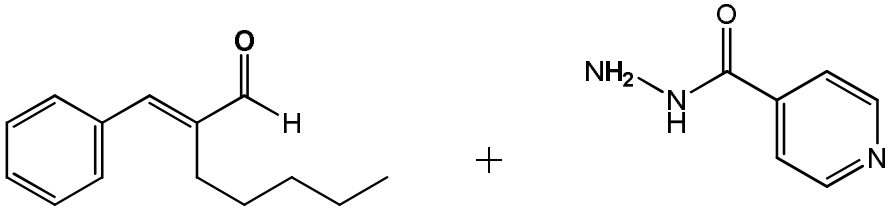
CHAPTER-4

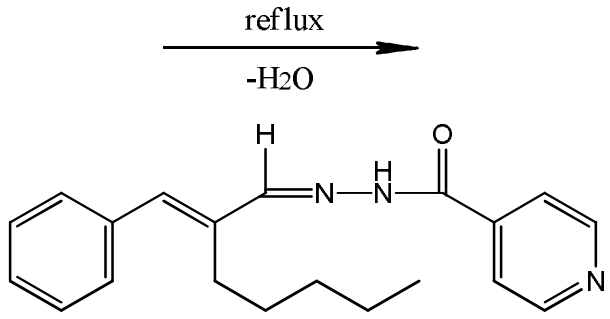
EXPERIMENTAL INVESTIGATIONS

4.1 Synthesis, Characterization and Analytical properties of α-Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH)

In a 250ml round bottom flask α-Amyl Cinnamaldehyde (2.022gm, 0.01M) was dissolved in hot methanol and hot methanol solution of Isonicotinoyl hydrazide (1.3714gm, 0.01M) were taken. By using water condenser the contents of the flask were refluxed for 4 hours. On cooling the reaction mixture, crystalline yellowish coloured product was formed. By filtration and washed with hot water and ethanol several times and the product was collected. This compound was recrystallised and dried in vacuo.



Alpha amyl cinnamic aldehyde Isonicotinyl hydrazide



alpha Amyl Cinnamaldehyde Isonicotinoyl Hydrazone

4.1.1 Characterization of α-Amyl Cinnamaldehyde Isonicotinoyl hydrazone

With the help of Infrared, ^{1}H-NMR and Mass spectral data α-Amyl Cinnamaldehyde Isonicotinoyl Hydrazone was characterized.

IR spectrum

In KBr medium by using Shimdzu FTIR-84005 infrared spectrum of ACINH was recorded. The IR spectrum of ACINH was incorporated in Fig.4.1.1. At 3314 cm^{-1} a medium absorption band is observed in the spectrum of ACINH was assigned to ν(NH) stretching. Two bands were observed at 1643.69 cm^{-1} and 1728 cm^{-1} in the spectrum respectively assigned to the presence of ν(C=O) and ν(C=N) moieties in the ligand. In Table 4.1.1 other important Infrared bands of ACINH and their assignments are presented.

Table 4.1.1: Infrared spectral bands (cm^{-1}) of ACINH

S.No.	Bands(cm^{-1})	Assignments
1	1643.96(s)	ν >C=O stretching
2	1728(s)	ν C=N stretching
3	3396.25(brs)	ν N-H stretching
4	2919(m)	ν C-H stretching
5	609(s)	ν N-H deformation
6	1511(s)	Aromatic C-N stretching
7	1359(s)	Aliphatic C-N stretching
8	2547(brs)	Aliphatic C-H stretching
9	2919(brs)	Aromatic C-H stretching
10	971.47-1238.55(m)	Aromatic C-H in plane bendings

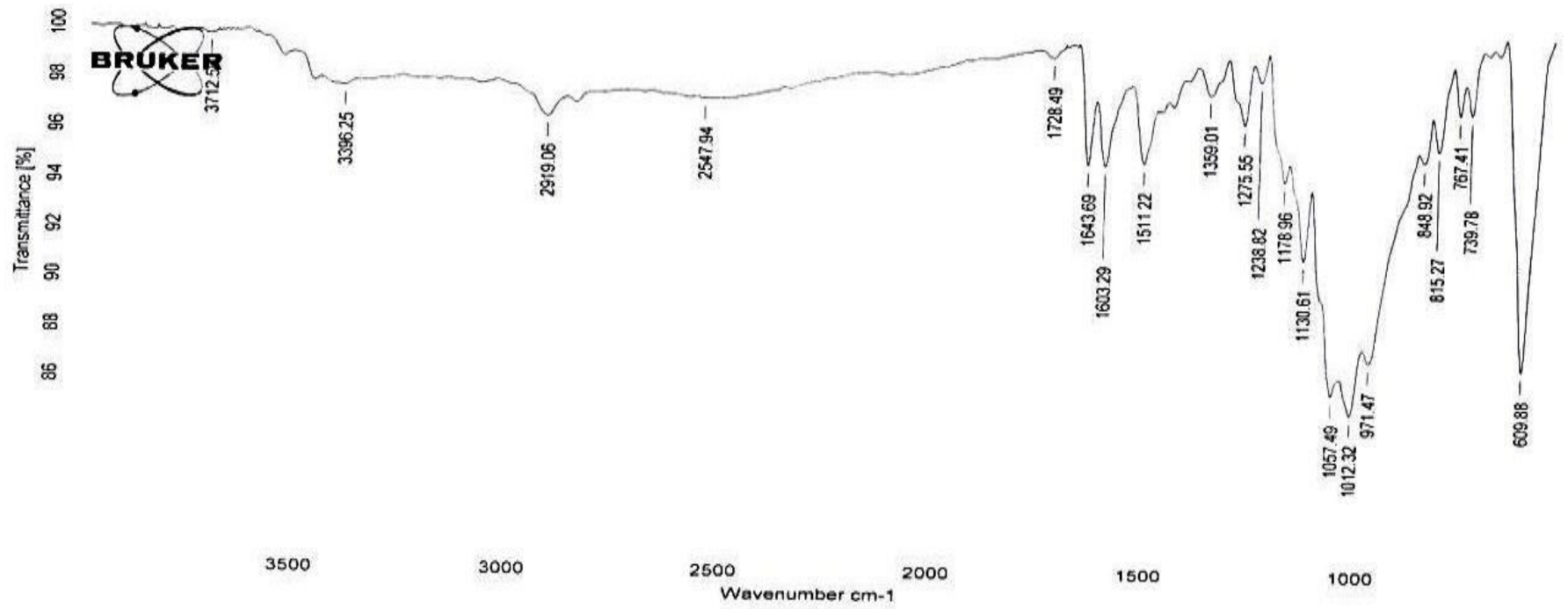


Fig: 4.1.1 IR spectrum of α-Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH)

NMR spectrum

^{1}H-NMR Spectrum of ACINH was recorded on New avance -300MHz in DMSO-d_6 (Dimethyl sulphoxide) solvent with TMS as an internal reference. ^{1}H-NMR spectrum of ACINH shown in Fig.4.1.2.

At 8.89 ppm doublet signal is observed at low field due to aromatic proton. At 7.10 one signal is observed due to N-H proton. The signals at 8.09 to 7.64 due to aromatic protons. In Table 4.1.2 assignment and spectral data of ACINH are represented.

