

Chapter V: Spectroscopic and thermodynamic study of charge transfer interaction between vitamin B₆ and *p*-chloranil in aqueous ethanol mixtures of varying composition

5.1 Introduction

Vitamin B₆ (pyridoxine hydrochloride) is needed in body system for the processing of amino acids, and to control the synthesis of dopamine, malatonin, and serotonin. It is essential to the regulation of mental processes and mood. Vitamin B₆ increases the absorption of magnesium. Coenzyme Q10, when used with iron and vitamin B₆, is reported to help prevent the progression of Alzheimers for up to two and one half years¹. Epidemiological research suggests that male smokers with higher serum levels of pyridoxine may have a lower risk of lung cancer. Owing to many such vital roles²⁻¹² of Vitamin B₆ in different biological processes, its deficiency or over doses create various disorder in living systems. It is well known² that drugs and enzymes most often act through the formation of molecular complexes as intermediates. Such complexes are mainly results of weak molecular interactions which are important in other physicochemical aspects also.¹³⁻¹⁵ Spectroscopic and thermodynamic study of its complexation with elrc tron acceptors should be useful for detection and quantification of Vitamin B₆. The objective of the work reported in this chapter is to show that vitamin B₆ acts as an electron

donor by studying its charge transfer (CT) complexes with a series of quinones which are well known electron acceptors.¹⁶ Among the quinones under study, menadione (2-methyl 1,4-naphthoquinone) is another bio-molecule, e.g., vitamin K₃. The present study, therefore, amounts to interaction between two vitamin molecules and is thus expected to have some pharmacological importance.

5.2 Materials and Method

Vitamin B₆ from Sigma were used without further purification. *p*-chloranil (i.e., 2,3,5,6-tetrachloro 1,4-benzoquinone) from Fluka, Swizerland, *p*-bromanil (i.e., 2,3,5,6-tetrabromo 1,4-benzoquinone) and *o*-chloranil (i.e., 3,4,5,6-tetrachloro-1,2-benzoquinone) from Sigma were further purified by sublimation just before use. Menadione (i.e., 2-methyl-1,4-naphthoquinone), and 2,3-dichloro 1,4-naphthoquinone from Sigma were used without further purification. Commercial grade absolute ethanol 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 mixture of varying composition, but not in pure ethanol or in pure water. This is because vitamin B₆ is soluble in water, while *p*-chloranil and the other electron acceptors are insoluble in water but soluble in organic solvents. All the spectral measurements were done on a UV

1601 PC model Shimadzu Spectrophotometer fitted with a Peltier controlled thermo bath.

5.3 Results and discussions

5.3.1 Observation of CT bands

In the present study CT bands were observed in case of complexes of vitamin B₆ with (i) *p*-chloranil (ii) *o*-chloranil (iii) *p*-bromanil (iv) menadione and (v) 2,3-dichloro 1,4-naphthoquinone. To obtain CT bands, spectrum of each of the solutions (in water-ethanol mixture) containing the vitamin B₆ as donor and an electron acceptor (from the above list) was recorded against the pristine acceptor solution as reference. It is a common experience that CT bands in solution are detected only by taking high concentration of donor compared to that of the acceptor. In the present case [vitamin B₆] $\approx 10^{-2}$ mol. dm⁻³ and concentrations of the acceptors were $\approx 10^{-4}$ mol. dm⁻³. The absorption spectrum of *p*-chloranil–vitamin B₆ mixture in 75% ethanol-water medium against the solution of *p*-chloranil at the same concentration as that in the mixture as reference is shown in Figure 5.1. The absorption bands of pristine acceptor (*p*-chloranil) solution is also shown in the same figure and the spectrum of vitamin B₆ is shown separately in Figure 5.2. Vitamin B₆ does not absorb above 360 nm but the two compounds have overlapping absorption band below 360 nm. Hence a difference spectrum (absorbance of mixture minus the sum of the individual absorbances of donor and acceptor, with concentrations as present in the mixture, over the wavelength range scanned) was constructed to get the CT absorption peak. The vertical electron affinities (E_A^v) of the

