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Study of Interaction of Dextromethorphan Hydrobromide with Deoxyribonucleic Acid by Fluorescence Quenching

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Abstract

Interactions with many clinically active therapeutic agents with deoxyribonucleic acid (DNA) are well studied and it expedites deciphering the structure of DNA and to investigate the pathological implication of those molecules in a living organism. The interaction of dextromethorphan hydrobromide (DEX) with calf thymus DNA (ct DNA) was studied employing UV absorption and fluorescence spectroscopic techniques. The binding affinity of DEX to DNA was calculated at different temperatures and the stoichiometry of binding was characterized to be about 1 dextromethorphan molecule per nucleotide. Hypochromic effect was found in the absorption spectra of dextromethorphan, and its wavelength had no shift in the presence of DNA indicating external binding mode of dextromethorphan to DNA. Quenching constants 3532 L/ mol and 12446L/ mol at 298 K and 308 K respectively with correlation co-efficient of 0.974 and 0.976, using Stern-Volmer equation and the quenching mechanism was found to be dynamic. Fluorescence spectroscopic results showed the quenching of fluorescence intensity of DEX in the presence of DNA, indicating the interaction between DEX and DNA. Based on this, hydrophobic interaction were found to play the dominating role in DEX-DNA binding and those binding forces also indicate the binding site of dextromethorphan to be in the minor groove of DNA.

Key words: Calf-thymus DNA, affinity, Stern volmer constant, hypochromic effect, minor groove.

Introduction

Deoxyribonucleic acids (DNA) are common targets for antiviral, anticancer and antibiotic drugs. The study on the interaction of small molecules with DNA is of great importance in many areas (Ivandini *et al*., 2007; Ward *et al*., 1965). Most of the investigations of the interactions of DNA with small molecules were conducted to elucidate the structure of DNA (Neidle *et al*., 1997; Geierstanger *et al*., 1995; Chaires *et al*., 1996; Coleman *et al*., 1995; Wemmer *et al*., 1997). Some other studies were conducted solely to determine the interactions to observe possible pathological implications in living system with dyes, drugs and toxic chemicals using different spectroscopic methods (Yuan *et al*., 1998; Ran *et al*., 2007; Li *et al*., 2009). It is significant not only in understanding the mechanism of interaction, but also for guiding the design of new drugs. So it is necessary to understand the modes and the factors affecting the binding of small molecules to DNA (Singh *et al*., 1992). Studying the binding mechanism is of great importance in terms of life science, chemicals, pharmaceuticals, and clinical medicines (Bera *et al*., 2008; Zhao *et al*., 2003). Although the exact mode of actions of many clinically proven therapeutic agents is not known, their interactions to DNA are well studied (Coleman *et al*., 1995).

Dextromethorphan or (+)-3-methoxy-17-methyl-9α, 13α, 14α-morphinan is the dextrorotatory enantiomer of levomethorphan, which is the methyl ether of levorphanol, both opioid analgesics. It is an uncompetitive *N*-methyl-D-aspartate receptor (NMDA)

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receptor antagonist and μ-, δ-, and κ-opioid receptor agonist (Burns *et al*., 2013; Codd *et al*., 1995). Instead of acting as a direct antagonist of the NMDA receptor itself, dextromethorphan likely functions as a pro-drug to its nearly 10-fold more potent metabolite dextromethorphan, and this is the true mediator of its dissociative effects (Chou *et al*., 1999). (+)-3-methoxymorphinan, dextromethorphan's other major metabolite, plays role in its effects, but it is not entirely clear (Schmider *et al*., 1997).

Various researchers have studied the interaction of small molecules with DNA but there has been no report on the interaction of DNA with dextromethorphan hydrobromide. In this regard, our study on the interaction of dextromethorphan hydrobromide with calf thymus (ct) DNA was a novel approach on this particular case, done by using fluorescence spectroscopy and UV-visible spectroscopy. Ct-DNA was used as DNA model mainly because of its alternate sugar phosphate sequence with lower protein and highly polymerized skeleton. Moreover, it is easily available and can be used without further purification.

