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Spectrophotometric determination of mercury (II) in environmental samples and synthetic mixtures using N'-(1-(pyridin-2-yl) ethylidene) isonicotinohydrazide as sensitive analytical reagent

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ABSTRACT

N'-(1-(Pyridin-2-yl)ethylidene)isonicotinohydrazide (2-ACPINH) is identified as a selective and sensitive analytical reagent for the determination of trace levels of mercury (II) in environmental samples and synthetic mixtures by spectrophotometry. 2-ACPINH reacts with mercury (II) forms greenish yellow colored 2:3 (Hg-2-ACPINH) complex at pH 5.5, in aqueous DMF. The Hg-2-ACPINH complex shows maximum absorbance at λ_{max} *357nm. The color reaction is immediate and the absorbance remains constant for about 24h.The molar absorptivity coefficient and Sandell's sensitivity are found to be* 5.48×10^{4} $L.mol^{-1}$ cm^{-1} *and 0.0010µg* cm^{-2} *of mercury (II) respectively. The linear regression coefficient is 0.99925 and standard deviation is 0.04175. The system obeys Beer`s law in the range of 0.2-2.0 µg* mL^{-1} *of Hg (II). Large number of cations, anions and complexing agents do not interfere in this method. This method is successfully applied for the determination of mercury in a number of environmental water samples (potable and polluted), food samples and synthetic mixtures.*

INTRODUCTION

Mercury can exist in several forms, and all forms are poisonous. The most common forms of mercury that occur naturally in the environment are metallic mercury. Microorganisms and various natural processes can change these forms of mercury, from one form to the other. Methyl mercury is the most common form produced by these natural processes. This compound is of particular concern because it can bio accumulate and biomagnify through the food chain[1]. Acute exposure of organic mercury affects the central nerves system which causes blindness, deafness, tremors, insomnia, memory loss, neuromuscular changes, headaches, slow sensory and slow motor nerve function and reduction in cognitive function. The consumption of inorganic mercury compounds causes metallic taste in the mouth, nausea, vomiting and severe abdominal pain[2]. Chronic exposure to mercury can result in several diseases, including, acrodynia (pink disease), neurological diseases, Hunter-Russell syndrome, mad hatters disease, leukemia and Minamata disease [3-8]. In contrast, some forms of mercury are relatively non-toxic and have been used as medicines e.g. for the treatment of syphilis [9]. Due to human activities major amount of mercury enters into the environment through chlor-alkali industries, plastic industries, electrical, paint and pharmaceutical industries [10-15]. In addition, Mercury compounds are used as catalysts, fungicides, pesticides and also add mercury to the environment *via* the natural resources like water, food and igneous rocks, in particular granite for living organisms and additionally increases the danger of mercury exposure, even at trace levels [16, 17]. The environmental contaminants such as Pb^{2+}, Cd^{2+} and Hg^{2+} have been exploited by a number of research groups [18]. All these findings cause great concern regarding public health, demanding an accurate determination of this metal ion at trace and ultra-trace levels.

Several authors have reported on the determination of mercury (II) by using various ligands by spectrophotometry. However, the reported spectrophotometric methods [19-24] suffer from one or more disadvantages such as severe interferences, less sensitivity, less selectivity and difficulty in the preparation of reagent etc. However, none has been reported on spectrophotometric determination of Hg(II) in environmental samples using N'-(1-(pyridin-2-yl)ethylidene) isonicotinohydrazide (2ACPINH) as analytical reagent.

Herein, we developed a highly efficient spectrophotometric method for the determination of mercury (II) in environmental water samples (potable and polluted), food samples and synthetic mixtures using N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide (2ACPINH) as sensitive and selective analytical reagent.

Table. 1. Comparison of present method with other reported spectrophotometric methods

Name of the reagent	λ_{max}	**pH**	**Linearity range µg/mL**	**εx10^{4} (L mol^{-1}cm^{-1})**	**M:L**[a]	**Ref.**
Diacetylmonoximeisonicitinoylhydrozone (DMIH)	375	8.0	0.8-8.0	1.8	1:1	[19]
4-hydroxy3,5- dimethoxy benzaldehyde -4- hydroxy benzoylhydrazone	351	5.5	1.0-12.0	2.29	-	[20]
Diphenylcarbazone(Dithiozone)	420	4.0	0.60-2.70	4.56	-	[21]
6-Hydroxy-3-(2-oxoindolin-3-ylideneamino)2-thioxo-2H-1,3-thiazin-4(3H)one	505	4-6	0.2-2.0	4.0	-	[22]
2-Acetylpyridine Thio semicarbazone	351	5.0-7.0	0.24-2.40	5.4	1:2	[23]
Isonitriso p-isopropyl Acetophenone Phenyl Hydrazone (HIPAPH)	395	10.0	1.0-20.0	2.678	1:2	[24]
N'-(1-(pyridin-2-yl)ethylidene) isonicotinohydrazide (2ACPINH)	357	5.5	0.2-2.0	5.48	2:3	Present Method