acceptors under study are collected from literature¹⁶⁻¹⁹ and they correlate well with the presently observed CT transition energies ($h\nu_{CT}$, Table 5.1) in accordance with the Mulliken²⁰ equation :

$$h\nu_{CT} = I_D^v - C_1 + \frac{C_2}{I_D^v - C_1} \quad \dots(5.1)$$

where I_D^v is the vertical ionisation potential of the donor (vitamin B₆) and C_1 is given by the equation

$$C_1 = E_A^v + G_1 + G_0 \quad \dots(5.2)$$

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 (5.1) is related to the resonance energy of interaction between the 'no-bond' and 'dative' states. A rearrangement of equation (5.2) yields

$$2C_1 + h\nu_{CT} = \frac{C_1(C_1 + h\nu_{CT})}{I_D^v} + \frac{C_2}{I_D^v} + I_D^v \quad \dots(5.3)$$

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 $e^2/4\pi\epsilon_0 r = 4.13$ eV. Thus C_1

is obtained from equation (5.2) 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 value of I_D^v of the donor can be obtained. In the present case, with the experimental CT transition energies shown in Table 5.1, the plot is fairly linear (Figure 5.3) and the linear regression equation is

$$2C_I + h\nu_{CT} = (0.1231 \pm 0.0008)[C_I(C_I + h\nu_{CT})] + (8.4950 \pm 0.049);$$

$$r^2 = 0.9604 \quad \dots(5.4)$$

The value of I_D^v of vitamin B₆ obtained from this correlation is 8.12 eV.

5.3.2 Determination of formation constants (*K*)

Figure 5.4 shows the absorption spectra of mixtures containing the same concentration of *p*-chloranil and varying concentrations of vitamin B₆ at 298K against the solvent as reference. It is found that the intensity of the overall spectrum of the mixture changes from that of pure *p*-chloranil. The systematic perturbation of intensities of the *p*-chloranil absorption bands by addition of vitamin B₆ are indicative of complex formation. The stoichiometry and formation constants (*K*) were determined at 375 nm by using the Benesi-Hildebrand²¹ equation for cells with 1 cm optical path length. To eliminate the absorbance due to the uncomplexed components, the observed absorbance data were corrected by subtracting the absorbance due to the latter as follows:

If a 1 : 1 complex is formed according to the equilibrium,



the observed absorbance (with a cell of 1 cm path length) is

$$\begin{aligned} d &= \varepsilon_A([A]_0 - c) + \varepsilon_D([D]_0 - c) + \varepsilon_c c \\ &= d_A^0 + d_D^0 + (\varepsilon_c - \varepsilon_A - \varepsilon_D) c \end{aligned} \quad \dots(5.6)$$

or
$$d' = d - d_A^0 - d_D^0 \quad \dots(5.7)$$

Here $[A]_0$ and $[D]_0$ are the initial concentrations of A and D respectively in the mixture before complexation, d is the absorbance of the mixture at some suitable wavelength in the perturbed absorption band of *p*-chloranil measured against the solvent as reference; d_A^0 and d_D^0 are the absorbances of A and D in solution with the same molar concentrations as in the mixture and at the wavelength of measurement. The quantity $\varepsilon' = \varepsilon_c - \varepsilon_A - \varepsilon_D$ means an effective molar absorptivity, ε_c is the molar absorptivity of the complex, and ε_A and ε_D are those of A and D respectively at the wavelength of measurement. When the present experimental data were plotted according to equation (5.8), which is the B-H equation²¹ for 1:1 stoichiometry, a very good linear correlation was observed. One typical B-H plot is shown in Figure 5.5. The absorbance data for mixtures of *p*-chloranil and vitamin B₆ at four different temperatures are given in Table 5.2.

$$\frac{[A]_0[D]_0}{d'} = \frac{[D]_0}{\varepsilon'} + \frac{1}{K\varepsilon'} \quad \dots(5.8)$$

Here, K is the formation constant of the complex. Equation (8) is valid under the condition $[D]_0 \gg [A]_0$. The formation constant K was calculated at four different temperatures for the complex of vitamin B₆ and *p*-chloranil in 75% ethanol-water mixture at 375 nm because at this wavelength the variation of

absorbance with gradual addition of vitamin B₆ was notable and the latter does not absorb appreciably at the said wavelength. Results are given in Table 5.3.

