Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(1):82-88
(http://www.derpharmachemica.com/archive.html)

Electrochemical and Spectroscopic Evaluation on Interaction of Anticancer Drug Bicalutamide with DNA

Umar J Pandit[1], Gowhar A Naikoo[2], Gulzar A Khan[3], Sneha Wankar[1], Imran Khan[1], Raj KK[1], Limaye SN[1]*

[1]Department of Chemistry, Rare Earth and Electroanalytical Research Laboratory, Dr. Harisingh Gour (Central) University, Sagar, Madhya Pradesh 470003, India

[2]Department of Mathematics and Sciences, College of Arts and Applied Sciences, Dofar University, Salalah, Oman

[3]Department of Chemistry, Heterocyclic Synthesis and Electroanalytical Laboratory, Dr. Harisingh Gour (Central) University, Sagar, Madhya Pradesh 470003, India

ABSTRACT

Differential Pulse (DP) voltammetry, spectrophotometry, fluorescence emission spectroscopy and docking simulations were employed to investigate the interaction of an anticancer drug Bicalutamide (BIC) with CT-DNA (Calf thymus-DNA). At modified single walled carbon nanotube carbon paste (SWCNT-CP) electrode the anticancer drug BIC produced a well-defined cathodic reduction peak which decreased on addition of CT-DNA. On increasing concentrations of CT-DNA no shift of peak potential of BIC was observed, characteristic of Groove binding mode of interaction. A binding constant (K) of 2.13 × 10^6 M^{-1} was determined by DP voltammetric method. In addition, the interaction of BIC with CT-DNA was further examined by spectrophotometric and fluorescent emission techniques. Interestingly, the binding constants (K) obtained through spectrophotometric method and voltammetric technique was in close agreement. However, the groove binding interaction of drug and DNA was also revealed by spectroscopic studies which were supported by theoretical docking studies.

Keywords: Bicalutamide, DNA, Groove binding, Binding constant, Spectrophotometry

INTRODUCTION

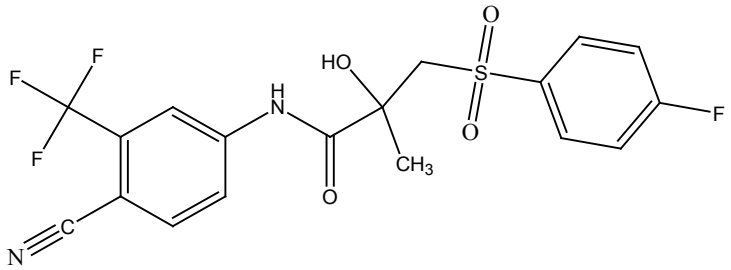
DNA is an important bio-macromolecule which plays main part in life processes *viz* storing, copying and transmitting genetic information through genes. From pharmacological point of view, DNA is target for many clinically practical drugs and drugs under advanced clinical trials. The binding nature of these pharmaceuticals with DNA is important in understanding the mechanism of these drugs and also helpful in designing new drugs [1-9].

Various analytical techniques like electrochemical [10-14], spectrophotometry [15], fluorescence [16], NMR [17], FT-IR [18] etc. have been used to study drug-DNA interactions and mechanisms. To understand the drug-DNA interaction mechanism, it is important to introduce techniques which are relatively simple, rapid and more importantly economical. DNA being electrochemically active with many other drug molecules exhibit redox activity. These electrochemical methods especially cyclic voltammetry, differential pulse voltammetry and stripping voltammetry have been widely employed to understand the mechanism of DNA-drug interactions due to their high sensitivity and economical nature.

Many binding mechanism are operative in drug-DNA interaction, the important of which are intercalative binding, covalent binding, non-covalent binding and external binding [3,19]. Among these, the intercalative binding which can occur as classical intercalation, threading intercalation or through groove binding results in insertion of aromatic ligand into adjacent base pairs on the DNA, which

results in distortion of DNA structure and thus thwarting its biological function [2,3]. The study of the drug-DNA mechanisms have considerably increased the knowledge for better understanding the pharmacokinetics of many anti-cancer drugs with the advent of developing more powerful DNA targeted pharmaceuticals.

