Spectroscopic and Thermodynamic Study of Charge Transfer Interaction of Doxycycline Hydrochloride with Riboflavin in Aqueous Ethanol Media of Varying Compositions

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Doxycycline hydrochloride has been shown to form charge transfer (CT) complexes with a number of electron acceptors in 50% (v/v) aqueous ethanol medium. CT absorption bands of complexes with four acceptors of known electron affinity have been determined, and they correlate well with the electron affinities of the acceptors. From this correlation, the electron affinity of riboflavin has been estimated to be 1.02 eV. The CT absorption bands have also been utilized to determine the vertical ionization potential of doxycycline hydrochloride (7.05 eV). The enthalpy and entropy of formation of the complex between riboflavin and doxycycline hydrochloride have been determined by estimating the formation constant (*K*) spectrophotometrically at five different temperatures in pure water. The pronounced effect of the dielectric constant of the medium on the magnitude of *K* has been observed by determining *K* in aqueous ethanol mixtures of varying compositions. This has been rationalized in terms of ionic dissociation of the hydrochloride DH^+Cl^-, hydrolysis of the cation DH^+, and complexation of the free base D with riboflavin.

Introduction

Physicochemical properties of a drug molecule in solution determine the mechanism of action of the drug. Investigation of spectroscopic and thermodynamic properties of drug molecules is of importance in pharmacokinetics[1] and in developing analytical methods for the detection and estimation of small quantities of specific drugs in the meat and milk of animals administered with such drugs.[2] Most analytical methods used for the latter purpose mainly involve HPLC or GC techniques.[3−5] Spectroscopic and thermodynamic investigations, on the other hand, should not only serve these purposes but also lead to a measure of the strength of the binding of the drug molecules to other substances present in living systems. It was indicated much earlier by Webb and Thompson[6] that electron donor−acceptor complexes possibly have some role of in drug−receptor binding. The electron donor ability of a drug molecule, which can be directly measured from its vertical ionization potential, is an important parameter in this respect and can be determined through the study of charge transfer (CT) complexes, as has been shown in a recent study with paracetamol.[7] CT complexation is also an important phenomenon in biochemical and bioelectrochemical energy transfer processes.[8−17] Moreover, the formation constant (*K*) of a drug−protein complex is an important parameter in pharmaceutical science,[18−20] particularly in the context of targeted drug delivery.[21,22] With such potential applications in view, we have carried out a detailed spectroscopic and thermodynamic study of CT complexation of the broad-spectrum antibiotic, doxycycline hydrochloride (structure b, Figure 1) which is generally used in the treatment of respiratory tract infection. With a phenolic aromatic ring and a tertiary amine group in the molecule, it is a potential electron donor. On the other hand, quinones are well-known electron acceptors.[23] So, there is a possibility of CT complex formation of doxycycline hydrochloride with quinones. In particular, menadione (i.e., 2-methyl-1,4-naphthoquinone, vitamin K_3) and riboflavin (structure a, Figure 1) are vitamin molecules. Study of the interactions of such molecules with doxycycline hydrochloride is, therefore, expected to have some relevance in physical pharmacology. Because the drug in the present study is ionic, variation of the dielectric constant of the medium is expected to have a pronounced effect on the stability of the drug−vitamin complex. This aspect of the complexation process has been studied in the present work by measuring *K* of the complex in aqueous ethanol media of varying compositions, and a rationale for the observed variation in *K* values has been proposed.

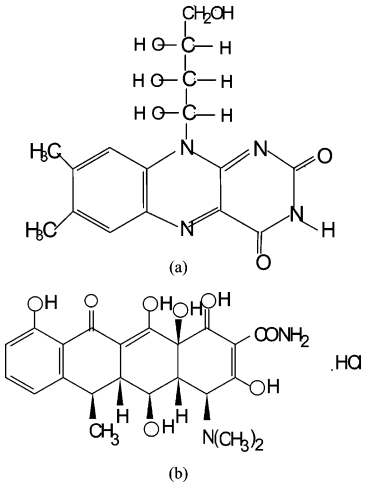

Figure 1. Structure of (a) riboflavin and (b) doxycycline hydrochloride.

