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Drug-Drug Interaction and DNA Binding Studies of Azathioprine – An Antileukemic Drug.

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ABSTRACT

The present study was aimed at providing valuable information on the interaction of AZA, an immunosuppressant, with calf-thymus ds-DNA and other common drugs viz., allopurinol, ascorbic acid, atorvastatin, glucose, insulin, metronidazole, paracetamol, ranitidine, sulfasalazine and warfarin. The cyclic voltammetric studies were carried out to study the interaction and the binding ability of the drug with calf-thymus ds-DNA. The analysis revealed that the drugs viz., ascorbic acid, atorvastatin, ranitidine, allopurinol, sulfasalazine and the ds-DNA interacted with AZA through intercalative mode. Hence these drugs are not to be recommended along with AZA medication. The DNA binding constant was evaluated as $1.207\times10^{4}M^{-1}$ indicating the high association of the drug with DNA. The negative value of ΔG (-23.285 $kJmol^{-1}$) indicated that the binding of AZA with DNA was a spontaneous process.

Keywords: Drug, interaction, cyclic voltammetry**,** peak potential, intercalative

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INTRODUCTION

Azathioprine (6-[(1-Methyl-4-nitro-1H-imidazol-5-yl)thio]-1H-purine) (Fig.1) is an immunosuppressant used in organ transplantation and autoimmune disease such as rheumatoid arthritis or inflammatory bowel disease or Crohn's disease [1,2]. Toxicity caused by the use of these drugs has been demonstrated in organs such as bone marrow, liver and gastrointestinal tract and pancreas [3,4]. Long-term use of AZA in combination with other immune-suppressing medications in transplant patients has been associated with a slightly elevated risk of cancer [5].

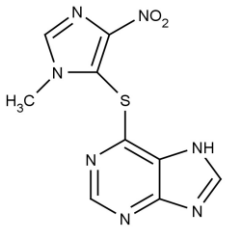
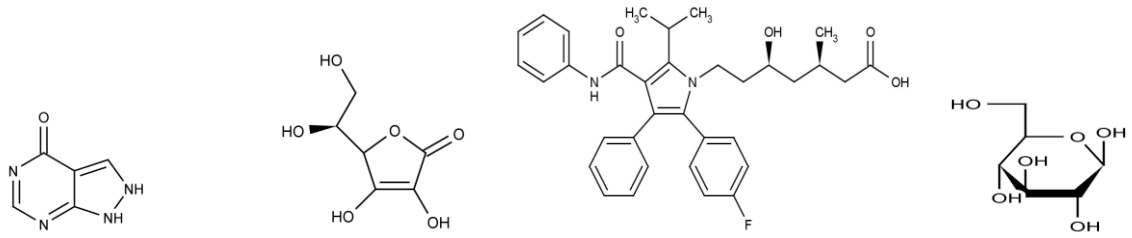


Figure 1: Structure of AZA

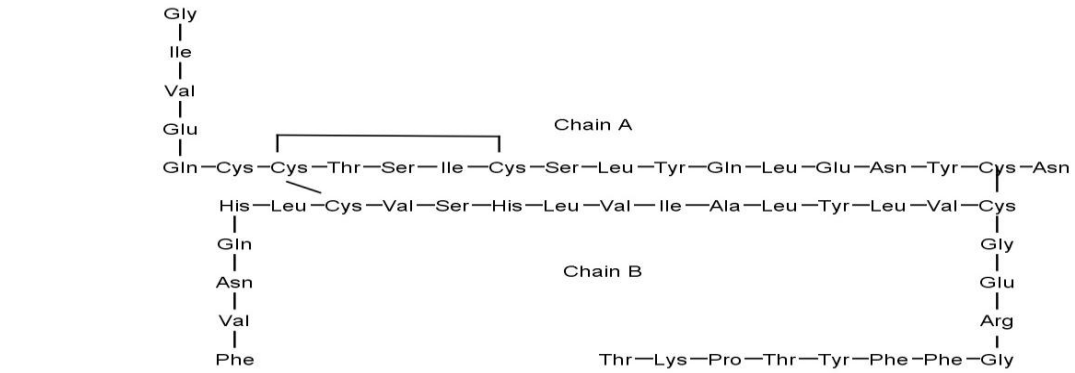
Drug-Drug Interactions (DDI) is possible whenever a person is administered with two or more medications simultaneously. The outcome may be either beneficial or harmful [6]. A recent study indicated that medications were commonly used together in older adults, with nearly 1 in 25 individuals potentially at risk of a major DDI [7].