The present work reveals the investigation on the interaction of an anticancer drug Bicalutamide (BIC) [N-(4-cyano-3-trifluoromethyl-phenyl)-3-(4-fluoro-phenylsulfonyl)-2-hydroxy-2-methyl-propionamide (Scheme 1)] with calf-thymus DNA (CT-DNA) by voltammetric technique along with the support of fluorescence, spectrophotometric techniques followed by theoretical studies.



Scheme 1: N-(4-Cyano-3trifluoromethyl-phenyl)-3-(4-fluoro-benzenesul fonyl)-2hydroxy-2-methyl-propionamide

MATERIALS AND METHODS

Chemical and materials

CT-DNA, Bicalutamide pure, graphite powder and SWCNT's (Sigma-Aldrich) were used without further purification. All other chemicals and reagents used were of analytical grade and purchased from Merck India. Stock standard solution of BIC was prepared by dissolving 1 mg BIC in 10 ml ethanol solution. 0.1 M phosphate buffer was prepared in ultrapure water. CT-DNA solution was prepared in 0.1 M phosphate buffer of pH 7.0 ± 0.2 by dissolving 1 mg CT-DNA in 100 ml buffer solution. The concentration of stock solution of CT-DNA was determined by spectrophotometry using molar absorption coefficient of 6600 $cm^{-1}M^{-1}$ [20]. Working solutions of drug and CT-DNA were prepared by diluting standard solutions with appropriate volumes of solvent or buffer solutions.

Preparation of modified carbon paste electrode

Bare and modified Carbon Paste Electrodes (CPE) was prepared according to literature reports [21,22] by hand-mixing Graphite powder and mineral oil (paraffin oil) in 70:30 ratios for bare CPE. Modified SWCNT-CPE was prepared in the same way by mixing SWCNT's: Graphite: Paraffin oil in the ratio 10:60:30. The resulting paste was filled in polyethylene syringe of 2 mm internal diameter with a pre-inserted copper wire to establish an external electric contact. Fresh electrode surface for each measurement was generated by mechanically pressing the paste from top and smoothed using weighing paper. Finally the electrodes were carefully washed with distilled water.

INSTRUMENTATION

Voltammetric measurements were performed with a computer-controlled Electrochemical Ion Analyzer model Ω 797 VA Computrace (Swiss made), assembled with a three electrode cell employing a bare and hand-made SWCNT-CP working electrode, an Ag/AgCl (saturated KCl) reference electrode and a platinum wire as counter electrode. Spectrophotometric experiments were carried out on Systronics 2201 double beam UV-Vis spectrophotometer. Fluorescence spectra were recorded on Shimadzu RF 5301 spectrofluorometer equipped with a Xe lamp as the excitation source. Excitation and emission slits were 5 nm each with the instrument operating in high sensitivity and super scan speed. All pH measurements were performed with Systronic digital μpH meter model-361. All experiments were performed at room temperature. Pure nitrogen gas was purged through test solutions for 5 minutes for oxygen free atmosphere.

RESULTS AND DISCUSSION

Surface study

Prior to voltammetric investigation on drug-DNA interactions, the fabricated bare and modified SWCNT-CPE were electrochemically characterized for their electroactive surface area by performing cyclic voltammetric measurements on 1.0 mM $K_3Fe(CN)_6$ probe in 0.1 M KCl electrolyte at different scan rates. The probe exhibited a pair of reversible couple at both electrodes. However at modified SWCNT-CPE the probe exhibited a reversible couple with smaller ΔE_p values, which is a consequence of faster electron transfer process at the modified electrode. The Randles–Sevick [23] equation for a reversible system is described as:

$$I_{pa} = 0.4463\left(\frac{F^3}{RT}\right)^{\frac{1}{2}} n^{3/2} A D^{1/2} v^{1/2} C \qquad (1)$$

Where I_{pa} is anodic peak current, A surface area of electrode, D diffusion coefficient, n number of electrons transferred, C

concentration of $K_3Fe(CN)_6$ and v (Vs^{-1}) is scan rate. For 1.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl, n=1 and D=7.6 × 10^{-6} cm^2 s^{-1} at T=298 K. The slope of the plot of I_{pa} (µA) *vs.* $\nu^{1/2}$ (Vs^{-1}) is then operated for calculating electroactive surface area electrodes. The surface area of bare electrode was calculated 0.057 cm^2 and for modified electrode the microscopic surface area was found to be 0.270 cm^2.