Experimental Section

Menadione (2-methyl-1,4-naphthoquinone), 2,3-dichloro-1,4-naphthoquinone, the drug doxycycline hydrochloride (compound **1**), and riboflavin from Sigma were used without further purification. The other chemicals, *p*-bromanil (2,3,5,6-tetrabromo-1,4-benzoquinone) from Sigma and *p*-chloranil (2,3,5,6-

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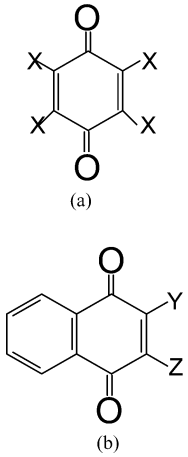

Figure 2. Structures of menadione (2-methyl-1,4-naphthoquinone), 2,3-dichloro-1,4-naphthoquinone, *p*-bromanil (2,3,5,6-tetrabromo-1,4-benzoquinone), and *p*-chloranil (2,3,5,6-tetrachloro-1,4-benzoquinone). (a) X = Cl for *p*-chloranil and Br for *p*-bromanil; (b) Y = Z = Cl for 2,3-dichloro-1,4-naphthoquinone and Y = $-CH_3$, Z = H for menadione.

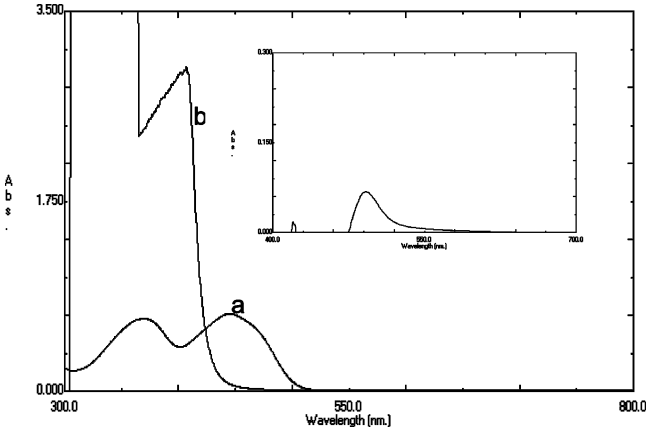

Figure 3. Absorption spectra of (a) riboflavin (4.769×10^{-5} mol dm^{-3}) and (b) doxycycline hydrochloride (9.747×10^{-3} mol dm^{-3}) against the solvent water as reference. Inset: CT absorption spectra of the complex between riboflavin (4.769×10^{-5} mol dm^{-3}) and doxycycline hydrochloride (1.449×10^{-2} mol dm^{-3}) obtained by difference method. In the ordinate, "Abs." means absorbance.

tetrachloro-1,4-benzoquinone) from Fluka, Switzerland, were further purified by sublimation just before use. Molecular structures of these compounds are shown in Figure 2. The solvent, ethanol, was purified by the method described in the literature[24,25] as follows: Commercial-grade absolute alcohol was dried over lime and distilled. The distillate was refluxed for 30 min with iodine-activated magnesium and then distilled under moisture-free conditions. The entire experiment was done in ethanol−water mixtures of varying composition. The experiment was not done in pure ethanol medium, because riboflavin and doxycycline hydrochloride are soluble in water but insoluble in pure ethanol. Moreover, such a medium is closer to a biological system than the nonpolar solvents which are generally used in the study of CT complexes. All optical measurements were done on a UV 1601 PC model Shimadzu spectrophotometer fitted with a Peltier-controlled thermal bath; a matched pair of quartz cells of 1-cm optical path length were used for the measurements.