[a] *M:L is Metal (Hg) and ligand (2-ACPINH) ratio*

MATERIALS AND METHODS

Apparatus

A Double beam UV-Visible spectrophotometer (Shimadzu model UV-1800) with a 1.0 cm quartz cell is used for absorbance studies and pH meter (Systronics model 3305) is used for measurement of pH respectively. Melting point is determined and is uncorrected. Flame atomic absorption spectrophotometer (Shimadzu model No: AA-6300). ^{1}H NMR spectrum is recorded on Jeol 400 MHz NMR Spectrometer (JNM-400) and Mass spectrum is recorded on Shimadzu-LCMS with ESI probe (LC-2010EV).

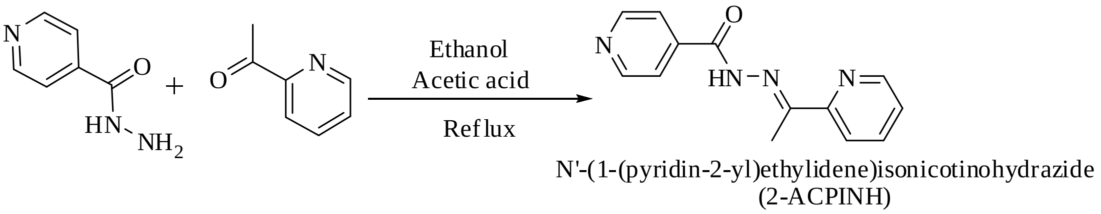
Reagents and solutions

All the chemicals used are of analytical reagent grade or the highest purity available (Aldrich ACS or Merck pro-analysis grade). DMF and Double distilled water are used though out.

Preparation of N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide (2-ACPINH)

Microwave method

Isonicotinic acid hydrazide [7.3 mmol, 1.0 gm] and 2-acetyl pyridine [7.3 mmol,1.0 gm] are dissolved in 10 mL of ethanol in a 100-mL beaker and then 5% glacial acetic acid [2.5 mL] is added. The beaker is placed in a domestic microwave oven at 200 watts for 30-45s. The progress of the reaction is monitored by TLC. After completion of the reaction, the reaction mixture is cooled to RT. The formed crude product is washed twice with cold ethanol and dried. Finally, the obtained product is re-crystallized from hot ethanol. The structure of the synthesized compound is confirmed by ^{1}H NMR and Mass Spectral data.



Scheme 1. Preparation of ligand, N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide (2-ACPINH)

Characterization data of N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide(2-ACPINH)

Yellow color solid; Yield: 1.88 g (95%); mp 220-222^o C.; ^{1}H NMR (400 MHz, DMSO-d$_{6,}$ δ/ppm): 11.12 (s, 1H, -NH), 8.78 (d, 2H, *J*=4.4 Hz, arom H), 8.13 (d, 1H, *J*=8.0, arom H), 7.89 (d, 1H, *J*=5.6 Hz, arom H), 7.82 (d, 2H, *J*=7.2 Hz, arom H)7.65 (t, 1H, *J*=6.0 Hz, arom H), 7.46 (t, 1H, arom H), 2.513 (s, 3H, -CH$_3$).; MS (ESI): *m/z* (M+H)$^+$ 240.80.

