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

4.1 Introduction

This chapter describes the studies on CT complexation between cloxacillin sodium and riboflabin (i.e., vitamin B_2). With a nitrogen containing heterocyclic ring in its molecule, cloxacillin sodium (structure (a), Figure 4.1) is a potential electron donor. On the other hand, riboflavin (structure (b), Figure 4.1) is a vitamin and a quinonoid ring in its molecular structure makes it a potential electron acceptor. Study of CT interaction between such molecules is, therefore, expected to have some relevance in physical pharmacy. The objective of the work reported in this chapter is to see whether a charge transfer complex is formed between these two compounds and if so, to determine the formation constant (K) of the complex. Variation of K with change in ethanol-water ratio of the medium will also be studied. The importance of formation constants in pharmacology (in the context of drug-protein complexes) was pointed out long ago by Pernarowski.[1,2]

4.2 Experimental

Cloxacillin sodium and riboflavin from Sigma were used without further purification. The solvent, ethanol was purified by the method described in the

experimental section of Chapter III. 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.

4.3 Results and discussion

4.3.1 *Observation of CT absorption band*

Figure 4.2 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 Figure 4.2.That this new band is due to the formation of a charge transfer complex is inferred from the fact that the transition energy (hν) 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 in chapter III of the present thesis. In Table 4.1 these transition energies are summarized for a ready reference together with the hν value which corresponds to the absorption peak observed in the present work. The correlation was tested in the light of a rearranged form[3] of the Mulliken[4] equation:

$$2C_1+h\nu_{CT}=\frac{C_1(C_1+h\nu_{CT})}{I_D^v}+\left(\frac{C_2}{I_D^v}+I_D^v\right) \qquad \ldots(4.1)$$

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

$$C_1 = E_A^v + G_1 + G_0 \qquad \ldots(4.2)$$

where 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_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 equation (4.1) 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 equation (4.2) 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 Figure 4.3, 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.

4.3.2 *Spectrophotometric study of formation equilibria of the complex of cloxacillin sodium with riboflavin*

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 cloxacillin sodium keeping that of riboflavin fixed. Experimental data are given in Tables 4.1 and 4.2. 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[5] (B-H) equation which, for cells with 1 cm optical path length, is:

$$\frac{[A]_0[D]_0}{d'} = \frac{[D]_0}{\varepsilon'} + \frac{1}{K\varepsilon'} \qquad \ldots(4.3)$$

with $$d' = d - d_A^{\,0} - d_D^{\,0} \qquad \ldots (4.4)$$

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 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. Equation (4.3) is valid under the condition $[D]_0 >> [A]_0$. If, however, the complex is of 2 : 1 (donor : acceptor) stoichiometry the B-H equation requires a modification to

$$\frac{[A]_0[D]_0^{\,2}}{d'} = \frac{[D]_0^{\,2}}{\varepsilon'} + \frac{1}{K\varepsilon'} \qquad \ldots(4.5)$$

with $d' = d - d_A^0 - d_D^0$ ...(4.6)

The quantity ε' now means ε_c - ε_A - $2\varepsilon_D$.

In the present case, when experimental data (Tables 4.2 and 4.3) were plotted according to equation (4.3) a very wide scatter was observed with a bad correlation. But when equation (4.5) was tried, an excellent linear plot was obtained at each of the temperatures studied. One such plot is shown in Figure 4.4. 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).

4.3.3 *Determination of enthalpy and entropy of formation of the riboflavin .1 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 Figure 4.5. The following regression equation was obtained for the riboflavin-cloxacillin sodium complex under study:

$$\ln K = (556.74 \pm 12.34)\frac{1}{T} + (4.79 \pm 0.04); \quad r^2 = 0.998 \qquad \ldots(4.7)$$

The values of enthalpy (ΔH_f^0) and entropy (ΔS_f^0) of formation obtained from such plots are given in Table 4.4. The positive entropy change indicates that much desolvation occurs during complexation.