Results and Discussion

Observation of CT bands. In the present study, CT bands were observed for complexes of compound **1** with (i) *p*-bromanil, (ii) *p*-chloranil, (iii) menadione, (iv) 2,3-dichloro-1,4-naphthoquinone, and (v) riboflavin. To obtain CT bands, spectra of each of the solutions (in 50% v/v ethanol) containing compound **1** as donor and the acceptors (i) to (iv) separately were recorded against the pristine acceptor solution as a reference. It is a common experience that CT bands in solution are detected only by taking a high concentration of donor compared to that of the acceptor. In the present case, [compound **1**] $\approx 10^{-2}$ mol dm^{-3}, and concentrations of the acceptors were $\sim 10^{-3}-10^{-4}$ mol dm^{-3}. One typical CT absorption band is shown in the inset of Figure 3; the absorption bands of the pristine acceptor solution and compound **1** are also shown in the same figure. Compound **1** does not absorb above 470 nm, but the two substances have overlapping absorption bands in the visible region below 470 nm. So, the difference spectra (absorbance of mixture minus the sum of the individual absorbances of compound **1** and riboflavin with concentrations as present in the mixture over the wavelength range scanned) give the CT absorption band (inset, Figure 3). The vertical electron affinities (E_A^v values) of the first four acceptors under study are known,[23,26−28] and they correlate well with the presently observed CT transition energies ($h\nu_{CT}$ values, Table 1) in accordance with the Mulliken[29] equation

TABLE 1: Charge Transfer Absorption Maxima (λ_{CT}), CT Transition Energy ($h\nu_{CT}$), Electron Affinity of the Acceptors (E_A^v), Vertical Ionization Potential of Doxycycline Hydrochloride (I_D^v), and Degree of Charge Transfer (α)

acceptor	λ_{CT}, nm	$h\nu_{CT}$, eV	E_A^v,eV	$10^3 \times \alpha$	I_D^v, eV
2,3-dichloro-1,4 naphthoquinone	560	2.216	2.38	4.47	7.05
menadione	434	2.860	2.18	4.32	7.05
p-bromanil	525	2.364	1.41	3.82	7.05
p-chloranil	520	2.387	1.37	3.79	7.05
riboflavin	490	2.533	1.02[a]	3.59	7.05

[a] This vertical electron affinity has been determined in present study.

$$h\nu_{CT} = I_D^v - C_1 + \frac{C_2}{I_D^v - C_1} \quad (1)$$

where I_D^v is the vertical ionization potential of the donor (compound **1**) and C_1 is given by the equation

$$C_1 = E_A^v + G_1 + G_0 \quad (2)$$

Here, E_A^v is the vertical electron affinity of the acceptor, G_0 is the sum of several energy terms (such as dipole−dipole, van der Waals interaction, etc.) in the no-bond state, and G_1 is the sum of a number of energy terms in the dative state. In most cases, G_0 is small and can be neglected, while G_1 is mainly the electrostatic energy of attraction between donor (D^+) and acceptor (A^-) in the dative state. The term C_2 in eq 1 is related to the resonance energy of interaction between the no-bond and dative states. Equation 1 is obviously nonlinear (quadratic) with respect to C_1 (consequently, also w. r. t. E_A^v), and the following regression equation was obtained with the present experimental data

$$E_A^v = 12.180\,077\,87(h\nu_{CT})^2 - 62.126\,728\,75h\nu_{CT} + 80.238\,380\,09 \quad (3)$$

A parabolic plot (Figure 4) was obtained for E_A^v against $h\nu_{CT}$. Putting the value of $h\nu_{CT}$ of the complex riboflavin with

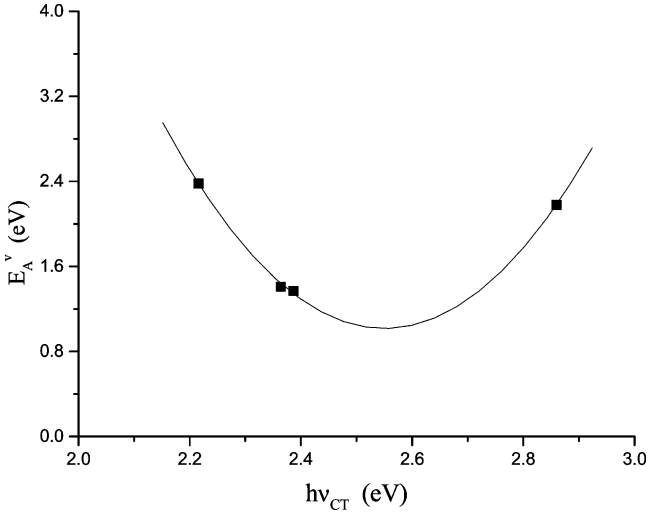

Figure 4. Plot of E_A^v against $h\nu_{CT}$ of complexes of doxycycline hydrochloride with some acceptors of known electron affinity.