The present study was aimed to provide an analytical tool to determine the *invitro* interaction of AZA with other commonly used drugs viz., allopurinol, ascorbic acid, atorvastatin, glucose, insulin, metronidazole, paracetamol, ranitidine, sulfasalazine and warfarin (Fig.2). The binding ability of the drug with DNA was also studied by electrochemical means.



(a) (b) (c) (d)

ISSN: 0975-8585



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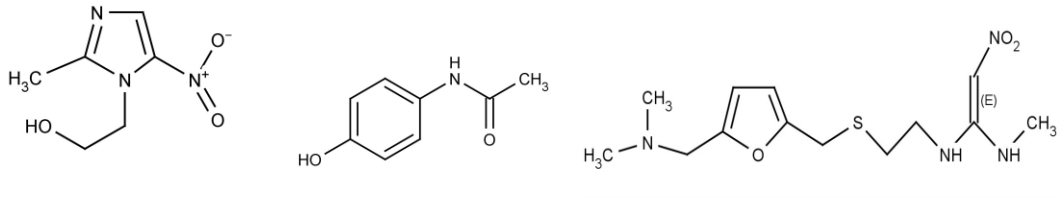
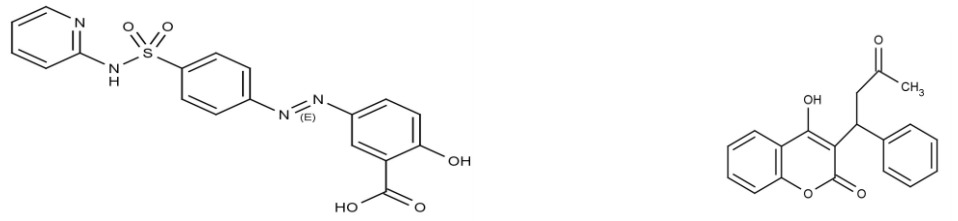


(f) (g) (h)



(i) (j)

Figure 2: Structure of commonly used drugs(a) Allopurinol (b) Ascorbic acid (c) Atorvastatin (d) Glucose (e) Insulin (f) Metranidazole (g) Paracetamol (h) Ranitidine (i) Sulfasalazine (j) Warfarin.

MATERIALS AND METHODS

The drugs used for the study was purchased from local pharmaceuticals. All reagents used were of analytical grade and used without further purification.

Instruments

Electrochemical measurements were carried out using CHI-600C Electrochemical Analyzer. The pH measurements were carried out with Coronation pH meter, Coronation instrument, India. UV-Visible The absorbance of solutions at the desired wavelength was determined using Thermo Scientific Helious Alpha UV-Visible spectrophotometer.

Experimental

ISSN: 0975-8585

The standard solution of 0.01M AZA and other drugs were prepared by dissolving the required quantity of the drugs in 50 ml of 50% aqueous acetonitrile.

For electrochemical measurements, a three-electrode system consisting of Ag/AgCl electrode as a reference electrode, a thin Pt wire of thickness 0.5 mm with an exposed end of 10 mm as the counter electrode and a bare glassy carbon electrode (GCE) with a geometric area of 0.126 cm^2 as the working electrode. Before starting the experiments, the GCE was polished with 0.05 µm alumina powder on a nylon buffing pad. The test solution was placed in a voltammetric cell of volume 15ml and deoxygenated by bubbling nitrogen for 15min for recording the voltammogram. All experiments were carried out at room temperature.

