Electrochemical studies and spectroscopic investigations on the interaction of an anticancer drug flutamide with DNA and its analytical applications

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**Abstract**

Flutamide (Flu) is a chemotherapeutic drug, used for the treatment of prostate cancer. It functions by interfering DNA in fast growing cells and preventing them from reproducing. The present work is focused on the interaction of Flu with single and double stranded DNA at different temperatures and at physiological pH 7.4 (human blood pH). Cyclic voltammetry, square wave voltammetry and UV–visible spectroscopy were used to analyze the interaction of Flu with DNA. The voltammetric results indicate Flu gets intercalated between dsDNA bases and the strength of interaction is independent on the ionic strength. The hyperchromic effect in absorption spectra of Flu-dsDNA complex affirmed the intercalative mode of binding between Flu and dsDNA. Comparison of the mode interaction of Flu with dsDNA and ssDNA was discussed. The binding constants, stoichiometric coefficients and thermodynamic parameters of Flu-dsDNA and Flu-ssDNA complexes were evaluated. The association between Flu and dsDNA is maximum at 308 K which depicts the most stable complexes are formed at near human body temperature. The decrease in the peak current of Flu resulting from its interaction with DNA was employed for determination of dsDNA and ssDNA concentration. The detection limits of dsDNA and ssDNA were found to be $4.27 \times 10^{-7}$ M and $1.87 \times 10^{-7}$ M, respectively.

**1. Introduction**

DNA is the pharmacological target of many of the drugs that are currently in clinical use or in advanced clinical trials [1]. Due to the central role of DNA in replication and transcription, DNA has been a major target for antibiotic, anticancer and antiviral drugs [2]. So the study of the interaction of drug and DNA plays a key role in pharmacology and it is of great significance for designing and synthesizing the new drugs targeted to DNA and their effectiveness depends on the mode and affinity of the binding [3]. The recognition of DNA binders involves a complex relationship of different interactive forces. It includes the intercalation between adjacent base pairs, intrusion into the minor groove and major groove, and electrostatic interaction [4,5]. The resulting DNA-drug complex is stabilized by a number of non-covalent interactions such as van der Waals interactions, hydrophobic forces and hydrogen bonds.

The interaction of some anticancer drugs with DNA has been studied by a variety of techniques including electrophoresis [6], X-ray crystallography [7], structural modeling [8], luminescence [9], NMR [10], quartz crystal microgravimetry [11], resonance Rayleigh light scattering [12], fluorescence [13–16], and UV–visible absorption spectroscopy [17–23]. In recent years, there has been a growing interest in electrochemical investigation of interactions between anticancer drugs and other DNA-target molecules and DNA [24–34]. The use of electrochemical techniques to increase the knowledge of drug molecules and their mechanism of action is one of the most important phases of drug discovery [35]. On the other hand, the electrochemical DNA-biosensors form a useful model for simulating nucleic acid interactions with cell membranes, potential environmental carcinogenic compounds and clarifying the mechanism of interaction with drugs and chemotherapeutics. However electrochemistry coupled with spectroscopy techniques could provide a relatively easy way to obtain useful insights into the molecular mechanism of drug-DNA interaction, which could be an important step in the development of a new anticancer drug.

Among anticancer drugs, Flutamide (Flu), chemically known as 2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]-propanamide (Scheme 1), is a potent nonsteroidal antiandrogen drug primarily used to treat prostatic cancer [36]. Flu competes with testosterone
and its powerful metabolite dihydrotestosterone for binding to androgen receptors in the prostate gland thereby, and it inhibits the prostate cancer to grow. Further, Flu may also be used to treat excess androgen levels in women especially those with polycystic ovarian syndrome. This drug and its primary hydroxyl metabolite decrease metabolism of C-19 steroids by cytochrome P-450 system at the target cells in the secondary sex organ [37]. To the best of our knowledge, no attention has been paid to the interaction between anticancer drug Flu with DNA at different temperatures and at physiological pH 7.4. The formed anticancer Flu-DNA adduct at different temperatures leads to change in the thermodynamic stability and structure properties of DNA. In this context the thermodynamic characterization of drug binding might be used, together with structural studies, in development of new strategies to interface with gene expression in living cells.

