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

3.1 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 the 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' (structure **1,** Fig. 3.1). 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 molecule (vitamin K_3). The study, therefore, is expected to have some relevance in pharmacology.

3.2 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-dicyano1,4-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 references[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%(by volume) 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 thermo bath.

3.3 Results and discussion

3.3.1 Observation of CT bands

In the present study CT bands were observed in case of complexes of cloxacillin sodium (Fig. 3.1) 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. 3.2. To obtain of 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) to (v) separately was recorded against the pristine acceptor solution as 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. 3.3. In the wavelength range shown in Fig.3.2 the absoption due to free cloxacillin does not interfere. Two important points must be mentioned here: (a) *o*-chloranil undergoes slow reduction by aqueous ethanol as was observed 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 dichhloro-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) to (iv) were collected from literature[21,24,25] and that of the fifth one has been recently determined.[26] These E_A^v values correlate well with the presently observed CT transition energies ($h\nu_{CT}$, Table 3.1) in accordance with the Mulliken[27] equation :

$$h\nu_{CT} = I_D^v - C_1 + \frac{C_2}{I_D^v - C_1} \qquad \ldots.(3.1)$$

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

$$C_1 = E_A^v + G_1 + G_0 \qquad \ldots.(3.2)$$

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_1 is the sum 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 (3.1) is related to the resonance energy of interaction between the 'no-bond' and 'dative' states. A rearrangement of equation (3.1) yields

$$2C_1 + h\nu_{CT} = \frac{C_1(C_1 + h\nu_{CT})}{I_D^v} + \left(\frac{C_2}{I_D^v} + I_D^v\right) \qquad \ldots(3.3)$$

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_1 is estimated to be $e^2/4\pi\varepsilon_0 r$ = 4.13 eV. Using these values C_1 is obtained from equation (3.2) 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 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 3.1, the plot is fairly linear (Fig.3.4) and the linear regression equation is

$$2C_1 + h\nu_{CT} = (0.127 \pm 0.004)[C_1(C_1 + h\nu_{CT})] + (8.055 \pm 0.260) \qquad \ldots(3.4)$$

with a correlation coefficient of 0.99. From the slope, I_D^v of cloxacillin sodium is found to be 7.89 eV.

3.3.2 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 ψ (D^o, A^o) and ionic ψ (D^+ , A^-) states,

$$\psi_g=\sqrt{1-\alpha}\ \psi(D^o,A^o)+\sqrt{\alpha}\ \psi(D^+,A^-) \qquad ...(3.5)$$

$$\psi_{ex}=\sqrt{1-\alpha}\ \psi(D^+,A^-)-\sqrt{\alpha}\ \psi(D^o,A^o) \qquad ... (3.6)$$

where α is the degree of charge transfer. The function ψ (D^+ , A^-) differs from ψ (D^o, A^o) by the promotion of an electron from donor to the acceptor. α is given[27,28] by

$$\alpha=\frac{C_2}{2(I_D^v-E_A^v+C_1)^2+C_2} \qquad ... (3.7)$$

The values of α, calculated by using equation (3.7) and shown in Table 3.1 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 3.1).

3.3.3 Spectrophotometric study of formation equilibria of the complexes of cloxacillin sodium with o-chloranil and DDQ

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 (Figures 3.5 & 3.6). This indicates complex formation. An isosbestic point at λ = 425 nm was obtained in case of the cloxacillin sodium-DDQ complex (inset, Figure 3.6). Stoichiometry and

formation constants of the complexes were determined by using Benesi – Hildebrand[29] equation for cells with 1 cm optical path length :

$$\frac{[A]_0[D]_0}{d'} = \frac{[D]_0}{\varepsilon'} + \frac{1}{K\varepsilon'} \qquad \ldots (3.8)$$

with $$d' = d - d_A^0 - d_d^0 \qquad \ldots (3.9)$$

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, 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. Equation. (8) is valid[29] under the condition $[D]_0 >> [A]_0$ for 1:1 donor-acceptor complexes. Absorbance data were taken at 558 and 575 nm respectively for the *o*-chloranil and DDQ complexes because 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 3.2 and 3.3. In all the cases very good linear plots according to equation (3.8) are obtained, one typical case being shown in Figure 3.7. 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 3.4. The enthalpies of formation were obtained by using van't Hoff equation. Plots of ln K against 1/T are shown in Figure 3.8. The following regression equations were obtained for the two complexes under study:

o-chloranil –cloxacillin sodium complex:

$$\ln K = \frac{1184 \pm 176}{T} + (0.64 \pm 0.58) \quad ...(3.10)$$

DDQ –cloxacillin sodium complex:

$$\ln K = \frac{2533 \pm 136}{T} + (-3.72 \pm 0.45) \quad ..(3.11)$$

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 3.5.

