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Spectroscopic and thermodynamic study of charge transfer complexes of cloxacillin sodium in aqueous ethanol medium

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Abstract

Cloxacillin sodium has been shown to form charge transfer (CT) complexes of 1:1 stoichiometry with a number of electron acceptors in 50% (v/v) aqueous ethanol medium. From the trends in the CT absorption bands, the vertical ionization potential of the drug molecule (cloxacillin sodium) has been estimated to be 7.89 eV. The enthalpies and entropies of formation of two such complexes have been determined by estimating the formation constants spectrophotometrically at five different temperatures. The oscillator strengths and transition dipole moments of these complexes have been determined. It has further been noted that the reduction of *o*-chloranil by aqueous ethanol is completely inhibited by cloxacillin sodium, a phenomenon that makes the present study of formation equilibrium possible.

Keywords: Charge transfer complexes; Cloxacillin sodium; Aqueous ethanol

1. Introduction

Study of the physicochemical properties of drug molecules in solution is of importance in pharmacokinetics and is being recently carried out [1]. Development of methods of analysis of small quantities of these drugs in meat and milk of animals administered with such drugs is another important purpose of studying the physicochemical properties of the drugs in vitro [2]. Most of such methods involve HPLC or GC techniques [3–5]. Spectroscopic and thermodynamic methods, on the other hand, should be useful not only for detection and quantification of these drugs in a given sample of tissue/blood, but also for understanding the mechanism of binding of the drug molecules to other substances present in living systems. The possible role of electron donor–acceptor complexes in drug–receptor binding was indicated much earlier by Webb and Thompson [6]. 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. This parameter 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]. Not only this, CT complexes of a drug molecule may absorb in the visible range and thus lead to easy detection and estimation of the drug. Moreover, the formation constant of a drug–protein complex (commonly called drug–protein association constant by pharmacokineticists) is an important parameter in the context of targeted drug delivery [18,19]. For this purpose we have carried out a detailed spectroscopic and thermodynamic study of CT complexation of the antibacterial drug, sodium (6*R*)-6-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido] penicillanate, which is commonly known as 'cloxacillin sodium' (Fig. 1). This drug is known to bind to human serum albumin [20]. With a nitrogen-containing heterocyclic ring, it is a potential electron donor. On the other hand, quinones are well-known electron acceptors [21]. So there is a possibility of CT complex formation of cloxacillin sodium with quinones. In particular, menadione, i.e., 2-methyl-1,4-napthoquinone, is a vitamin

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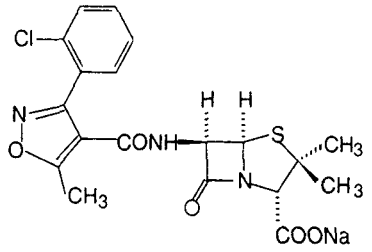

Fig. 1. Structure of sodium (6*R*)-6-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido] penicillanate (cloxacillin sodium).

molecule (Vitamin K_3). The study, therefore, is expected to have some relevance in pharmacology.

2. Experimental

Menadione (i.e., 2-methyl-1,4-napthoquinone), 2,3-dichloro-1,4-napthoquinone and the drug cloxacillin sodium from Sigma and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) from Aldrich, were used without further purification. The other chemicals, *o*-chloranil (i.e., 3,4,5,6-tetrachloro-1,2-benzoquinone) from Sigma and *p*-chloranil (i.e., 2,3,5,6-tetrachloro-1,4-benzoquinone) from Fluka, Switzerland, were further purified by sublimation just before use. The solvent, ethanol was purified by the method described in [22,23] as follows: commercial grade absolute alcohol was dried over lime and distilled. The distillate was refluxed for half an hour with iodine-activated magnesium and then distilled under moisture-free conditions. The entire experiment was done in a medium containing 50% (v/v) of conductivity water and 50% of the purified ethanol. Such a medium was chosen because cloxacillin sodium is soluble in water but insoluble in ethanol while the acceptors used are soluble in ethanol but insoluble in water. Moreover, such a medium is closer to biological system than non-polar solvents which are generally used in the study of charge transfer complexes. All optical measurements were done on a UV 1601 PC model Simadzu spectrophotometer fitted with a Peltier controlled thermobath.

3. Results and discussion

3.1. Observation of CT bands

In the present study, CT bands were observed in case of complexes of cloxacillin sodium (Fig. 1) with: (i) *o*-chloranil; (ii) *p*-chloranil; (iii) menadione; (iv) DDQ; and (v) 2,3-dichloro-1,4-napthoquinone. In 50% aqueous ethanol medium, the spectrum of cloxacillin sodium itself is peaked at 341 nm and it does not absorb beyond 380 nm as shown in Fig. 2. To obtain CT bands of the drug–quinone complexes, spectrum of each of the solutions (in 50% ethanol) containing cloxacillin sodium as donor and the acceptors (i)–(v) separately was recorded against the pristine acceptor solution as

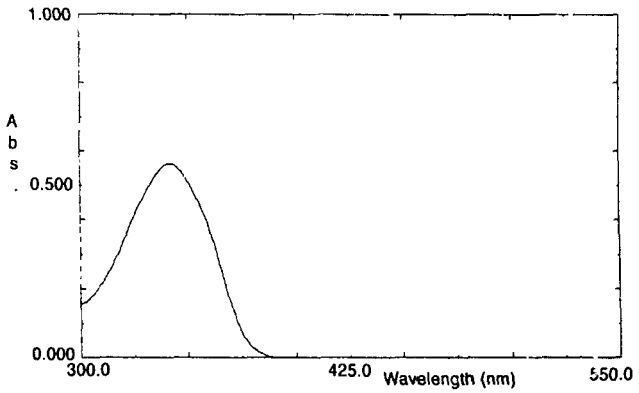

Fig. 2. Absorption spectrum of cloxacillin sodium (1.275×10^{-3} mol dm^{-3}) against the solvent (50% ethanol–water (v/v)) as reference. In the ord nate, 'Abs.' means 'absorbance'.