In the present work, the cyclic voltammetry, square wave voltammetry and UV–visible absorption spectroscopy were used to delineate the interaction of anticancer drug Flu with single and double stranded DNA in phosphate buffer solution of physiological pH 7.4 and at different temperatures. The aim of the work is to characterize binding constant, stoichiometric coefficients, thermodynamic parameters and the interaction mechanism of anticancer drug Flu with single and double stranded DNA. It is suggestive for further fruitful research to design novel anticancer drugs and diagnosis diseases. This paper also reports analytical applications of the electrochemical studies.

2. Materials and methods

2.1. Apparatus

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were performed using an EG&G PAR 384 B (Princeton Applied Research, Oak Ridge, TN, USA) polarographic analyzer controlled by 394 software in conjunction with a PAR Model 303A HMDE. The three electrode system was completed by a Ag/AgCl (saturated KCl) reference electrode and a Pt wire auxiliary electrode. A PAR Model 305 stirrer was used for the SWV. The ultraviolet and visible absorption spectra measurements were obtained using PerkinElmer (Lambda) spectrophotometer.

2.2. Chemicals

Calf thymus double stranded deoxyribonucleic acid (dsDNA) was purchased from Sigma–Aldrich chemicals (St. Louis, Mo, USA) and was used as received without further purification. Stock solutions of dsDNA in 100 ml autoclaved deionized water and stored at 4 °C and discarded after no more than four days. The concentration of the stock solution of dsDNA (1.86 × 10⁻³ M in nucleotide phosphate, NP) was determined by UV absorbance at 260 nm using the molar extinction coefficient (ε) at 6600 M⁻¹ cm⁻¹. Denatured single-stranded DNA was prepared by heating native double-stranded DNA in water bath at a 100 °C for 30 min, followed by promptly cooling in ice bath [38]. The formation of ssDNA from dsDNA is checked by gel electrophoresis measurements. Images gel electrophoresis of ssDNA and dsDNA are provided in Fig. 1. Stock solution of Flu was prepared by dissolving the appropriate amount of the drug in ethanol and storing the solution in the dark at 4 °C. The supporting electrolyte was phosphate buffer prepared by mixing stock solution of 0.2 M NaH₂PO₄ and 0.2 M Na₂HPO₄ and adjusting the pH with H₃PO₄ or NaOH to obtain buffers in pH range 2–12. All chemicals were of reagent grade (Merck, Darmstadt, Germany). Double-distilled deionized water was used to prepare the solutions.

2.3. Voltammetric measurements

For voltammetric measurements, the test solution was placed in a polarographic cell of volume 10 ml and deoxygenated by bubbling nitrogen for 15 min. During measurements a steam of nitrogen was passed over the solution. SWV was performed using a pulse amplitude of 50 mVpp; a frequency of 100 Hz; a scan increment of 6 mV; an adsorption time of 60 s and equilibrium time of 15 s. CVs were recorded at a scan rate of 100 mV s⁻¹ (unless otherwise stated). The peak potentials were reproducible to at least ±5 mV in the CVs and ±2 mV in the SWV. Concentrations of Flu and the total volume solution constant (5 ml) were kept constant while the concentrations of dsDNA and ssDNA were varied. The equilibrium binding constant for dsDNA-Flu and ssDNA-Flu was determined by voltammetric titrations at different temperatures. All experiments were carried out at 25 ± 0.05 °C.

3. Results and discussion

3.1. Interaction of Flu with dsDNA

Cyclic voltammetric studies of Flu at a HMDE in phosphate buffer solutions showed only one cathodic peak corresponding to the direct reduction of the nitroaromatic moiety in the molecule [39]. No anodic peaks were observed at any scan rate, indicating that the reduced form of Flu is electrochemically irreversible. The decrease in peak current of Flu upon the addition of ds-DNA into the Flu solution is the maximum at pH 7.4. Therefore pH 7.4 was selected as the optimum pH to study the interaction between Flu and ds-DNA. Under these conditions the dramatic changes of CV behavior of Flu in the presence of dsDNA were occurred (Fig. 2).

