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Spectrophotometric and thermodynamic study on reaction of tetra (p-sulphnaphthyl) porphyrin Iron (II) complex with thiols and glycine ethyl ester as amodel for cytochrome (p-450) state c and haemochrome.

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ABSTRACT

Studies using spectrophotometric titrations on dilute solution of tetra (p-sulphonaphthyl) porphinato Iron (II) [$TNPS_4$ Fe (II)] in the presence of a large excess of thiols and glycine ethyl ester at high (PH=12.8) are reported. evidence for high spin five coordinate Iron (II) and low spin six coordinate Iron (II) complexes were found . Thermodynamic parameters and stability constants were also recorded, refer to exothermic reaction with negative values of ΔH and ΔG for both ligands thiols and glycine ethyl ester . $LogK_F$, Log K_D and (n) number of bounded ligands were calculated, to be found for (n=1-1.3) for thiol ligand and (n=1.8-2.1) for glycine ethyl ester ligand, which were assigned to five and six coordinate to the Iron (II) atom, respectively. These results are discussed in relation to the high spin Iron (II) state in the catalytic cycle of cytochrome (p-450) and to the low spin Iron (II) haemochrome.

Introduction

Cytochrome p450 are a unique class of haem proteins that catalyze the hydroxylation of a wide variety of organic compounds through the activation of molecular oxygen (1,2). The enzymes are found in most organisms, covering the entire range of the animal, plant and bacterial kingdoms where they have various metabolic functions (3-7) . Cytochrome p450 is unique among other haem proteins for the following two reasons :First, its ferrous carbonyl aduct absorbs light at the unusually long wavelength of approximatelly 450nm, but other CO-haem proteins, such as CO – haemoglobin and CO – myoglobin show a single soret band at about 420nm . The second reason is that only one other haem protein (8,9) is capable of activating oxygen for insertion into organic molecules, but this enzyme loses its catalytic activity upon treatment with various compounds such as organic solvents, detergents, sulfhydryl reagents, and salts which convert it to an inactive form called cytochrome p420 (10,11).

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There are four states associated with the catalytic cycle of this enzyme, these are shown in scheme-1, and can be describes as follows (10,11).

1 . State A : The resting form, this state is easily isolated and stable in the absence of a reducing agent or a substrate . It is a six coordinate low spin Iron (III) protoporphyrin IX complex as indicated by its absorption spectrum with maxima at 417nm,535nm and 571nm (12).

2. State B : Addition of substrate to state (A) convert the spin state of the iron (III) complex from a low spin to a high spin state that is five coordinate (13) .

3.State C : This state is produced by the reduction of state (B), it is a five coordinate hiqh spin Fe(II) PPIX complex as indicated by its absorption spectrum bands at 408nm and 540nm (14-15) .

4.State D : This is a six coordinate low spin iron (II) porphyrin complex . It is formed by oxygen adduction to state (C) as indicated by its absorption spectrum (14)and are very similar to the corresponding haemoglobin and myoglobin complexes (15) .

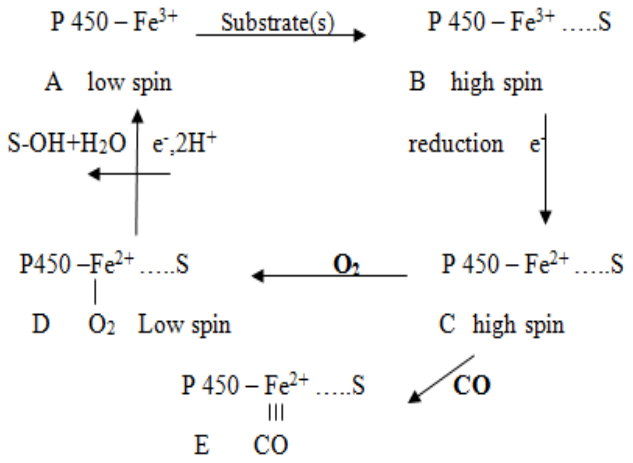
5.State E : State (E) is a carbonyl adduct of state (E) and characterized by its unusual absorption

spectrum which shows two soret bands at 360nm and 450nm . The infrared stretching frequency for this CO adduct are very similar to those of the CO – haemoglobin complexes (14,16) .

