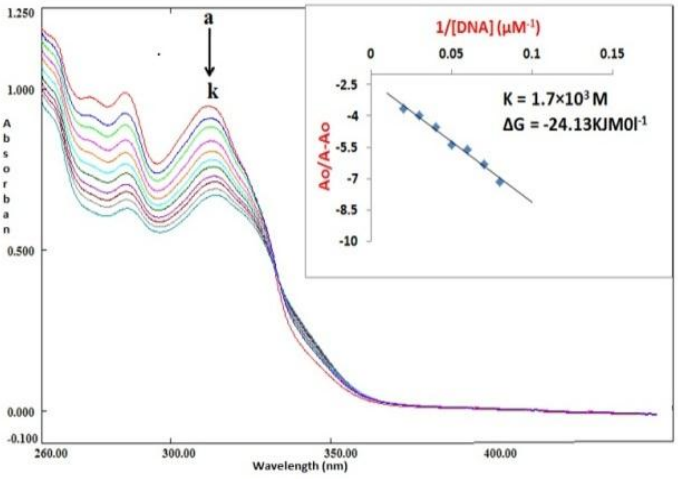



**Figure 3**: **UV Spectrum of B in the presence and absence of DNA.**

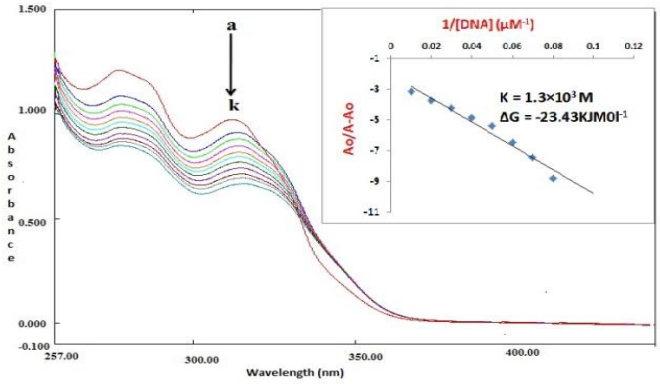



**Figure 4**: **UV Spectrum of C in the presence and absence of DNA.**

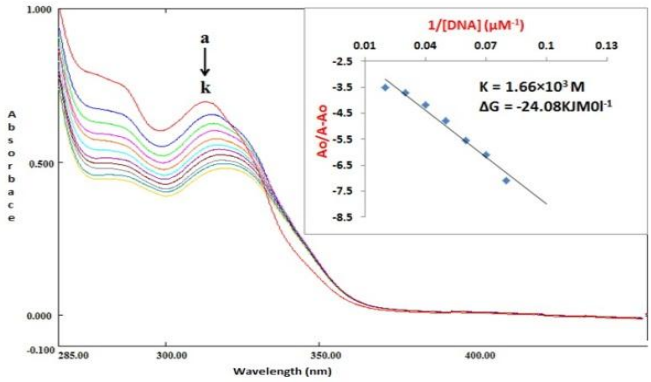



**Figure 5**: **UV Spectrum of D in the presence and absence of DNA.**

In current study the UV spectra of screened compounds showed that upon addition of different concentrations of DNA, the absorbance at each wavelength decreased. This hypochromic effect was the result of contraction of helix axis as well as from the conformational changes of DNA.[16] **Figure2** showed a UV spectrum of compound **A.** It showed a hypochromic effect and a blue shift of about 1nm which are the indicative of non-intercalative binding mode such as electrostatic interactions. In the structures of **A** only OH group can form electrostatic interactions if we consider the deprotonation of OH group then its interaction with sugar groups of the DNA instead of base pairs. **A-DNA** binding constant, **Kb**, was calculated to be $1.35*10^{3}M^{-1}$ which showed a moderate interaction of **A** with CT-DNA. In literature the stronger interactions showed a binding constant of $2.22*10^{6}M^{-1}$.[16]