Voltammetric study of BIC-DNA interaction

The differential pulse (DP) voltammetric behavior of BIC yielded a well-defined, cathodic reduction peak at -0.840 V in 0.1 M phosphate buffer (pH=7.0 ± 0.2) at modified SWCNT-CPE. Various instrumental and experimental conditions were optimized for the selective determination of the drug. The analytical parameters of the developed method for the drug are presented in Table 1 [24]. Under these optimized parameters it was found that the peak current intensity of BIC decreased upon the addition of CT-DNA solution (Figure 1). To confirm whether the decrease in peak current of BIC is solely due to formation of BIC-DNA adduct or due to adsorption of DNA on electrode surface or due to effect of DNA on viscosity of solution, a cyclic voltammetric measurement was carried on $K_4Fe(CN)_6$ solution in absence and presence of increasing amounts of CT-DNA. A negligible change in peak current of $K_4Fe(CN)_6$ was observed which supports the fact that CT-DNA does not get adsorbed at the electrode surface and has minor effect on viscosity of the solution.

Table 1: Analytical parameters for voltammetric determination of bicalutamide using differential pulse voltammetry

Parameters	Value
Peak potential Ep (V)	-0.84
Linearity range (mM)	0.01-1
Slope (mA/mM)	5.783
Intercept	5.841
Correlation Coefficient (R2)	0.982
LOD (mM)	0.052 (±0.005)
LOQ(mM)	0.174 (±0.005)
Repeatability of peak current (RSD%)	1.21
Repeatability of peak potential (RSD%)	0.82

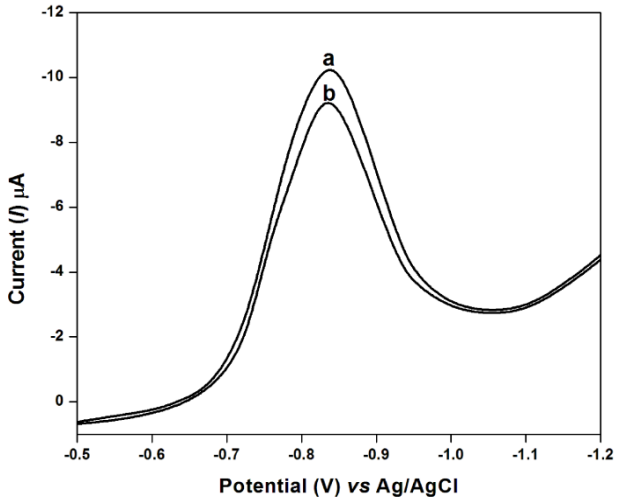


Figure 1: DP voltammograms of 2 × 10^{-6} M BIC in (a) absence and (b) presence of 5 × 10^{-6} M DNA at SWCNT-CP electrode

The DP voltammetric behavior of BIC upon increasing concentrations of CT-DNA (Figure 2A) resulted in shift of peak potential to more positive values, ratifying to the fact that BIC-DNA interact through intercalative mode [25]. Effect of scan rate on BIC in presence of CT-DNA (Figure 2B) resulted in linear plot between peak current of BIC-DNA complex and square root of scan rate which is a property shown by diffusion controlled species [26]. The binding constant (K) between DNA and BIC was evaluated through literature reports [27,28] from the following equation.

$$\log\left(\frac{1}{[DNA]}\right) = \log K + \log\left(\frac{I}{I_0 - I}\right) \qquad (2)$$

Where K is binding constant, I and I_0 are peak currents of BIC in presence and absence of CT-DNA respectively. A plot of $\log\left(\frac{1}{[DNA]}\right) vs. \log\left(\frac{I}{I_0 - I}\right)$ as shown in Figure 3 was linear. The intercept of this plot provides binding constant (K) between BIC and CT-DNA, which in present case was calculated to be 2.13 × 10^6 M^{-1}.