RESULTS AND DISCUSSION

Electrochemical behaviour of AZA

Cyclic voltammetric behaviour of 0.01M AZA was studied using Glassy carbon electrode (GCE) as working electrode. Acid, acetate, phosphate and Britton-Robinson buffers have been chosen for the study. Among these buffers used, the best electroanalytical signal was noticed in acid buffer. The pH was also varied using the above mentioned buffers and best signal was noticed at pH1. AZA was electroactive only in acid buffer at pH 1 and therefore this buffer was chosen for the study. The cyclic voltammogram of AZA on a bare GCE in acid buffer at pH1 is shown in fig. 3.

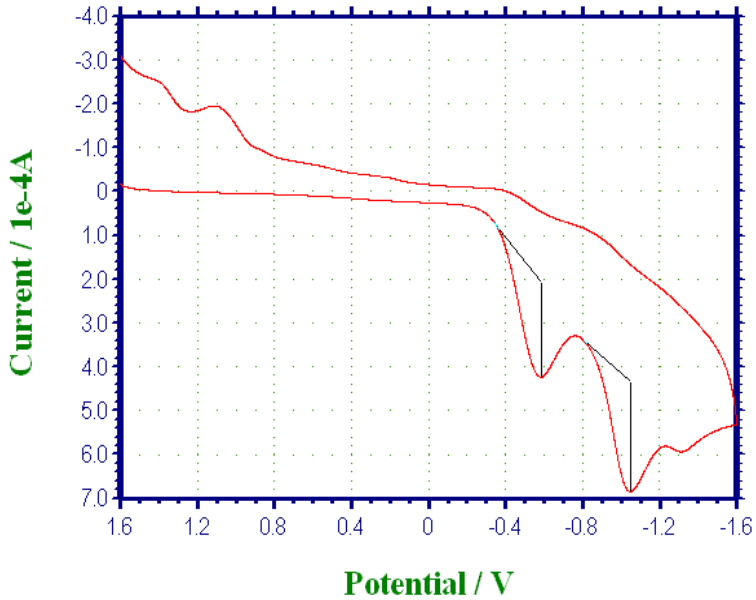


Figure 3: Cyclic voltammogram of 0.1M AZA in acid buffer at pH1

It is clear that AZA on the bare GCE shows an oxidation peak at E_p = 1.098V and three reduction peaks at potentials E_p = -0.582V, -1.046V and -1.248V. The reduction peaks obtained are mainly due to the reduction of -NO_2 group present in AZA.

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Electrochemical investigation into drug-drug interaction

The interference of AZA with other common drugs like allopurinol, ascorbic acid, atorvastatin, glucose, insulin, metronidazole, paracetamol, ranitidine, sulfasalazine and warfarin was studied using cyclic voltammetry. The cyclic voltammetric experiments were carried out using equimolar solutions of AZA and the above mentioned drug solutions. The respective cyclic voltammograms were recorded.

When allopurinol was added to AZA, reduction in cathodic peak current followed by negative shift in peak potential was noticed. This indicated the intercalative mode of interaction of allopurinol with AZA.

Addition of ascorbic acid to AZA resulted in a slight shift in reduction peak potentials from -0.582V to -0.621V and from -1.046V to -1.098V. The negative shift in peak potentials suggested intercalative mode of interaction of ascorbic acid with AZA.

The cyclic voltammogram was recorded when atorvastatin, an anti-diabetic drug was added to AZA (fig.4). The reduction peak potentials were shifted from -0.582V to -0.658V and from -1.046V to -1.137 V. The vast shift in peak potential to the negative side indicated the strong intercalative mode of interaction between AZA and atorvastatin.