When dsDNA is added to a solution of a Flu, the peak current height decreases and the peak potential shifts to more negative values are observed. The cathodic peak current of Flu decreased by Ca. 77.8% in presence of 1.04 × 10⁻⁵ M dsDNA. In this context, the pure dsDNA is electrochemically inactive in the potential range

![Scheme 1. Molecular structure of Flutamide.](image)

**Fig. 1.** Images of agarose gel electrophoresis of ssDNA and dsDNA. Lane M: DNA marker; concentrations of ssDNA: 1.0 (Lane 1), 0.5 (Lane 3) and 0.25 (Lane 5) mg/ml. Concentrations of dsDNA: 1.0 (Lane 2), 0.5 (Lane 4) and 0.25 (Lane 6) mg/ml.
Fig. 2. CV of 38.5 µM Flu at HMDE in phosphate buffer solution (pH= 7.4) in absence (1) and presence of (2) 0.84, (3) 2.49, (4) 3.31, (5) 4.92, (6) 5.72, (7) 7.29, (8) 8.85 and (9) 10.4 µM dsDNA. Adsorption potential, −0.2 V; adsorption time, 30 s; and scan rate, 120 mV s−1.

from 0.0 V to −0.7 V (versus Ag/AgCl) at the HMDE in phosphate buffer solution at pH 7.4. In terms of this result, it seems that binding of Flu to the large, slowly diffusing dsDNA, caused the decrease in the peak current of Flu in the presence of a $3.85 \times 10^{-5}$ M of dsDNA, which results in the considerable decrease in apparent diffusion coefficient of the Flu–dsDNA adduct. This is emphasized from the decrease in slope of the linear $i_l^{-0.5}$ plots ($R \approx 0.997$), where the slope values are 0.22 and 0.15 µA mV−1 s0.5 in absence and presence of dsDNA respectively. From these values, the diffusion coefficient of the free Flu ($D_0$) was found to be $1.94 \times 10^{-6}$ cm2 s−1, whereas $D_0 = 7.63 \times 10^{-2}$ cm2 s−1 for the bound Flu (in presence of $3.85 \times 10^{-5}$ M dsDNA, NP). Accordingly to these observations it seems that the decrease of peak current of Flu after an addition of excess of dsDNA is caused by the intercalation of Flu to the bulk, slowly diffusing dsDNA.

Ionic strength dependence of the interaction between Flu and dsDNA was also investigated. When the ionic strength of the solution increased after a salt was added, charge compensation of the nucleotide phosphates caused by cations would hinder the electrostatic binding effect. Consequently, complex formation through electrostatic binding would decrease, where the binding based on an intercalation mechanism should virtually unaffected [40]. The ionic strength of reaction medium was varied by changing the concentration of NaCl from 0.05 to 0.3 M. Square wave signals in those solutions with different ionic strength were recorded. No significant change in square wave signals was observed when ionic strength increased. The obtained result indicates that the interaction process of Flu with dsDNA is independent on salt concentration and was mainly intercalation mode. The interaction might be attributed to Flu that intercalates into the DNA double helix between stacked base pairs of dsDNA.

The changes in current upon addition of dsDNA can be used to quantify the binding of Flu to dsDNA. In this context, current titrations were performed by keeping the concentration of Flu constant while varying the concentration of dsDNA using CV and SWV at pH 7.4. The current titration was described by the following Eq. (1) [28].

$$\log(1/[DNA]) = \log K + \log(i_{H-c}/i_c - i_{H-c})$$  \hspace{1cm} (1)

where $K$ is the apparent binding constant, $i_c$ and $i_{H-c}$ are the peak current of the free guest (G) and the complex (H–C) respectively. Under the assumptions of irreversible, diffusion-controlled electron transfer and a 1:1 association complex between the drug and dsDNA (in nucleotide phosphate), the plot of $\log([1/DNA])$ versus $\log(i_{H-c}/i_c - i_{H-c})$ is linear with the intercept of $\log K$. The binding constant of this complex was evaluated according to Eq. (1) and the result is cited in Table 1. The value of $K$ demonstrated that Flu binds to dsDNA with a high association constant. The aforementioned results indicate the large binding constant for Flu–dsDNA adducts revealing the high affinity of the Flu for the dsDNA base pairs, i.e. a strong overlap between electronic states of the Flu chromophore and that of dsDNA base occurs. The voltammetric changes unequivocally suggest that Flu molecule, acting as an intercalator, is inserted into the base-spacing domain of dsDNA double helix [32].