Many workers (17-20) have reported models for states B,C and D using iron porphyrin thiolate complexes . An iron (II) picket fence porphyrin thiolate complex has been synthesized (21,22) which could bind an oxygen molecule reversibly as a model for state D of ctochrome p450.

Such models have been prepared using Fe(II)PPXCl solutions and non-naturally occurring iron porphyrins solutions containing mercaptans (23-26) in both aqueous and non-aqueous solutions . Other workers (27) have been reported models for state C high spin five coordinate complexes of cytochrome p450 by using Fe(II) PPIX solutions and non-naturally occurring Fe(II)$TPPS_4$ solutions containing various mercaptan (at high pH) using visible absorption spectroscopics . Other workers (27-29) have studied the binding of nitrogen ligands (amines) and thiols to the Fe(II)PPIX, Fe(II)$TPPS_4$ and Fe(II)$TNPS_4$ in aqueous solutions at high pH ≈12.8 preparing models for state C high spin with thiols and haemochrome complexes low spin with amines and glycine ethyl ester ligand .

The main aim of this work was to examine high concentrations of non – naturally occurring Fe(II)TNPS solutions containing varions mercaptans and glycine ethyl ester at high pH ≈ 12.8 to understanding its chemical and physical properties using visible absorption titrations.



Scheme-1 : The catalytic cycle of cytochrome p450

Experimental

Preparation of $FeTNPS_4$ complex. $TNPS_4Fe$ was prepared according to the method of Fleischer (30), chemical analysis of TNP, $TNPS_4$ and $FeTNPS_4$ are listed in (table-1).

Electronic absorption titration experiments.

Fe (III) $TNPS_4$ was prepared in buffer solution 0.1M KCL, 26ml of 0.1 M NaOH to form buffer pH=12.81 . For electronic absorption spectra cells of at 1cm path length, containing 2.5ml of solution were used . The cells were quartz were fitted with tap tops enabling the solutions to be kept under an N_2 atmosphere. All electronic spectra were recorded at 23and33^{0}C. A few drops of concentrated sodium dithionite were added to the cell, then the ligand was injected, using a micro syringe. The concentration of $TNPS_4Fe$ (II) used was in the range (5-8×$10^{-5}M$),depending upon which absorption band was involved in the study (the ionic strength used was 0.1M $NaNO_2$)

Results and discussion

Electronic absorption spectra

It is convenient to first briefly describe the spectra of $TNPS_4$ Fe (II) glycine ethyl ester in aqueous solution at high pH. The visible absorption spectrum of this complex is very similar to those of other Fe (II) porphyrin and Fe(II) $TPPS_4$ with other amines as axial ligands(27-29) suggested that six – coordinate low spin complexes. It has a sort band at 425nm and to visible bands at 530nm and 562nm . The electronic absorption spectra in dilute solutions of $TNPS_4Fe$(II) – thiols at high pH have soret bands around 412nm and 444nm as a shoulder (see table – 2) which identical to those of reduced cytochrome (p-450) (33,34) . It has been suggested (33,34) that the former complex is a penta coordinate haem complex, and thus the solutions reported here must also contain such high spin five coordinate iron (II) complexes. Similar soret bands were found by Chang et al (35) for PPDMEFe(II) – thiol complexes in to toluene at 23^{0}C around 408nm and by Silver et al (26,27) for PPIXFe (II) – thiol at 405nm as a shoulder . All suggest that the $TNPS_4$ Fe (II) – thiol- species in this work are penta coordinate high spin Iron (II) complexes. For the $TNPS_4$ Fe (II)-glycine ethyl ester comparning to the workers (37-38) suggest that are six coordinate low spin complex haemochrome.

Spectrophotometric titrations.

The spectrophotometric titration data of $TNPS_4$ Fe (II) in aqueous solution at high pH=12.81 with thiols and glycine ethyl ester shows evidence that only one molecule of thiols per $TNPS_4Fe$ (II) and two molecule of glycine ethyl ester per haem bind as described in eqn (1) (39).

$TNPS_4Fe(II) + nL \overset{Kf}{\rightleftharpoons} TNPS_4Fe(II)(L)_n$ ……..(1) K_f is stability constant .