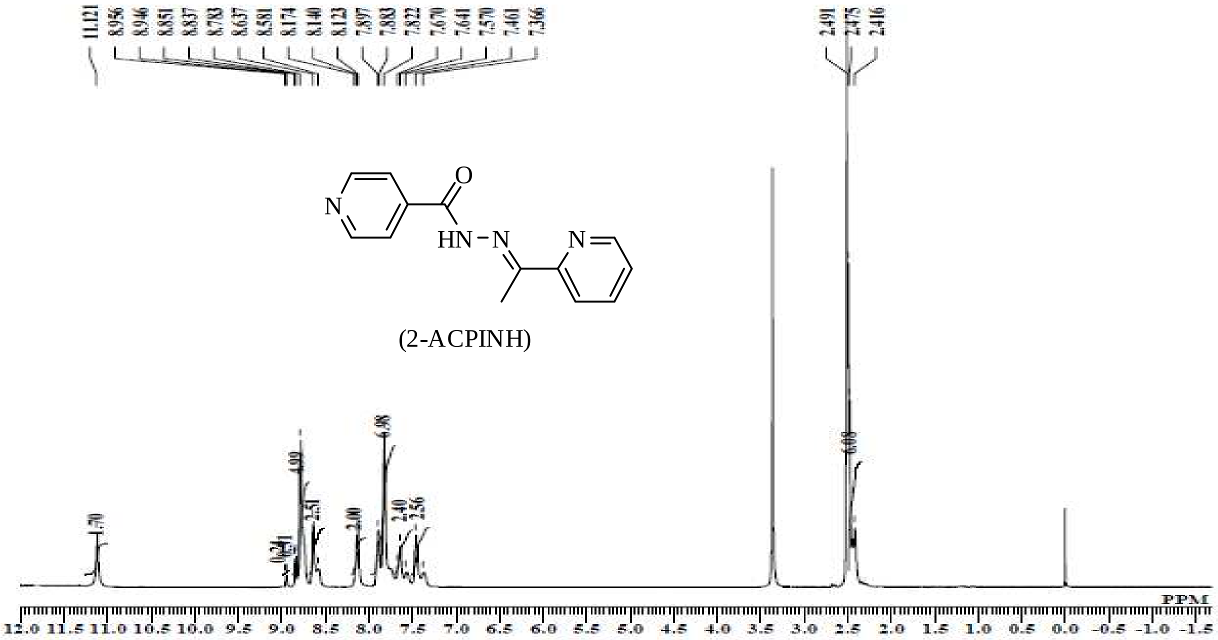


Figure 1. ^{1}H NMR spectrum of ligand, N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide (2-ACPINH)

Preparation of 0.01 M of ligand, 2-ACPINH solution

0.01M of 2ACPINH reagent is prepared by dissolving 0.240 gm of 2-ACPINH in 100 mL of DMF. This stock solution is diluted further, whenever necessary with DMF.

Preparation of mercury (II) stock solution

0.01M Mercury solution is prepared by dissolving 271.5mg of mercury chloride(Merck Darmstadt) in de-ionised water containing few drops of concentrated Hydrochloric acid and made up to the mark in a 100-mL volumetric flask. Aliquots of this solution are standardized with EDTA using xylenol orange as an indicator. Dilute solutions are prepared from this stock solution. Solutions of large number of inorganic ions and complexing agents are prepared from their analytical grade or equivalent grade, water soluble salts [25].

Preparation of buffer solutions

Hydrochloric acid (1M) and Sodium acetate (1M) are mixed to get the required pH (1-3.5); 0.2M Sodium acetate and 0.2M Acetic acid are mixed to get the required pH (4-6) and Ammonium acetate buffer (pH 7) solutions are used.

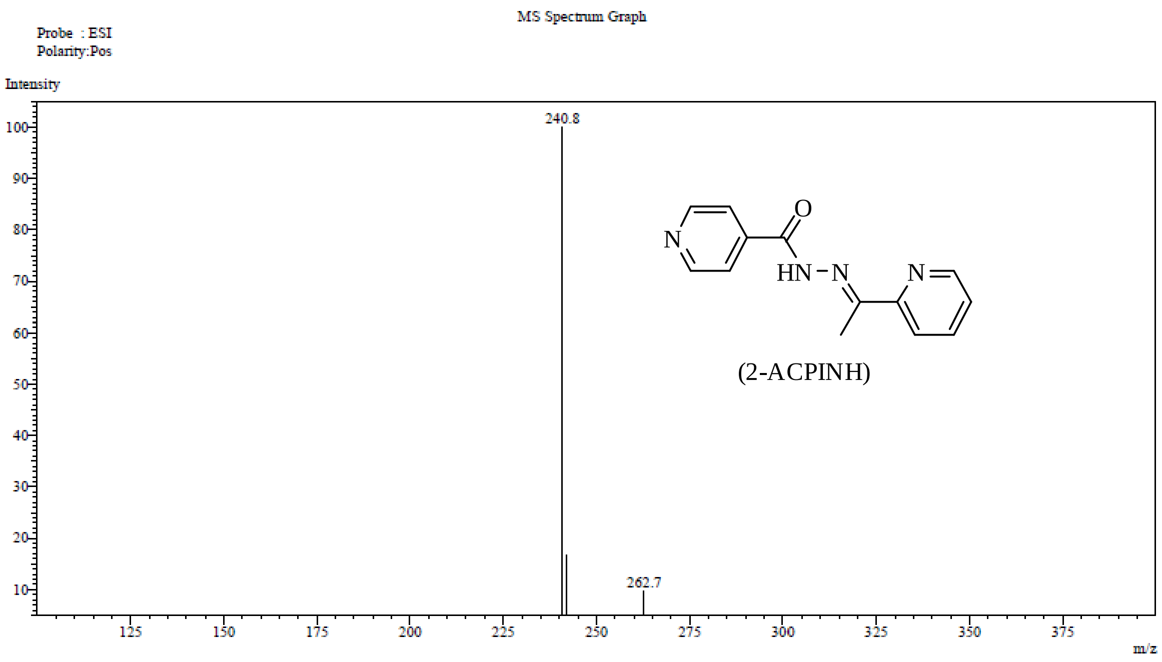


Figure 2. Mass spectrum of ligand, N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide (2-ACPINH)

Acetic acid and sodium acetate buffer solution of pH 5.5 is prepared by mixing 25 mL of 0.2 M acetic acid and required quantity of 0.2 M sodium acetate solutions. The pH of the above buffer solution is measured by a pH meter and finally adjusted suitably.