Table 4.1.2: ^{1}H-NMR spectral data of ACINH

S.No.	δ Value	Assignment
1	8.89 (d, H)	ArH
2	8.09 (d, H)	ArH
3	7.70 (d, H)	ArH
4	7.64 (t, H)	ArH
5	7.62 (s, H)	CH
6	7.21 (d, H)	CH
7	7.10 (s, H)	NH
8	5.95 (s, H)	CH
9	2.1 (t, 2H)	CH_2
10	1.65 (sextet, 2H)	CH_2
11	1.52 (p, 2H)	CH_2
12	1.52 (p, 2H)	CH_2
13	0.75 (t,3H)	CH_3

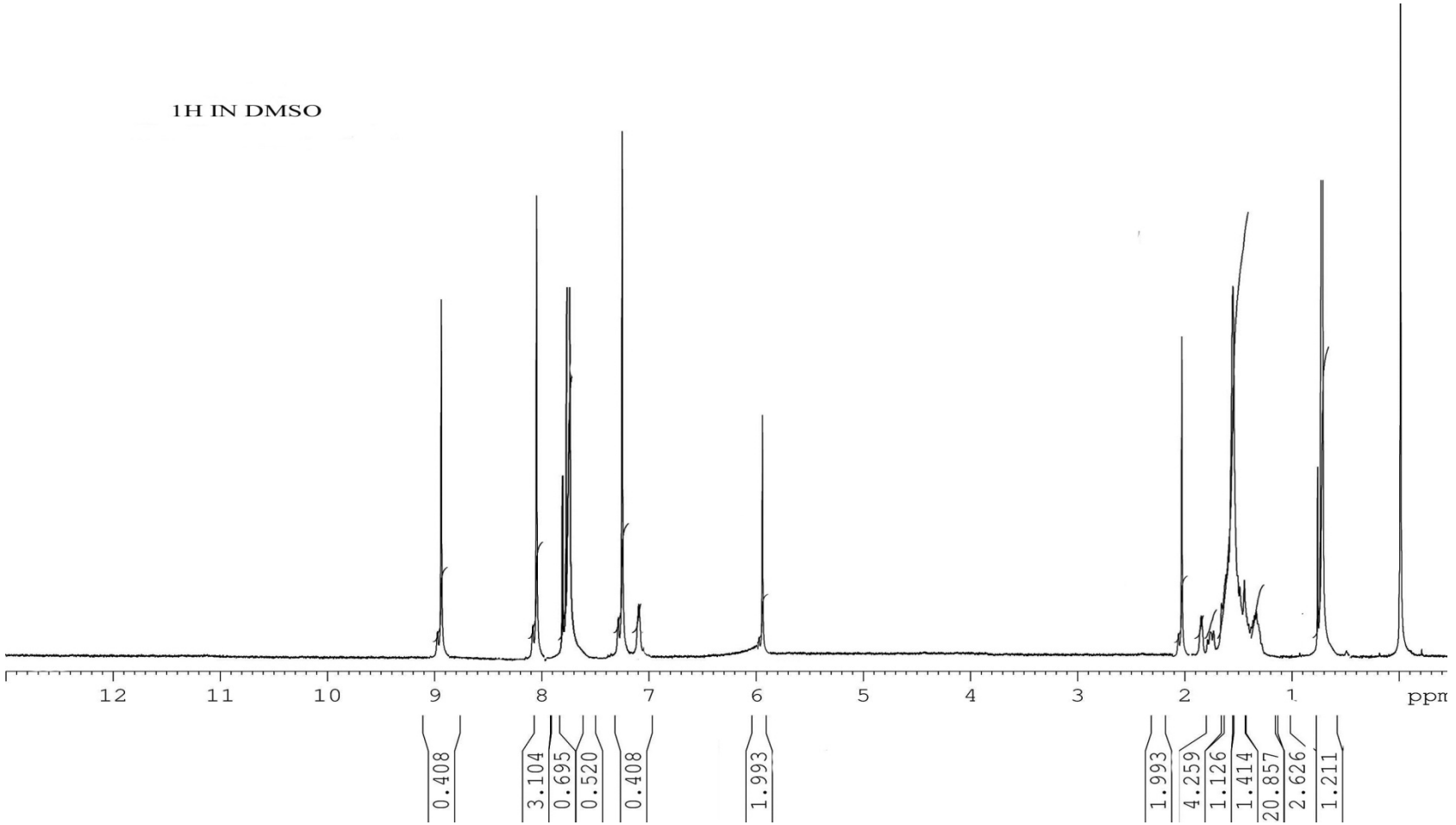


Fig: 4.1.2 NMR spectrum of α-Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH)

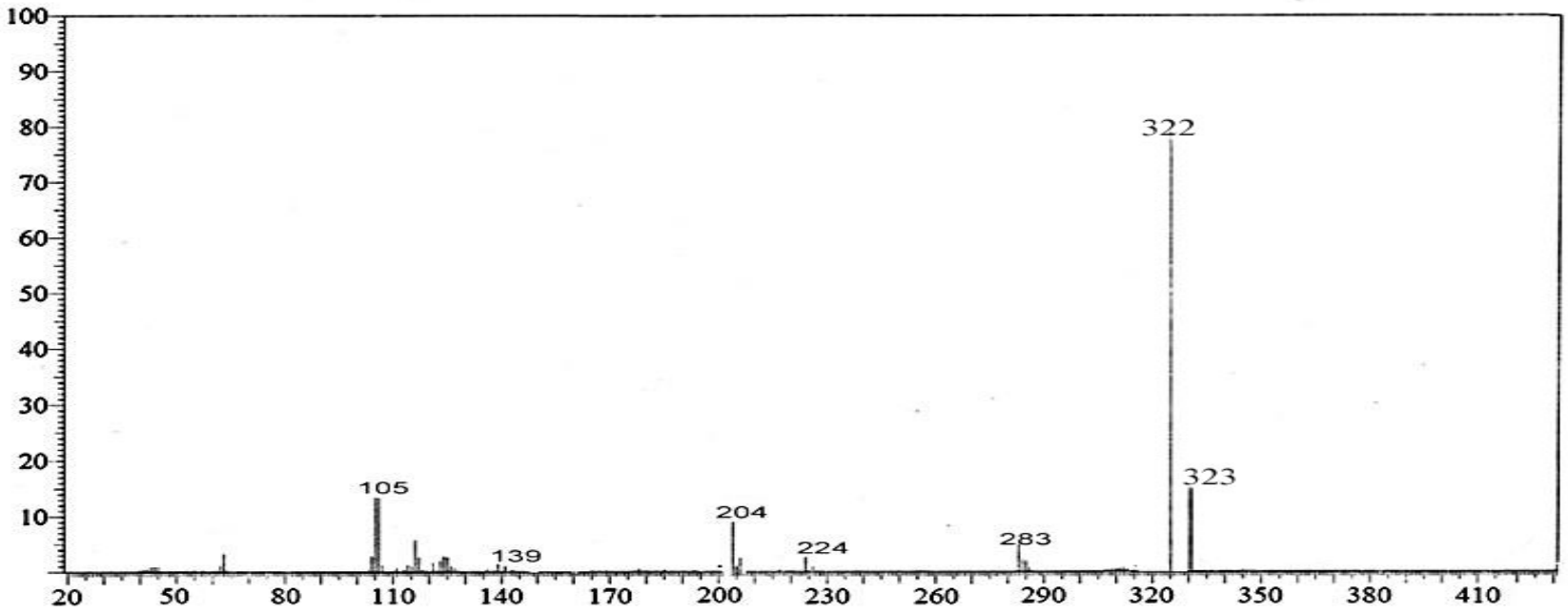


Fig: 4.1.3 Mass spectrum of α-Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH)

Mass Spectrum

M+1 peak of ACINH shows at 322 (m/z) in mass spectrum corresponding to its molecular weight. The Mass spectrum of ACINH is incorporated in the Fig.4.1.3.