reference. The CT bands in solution were detected by taking high concentration of the donor, viz. [cloxacillin sodium] $\approx 10^{-2}$ mol dm^{-3} compared to that of each acceptor ($\approx 10^{-3}$ to 10^{-4} mol dm^{-3}). CT absorption bands for four typical cases under study are shown in Fig. 3. As evident from Fig. 2, the absorption due to free cloxacillin does not interfere with the CT bands. Two important points must be mentioned here: (a) *o*-Chloranil undergoes slow reduction by aqueous ethanol as was observed by the gradual spectral change of the aqueous ethanolic solution with time. But this reduction was completely inhibited in the presence of cloxacillin sodium, and this fact makes the present study of formation equilibrium of the drug–*o*-chloranil complex possible. (b) 2,3-Dichloro-1,4-napthoquinone slowly reacts with cloxacillin sodium in aqueous ethanolic medium; the wavelength (λ_{CT}) of the CT absorption peak remains unchanged but the intensity of the absorption gradually changes with time. For this reason, we have used only the λ_{CT} for analysis but have not carried out formation equilibrium study of this complex in present work. The vertical electron affinities (E_A^v) of the acceptors (i)–(iv) were collected from literature [21,24,25] and that of the fifth

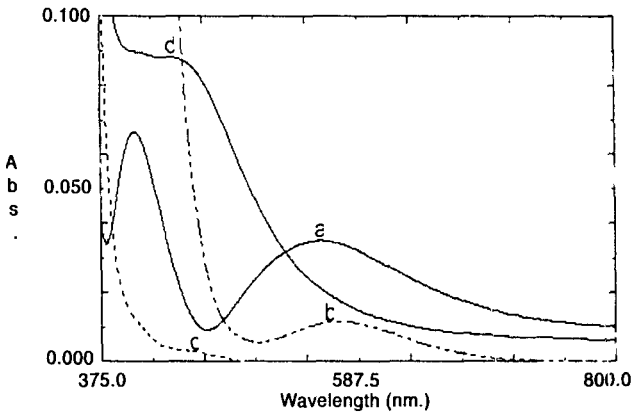

Fig. 3. CT absorption spectra of mixtures containing cloxacillin sodium (10^{-2} mol dm^{-3}) and (a) *o*-chloranil (10^{-4} mol dm^{-3}), (b) DDQ (10^{-4} mol dm^{-3}), (c) menadione (10^{-3} mol dm^{-3}) and (d) 2,3-dichloro-1,4-napthoquinone (10^{-3} mol dm^{-3}) against the pristine acceptor solutions as reference. In the ordinate, 'Abs.' means 'absorbance'.

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 cloxacillin sodium (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)
DDQ	575	2.158	3.27	4.25	7.89
o-Chloranil	558	2.224	2.87	3.99	
2,3-Dichloro-1,4-napthoquinone	445	2.789	2.38	3.71	
Menadione	446	2.783	2.18	3.56	
p-Chloranil	454	2.734	1.37	3.21	

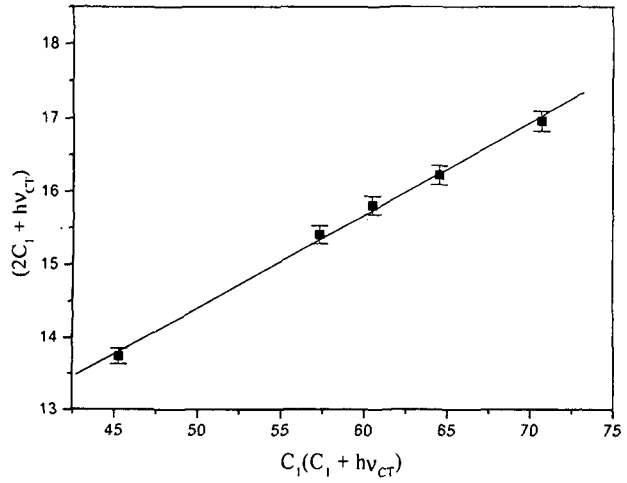

Fig. 4. Plot for determination of vertical ionization potential of cloxacillin sodium according to Eq. (4).

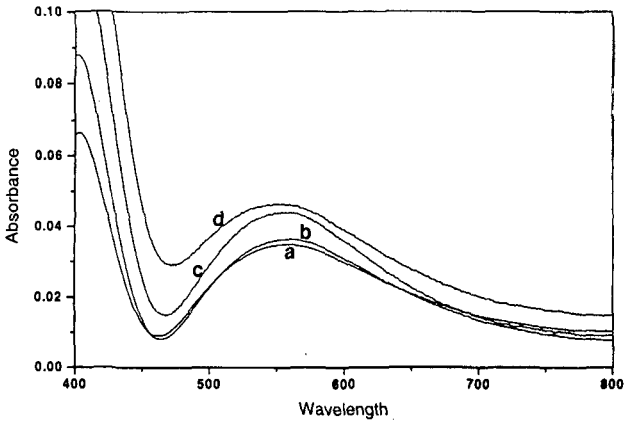

Fig. 5. CT absorption spectrum (calculated by subtracting the absorption due to *o*-chloranil) of mixtures containing *o*-chloranil (4.553×10^{-4} mol dm^{-3}) and cloxacillin sodium: (a) 1.408×10^{-2} mol dm^{-3}, (b) 1.849×10^{-2} mol dm^{-3}, (c) 3.901×10^{-2} mol dm^{-3} and (d) 4.783×10^{-2} mol dm^{-3} progressively upwards.

one has been recently determined [26]. These E_A^v values correlate well with the presently observed CT transition energies ($h\nu_{CT}$, Table 1) in accordance with the Mulliken [27] equation:

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

where I_D^v is the vertical ionization potential of the donor (cloxacillin sodium) and C_I is given by the equation:

$$C_I = E_A^v + G_I + G_0 \quad (2)$$

Here, E_A^v is the vertical electron affinity of the acceptor, G_0 is the sum of several energy terms (like dipole–dipole, van der Waals interaction, etc.) in the 'no-bond' state and G_I is the sum of several energy terms in the 'dative' state. In most cases, G_0 is small and can be neglected while G_I is mainly the electrostatic energy of attraction between D^+ and 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. A rearrangement of Eq. (1) yields:

$$2C_I + h\nu_{CT} = \frac{C_I(C_I + h\nu_{CT})}{I_D^v} + \left(\frac{C_2}{I_D^v} + I_D^v\right) \quad (3)$$

Neglecting G_0 and taking the typical D–A distance in π-type EDA complexes to be 3.5 Å, the major part of G_I is estimated to be $e^2/4\pi\varepsilon_0 r = 4.13$ eV. Using these values, C_I is obtained from Eq. (2) for each of the acceptors. A plot of $2C_I + h\nu_{CT}$ against $C_I(C_I + h\nu_{CT})$ for a given donor and various acceptors should yield a slope of $1/I_D^v$ from which the values 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 (Fig. 4) and the linear regression equation is:

$$2C_I + h\nu_{CT} = (0.127 \pm 0.004)[C_I(C_I + h\nu_{CT})] + (8.055 \pm 0.260); \quad \text{correlation coefficient} = 0.99 \quad (4)$$

From the slope, I_D^v of cloxacillin sodium is found to be 7.89 eV.