3.3.4 Oscillator and transition dipole strengths

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

$$f = 4.32 \times 10^{-9} \varepsilon_{max} \Delta \nu_{1/2} \quad ...(3.12)$$

$$\mu_{EN} = 0.0958 [\varepsilon_{max} \Delta \nu_{1/2} / \nu]^{1/2} \quad ...(3.13)$$

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 3.5.

3.4 Conclusion

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 e.V. 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.

Table 3.1

Charge transfer absorption maxima(λ_{CT}), CT transition energy ($h\nu_{CT}$), electron affinity of the acceptors (E_A^v), vertical ionisation 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-naptho-quinone	445	2.789	2.38	3.71	
Menadione	446	2.783	2.18	3.56	
p-chloranil	454	2.734	1.37	3.21	

Table 3.2

Absorbance data of mixtures containing *o*-chloranil (acceptor) and cloxacillin sodium (D) at five different temperatures against the pristine acceptor solution as reference. Concentration of acceptor in mixture = 4.553×10^{-4} mol dm^{-3}

$10^2[D]_0$ (mol dm^{-3})	Absorbance at 558 nm				
	293K	298K	303K	308K	313K
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
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	0.0426

Table 3.3

Absorbance data of mixtures containing DDQ (acceptor) and cloxacillin sodium (D) at five different temperatures against the pristine acceptor solution as reference (medium: 50% v/v aqueous ethanol). Concentration of acceptor in mixture = 4.075×10^{-4} mol dm^{-3}

$10^2[D]_0$ (mol dm^{-3})	Absorbance at 575 nm				
	293K	298K	303K	308K	313K
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 3.4 .

Formation constants of the complexes of cloxacillin sodium with *o*-chloranil and DDQ at five different temperatures

Acceptor	Temperature (K)	Formation Constant (*K*) mol^{-1} dm^3
o-chloranil	293	111 ± 0.6
	298	98 ± 0.7
	303	95 ± 0.8
	308	90 ± 0.8
	313	85 ± 0.1
DDQ	293	140 ± 0.1
	298	118 ± 0.2
	303	100 ± 1.0
	308	91 ± 0.4
	313	80 ± 0.7

Table 3.5:

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.

ΔH_f^0(kJ mol^{-1})	ΔS_f^0 ($JK^{-1} mol^{-1}$)	ε'(dm^3 mol^{-1} cm^{-1})	$10^3 \times f$	μ_{EN} (Debye)
-9.84 ± 1.47	5.39 ± 4.85	117 ± 0.5	3.01 ± 0.03	0.60 ± 0.01
-21.06 ± 1.14	-30.96 ± 3.75	38± 0.1	1.50 ±0.02	0.40 ± 0.03