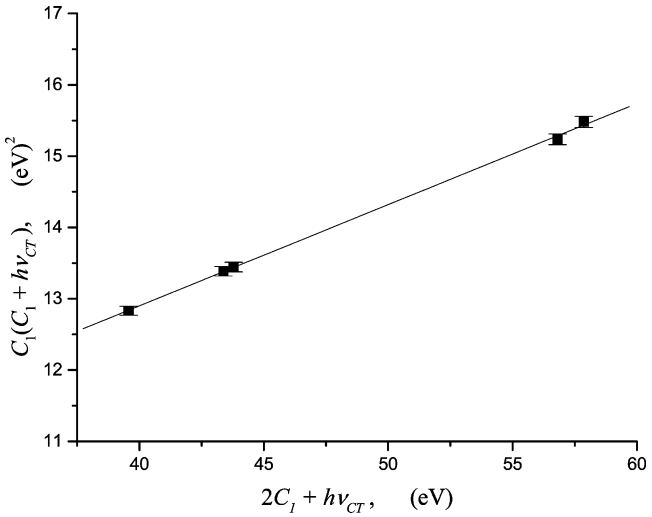

Figure 5. Plot for determination of vertical ionization potential of doxycycline hydrochloride according to eq 4.

compound **1** in eq 3, the E_A^v of riboflavin (i.e., acceptor (v)) was found to be 1.02 eV. A rearrangement of eq 1 yields

$$2C_1 + h\nu_{CT} = \frac{C_1(C_1 + h\nu_{CT})}{I_D^v} + \left(\frac{C_2}{I_D^v} + I_D^v\right) \qquad (4)$$

By neglecting G_0 and taking the typical D−A distance in π-type electron donor−acceptor (EDA) complexes to be 3.5 Å, the major part of G_1 is estimated to be $e^2/4\pi\epsilon_0 r = 4.13$ eV. By using these values, C_1 is obtained from eq 2 for each of the acceptors. A plot of $2C_1 + h\nu_{CT}$ against $C_1(C_1 + h\nu_{CT})$ for a given donor and various acceptors should be a straight line with a slope of $1/I_D^v$, from which the value of I_D^v of the donor can be obtained. In the present case, with the experimental CT transition energies shown in Table 1, the plot is fairly linear (Figure 5), and the linear regression equation is

$$2C_1 + h\nu_{CT} = (0.142 \pm 0.002)[C_1(C_1 + h\nu_{CT})] + (7.225 \pm 0.113);\ r^2 = 0.99 \qquad (5)$$

From the slope, I_D^v of compound **1** is found to be 7.05 eV.

Degree of Charge Transfer (α). In a Mulliken two-state model,[29] the ground (ψ_g) and excited (ψ_{ex})-state wavefunctions of the CT complexes are described by a linear combination of dative $\psi(D^\circ, A^\circ)$ and ionic $\psi(D^+, A^-)$ states

$$\psi_g = \sqrt{1-\alpha}\,\psi(D^\circ, A^\circ) + \sqrt{\alpha}\,\psi(D^+, A^-) \qquad (6)$$

$$\psi_{ex} = \sqrt{1-\alpha}\,\psi(D^+, A^-) - \sqrt{\alpha}\,\psi(D^\circ, A^\circ) \qquad (7)$$

where α is the degree of charge transfer. The function $\psi(D^+, A^-)$ differs from $\psi(D^\circ, A^\circ)$ by the promotion of an electron from the donor to the acceptor. The value of α is given[29,30] by

$$\alpha = \frac{C_2}{2(I_D^v - E_A^v + C_1)^2 + C_2} \qquad (8)$$

The values of α, calculated by using eq 8 and shown in Table 1, are small and indicate that very little charge transfer occurs in the ground state. However, as expected with a fixed donor, α increases with an increase in the electron affinity of the acceptors (Table 1).