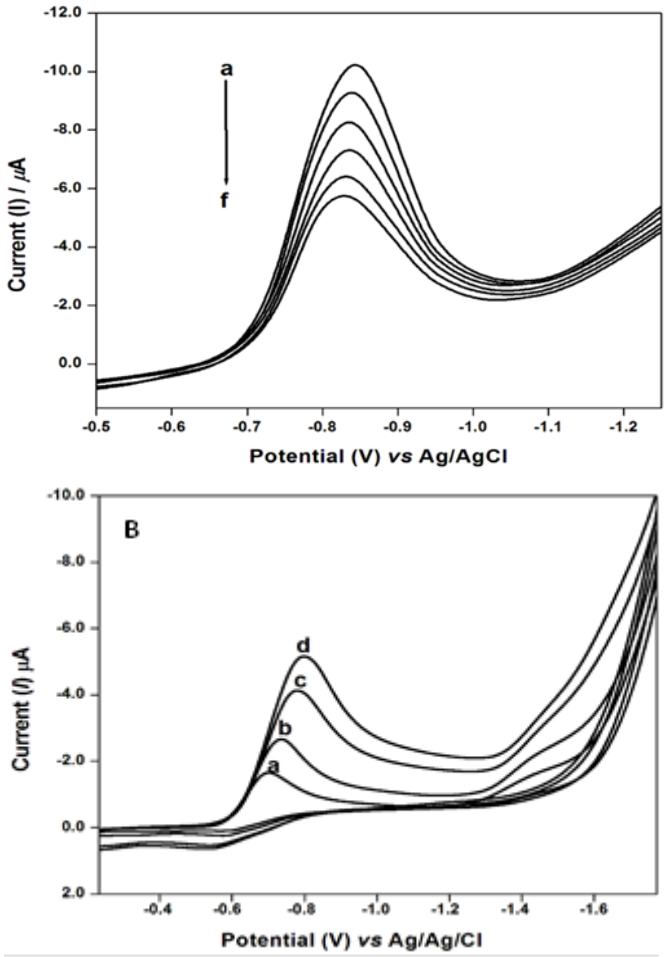


Figure 2: (A) DP voltammograms of 2 × 10^{-6} M BIC in a) absence and presence of b) 5 × 10^{-6} M, c) 10 × 10^{-6} M, d) 20 × 10^{-6} M, e) 30 × 10^{-6} M, f) 40 × 10^{-6} M DNA at SWCNT-CP electrode. (B) Cyclic voltammograms of 2 × 10^{-6} M BIC and 5 × 10^{-6} M DNA adduct at scan rate a) 25 mVs^{-1}, b) 50 mVs^{-1}, c) 75 mVs^{-1}, d) 100 mVs^{-1} on SWCNT-CP electrode

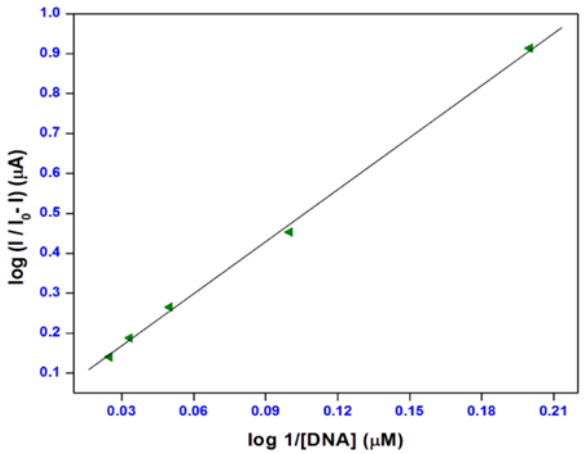


Figure 3: Plot of log(1/[DNA]) *vs.* log(I/(I_0-I))

UV-Vis spectroscopic study of BIC-DNA complex

UV-Vis spectrophotometry is the most common and simplest technique used to study the drug-DNA interactions and also to evaluate the binding constant (*K*). The maximum absorption of CT-DNA was located at 260 nm and for BIC maximum absorption is at 273 nm (Figure 4A). On addition of increasing concentrations of CT-DNA to BIC solution a consistent decrease in absorption maxima of BIC was observed as shown in Figure 4B with a slight bathochromic shift. Both hypochromism and bathochromism are considered an indication of intercalative interaction between CT-DNA and BIC. While the extent of hypochromism [29] and magnitude of red-shift [30] could be interpreted for the strength of interaction between DNA and drug. As observed from Figure 4B the drug-DNA adduct display a smaller red shift ($\Delta\lambda \leq 8$ nm) [31] which further sub divides that interaction occurs probably through