4.3.4 *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[6] in the medium at constant temperature

(298K) and by using pure water as the medium and causing its dielectric constant to change by changing the temperature.[7] Results are shown in Table 4.4 and Figure 4.6. It is found that K decreases linearly with the reciprocal of dielectric constant in both the types of study. A plausible explanation for this is as follows. 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):

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

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

$$2DH + A \rightleftharpoons \text{complex (DH...A...DH) ; equilibrium constant} = K'$$

Overall reaction,

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

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 Figure 4.6b, the linear decrease of K with reciprocal dielectric constant is much steeper than in Figure 4.6a. This is because in this case decrease of K results not only from decrease of dielectric constant but also from increase of temperature.

4.4 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.

References

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6. G. Åkerlöf, *J. Am. Chem. Soc.*,**1932,** *54,* 4125
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Table 4.1

Charge transfer absorption maxima(λ_{CT}), CT transition energy ($h\nu_{CT}$), electron affinity of the acceptors (E_A^{ν}), vertical ionisation potential of cloxacillin sodium (I_D^{ν}) and degee of charge transfer (α).

Acceptor	λ_{CT}, nm	$h\nu_{CT}$, eV	E_A^{ν},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

Table 4.2

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. 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.

$10 \times$ [D] (mol dm^{-3})				Absorbance at 491 nm at 298K			
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	0.0031	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	0.0051	0.0187
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	0.0217
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	----	0.0303

Table 4.3

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.

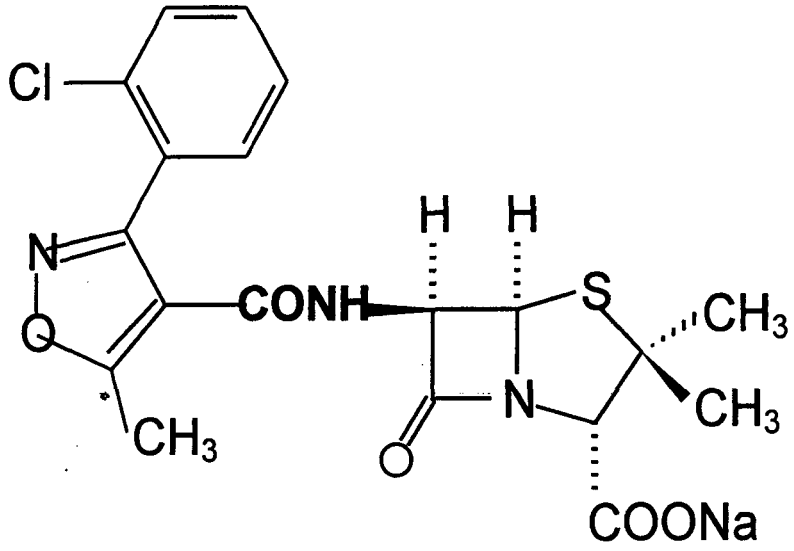
10^5[riboflavin] (mol dm^{-3})	10^2 [cloxacillin sodium] (mol dm^{-3}) [in water]	Absorbance at 491 nm				
		298K	303K	308K	313K	318K
1.170	1.506	0.0233	0.0211	0.021	0.019	0.0182
	2.794	0.0394	0.0362	0.0394	0.0372	0.0367
	4.286	0.0527	0.0509	0.0489	0.046	0.046
	4.923	0.1056	0.1034	0.0834	0.0961	0.0906
	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

Table 4.4

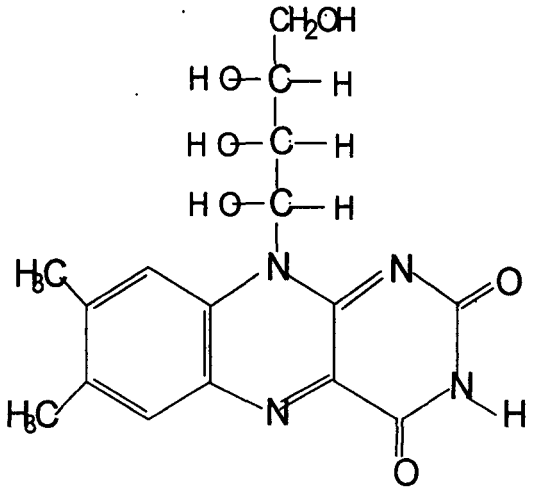
Formation constants, enthalpy and entropy of formation of the complex of cloxacillin sodium with riboflavin in pure water and in different aqueous ethanol media. Compositions of the aqueous ethanol mixtures are expressed in % of ethanol (v/v).