Based on the Infrared, Proton NMR and Mass spectral data of ACINH, the following structure Fig (4.1.4) was affirmed for the compound in solid state.

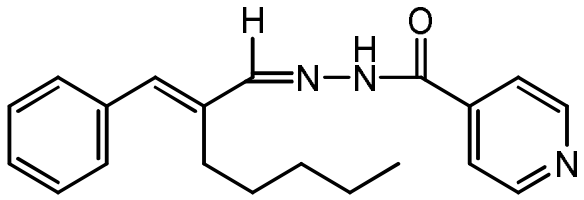


Fig: 4.1.4: Structure of ACINH

4.2 Direct Spectrophotometric determination of Hg (II) using ACINH

The reagent α-Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH) reacts with Mercury(II) in basic buffer pH 10.0 medium to give water soluble yellow coloured species. The absorbance of the complex was stable for five hours. For the determination of Mercury(II) by spectrophotometric method the complex was studied systematically in aqueous medium.

4.2.1 Absorption spectra of reagent solution and Hg (II)-ACINH complex

At the wavelength region 300-650 nm the absorption spectra of Mercury (II)-ACINH complex and reagent solution was calculated by employing the procedure described in 3.2.1 is adopted. A plot between wavelength and absorption is presented in Fig.4.2.1. The metal complex show maximum absorbance at 391nm as shown in figure.

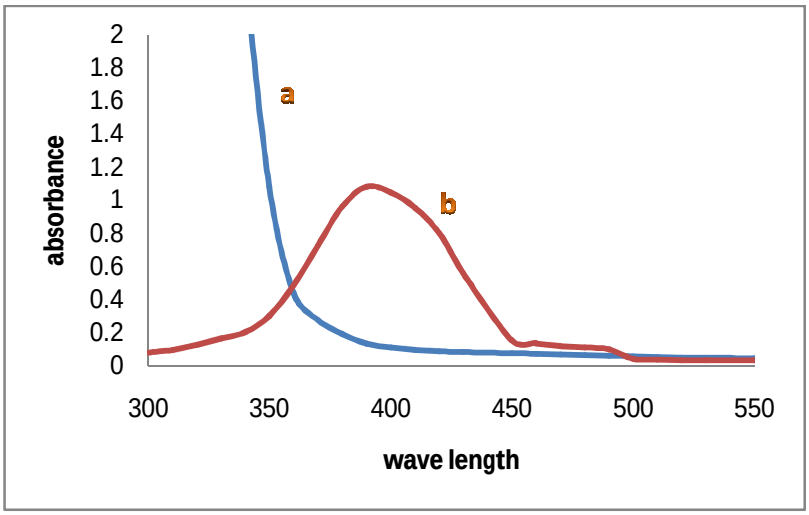


Fig: 4.2.1 Absorption spectra of

a. ACINH vs buffer blank
b. [Hg (II)-ACINH] complex vs reagent blank
[Hg (II)] = 1.0×10^{-5} M
[ACINH] = 1.0×10^{-4} M
pH = 10.0

4.2.2 Effect of pH on the absorbance of Hg (II)-ACINH complex

To arrive the optimum pH required for getting the maximum and constant absorbance by employing the procedure described in 3.2.2 is adopted. The effect of pH on the absorbance of the Hg (II)-ACINH complex was studied. A plot was drawn between absorbance and pH of the complex and given in Fig.4.2.2.

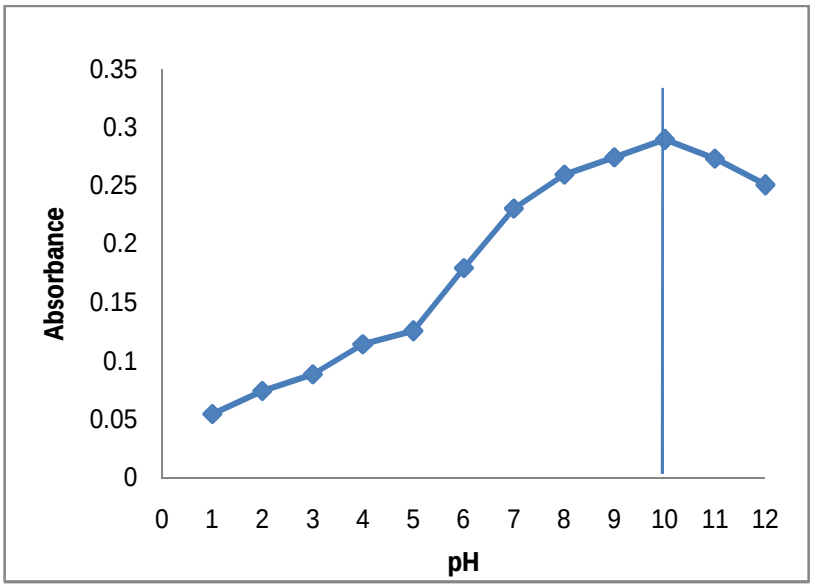


Fig: 4.2.2 Effect of pH on the absorbance [Hg (II)-ACINH] system

[Hg (II)] = 1.0×10^{-5}M
[ACINH] = 1.0×10^{-4}M

4.2.3 Effect of reagent on the absorbance of the metal complex

By employing the procedure described in 3.2.3 how much amount of reagent is necessary for full colour development was established and the results are shown in Table 4.2.1.

Table 4.2.1: Effect of ACINH on the absorbance of the metal complex

[Hg (II)] = 1.0×10^{-5}
pH = 10.0
Wavelength = 391 nm

Hg (II) : ACINH	Absorbance
1:1	0.764
1:3	0.786
1:5	0.790
1:10	0.801
1:15	0.797
1:20	0.780
1:25	0.774

4.2.4 Effect of time on the absorbance of Hg (II)-ACINH complex

At different time intervals the absorbance of Mercury (II)-ACINH was measured, for the determination of the time stability of the complex the procedure described in 3.2.4 was adopted.

4.2.5 Order of addition

The order of addition of metal, reagent and buffer solution has no adverse effect on the absorbance of reaction mixture

4.2.6 Applicability of Beer's law

By employing the procedure described in 3.2.5 the applicability of Beer's law for the present system was examined. A calibration plot (Fig.4.2.3) was drawn between the amount of Hg (II) and absorbance. System obeys Beer's law. The optimum concentration range, Beer's law absorbance, molar absorptivity and Sandel's sensitivity results are presented in section 5.2.6.

