

## Complex Formation Between Iron(III) and Isonicotinohydroxamic Acid and Its Microbial Studies

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**ABSTRACT:** Complex of Fe(III) with isonicotinohydroxamic acid (INHA) has been investigated by using spectrophotometric method. Fe(III) in aqueous solution revealed the sole formation of the 1:3 complexes at equilibrium. The spectra and magnetic studies of the isolated complex indicated octahedral coordination. The bonding mode proposed for the Fe(III) hydroxamate complex is N-bonding mode. The complex is biologically active against *Staphylococcus.aureus*, *Escherichia-coli*, *Salmonella-typhylidium*, *Klebsiella*, *a-heamolytic Streptococcus*, *Pseudomonias*, *Corrynebacterium* and *Neisseria*.

**KEYWORDS:** Hydroxamic acid, Fe(III) complex, N-bonding mode, microorganism and octahedral coordination.

### INTRODUCTION

Hydroxamic acids having one or more CONHOH- groups have been extensively studied as a consequence of their biological importance which is related with their ability to form metal ion complexes (Fernandes *et al.*, 1997). Hydroxamic acids and other compounds containing the hydroxamate group are ubiquitous in nature and are intimately associated with iron transport in bacteria (Nwabueze 1997). Iron(III) complexes of naturally occurring hydroxamate acids called siderophores, are involved in the processes of iron transport from the environment into the living organisms (Nigovic *et al.*, 2002). Naturally occurring hydroxamic acids such as desferrioxamine B, have typically three bidentate binding sites, making them hexadentate ligands. However, a small group of these compounds have only two hydroxamate binding sites. The most well-known example is rhodotorulic acid which forms a highly stable Fe (III) complex between pH 3 and 12 (Biruset *et al.*, 1998).

Monohydroxamic acids (such as acetohydroxamic acids  $\text{CH}_3\text{CONHOH} = \text{Aha}$ ) after deprotonation act as bidentate ligands forming octahedral complexes with a series of metal ions via co-ordination through the two oxygen atoms of the -CONHO- group. This type of co-ordination has been observed for complexes formed with  $\text{Fe}^{\text{III}}$ ,  $\text{Cr}^{\text{III}}$ ,  $\text{Ni}^{\text{II}}$ ,  $\text{Co}^{\text{II}}$  and  $\text{Zn}^{\text{II}}$  ions, which exhibited octahedral configuration both in the solid state and in solution (Kurzak *et al.*, 1992). This was

confirmed by x-ray diffraction studies carried out on complexes of tris (benzohydroxamic acid) with Fe(III) dihydrate and tris (benzohydroxamic acid) with chromium(III) (Brown *et al.*, 1979).

They are known to have antibacterial and antifungal properties and are inhibitors of enzymes such as prostaglandin H synthase, peroxidases, urease, and matrix metalloproteinases (MMP) which degrade the barriers holding cells in place and are involved in tumor growth. Their ability to inhibit enzymes make them ideal as drug candidates e.g Marimastat is a hydroxamic acid which is an MMP inhibitor and is at an advantage of clinical development as an anticancer drug (Celine 1972).

The biological activity of hydroxamic acids appeared to be related to their iron-chelating ability. The different but related, problems of iron-deficiency anaemia and iron overload resulting from the treatment of thalassaemia (Bates *et al.*, 1972 and Dobbin and Hider 1990). are still serious, especially in tropical countries. There is therefore, a continuous search for iron complexes which can act as suitable oral sources to counter iron deficiency and suitable ligands with high affinities for iron to relieve overload.

While there are reports of studies involving iron complexes of some monohydroxamic acids, nothing is known about those derived from isonicotinohydroxamic acids despite report that

other derivatives of these acids are bioactive (Nwabueze, 1996). However, complexes of isonicotinohydroxamic acid with Ni(II), Co(II) and VO<sup>IV</sup> have been reported (Nwabueze and Aliyu, 2007; Aliyu and Nwabueze, 2008; Aliyu and Nwabueze 2009;).