5.3.3 Enthalpies ΔH_0^f and entropies of formation ΔS_0^f of the complexes

The enthalpy ΔH_0^f and entropy of formation ΔS_0^f of the complex have been determined by using equation. (5.9). As measured, these terms will represent the net change in enthalpy and entropy for the solvated species.

$$\ln K \equiv -\Delta H_0^f / RT + \text{constant} \quad \dots (5.9)$$

Plot of $\ln K$ vs $1/T$ for the complex of vitamin B₆ with *p*-chloranil is linear and have positive slopes. The plot is shown in Figure 5.6 of supplementary document.

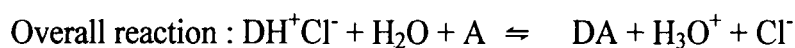
The linear regression relation according to equation (9) is as follows.

$$\begin{aligned} \ln K &= (1263.27 \pm 25.21) / RT - (0.78 \pm 0.08); \\ \text{corrln. coeff.} &= 0.99 \quad \dots (5.10) \end{aligned}$$

The positive slope in each case indicates that the complexation process is exothermic and thus driven by favorable enthalpy changes. The values of ΔH_0^f and ΔS_0^f of the complex are listed in Table 5.3.

5.3.4 Effect of dielectric constant on the magnitude of formation constant of the complex

The ethanol-water ratio in the medium at constant temperature (303 K) was varied to prepare media²² of varying dielectric constant (ϵ) and variation of formation constant of the complex with change in ϵ of the medium was studied. Results are shown in Figure 5.7. It is found that K decreases linearly with $1/\epsilon$. A plausible explanation for this is as follows: The hydrochloride form of vitamin B₆ (abbreviated as DH^+Cl^-) at first dissociates into ions; then the cation (DH^+) undergoes hydrolysis to form the corresponding free base which forms a molecular complex with *p*-chloranil (A):



The quantity $K_d K_h K'$ is the apparent formation constant (K) of the complex. With increasing dielectric constant of the medium at a particular temperature, the values of K' and K_h do not change appreciably, but K_d undergoes a substantial increase. This explains the observed increase in the apparent K with an increase in dielectric constant (more specifically, increase in water content)

of the medium. A similar explanation was put forward²³ in the study of complexation of doxycycline hydrochloride in aqueous ethanol medium.

5.4 Conclusion

The present study shows that vitamin B₆ forms a charge transfer complex of 1:1 stoichiometry with *p*-chloranil in aqueous ethanol medium. The enthalpy and entropy of complexation are, in magnitude, like those of typical weak molecular complexes. The formation constant decreases regularly with increase in dielectric constant (water content) of the medium. This has been explained by assuming that vitamin B₆ (pyridoxine hydrochloride) ionizes and then hydrolyses and the free pyridoxine base acts as the donor in forming the CT complex. From the trends in CT absorption bands of complexes of vitamin B₆ with a series of electron acceptors the vertical ionization potential of vitamin B₆ has been estimated to be 8.12eV.

References

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Table 5.1. Charge transfer absorption maxima (λ_{CT}), CT transition energy ($h\nu_{CT}$), electron affinity of the acceptors (E_A^V) and vertical ionization potential (I_D^V) of vitamin B₆

Acceptors	E_A^V , (eV)	λ_{CT} , (nm)	$h\nu_{CT}$, (eV)	I_D^V , (eV)
<i>p</i> -chloranil	1.37	326	3.803	8.12
<i>o</i> -chloranil	2.87	330	3.757	
2,3- dichloro 1,4- naphthoquinone	2.38	352	3.522	
menadione	2.18	348	3.563	
<i>p</i> -bromanil	1.41	335	3.701	

Table 5.2. Absorbance data of mixtures containing vitamin B₆ (donor) and *p*-chloranil (acceptor) in water ethanol media of different compositions against solvent as reference

Concentration of *p*-chloranil: i) $5.96205 \times 10^{-4} \text{ mol dm}^{-3}$ (at 66%), ii) $5.28455 \times 10^{-4} \text{ mol dm}^{-3}$ (at 69%), iii) $6.30081 \times 10^{-4} \text{ mol dm}^{-3}$ (at 82%) and iv) $4.7686 \times 10^{-4} \text{ mol dm}^{-3}$ (at 90%)