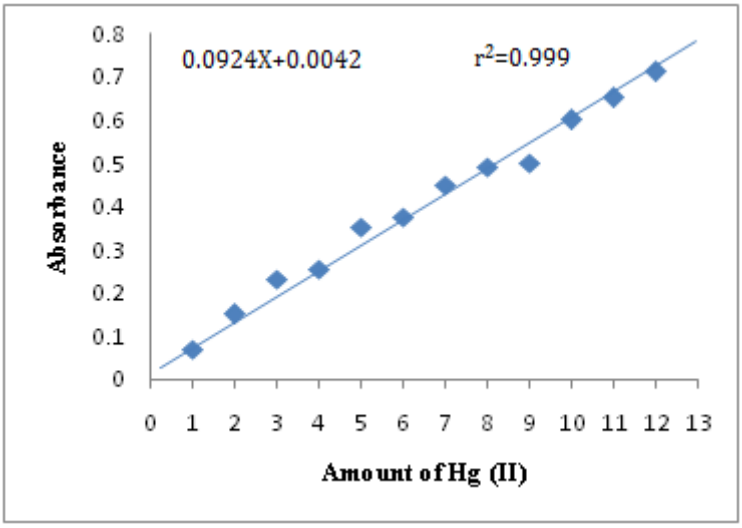


Fig: 4.2.3 Absorbance vs Amount of Hg (II) µg/ml

[ACINH] = 1.0×10^{-4}
pH = 10.0
Wavelength = 391 nm

4.2.7 Tolerance limit of foreign ions

By employing above established optimum conditions with a view to examine the selectivity of reagent, the effect of various foreign ions was studied. This experiment was also intended for the determination of tolerance limit of various associated ions. By employing the procedure described in 3.2.6 the interference of foreign ions was studied with 5.01475 µg/ml of mercury (II). The results are summarized in Table 4.2.2.

Table 4.2.2: Tolerance limits of foreign ions in the determination of 5.01475 µg/ml of mercury (II)

Ion Added	Tolerance Limit (µg/mL)
	Zero Order
Urea	31
Phosphate	93
Nitrate	63
Oxalate	133
Tartarate	140
Fluoride	20
Bromide	79.9
Iodide	126.9
Mn^{+2}	55
Bi^{+3}	209
Zn^{+2}	64
Zr^{+2}	135
W^{+6}	181
Ag +	107
Pd^{+2}	106.5
V^{+5}	52
Sr^{+2}	87

4.2.8: Applications

To the determination of Mercury (II) in water samples the present method was applied.

Determination of Mercury (II) in water samples

The recommended procedures given in 3.2.9.1 water samples was prepared and analyzed for the determination of Mercury (II). The results are presented in Table 4.2.3.

Table 4.2.3: Determination of Mercury (II) in water samples

Name of the sample	Amount of Mercury (II) found (μg ml^{-1})		
	Present Method*	AAS method	Error (%)
Laboratory water	1.94	1.87	+3.61
Tap water	0.68	0.69	-1.47
Waste water	3.27	3.28	-0.31
Sea water	0.99	1.02	-3.03
River water	0.63	0.62	+1.58
Drain water	1.16	1.18	-1.72
Well water	0.93	0.84	+9.67

* Average of best three determinations among five determinations

4.2.9 Composition and stability constant of the complex

By using Job's continuous variation method the composition of the complex was determined and confirmed by molar ratio method. By using the data obtained from the job's plot (Fig.4.2.4) stability constant of the complex was calculated.

4.2.9.1 Job's continuous variation method

By applying described procedure given in 3.2.7.1, the composition and stability of the metal complex was studied. A graph (Fig.4.2.4) was drawn between mole fraction of Mercury (II) and absorbance of the complex. Job's curve shows that one mole of metal ion reacts with one mole of reagent.

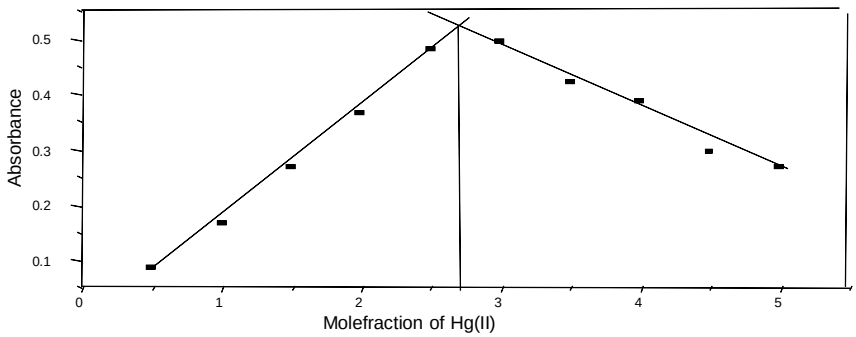


Fig: 4.2.4 Job's curve of [Hg (II)-ACINH] complex

[Hg (II)] = [ACINH]	=	$1.0x10^{-5}$
pH	=	10.0
Wavelength	=	391 nm

4.2.9.2 Molar ratio method

By Employing described procedure of molar ratio in 3.2.7.2 the composition of the complex was examined. The plot was drawn between the number of moles of reagent and absorbance at 391nm in Fig.4.2.5.

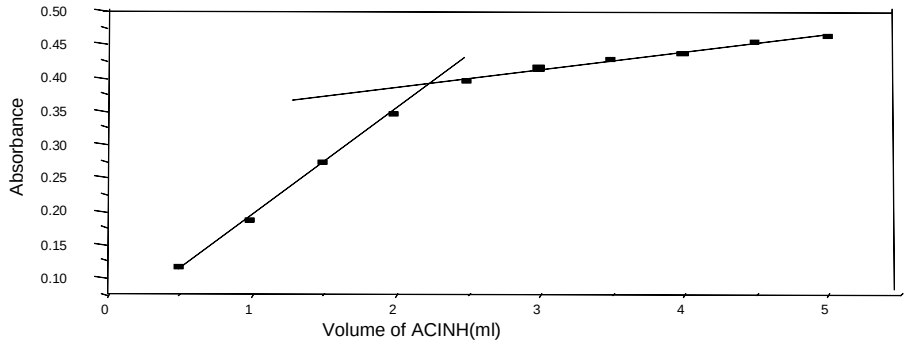


Fig: 4.2.5 Molar ratio plot of [Hg (II)-ACINH] complex

[Hg (II)] = [ACINH]	=	$1.0x10^{-5}$
pH	=	10.0
Wavelength	=	391 nm

4.3 Derivative spectrophotometric determination of Hg (II)-ACINH complex

Mercury (II) forms water soluble yellow coloured complex with an α-Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH). The coloured complex was stable for five hours, zero order spectrum shows λ_{max} at 391 nm. For the Determination of Mercury (II) in microgram quantities first order derivative spectrophotometric method was developed. The detailed investigation is presented in this section.

4.3.1 Derivative spectra of Hg (II)-ACINH complex

The first order derivative spectrum of Hg (II)-ACINH complex was recorded at pH 10.0 by following the procedure described in3.2.10. In Fig.4.3.1 maximum amplitude of first order derivative spectrum was observed at 440nm. Therefore determination of Mercury (II) by first order derivative spectrophotometric method was carried out at 440nm.