The UV spectra of compounds **B** and **C** are represented in **figure 2** and **figure 3.** The UV peaks of these compounds were shifted to higher wavelengths. This was because the substitution of 4-Hydroxycoumarin with chalcone introduces another benzene ring in the molecule. It had increased conjugation due to extensive delocalization of benzene ring which decreased energy gaps between π-π* transitions causing a shift of UV-absorption peak

towards a longer wavelength. In **B** the bathochromic shift from 311nm to 312nm took place. Similarly the absorbance at 320nm was shifted to 322nm. It means that the red shift of ~ 2nm spectrum took place in the maximum concentration of DNA ~$1*10^{-3}$M. Similarly compound **3** showed a red shift of 3nm (313-316) due to interaction with DNA. As the red shifts in both the cases were less than 8nm so the possible suggested interaction of chalcone and bromochalcone substituted products were groove bindings.[17]

The UV spectra of compounds **C** is represented in **figure 4**. The analysis of this spectrum showed both hypochromic and bathochromic shifts. The compound **D** caused a maximum red shift of 15nm (310nm-325nm). The compound **D** contained $NO_2$ group at $4^{th}$ position of chalcone, nitro group also took part in delocalization due to double bonds. This maximum red shift value of 15nm was the indicative of interaction of **D** with DNA through intercalation.[18]

Decrease in absorbance at $\lambda_{max}$ of all the studied compounds reflected the interaction of these compounds with DNA. It also indicated the interaction of the electronic states of the intercalating chromophores of the compounds and those of the stacked base pairs of CT-DNA.[19] Furthermore the red shift could be directly linked with improper coupling (conformational changes) of π*-orbital of intercalated ligands with the π-orbital of the base pair.

It had been observed that the absorbance varied after the addition of CT-DNA, so the binding constants "$K_b$" of compounds-DNA complex could be determined from the variation in absorbance in UV-visible spectra. Binding constants could be determined spectrophotometrically by applying Benesi-Hildebrand equation.

$$\frac{Ao}{A-Ao} = \frac{\varepsilon o}{\varepsilon(H-G)-\varepsilon G} + \frac{\varepsilon}{\varepsilon(H-\varepsilon)-\varepsilon G} 1/Kb[DNA] \qquad (1)$$

Where $A_o$ and A were the absorbance of compounds and complexes respectively. $\varepsilon_G$ and $\varepsilon_{H\text{-}G}$ are the molar extinction coefficients of compounds and complexes respectively. From the plot of Ao/A-Ao to 1/[DNA], the ratio of intercept to the slop gave binding constants "$K_b$". Their plots are given along their absorption spectra.

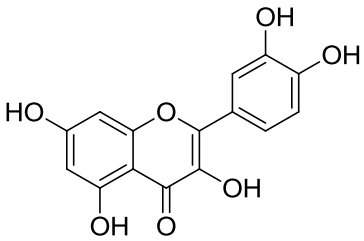
After the calculations for binding constants of compounds-DNA complexes, their free energies ΔG (kJ/mol) were calculated as.

$$\Delta G = -RTlnK \qquad (2)$$

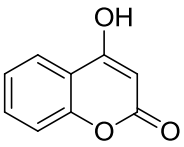
Where K is the binding constants, R is the General Gas constant and T is the temperature (298K). The values of these energies are also represented in **table 2**.

Binding constants are the measure of stability of compound-DNA complexes while free energy "ΔG" indicates the spontaneity of the compounds-DNA complexes. The values of binding constants "$K_b$" represented that all the compounds bind to the DNA strongly. But the compounds **5** and **7** binded more strongly than other compounds. The values of free energies for **5** and **7** were minimum, reflecting the more stability of these complexes as compared to others.[20]

Compounds **A**, **B**, **C** and **D** were the derivatives of 4-Hydroxycoumarin. Much of the work related to the studies of DNA-gyrase interaction with coumarin nucleus based drugs has been reported.[21-23] but little studies related to the DNA interaction had been carried out. The current research related to these coumarin based compounds suggested the formation of complexes of these drugs with DNA. The structures of coumarins quite resemble with the flavonoids (quricitin) and their interaction pattern also showed same resemblance and proposed that the complexes of compounds **B-D** binds to CT-DNA through groove bindings as well as intercalations while compound **A** interacts through electrostatic interactions.[24]