General analytical procedure for the determination of mercury (II)

An aliquot (2.0 mL) of the solution containing known amount of mercury (II), 4.0 mL of sodium acetate-acetic acid buffer solution (pH5.5) and 3.0 mL of 2ACPINH reagent of required concentration are mixed in a 10-mL volumetric flask and the resulting solution is diluted up to the mark with double distilled water. The absorbance of this solution is measured at 357nm, against ligand blank.

RESULTS AND DISCUSSION

N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide reacts with Mercury (II) forms a greenish yellow colored complex in aqueous DMF at pH 5.5. The Hg-2ACPINH complex shows maximum absorbance at λ_{max} 357nm against the reagent blank. Hence, a detailed study has been undertaken for the determination of Mercury (II) using 2-ACPINH by spectrophotometric method. The optimized method is successfully applied for the determination of mercury in water samples, food samples and synthetic mixture alone or in presence of diverse ions.

Absorption spectra of ligand, 2-ACPINH and Hg(II)-2ACPINH complex (MLC)

Absorption spectrum of the 2ACPINH is recorded against the solvent blank initially. The absorption spectrum of Hg(II)-2ACPINH complex is recorded against the reagent blank. The absorption spectrum of both complex and ligand are shown in Figure 3. From the absorption spectra, it is clear that the ligand shows maximum absorption at 284 nm where as the complex shows maximum absorption at 357 nm. Therefore, all the spectral measurements are carried out at 357 nm.

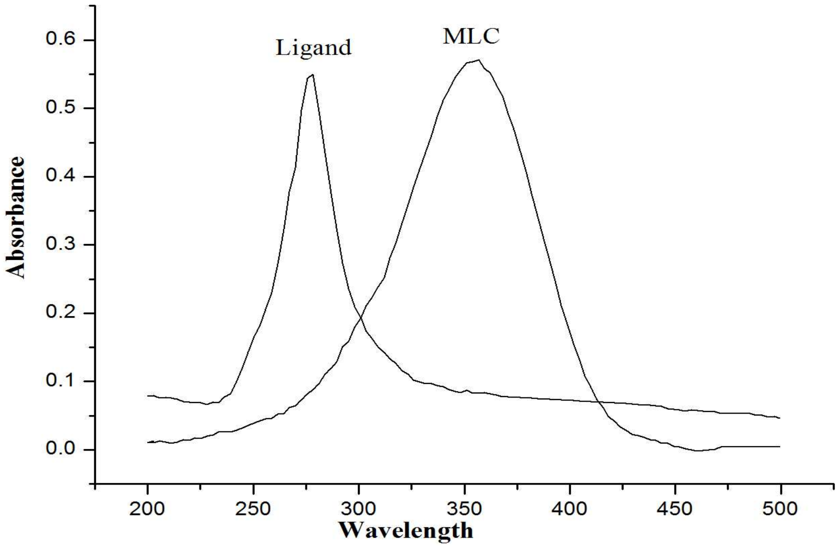


Figure 3. (A) Absorption spectrum of ligand (2ACPINH) Vs. solvent (DMF) blank (B) Absorption spectrum of Hg(II)-2ACPINH complex (MLC) Vs. Ligand blank; Hg(II) = 2.0 mL of 1.0x10^{-4}M, 2ACPINH = 3.0 mL of 1.0x10^{-4}M and pH = 5.5.