3.2. Degree of charge transfer (α)

In a Mulliken two-state model [27], the ground (ψ_g) and excited (ψ_{ex}) state wave functions of the CT complexes are described by a linear combination of dative $\psi(D^0, A^0)$ and ionic $\psi(D^+, A^-)$ states:

$$\psi_g = \sqrt{1-\alpha}\psi(D^0, A^0) + \sqrt{\alpha}\psi(D^+, A^-) \quad (5)$$

$$\psi_{ex} = \sqrt{1-\alpha}\psi(D^+, A^-) - \sqrt{\alpha}\psi(D^0, A^0) \quad (6)$$

where α is the degree of charge transfer. The function $\psi(D^+, A^-)$ differs from $\psi(D^0, A^0)$ by the promotion of an electron

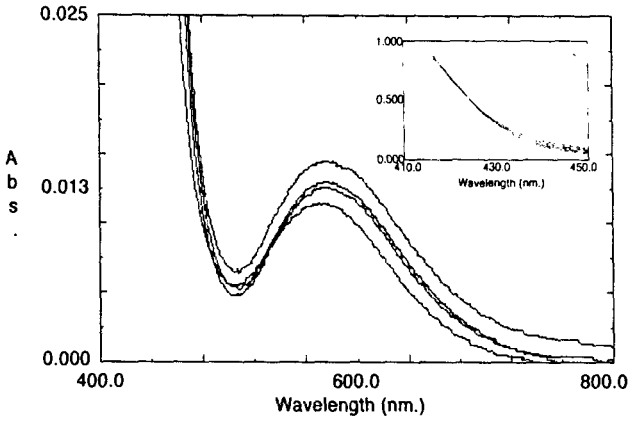

Fig. 6. CT absorption spectra of mixtures containing DDQ (4.075×10^{-4} mol dm^{-3}) and cloxacillin sodium (2.289×10^{-2}, 4.123×10^{-2}, 4.559×10^{-2} and 5.441×10^{-2} mol dm^{-3} progressively upwards) all taken against pristine acceptor solution as reference. Inset: 410–450 nm wavelength range and 0.0–1.0 absorbance expanded to show the isosbestic point.

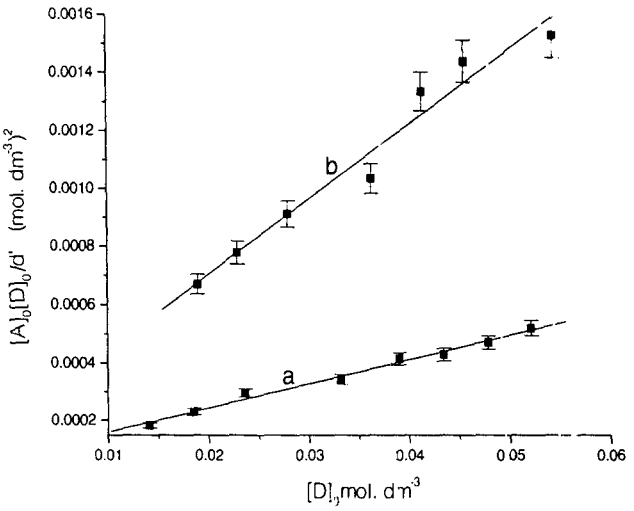

Fig. 7. Benesi–Hildebrand plot for cloxacillin sodium with (a) *o*-chloranil and (b) DDQ complex at 304 K.

from donor to the acceptor. α is given [27,28] by:

$$\alpha = \frac{C_2}{2(I_D^v - E_A^v + C_1)^2 + C_2} \tag{7}$$

The values of α, calculated by using Eq. (7) 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 increase in the electron affinity of the acceptors (Table 1).

3.3. Spectrophotometric study of formation equilibria of the complexes of cloxacillin sodium with o-chloranil, DDQ and 2,3-dichloro-1,4-napthoquinone

The intensity in the visible portion of the absorption band, measured against the pristine acceptor solution as reference, increases systematically with gradual addition of cloxacillin sodium (Figs. 5 and 6). This indicates complex formation. An isosbestic point at λ = 425 nm was obtained in case of the cloxacillin sodium–DDQ complex (Fig. 6, inset). Stoichiometry and formation constants of the complexes were determined by using Benesi–Hildebrand [29] equation for cells with 1 cm optical path length:

$$\frac{[A]_0[D]_0}{d'} = \frac{[D]_0}{\varepsilon'} + \frac{1}{K\varepsilon'} \tag{8}$$

with

$$d' = d - d_A^0 - d_D^0 \tag{9}$$

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 some suitable λ against the solvent as reference, and d_A^0 and d_D^0 are the absorbances of the acceptor and donor solutions with same molar concentrations as in the mixture at the same wavelength (i.e., λ). The quantity $\varepsilon' = \varepsilon_c - \varepsilon_A - \varepsilon_D$ means the molar absorptivity of the complex, ε_A and ε_D being those of the acceptor and the donor, respectively, at λ. K is the formation constant of the complex. Eq. (8) is valid [29] under the condition $[D]_0 \gg [A]_0$ for 1:1 donor–acceptor complexes. Absorbance data were taken at 558 and 575 nm, respectively, for the *o*-chloranil and DDQ complexes because at these wavelengths the variation of absorbance with gradual addition of cloxacillin sodium was notable and the latter does not absorb appreciably at these two wavelengths. Experimental data are given in Tables 2 and 3.