Quricitine




Coumarin

**Table3**: **Binding Constants and Free Energies of studied compounds.**

| Sr.No | Compound | Binding Constant "$K_b$ $M^{-1}$" | Free Energy (ΔG (kJ/mol)) |
|---|---|---|---|
| 1 | **A** | $1.25*10^3$ | -23.36 |
| 2 | **B** | $1.70*10^3$ | -24.13 |
| 3 | **C** | $1.30*10^3$ | -23.43 |
| 4 | **D** | $1.66*10^3$ | -24.03 |

**CT-DNA Binding Studies using Cyclic Voltammetry**

The electrochemical investigations of compound–DNA interactions can also provide a useful supplement to spectroscopic methods. The cyclic voltammetric measurements of 0.1M

strength solution of the compounds (**A-D**) were performed in the absence and presence of Calf Thymus DNA. (CT-DNA) and their voltammogarams were recorded at 298K. The scan rate was 0.2volt/sec at glassy carbon electrode.

The redox couple for each compound had been studied with addition of CT- DNA and the corresponding potentials with their shifts are given in **table 3**. Generally, positive shift in an electrochemical potential of a small molecule was observed when mode of interaction was intercalative, while negative shift was observed in the case of electrostatic interaction mode with DNA.[25]

In case of these selected compounds, no new redox peaks appeared after the addition of CT-DNA to each compound, but the current of all the peaks vary significantly, suggesting the existence of an interaction between each compound and CT- DNA.

All the compounds showed irreversible reduction processes and a single peaks were observed. Addition of CT-DNA to compound solution resulted a shift to negative potential and drop in the current in case of **A**. Similarly, in case of **B** and **C** a positive shift in potential and drop in current had observed (**table 3**). The drop in current was attributed to diffusion of drug into double helical DNA resulting in the formation of supramolecular complex. The shift in formal potential revealed the mode of interaction between drug and DNA.[26]