This paper is therefore a report on the work carried out on the synthesis, characterization and study of the interaction of iron(III) in aqueous solution with hydroxamic acid with special emphasis on the structure and bonding involved. In addition, some physico-chemical properties and the microbial activity of the complex is investigated and included in this report.

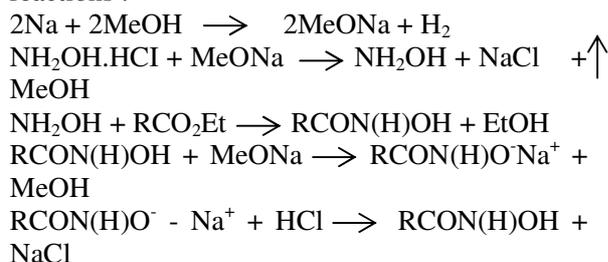
## EXPERIMENTAL

Ethylisonicotinate was obtained from Aldrich.UK All other reagents were of AnalaR-grade. NaNO<sub>3</sub> was used for the preparation of the background electrolyte and stock solutions. Water was doubly distilled, degassed using purified N<sub>2</sub> and stored in glass stoppered flasks. KOH and HNO<sub>3</sub> used for adjusting pH were stored in glass ampoules and were standardized with potassium hydrogen phthalate and tris (hydroxymethyl) methyl amine respectively. The pH measurements were made using a radiometer Copenhagen Research pH meter calibrated with standard buffer tables (2, 4 and 9).

Electronic spectra were recorded on ATI Maltson Genesis series. FT-IR<sup>TM</sup> machine as Nujol mull in the 4000-200cm<sup>-1</sup> spectra region. Room temperature magnetic susceptibility measurements were made on MSB Auto magnetic susceptibility balance.

### Preparation of the ligands

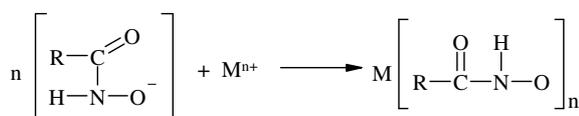
Isonicotinohydroxamic acid (INHA) was prepared as described by Jones and Scott (1922) The various stages involved in the preparation of NHA are as represented by the following reactions :



where R = Pyridine ring for the complex of INHA model.

### Preparation of the complex (Fe (INHA)<sub>3</sub>2H<sub>2</sub>O)

Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O (0.8080g, 0.002 mol) in cold water was added with stirring to a solution of INHA (0.843g, 0.006 mol) in water (20cm<sup>3</sup>). To the resulting purple solution, a 10% solution of NaHCO<sub>3</sub> was added dropwise until precipitation occurred. The dark red precipitate was removed by filtration, washed with small aliquots of Et<sub>2</sub>O and dried over silica gel in a vacuum desiccator. Yield was 58%.



Where n = the number of moles of a neutral monodentate ligand.

### Equilibrium Studies

The pK<sub>a</sub> value for the ligand was determined spectrophotometrically by the method of Albert and Sergent (1971) using boric acid and borax of ionic strength 0.1 mol dm<sup>-3</sup> and 0.025 mol dm<sup>-3</sup> as buffer for INHA ligand. In each case, the ligand stock solution was 5.0 x 10<sup>-4</sup> mol dm<sup>-3</sup> diluted five folds in the buffer solution for INHA. Measurements were made in eight boric/borax buffer solutions at 215nm on a Unicam SP800 spectrophotometer. The number of complexes present in solution at equilibrium was determined by the isobestic point method and graphical Matrix rank analysis using nine solution containing 1:1 – 1:5 metal: ligand ratio ( ligand concentration increasing in unit of 0.5 ). Fifteen solutions containing 1:1 – 1:5 metal: ligand ratios and pH values between 1 and about 3 were used to generate the absorbance data. A solution of I= 0.1 mol dm<sup>-3</sup> made up of 0.01 mol dm<sup>-3</sup> HNO<sub>3</sub> and 0.09 mol dm<sup>-3</sup> NaNO<sub>3</sub> was used to prepare equimolar stock solution of Fe<sup>3+</sup> and the ligand of 2.5 x 10<sup>-3</sup> mol dm<sup>-3</sup>. The same solution was used for all dilutions. Solutions of 0.01 and 0.1 mol dm<sup>-3</sup> of NaOH and HNO<sub>3</sub> respectively, were used to adjust the pH of the reaction mixture. In all case, the solution were thermostated at 25°C for 2hrs in ultrasonic bath.