Where n=1 for thiol, n=2 for amine or glycine ethyl ester. The hill plot(25) equation (2) is used to calculate the binding constants .

$\text{Log } A-A_0 / A_\infty - A = \text{Log } K + n \text{ Log } [L]$……(2)

- Where A is the absorbance at the wave length of study of mixed species, A_0 is the absorbance of Fe(II) porphyrins in the absence of L, and A_∞ is the absorbance in the presence of alarge excess of L, L = Ligand .

from the slope formation of 1:1 or 1:2 complexes can be established .

On addition of the thiols ligands to the $TNPS_4$ Fe (II) solution, the absorption spectra in the visible region showed changes in the soret band at 444nm. and other visible bands at 567 and 608nm decrease in intensity, and a new band occurs in the soret band at 412nm, which assigned to the $TNPS_4Fe$ (II) (thiol) complex (26). As the visible bands disappear due to the formation of the 1:1 complex there is little evidence of new bands replacing them (Fig-1,2).

Addition of glycine ethyl ester to the $Fe(II)TNPS_4$ solution at high pH in aqueous solvent induces spectral changes. Well define isosbestic points are observed, typical spectrophotometric titration are presented in figure-3 . The reaction are rapid and quickly reach a point at which no further change in absorbance occurs, suggesting strong binding constants . Such spectral changes were similar for nitrogen ligands with $Fe(II)TPPS_4$ in aqueous solvent at high pH(29) . Hill plots (25) were constructed to analyze these date, to measure the K_f,K_D and (n=slope) the number of ligand bind to the Fe(II) $TNPS_4$ complex at temparatures ranges (23-33^{o}C) see figure-4 . Saturation curve were plotted see figure-6,7.

A plot of ΔA vs the concentration of the ligand (the saturation curve) figure – 5 is presented . The results curve indicates that the ligands bind to the haem cooperatively.

Dissociation and binding constants with , thermodynamic parameters, 50% saturation and Log K_F, Log K_D and n are listed in (table-3) . Solpe that are slightly higher than 1.0 result from water solvent effects (polar solvents) (13,40). though they do not greatly affect the Log K_F, since OH^- and H_2O can bind to the haem (41). The low value of Log K_f for thiol ligands in aqueous solution must be due to the polar solvent (solute- solvent interaction). The aggregation and polymerization of the porphyrin in aqueous media will lower the values of K_f (42). Steric effects are aslo known to lower the value of K_f .

ΔH, ΔG and ΔS values were calculated by using eqn.3-5. Low values results were published by other workers (31,38,43) with negative value for ΔH and ΔG at different temperatures under study and the reaction was fast and exothermic, are listed in table-4.These values were higher than that recorded for Fe(II)TPPS, Fe(II)PPIX and $Fe(II)TNPS_4$ (27,29) porphyrins complexes with strong amines ligands might be due to increase in the $Fe(II)TNPS_4$ – gly and $Fe(II)TNPS_4$ – thiols bond energy with increase sigma donating ability of the ligands .

$\Delta H=19.14T_1T_2(\text{Log } K_2 - \text{Log } K_1) / T_1T_2$ (Kcal/mol) ----------eqn (3)

$\Delta G=4.576T\text{Log}K$ (cal /mol) --------eqn (4)

$\Delta S=\Delta H- \Delta G / T$ (cal /mol) -------eqn (5)

Conclusions

Electronic absorption spectra on dilute solutions of $TNPS_4$ Fe (II) with thiols all show spectra that can be assigned to high spin Iron (II) at 23 and 33^{0}C. Spectrophotometric titrations of $TNPS_4Fe$ (II) with thiols show results where n=1 to 1.3, these values are assigned to one molecule being bound to the Fe (II) ion and which agrees with high spin five coordinate complexes figure-8 a,b . The higher values of n when thiols were used are due to the aggregation and stacking of the Iron porphyrins (44). When glycine ethyl ester was used as a ligand the slope was found to be around n=2.0 .This was assigned to a low spin Iron (II) six. coordinate complex which is similar to that recorded for $TPPS_4Fe$ (II) with glycine ethyl ester and amines (31,38,43).Polar solvents have an effect on the binding constants and OH^-, H_2O can react as axial ligands (40,41,45,46) . Stability constants for these complexes with glycine ethyl ester and thiols were higher than that recorded for $Fe(II)TPPS_4$ (27) complexes with the same ligands, that suggested the former complexes are more stable due to the size of methene substituents (sulphonaphthyl group) on

porpyrin ligand comparing to Fe(II)$TPPS_4$ carry only sulphophenyl group to each CH methine(48-50). The binding constant is determined from eqn (2) by plotting Log $(A-A_0) / (A_\infty-A)$ versus Log [L] (Fig-4),