Medium	Temperature (K)	Formation constant (*K*) (mol^{-1} dm^3)	Dielectric constant (*d*)	ε' (dm^3 mol^{-1} cm^{-1})
Pure water	298	775 ± 1	78.36	910
	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

$\Delta H_f^0 = -4.63 \pm 0.10$ kJ mol^{-1}, $\Delta S_f^0 = 39.78 \pm 0.04$ J K^{-1} mol^{-1}



(a)



(b)

Figure 4.1 Structure of (a) cloxacillin sodium and (b) riboflavin

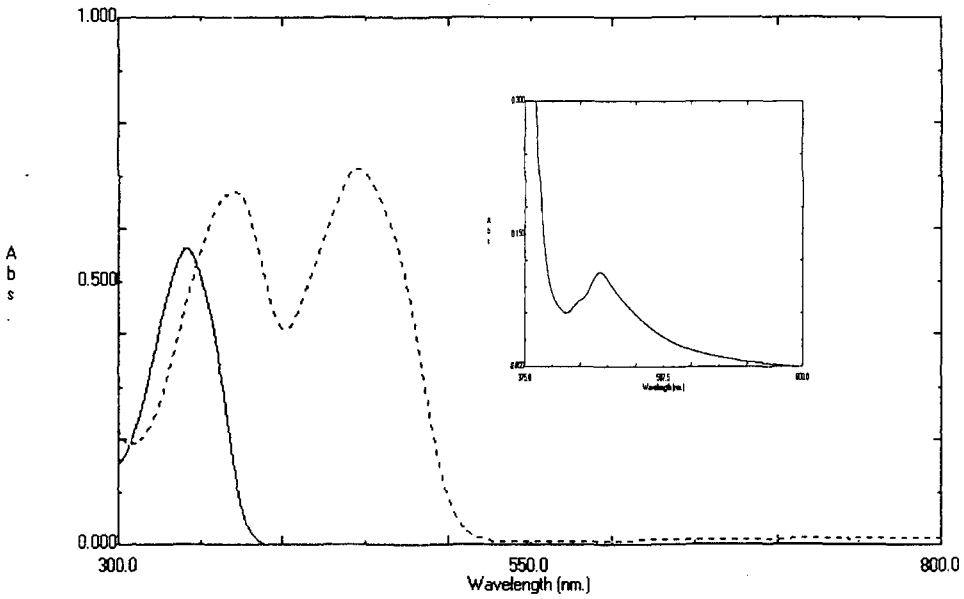


Figure 4.2

Absorption spectra of (a) riboflavin (4.769×10^{-5} mol dm^{-3}) and (b) 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'.