As no new electrochemical signals appeared after dsDNA Flu interaction, it would be possible to assume that only one complex is formed, [dsDNA(Flu)n], according to [41]:

$$\text{dsDNA} + n\text{Flu} = \text{dsDNA(Flu)}_n$$  \hspace{1cm} (2)

Thus, the binding constant is as follows:

$$\beta_n = [\text{dsDNA(Flu)}_n]/[\text{dsDNA}][\text{Flu}]^n$$  \hspace{1cm} (3)

where $\beta_n$ is the molar relation of Flu that interacts with a molar quantity of dsDNA bases. Thus, on assessing the change in the current due to the presence of a constant concentration of dsDNA over different concentrations of Flu, the following equation was used:

$$\log(|\Delta I/(\Delta I_{max} - \Delta I)|) = n \log(\beta_n/M^{-1}) + n \log[\text{Flu}]$$  \hspace{1cm} (4)

The plot of $\log(|\Delta I/(\Delta I_{max} - \Delta I)|) versus \log[\text{Flu}]$ becomes linear with the slope of $\beta_n$ and the intercept of $n \log(\beta_n)$. Fig. 3A shows the effect of a constant dsDNA concentration on the electrochemical response of a series of Flu concentration solutions and Fig. 3B shows a linear logarithmic relation with a regression coefficient 0.997. The fact that there is one slope in plot represented in Fig. 3B confirms the single complex formation, thus the molar value of $\beta_n$ and binding constant for dsDNA–nFlu complex are determined. There is good agreement between $K$ values of Flu–dsDNA obtained from Eqs. (1) and (4) which are $1.70 \times 10^4$ M−1 and $1.9 \times 10^5$ M−1 respectively. The value obtained for $\beta_n$ is 1.68 and smaller than those obtained for bigger molecules such as metallic complexes with reported values up to 25 [42]. This is probably due to the smaller size of Flu compared with metallic complexes.

3.2. UV–vis spectral studies of Flu–dsDNA complex

In order to investigate the mode of Flu binding to dsDNA, the absorption spectra has been employed in examination of the reaction, as Flu binds to dsDNA by intercalation. The UV–visible absorption spectrum of dsDNA (Fig. 4A) showed a maximum absorption at 260 nm. This maximum is a consequence of the electronic transitions. With the addition of various concentrations of anticancer drug Flu into dsDNA solution, the absorption spectrum of dsDNA (Fig. 4A) showed a maximum absorption at 260 nm. This maximum is a consequence of the electronic transitions. With the addition of various concentrations of anticancer drug Flu into dsDNA solution, the absorption spectra in significantly different from that of Flu or dsDNA. When the Flu interacts with dsDNA, it causes hyperchromic effect (increase in absorbance of dsDNA) with increasing concentration of Flu in solution (Fig. 4A). The increase in the absorbance intensity of dsDNA is attributed to stacking interaction between the aromatic chromophore and dsDNA base pair. This indicated that Flu might have intercalated between the two strands of dsDNA thereby increasing that absorption of dsDNA due to the unwinding of dsDNA double helical structure to a little extent. Due to this slight unwinding of
Table 1
Values of binding constant ($K$), the standard Gibbs free energy ($\Delta G^0$), the enthalpy ($\Delta H^0$) and the entropy ($\Delta S^0$) for the binding of flutamide with dsDNA and ssDNA at different temperatures at pH 7.4 using SWV.

<table>
<thead>
<tr>
<th>$T$(K)</th>
<th>$K \times 10^5$ (M$^{-1}$)</th>
<th>$-\Delta G^0$ (kJ mol$^{-1}$)</th>
<th>$\Delta H^0$ (kJ mol$^{-1}$)</th>
<th>$\Delta S^0$ (JK$^{-1}$ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu-dsDNA</td>
<td>278.00 ± 0.05 1.01</td>
<td>26.63</td>
<td>19.87</td>
<td>166.94</td>
</tr>
<tr>
<td>288.00 ± 0.05 1.28</td>
<td>28.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>298.00 ± 0.05 1.70</td>
<td>29.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>308.00 ± 0.05 2.34</td>
<td>31.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flu-ssDNA</td>
<td>278.00 ± 0.05 2.10</td>
<td>28.33</td>
<td>20.84</td>
<td>176.67</td>
</tr>
<tr>
<td>288.00 ± 0.05 2.90</td>
<td>30.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>298.00 ± 0.05 3.80</td>
<td>31.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>308.00 ± 0.05 4.92</td>
<td>33.56</td>
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</table>

dsDNA, the aromatic base get more exposed to UV radiation and resulted in increased intensity of absorption.