Table -1: The chemical analysis of TNP, $TNPS_4$ and $FeTNPS_4$

Compound	M.Wt	Compound formula	Elemental Calculated (%)			
			C Calc. found	H Calc. found	O Calc. found	
TNP	814.944 g/ mol	$C_{60}H_{38}N_4$	88.4 85.80	4.7 4.66	6.88 6.90	
$TNPS_4$	1139.216 g/ mol	$C_{60}H_{42}N_4O_{12}S_4$	63.21 63.11	3.68 3.55	16.85 16.17	
$FeTNPS_4$	1194.6 g/ mol	$C_{60}H_{40}N_4O_{12}S_4$	60.27 60.20	3.34 3.41	16.07 15.98	

Table -2 : Electronic absorption of porphyrin ligands and Fe-porprins complexes at room temperature .

Compound	Aλ1 nm	Aλ2 nm	Aλ3 nm	Aλ4 nm	Aλ5 nm	Refs
TNP	418	512	544	590	654	
$TNPS_4$ (a)	418	516	580	646		
$FeTNPS_4$ (c)	396	530	---	---		
Fe(II) $TNPS_4$(a)	444	567	608	---		
Fe(II) $TNPS_4$ (a)	444	567	608	---		(28)
Fe(II) $TPPS_4$ (a)	438	568	608	---		(29)
Fe(II) $TNPS_4$ (a)+SR	412, 444 (b)	---	---	---		
Fe(II) $TNPS_4$ (a)+gly	425	530	562	---		
Fe(II) $TPPS_4$ (a)+SR	409, 440 (b)	---	---	---		(27,29)
Fe(II) $TPPS_4$ (a)+gly	423	532	563	---		(27,29)
Fe(II) $TPPS_4$ (a)+Py	424	529	562	---		(29)
Fe(II) $TNPS_4$ (a)+Py	426	532	562	---		(28)

(a)pH = 12.81, (b)These bands appear as a shoulders in the spectra, (c) pH= 3.9, SR = thiols, gly = glycine ethyl ester, Py = pyridine .

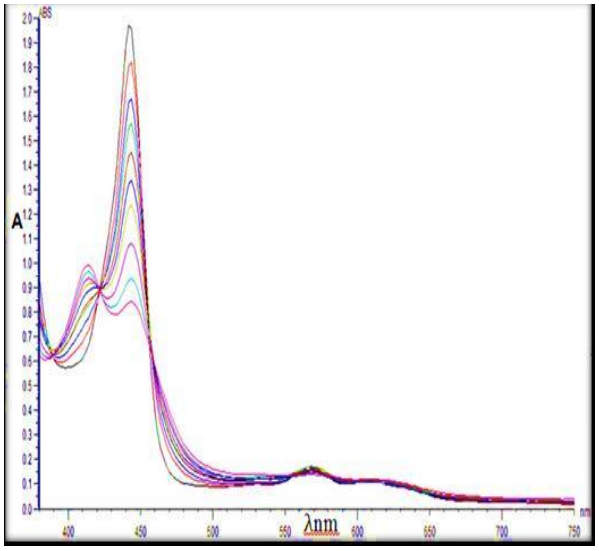


Figure -1 : The visible spectrum of the titration Fe(II) TNPS4 with 2-mercapto ethanol at 230C.