### Evaluation of the antimicrobial activity

The antimicrobial activity of the test compound was assayed against four Gram +ve bacteria

(*Staphylococcus aureus*, *Salmonella typhylimum*, *a-heamolytic Streptococcus* and *Corryne bacterium*) and four Gram -ve bacteria (*Escherichia coli*, *Klebsiella*, *Pseudomonias* and *Neisseria*). All are regarded as pathogenic to humans and animals. All media and bacteria suspensions were prepared using a method adapted from that of Cruickshank (1965).

About 15-20cm<sup>3</sup> of molten nutrient agar was poured into a 10cm diameter sterile Petri plates. After solidification of the agar, three cups (10mm in diameter and 5mm deep) were removed from each agar dish and fresh bacteria suspension was then uniformly spread on each cup. At this point, each of the cups was spotted three times with test solution at concentration of 50, 100 and 200 µg/cm<sup>3</sup> in dimethyl sulphoxide (DMSO). After incubating the plates at 37°C overnight, the diameter of the zone of inhibition of the bacteria growth was then recorded.

A 5% phenol solution was used as a positive control and DMSO as solvent control each time the experiments were performed.

**RESULTS AND DISCUSSION**

The composition of the solutions used for the equilibrium studies are shown in Table 1. HNO<sub>3</sub> (0.01 mol dm<sup>-3</sup>) was used in the preparation of the back ground electrolyte solution to suppress the hydrolysis of iron(III). At pH values above 3.5, precipitation (accompanied by the formation of some hydrolysis products) occurred, rendering such solution unsuitable for absorbance measurements.

**Table 1:** Composition of Solution used for Equilibrium Studies (Fe<sup>3+</sup>/INHA)

[Fe <sup>3+</sup> ]	[INHA]	pH
0.005	0.0005	2.12
0.005	0.0005	2.23
0.005	0.0005	1.45
0.005	0.001	2.77
0.005	0.001	3.48
0.005	0.00125	1.36
0.005	0.00125	2.19
0.005	0.00125	2.59
0.005	0.0015	2.04
0.005	0.0015	2.86

0.005	0.0015	2.92
0.005	0.002	1.57
0.005	0.002	2.30
0.005	0.0025	1.97
0.005	0.0025	3.02

The spectral data were digitized at 5nm intervals and processed by the computer program SQUAD (Biruset *al.*, 1998). The program calculates the composition, stability constants and molar absorptivities of the complexes by minimizing the sum of the squared residuals, S, defined as:

$$S = \sum (A_{obs} - A_{calcd})^2$$

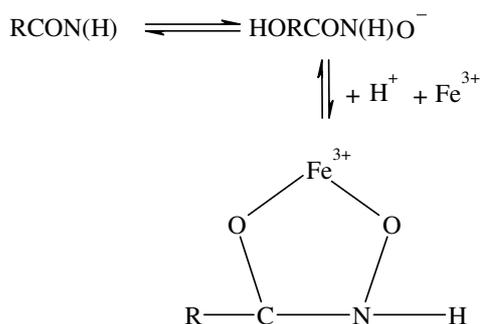
Where A<sub>obs</sub> is measured absorbance and A<sub>calcd.</sub> is the absorbance calculated by the program from the equation:

$$A_{calcd} = \sum B_{p, q, r} [Fe]^p [H] q [L]^r E_{p_1 q_1 r}$$

The results of the calculations indicated the formation of [Fe (INHA)<sub>3</sub>.2H<sub>2</sub>O with a stability constant log B<sub>3</sub> + 34.22+0.06 where B<sub>3</sub> = [FeL<sub>3</sub>]/[Fe<sup>3+</sup>][L]<sup>3</sup>.