$10^2 [\text{vitamin B}_6], (\text{mol} \cdot \text{dm}^{-3})$				Absorbance at 375 nm and 303 K			
66%	69%	82%	90%	66%	69%	82%	90%
1.614	1.663	1.541	1.456	0.1302	0.1301	0.1880	0.1353
2.464	2.124	2.112	2.209	0.1637	0.1323	0.1871	0.1383
3.010	2.573	2.439	2.876	0.1635	0.1320	0.1858	0.1436
3.532	3.083	2.985	3.398	0.1664	0.1327	0.1871	0.1440
4.332	3.519	3.447	3.896	0.1691	0.1330	0.1902	0.1438
4.660	3.968	3.701	4.430	0.1726	0.1328	0.1934	0.1468
4.976	4.490	4.430	4.757	0.1732	0.1340	0.1914	0.1485
5.655	5.049	4.757	5.40	0.1740	0.1339	0.1918	0.1487

Table 5.3. Absorbance data of mixtures containing vitamin B₆ (donor) and *p*-chloranil (acceptor) (at 7.85907×10^{-4} mol dm⁻³) in 75% ethanol-water medium at four different temperatures against solvent as reference.

10 ² [vitamin B ₆] (mol.dm ⁻³)	absorbance at 375nm			
	298 K	303 K	308 K	313 K
1.917	0.2181	0.2195	0.2202	0.2197
2.282	0.2162	0.2147	0.2157	0.2152
2.731	0.2190	0.2191	0.2200	0.2191
3.495	0.2181	0.2188	0.2200	0.2186
3.677	0.2189	0.2183	0.2201	0.2194
4.296	0.2194	0.2186	0.2206	0.2198
4.636	0.2201	0.2203	0.2209	0.2200
4.976	0.2196	0.2197	0.2210	0.2206

Table 5.4. Formation constants of the complex of vitamin B₆ and *p*-chloranil in ethanol-water media of varying composition and their dielectric constants at 303K. Enthalpy and entropy of the complex are obtained by temperature variation experiment in 75% ethanol-water medium.

Medium	Formation constant(K) (mol ⁻¹ .dm ³)	Dielectric constant (ϵ)	ΔH_f^0 (kJ mol ⁻¹)	ΔS_f^0 JK ⁻¹ mol ⁻¹
75%	141	35.03	10.50 ± 0.21	6.50 ± .003
66%	156	39.64		
69%	120	37.86		
82%	95	31.25		
90%	41	27.70		