Table 2
Absorbance data of mixtures containing *o*-chloranil (acceptor) and cloxacillin sodium at five different temperatures against the pristine acceptor solution as reference

Acceptor	[Acceptor] ($\times 10^4$ mol dm^{-3})	[Cloxacillin sodium] ($\times 10^2$ mol dm^{-3})	Absorbance at 558 nm				
			293 K	298 K	303 K	308 K	313 K
		1.408	0.0349	0.0334	0.033	0.0316	0.0306
		1.849	0.0364	0.0355	0.0339	0.0328	0.0322
		2.360	0.0363	0.0342	0.0334	0.0321	0.0309
o-Chloranil	4.553	3.312	0.0439	0.0429	0.0425	0.0418	0.0412
		3.901	0.0426	0.0412	0.0405	0.0397	0.0386
		4.342	0.0459	0.0442	0.0432	0.0414	0.0421
		4.783	0.0461	0.0456	0.0436	0.0421	0.0414
		5.210	0.0454	0.0448	0.0444	0.0437	0.0425

Table 3
Absorbance data of mixtures containing DDQ (acceptor) and cloxacillin sodium at five different temperatures against the pristine acceptor solution as reference (medium: 50% (v/v) aqueous ethanol)

Acceptor	[Acceptor] ($\times 10^4$ mol dm^{-3})	[Cloxacillin sodium] ($\times 10^2$ mol dm^{-3})	Absorbance at 558 nm				
			293 K	298 K	303 K	308 K	313 K
DDQ	4.075	1.891	0.0115	0.0106	0.0105	0.0098	0.0092
		2.289	0.0120	0.0117	0.0103	0.0093	0.0089
		2.794	0.0125	0.0120	0.0117	0.0112	0.0100
		3.627	0.0143	0.0161	0.0137	0.0135	0.0122
		4.132	0.0126	0.0122	0.0121	0.0110	0.0104
		4.559	0.0129	0.0134	0.0128	0.0117	0.0110
		5.441	0.0145	0.0139	0.0133	0.0128	0.0122

Table 4
Formation constants, enthalpies and entropies of formation of the complexes of cloxacillin sodium with *o*-chloranil and DDQ: oscillator strengths and transition dipole moments of the complexes

Acceptor	Temperature (K)	Formation constant (K, mol^{-1} dm^3)	ΔH_f^0 (kJ mol^{-1})	ΔS_f^0 (J K^{-1} mol^{-1})	ε' (dm^3 mol^{-1} cm^{-1})	$10^3 \times f$	μ_{EN} (Debye)
o-Chloranil	293	111±0.6					
	298	98 ± 0.7	−9.84	5.39	117	3.01	0.60
	303	95 ± 0.8	±1.47	±4.85	±0.5	±0.03	±0.01
	308	90 ± 0.8					
	313	85 ± 0.1					
DDQ	293	140 ± 0.1					
	298	118 ± 0.2	−21.06	−30.96	38	1.50	0.40
	303	100 ± 1.0	±1.14	±3.75	±0.1	±0.02	±0.03
	308	91 ± 0.4					
	313	80 ± 0.7					

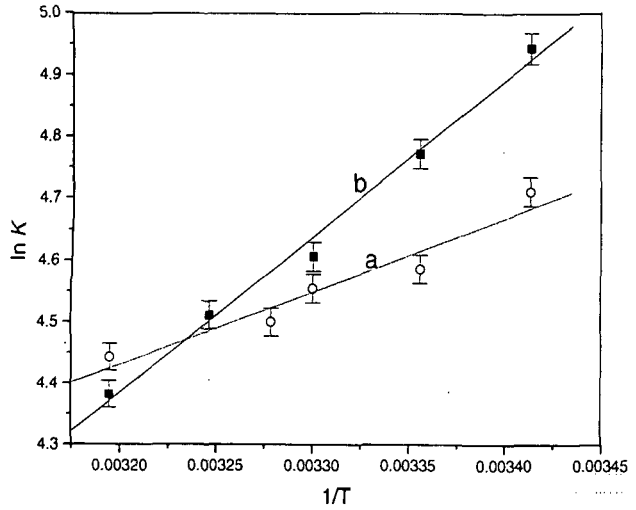

Fig. 8. van't Hoff plots for complexes of cloxacillin sodium with (a) *o*-chloranil and (b) DDQ.

In all the cases, very good linear plots according to Eq. (8) are obtained, one typical case being shown in Fig. 7. The correlation coefficients of all such plots were above 0.98. Values of K of the complexes obtained from such plots are shown in Table 4. The enthalpies of formation were obtained by using van't Hoff equation. Plots of ln K against $1/T$ are shown in Fig. 8. The following regression equations were obtained for the two complexes under study:

o-Chloranil–cloxacillin sodium complex:

$$\ln K = \frac{1184 \pm 176}{T} + (0.64 \pm 0.58) \quad (10)$$

DDQ–cloxacillin sodium complex:

$$\ln K = \frac{2533 \pm 136}{T} + (-3.72 \pm 0.45) \quad (11)$$

In each case, |correlation coefficient| is 0.98 or above. The values of enthalpy (ΔH_f^0) and entropy (ΔS_f^0) of formation obtained from such plots are given in Table 4.

3.4. Oscillator and transition dipole strengths

The oscillator strengths (f) and the transition dipole strengths (μ_{EN}) were calculated by using the equations:

$$f = 4.32 \times 10^{-9} \varepsilon_{max} \Delta\nu_{1/2} \quad (12)$$

$$\mu_{EN} = 0.0958 \left[\frac{\varepsilon_{max} \Delta\nu_{1/2}}{\nu_{1/2}} \right]^{1/2} \quad (13)$$

where ε_{max} is the maximum extinction coefficient of the band and $\Delta\nu_{1/2}$ is the half-width, i.e., width of the band at half the maximum extinction. In these two equations, ν has been expressed in cm^{-1} unit. Results are shown in Table 4.

4. Conclusions

Cloxacillin sodium forms charge transfer complexes of 1:1 stoichiometry with DDQ, *o*-chloranil, *p*-chloranil, 2,3-dichloro-1,4-napthoquinone and menadione (Vitamin K_3) in 50% (v/v) aqueous ethanol medium. From the trends in the CT absorption bands, the vertical ionization potential (I_D^v) of the drug molecule (cloxacillin sodium) has been estimated to be 7.89 eV. The enthalpies and entropies of formation of two complexes, viz. cloxacillin sodium–*o*-chloranil and cloxacillin sodium–DDQ have been determined by estimating the formation constants spectrophotometrically at five different temperatures. In the case of the drug–DDQ complex, an isosbestic point could be detected. The oscillator strengths and transition dipole moments of these complexes have also been determined. The medium used in the experiment is bio-friendly. The I_D^v of the drug molecule and the heat of complexation data may be useful in understanding the binding of drug molecules in real pharmacokinetic study. It has also been noted that the reduction of *o*-chloranil by aqueous ethanol is completely inhibited by cloxacillin sodium, a phenomenon that makes the present study of formation equilibrium possible.