To confirm the mode of interaction between the anticancer Fl and dsDNA, absorption spectra of the investigated drug at constant concentration (0.1 mM) in the absence and presence of dsDNA are taken (Fig. 4B). Absorbance maxima of Fl in the absence of dsDNA are located at 228 nm and 306 nm. Important changes in the absorption spectrum of Fl were observed upon the addition of dsDNA. With the addition of various concentrations of dsDNA into Flu solution, the absorption band of Fl at 228 nm disappeared whereas the broad band at 306 nm appeared as a strong shoulder indicating the strong interaction between Fl and dsDNA. At the same time a new band developed at approximately of 275 nm with increasing dsDNA concentration and has been assigned to FludsDNA complex. In this case the increase in the concentration of added dsDNA enhanced the absorption peak height i.e. hyperchromic effect that could be related with enhanced intercalation of Fl into dsDNA and the complex formed accordingly may become more compact. The increase in intensity of the absorption maxima (hyperchromic effect) of drug or dsDNA binding molecules is a typical characteristic of dsDNA intercalation [43]. This hyperchromic effect provided the evidence for Fl intercalation in dsDNA. The aforementioned spectral characteristics are attributed to the strong intercalation of the anticancer Flu chromophore with dsDNA bases. Thus, the spectroscopic studies complimented the electrochemical results to propose the intercalation of binding between the anticancer drug Fl and dsDNA.

3.3. Binding of Fl with ssDNA

The binding of the anticancer drug Fl to ssDNA was investigated by means of CV in the presence and absence of ssDNA (Fig. 5). When ssDNA is added to a solution of Fl, marked decreases in the peak current heights are observed. The cathodic peak current of Fl decreased by Ca. 76.67% in presence of 4.45 × 10$^{-6}$M ssDNA and the peak potential shifted to more negative potential. Accordingly to these observations it seems that the decrease of peak current of Fl after an addition of excess ssDNA is cased by the electrostatic attraction of Fl to the bulk, slowly dispersion ssDNA, which results in considerable decrease in the apparent diffusion coefficient. This is emphasized from the decrease in the slope of the linear $i_p$–$V^{1/2}$ plots ($R > 0.995$), where the slope values are 0.22 and 0.13 $\mu$A mV$^{-1}$ s$^{-1/2}$ in absence and...
presence of ssDNA respectively. From these values, the diffusion coefficient ($D_i$) of the free Flu was found to $1.94 \times 10^{-6}$ cm$^2$ s$^{-1}$, whereas $D_b = 8.83 \times 10^{-7}$ cm$^2$ s$^{-1}$ for the bound Flu (in presence of $1.27 \times 10^{-6}$ M ssDNA, NP).

Current titrations were performed by means of CV at pH 7.4 keeping Flu concentration constant while varying the concentration of ssDNA. Assuming an irreversible diffusion controlled electron transfer and a 1:1 association complex between Flu and ssDNA, the plot of log[1/[[ssDNA]]] versus log([H$_i$-c]/[H$_i$-c]) is linear with the intercept of logK. The binding constant was evaluated according to Eq. ($1$) and the result is shown in Table 1 demonstrated that Flu binds to ssDNA with a high association constant. The interaction mode of Flu molecules with ssDNA is electrostatic attraction via negative phosphate on the exterior of the ssDNA with Flu. In addition, no intercalative interactions are expected if the bind is with single stranded DNA.

The effect of a constant ssDNA concentration on the SWV response of a series of concentration of Flu was studied. In this context the plot of log[$\Delta I/([\Delta I]_{\text{max}} - \Delta I)$] versus log[Flu] is linear and confirms the single complex formation. Thus the molar value of $n_i$ and binding constant $K_i$ Flu with ssDNA are determined.

The values for $n_i$ and $K_i$ are $3.30 \times 10^2$ M$^{-1}$ and 1.61 respectively. There is good agreement between the values of stability constant obtained from Eqs. ($1$) and ($4$) which are $3.80 \times 10^3$ M$^{-1}$ and $3.30 \times 10^2$ M$^{-1}$ respectively.