Several equilibrium models were tried but it was only with ML<sub>3</sub> that convergence was achieved. Models in which ML<sup>2+</sup> and ML<sup>+</sup> included failed to achieve convergence due to persistently high standard derivation of the constant, this indicates that the species are not present in any significant concentration at equilibrium (Cruckshank, 1965). The standard deviation in the absorbance data is 3.7x10<sup>-3</sup> for [Fe(INHA)<sub>3</sub>.2H<sub>2</sub>O]. This statistical parameter, together with the standard deviation in the refined constant, indicates the consistency of data (Braibanti and Bruschi, 1977 and Legget and Legget, 1985).

Analyses of the calculated spectrum of the complex shows a pH dependence of the absorption maxima. Fe(INHA)<sub>3</sub>.2H<sub>2</sub>O exhibits an absorption maxima centered at about 500nm (Σ = 614) for solution of pH less than 2; this is shift to 470nm (Σ = 631) for pH >2. This pH dependence is due to the shift of equilibrium.



A shift to the right results from deprotonation of the ligand as basicity increases.

The ligand shows comparable affinity for iron(III) ion. The low stability constant of  $[\text{Fe}(\text{INHA})_3] \cdot 2\text{H}_2\text{O}$  despite the high pka value of INHA may be due to steric effects of its large ring (Nwabueze, 1996).

The pka value for the ligand is  $8.68 \pm 0.05$ . The high basicity of the ligand may be ascribed to the positive inductive effect of the larger pyridine ring attached to it. Figure 1 shows the absorption spectra of solutions containing a constant metal but variable ligand molar concentration of the INHA system; while figure 2 shows the graphical matrix rank analysis for the absorbance data generated from similar solutions for the INHA system. In this regard, the two systems show similar behaviour. The absence of an isosbestic point and the shape of the graph is typical of systems containing only one complex species (Legget and Bryde, 1975). The composition of the complex in solution was determined as a 1:3 M:L species by Job's plot as shown in figure 3 (Hartley *et al.*, 1980).

The analytical data and some physico-chemical properties of the ligand and its isolated complex are shown in Table 2.

**Table 2:** Analytical data and some physico-chemical properties of the ligand and its Isolated Complex.

	INHA	Compound $\text{Fe}(\text{INHA})_3 \cdot 2\text{H}_2\text{O}$
<b>Formula</b>	$\text{C}_6\text{H}_7\text{N}_2\text{O}_2$	$\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_8\text{Fe}$
<b>FW</b>	139	477.90

<b>Mp/Dec °C</b>	96	210
<b>Colour</b>	Cream	Dark red
<b>Neff (B.M) 298K</b>	-	5.85
<b><math>\lambda_{\text{Max}}</math> nm</b>	-	410
<b>Found (calcd) (%)</b>	-	11.07 (11.66)
<b>Assignment</b>	-	${}^6\text{A}_{1g} \longrightarrow {}^4\text{A}_{1g}, {}^4\text{E}_g$

M = metal, FW = Formula weight, MP/Dec = Melting Point/Decomposition, B.M = Bohr Magnetron,  $\lambda_{\text{max}}$  = Wavelength

The visible Spectrum of the complex shows a broad band centered at about 410nm following similar earlier observation and has been assigned to  ${}^6\text{A}_{1g} \longrightarrow {}^4\text{A}_{1g}, {}^4\text{E}_g$  transition (Nicholls,

1974). The position of the band together with the observed magnetic moments is consistent with an octahedrally-coordinated high spin iron(III) complex (Nicholls, 1974).