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Spectroscopic and thermodynamic study of charge transfer complex formation between cloxacillin sodium and riboflavin in aqueous ethanol media of varying composition

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Abstract

Cloxacillin sodium has been shown to form a charge transfer complex of 2:1 stoichiometry with riboflavin (Vitamin B_2) in aqueous ethanol medium. The enthalpy and entropy of formation of this complex have been determined by estimating the formation constant spectrophotometrically at five different temperatures in pure water medium. Pronounced effect of dielectric constant of the medium on the magnitude of K has been observed by determining K in aqueous ethanol mixtures of varying composition. This has been rationalized in terms of ionic dissociation of the cloxacillin sodium (D^-Na^+), hydrolysis of the anion D^- and complexation of the free acid, DH with riboflavin.

Keywords: Cloxacillin sodium; Riboflavin; Ethanol

1. Introduction

The mechanism of action of a drug is largely determined by its physicochemical properties in solution. Thus, spectroscopic and thermodynamic studies on drug molecules are of great relevance in pharmacokinetics [1] and in developing analytical methods for detection and estimation of small quantities of specific drugs in meat and milk of animals administered with such drugs [2]. For the latter purpose mainly HPLC or GC techniques [3–5] are in use. On the other hand, spectroscopic and thermodynamic investigations not only serve these purposes but also lead to a measure of the strength of binding of the drug molecules to other substances present in living systems. Webb and Thompson [6] indicated much earlier that electron donor–acceptor complexes possibly have some role in binding. An important parameter in this respect is the electron donor ability of a drug molecule, which can be directly measured from its vertical ionization potential; this 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]. For this purpose, a detailed spectroscopic and thermodynamic study of CT complexation of the antibacterial drug, sodium (6*R*)-6-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido] penicillanate, which is commonly known as 'cloxacillin sodium' (structure (a), Fig. 1) has been carried out in the present work. This drug is known to bind to human serum albumin [23]. With a nitrogen-containing heterocyclic ring, it is a potential electron donor. On the other hand, riboflavin (structure (b), Fig. 1) is a Vitamin and a quinonoid ring in its molecular structure makes it a potential electron acceptor. Study of interactions of such a molecule with cloxacillin sodium is, therefore, expected to have some relevance in physical pharmacy.

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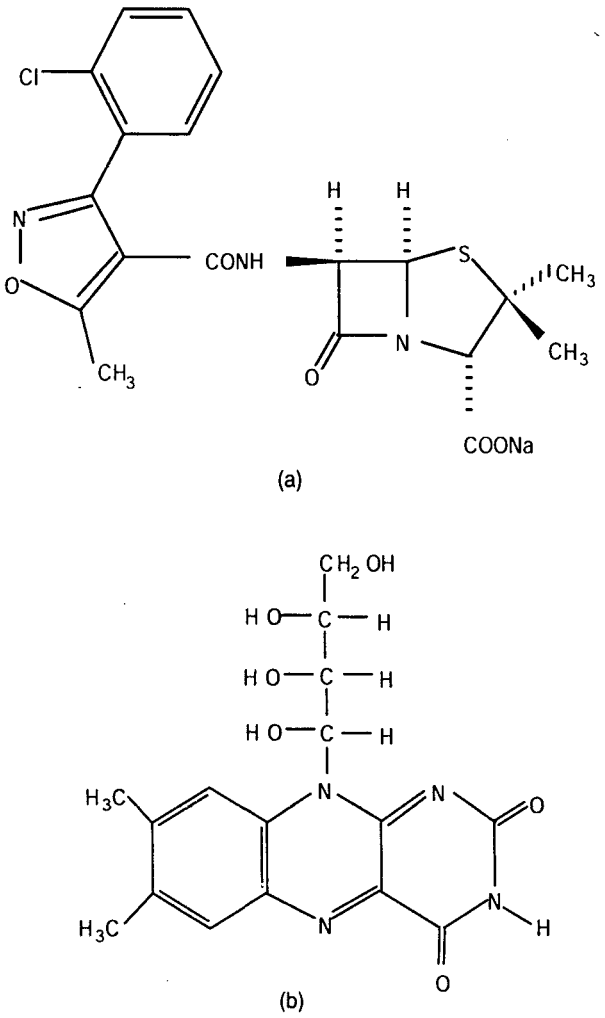

Fig. 1. Structure of (a) cloxacillin sodium and (b) riboflavin.

2. Experimental

Cloxacillin sodium and riboflavin from Sigma were used without further purification. The solvent, ethanol was purified by the method described in references [24,25] as follows: commercial grade absolute alcohol was dried over lime and distilled. The distillate was refluxed for half an hour with iodine-activated magnesium and then distilled under moisture free conditions. The entire experiment was done in pure water and in ethanol–water mixtures of varying composition. Such media are closer to biological system than non-polar solvents, which are generally used in the study of charge transfer complexes. Absorbance measurements were done on a UV 1601 PC model Shimadzu spectrophotometer fitted with a Peltier controlled thermo bath.

3. Results and discussion

3.1. Observation of CT absorption band

Fig. 2 shows the electronic absorption band of riboflavin and cloxacillin sodium in pure water. A new absorption band, which is obtained by subtracting the component absorbances from the absorption spectrum of a mixture of the two, is shown in the inset of Fig. 2. That this new band is due to the formation of a charge transfer complex is inferred from the fact that the transition energy ($h\nu$) corresponding to the peak of this band correlates very well with the CT transition energies of a number of complexes of cloxacillin sodium with a series of electron acceptors recently studied by our group [26]. In Table 1, these transition energies are summarized for a ready reference together with the $h\nu$ value which corresponds to the presently observed new absorption peak. The correlation was tested in the light of a rearranged form [27] of the Mulliken [28] equation:

Table 1
Charge transfer absorption maxima(λ_{CT}), CT transition energy ($h\nu_{CT}$) and electron affinity of the acceptors (E_A^v)

Acceptor	λ_{CT} (nm)	$h\nu_{CT}$ (eV)	E_A^v (eV)
DDQ	575	2.158	3.27
o-Chloranil	558	2.224	2.87
2,3-Dichloro-1,4 napthoquinone	445	2.789	2.38
Menadione	446	2.783	2.18
p-Chloranil	454	2.734	1.37
Riboflavin	491	2.528	1.02

$$2C_1 + h\nu_{CT} = \frac{C_1(C_1 + h\nu_{CT})}{I_D^v} + \left(\frac{C_2}{I_D^v} + I_D^v\right) \quad (1)$$

Here, I_D^v is the vertical ionization potential of the donor (cloxacillin sodium) and C_1 is given by the equation

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

where E_A^v is the vertical electron affinity of the acceptor, G_0 the sum of several energy terms (like dipole–dipole, van der Waals interaction, etc.) in the 'no-bond' state and G_1 is composed of several 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 D^+ and 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. Neglecting G_0 and taking the typical D–A distance in π-type EDA complexes to be 3.5 Å, the major part of G_1 is estimated to be $e^2/4\pi\varepsilon_0 r = 4.13$ eV. 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 a series of acceptors should be linear. In the present case, a very good linear plot with a correlation coefficient of 0.99 was obtained. The linearity is shown in Fig. 3, where the point corresponding to the presently studied complex with riboflavin is enclosed in a circle, and the other points correspond to CT transition energies of cloxacillin sodium complexes with other acceptors [26].

