# Ferrocene derivatives: Potential anticancer material

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**Summary:** The presented work is focused on the investigation of ferrocene and its derivatives (ferrocene benzoic acid and ferrocene dicarboxylic acid) via electrochemical, and spectrophotometric for biological activity. DNA binding capacity was studied by cyclic voltammetry and UV-Vis spectroscopy with evaluation of kinetic and thermodynamic parameters. Heterogeneous rate constant and diffusion coefficients were estimated for pure compounds and the compound bound with ds.DNA (double-stranded DNA). The voltammetric investigation suggested that the interaction between the ferrocene derivatives and ds.DNA was present. Oxidation potential ($E_p$) values showed that the interactions of ferrocene dicarboxlic acid (FC-2) with ds.DNA was stronger than that of ferrocene benzoic acid (FC-1). Diffusion coefficient and heterogeneous rate constant values also corresponded to the structural dependent interactions of these compounds with ds.DNA.

**Keywords**: Ferrocene derivatives; DNA interactions; Cyclic voltammetry; Spectrophotometry.

## Introduction

The growing application of metallocenes in the treatment of numerous human diseases especially in cancer is a vigorously expanding area in biomedical sciences [1,2]. Discovery of the cisplatin as an anticancer material moved the research towards the synthesis and investigation of cytotoxic compounds with more expansion of activity [1, 2]. Molecules containing other heavy transition metals have frequently been assessed as potential anticancer agents in many human tumor cells as they have variation in geometries, coordination number, accessible redox states and thermodynamics that offers medicinal chemists to employ different strategies for their exploitation. Thus, much attention has been diverted to utilize the new organometallics especially metallocenes and their derivatives in medicine [1-3]. These organometallics find potential in biosensors with a provision for variation in the substituents. Ferrocene derivatives have versatile character for use in medicinal chemistry due to the ferrocene electrophore which gives ideal and neat signal in case of voltammetric studies [3]. Many ferrocene derivatives have wide application in biological and medicine field such as anti-malarial [4], anti-tumor [5], anti-bacterial [6], anti-inflammatory, in treatment of anemia [7], and inhibition of enzymatic activity [8].

Deoxyribonucleic acid, ds.DNA is important being building block of the living organisms [9]. Drug–DNA interactions are of three main types a) intercalation b) groove bonding, and c) electrostatic [10]. Various analytical techniques like UV-Vis, NMR, FTIR, circular dichroism, fluorescence, and voltammetry have been used for investigating the binding modes and extent of binding of small drug-like molecule with ds.DNA [11]. Electrochemical (EC) method has importance in that as it could be used with quite clarity for assignment of peak potential variation with the change in molecular configuration upon interaction with DNA. EC data can be used to relate with the potentiality of drug for biosensing, anticancer, or other biological studies, thus posing a facility for in-vitro studies [12].

In the present study, ferrocene (FC) and carboxylic acid substituted derivatives (ferrocene benzoic acid, FC-1, ferrocene dicarboxlic acid, FC-2) were selected for electrochemical (EC) and spectrophotometric. These compounds were investigated because of the ferrocene redox couple in their structures which is always an interesting electrophore to study. The ds.DNA−binding capacity of these FC derivatives was studied at room temperature and an understanding of the substituent effect was developed. The estimated values of heterogeneous rate constant and diffusion coefficient pointed to the structure dependent interactions of ferrocene derivatives with ds.DNA.

## Experimental

Ferrocene benzoic acid (FC-1), ferrocene dicarboxlic acid (FC-2), and pure ferrocene were

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procured from local source and used without further purification. Cyclic voltammetric measurements were carried out by Eco Chemie Auto lab PGSTAT 12 potentiostat/galvanostat (Utrecht, The Netherlands) with the electrochemical software package GPES 4.9. Glassy carbon electrode (GC, PINE Company, part # AFE1XFP030GCR) with surface area 0.071cm$^2$, saturated calomel electrode (SCE), (Fisher scientific company cat # 1363951) and Beckmann platinum wire of 1mm diameter, were used as working, reference electrode and counter electrodes, respectively. All electrodes were inserted in electrochemical cell provided with an Argon gas inlet in the cyclic voltammetry experiments. Tetrabutylammonium perchlorate (TBAP, Fluka Chemical Co.) was used as supporting electrolyte in these electrochemical studies. UV-Visible 1601 Shimadzu spectrophotometer with measurement wavelength range of 190-1100 nm was used for DNA concentration as well as for the study of drug-DNA interactions.