Spectrophotometric Study of Formation Equilibria of the Complexes of Doxycycline Hydrochloride with Riboflavin. Irrespective of the medium, be it pure water or a water−ethanol mixture, it was found that the intensity in the visible portion of the absorption band of a mixture of compound **1** and riboflavin, measured against the pristine riboflavin solution as reference, increases systematically with an increase in concentration of compound **1**, keeping that of riboflavin fixed. Experimental data are given in Tables 2 and 3. With an increase in the percentage of ethanol in the medium, λ_{CT} does not change, but the absorbance decreases, while the overall spectral feature with respect to the increase in donor concentration remains the same. Stoichiometry and formation constants of the CT complexes were determined by using the Benesi−Hildebrand[31] equation for cells with 1-cm optical path length

$$\frac{[A]_0[D]_0}{d'} = \frac{[D]_0}{\epsilon'} + \frac{1}{K\epsilon'} \qquad (9)$$

with

$$d' = d - d_A^0 - d_D^0 \qquad (10)$$

Here, $[A]_0$ and $[D]_0$ are the initial concentrations of the acceptor and donor, respectively, d is the absorbance of the donor−acceptor mixture at λ_{CT} against the solvent as reference, and d_A^0 and d_D^0 are the absorbances of the acceptor and donor solutions with the same molar concentrations as in the mixture at the same wavelength (i.e., λ_{CT}). The quantity $\epsilon' = \epsilon_c - \epsilon_A - \epsilon_D$ means the molar absorptivity of the complex, with ϵ_A and ϵ_D being those of the acceptor and the donor, respectively, at λ_{CT}. K is the formation constant of the complex. Equation 9 is valid[29] under the condition $[D]_0 \gg [A]_0$ for 1:1 donor−acceptor complexes. Experimental data for the riboflavin·**1** complex at λ_{CT} are given in Tables 2 and 3. In all cases, very good linear plots according to eq 9 were obtained. Four typical Benesi−Hildebrand plots, with experimental data taken in varying water−ethanol compositions, are shown in Figure 6. The correlation coefficients of all such plots were >0.98. Values of K of the complexes obtained from such plots are shown in Table 4.

Determination of Enthalpy and Entropy of Formation of the Riboflavin·1 Complex in Pure Water. The enthalpies of formation were obtained by using the van't Hoff equation. Plots of ln K (in pure water) against $1/T$ are shown in Figure 7. The

TABLE 2: Absorbance Data of Mixtures Containing Riboflavin (Acceptor) and Doxycycline Hydrochloride (Donor) in Aqueous Ethanol Media of Different Compositions against the Pristine Acceptor Solution as Reference.

10^5 [riboflavin] (mol dm^{-3})	10^2 [doxycycline hydrochloride] (mol dm^{-3})				absorbance at 490 nm and 293 K			
	25% ethanol	50% ethanol	75% ethanol	90% ethanol	25% ethanol	50% ethanol	75% ethanol	90% ethanol
3.891	0.598	0.552	0.559	0.565	0.0501	0.0406	0.0216	0.0166
3.891	0.721	0.630	0.624	0.689	0.0593	0.0437	0.0234	0.0194
3.891	0.806	0.773	0.708	0.806	0.0639	0.0485	0.0247	0.0208
3.891	0.910	0.877	0.806	0.890	0.0657	0.0507	0.0271	0.0226
3.891	0.994	1.007	0.890	0.968	0.0696	0.0557	0.0294	0.0243
3.891	1.124	1.098	1.033	1.072	0.0717	0.0581	0.0308	0.0256
3.891	1.235	1.189	1.150	1.209	0.0780	0.0597	0.0326	0.0269
3.891	1.313	1.293	1.241	1.345	0.0755	0.0619	0.0341	0.0293
3.891	1.566	1.436	1.397		0.0781	0.0641	0.0361	

TABLE 3: Absorbance Data of Mixtures Containing Riboflavin (Acceptor) and Doxycycline Hydrochloride (Donor) in Water Medium at Five Different Temperatures against the Pristine Acceptor Solution as Reference