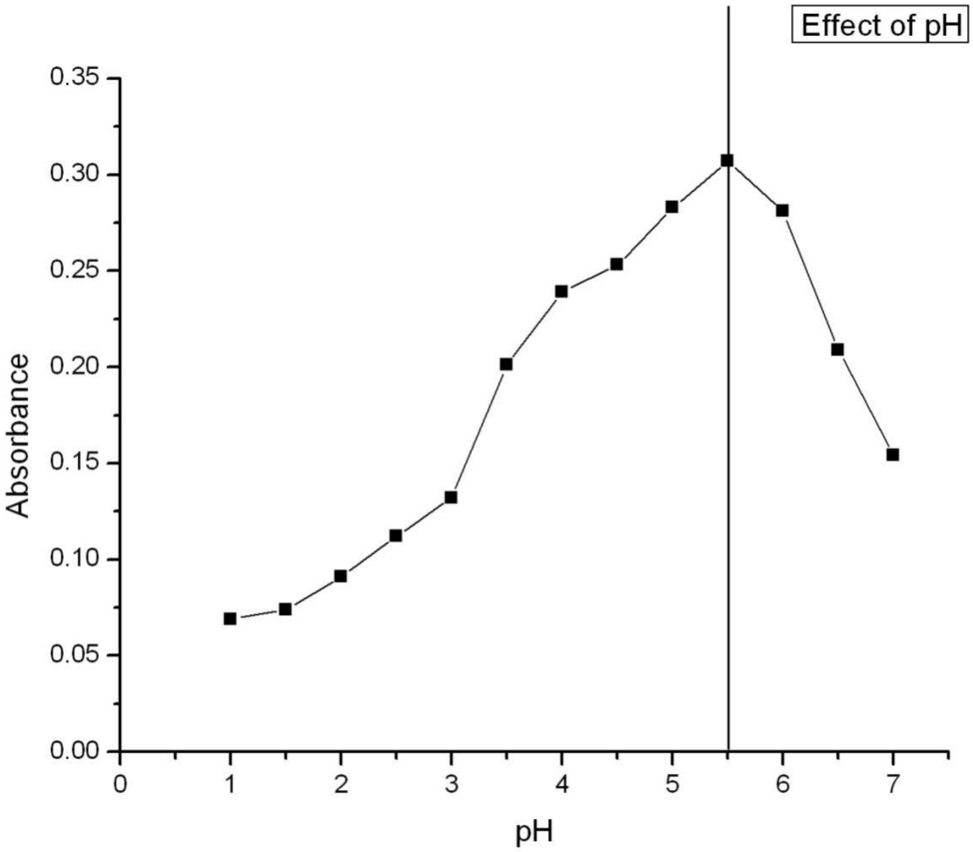


Figure 4: Effect of pH on the absorbance of Hg (II)-2ACPINH complex; Hg (II) = 2.0 mL of 1.0x10^{-4} M; 2ACPINH=3.0 mL of 1.0x10^{-4}M; λ_{max} = 357 nm

Effect of pH

Into a series of 10-mL volumetric flasks, 2.0 mL of Hg (II) solution ($1.0x10^{-4}$M), 3.0 mL of Ligand solution ($1.0x10^{-4}$M), and 4.0 mL of buffer of varying pH (1.0-7.0) are added and made up to the mark with double distilled water and the absorbance is measured against ligand blank at 357 nm. The absorbance increases from pH 1.0 to 5.5 and then decreases. Hence, pH5.5 is optimized for further studies (Figure 4).

Applicability of Beer's law

The known aliquots of 10.0 mL solutions, each containing constant volumes of 4.0 mL of buffer (pH 5.5), 3.0 mL of 1.0×10^{-4}M of reagent and 2.0 mL of mercury (II) solutions of concentrations in the range from 0.10×10^{-4} to 1.0×10^{-4}M (0.2-2.0 µg mL^{-1}) are prepared. The absorbance of these solutions is measured at 357 nm. A graph plotted between the amount of Mercury (II) and its absorbance is shown in Figure 5. It can be observed from the graph that, a linear plot passing through the origin obeys Beer's law in the range from 0.2-2.0 µg mL^{-1} of Mercury (II). The correlation coefficient is 0.99925 which indicates the linearity between the two variables. The molar absorptivity coefficient and sandal's sensitivity of the complex are found to be $5.48x10^{4}$ L mol^{-1} cm^{-1} and 0.0010µg cm^{-2}, respectively.