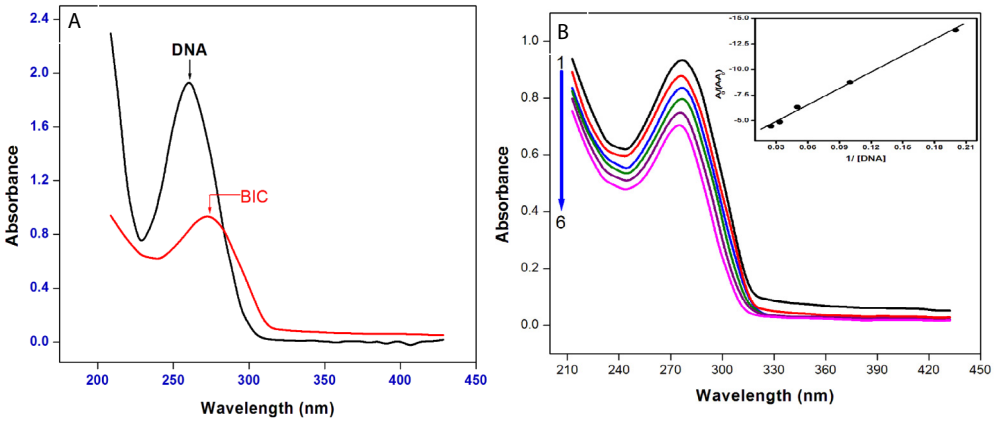


Figure 4: (A) Absorption spectra of DNA and BIC in 0.1 M phosphate buffer. (B) Absorption spectra of 2 × 10^{-6} M BIC in 0.1 M phosphate buffer (pH 7.0 ± 0.2) with increasing concentrations of CT-DNA (1-6) 5 × 10^{-6} M, 10 × 10^{-6} M, 20 × 10^{-6} M, 30 × 10^{-6} M, 40 × 10^{-6} M (Insert plot of $\frac{A_0}{A-A_0}$ *vs.* 1/[DNA])

groove binding. The binding constant (*K*) of the drug with DNA is based on the variation in absorbance of drug upon binding with DNA and employing Benesi-Hilderbrand equation [32].

$$\frac{A}{A-A_0}=\frac{\varepsilon G}{\varepsilon_{H-G}-\varepsilon G}+\frac{\varepsilon G}{\varepsilon_{H-G}-\varepsilon G}\times\frac{1}{K[DNA]} \qquad (3)$$

Where *K i*s binding constant A_0 and A are absorbances of pure BIC and BIC-DNA complex respectively. εG and ε_{H-G} are absorption coefficients of drug and drug-DNA complex respectively. The binding constant is evaluated from the intercept to slope ratio of $\frac{A_0}{A-A_0} vs. 1/[DNA]$ (Figure 4B). In the present study the binding constant obtained from spectrophotometric measurements is 1.78 × 10^6 M^{-1}.

Fluorescence study of BIC-DNA complex

BIC-DNA interaction was further examined by fluorescence titration technique. Fluorescence emission spectroscopy is probably the most common to study drug-DNA interaction, the advantages being its high sensitivity, large linear concentration range and selectivity [3]. BIC emits at 328 nm upon excitation at 272 nm in fluorescence emission spectra. As evident from Figure 5 the fluorescence emission spectra of BIC constantly decreased with increasing concentrations of CT-DNA and reached BIC-DNA binding saturation at 4 × 10^{-5} M CT-DNA concentration. This observation confirms the fact that BIC fluorescence is efficiently quenched upon binding with DNA. The fluorescence titration results are subjected to Stern-Volmer analysis using the equation [33].