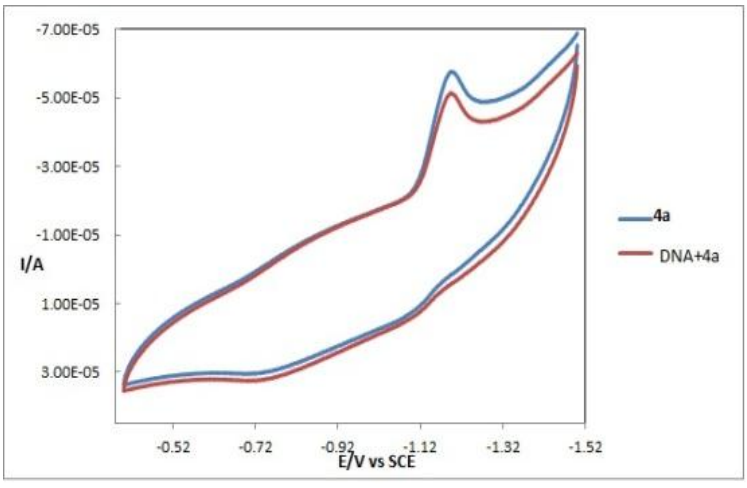



**Figure 6: Cyclic Voltammogram for (0.1M) A at Scan Rate of 0.2V/s in Ethanol Water Mixture (8:2) at pH 7.4.**

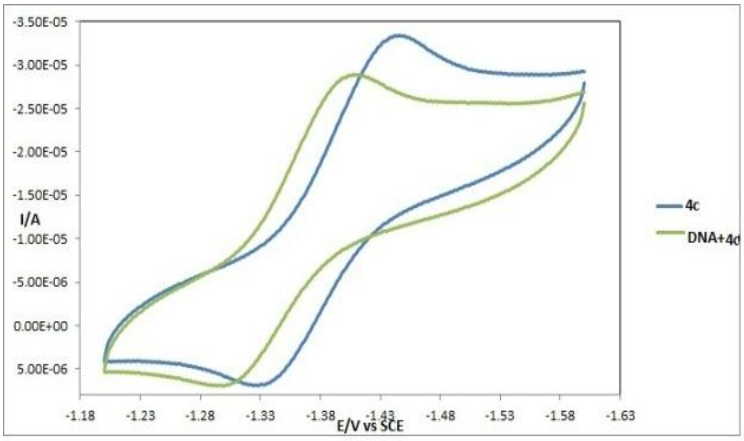



**Figure 7: Cyclic Voltammogram for (0.1M) B at Scan Rate of 0.2V/s in Ethanol Water Mixture (8:2) at pH 7.4.**

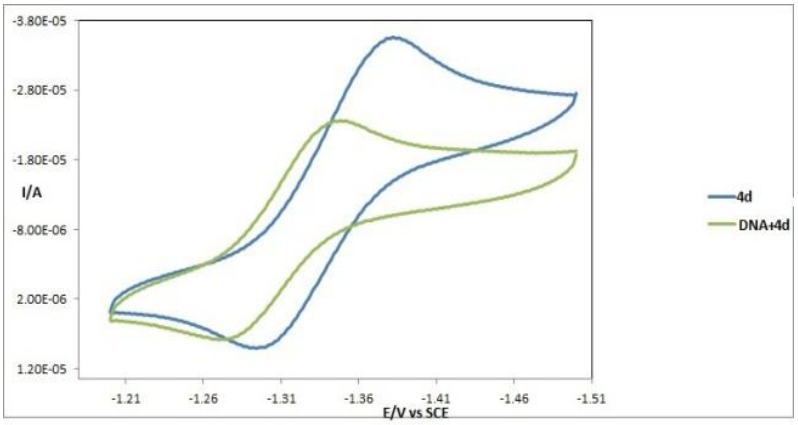



**Figure 8: Cyclic Voltammogram for (0.1M) C at Scan Rate of 0.2V/s in Ethanol Water Mixture (8:2) at pH 7.4.**

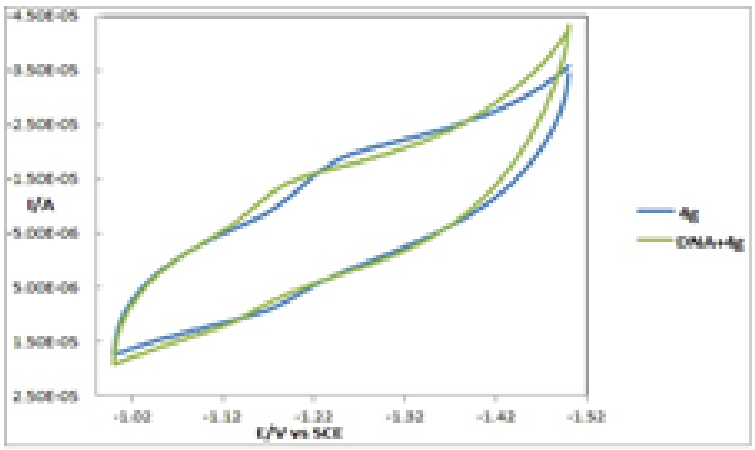



**Figure 9: Cyclic Voltammogram for (0.1M) D at Scan Rate of 0.2V/s in Ethanol Water Mixture (8:2) at pH 7.4.**

Voltammogram of compound **D** showed interesting results. This showed an increase in peak current at potential of -1.17V, a small decrease at -1.32V while again an increase of current at -1.47V. As it was evident from UV spectral analysis that **D** interacted strongly with DNA through intaercalation.

In our compounds **B, C** and **D,** a gradual shift in the positive potential was observed with addition of DNA in the voltammetric wave accompanied by decrease in peak current. The positive shift in peak potentials represented groove bindings as well as intercalative mode of bindings.[27-28] While negative shifts were the indicative of electrostatic interactions.[29-31] Intercalative and groove bindings were dependent upon DNA double helix. But the electrostatic bindings took place outside the groove of DNA. The peak current decreased as the free compound molecules were used in the formation of compound-DNA adducts. Assignments in **Table 3** showed that with increasing amounts of CT- DNA, one of the cathodic (Epc) or the anodic (Epa) potentials of compounds showed a positive shift.