*Materials*

All material was purchased from Sigma Aldrich grade and used as received. 5mM concentration of pure ferrocene, ferrocenedicarboxylic acid and ferrocenyl benzoic acid in solvents, acetonitrile and doubly distilled deionized water (80:20 v/v) were used for electrochemical and binding studies. Nuclear cell lysis method was used to extract double stranded DNA (ds.DNA) from chicken blood. UV-Vis spectroscopy was used to determine the concentration of DNA stock solution which was 182µM and the ratio of absorbance, $A_{260}/A_{280}$ is greater than 1.8 which reflects the purity of extracted ds.DNA.

**Results and Discussion**

The voltammetric technique is an easy way which could provide insight into the molecular level interactions in such a manner that is easily comprehensible [13]. The ferrocene redox couple is often studied for its ideal signal and also due to the medicinal use as anti-cancer drug [14]. The carboxylic derivatives of ferrocene are of medicinal interest as they are thought to be responsible for the increase in the aqueous solubility and enhance drug-potentiality of ferrocene in terms of targeting and decreasing other related problems [4].

The electrochemical behaviors of pure ferrocene, benzoic acid, ferrocene dicarboxylic acid was studied by the cyclic voltammetry in aqueous acetonitrile medium (acetonitrile/water in 8:2 v/v) for the comparison purpose. Fig. 1 and 2 show the voltammetric plots of ferrocene derivatives at different scan rates from 100 to 800 mV s$^{-1}$.

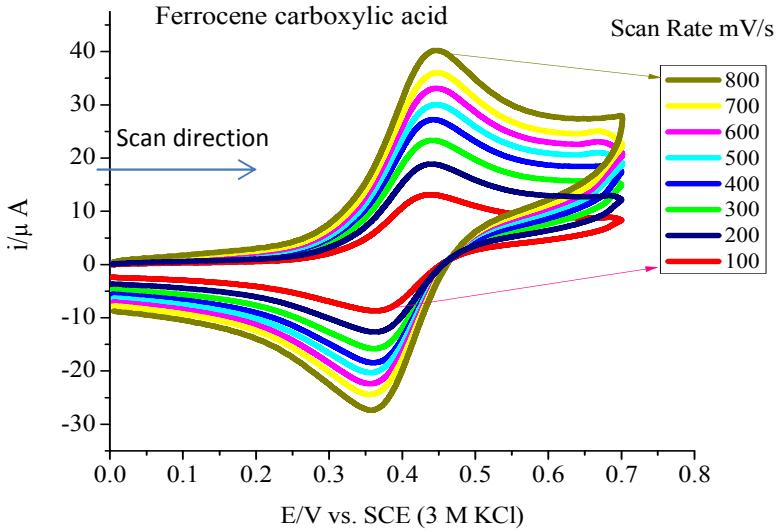


Fig.1: Cyclic voltammetric profiles of FC-1 in acetonitrile-water mixture.

These voltammograms at different scan rates were used to calculate electrochemical parameters such as transfer coefficient α, found by the equation:$\alpha = (E_{1/2}-E_p^c)/(E_p^a-E_p^c)$ in which $E_{1/2}$ is the half wave potential, $E_p^a$ and $E_p^c$ are the anodic and cathodic peak potentials [13]. Half wave potential and standard potential for totally reversible reaction are equal and was measured by the relation: $E½ (= E^o = (E_p^c + E_p^a)/2)$ [14] which is true only for a completely reversible redox process. The electrochemical (EC) data was collected for FC-1 at different scan rates and is given in Table 1 whereas the Table 2 comprises EC data for all the three compounds for comparison.

Kinetic parameters like diffusion coefficients and heterogeneous rate constants were also derived by using the data given in Tables 1 and 2. Randles-Sevcik equation was used for calculating diffusion coefficient for the reversible redox process [13, 14]:

$$i_p = (269{,}000)\, n^{3/2} A\, D^{1/2}\, C\nu^{1/2} \qquad (1)$$

where $i_p$ is the peak current (A), n is the number of electron transfer (1 in present study), D is diffusion coefficient, A is the area of electrode (0.071 $cm^2$), C is the concentration (5 x $10^{-3}$ mol $cm^{-3}$) and ν is the scan rate in V $s^{-1}$.