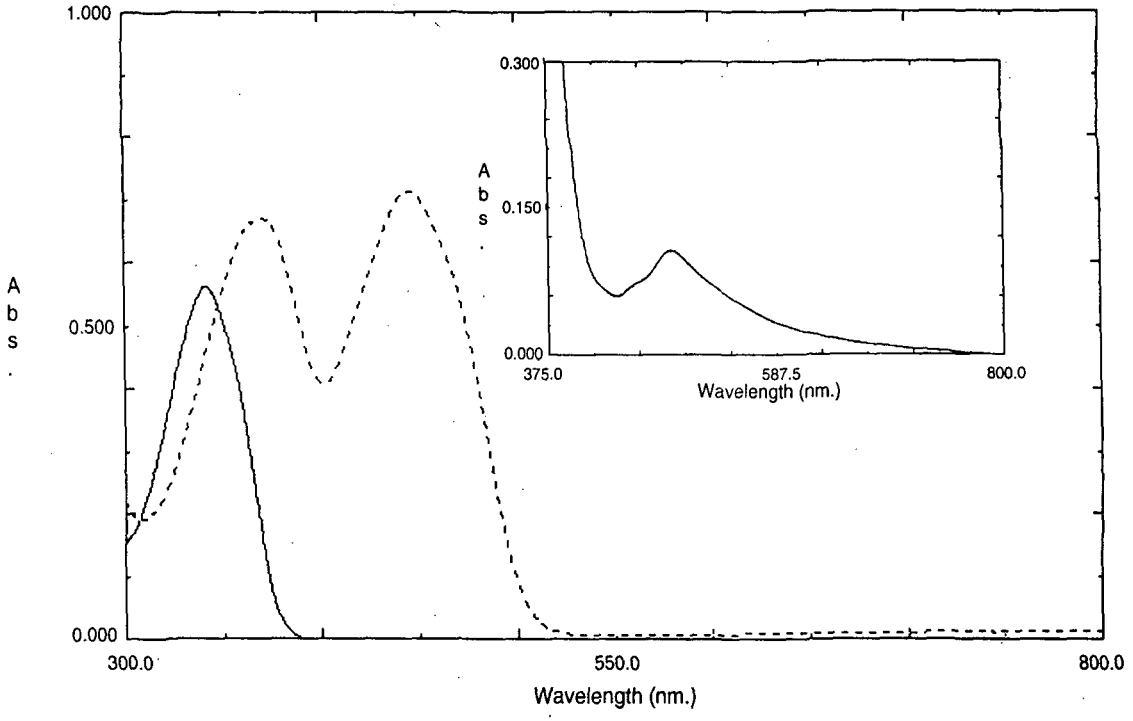

Fig. 2. Absorption spectra of: (solid curve) riboflavin (4.769×10^{-5} mol dm^{-3}) and (dotted curve) cloxacillin sodium (1.275×10^{-2} mol dm^{-3}) against the solvent water as reference. Inset: CT absorption spectra of the complex between riboflavin (4.769×10^{-5} mol dm^{-3}) and cloxacillin sodium (1.449×10^{-2} mol dm^{-3}) obtained by difference method. In the ordinate, 'Abs.' means 'absorbance'.

3.2. Spectrophotometric study of formation equilibria of the complex of cloxacillin sodium with riboflavin

Job's method of continuous variation [29] was employed to determine the stoichiometry of the complex, in pure ethanol medium. Keeping the sum of the molar concentrations of cloxacillin sodium and riboflavin fixed, the ratio of the concentrations of the two in the mixture was varied and the absorbances of the mixtures were recorded at 491 nm against the solvent as reference. The observed absorbance values were corrected for the riboflavin absorption in each experimental set. The maximum absorbance, as is well known, corresponds to the stoichiometric donor–acceptor ratio in the complex. In the present case, the stoichiometry was found to be 2:1 (cloxacillin sodium:riboflavin) as shown in Fig. 4. In both pure water and in water–ethanol mixtures, it was found that the intensity of the 491 nm absorption band (i.e., the CT band) of a mixture of cloxacillin sodium and riboflavin, measured against the pristine riboflavin solution as reference, increases systematically with increase in concentration of

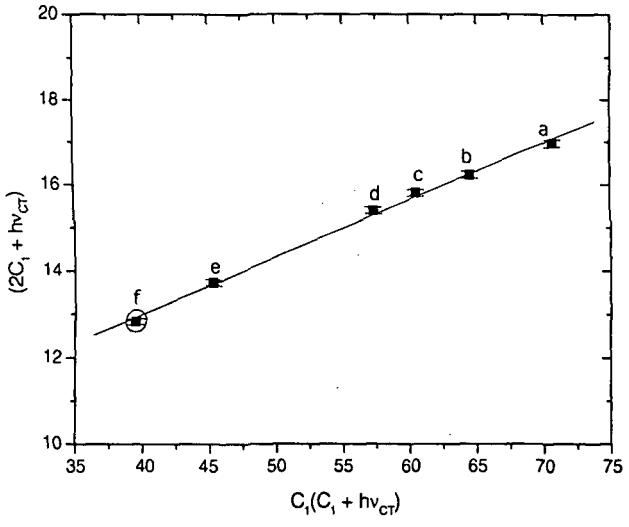

Fig. 3. Linear plot according to Eq. (1) with the transition energies of CT complexes of cloxacillin sodium with: (a) DDQ, (b) *o*-chloranil, (c) 2,3 dichloro-1,4-napthoquinone, (d) menadione, (e) *p*-chloranil and (f) riboflavin.