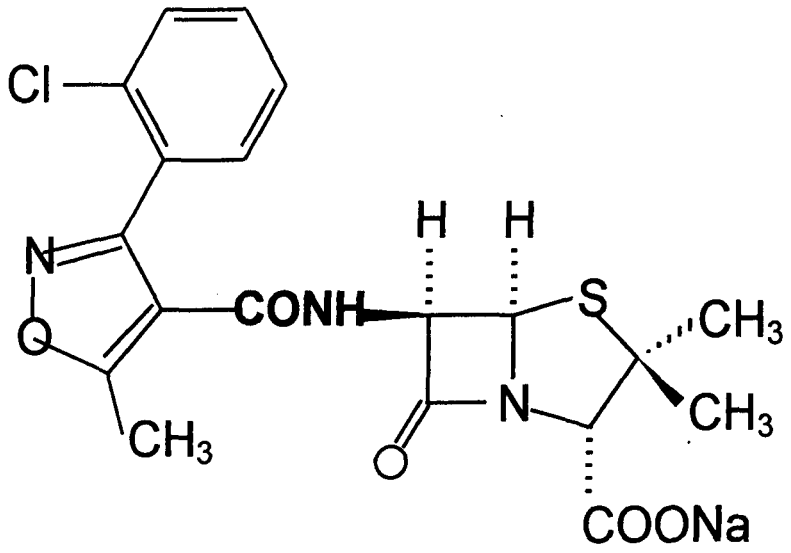


Figure 3.1

Structure of Sodium (6*R*)-6-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido] penicillanate (i.e., cloxacillin sodium)

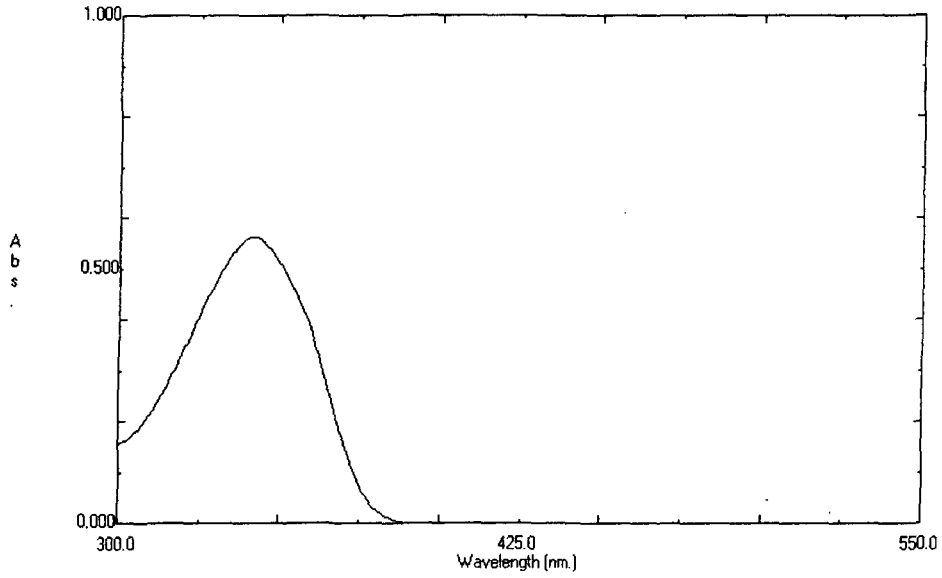
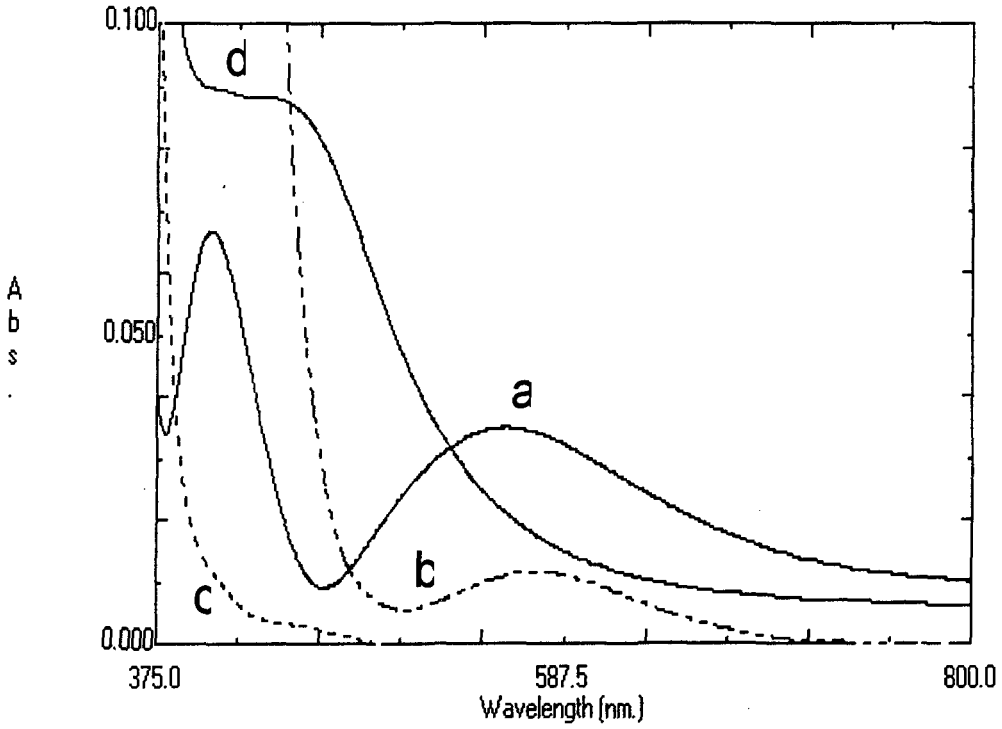