From the slope of Randles-Sevcik plot between $(\text{scan rate})^{1/2}$ and peak current, the diffusion coefficient was determined as depicted in Fig. 3.

With the help of the graph given above, diffusion coefficient for ferrocene benzoic acid was found to be $1.337\times10^{-7} cm^2 s^{-1}$ for oxidation process ($D_{Oxid}$) and $1.171\times10^{-7} cm^2 s^{-1}$ for reduction process ($D_{red}$). A carbon paste electrode modified with ferrocene carboxylic acid was used to sense ascorbic acid in buffered aqueous solution. A carbon paste electrode modified with ferrocene carboxylic acid was used by Raoof and coworkers and found to sense ascorbic acid in buffered aqueous solution [15].

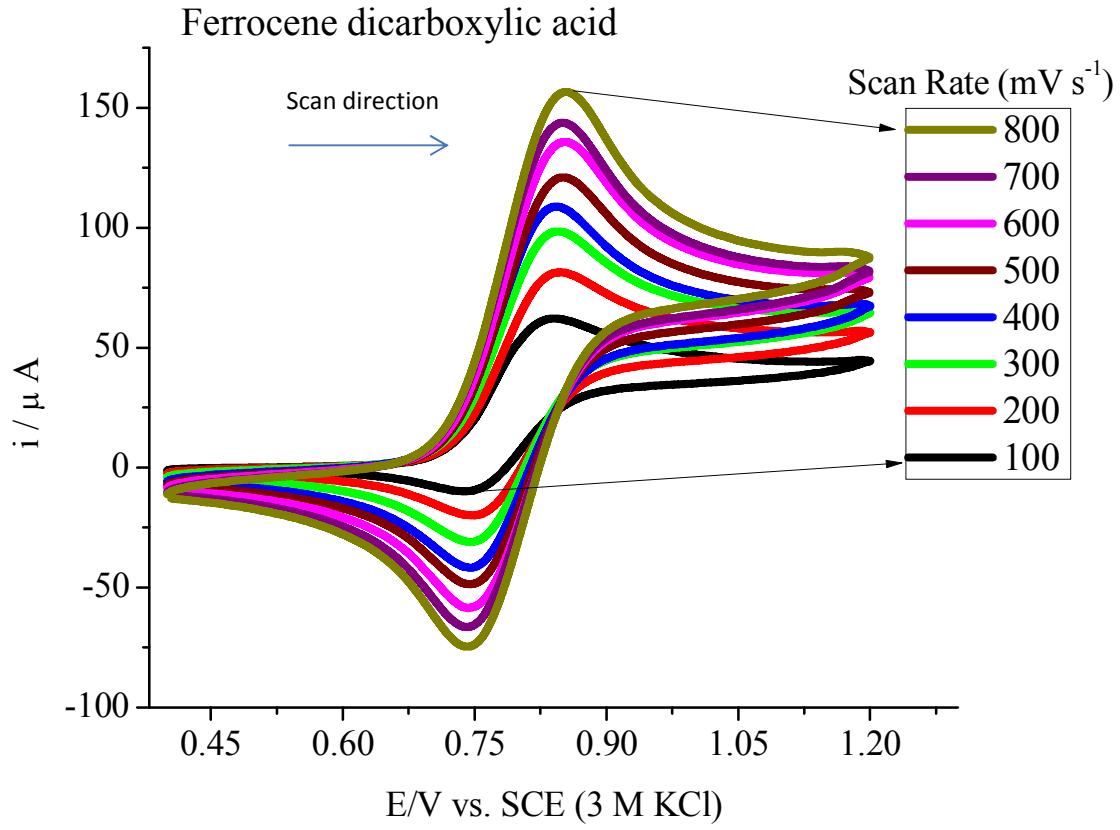


Fig. 2: Cyclic voltammetric profiles of FC-2 in acetonitrile-water mixture.