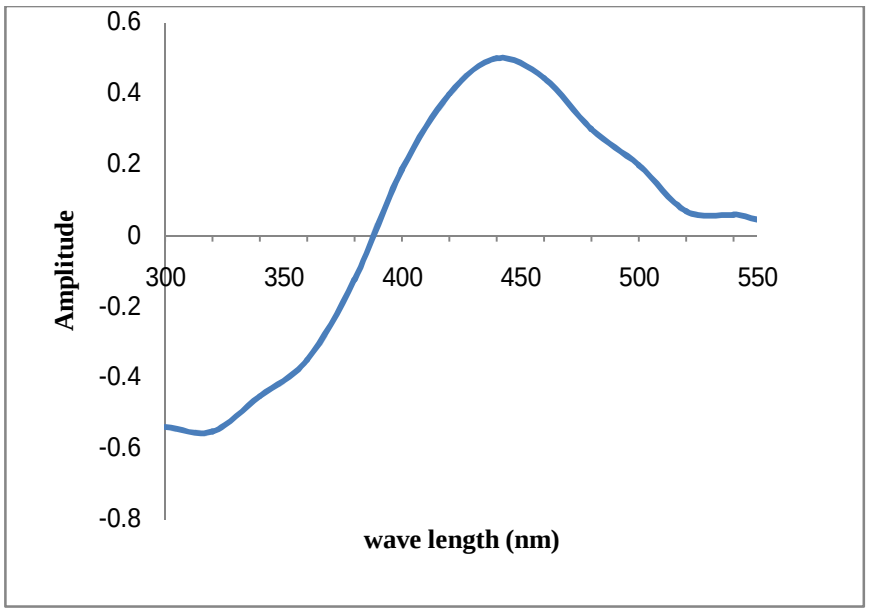


Fig: 4.3.1 First derivative spectrum of [Hg (II)-ACINH] vs reagent

[Hg(II)] = 1.0×10^{-5}M
[ACINH] = 1.0×10^{-4}M
pH = 10.0

4.3.2 First order derivative method (Verification of Beer's law)

At different concentrations of Mercury (II), the first order derivative spectra of the Hg (II)-ACINH was recorded and shown in Fig 4.3.2. For the determination of the amount of Mercury (II), a calibration plot was drawn between the derivative amplitude at 440nm and the amount of Mercury (II) (Fig.4.3.3) was liner and obeys the Beer's law.

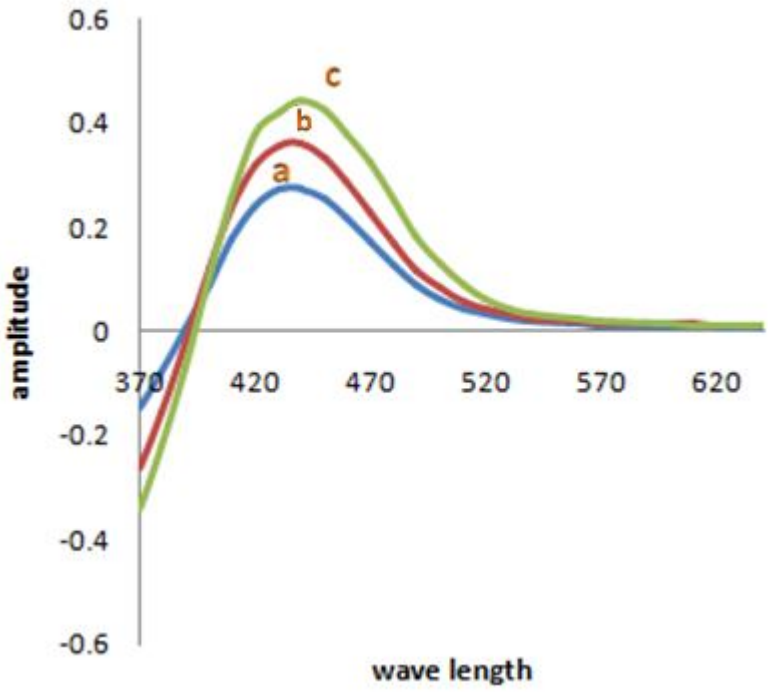


Fig: 4.3.2 First derivative spectra of [Hg (II)-ACINH] vs reagent

Hg (II)(µg/ml) = a) 4.0118; b) 6.0177; c) 8.0236
[ACINH] = 1.0×10^{-4} M
pH = 10.0

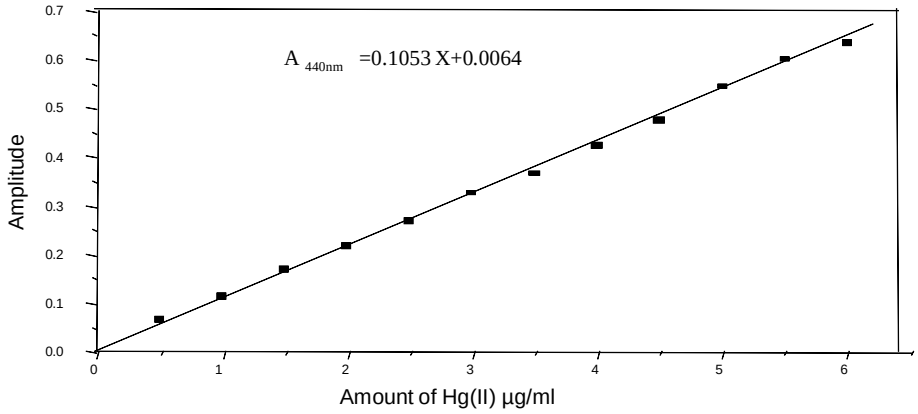


Fig: 4.3.3 First derivative amplitude vs Amount of Hg (II) µg/ml

[ACINH]	=	1.0×10^{-4}M
pH	=	10.0
Wavelength	=	440nm

4.3.3 Effect of Foreign ions

By employing the method described in 3.2.6, the effect of various ions on the derivative amplitude was studied. The interference of various foreign ions was studied in the determination of 5.01475 µg/ml of Mercury (II). For each solution first order derivative spectrum was recorded against reagent blank solution and amplitude was measured at selected wavelength at 440nm from which the tolerance limits of foreign ion were determined. All ions that do not interfere in determination of Mercury (II) (Table 4.2.2), in zero order but also do not interfere in the first order derivative spectrophotometric determination of Mercury (II) (Given in Table 4.3.1)

Table 4.3.1: Tolerance limit of foreign ions in the determination of 5.01475 µg/ml of Mercury (II)

Ion Added	Tolerance Limit (µg/mL)
	First Order derivative
Urea	62
Phosphate	93
Nitrate	92
Oxalate	134
Tartarate	142
Fluoride	28
Bromide	79.9
Iodide	126.9
Mn^{+2}	56
Bi^{+3}	105
Zn^{+2}	92.6
Zr^{+2}	136
W^{+6}	183
Ag +	161
Pd^{+2}	159
V^{+5}	75.3
Sr^{+2}	87

4.3.4 Applications

Determination of Mercury (II) in water samples

For the determination of Mercury (II) in water samples the present derivative method was employed. The water samples preparation and analyzed by the adopting procedure in 3.2.9.1 and the results are summarized in Table 4.3.2.

Table 4.3.2: Determination of Mercury (II) in water samples

Name of the sample	Amount of Mercury(II) found (μg ml^{-1})		
	First order*	AAS method	Error(%)
Laboratory water	1.91	1.87	+2.61
Tap water	0.67	0.69	-2.89
Waste water	3.26	3.28	-0.60
Sea water	1.00	1.02	-1.96
River water	0.65	0.62	+4.83
Drain water	1.15	1.18	-2.54
Well water	0.93	0.84	+10.71

* Average of best three determinations among five determinations

4.4 Direct spectrophotometric determination of Cd (II) using ACINH

The reagent α-Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH) reacts with Cadmium (II) in basic buffer pH 9.0 medium to give water soluble yellow coloured species. The absorbance of the complex was stable for five hours. For the determination of Cadmium (II) by spectrophotometric method the complex was studied systematically in aqueous medium.