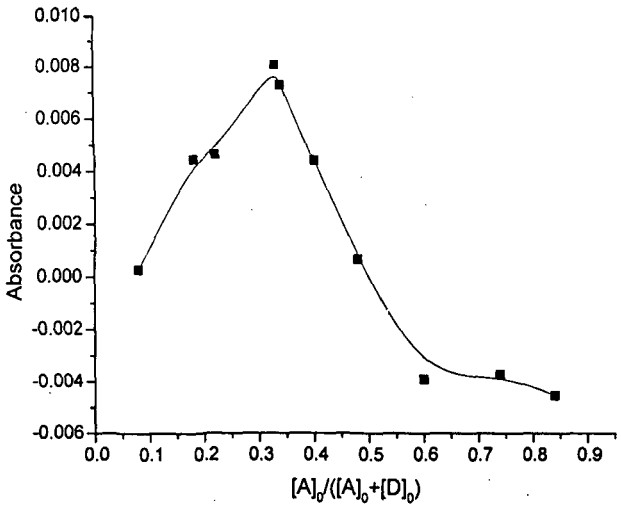

Fig. 4. Continuous variation plot for determination of stoichiometry (A = riboflavin, D = cloxacillin sodium).

Table 2
Absorbance data of mixtures containing riboflavin (acceptor) and cloxacillin sodium (donor) in aqueous ethanol media of different composition against the pristine acceptor solution as reference

$10 \times [\text{cloxacillin-Na}]$ (mol dm^{-3})				Absorbance at 491 nm at 298 K			
80% Water	60% Water	40% Water	20% Water	80% Water	60% Water	40% Water	20% Water
1.324	1.358	1.309	1.338	0.0077	0.009	0.0029	0.0033
2.1781	2.829	2.094	2.136	0.0254	0.0229	0.003	0.0132
3.620	3.543	2.941	2.721	0.033	0.0308	0.0026	0.012
4.167	4.089	4.132	3.480	0.0376	0.0409	0.005	0.0167
4.951	4.979	4.524	4.167	0.0594	0.047	0.0035	0.0188
5.658	5.518	5.245	4.853	0.0483	0.052	0.0062	0.027
6.541	6.394	6.106	5.560	0.0555	0.0544	0.009	0.0316
7.219	7.437	-	6.232	0.0593	0.0559	-	0.0305

The concentrations of riboflavin in 20, 40, 60 and 80% water media are 1.170×10^{-4}, 1.170×10^{-4}, 1.011×10^{-4} and 1.223×10^{-4} mol dm^{-3}, respectively.

cloxacillin sodium keeping that of riboflavin fixed. Experimental data are given in Tables 1 and 2. With increase in percentage of ethanol in the medium, λ_{CT} does not change but the absorbance decreases while the overall spectral feature with respect to increase in donor concentration remains the same. Formation constants of CT complexes with 1:1 (donor:acceptor) stoichiometry, are usually determined by using the Benesi–Hildebrand [30] (B–H) equation which, for cells with 1 cm optical path length, is:

$$\frac{[A]_0[D]_0}{d'} = \frac{[D]_0}{\varepsilon'} + \frac{1}{K\varepsilon'} \tag{3}$$

with

$$d' = d - d_A^0 - d_D^0 \tag{4}$$

Here, $[A]_0$ and $[D]_0$ are the initial concentrations of the acceptor and donor respectively, d the absorbance of the donor–acceptor mixture at some suitable wavelength (λ) against the solvent as reference d_A^0 and d_D^0 are the absorbances of the acceptor and donor solutions with same molar concentrations as in the mixture at the same wavelength (λ). The quantity $\varepsilon' = \varepsilon_c - \varepsilon_A - \varepsilon_D$ means the apparent molar absorptivity of the complex, ε_A and ε_D being those of the acceptor and the donor, respectively, at λ. K is the formation constant of the complex. Eq. (3) is valid under the condition $[D]_0 \gg [A]_0$. If, however, the complex is of 2:1 (donor:acceptor) stoichiometry the B–H equation requires a modification to

$$\frac{[A]_0[D]_0^2}{d'} = \frac{[D]_0^2}{\varepsilon'} + \frac{1}{K\varepsilon'} \tag{5}$$

with

$$d' = d - d_A^0 - d_D^0 \tag{6}$$

The quantity ε' now means $\varepsilon_c - \varepsilon_A - 2\varepsilon_D$.

In the present case, when experimental data (recorded at 491 nm and presented in Tables 2 and 3) were plotted according to Eq. (3) a very wide scatter was observed with a bad correlation. But when Eq. (5) was tried, an excellent linear plot was obtained at each of the temperatures studied. One such plot is shown in Fig. 5. From the slopes and intercepts of such plots, the formation constants were determined at five different temperatures. The stoichiometry of the complex is, therefore, 2:1 (cloxacillin sodium:riboflavin); this is a further support to the observation of the continuous variation experiment stated earlier.

3.3. Determination of enthalpy and entropy of formation of the riboflavin complex in pure water

The enthalpies of formation were obtained by using van't Hoff equation. Plots of $\ln K$ (in pure water) against $1/T$ are shown in Fig. 6. The following regression equation was obtained for the riboflavin–cloxacillin sodium complex under

Table 3
Absorbance data of mixtures containing riboflavin (acceptor) and cloxacillin sodium (donor) in water medium at five different temperatures against the pristine acceptor solution as reference

10^5 [riboflavin] (mol dm^{-3})	10^2 [cloxacillin sodium] (mol dm^{-3}) (in water)	Absorbance at 491 nm				
		298 K	303 K	308 K	313 K	318 K
1.170	1.506	0.0233	0.0211	0.021	0.019	0.0182
	2.794	0.0394	0.0362	0.0394	0.0372	0.0367
	4.286	0.0527	0.0509	0.0489	0.046	0.046
	4.923	0.1056	0.1034	0.0834	0.0961	0.0906
	5.777	0.0794	0.0746	0.0762	0.076	0.0732
	6.638	0.094	0.0908	0.0871	0.0877	0.0851
	7.199	0.0923	0.0869	0.0879	0.084	0.084

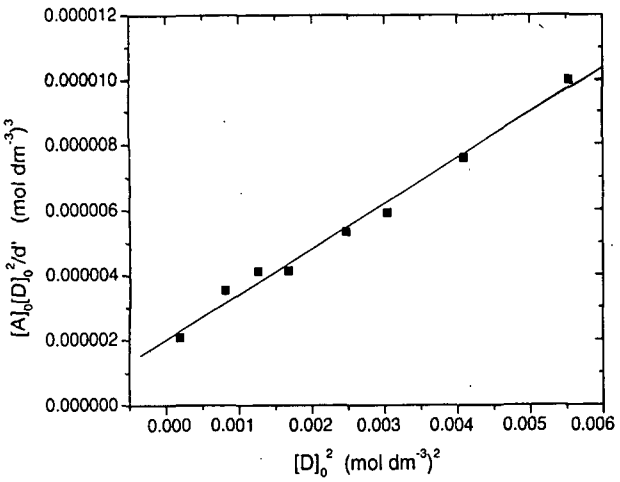

Fig. 5. Benesi–Hildebrand plots for riboflavin–cloxacillin sodium complex in water–ethanol mixture at 298 K (composition of medium: 60% of water).