Figure 3.2

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

Figure 3.3



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}) (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'.

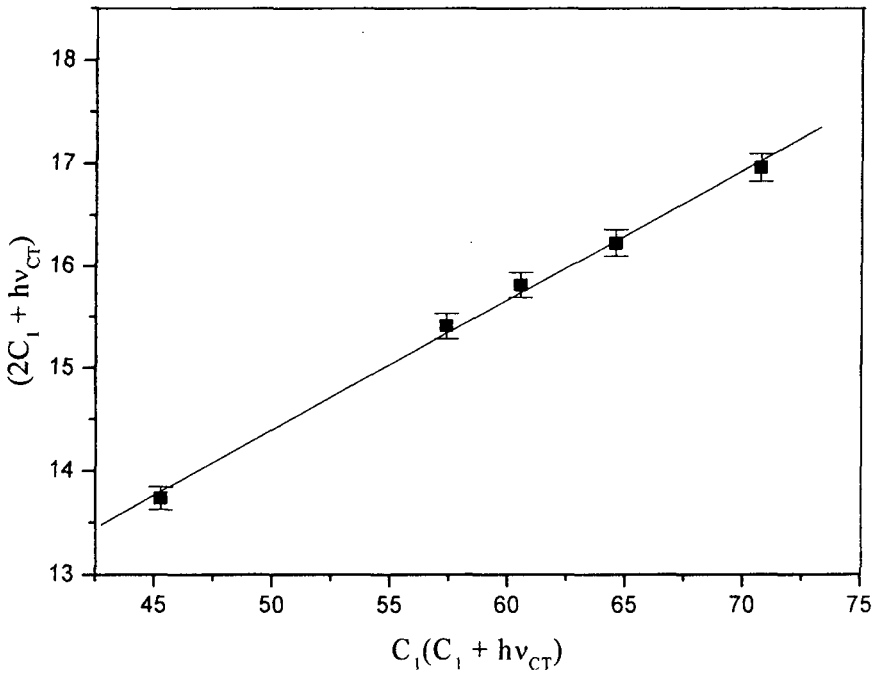


Figure 3.4

Plot for determination of vertical ionization potential of cloxacillin sodium according to equation (3.4)