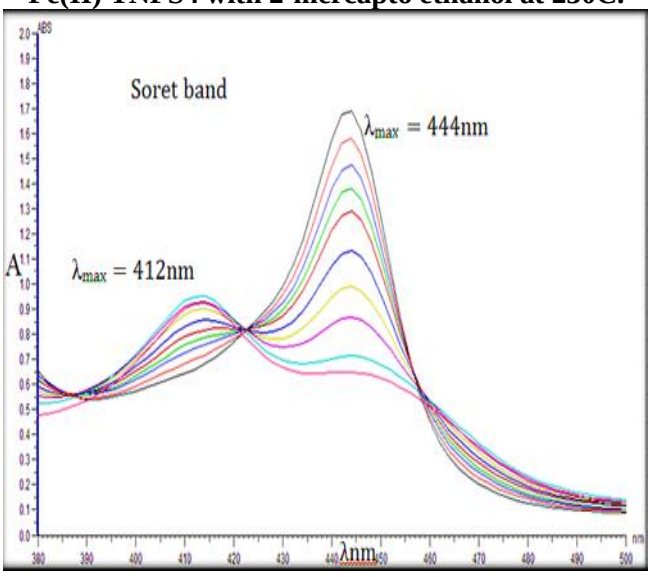


Figure -2 : The Soret band region of the titration Fe(II)TNPS4 with 2-mercapto ethanol at 230C .

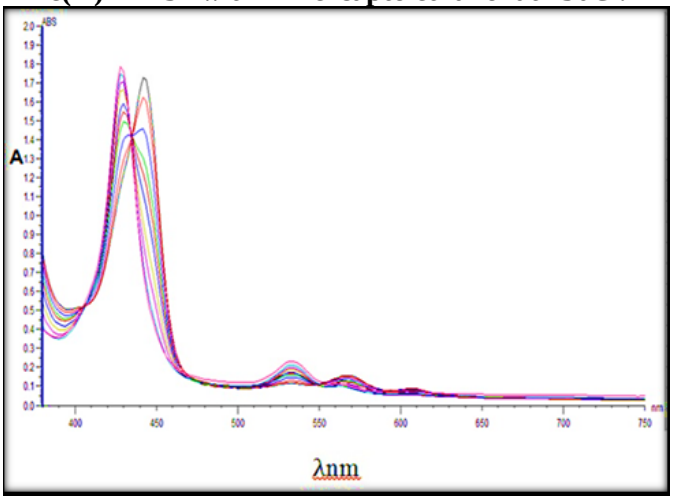


Figure -3 : Spectral changes occurring upon titration of a TNPS4 Fe(II) with glycine ethyl ester at 230C .

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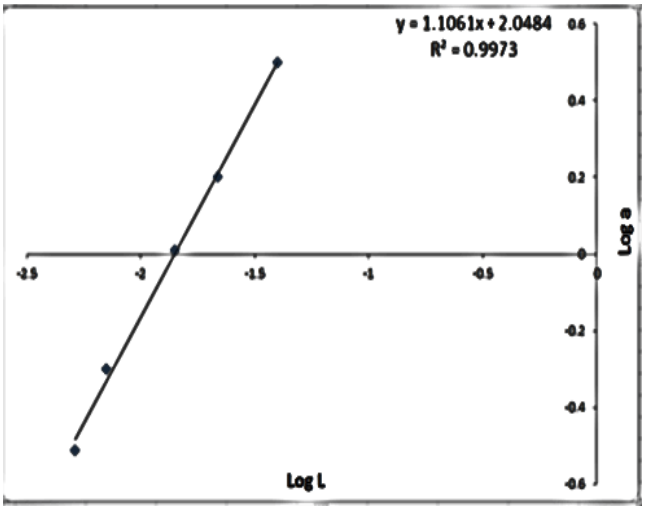


Figure -4 : Hill plot for Fe(II)TNPS4 with 2-mercapto ethanol at 230C .

Table -3 : Dissociation,50% saturation and stability constants for Fe(II)TNPS4 complexes containing thiols and glycine ethyl ester .