The voltammograms of compounds **B** and **C** seemed to be reversible. For **A** the $E_{red}$ was find out to be -1.43V and $E_{oxd}$ was find out to be -1.33V. The ΔE was given as,

$\Delta E = E_{red} - E_{oxd}$

$= 0.10/n$ V

If we consider to be single electron transfer process then n=1 and its value will be 0.10V which would not fulfil the condition of reversibility. For a reversible system its value should be equal to 0.059V. Also for a reversible systems the ratio of cathodic and anodic peak current should be equal to 1.Here ipr/ipf=1.69 so this system was not reversible.

The voltammogram of **C** gave values of $E_{red}$ and $E_{oxd}$ -1.39V and -1.30V respectively. For a single electron system the values of ΔE could be 0.09V and again did not fulfil the criterion of reversibility. Simillarly ipr/ipf=1.24 and were not equal to 1. Moreover, **Table 3** reflected that the compound-DNA adduct formation was a spontaneous process and both techniques spectroscopic and voltammetric assignments agreed well with each other.

The binding constant "K" was calculated according to the following equation.[32]

$$\log\left(\frac{1}{[DNA]}\right) = \log K + \frac{\log(I(H-G))}{[IG-(I(H-G))]} \qquad (3)$$

Where, K was the binding constant, $I_G$ and $I_{H-G}$ are the peak currents of the free guest (G) and the complex (H–G), respectively.

While standard Gibbs free energy was calculated by using equation 2. The value of "K" represented binding strength of compound with DNA while ΔG represented the spontaneity of adduct (compound +DNA complex).

Current research was based on the synthesis of derivatives of α, β unsaturated systems. Literature had showed the DNA binding studies of such compound, 1-(4'-aminophenyl)-3-(4-*N*, *N*-dimethylphenyl)-2-propen-1-one (chalcone) in which α, β unsaturated system was intact.[14] Both UV and CV studies showed a stronger interaction of chalcone with DNA through intercalation. The binding constant calculated was about $6.15*10^5 M^{-1}$. But in our case both the techniques showed weaker interactions. This could be proposed in terms of absence of α, β unsaturated system which were responsible for reversible electron transfer and existence of different resonance structures and dimerization of the molecule. With the reaction at these centers these resonance structures were disappeared thus making difficulties in electron transfers processes so giving irreversible peaks. The interactions in our case could be proposed to be due to the presence of substituents. Stronger bathochromic shifts were observed for **D,** which was 4-Nitrochalcone derivative of 4-Hydroxycoumarin.

**Table 3**: **Values of Voltage and Current in CV Measurements.**

| Compound Name | Only Compound | | Compound+DNA | | Shift in potential |
|---|---|---|---|---|---|
| | Current | Voltage | Current | Voltage | |
| **1** | $-1.02\times10^{-5}$ | -0.606 | $-8.7\times10^{-6}$ | -0.626 | To negative |
| **2** | $-3.57\times10^{-5}$ | -1.38 | $-2.37\times10^{-5}$ | -1.348 | To Positive |
| **3** | $-3.34\times10^{-5}$ | -1.44 | $2.9\times10^{-5}$ | -1.408 | To Positive |
| **4** | $-1.9\times10^{-5}$ | -4.49 | $-1.19\times10^{-5}$ | -3.50 | To positive |

**Table 4: Calculated Binding parameters by Cyclic Voltammetric Studies.**

| Compound | K (Binding constant) | -ΔG(Binding energy) |
|---|---|---|
| **1** | $1.31\times10^2$ | 22.36 |
| **2** | $1.71\times10^3$ | 25.50 |
| **3** | $1.60\times10^3$ | 23.43 |
| **4** | $1.58\times10^3$ | 24.27 |

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