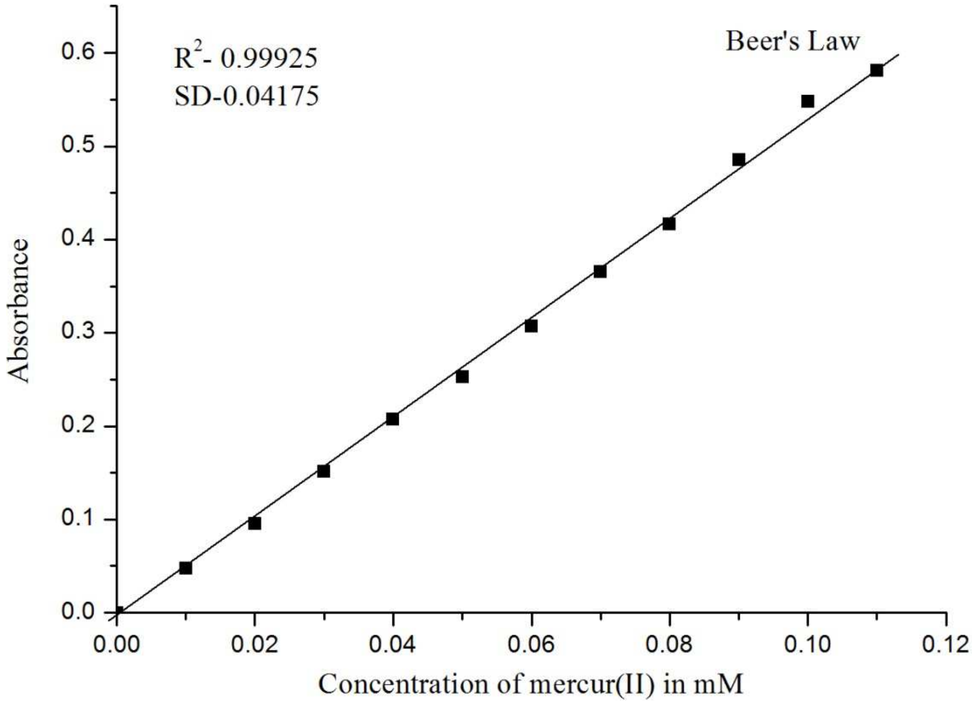


Figure 5. Effect of Metal concentration- 2ACPINH = 3.0 mL of 1.0x 10^{-4}M, Hg(II) = 2.0 mL of ($0.1x10^{-4}$M - 1.0x 10^{-4} M), pH = 5.5 and λ_{max}= 357 nm

Job's method of continuous variation

Equi-molar solutions of mercury (II) ion and ligand 2ACPINH (1.0×10^{-4}M concentration each) is prepared. The metal and reagent solutions are mixed in different proportions, keeping the total volume of metal and ligand is constant at 5.0 mL. In each case, 4.0 mL of sodium acetate-acetic acid buffer (pH 5.5) is added to the mixture and the total volume of the solution is made up to 10.0 mL with double distilled water. The absorbances of all the solutions are measured at 357nm against their reagent blanks. The corresponding graph (Figure 4) is drawn between absorbance and V_M/V_L+V_M (where V_L and V_M are the volumes of the reagent and the metal, respectively) indicates that two moles of Mercury(II) reacts with three moles of ligand and the composition of metal to ligand complex as 2:3 ratio (Figure 6). The composition of the M-L complex is further confirmed by Molar ratio method (Figure 7).

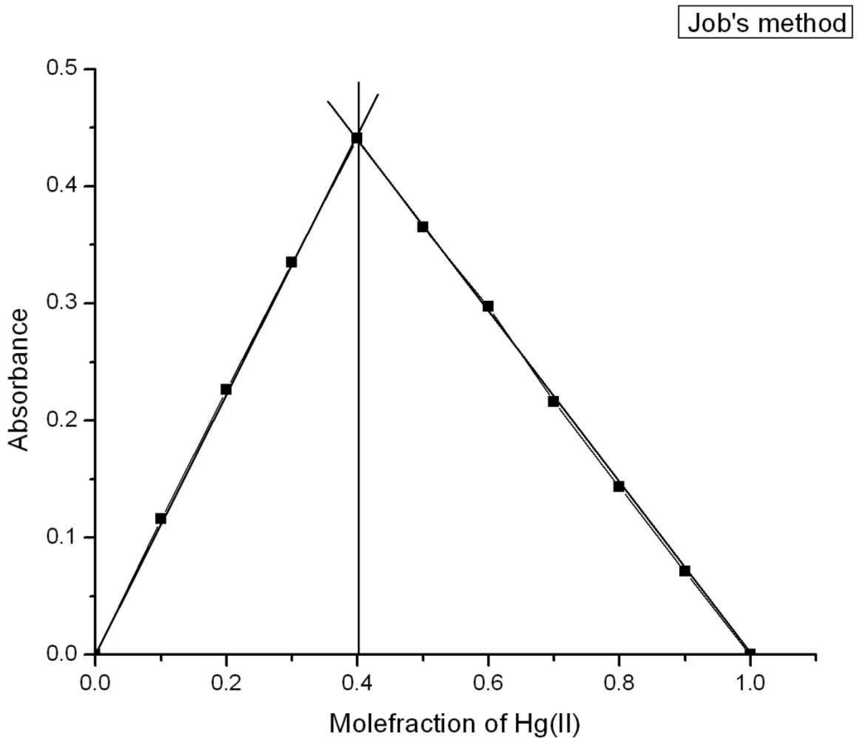


Figure 6. Jobs method of continuous variations for stoichiometric ratio between Hg(II) and 2-ACPINH (1.0×10^{-4}M concentration each); pH = 5.5 and λ_{max} = 357 nm