Table-1: Cyclic voltammetric data for FC-1 in acetonitrile-water mixture.

| Scan rate ($mVs^{-1}$) | $i_p^a$ (µA) | $i_p^c$ (µA) | $E_p^a$ (V) | $E_p^c$ (V) | $(E_p-E_{p/2})$ (V) |
|---|---|---|---|---|---|
| 100 | 8.80 | -10.3 | 0.435 | 0.366 | 0.058 |
| 200 | 12.4 | -14.8 | 0.437 | 0.366 | 0.057 |
| 300 | 15.3 | -18.25 | 0.437 | 0.366 | 0.059 |
| 400 | 17.5 | -21.1 | 0.439 | 0.366 | 0.062 |
| 500 | 18.8 | -23.0 | 0.439 | 0.361 | 0.062 |
| 600 | 21.0 | -25.1 | 0.439 | 0.361 | 0.062 |
| 700 | 23.1 | -27.1 | 0.439 | 0.361 | 0.062 |
| 800 | 26.2 | -30.3 | 0.439 | 0.361 | 0.062 |

Table-2: Electrochemical parameters for ferrocene and its derivatives at 400 mV $s^{-1}$.

| Compound | $i_p^a$/µA | $-i_p^c$/µA | $i_p^a/i_p^c$ | $E_p^a$/V | $E_p^c$/V | $\Delta E_p$ /V | α |
|---|---|---|---|---|---|---|---|
| FC | 401 | 314 | 1.27 | 0.46 | 0.23 | 0.11 | 0.50 |
| FC-1 | 84.0 | 71.1 | 1.15 | 0.84 | 0.75 | 0.09 | 0.49 |
| FC-2 | 17.5 | 21.1 | 0.85 | 0.43 | 0.37 | 0.06 | 0.50 |

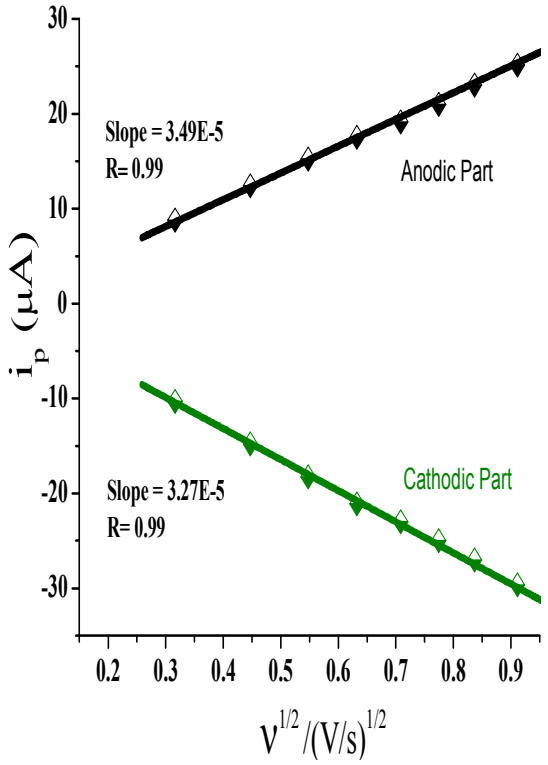


Fig. 3: Randles-Sevcik plots for FC-1 redox systems in acetonitrile-water mixture with TBAP as a supporting electrolyte.

Heterogeneous rate constants for the pure ferrocene and its derivatives were calculated by using relationship between the rate constant for heterogeneous electron transfer and peak separation formulated as given below [14].

$$k_{s,h} = 2.18\left[\frac{D_o \alpha n F v}{RT}\right]^{1/2} \exp\left[-\frac{\alpha^2 nF}{RT}\left(E_p^a - E_p^c\right)\right] \quad (2)$$

The values of diffusion constants and heterogeneous rate constants are given in the Table 3 while the comparison of voltammograms for ferrocene and its derivatives are shown in Fig. 5.

From this comparative graph, it can be easily seen that how much the diffusion coefficient decrease down with substituent group and what is the extent of shift in reduction and oxidation potentials (and extent of shift in oxidation and reduction potentials). Oxidation potential ($E_p^a$) for pure ferrocene at scan rate of 400 $mVs^{-1}$is 0.46V whereas for FC-1 and FC-2, $E_p^a$ values are 0.43 and 0.84 V, respectively.

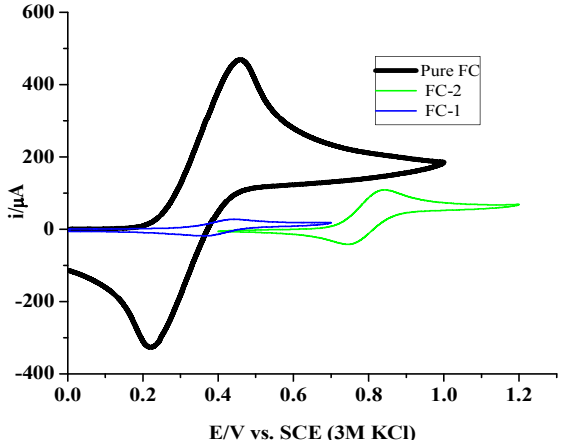


Fig. 4: Voltammograms for the comparison of pure ferrocene and its derivatives at scan rate of 400 mV $s^{-1}$.