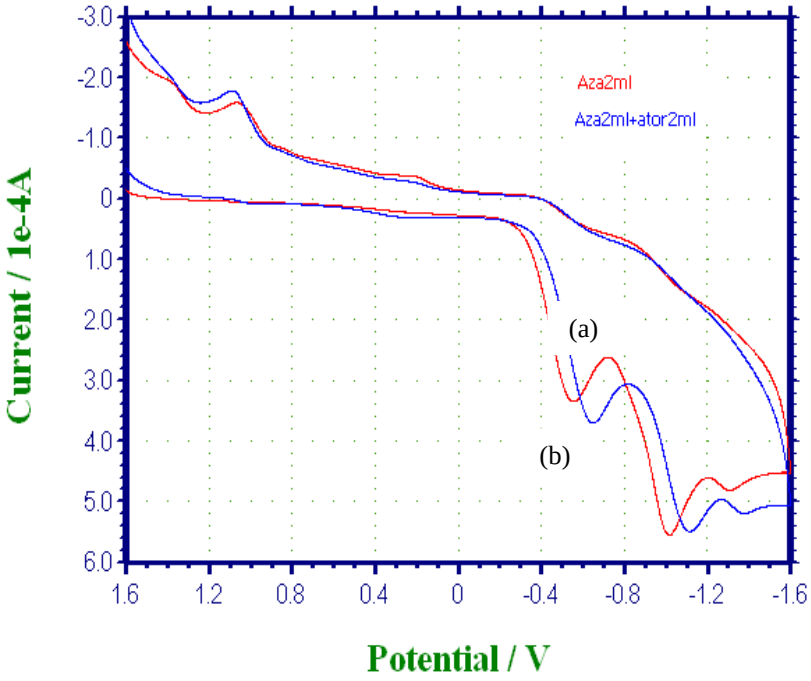

Figure 4: Cyclic voltammogram of (a) AZA (b)AZA + atorvastatin

A reduction in peak current with no shift in peak potential was noticed, when glucose was added to AZA (fig.5). This suggested that glucose did not interact with AZA. Hence, simultaneous intake of glucose and AZA may be advisable.

In the presence of insulin, the electrochemical behaviour of AZA depends on the concentration of insulin. At 0.03M concentration, the reduction peaks of AZA disappeared

and this indicated the interference of insulin with AZA. So the interference can be minimized by optimizing the dosage level of insulin [3].

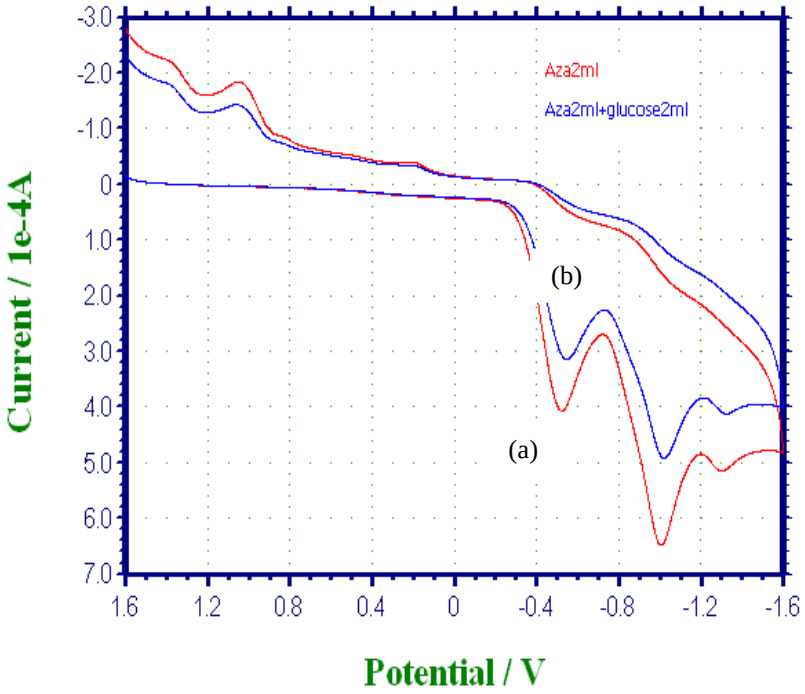

Figure 5: Cyclic voltammogram of (a) AZA (b) AZA + glucose

When metronidazole was added to AZA, because of the presence of $–NO_2$ group in metronidazole, the intensity of the reduction peak corresponding to the reduction of $–NO_2$ group in AZA was increased. No considerable shift in peak potential was noticed and this indicated the non-interactive nature of metronidazole with AZA.