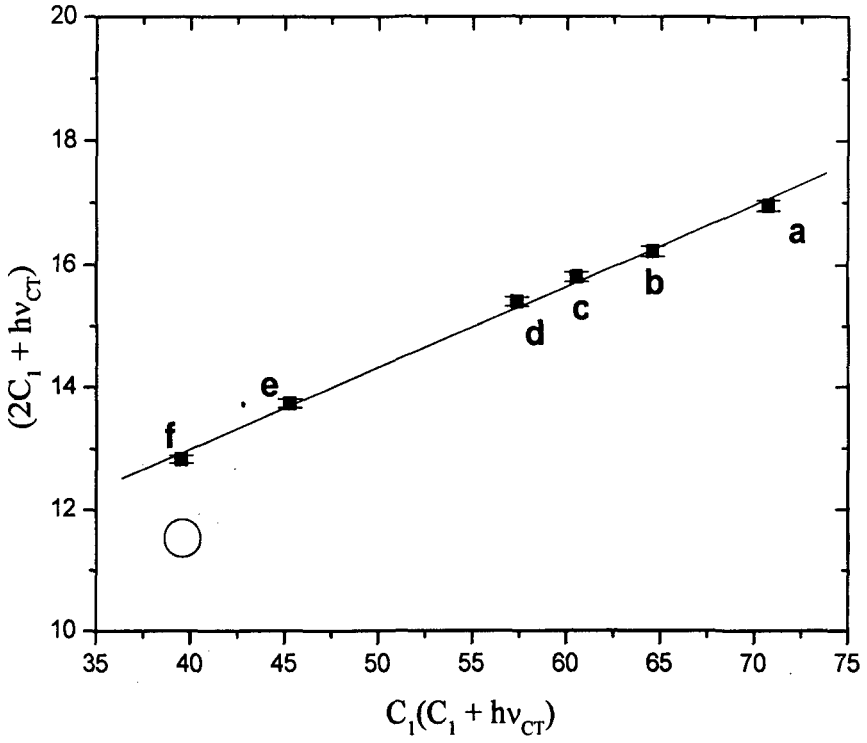


Figure 4.3

Linear plot according to equation (4.1) 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.