4.4.1 Absorption spectra of reagent solution and Cadmium (II)-ACINH complex

At the wavelength region 300-650 nm the absorption spectra of Cadmium(II)-ACINH complex and reagent solution was calculated by employing the method described in 3.2.1 is adopted. A plot between wavelength and absorption is presented in Fig.4.4.1. In figure at 380nm the metal complex has shown maximum absorbance.

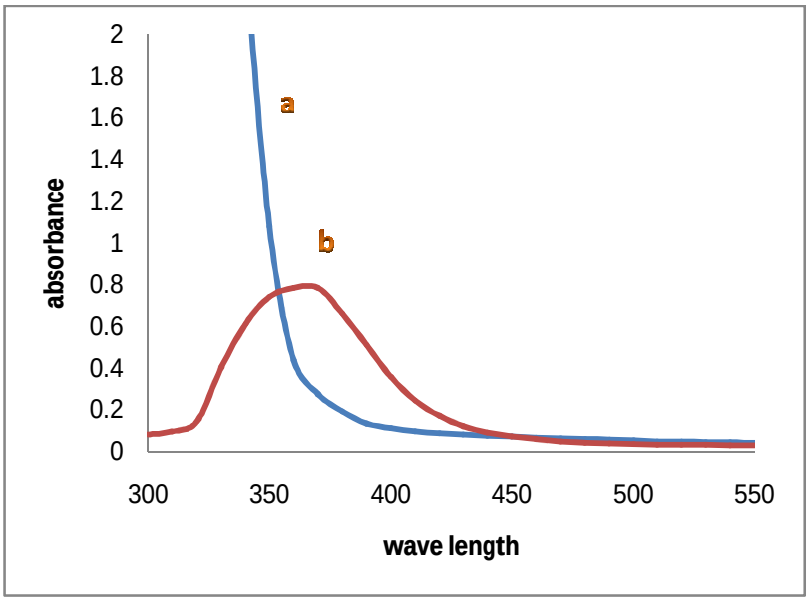


Fig: 4.4.1 Absorption spectra of

a. ACINH vs buffer blank

b. [Cd(II)-ACINH] complex vs reagent blank

[Cd(II)] = 1.0×10^{-5}M

[ACINH] = 1.0×10^{-4}M

pH = 9.0

4.4.2 Effect of pH on the absorbance of Cd (II)-ACINH complex

To arrive the optimum pH required for getting the maximum and constant absorbance by employing the procedure described in 3.2.2 is adopted. The effect of pH on the absorbance of the Cd (II)-ACINH complex was studied. A plot was made between absorbance and pH of the complex and given in Fig.4.4.2.

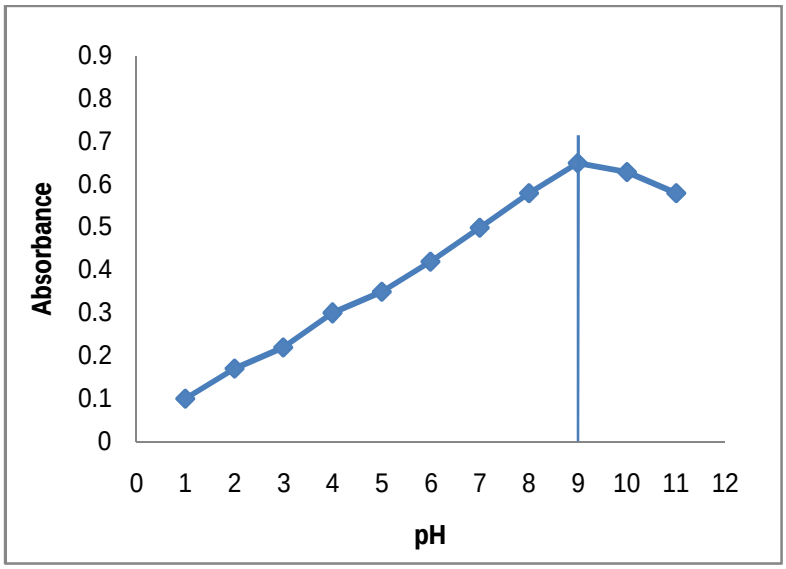


Fig: 4.4.2 Effect of pH on the absorbance [Cd (II)-ACINH] system

[Cd (II)] = 1.0×10^{-5}M
[ACINH] = 1.0×10^{-4}M

4.4.3 Effect of reagent on the absorbance of the metal complex

By employing the procedure described in 3.2.3 how much amount of reagent is necessary for full colour development was established and the results are shown in Table 4.4.1.

Table 4.4.1: Effect of ACINH on the absorbance of the metal complex

[Cd (II)] = 1.0×10^{-5} M
pH = 9.0
Wavelength = 380 nm

Cd (II) : ACINH	Absorbance
1:5	0.416
1:10	0.418
1:15	0.415
1:20	0.416
1:25	0.414
1:30	0.413
1:40	0.412

4.4.4 Effect of time on the absorbance of [Cd (II)-ACINH]

At different time intervals the absorbance of Cadmium (II)-ACINH was measured, for the determination of the time stability of the complex as procedure described in 3.2.4.

4.4.5 Order of addition

The order of addition of metal, reagent and buffer solution has no adverse effect on the absorbance of reaction mixture.

4.4.6 Applicability of Beer's law

By employing the procedure described in 3.2.5 the applicability of Beer's law for the present system was examined. A calibration plot (Fig.4.4.3) was drawn between the amount of Cd (II) and absorbance. System obeys Beer's law. The optimum concentration range, Beer's law absorbance, molar absorptivity and Sandel's sensitivity results are presented in 5.2.6.

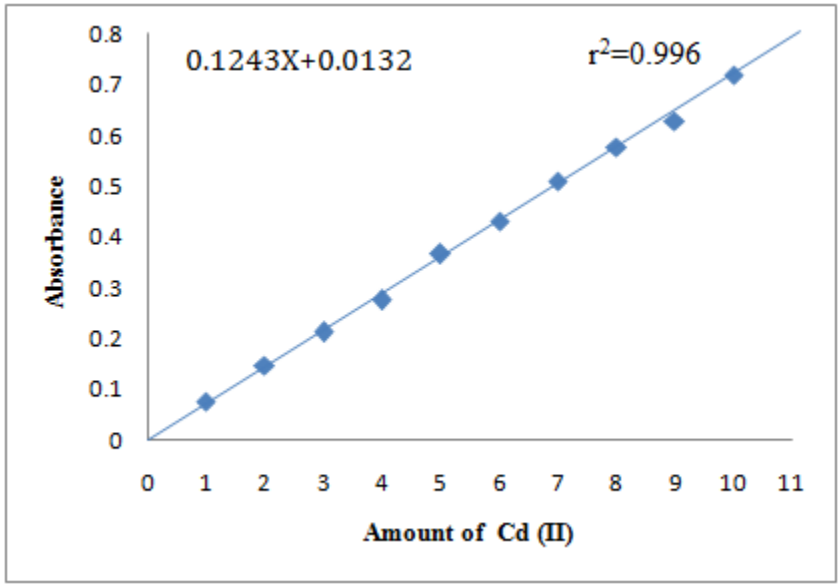


Fig: 4.4.3 Absorbance vs Amount of Cd (II) µg/ml

[ACINH]	=	1.0×10^{-4}
pH	=	9.0
Wavelength	=	380 nm

4.4.7 Tolerance limit of foreign ions

By employing above established optimum conditions with a view to examine the selectivity of reagent, the effect of various foreign ions was studied. This experiment was also intended for the determination of tolerance limit of different associated ions. By employing the procedure described in 3.2.6 the interference of foreign ions was studied with 1.5888 µg/ml of Cadmium(II). The results are summarized in Table 4.4.2.