When paracetamol was added to AZA considerable difference in peak potential was not noticed indicating the non-interactive nature of paracetamol with AZA.

Ranitidine, an anti-ulcer drug which when added to AZA, the reduction peak corresponding to the reduction of $–NO_2$ group was vanished. This indicated the strong interactive nature of ranitidine with AZA.

When sulfasalazine was added to AZA, a negative shift in peak potential was noticed. Sulfasalazine intake along with AZA is therefore not advisable.

No shift in peak potential was noticed when warfarin was added to AZA. This indicated the non-interactive nature of the drug with AZA**.**

The potential, mode of interaction and recommendations based on the electrochemical studies were summarized in Table 1.

ISSN: 0975-8585

Table 1: Interaction of Azathioprine with Common Drugs.

Drugs	Potential shift	Mode of interaction	Recommendation*
AZA + allopurinol	-ve	Intercalative	x
AZA + ascorbic acid	-ve	Intercalative	x
AZA + atorvastatin	-ve	Intercalative	x
AZA + glucose	No shift	No interaction	✓
AZA + Metronidazole	No shift	No interaction	✓
AZA + paracetamol	No shift	No interaction	✓
AZA + Ranitidine	Reduction peak vanished	Strong interaction	x
AZA + sulfasalazine	-ve	Intercalative	x
AZA + warfarin	No shift	No interaction	✓

✓ -Recommended , x- not recommended

Electrochemical investigation of drug-DNA interaction

The investigation of surface properties viz., surface concentration, Diffusion coefficient and rate of electron transfer process, will provide valuable information regarding drug-DNA interaction. The scan rate was varied from 25 to 500 mVs^{-1} in order to study the surface properties. The peak currents for the oxidation and reduction of AZA increased with increasing the scan rate (Table 2.). The variation of oxidation peak current (i_p) with scan rate (ν) was found to be linear with very with very high correlation coefficient (R^2= 0.9905) which indicated that the electron transfer process was a diffusion controlled process. This was confirmed by the slope value (0.4393) obtained from the plot of log i_p vs. log ν (fig.6) which was closer to the theoretical value of 0.5 for an ideal reaction diffusion-controlled electrode process [8]. The surface concentration (Γ), of the reactant was calculated using the formula [9],

$$i_p = n^2 F^2 A \Gamma \nu / 4RT \qquad (1)$$

where R is the gas constant (8.314 $JK^{-1}mol^{-1}$), T is temperature (K), F is the faraday constant (96487 C mol^{-1}), n is the number of electrons transferred in the reaction, A is the surface area of the working electrode (0.126 cm^2) and ν is the scan rate, and was found to be 5.3183×10^{-7} $mol^{-1}cm^{-2}$.

Table 2: Voltammetric Behaviour of AZA and AZA-DNA Complex

Scan rate ν (V/s)	E_p(V)		i_p (μA)	
	AZA	AZA-DNA complex	AZA	AZA-DNA complex
0.025	-0.517	-0.459	116.6	53.76
0.050	-0.539	-0.468	155.9	79.03
0.100	-0.557	-0.5200	215.8	96.61
0.200	-0.6111	-0.568	281.0	128.0
0.300	-0.646	-0.512	336.5	143.6
0.400	-0.652	-	382.0	-
0.500	-0.652	-	429.7	-