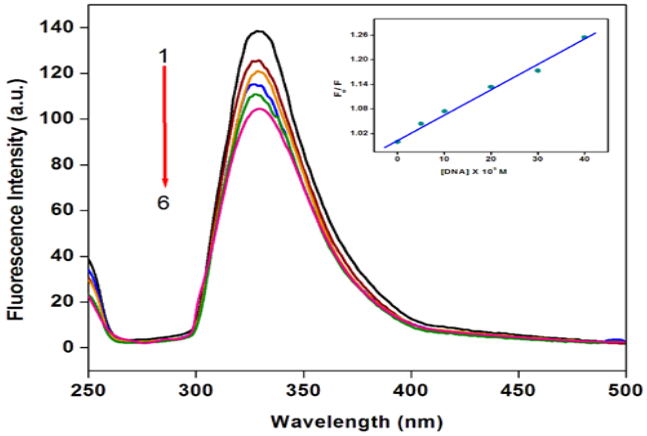


Figure 5: Fluorescence emission spectra of 2 × 10^{-6} M BIC in presence of (1-6) 0 µM, 5 µM, 10 µM, 20 µM, 30 µM and 40 µM CT-DNA. (Insert: Stern-Volmer plot of F_0/F *vs.* [DNA])

$$\frac{F_0}{F}=1+K_q\tau_0[DNA]=1+K_{sv}[DNA] \qquad (4)$$

Where F_0 and F are fluorescence intensities of BIC in absence and presence of quencher (DNA) respectively, Kq is the quenching rate constant, KSV is Stern-Volmer quenching constant and τ_0 is average life-time of biomolecule (fluorophore) without quencher. A linear Stern-Volmer plot is obtained between F_0/F *vs.* [DNA] shown in Figure 5 insert, this elucidates that only one type of

quenching occurs between BIC-DNA, either static or dynamic [33]. The Stern-Volmer quenching constant (KSV) calculated in present study is 2.24×10^3 M^{-1}.

Docking studies of BIC-DNA interaction

Molecular docking studies aid in understanding molecular level interactions between drugs and their targets [34]. Keeping this in mind molecular docking simulations were carried out to predict binding affinity of BIC towards DNA and evaluate the molecular level interactions. Crystal structure of double stranded DNA was downloaded from the RCSB PDB website (PDB ID: 1BNA) [35]. The crystal structure was chosen because it contains all the four bases (A, T, G and C) and both minor and major grooves. The Auto Dock Tools (ADT) graphical user interface was used to add Kollman charges and polar hydrogens. BIC was prepared for docking by minimizing its energy with DFT at B3LYP-6-31G (d,p) level of theory. Partial charges were added by Geistenger method. The grid size 40 Å × 40 Å × 40 Å was defined in a way so as to include both minor and major grooves of DNA. All molecular docking simulations were carried with AutoDock-Vina software [36]. The most popular algorithm, Lamarckian Genetic Algorithm (LGA) available in Autodock was employed for docking. The poses were visualized in Discovery Studio Visualizer [37] and Pymol molecular graphic program available at http://pymol.sourceforge.net.

As can be seen in Figure 6a, docking simulations predict groove binding for BIC. Figure 6b, shows the interactions between BIC and DNA, here we find BIC is surrounded by DG17, DG22, DG23 and DA5. Carbonyl oxygen of amide forms two H-bonds with DG22. Binding affinity of -8.4 kcal mol^{-1} for the top scored pose predicts high binding affinity of BIC towards DNA. Overall docking study predicts the molecule under examination as minor groove binder which could bind with the DNA and interfere with its replication.