The aim of this investigation was to divulge the binding characteristics between dextromethorphan and DNA by fluorescence quenching spectroscopy. In this regard Stern-Volmer constant which assumed as equivalent to binding constant and binding points per DNA at different temperatures were determined. Besides, the nature of the binding forces was analyzed based on the calculated thermodynamic parameters like enthalpy change (ΔH), entropy change (ΔS) and Gibb's free energy (ΔG)).

Materials and Method

Reagents and chemicals: Calf-thymus DNA was commercially obtained from Sigma–Aldrich Chemicals Co. (USA) and used without further purification. Its stock solution was prepared by dissolving an appropriate amount of DNA in nuclease free water overnight at a final concentration of 3.35×10^{-3} mol/L and stored at 4 ºC. The lyophilized DNA should be reconstituted overnight at 2-8 ºC to ensure that all materials go into solution. When the product was fully dissolved, the DNA concentration was determined by measuring its UV absorption at 260 nm using a molar absorption coefficient of 6600 L mol^{-1} cm^{-1}(Wang *et al*., 2007). Purity of DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of A_{260}/A_{280} in the range of 1.8- 1.9, indicating that the DNA was sufficiently free from protein (Joly *et al*., 2016). Tris-HCl buffer (pH 7.40) was used in this study. 0.05 M buffer was prepared by adding appropriate amount of tris powder (M.W. 121.14) in nanopure water and pH was adjusted by using concentrated HCl which was used for later experiments. 2×10^{-3} M solution of dextromethorphan hydrobromide (Mol. wt. 370.3) was prepared by dissolving stock solution in nanopure water.

Apparatus: All fluorescence spectra were recorded on F-7000 spectrophotometer (Hitachi, Japan). The absorbance measurements were performed on a UV-1800 spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm quartz cells. Measurement of pH values of the solutions was performed by using pH meter (Hanna, Portugal).

Procedures

Selection of appropriate concentration of drug: At first, different concentrations of dextromethorphan hydrobromide were screened in UV spectrophotometer to determine maximum wavelength which was found to be 308 nm. In order to determine the effects of different [DEX] concentration on the interaction of dextromethorphan hydrobromide with ct DNA, a constant concentration of DNA was treated with different concentrations of the drug. Then fluorescence intensities of different concentration of drug were measured at the excitation wavelength of 308 nm in the presence of fixed DNA concentration at 298 K.

Measurement of absorbance: During absorbance measurement a constant concentration of the drug was treated with increasing concentration of the DNA. In order to observe the effect of DNA on absorption spectra of drug, the absorbance of drug was measured in the presence of different DNA concentrations in 1cm path length matched quartz cells with continuous stirring throughout the course of the titration.

Effect of DNA on the fluorescence spectra of dextromethorphan: Fluorescence spectra of drug in presence of various concentration of DNA were

measured at the excitation wavelength of 308 nm. Aliquots of DNA were added to the solution of dextromethorphan hydrobromide in Tris-HCl buffer of pH 7.4 and mixed by stirring the complex for 2-3 minutes.

Fluorescence quenching measurements: A decrease in intrinsic fluorescence intensity of a fluorophore due to various molecular interactions with other molecules is called fluorescence quenching. Dynamic quenching refers to a process in which the fluorophore and the quencher come into contact during the lifetime of the excited state, whereas static quenching refers to fluorophore - quencher complex formation. To assess the interaction between drug and DNA, the fluorescence quenching data are analyzed by Stern-Volmer equation (Lacowicz *et al*., 1999):

$$F_0/F = 1 + K_{SV}[Q]$$

Here F_0 and F are the fluorescence intensities of drug in the absence and presence of quencher, respectively. [Q] represents the concentration of quencher and K_{SV} is the Stern-Volmer quenching constant.