### 3.4. Thermodynamic parameters of Flu-dsDNA and Flu-ssDNA

The binding constants of Flu-dsDNA adducts at different temperatures and at a physiological pH 7.4 were studied by means of SWV. Inspection of Table 1 unequivocally reflects an increase in the value of binding constant with the increase in temperature. This suggests that the binding reaction of Flu with dsDNA is endothermic, and the increase temperature benefits Flu binding with dsDNA. It can be manifested that increasing temperature from 278.00 ± 0.05 K to 308.00 ± 0.05 K maximizes the association between Flu and dsDNA which corresponds to more stable Flu-dsDNA complex formed at 308.00 ± 0.05 K. Obviously, the conformation of the Flu between the stacked base pairs of the dsDNA and hence intercalation is optimal near the human body temperature helping Flu to act as anticancer drug.

For the evaluation of the thermodynamic parameters of Flu-dsDNA adduct, the binding constant values were converted to Gibbs energy changes by the Eq. ($5$):

$$
\Delta G^\circ = -RT \ln K
$$

where the $R$ is universal gas constant $(8.31 \text{ J K}^{-1} \text{ mol}^{-1})$, $T$ the absolute temperature and $K$ the binding constant. The calculated values of $\Delta G^\circ$ are negative, suggesting a thermodynamically favorable binding process. The integrated form of van’t Hoff Eq. ($6$) permits to calculate the values of enthalpy ($\Delta H^\circ$) and entropy ($\Delta S^\circ$) depending on variation of the stability constant with temperature.

$$
\ln K = -\Delta H^\circ / RT + \Delta S^\circ / R
$$

The van’t Hoff plots for the Flu-dsDNA adduct shows a linear behavior (Fig. 6) and allows to derive the thermodynamic parameters.

The relative thermodynamic parameters of Flu-dsDNA showed the following tendencies (Table 1). The positive value of $\Delta S^\circ$ indicates that Flu interactions with dsDNA are entropy driven. The value of $\Delta H^\circ$ is positive which proves that the binding reaction of Flu with dsDNA is endothermic. The free energy values $\Delta G^\circ$ of this interaction at different temperatures and pH 7.4 are negatively large due to their strong association and this is consistent with spontaneously reaction. In this context Ross and Subramanian [44] reported that when $\Delta H^\circ < 0$ or $\Delta H^\circ \approx 0$ and $\Delta S^\circ > 0$, the electrostatic force demonilates the interaction; when $\Delta H^\circ < 0$ and $\Delta S^\circ < 0$, van der Waals interactions or hydrogen bonds demonilate

![Fig. 5. CV of 38.5 μM Flu at HMDE in phosphate buffer solution (pH = 7.4) in absence (1) and presence of (2) 0.66, (3) 1.32, (4) 1.96, (5) 2.60, (6) 3.23, (7) 3.85, and (8) 4.45 μM ssDNA. Other conditions as in Fig. 2.](image)

![Fig. 6. (A) Plot of ln K versus 1/T for the Flu-dsDNA complex. (B) Plot of ln K versus 1/T for the Flu-ssDNA complex.](image)
the reaction; and when $\Delta H^r > 0$ and $\Delta S^r > 0$, hydrophobic interactions dominate the binding process. By applying this analysis to the binding system of Flu-dsDNA, we determine that non-bonded (hydrophobic) interaction was the most important factor contributing to the observed positive $\Delta H^r$ and $\Delta S^r$ and hence to the stability of dsDNA association complex.

The temperature dependence of the binding constant for the interaction of Flu with ssDNA was studied at pH 7.4. The interaction of Flu with ssDNA exhibits a positive $\Delta H^r$ value and a positive $\Delta S^r$ value (Table 1). $\Delta G^r$ is negative at different temperatures revealing that the binding process of Flu with ssDNA is favorable and spontaneous.

### 3.5. Comparison of the interaction of anticancer Flu with dsDNA and ssDNA

Comparison of the interaction of Flu upon the addition of dsDNA and ssDNA at pH 7.4 indicated that the decrease in peak current ($\Delta I$) decreases more sharply with the addition of ssDNA than in the presence of dsDNA as shown in Fig. 7.