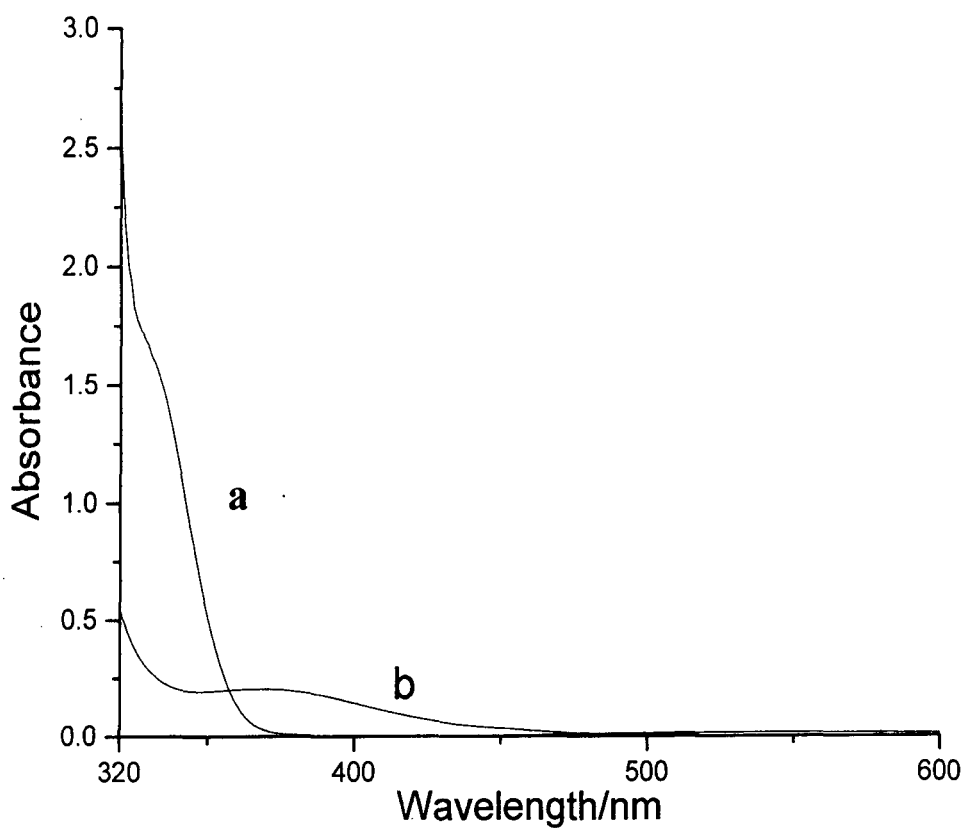


Figure 5.1

Absorption Spectra of (a) *p*-chloranil ($7.8591 \times 10^{-4} \text{ mol.dm}^{-3}$) solution against solvent and (b) the mixture of vitamin B₆ ($2.7306 \times 10^{-2} \text{ mol.dm}^{-3}$) and *p*-chloranil ($7.8591 \times 10^{-4} \text{ mol.dm}^{-3}$) against *p*-chloranil solution of the same concentration as that in the mixture.

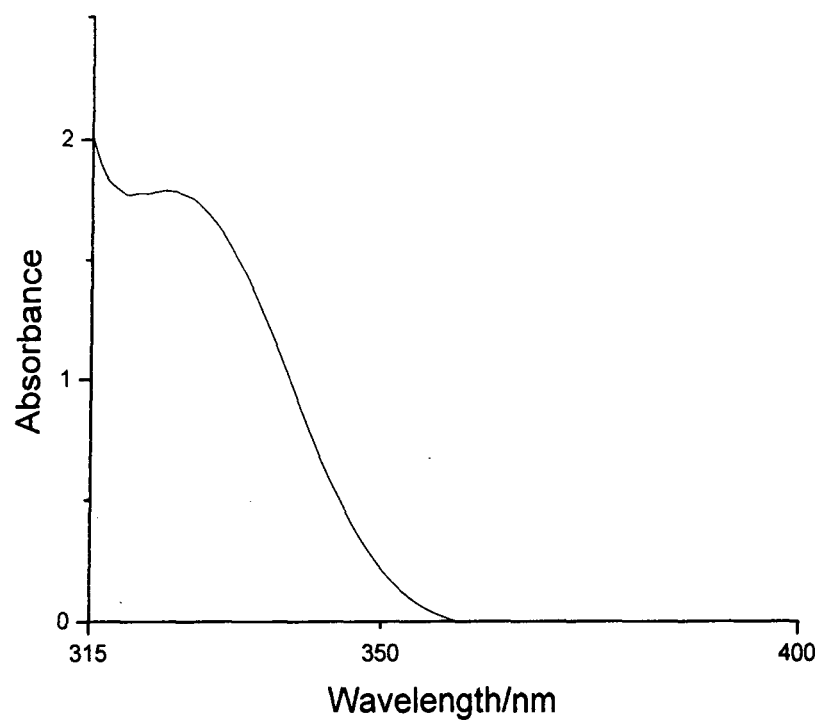


Figure 5.2

Absorption spectrum of vitamin B₆ (3.4951×10^{-3} mol. dm⁻³) against solvent as reference.