The lowering of peak potential in monocarboxylic derivative may be due to the electron density resonance effect on the iron atom and its ferrocene/ferrocenium oxidation is facilitated as compared to that in pure ferrocene, Fig. 4 [16]. Two strong electron withdrawing groups are directly attached to η-rings in the dicarboxlic acid derivative and these apparently seem to be hydrogen bonded; therefore, the redox process is hurdled [17]. These redox potentials are ca. 380 mV higher than the redox potential of the neat ferrocene/ferrocenium redox couple [18, 19]. This increasing redox potential reflects the electron withdrawing properties of heterocyclic pyranose rings.

The diffusion coefficient and heterogeneous rate constant directly predict that how electrochemically active molecules diffuse towards the electrode interfaces during the redox process. It is presumed that as the size of the substituents attached with the ferrocene ring increases, its velocity towards the electrode surface decreases as also perceived from the parameters gathered in Table 3.

Table-3: Diffusion coefficients and heterogeneous rate constants for all compounds with and without DNA at 400 $mVs^{-1}$.

| Compound | $D_{Oxid}/cm^2s^{-1}$ | $k_{s,h}/cm \cdot s^{-1}$ | $D_{oxid}/cm^2s^{-1}$ with ds.DNA |
|---|---|---|---|
| FC | $2.51\times10^{-5}$ | $3.27\times10^{-3}$ | $1.41\times10^{-5}$ |
| FC-1 | $2.05\times10^{-6}$ | $1.84\times10^{-3}$ | $6.24\times10^{-7}$ |
| FC-2 | $1.33\times10^{-7}$ | $5.66\times10^{-4}$ | $3.34\times10^{-8}$ |

The values of diffusion coefficient and heterogeneous rate constant decreased with the size of the group attached with ferrocene. In all cases, diffusion coefficient for oxidizing current was found to be larger than that for reduction process that may be due to enhanced $Fe^{+}$-centre interactions with solvent. The adsorbed species in the forward reaction that is the oxidation process, may pose some hinderance thus decreasing the current profile in the backward direction and resulting in lower values of the kinectic parameters. In all the studies, there was a correspondence between the structures and the resultant interactional parameters of the compounds.

*Interactional studies via voltammetry*

Upon the sequential addition of ds.DNA, cyclic voltammetry profiles of the ferrocene derivatives change prominently by shift in the peak position and decrease in peak height. With gradual addition of different concentration of ds.DNA, a negative (cathodic) shift in the peak position was observed revealing some interaction with ferrocene-redox system. The oxidation process is observed at lower potentials which is due to the electrostatic interaction of cationic drug (the ferrocene derivative) with anionic phosphate group of ds.DNA backbone. Any changes in the formal potential predicts about the mode of interaction [18-21]. Positive shift or anodic shift in peak potential points to the intercalation type of interaction in which the drug stacks between the bases of double helical ds.DNA. In case of FC-1, a negative peak shift of 27 mV was observed upon 18.5 µM thus clearly indicating the electrostatic mode of interaction beween the ferrocene derivative and the ds.DNA. Whereas, in FC-2, the strong intractional indicators were observed from a negative peak shift of 27 mV upon 18.5 µM ds.DNA addition into FC-2 solution. It clearly shows the electrostatic mode of interaction beween the ferrocene derivative and the ds.DNA.. A negative or cathodic peak shift (as in case of ferrocene and its other derivatives) reveals that the electrostatic mode of interaction in which negatively charged phosphate group of double helix makes electrostatic interaction with the electropositive metal atom/ion/molecular species.

The binding constant, $K_f$ values for ferrocene benzoic acid and ferrocene dicarboxylic acid were obtained as $2.12\times10^{4}$ $M^{-1}$ and $2.48\times10^{4}$ $M^{-1}$, respectively. Recently, Kowalski et al. have demonstrated the structure dependent activities of ferrocene derivatives which have also been verified through voltammetric study, as well [22].