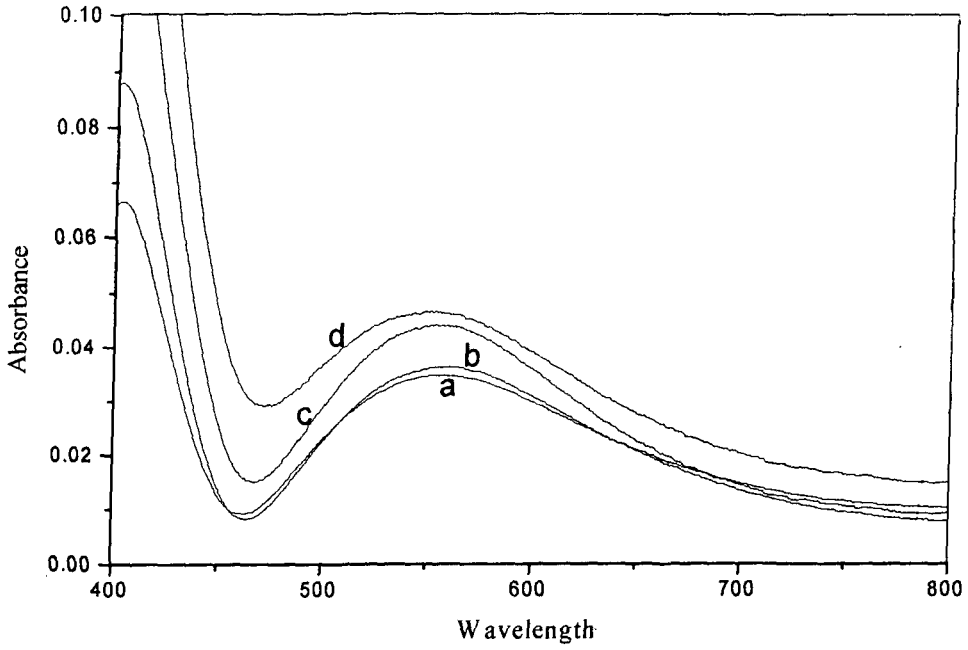


Figure 3.5

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}, (b)1.849×10^{-2}, (c) 3.901×10^{-2} and (d) 4.783×10^{-2} mol dm^{-3} progressively upwords.

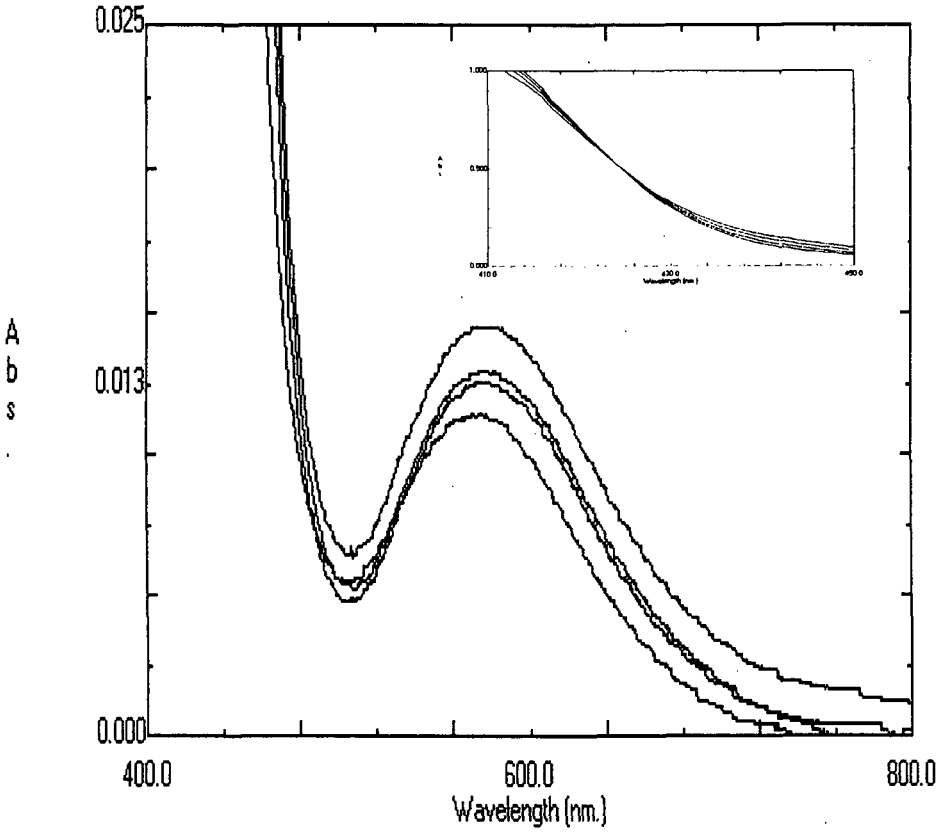


Figure 3.6

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}, 5.441×10^{-2}, mol dm^{-3} progressively upwords) all taken against pristine acceptor solution as reference. Inset: 410 to 450 nm wavelength range and 0.0 to 1.0 absorbance expanded to show the isosbestic point.