Donor	Slope(n)		Log K_{eq}		Log K_D		50% saturation	
	296K	306K	296K	306K	296K	306K	296K	306K
2-Mercapto ethanol	1 .183	1 .1061	2 .1373	2 .0484	0 .4679	0 .4882	1 4	1 0
	* 1.39		* 1.28		* 1.72			
Glycine ethyl ester hdro chloride	2 .1705	1 .8566	6 .3888	5 .2352	0 .1565	0 .1910	1 .4	1 .7
	* 1.93		* 3.5		* 0.285			
Ethyl-2- mercapto acetate	1 .300	1 .2633	3 .150	3 .0589	0 .3175	0 .3269	5 .5	4
	* 1.20		* 0.88		* 2.07			

- **Fe (II)$TPPS_4$ (27).**

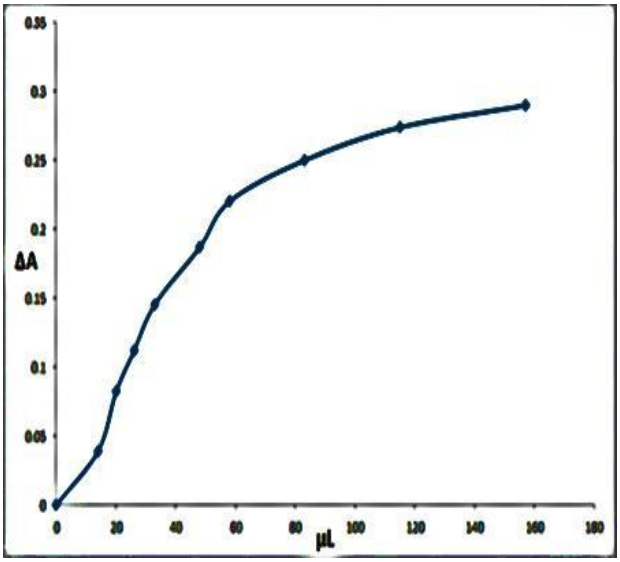


Figure -5 : Plot Δ*A* vs µL ligand of 2-mercapto ethanol at 23^0C .

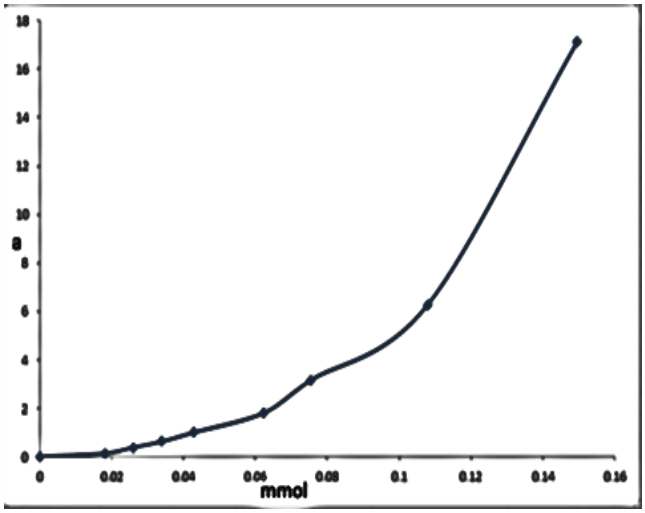


Figure -6 : Polt a = ΔA/ ΔA∞ vs mmole of 2-mercapto ethanol at 230C .

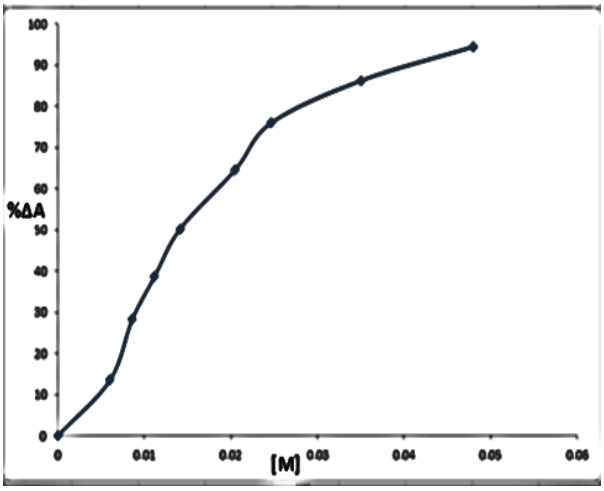
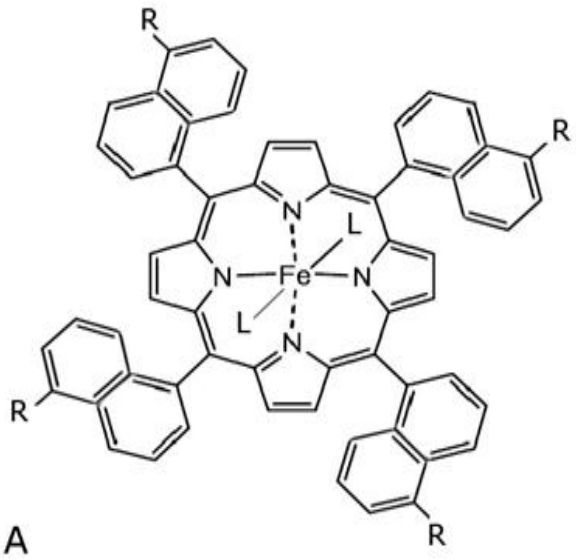