ISSN: 0975-8585

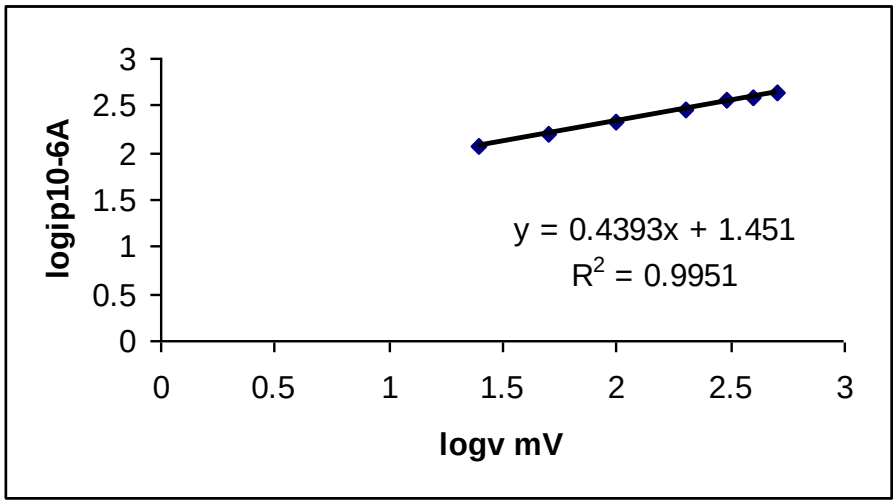

Figure 6: A plot of log ν vs. log i_p for 0.1M AZA

The diffusion coefficient of the drug was determined by using the following formula [10],

$$i_p = (2.69 \times 10^5)\, n^{3/2}\, A\, D_0^{1/2}\, \upsilon^{1/2}\, C_0 \qquad (2)$$

where i_p is the peak current (μA), A is the surface area of the electrode (cm^2), C_0 is the bulk concentration of the electroactive species (mol cm^{-3}), D_0 is the diffusion coefficient ($cm^2\ s^{-1}$) and ν is the scan rate and n is the number of electrons transferred. From the slope (19.616) value of the plot of i_p vs. $\nu^{1/2}$ (fig.7), the D_0 value was calculated as 6.398×10^{-6} cm^2s^{-1}.

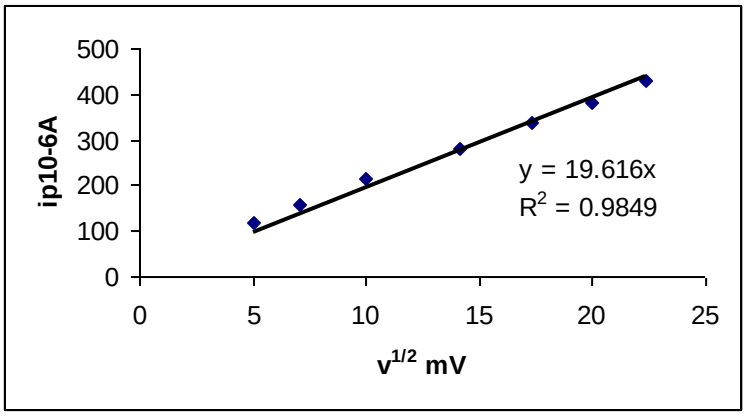

Figure 7: The plot of $\nu^{1/2}$ vs. i_p (A) for 0.1M AZA

The cyclic voltammetric experiments exhibited that both peak potential (E_p) and peak current (i_p) of AZA was in direct relation with the scan rate (ν). The relationship between E_p and ln $\nu^{1/2}$, over the range of scan rate from 25 to 500 mVs^{-1} was discussed and for an irreversible cathodic reaction, the following equation may be used [9],

$$E_p = E^0 - RT/[(\alpha)\, nF]\, \{0.780 + \ln (D_0^{1/2}/k^0) + \ln [(\alpha)\, nF\nu/(RT)]^{1/2}\} \qquad (3)$$

where, (α) corresponds to the transfer coefficient for the process, k^0 is the standard heterogeneous rate constant and E^0 is the formal potential of the electrode. The

heterogeneous rate constant (k^0) was calculated from the intercept of the linear regression equation obtained by plotting E_p (V) vs. $\ln\nu^{1/2}$ was found to be 1.742×10^{-2} cm s^{-1}.