Thermodynamic parameters and nature of the binding forces: The interaction forces between quencher and bio- molecules may include hydrophobic forces, electrostatic interactions, Vander Waals interactions, hydrogen bonds etc. (Papadopoulou *et al*., 2005). The thermodynamic parameters namely the enthalpy change, the entropy change and Gibb's free energy are calculated in order to elucidate the interaction forces between the DNA and dextromethorphan. The basic reason for carrying out thermodynamic studies of dextromethorphan - DNA interaction is to determine the factors responsible for overall binding affinity and specificity of the drug (Haq *et al*., 2002). The thermodynamic parameters can be determined from the Van't Hoff equation:

$$\ln K_a = -(\Delta H/RT) + (\Delta S/R)$$

Where ΔH and ΔS are the enthalpy change and the entropy change respectively; constant K_a is analogous to the Stern-Volmer quenching constants K_{SV} at the corresponding temperature (Sun *et al*., 2006) , whereas R is the gas constant. The enthalpy change (ΔH) and the entropy change (ΔS) can be determined from the slope and intercept of the fitted curve of ln K_{SV} against 1/T, respectively.

The Gibb's free energy (ΔG) can be estimated from the following relationship:

$$\Delta G = \Delta H - T\Delta S$$

Binding constant and binding points: When small molecule bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecule is given by the following equation (Wu *et al*., 2009):

$$\log [(F_0 - F)/F] = \log K + n \log [Q]$$

Where, K and n represent the binding constant to a site and the number of binding per molecule respectively. The values of K and n are calculated from the values of intercept and slope of the plot of log $[(F_0 - F)/F]$ versus log [Q], respectively.

Data analysis: All the data were analyzed by using the software Microsoft Excel (MS Excel. 2013).

Results and Discussion

Effect of [DEX] concentration on interaction of dextromethorphan with DNA: Interaction of DNA with [DEX] was studied first at a fixed DNA concentration of 1×10^{-6} where concentration of dextromethorphan varied from 6.3×10^{-6} to 6.4×10^{-4} in order to observe the effect of [DEX] concentration on interaction. The result shows (Figure 1) that when the concentration of dextromethorphan reaches to 6.4×10^{-5} mol/L, the change of the fluorescence intensity is the most obvious. So, this concentration of dextromethorphan was chosen for further experiments.

Effect of DNA on absorption of dextromethorphan: When a small molecule interacts with DNA and forms a new complex, a shift in the absorbance wavelength and/ or a change in the molar absorptivity may occur. In order to determine the effect of increasing concentration of DNA on the absorption spectra of dextromethorphan, the absorption of [DEX] in the presence of different DNA concentrations were measured at the excitation wavelength of 308 nm. Here the interaction of the dextromethorphan with DNA

results in changes with hypochromic effect that essentially indicates strong intermolecular interaction (Table 1). At the same time, no bathochromism (red-shift) effect occurs when [DEX] bound to DNA. If the binding of small molecules and DNA involves a typical intercalative mode, a hypocromism effect coupled with obvious bathocromism for the characteristic peaks of the small molecules will be found due to strong stacking between the chromophore and the base pairs of DNA (Long *et al*., 1990). Therefore, the hypochromic effect could be the indication of noncovalent groove binding.

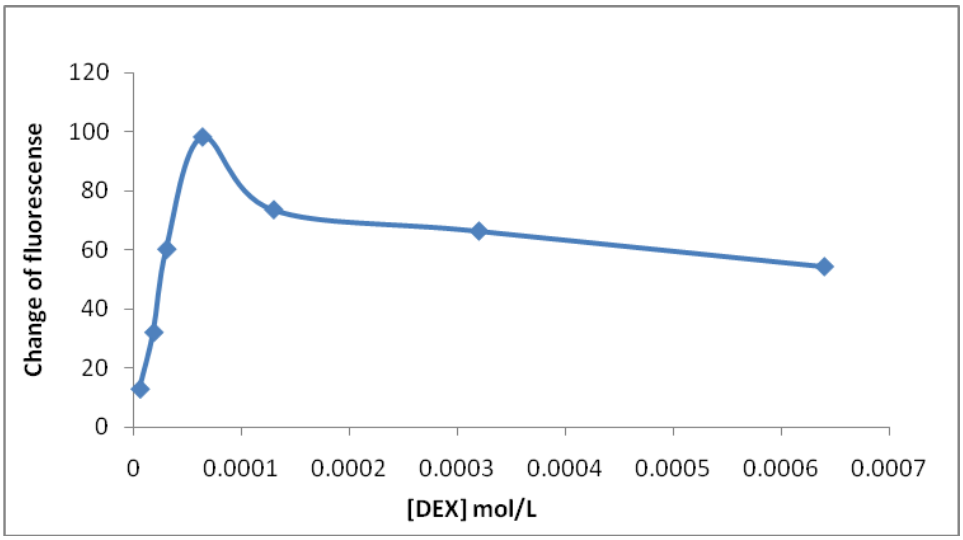

Figure 1. Fluorescence titration curve of effect of dextromethorphan concentration on the interaction of DEX with ct DNA.