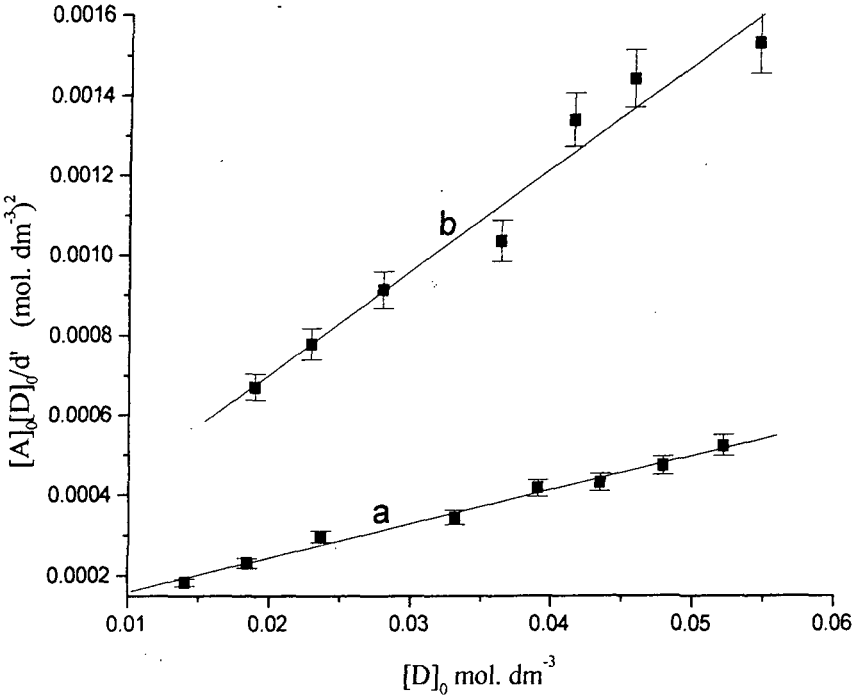


Figure 3.7

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

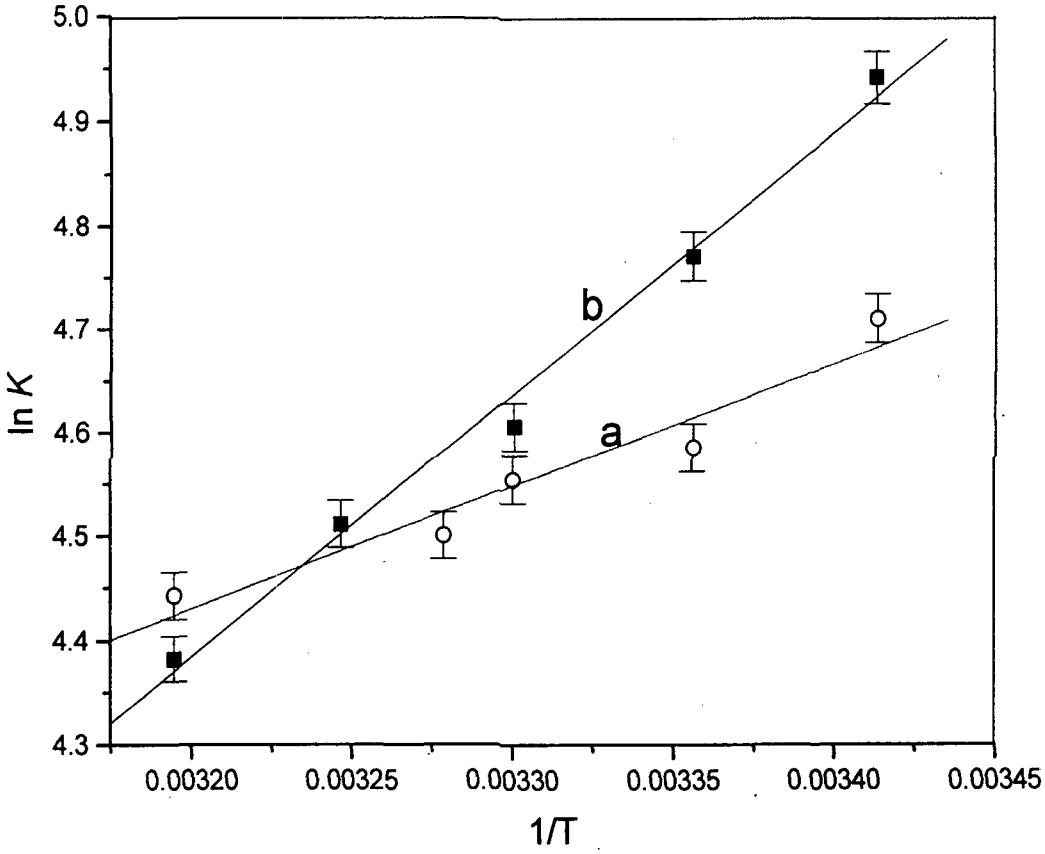


Figure 3.8

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

References

1. P. Taboada, M. Gutierrez-Pichel, S. Barbosa, D. Attwood and V. Mosquera, *Phys. Chem. Chem. Phys.*, **2003,** *5*, 703