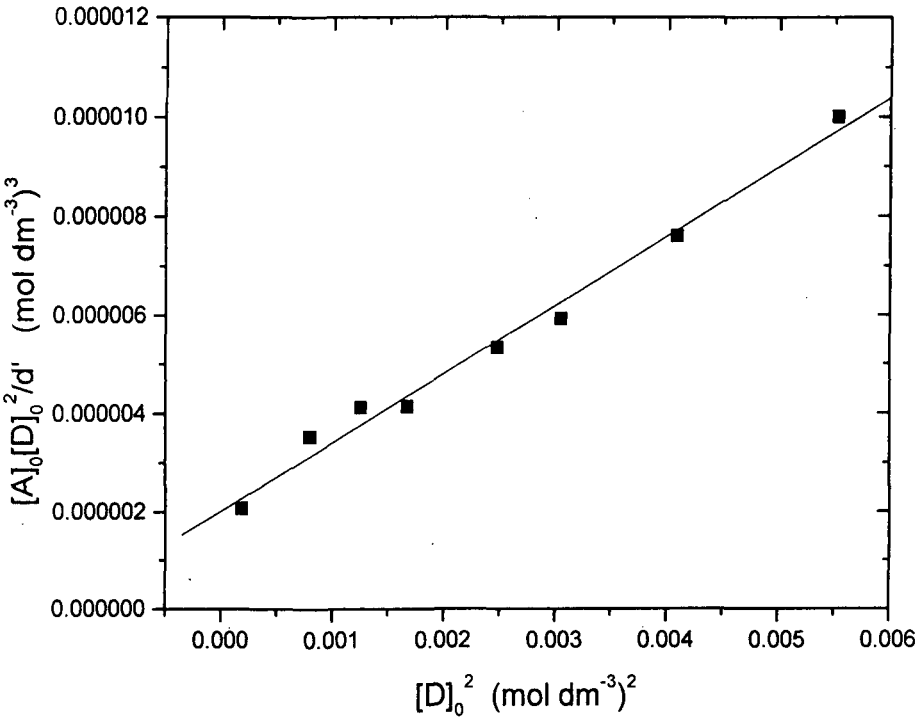


Figure 4.4

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

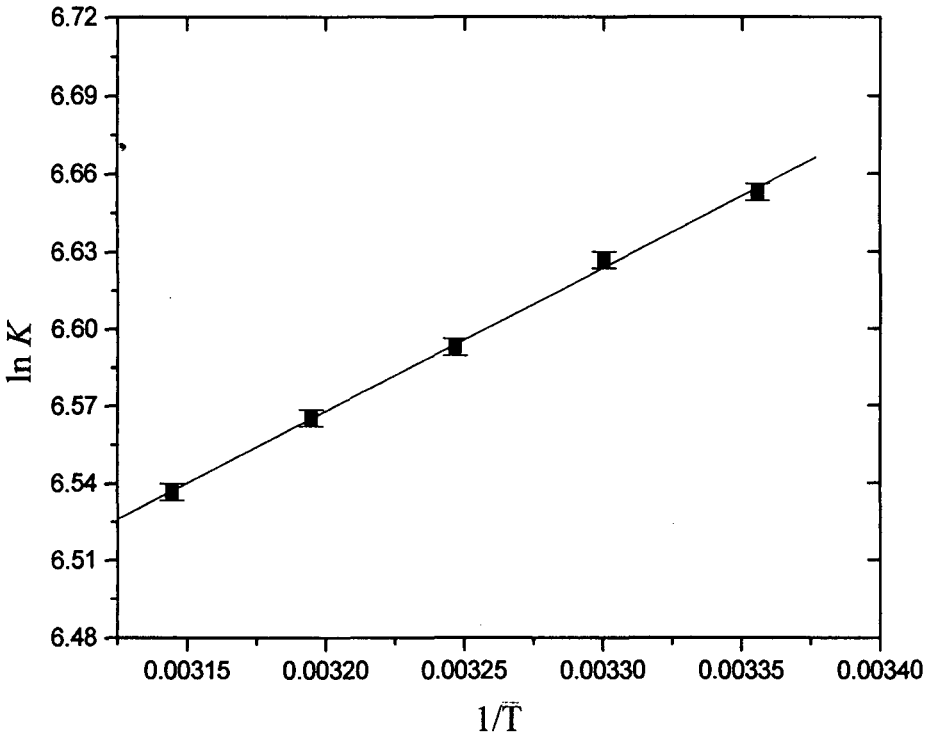


Figure 4.5

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

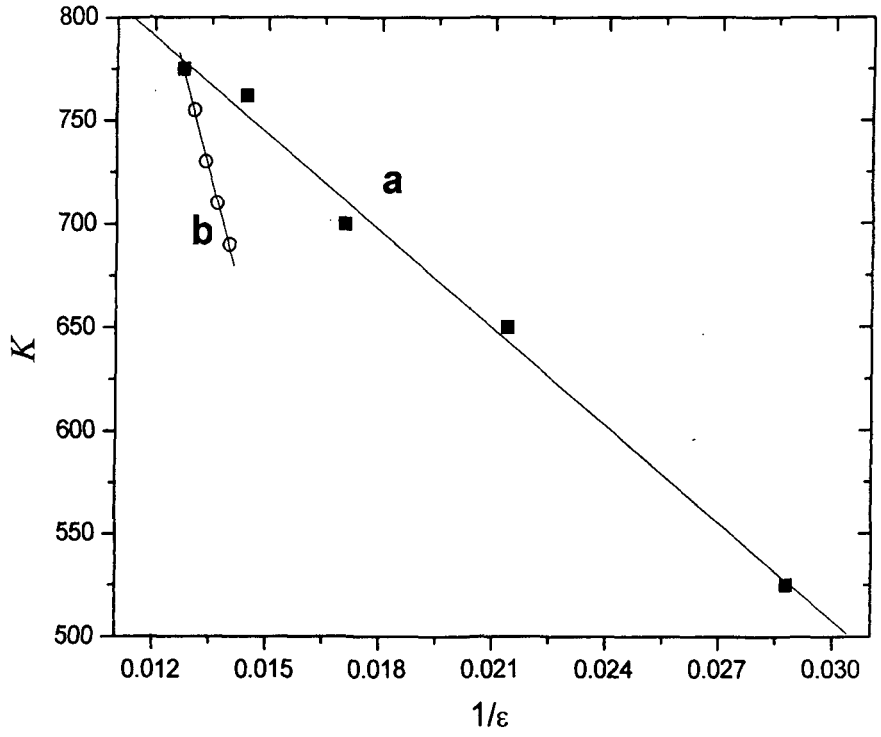


Figure 4.6

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