10^5 [riboflavin] (mol dm^{-3})	10^2 [doxycycline hydrochloride] (mol dm^{-3}) [in water]	absorbance at 490 nm				
		293 K	298 K	303 K	308 K	313 K
3.891	0.572	0.0434	0.0406	0.0379	0.0343	0.0316
3.891	0.656	0.0456	0.0430	0.0403	0.0359	0.0330
3.891	0.793	0.0502	0.0470	0.0440	0.0402	0.0370
3.891	0.890	0.0534	0.0501	0.0469	0.0414	0.0388
3.891	0.994	0.0551	0.0522	0.0491	0.0451	0.0418
3.891	1.098	0.0584	0.0544	0.0519	0.0477	0.0440
3.891	1.222	0.0601	0.0574	0.0520	0.0493	0.0460
3.891	1.319	0.0621	0.0587	0.0547	0.0499	0.0461
3.891	1.449	0.0637	0.0611	0.0591	0.0526	0.0496

TABLE 4: Formation Constants, Enthalpy, and Entropy of Formation of the Complex of Doxycycline Hydrochloride with Riboflavin in Pure Water and in Different Aqueous Ethanol Media[a]

medium	temp (K)	formation constant (K) (mol^{-1} dm^3)	dielectric constant (d)	ϵ' (dm^3 mol^{-1} cm^{-1})	ΔH_f^0 (kJ mol^{-1})	ΔS_f^0 (J K^{-1} mol^{-1})
pure	293	147.0 ± 0.2	80.18	1830	−10.5 ± 0.3	5.6 ± 0.9
water	298	135.0 ± 1.2	78.36			
	303	127.0 ± 0.3	76.58			
	308	119.0 ± 0.5	74.85			
	313	111.0 ± 0.1	73.15			
25%	293	138.0 ± 0.9	68.27	1844		
50%	293	120.0 ± 3.0	54.09	1927		
75%	293	90.0 ± 0.3	38.65	1215		
90%	293	62.0 ± 0.3	30.39	1197		

[a] Compositions of the aqueous ethanol mixtures are expressed in % of ethanol (v/v).

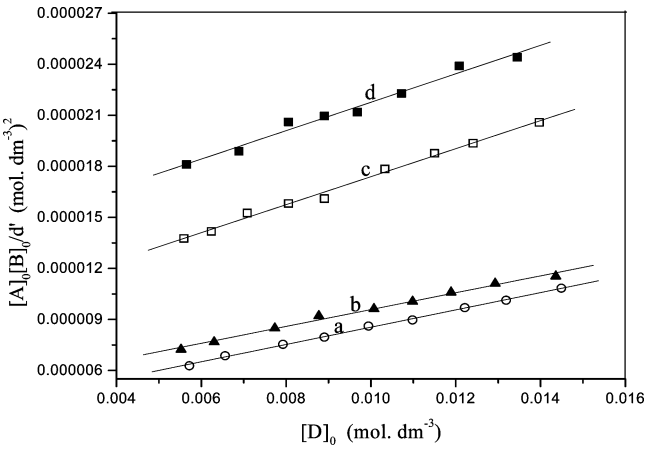

Figure 6. Benesi−Hildebrand plots for riboflavin−doxycycline hydrochloride complex in different media at 293 K. (a) pure water, (b) 50%, (c) 75%, and (d) 90% ethanol (v/v).

following regression equation was obtained for the riboflavin·**1** complex under study

$$\ln K = (1262.40 \pm 33.55)\frac{1}{T} + (0.68 \pm 0.11);\ r^2 = 0.99 \quad (11)$$

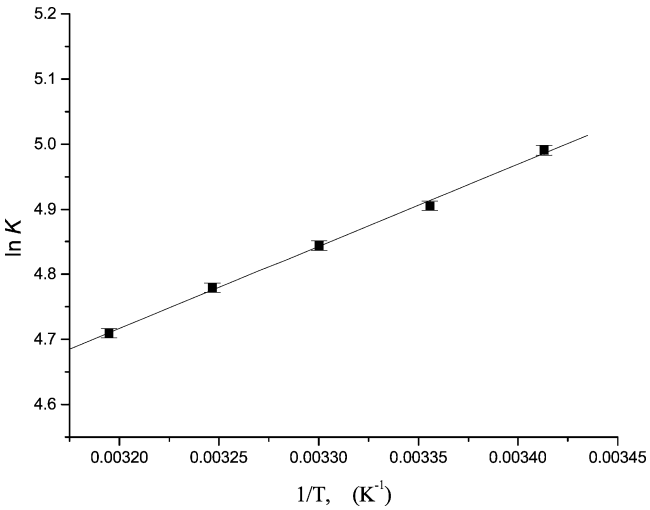

Figure 7. van't Hoff plots for complexes of riboflavin with drug **1** in pure water medium.