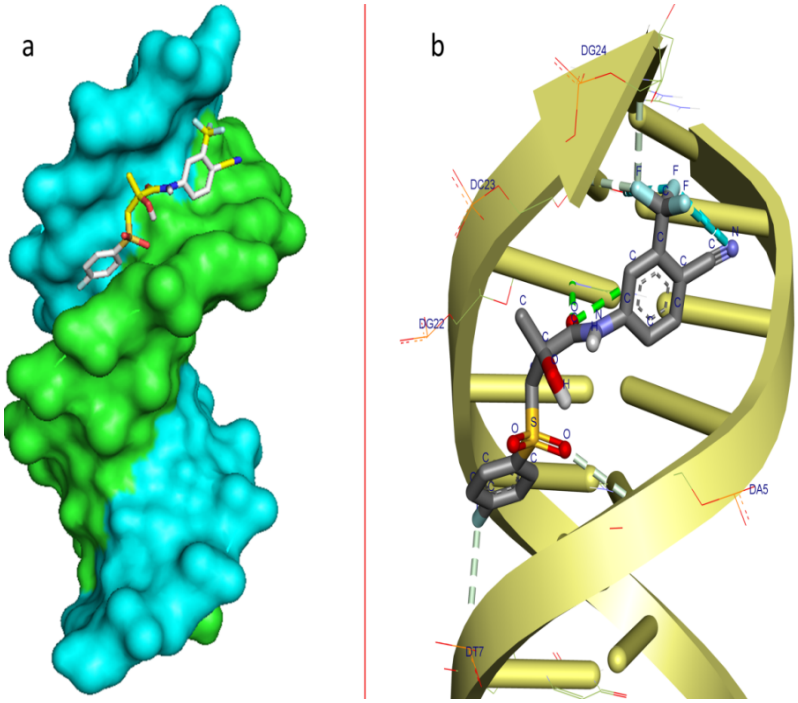


Figure 6: Docking structure between d(CGCGAATTCGCG) (PDB 1BNA-DNA) and BIC. (a) Surface representation of d(CGCGAATTCGCG) complexes with the BIC. (b) Close up view of d(CGCGAATTCGCG) complexes with BIC showing the interactions. Green dotted lines represent H-bonds

CONCLUSION

The work describes interaction of anticancer drug bicalutamide with calf thymus-DNA. Electrochemical and spectroscopic methods were used to study the drug-DNA interaction. The results were used to elucidate the binding constant (K) and Stern-Volmer quenching constant (K_{SV}). The slight higher value of binding constant (K) obtained through voltammetric technique over that obtained in spectrophotometric technique may be a consequence of higher sensitivity of electrochemical techniques. The Stern-Volmer quenching constant obtained in Fluorescence emission spectroscopy, supports that BIC is an efficient DNA intercalating agent. Electrochemical technique suggested intercalative mode of interaction between BIC and DNA, while spectroscopic techniques proved that groove-binding type of interaction is mainly operating between the drug and CT-DNA. The groove-binding mode of interaction was also supported by docking simulation studies.

ACKNOWLEDGMENTS

Authors are highly thankful to UGC-SAP for electrochemical instrumentation facilities. Authors also acknowledge Department of Pharmaceutical sciences and Department of Chemistry, Dr. Harisingh Gour (Central) University Sagar (M.P.) India for providing necessary laboratory facility.

REFERENCES

[1] X. Lu, Y. Chen, J. Chen, Y. Zhang, L. Zhang, M. Li, *Int. J. Electrochem. Sci.,* **2006,** 1, 130.

[2] Q. Wang, X. Wang, Z. Yu, X. Yuan, K. Jiao, *Int. J. Electrochem. Sci.,* **2011,** 6, 5470.

[3] M. Sirajuddin, S. Ali, A. Badshah, *J. Photochem. Photobio. B: Biol.,* **2013**, 124, 1.

[4] X. Tian, Y. Song, H. Dong, B. Ye, *Bioelectrochemical.,* **2008**, 73, 18.

[5] L. Wang, L. Lin, Y. Baoxian, *J. Pharm. Biomed. Anal.,* **2006,** 42, 625.

[6] S.S. Kalanur, U. Katrahalli, J. Seetharamappa*, J. Electroanal. Chem.,* **2009,** 636, 93.

[7] C. Xia, L.S. Guo, J.H. Jiang, T.F. Kang, B. Xiong, Y. Ru-Qin, *Anal. Chim. Acta.,* **1998**, 373, 29.

[8] S.C.B. Oliveira, A.M. Chiorcea-Paquim, S.M. Ribeiro, A.T.P. Melo M. Vivanc, A.M. OliveiraBrett, *Bioelectrochemical.*, **2009,** 76, 201.

[9] S. Raufa, J.J. Gooding, K. Akhtar, M.A. Ghauria, M. Rahman, M.A. Anwar, A.M. Khalid*, J. Pharm. Biomed. Anal.*, **2005**, 37, 205.