Table 4.4.2: Tolerance limit of foreign ions in the determination of 1.5888 µg/ml of Cadmium (II)

Ion added	Tolerance limit (µg/ml)
	Zero order
Chlorides	345
Tartarate	521
Iodide	469
Phosphate	41
Ascorbic acid	90
Citrate	655
Bromide	242
Tetra borate	135
Nitrate	57
Acetate	163
Zn(II)	6.4
Ru(III)	5.4
Hg(II)	1.7
V(V)	11.1
Co(II)	6.5
Se(IV)	30
Ba(II)	10
Pb(II)	2
Ag(II)	12
U(VI)	63
Zr(IV)	24
Bi(III)	5
Pd(II)	4
Sn(II)	38

4.4.8 Applications

To the determination of Cadmium (II) in soil samples the present method was applied.

Determination of Cadmium (II) in Soil samples

The recommended procedures given in 3.2.9.1 soil samples were prepared and analyzed for the determination of Cadmium (II). The results are presented in Table 4.4.3.

Table 4.4.3: Determination of Cadmium (II) in soil samples

Name of the sample	Amount of Cadmium (II) foundμg/g		
	AAS method	Present method	Error (%)
Road side sample	1.2	1.1	-8.33
Industrial Sample	0.32	0.26	-18.75
Agriculture sample	0.52	0.57	9.61

* Average of best three determinations among five determinations

4.4.9 Composition and stability constant of the complex

By using Job's continuous variation method the composition of the complex was determined and confirmed by molar ratio method. By using the data obtained from the job's plot (Fig.4.4.4) stability constant of the complex was calculated.

4.4.9.1 Job's continuous variation method

By applying described procedure given in 3.2.7.1 the composition and stability of the metal complex was studied. A graph (Fig.4.4.4) was drawn between mole fraction of Cadmium (II) and absorbance of the complex. Job's curve shows that one mole of metal ion reacts with one mole of reagent.

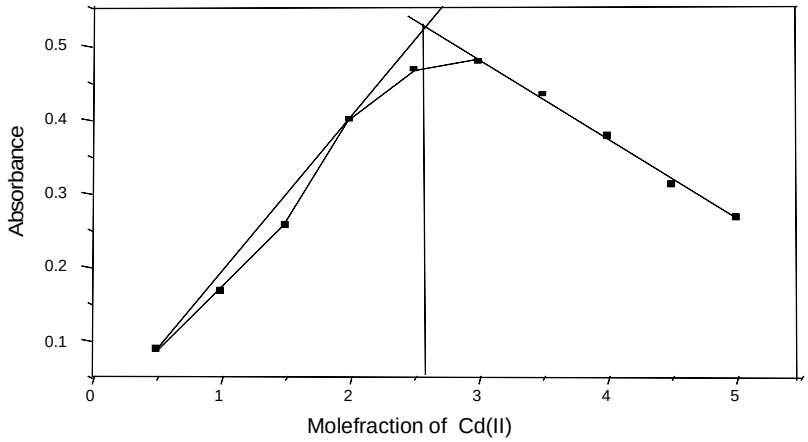


Fig: 4.4.4 Job's curve of [Cd (II)-ACINH] complex

[Hg (II)] = [ACINH] = $1.0x10^{-5}$
pH = 9.0
Wavelength = 380 nm

4.4.9.2: Molar ratio method

By Employing described procedure of molar ratio in 3.2.7.2 the composition of the complex was examined. The plot was drawn between the number of moles of reagent and absorbance at 380nm in Fig.4.4.5.

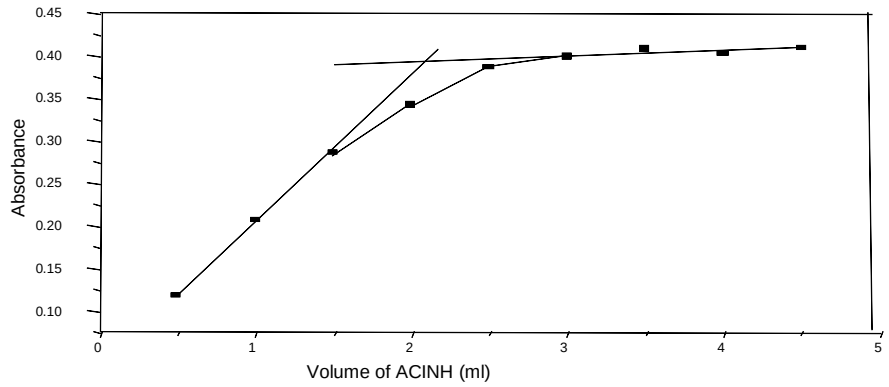


Fig: 4.4.5 Molar ratio plot of [Cd(II)- ACINH] complex

[Cd (II)] = [ACINH] = $1.0x10^{-5}$
pH = 9.0
Wavelength = 380 nm

4.5 Derivative spectrophotometric determination of Cd (II)-ACINH

Cadmium (II) forms water soluble yellow coloured complex with an α-Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH). The coloured complex was stable for five hours, zero order spectrum shows λ_{max} at 380 nm. For the determination of Cadmium (II) in microgram quantities first order derivative spectrophotometric method was developed. The detailed investigation is presented in this section.

4.5.1 Derivative spectra of Cd (II)-ACINH complex

The first order derivative spectrum of Cd (II)-ACINH complex was recorded in pH 9.0 by following the procedure described in 3.2.10. In Fig.4.5.1 maximum amplitude of first order derivative spectrum was observed at 435nm. Therefore determination of Cadmium (II) by first order derivative spectrophotometric was carried out at 435nm.