In comparing the values of the binding affinity and thermodynamic parameters for the interaction of anticancer Flu with dsDNA and ssDNA we observed the following tendencies. The interaction of Flu with ssDNA displaced high affinity in solution than with dsDNA (Table 1). At the same time the magnitude of changes in $\Delta G^r$, $\Delta H^r$ and $\Delta S^r$ supported that binding process of Flu with ssDNA is more favorable and spontaneous than that with dsDNA. This result indicates a different interaction mechanism of Flu with ssDNA than with dsDNA. The interaction of Flu with dsDNA might be attributed to their intercalation into base stacking domain of DNA double helix. The interaction of Flu with ssDNA indicated that the backbone of ssDNA is negatively and can easily attract to the investigated drug via electrostatic attraction. This reflected the more negative value of $\Delta G^r$ in interaction of Flu with ssDNA than that with dsDNA. The results offer an opportunity to understand how the kind of DNA affects its binding and affinity of binding to the anticancer drug Flu.

### 3.6. Analytical aspects of DNA-Flu interaction

The decrease in peak current of Flu resulted from the addition of ssDNA or dsDNA into the Flu solution can be employed to determine the concentration of dsDNA and ssDNA. Under the optimum experimental conditions the decreases in peak current of Flu were linearly related to dsDNA or ssDNA concentration in the range of $6.60 \times 10^{-7}$ M–$4.45 \times 10^{-6}$ M when the Flu concentration was fixed at $3.85 \times 10^{-5}$ M (Fig. 8).

The variation of decrease in peak current versus the dsDNA or ssDNA concentration was represented by a straight line followed the equations $I_d(\mu A) = 1.04 - 6.72 \times 10^6 C (M) (dsDNA)$ and $I_s(\mu A) = 1.06 - 1.60 \times 10^5 C (M) (ssDNA)$, with regression coefficients of 0.993 and 0.990 respectively. The limits of detection (LOD) and quantitation (LOQ) were computed and the following results obtained: LOD (dsDNA) = $4.27 \times 10^{-7}$ M, LOQ (dsDNA) = $1.40 \times 10^{-6}$ M, LOD (ssDNA) = $1.87 \times 10^{-2}$ M, LOQ (ssDNA) = $6.25 \times 10^{-2}$ M. Interferences of the coexisting metal ions forming complexes with Flu were evaluated. Metal ions tested at 0.39 $\mu$M and found not to interfere with the reduction of the Flu were Mg$^{2+}$, Pb$^{2+}$, Co$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$. However in presence of 0.39 $\mu$M of Cd$^{2+}$, Ca$^{2+}$ and Ba$^{2+}$ the peak height of Flu reduction decreased by 0.71%, 1.12%, and 1.22% respectively. In the presence of some organic compounds such as alanine, phenylalanine, and oxalic acid the reduction current of Flu decreased by 2.81%, 1.12%, and 1.77% respectively. The results offer an opportunity to use Flu drug as a new indicator measuring DNA concentration. The proposed method is simple, sensitive and rapid, and hence, can be applied for determination of dsDNA and ssDNA concentrations in the clinical samples.
4. Conclusions

The single and double stranded DNA properties of anticancer, flutamide at different temperatures and at physiological pH 7.4 were studied. The voltammetric results indicated that the planar of Flu intercalated to the dsDNA. The hyperchromic effect in absorption spectra of Flu-dsDNA complex affirmed the intercalative mode of binding between Flu and dsDNA. The interaction mode of Flu molecules with ssDNA is electrostatic attraction via negative phosphate on the exterior of the ssDNA with Flu. The complete thermodynamic profiles for the binding of Flu to DNA are also constructed and the results show the binding of Flu to DNA is endothermic and a spontaneous process of association. The association between Flu and dsDNA is maximum at 308 K which depicts the most stable complexes are formed at near human body temperature. Thus the human body temperature provides the most favorable conformation of dsDNA which binds to the anticancer Flu, helping this drug to hinder dsDNA replication under the physiological conditions. The obtained results are of potential importance in understanding the mechanism of interaction of the drug with DNA in the living body. Simple, rapid and sensitive voltammetric method is proposed for the determination of dsDNA and ssDNA concentration.

Conflict of interest

There is no conflict of interest.

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