In order to study the interaction of AZA with DNA, 1ml of 0.2354×10^{-4} M solution of DNA was mixed with 1ml of 0.017M AZA. When DNA was added to a solution of AZA, the peak current decreases and peak potentials shift from -0.6703V to more negative values are observed. The cathodic peak current decreased to 25.8% in the presence of DNA and a dramatic shift in peak potential towards negative side upon addition of DNA was noticed and this was due to the intercalation of DNA to AZA[10]. The decrease in peak current leads to the decrease in the slope of the linear i_p Vs.. $\nu^{1/2}$ plot (R^2 = 0.9036), shows the bulky and slowly diffusing DNA which results in considerable decrease in the surface concentration and apparent diffusion coefficient [11]. The Γ and D_0 of DNA bound AZA was found to be 3.0858×10^{-7} mol^{-1} cm^{-2} and 2.2476×10^{-6} cm^2 s^{-1}, which was lower than that of free AZA (5.3183×10^{-7} mol^{-1} cm^{-2} and 6.3980×10^{-6} cm^2 s^{-1}). The k^0 obtained was 3.780×10^{-2} cm s^{-1} and was found to be lower than the k^0 of free AZA (6.44×10^{-2} cm s^{-1}).

The changes in current upon addition of DNA can be explained in terms of diffusion of an equilibrium mixture of free and bound AZA to the electrode and is used to quantify the binding of AZA to DNA. The expression used to study the binding ability was,

$$\log (1 / [DNA]) = \log K + \log (I_{H\text{-}G} / I_G\text{-}I_{H\text{-}G}) \qquad (4)$$

where K is the apparent binding constant, I_G and $I_{H\text{-}G}$ are the peak currents of free guest (G) and the complex (H-G) respectively. The straight line obtained by plotting log ($I_{H\text{-}G}/I_G\text{-}I_{H\text{-}G}$) vs. log (1/ [DNA]) is log (1/ [DNA]) = 0.6811 log ($I_{H\text{-}G}/I_G\text{-}I_{H\text{-}G}$) + 4.0818, ($R^2$=0.9995) and the binding constant of this complex was evaluated as 1.207×10^4 M^{-1}. The value of K demonstrated that AZA binds to DNA with a high association constant.

Spectral investigations into drug-DNA interaction

The maximum absorbance of AZA was located around 278 nm. The maximum absorbance band was attributed to the charge transition from the nitro substituent on the ring itself. UV-Visible absorption spectra of AZA in the presence of different concentrations of DNA were shown in Fig.8. A continuous decrease in the absorbance of AZA was observed with gradual increase in concentration of DNA. This hypo chromic effect may be due to the interaction between the electronic states of the intercalating chromophore and those of the DNA bases. It is expected that the strength of this electronic interaction would decrease as the cube of the distance of separation between chromophore and the DNA bases. The AZA solution exhibited peculiar hypo chromic effect in UV-visible spectra upon binding to DNA, a typical characteristic of an intercalating mode.

The binding constant of AZA-DNA at different temperatures was studied by using cyclic voltammetry. It was observed that the value of K increases as the temperature increases for AZA-DNA. Fig.9 indicated the linear relationship between ln K and 1/T and the thermodynamic parameters like ΔH and ΔS were calculated from the slope and intercept, respectively. The results were consolidated in Table 3. The positive value of ΔH indicates

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that the nature of the binding process is endothermic [12]. The change in entropy (ΔS) was positive and this again confirms the spontaneity of the process. The change in Gibbs free energy value (ΔG) for the binding of AZA with DNA were calculated from the ΔH and ΔS values and was found to be negative indicating that the binding of AZA with DNA is a spontaneous process[13,14].