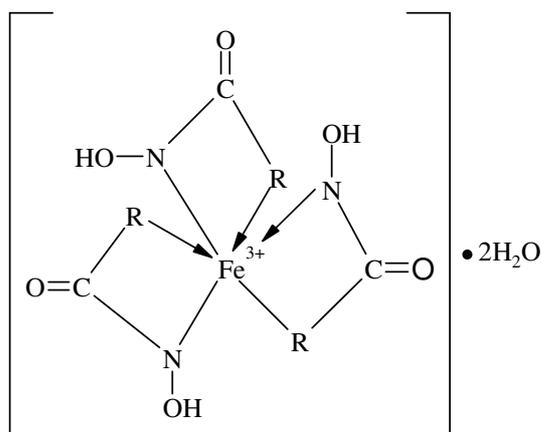
The i.r bands in the ligand and its position in the corresponding complex is reported in Table 3. The spectrum of the ligand shows a band at  $3422.59\text{cm}^{-1}$  and a broad band at  $1605.01\text{cm}^{-1}$ , which were assigned to  $\nu(\text{NH})$  and  $\nu(\text{C}=\text{O})$  vibrations respectively. The position of the bands and the broadening of the  $\nu(\text{C}=\text{O})$  band indicates hydrogen bonding in the molecules. In the complex, the  $\nu(\text{C}=\text{O})$  band is lowered to about  $1602.77\text{cm}^{-1}$  while the  $\nu(\text{C}-\text{N})$  vibration observed at  $1125.95\text{cm}^{-1}$  in the ligand is shifted to  $1157.98\text{cm}^{-1}$  in the complex. These observations indicated N- bonding mode.

**Table 3:** Diagnostic I.R Data for the complex

I.R Data	INHA	$\text{Fe}(\text{INHA})_3 \cdot 2\text{H}_2\text{O}$
$\nu(\text{NH}) \text{ cm}^{-1}$	3422.59	3382.12
$\Delta \nu(\text{NH}) \text{ cm}^{-1}$	-	-40.47
$\nu(\text{C}=\text{O}) \text{ cm}^{-1}$	1605.01	1602.77
$\Delta \nu(\text{C}=\text{O}) \text{ cm}^{-1}$	-	-3.24
$\nu(\text{CN}) \text{ cm}^{-1}$	1125.95	1157.98
$\Delta \nu(\text{CN}) \text{ cm}^{-1}$	-	+31.33

INHA = Isonicotinohydroxamic acid

On the basis of the observed physico-chemical properties, the structure (Scheme 1) is proposed for the complex.



Key: R = Pyridine.

**Scheme 1:** Proposed structure having N- bonding mode and is an octahedrally coordinated complex.

The results of the microbial sensitivity test carried out on the ligand and its isolated Fe(III) complex are shown in Tables 4 and 5. It is evident from Table 4 that Fe(III) complex exhibit moderate activity against *S. Typhlium*, *Klebsiella*,

*Pseudomonas* and *corynebacteria*, while maximum activity was exhibited against *S.*

*aureus*, *E. Coli*, *a-heamolytic strep.* and *Neisseria*.

**Table 4:** Diameter of Zone of Inhibition

Diameter (mm)	Symbol	Comment on Activity
12-15	+	Insignificant
16-20	++	Minimum
21-25	+++	Moderate
26-35	++++	Maximum

**Table 5:** Microbial Sensitivity Test for the Ligand and its isolated Fe(III) Complex.

Bacteria	INHA	Fe(INHA) <sub>3</sub> 2H <sub>2</sub> O
<i>Staph. Aureus</i>	++	++++
<i>S. Typhylum</i>	++	+++
<i>E. Coli</i>	++	++++
<i>Klebsiella</i>	++	+++
<i>a-hemolytic Strep</i>	++	++++
<i>Neisseria</i>	++	++++
<i>Pseudo-cnonias</i>	++	+++
<i>Coryne-bacteria</i>	++	+++