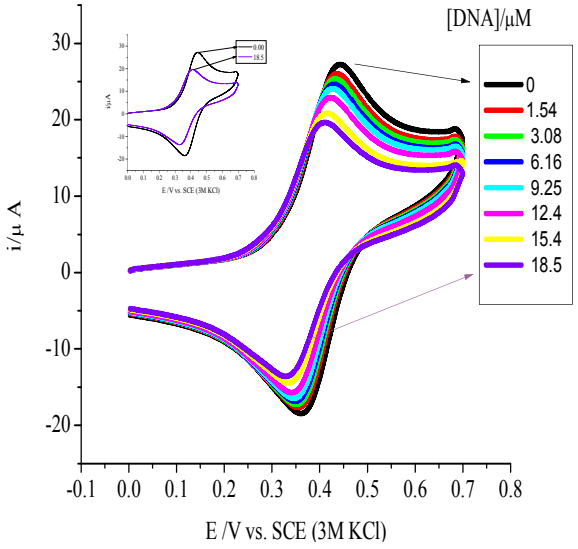


Fig. 5: Cyclic voltammetric titration of FC-1 (5mM) with [ds.DNA] at 400 $mVs^{-1}$ Inset: Comparative cyclic voltammetry with 18.5 µM ds.DNA.

The change in peak current height also predicts about which amount of drug has interacted with the ds.DNA. By adding 18.5 µM ds.DNA, the anodic peak current decreased from 17.5 to 12.49 µA and the cathodic current also decreased from 21.1 to 14.45 µA. On the average, a 30% decrease in peak current is observed upon ds.DNA addition. The substantial decrease in peak current points to the formation of a complex between FC-1 and ds.DNA thus, lowering the activity of the electrophore. By using the following equation, formation or binding constant, $K_f$ was determined, Fig. 5, 6 and 7 [21]:

$$ip^{2} = \frac{1}{K_f[DNA]}(ip_o^{2} - ip^{2}) + ip_o^{2} - [DNA] \quad (3)$$

where $i_p$ and $ip_o$ is the peak currentswith and without [DNA] and $K_f$ is the formation constant.

*UV-Visible measurements*

UV-Vis spectroscopy is also very interesting and useful technique for the exploration of drug-

DNA interaction. Spectral subtleties such as hyperchromic effect, hypsochromic red shift and blue shift may predict the behavior of the drug towards ds.DNA [18, 19, 21]. A strong peak which appeared at 304 nm (due to π→π* transition in the conjugated ring of ferrocene moiety) lowered in intensity upon titration of FC-1 with ds.DNA, Fig. 8.

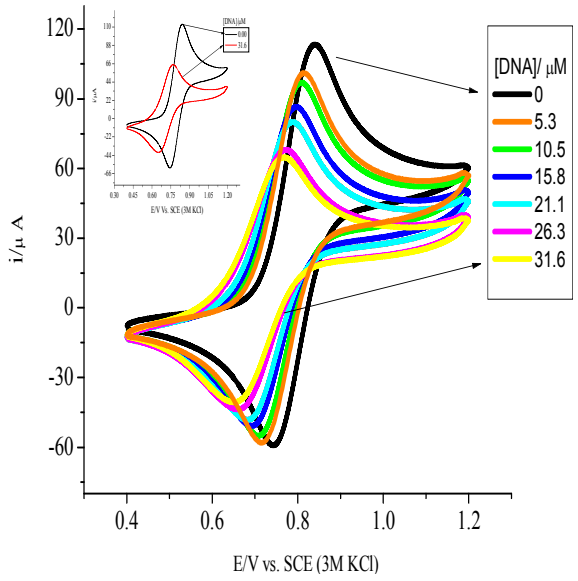


Fig. 6: Cyclic voltammetric titration of FC-2 (5 mM) without and with different concentration of DNA at scan rate of 400 mV/s. Inset: Comparative cyclic voltammetry with 31.6 μM ds.DNA.