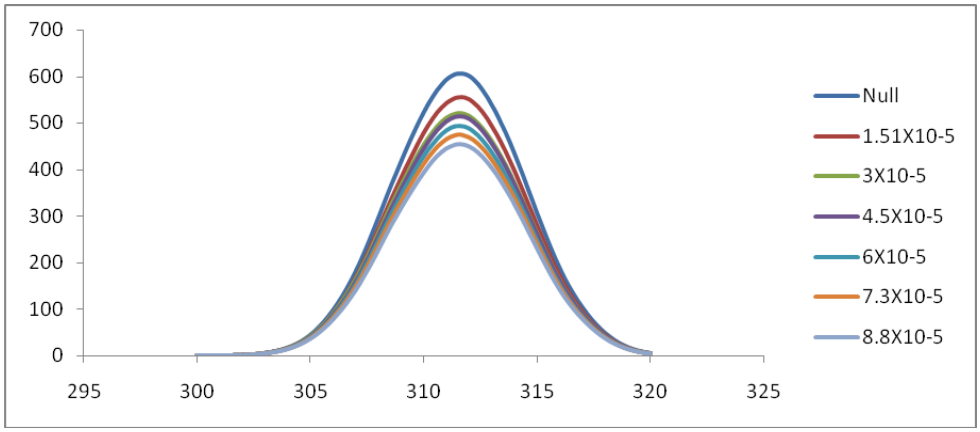

Figure 2. Fluorescence emission spectra of dextromethorphan (6.4×10^{-5} mol/L) in the presence of different concentration of DNA (λex_{max} = 308 nm, T= 298).

Analysis of fluorescence quenching mechanism: A decrease in intrinsic fluorescence intensity due to various molecular interactions with other molecules is called fluorescence quenching. Molecular rearrangement, energy transfer, ground state complex formation, coalitional quenching etc. are the various molecular interactions that can result in fluorescence quenching of excited state fluorophores. To further asses the interaction between [DEX] and DNA, the fluorescence quenching data are analyzed by Stern-Volmer equation:

$$F_0/F = 1 + K_{SV}[Q]$$

Figure 2 shows the fluorescence spectra of [DEX] in the absence and presence of DNA. The quenching of fluorescence of [DEX] in the presence of different DNA concentration was observed. The drug has an emission spectrum with maxima centered 311.6 nm when excited at 308 nm. No shift in emission wavelength was found. Here, DNA works as a quencher. This is an indication of strong interaction and energy transfer between [DEX] and DNA at 298 K. From the value of Stern-Volmer constant (K_{SV}), the pattern of quenching can be determined. K_{SV} is obtained from the slope of the plot of F_0/F against [Q] based on the fluorescence data at different temperatures (298 K and 308 K). Binding constants and binding points are showed in Table 2 and Table 3 at two different temperatures. Static and dynamic quenching are the two quenching process. In static quenching, formation of non fluorescent complex with quencher molecule is responsible for quenching of fluorescence intensity of a