[10] B.X. Ye, L.J. Yuan, C. Chen, J.C. Tao, *Electroanalysis,* **2005**, 17, 1523.

[11] G. Congur, A. Erdem, F. Mese, *Bioelectrochemistry,* **2015**, 102, 21.

[12] A.D.R. Pontinha, S. Sparapani, S. Neidle, A.M. Oliveira-Brett, *Bioelectrochemistry,* **2013**, 89, 50.

[13] X. Tian, F. Li, L. Zhu, B. Ye, *J. Electroanal. Chem.,* **2008,** 621, 1.

[14] F.P. Zhang, Q. Cao, J.J. Cheng, C.H. Zhang, N. An, S.P. Bi, *Anal. Sci.,* **2009,** 25, 1019.

[15] C.V. Kumar, J.K. Barton, N.J.J. Turro, *J. Am. Chem. Soc.*, **1985**, 107, 5518.

[16] C.V. Kumar, E.H. Asuncion, *J. Am. Chem. Soc.,* **1993**, 115, 8547.

[17] L. Scaglioni, S. Mazzini, R. Mondelli, S. Dallavalle, S. Gattinoni, S. Tinelli, G.L. Beretta, F. Zunino, E. Ragg, *Bioorg. Med. Chem.,* **2009,** 17, 484.

[18] S.T. Saito, G. Silva, *J. Photochem. Photobio., B: Biol.,* **2012**, 111, 59.

[19] X.H. Zhang, L.Y. Wang, Z.X. Nan, S.H. Tan, Z.X. Zhang, *Dyes and Pigments.*, **2008,** 79, 205.

[20] R.A. Dar, P.K. Brahman, S. Tiwari, K.S. Pitre, *J. Appl. Electrochem.,* **2011**, 41, 1311.

[21] B.H.M. Hussein, H.A. Azab, W. Fathalla, S.A.M. Ali, *J. Luminescence*, **2013,** 134, 441.

[22] J. Wang, Analytical Electrochemistry, Willey, NY, USA, **2001,** 2nd Ed., 115.

[23] N.P. Shetti, S.J. Malode, S.T. Nandibewoor, *Bioelectrochemistry,* **2012**, 88, 76.

[24] U.J. Pandit, I. Khan, S. Wankar, K.K. Raj, S. N. Limaye, *Anal. Methods.,* **2015**, 7, 10192.

[25] M.T. Carter, A.J. Bard, *J. Am. Chem. Soc.,* **1987**, 109, 7528.

[26] N. Li, Y. Ma, C. Yang, L. Guo, X. Yang, *Biophys. Chem.,* **2005**, 116, 199.

[27] H. Nawaz, S. Rauf, K. Akhtar, A.M. Khalid, *Anal. Biochem.,* **2006**, 354, 28.

[28] F. Jalali, P.S. Dorraji, *J. Pharm. Biomed. Anal.,* **2012,** 70, 598.

[29] J. Liu, T. Zhang, T. Lu, L. Qu, H. Zhou, Q. Zhang, L. Ji, *J. Inorg. Biochem.,* **2002,** 91, 269.

[30] J. Jaumot, R. Gargallo, *Curr. Pharmaceut. Des.,* **2012**, 18, 1900.

[31] Y.J. Guo, J.B. Chao, J.H. Pan, *Spectrochim. Acta. Part A*, **2007**, 68, 231.

[32] H.A. Benesi, J.H. Hildebrand, *J. Am. Chem. Soc.,* **1949,** 71, 2703.

[33] J.R. Lakowicz, Springer, NY, USA, **2006,** 3rd ed., 241.

[34] R.D. Taylor, P.J. Jewsbury, J. W. Essex, *Journal Computer Aided Drug Design.,* **2002**, 16, 151.

[35] H.R. Drew, R.M. Wing, T. Takano, C. Broka, S. Tanaka, K. Itakura, R.E. Dickerson, *Proc. Natl. Acad. Sci. USA.,* **1981**, 78, 2179.

[36] O. Trott, A.J. Olson, *J. Comput. Chem.*, **2010**, 31, 455.

[37] Accelrys Software Inc., Release 4.0, *San Diego: Accelrys Software Inc*., **2013**.