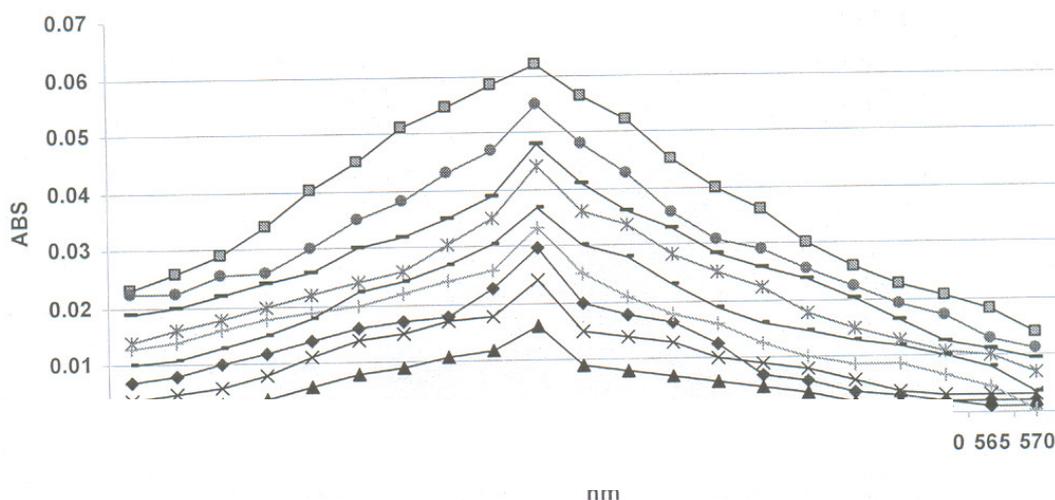
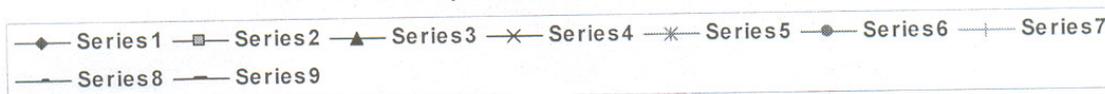


FIG.1: Isosbestic point search for Fe<sup>III</sup>-NHA system



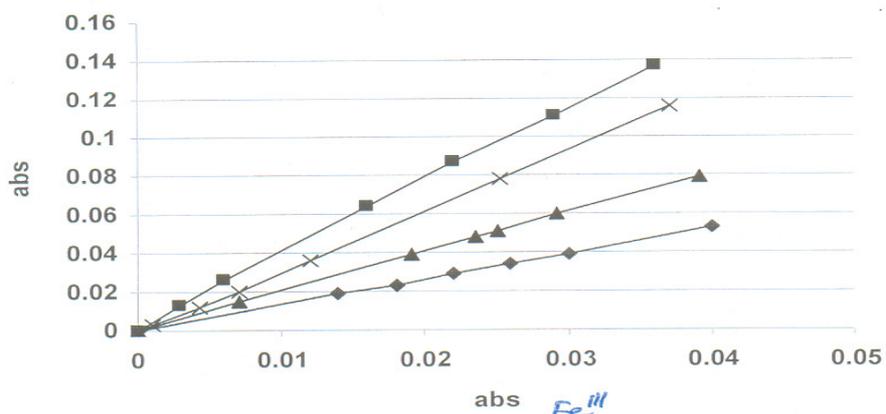


Fig 2: Graphical rank Matrix analysis for  $Fe^{III}$ -NHA system (one specie test)