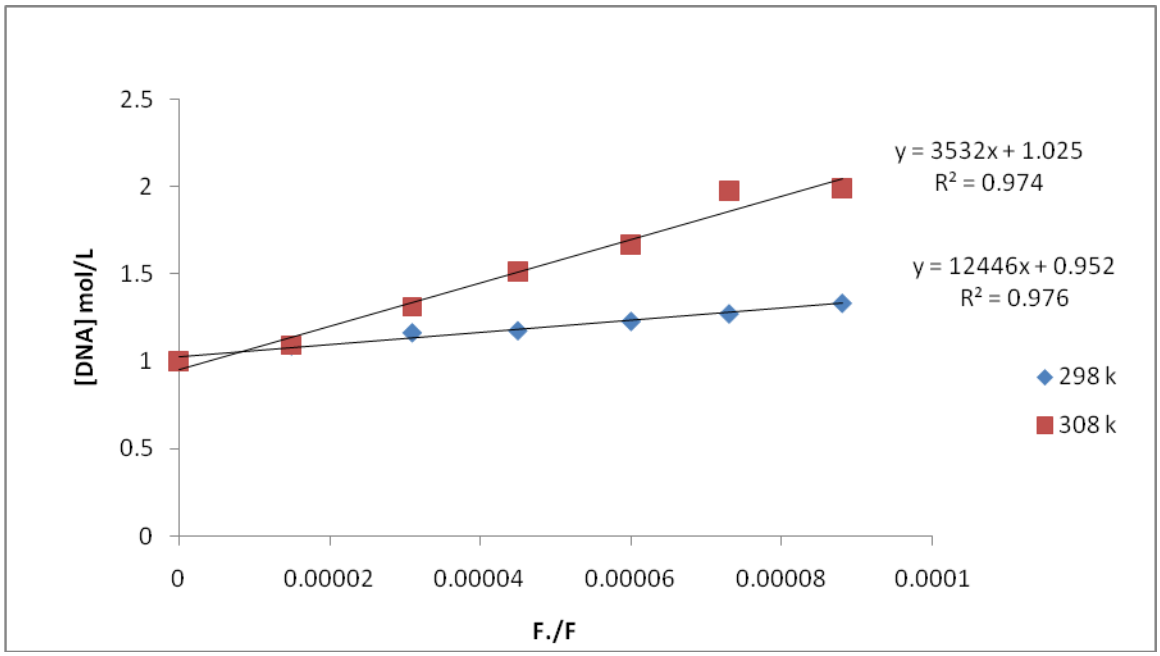

Figure 3. The Stern-Volmer plot for the quenching of DEX by DNA at two different temperatures.

Table 1. Data of binding constant and binding points for DEX-DNA system at 298 K.

[DEX] mol/l	[DNA] mol/l	Change of fluorescence intensity
6.3×10^{-6}	1×10^{-6}	12.81
1.9×10^{-5}	1×10^{-6}	32.00
3.1×10^{-5}	1×10^{-6}	60.14
6.4×10^{-5}	1×10^{-6}	98.10
1.3×10^{-4}	1×10^{-6}	73.51
3.2×10^{-4}	1×10^{-6}	66.28
6.4×10^{-4}	1×10^{-6}	54.24

Table 2. Data of binding constant and binding points for DEX-DNA system at 298 K.

F_o	F	Fo-F	Fo-F/F	log(Fo-F/F)	[DNA]mol/l	log [DNA]
607.1	556.3	50.8	0.091317634	-1.039445348	1.51 x10-5	-4.824
607.1	520.4	86.7	0.166602613	-0.77831819	3 x10-5	-4.523
607.1	515.3	91.8	0.178148651	-0.749217461	4.5 x10-5	-4.346
607.1	492.9	114.2	0.231689998	-0.635092714	6 x10-5	-4.222
607.1	476	131.1	0.275420168	-0.560004261	7.3 x10-5	-4.136
607.1	454.4	152.7	0.336047535	-0.473599286	8.8 x10-5	-4.055

Table 3. Data of binding constant and binding points for DLX-DNA system at 308 K.

F_o	F	Fo-F	Fo-F/F	log(Fo-F/F)	[DNA]mol/l	log [DNA]
1059	970.2	88.8	0.09152752	-1.038448305	1.51 x10-5	-4.824
1059	952.6	106.4	0.11169431	-0.951968949	3 x10-5	-4.523
1059	700	359	0.512857143	-0.290003591	4.5 x10-5	-4.346
1059	634.2	424.8	0.669820246	-0.17404173	6 x10-5	-4.222
1059	536.1	522.9	0.975377728	-0.010827165	7.3 x10-5	-4.136
1059	531.8	527.2	0.991350132	-0.003772931	8.8 x10-5	-4.055