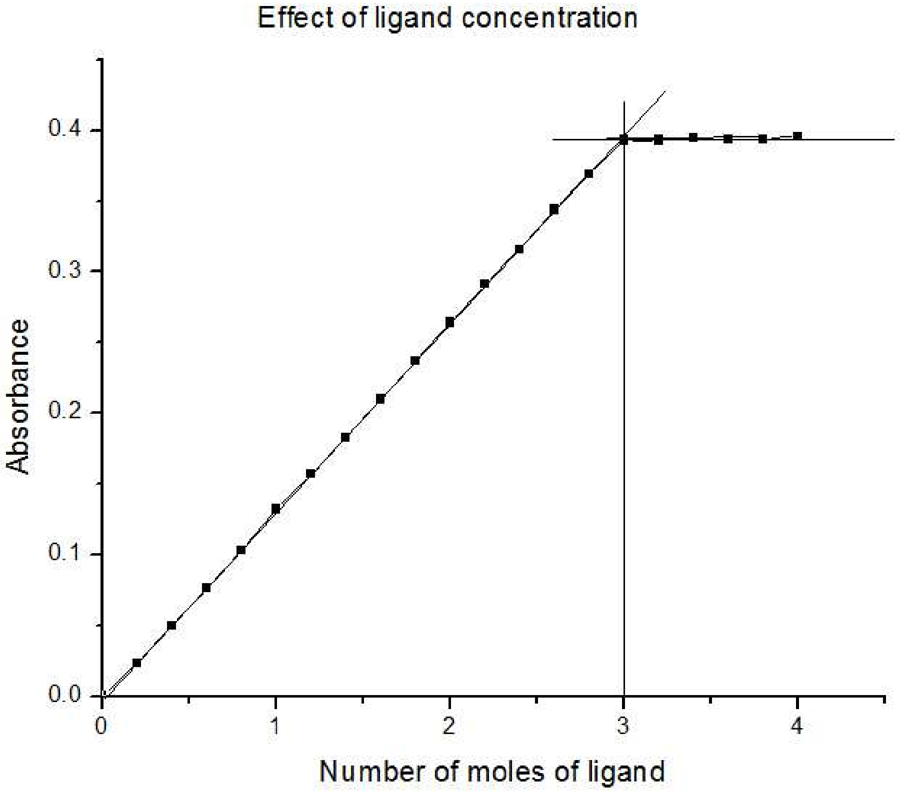


Figure 7. Effect of Ligand concentration: 2ACPINH=3.0mL of ($1.0x\ 10^{-5}$ -$3x10^{-4}$M), Hg(II)=2.0 mL of ($1.0x\ 10^{-4}$ M), pH=5.5 and λ_{max}=357nm

Mole ratio method

Effect of ligand concentration

In to a 10-mL volumetric flask 2.0 mL of $1.0x10^{-4}$M Hg(II) solution,4.0 mL of buffer pH5.5 and 3.0 mL of varying amounts of ($1x10^{-5}$ to $3.0x10^{-4}$M) ligand (2-ACPINH) are added and made up to the mark with water and measured the absorbance at 357 nm against the corresponding ligand blanks. From the results, it is clear that 3.0 moles of ligand is necessary for the maximum recovery of 2.0 moles of Hg (II). The results are plotted in Figure 7.

Effect of metal ion concentration:

Different molar excesses of Hg(II) are added to the fixed amount of 2-ACPINH and absorbance is measured according to the standard procedure. It is observed that the reagent and the Hg(II) metal molar ratio is 2:3. Based on the above two methods the composition of the Hg(II)- 2ACPINH complex is confirmed as 2:3 ratio (Figure 8).

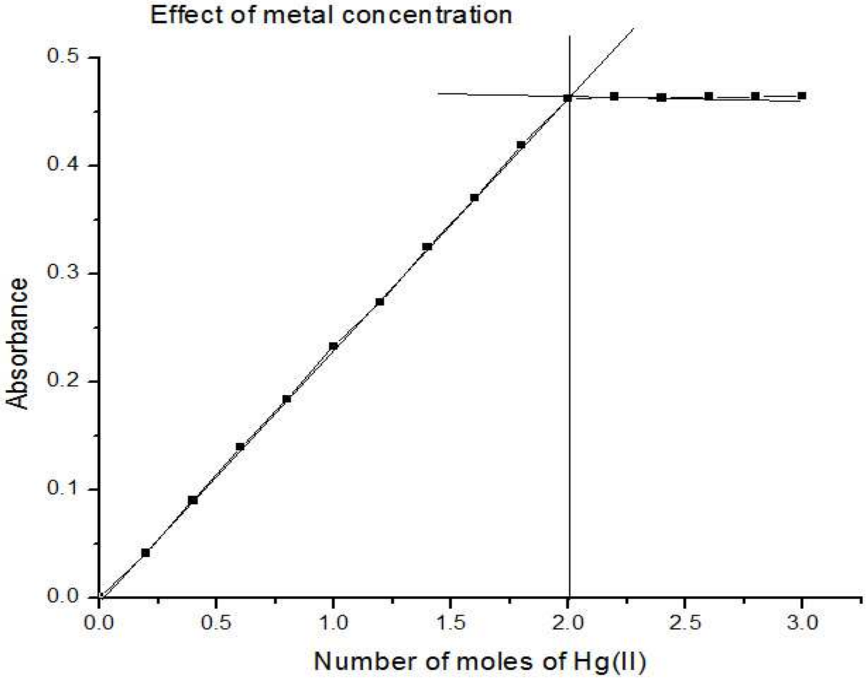


Figure 8: Effect of Metal concentration: 2ACPINH=3.0 moles of 1.0x 10^{-4} M, Hg(II)=2.0 moles of ($0.1x10^{-4}$M-1.0x 10^{-4} M), pH=5.5 and λ_{max}=357nm.