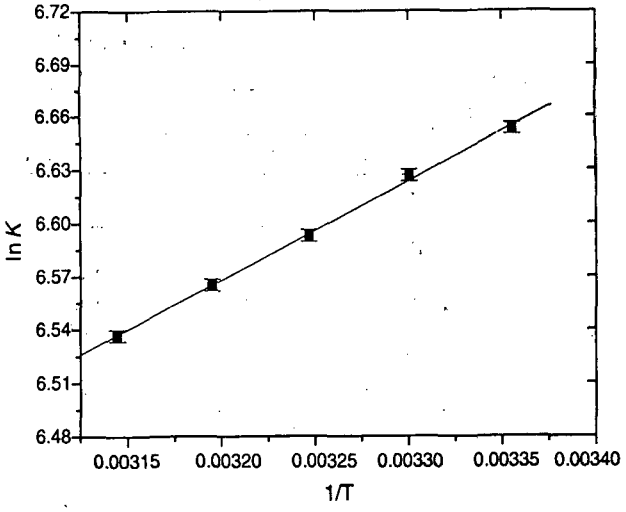

Fig. 6. van't Hoff plots for complexes of riboflavin with cloxacillin sodium in pure water medium.

study:

$$\ln K = (556.74 \pm 12.34)\frac{1}{T} + (4.79 \pm 0.04); \quad r^2 = 0.998 \tag{7}$$

The values of enthalpy (ΔH_f^0) and entropy (ΔS_f^0) of formation obtained from such plots are given in Table 4. The positive entropy change indicates that much desolvation occurs during complexation.

3.4. Effect of dielectric constant on the magnitude of K of the complex

In the present work, two ways have been adopted for studying the variation of the formation constant of the complex with dielectric constant of the medium—by varying the ethanol:water ratio [31] in the medium at constant temperature (298 K) and by using pure water as the medium and causing its dielectric constant to change by changing the temperature [32]. Results are shown in Table 4 and Fig. 7. It is found that K decreases linearly with the reciprocal of dielectric constant in both the types of study. A plausible

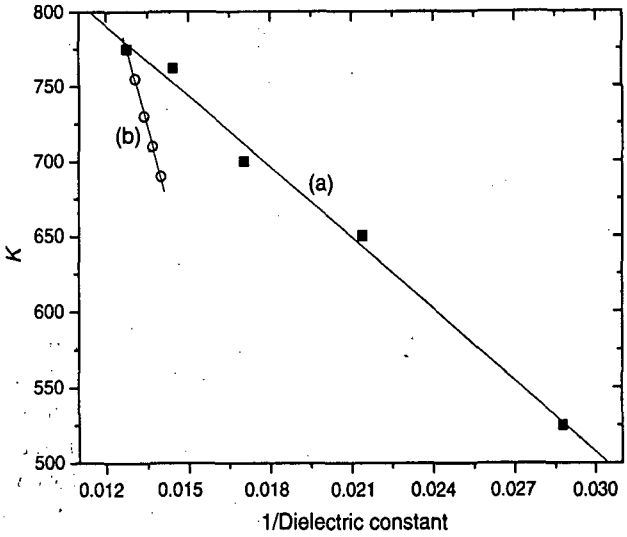

Fig. 7. Plot of formation constant against reciprocal of dielectric constant: (a) varying ethanol:water ratio and (b) varying the temperature of pure water.

Table 4
Formation constants, enthalpy and entropy of formation of the complex of cloxacillin sodium with riboflavin in pure water and in different aqueous ethanol media

Medium	Temperature (K)	Formation constant, K (mol^{-1} dm^3)^2	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 water	298	775 ± 1	78.36	910	−4.63 ± 0.10	39.78 ± 0.04
	303	755 ± 1	76.58			
	308	730 ± 2	74.85			
	313	710 ± 3	73.15			
	318	690 ± 2	71.45			
80%	298	760 ± 2	69.24	600	–	–
60%	298	690 ± 3	58.65	520	–	–
40%	298	650 ± 5	46.76	425	–	–
20%	298	525 ± 5	34.75	385	–	–

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

explanation for this is as follows. The cloxacillin sodium (which may be abbreviated as D^-Na^+) at first dissociates into ions; then the anion (D^-) undergoes hydrolysis to form the corresponding free acid (DH), which forms molecular complex with riboflavin (A):

$$D^-Na^+ \rightleftharpoons D^- + Na^+; \quad \text{dissociation constant} = K_d$$

$$D^- + H_2O \rightleftharpoons DH + OH^-; \quad \text{hydrolysis constant} = K_h$$

$$2DH + A \rightleftharpoons \text{complex}\,(DH \cdots A \cdots DH);$$

$$\text{equilibrium constant} = K'$$

Overall reaction,

$$2D^-Na^+ + 2H_2O + A \rightleftharpoons \text{complex}\,(DH \cdots A \cdots DH) + 2Na^+ + 2OH^-$$

Apparent formation constant (K) of the complex = $K_d^2 K_h^2 K'$.

With 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 increase in dielectric constant of the medium. In Fig. 7b, the linear decrease of K with reciprocal dielectric constant is much steeper than in Fig. 7a. This is because in this case decrease of K results not only from decrease of dielectric constant but also from increase of temperature.

4. Conclusion

The present study shows that cloxacillin sodium forms charge transfer complex with riboflavin in aqueous and aqueous-ethanol media, which are close to biological systems. Temperature and dielectric constant of the medium have pronounced effect on the formation constant of the complex. Since 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 pharmacy. It has further been established that the free acid form (DH) of the drug actually binds to the riboflavin molecule.

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