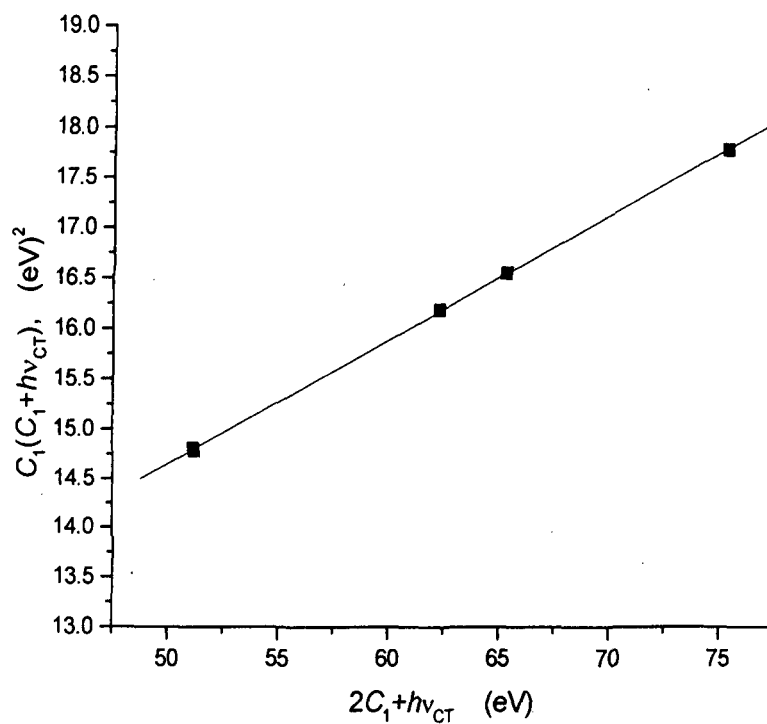


Figure 5.3

Plot for determination of vertical ionization potential of vitamin B₆ according to equation (5.4).

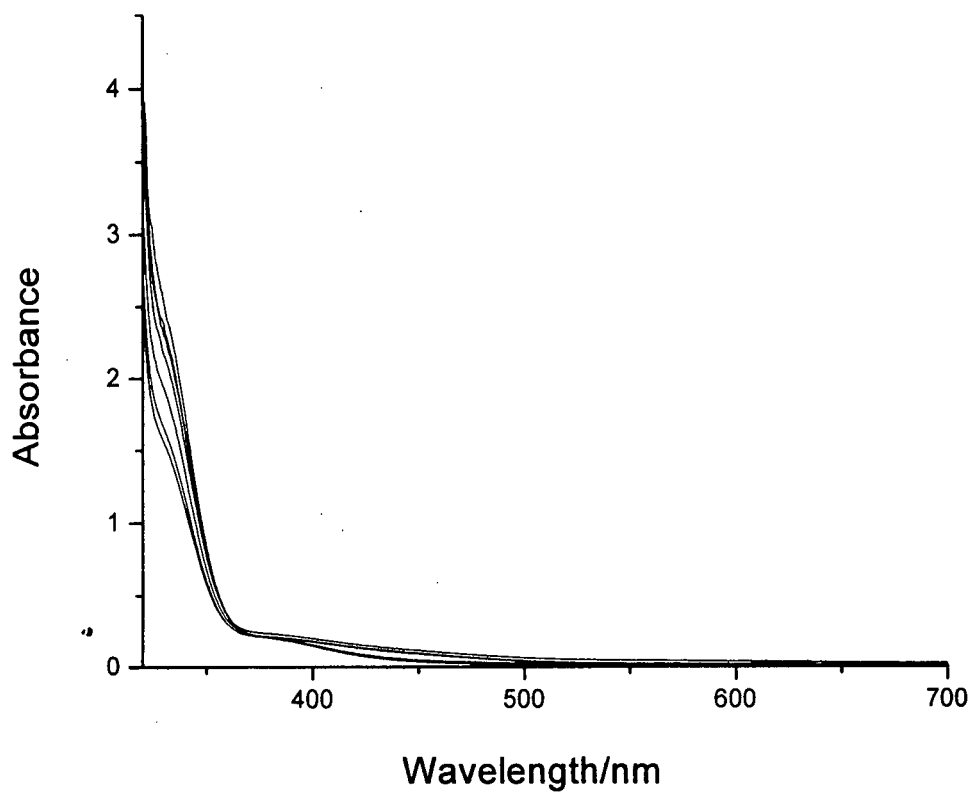


Figure 5.4

Variation of the intensity of the absorption spectrum of p-chloranil on addition of vitamin B₆