2. S. Croubels, K. Baert, J. D. Busser and P. D. Backer, *Analyst*, **1998,** *123*, 2733

3. M. C. Carson, P. –S. Chu and J. Bredow, in Verterinary Drug Resues: Food Safety, Ed. W. A. Moats and M. B. Medina, ACS symposium series 636, Washington, USA, **1996**

4. R. Dietrich, E. Usleber and E. Märtlbauer, *Analyst*, **1998,** *123*, 2749

5. K. D. Wasch, L. Okerman, S. Croubels, H. D. Brabander, J. V. Hoof and P. D. Backer, *Analyst*, **1998,** *123*, 2737

6. N. E. Webb, C. C. Thompson, *J. Pharm. Sci.*, **1978,** 67, 165

7. A. Saha, A. K. Mukherjee, *Spectrochim. Acta A,* **2004**, *60*, 1731

8. M. J. Frost, *Phys. Chem. Chem. Phys.*, **2003,** *5*, 3169

9. Y-B. Wang, Z. Lin, *J.Am.Chem.Soc.*, **2003,** *125*, 6072

10. B, Rosenberg, J. F. Camiscoli, *J. Chem. Phys.*, **1961,** *35*, 982

11. A. Epstein, B. S. Wildi, *J. Chem. Phys.*, **1960,** *32*, 324

12. M. A. Slifkin, *Biochim. Biophys. Acta*, **1965,** *109*, 617

13. C. A. Langhoff, C. J. Fritchie, *Chem. Comm.*, **1970,** 20

14. C. A. Bear, J. M. Waters, T. M. Waters, *J. Chem. Soc., Chem. Commun.*, 1970, 702

15. D. M. Guldi, C. Luo, M. Prato, A. Troisi, F. Zerbetto, M. Scheloske, E. Dietel, W. Bauer, A. Hirsch, *J. Am. Chem. Soc.*, **2001,** *123*, 9166

16. D. M. Guldi, C. Luo, M. Prato, E. Dietel, W. Bauer, A. Hirsch, *J. Chem. Soc., Chem. Commun*, **2000**, 373

17. H. Nakayama, H. Ohno, Y. Okahata, *J. Chem. Soc., Chem. Commun*, **2001,** 2300

18. Y. W. Chien, *Novel Drug Delivery Systems,* 2 nd edition, New York, Marcel Dekker, Inc., **1992**

19. A. Kydoneus, Treatise on Controlled Drug Delivery, New York, Marcel Dekker, Inc., **1992**

20. J. C. Mc Elnay, in Encyclopedia of Pharmaceutical Technology, (J. Swarbrick and J. C.Boylan, eds) New York, Marcel Dekker, Inc., vol. 2, **1990**

21. R. Foster, Organic charge transfer complexes, Academic Press, **1969**

22. A. Weissberger, Technique of Organic Chemistry, Interscience, New York, **1969**

23. A. I. Vogel, A Text Book of Physical Organic Chemistry, Longmans, New York **1978**

24. M. E. Peover, *J. chem. Soc*, **1962,** 540

25. G. Briegleb, *Angew. Chem.*, **1964,** 76, 326

26. A.Saha, S. K. Nayak, S. Chattopadhyay, A. K. Mukherjee, *J. Phys.Chem.* B, **2003,** *107*, 11889

27. R. S. Mulliken, *J. Am. Chem. Soc.*, **1952,** *74*, 811

28. M. Ichida, T. Sohda, A. Nakamura, *Chem. Phys. Lett.*, **1999,** 310, 373

29. H.A.Benesi and J. H. Hildebrand, *J. Am. Chem.Soc.*, **1949,** 71, 2703