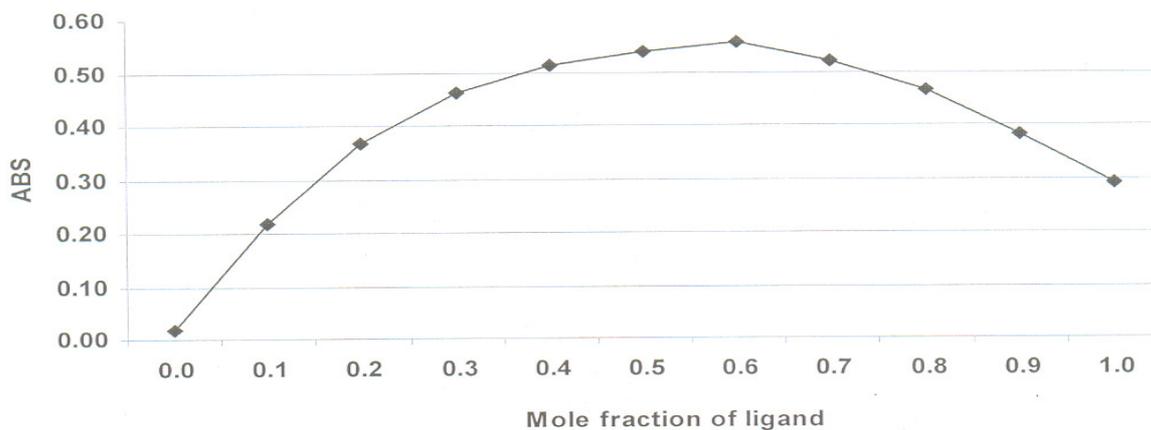
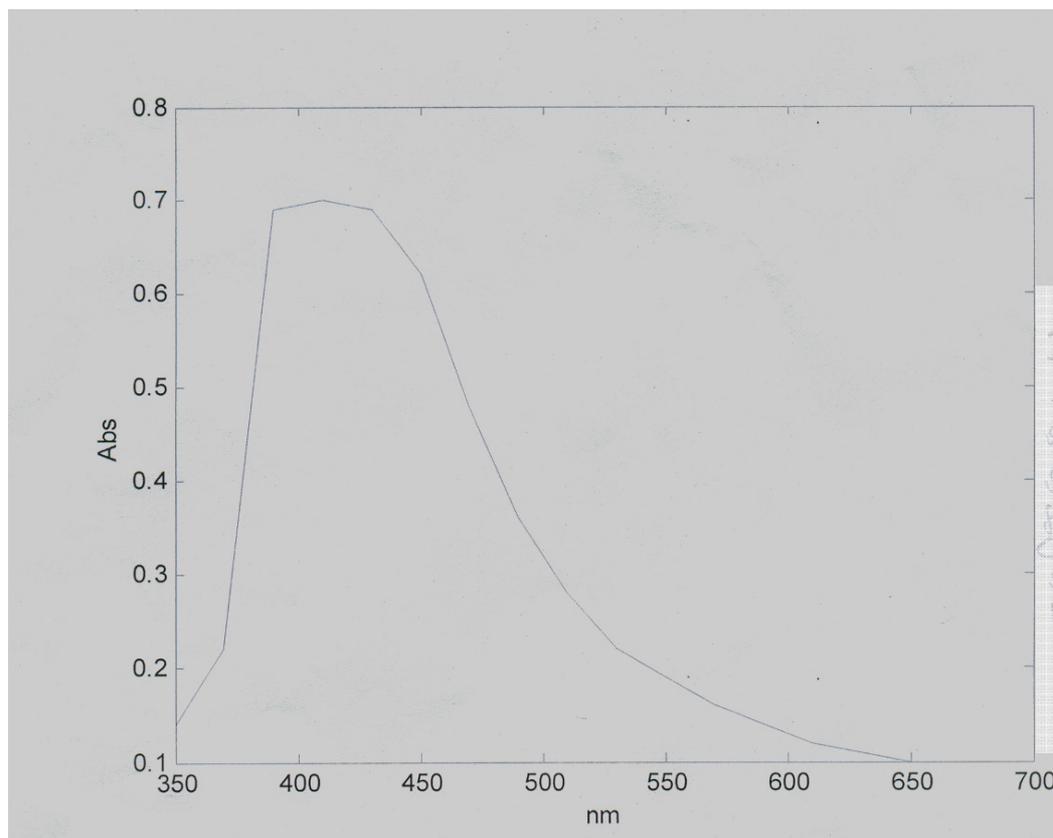


Fig 3: Continuous variations (Job's plot) method for  $Fe^{III}$ -NHA system



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