The values of enthalpy (ΔH_f^0) and entropy (ΔS_f^0) of formation obtained from such plots are given in Table 4.

Effect of Dielectric Constant on the Magnitude of Formation Constant of the Complex. Variation of the formation

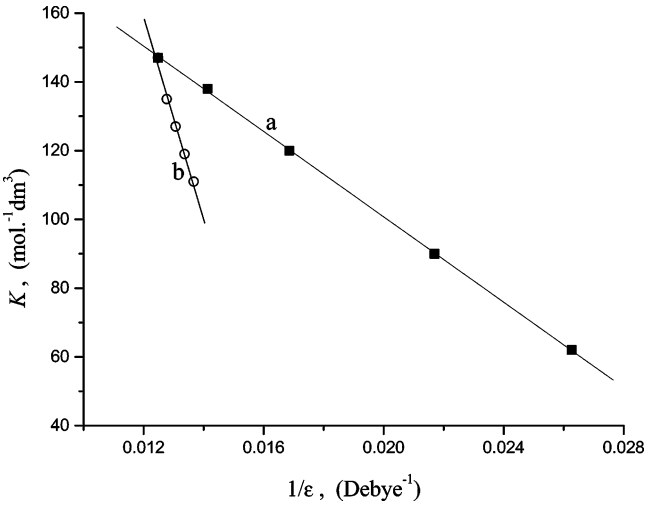

Figure 8. Plot of formation constant against reciprocal of dielectric constant: (a) varying ethanol−water ratio and (b) varying the temperature of pure water.

constant with the dielectric constant (ε) of the medium was studied in two ways: by varying the ethanol−water ratio[32] in the medium at constant temperature (293 K) and by using pure water as the medium and causing its dielectric constant to change by changing the temperature.[33] Results are shown in Table 4 and Figure 8. It is found that K decreases linearly with $1/\varepsilon$ in both types of study. A plausible explanation for this is as follows. The hydrochloride compound **1** (which may be abbreviated as DH^+Cl^-) at first dissociates into ions; then, the cation (DH^+) undergoes hydrolysis to form the corresponding free base (D) which forms a molecular complex with riboflavin (A):

$DH^+Cl^- \rightleftharpoons DH^+ + Cl^-$; dissociation constant $= K_d$

$DH^+ + H_2O \rightleftharpoons D + H_3O^+$; hydrolysis constant $= K_h$

$D + A \rightleftharpoons DA$; equilibrium constant $= K'$

Overall reaction: $DH^+Cl^- + H_2O + A \rightleftharpoons DA + H_3O^+ + Cl^-$

Apparent formation constant (K) of the complex $= K_dK_hK'$

With an increase in dielectric constant at a particular temperature, the values of $K'$and K_h do not change appreciably, but K_d increases remarkably. This explains the observed increase in the apparent K with an increase in dielectric constant of the medium. In Figure 8b, the linear decrease of K with $1/\varepsilon$ is much steeper than in Figure 8a. This is because, in this case, a decrease of K results not only from a decrease of ε but also from an increase of temperature.

Conclusion

The present study shows that doxycycline hydrochloride forms a charge-transfer complex with riboflavin in aqueous and aqueous−ethanol media which are close to biological systems. The temperature and dielectric constant of the medium have a pronounced effect on the formation constant of the complex. Because a change in body temperature also changes the dielectric constant of the body fluid, the results obtained in the present work may have some relevance in physical pharmacology. Moreover, the ionization potential of the drug **1** and the electron affinity of riboflavin determined in the present work may be utilized in theoretical modeling of the mechanism of drug action. It has further been established that the free base form (D) of the drug actually binds to the riboflavin molecule.

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