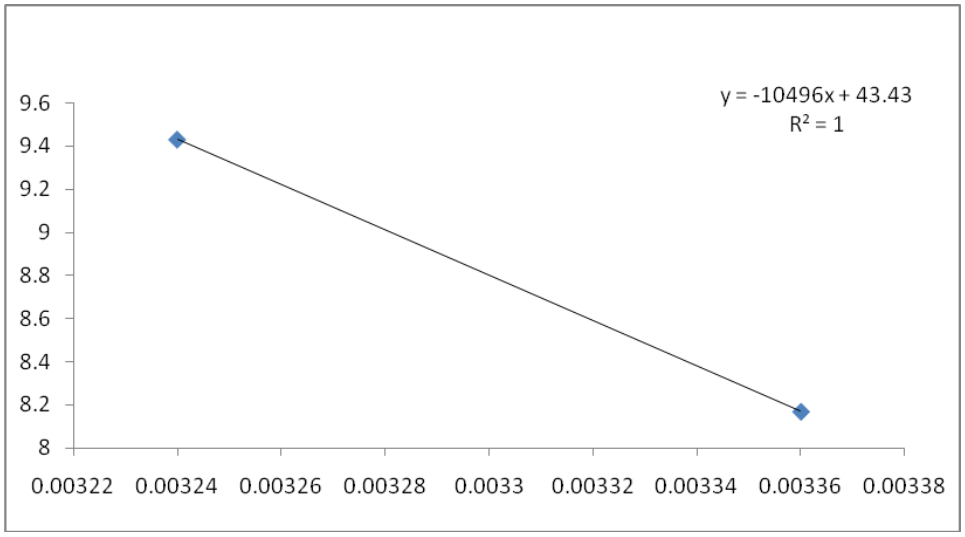

Figure 4. The Van't Hoff plot for DEX-DNA system. The curve is found by placing 1/T vs ln Ksv.

fluorophore, whereas dynamic quenching refers to a process in which the fluorophore and the quencher comes into contact during the lifetime of the excited state (Wang *et al*., 2006). They can be distinguished by their different temperature dependence. Higher temperatures can lead to faster diffusion and extended collisional quenching, so K_{SV} increases along with increasing temperature. For static quenching, higher temperatures will typically cause the dissociation of weakly bound complexes and *Ksv* decreases with increasing solvent temperature.

The fluorescence quenching data at 298 K and 308 K were plotted according to Stern-Volmer equation (Figure 3). Stern-Volmer constant was found to be 3532 L/ mol and 12446 L/ mol at 298 K and 308 K respectively, with correlation co-efficient of 0.974 and

0.976. As the K_{SV} increases with increase of temperature , the probable quenching mechanism of the DEX-DNA binding reaction is due to dynamic quenching instead of static quenching.

Thermodynamic parameters and nature of the binding forces: In order to elucidate the forces responsible for overall binding affinity and specificity of the drug, thermodynamic parameters are calculated. The value of enthalpy change (ΔH) and the entropy change (ΔS) were determined from the slope and intercept of the fitted curve of ln K_{SV} against 1/T, respectively (Figure 4). The Gibb's free energy (ΔG) can be estimated from the following relationship:

$$\Delta G = \Delta H - T\Delta S$$

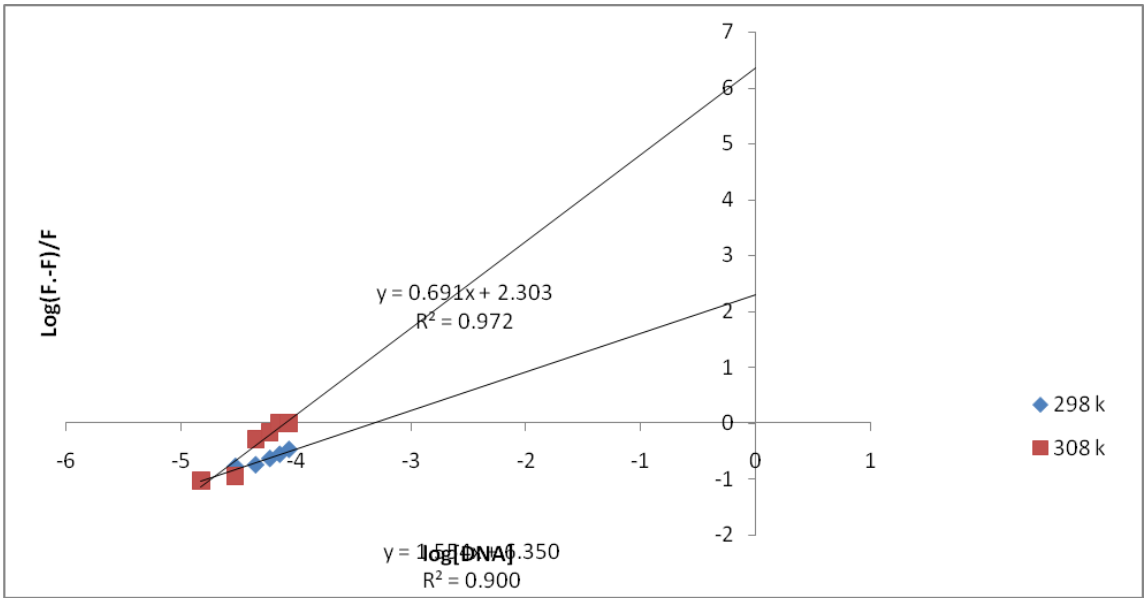

Figure 5. Plot for binding constant and binding points for DEX-DNA system at 298 and 308 K.

The enthalpy change (ΔH) and the entropy change (ΔS) and Gibb's free energy (ΔG) are the main evidence to characterize the binding mode. The value of ΔH, ΔS and ΔG were 87.28KJ/ mol, 361.16 KJ/ mol and -20.34 KJ/ mol respectively. Here the enthalpy change (ΔH) and the entropy change (ΔS) are positive and the Gibb's free energy (ΔG) is negative. The positive ΔS value is considered evidence for typical hydrophobic interaction from the point of H_2O structure and positive ΔH implies the possibility of hydrophobic interaction. The negative sign for ΔG indicates the spontaneity of the binding process (Ross *et al.*, 1981). Thus, hydrophobic interactions in low dielectric media are present in the DEX-DNA binding. As hydrophobic hydrophilic interaction is involved in the binding of DEX with DNA, so it can be said probably dextromethorphan binds in the minor groove of DNA.

Binding constant and binding points: From the plot of log [(F_0- F)/ F] versus log [DNA] (Figure 5), binding constant and binding number for dextromethorphan - DNA system were determined. Binding points were found to be 0.691 and 1.554 at 298 K and 308 K respectively. From corresponding binding constant 2×10^2 L/ mol and 2.28×10^6 L/ mol, it was observed that the binding constant increases with the increase in temperature of the DEX-DNA complex resulting in the increase stability of the DEX-DNA complex which proved further that the quenching mechanism of DEX-DNA binding reaction is dynamic. The values of *n* remained nearly constant at different temperatures. The DEX-DNA mol ratio of the system is 1:1. That means 1 mol of DEX binds with per mol of DNA. From these findings new pharmacological activity of dextro-methorphan can be discovered by exploiting its DNA binding property as it binds so significantly with DNA.

Discussion

In this study the nature and magnitude of the interaction of dextromethorphan hydrobromide to DNA was investigated in physiological buffer (pH 7.4) by fluorescence and UV absorption spectroscopy. The fluorescence quenching mechanism usually involves static or dynamic quenching. The experimental results demonstrate that dynamic quenching is involved in the DEX-DNA complex (1:1) formation. The thermodynamic parameters indicated that the interaction between [DEX] and DNA was spontaneous and hydrophobic interactions were present as major forces in the DEX-DNA binding that dominates the minor groove of binding. Similar observation was found in some other studies (Dorraji *et al*., 2013; Lv *et al*., 2014; Sultana *et al*., 2013).

Conclusion

Fluorescence absorption and quenching of the interaction between dextromethorphan and DNA at two different temperatures was observed in this research work. It showed that, dextromethorphan binds to DNA molecule with high affinity through hydrophobic interaction. The quenching pattern was found to be dynamic in this interaction and it was found that dextromethorphan binds with DNA with a mol ratio of 1 : 1.

Conflict of interest

The authors declare that there is no conflict of interest regarding this research.

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