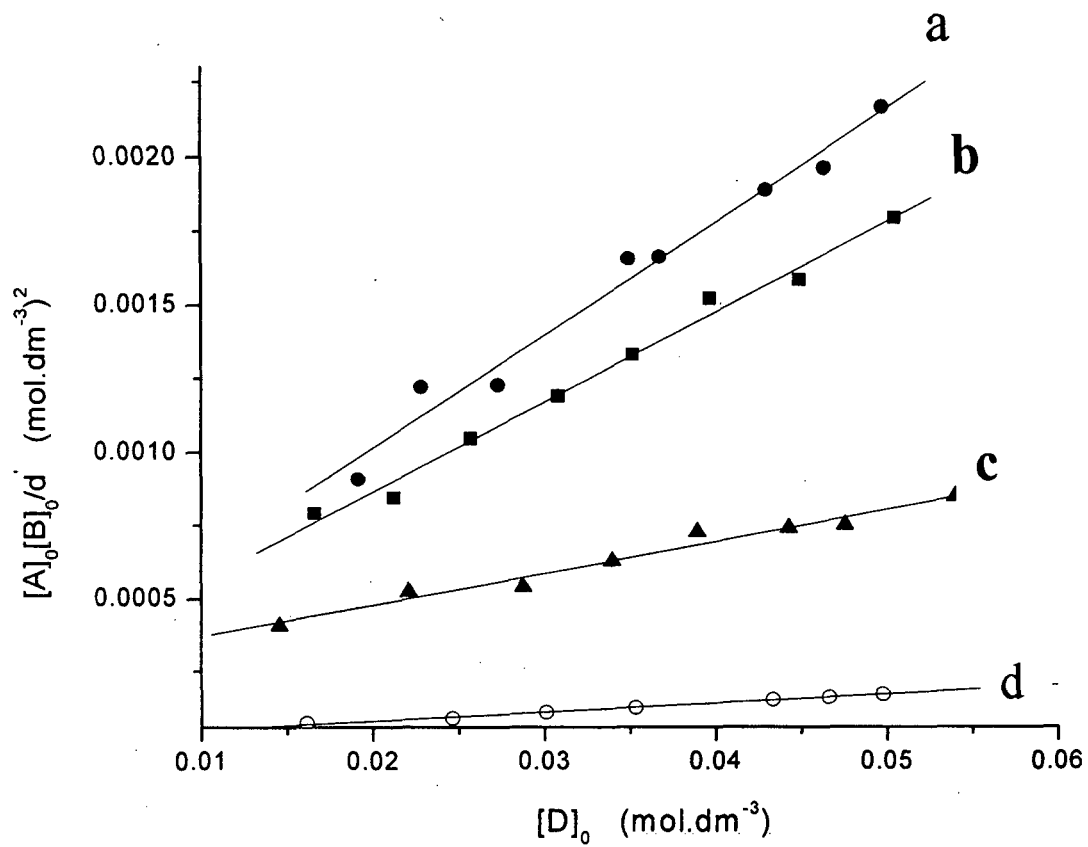


Figure 5.5

Benesi-Hildebrand plots for vitamin B₆ - *p*-chloranil complexes in different ethanol-water media (a) 75% (b) 82% (c) 69% and (d) 66%