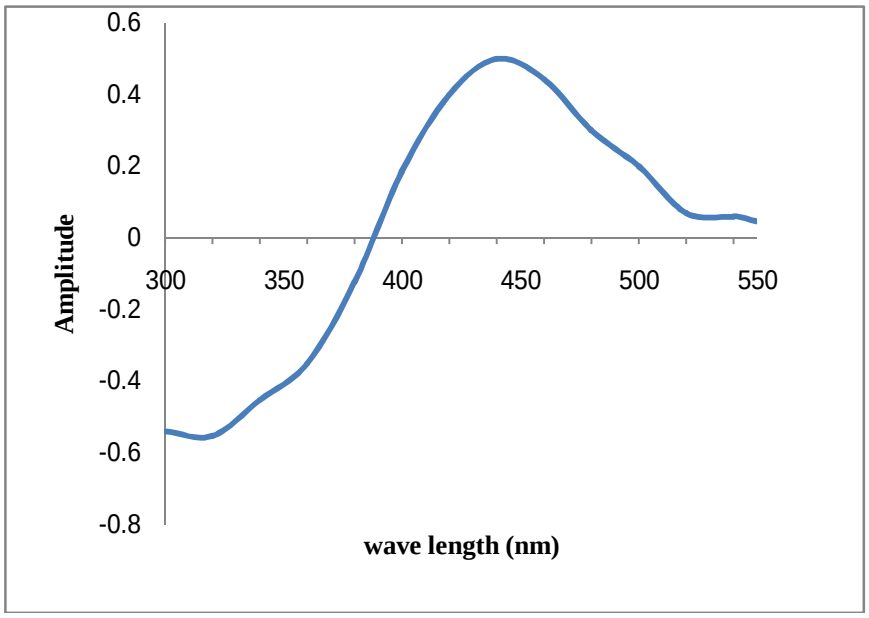


Fig: 4.5.1 First derivative spectrum of [Cd (II)-ACINH] vs reagent

[Cd (II)] = 1.0×10^{-5} M
[ACINH] = 1.0×10^{-4} M
pH = 9.0

4.5.2 First order derivative method (Verification of Beer's law)

At different concentrations of Cadmium (II), the first order derivative spectra of the Cd (II)-ACINH was recorded and shown in Fig.4.5.2. To determine the amount of Cadmium (II), at 435nm a calibration plot was drawn between the derivative amplitude and the amount of Cadmium (II) (Fig.4.5.3) was liner and obeys the Beer's law.

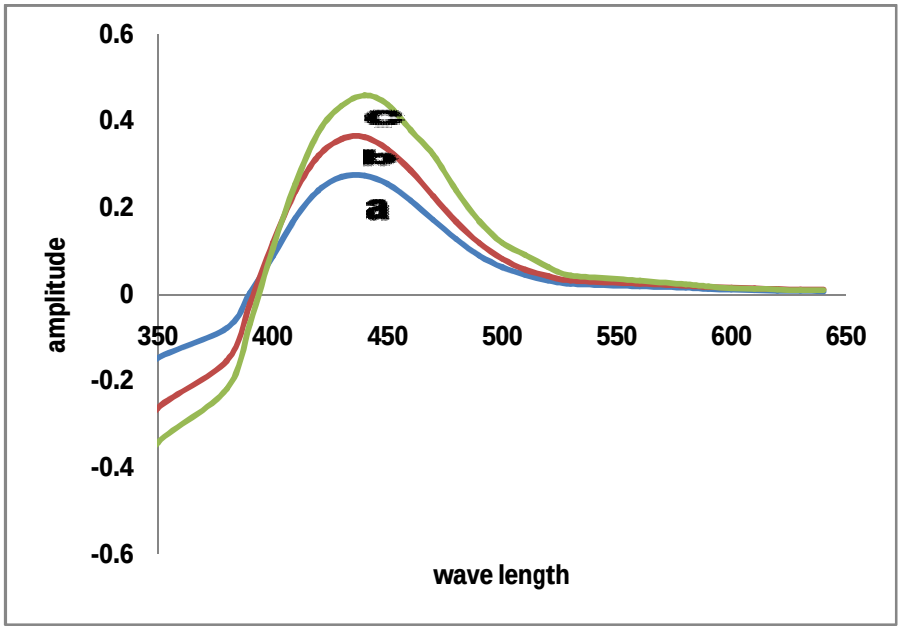


Fig: 4.5.2 First derivative spectra of [Cd (II)-ACINH] vs reagent

Cd (II) (μg/ml)	=	a) 0.232; b) 0.652; c) 0.891
[ACINH]	=	1.0×10^{-4}M
pH	=	9.0

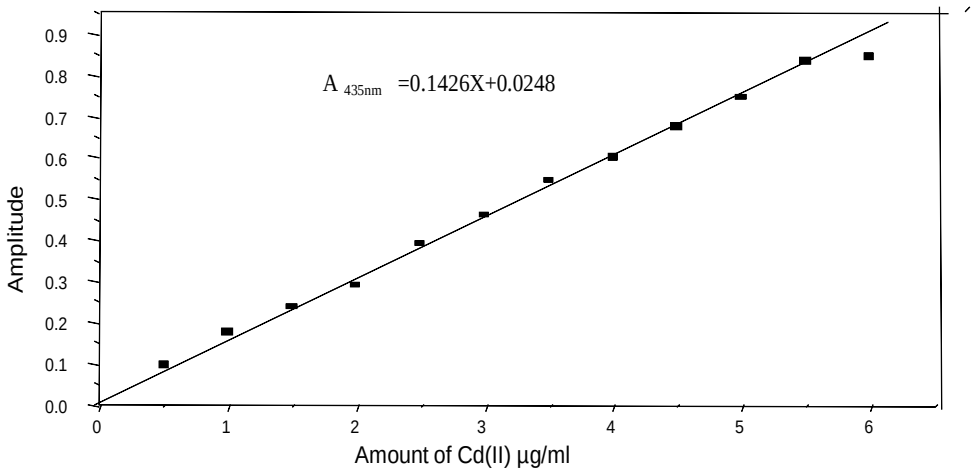


Fig: 4.5.3 First derivative amplitude vs Amount of Cd (II) µg/ml

[ACINH]	=	1.0×10^{-4} M
pH	=	9.0
Wavelength	=	435nm

4.5.3 Effect of Foreign ions

By employing the procedure described in 3.2.6 the effect of various ions on the derivative amplitude was studied. The interference of different foreign ions was studied in the determination of 1.5888 µg/ml of Cadmium (II). For each solution first order derivative spectrum was recorded against reagent blank solution and amplitude was measured at selected wavelength at 435nm from which the tolerance limits of foreign ion were determined. All ions that do not interfere in determination of Cadmium (II) (Table 4.4.2), in zero order also do not interfere in the first order derivative spectrophotometric determination of Cadmium (II) (Given in Table 4.5.1).

Table 4.5.1: Tolerance limit of foreign ions in the determination of 1.5888 µg/ml of Cadmium (II)

Ion added	Tolerance limit (µg/ml)
	First Order
Chlorides	343
Tartarate	517
Iodide	471
Phosphate	39
Ascorbic acid	95
Citrate	640
Bromide	240
Tetra borate	139
Nitrate	60
Acetate	160
Zn(II)	6.5
Ru(III)	5.1
Hg(II)	1.5
V(V)	12.3
Co(II)	6.1
Se(IV)	25
Ba(II)	13
Pb(II)	8
Ag(II)	10
U(VI)	65
Zr(IV)	20
Bi(III)	6
Pd(II)	8
Sn(II)	35

4.5.4 Applications

Determination of Cadmium (II) in soil samples

For the determination of Cadmium (II) in soil samples the present derivative method was employed. The soil samples preparation and analyzed by the adopting procedure in 3.2.9.1 and the results are summarized in Table 4.5.2.

Table 4.5.2: Determination of Cadmium (II) in soil samples

Name of the sample	Amount of Cadmium (II) found µg/g		
	AAS method	Present method	Error (%)
Road side sample	1.2	1.1	-8.33
Industrial Sample	0.32	0.26	-18.75
Agriculture sample	0.52	0.57	9.61

* Average of best three determinations among five determinations