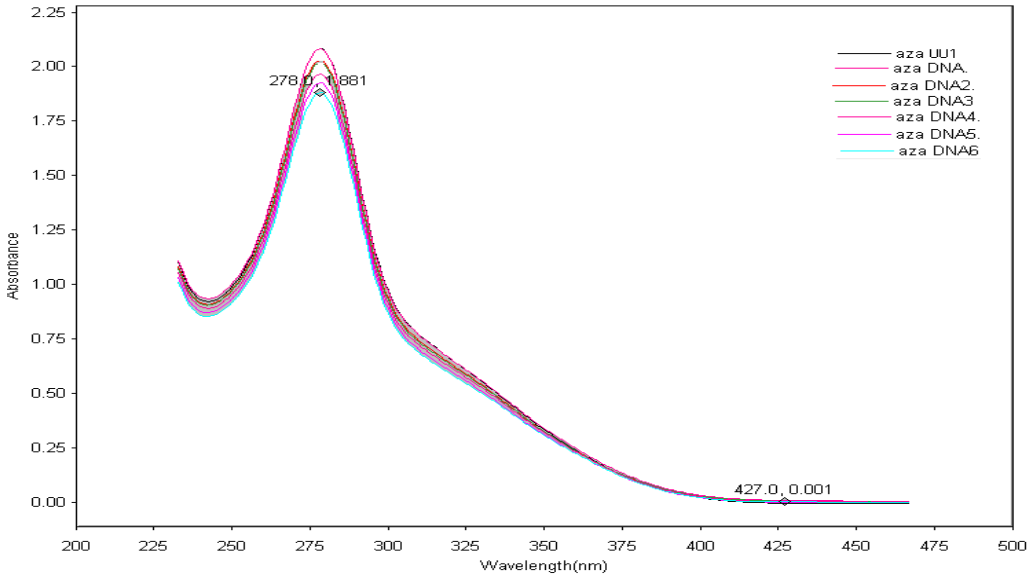

Figure 8: Cyclic voltammogram of 0.1M AZA in the presence of ds-DNA

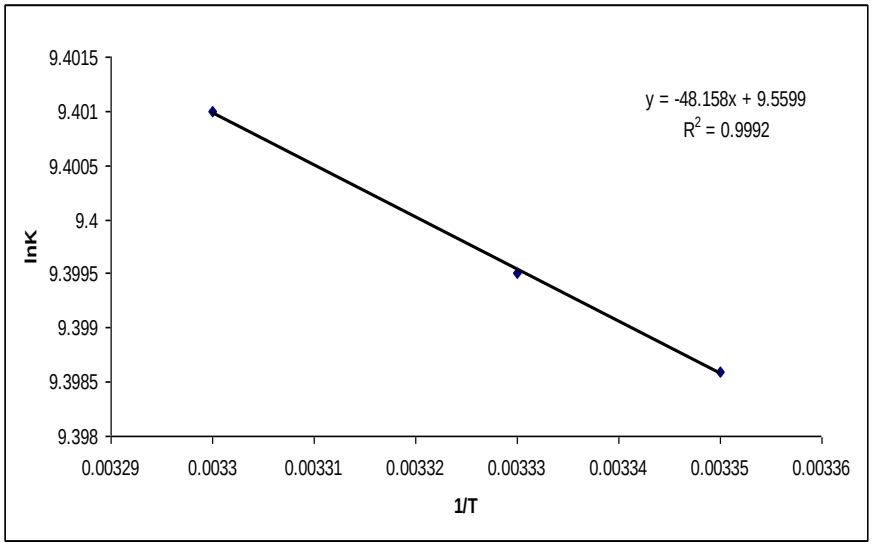

Figure 9: The plot of ln K vs. 1/T (K^{-1}) for AZA-DNA complex

Table 3: Thermodynamic Parameters for the Binding Of AZA With DNA

ΔG (kJ mol^{-1})			ΔH (J mol^{-1})	ΔS (J K^{-1} mol^{-1})
298 K	**300 K**	**303 K**		
-23.36	-23.52	-23.76	400.4	79.7

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CONCLUSION

In the present study, the electrochemical behaviour of AZA and its interaction with DNA and other commonly used drugs were investigated. The experimental results indicated that mode of interaction of AZA with DNA and the drugs viz., ascorbic acid, atorvastatin, ranitidine, allopurinol and sulfasalazine were intercalative. Azothioprine shows a strong interaction with DNA and this was confirmed from the surface properties and the binding constant value. Among the drugs studied warfarin, paracetamol, glucose and metronidazole were found to have no interaction with AZA. Insulin was found to interact with AZA at concentrations above 0.02 M. So, this study finds applications in clinical procedures.

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