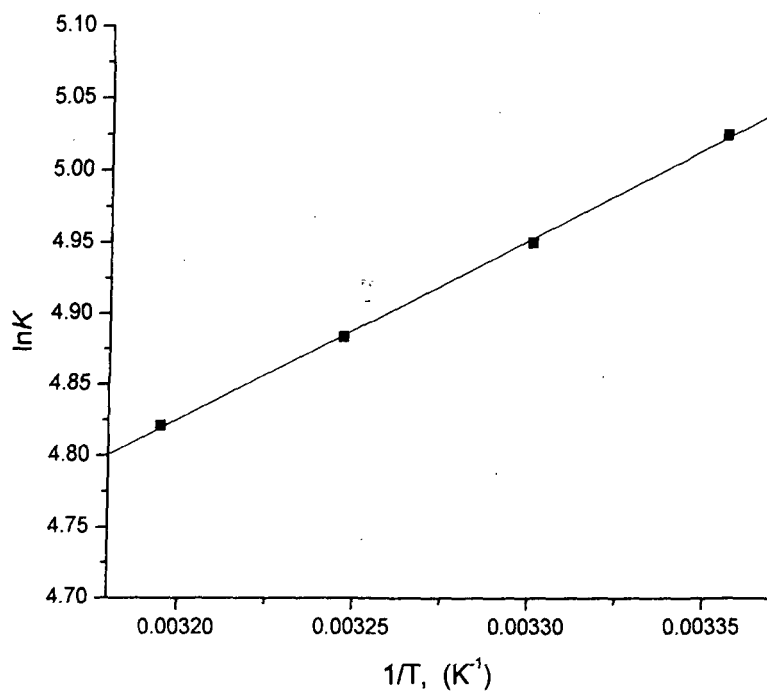


Figure 5.6

van't Hoff plots for complexes of vitamin B₆ and p-chloranil in
75% ethanol- water mixture

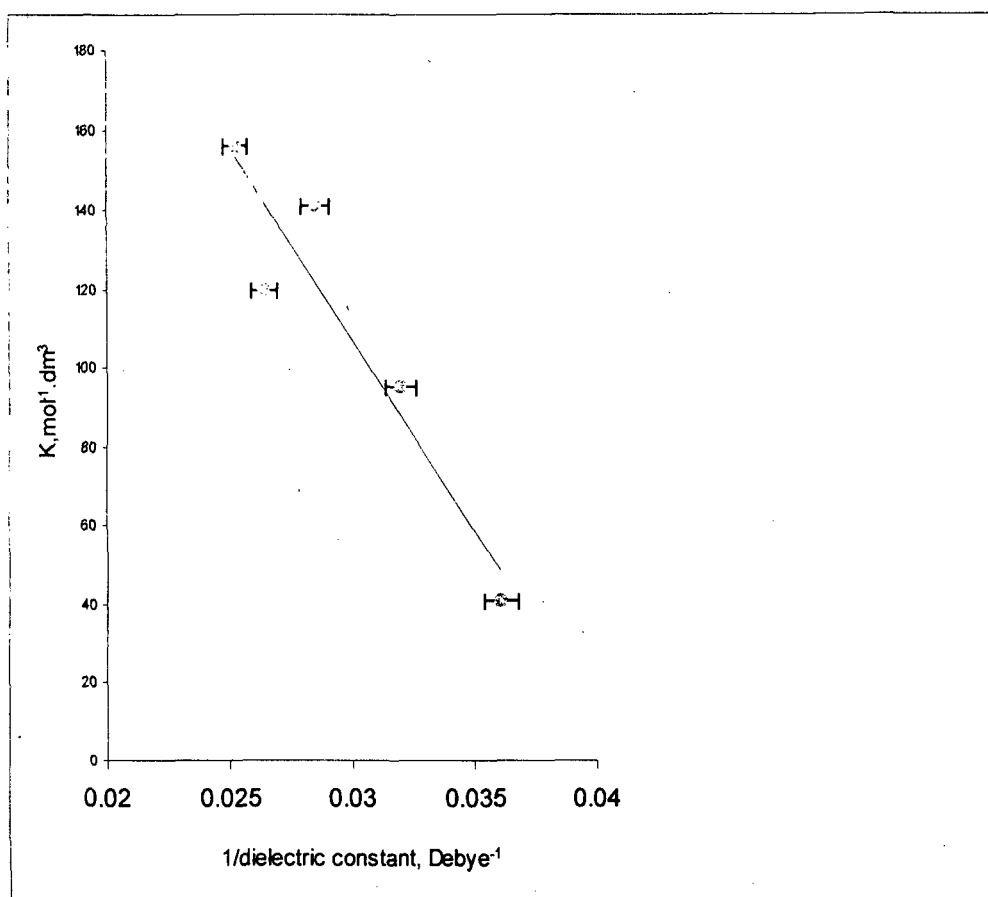


Figure 5.7

Plot of formation constant against reciprocal of dielectric constant by varying ethanol-water ratio