Figure -7 : Polt ΔA(%) vs ligand concentration of 2-mercapto ethanol at 230C.

Table – 4 : Thermodynamic parameters for ligands binding in aqueous solutions of Fe(II)TNPS4 complex .

L igands	Δ H (K.cal/mol)	Δ G(K.cal/ mol)		Δ S (cal/mol)	
		2 96K	3 06K	2 96K	3 06K
2- Mercapto ethanol	- 15.41	- 12.12	- 11.99	- 11.1	- 11.2
Glycine ethyl ester hdro chloride	- 199.99	- 36.2	- 30.66	- 553.3	- 553.4
Ethyl-2- mercapto acetate	- 15.79	-17.85	-17.92	6 .959	6 .961
P yridine (a) 288K (28)	- 11.4	- 8.5	- --	- 10.0	- --
4-methyl Pyridine 288K (29)	- 7.98	- 8.3	- --	1 .38	- --

(a) Fe(II)TPPS$_4$



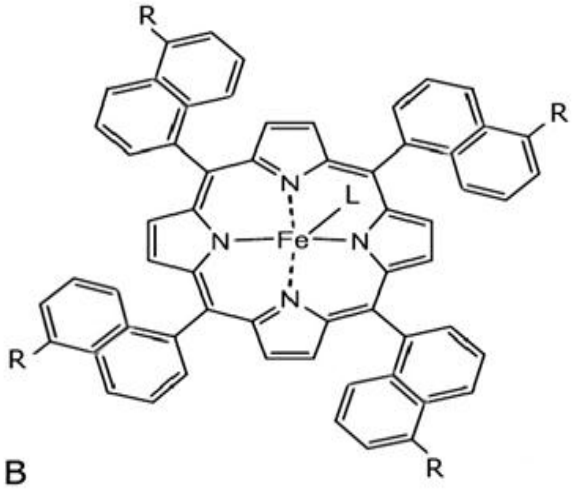


Figure-8 : Models structure of (A) haemochrome six coordinate L = N-gly (glycine ethyl ester, (B) cytochrome state C P-450 five coordinate L = SR(thiol), R = SO_3Na.

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دراسة طيفية وثرموديناميكية لتفاعل معقد بارا سلفونفثيل بورفرين حديد ثنائي مع الثايولات وكلايسين اثيل ايستر كنموذج للسايتوكروم P-450 نوع C والهيموكروم .

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الخلاصة:

تم توثيق دراسة طيفية لمعقد بارا سلفو نفثيل بورفرين حديد ثنائي في المحاليل المخففه بوجود زياده من الثايولات وكلايسين اثيل استر في PH عاليه ١٢.٨. وجدت دلائل على تكون معقدات خماسي التناسق عالي البرم وسداسي التناسق واطئ البرم لذرة الحديد الثنائية التكافؤ. القيم الثرمو ديناميكية ΔH, GΔ وثوابت الاستقرار والتفكك، LogKf, log Kd, n (عدد الليكندات المتعاضدة) تم حسابها وكانت جميع التفاعلات باعثة للحراره وذات قيم سالبة بالنسبة H, GΔ ولجميع الليكندات، حيث وجدت n=1-1.3 لليليكاند الثايول و n=1.8 -2.1 لليليكاند كلايسين اثيل استر وهذا دليل على تكون معقدات خماسية وسداسية التناسق مع ايون الحديد الثنائية على التوالي. هذه النتائج تم مناقشتها كعلاقة الى البرم العالي للحديد الثنائي في حلقة التحفيز للسايتوكروم P – 450 والهيموكروم واطئ البرم.