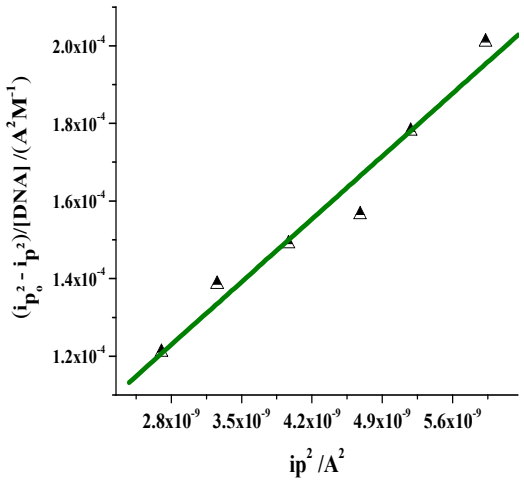


Fig.7: Functional plot for the calculation of binding constant for FC-2–DNA complex.

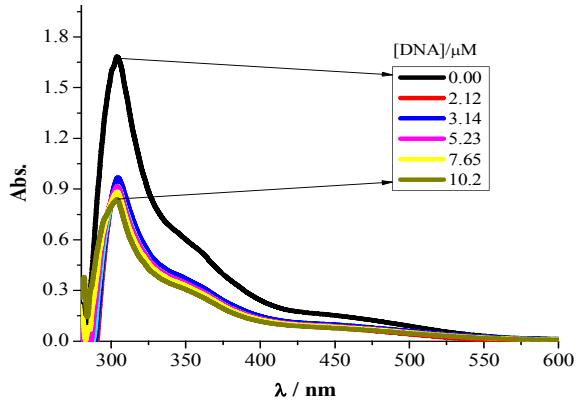


Fig. 8: UV-Vis spectra of 40 μM FC-1 with and without [ds.DNA].

Another very low peak in ferrocene benzoic acid but prominent in ferrocene dicarboxylic acid appeared at 454 nm which is due to the n→π* transition of carbonyl group. By the addition of different amount of [ds.DNA], two different behavior were observed; hypochromic effect that is due to the interaction of electronic state of binding chromophore and ds.DNA bases (π* of the binding drugs and π of the nucleotide bases couple) which ultimately reduces the probability of transition and hence hypochromic effect observed in both the ferrocene derivatives.

Strong hypochromic effect is observed for FC-1 upon addition of ds.DNA which may be due to the electrostatic interaction between the negatively charged phosphate.[18].

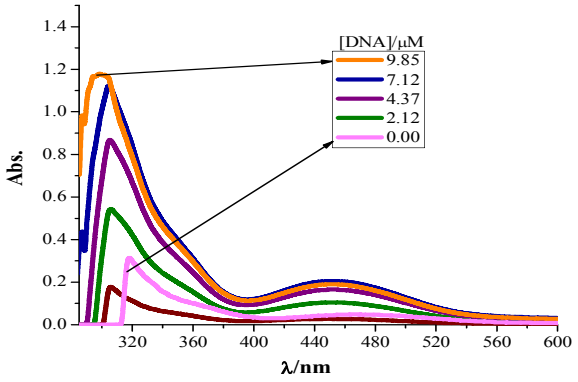


Fig. 9: UV-Vis spectra of 40μM FC-2 with and without [ds.DNA].

The observed hypsochromic and hyperchromic effects in the absorbance pointed to strong interactions between the host (ds.DNA) and

the guest, FC-2. Here, the substituent effect is prominent as both the acid groups may contribute to binding of FC-2 to ds.DNA, Figure 9. The spectral profiles were used to evaluate the formation constant, $K_f$ on quantitative basis by using Benesi-Hildebrand equation_ENREF_18_ENREF_18 [19-21]:

$$\frac{A_o}{A-A_o}=\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}+\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K_f[DNA]} \quad (4)$$

From the plots of [ds.DNA] versus absorbance factor, the $K_f$ values for both the derivatives were found to be $7.45\times10^5$ $M^{-1}$and $6.21\times10^5$ $M^{-1}$ for FC-1 and FC-2, respectively.

**Conclusions**

The presented work is focused on the study of DNA binding capacity of the ferrocene and its derivatives by using cyclic voltammetry and UV-Vis spectroscopy to evaluate the kinetic and thermodynamic parameters. The estimated values of heterogeneous rate constant and diffusion coefficients showed the presence of strong interactions between the ferrocene derivatives and ds.DNA. Oxidation potential ($E_p$) values showed that interactions of FC-2 are stronger compared to FC-1 with ds.DNA and all other parameters also corresponded to the structural dependent interactions of the compounds with ds.DNA. The strong interactions of the studied materials with ds.DNA indicate that the compounds could be studied further as potential drugs.

**Acknowledgement**

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