Effect of foreign Ions

The effect of various cations and anions which are generally associated with the metal ion on the determination of the mercury (II) is studied by measuring the absorbance of the mercury complex containing 1µg mL^{-1} of mercury (II) in solution.

The effect of foreign ions on complexation is studied by taking 1.0 mL of Hg(II) solution, 1.0 mL of required concentration of the foreign ion solution, 4.0 mL of sodium acetate acetic acid buffer (pH 5.5) and 3.0 mL of 2ACPINHsolution in a 10-mL standard flask. The total volume of the solution is brought to 10.0 mL with double distilled water. The experiment is repeated by changing the concentration of the diverse ion. The absorbance is measured at 357 nm. A change of ± 0.02 is taken as the tolerance limit for interference.

The results indicated that Ca(II), Mg(II), Pb(II) and Mn(II) do not interfere even when present up to 10000 µg. Interference due to Al(III) and Cr(III) can be tolerated up to 5000 µg, whereas Mo(VI)and W(V) can be tolerated up to 2500 µg only. Extraction of copper (II) is not possible in the presence of Co(II), Ni(II), Fe (II)/Fe(III), Zn(II), Pd(II), and Cd(II), due to their severe interference, even when present in trace amount. Anions such as fluoride, bromide, chloride, nitrate, sulfate, thiosulfate and acetate do not affect the extraction of mercury (II), even when present up to 5000 µg. In the presence of thiocyanate and oxalate, extraction of mercury(II) is not possible. 1.0 mL of 0.2% sodium fluoride has been used as a masking agent for Fe(III) and 1% potassium iodide for Ni(II), Co(II). Zn(II) and Cd(II) do not interfere in the pH range studied.

Table. 2: Physico-chemical and analytical characteristics of Hg(II)-ACPINH complex (MLC)

Characteristics of MLC[a]	Obtained Results
λ_{max}(nm)	357
pH range (optimized)	5.5
Mole ratio of M:L	2:3
Beer's law validity range(μg/mL)	0.2-2.0
Molar absorptivity coefficient (lit mol^{-1} cm^{-1})	5.48×10^4
Sendell's sensitivity (μg of Hg(II) cm^{-2})	0.0010

[a]*MLC is Metal-ligand complex*

SCOPE OF THE METHOD

The present method is successfully applied to the determination of mercury when present alone or in presence of diverse ions. The method is also extended to the determination of mercury in environmental samples, food samples and synthetic mixtures.

Determination of mercury in environmental water samples

Different water samples are collected from in and around KADAPA, A.P. India. Each filtered water samples is evaporated nearly to dryness with a mixture of 5.0 mL of concentrated H_2SO_4 and 10.0 mL of concentrated HNO_3 in a fume cupboard and then cooled to room temperature. The residue is then heated with 10.0 mL of double distilled water, in order to dissolve the salts. The solution is cooled and neutralized with dilute NH_4OH in the presence of 1–2 mL of 0.01% (W/V) tartarate solution. The resulting solution is filtered and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with double distilled water [26].

Table 3. Determination of mercury(II) in water samples

Sample	Mercury (μg/L)- (Spiked) added	Concentration of Hg(II) by AAS Method	Found concentration of Hg(II)	Recovery (%)
Waste water[1]	100	100.028	100.03	100.03%
	500	500.16	500.15	100.03%
Sea water[2]	100	100.036	100.034	100.034%
	500	500.19	500.17	100.034%
Tap water[3]	100	100.0	100.0	100.0%
	500	500.01	499.98	99.99%

[1]*Collected from industrial area, Kadapa.;* [2]*Bay of Bengal,Nellore.;* [3]*Yogi Vemana University, Kadapa.*

Determination of mercury in food samples

Food samples are collected from various villages around Kadapa, A.P. India. The samples are cleaned and dried in open air, protecting them from mineral contamination. The dried sample is pulverized in a mortar for the purpose of analysis, to a convenient size. One gram of powdered food sample is taken in a 100-mL beaker and digested with 10.0 mL of concentrated Nitric acid and Hydrochloric acid [27]. Further add 10.0 mL of double distilled water and then filtered by using Whatmann No.41 filter paper. The filtrate is taken in a 10-mL standard flask made up to the mark with double distilled water and analyzed as per the general procedure.

Table. 4 : Concentration levels of mercury in food samples

S. No.	Name of the food sample	Concentration of mercury (Hg) determined by AAS Method (ppm)	Concentration of mercury (Hg) in the present method (ppm)*	Standard Deviation
1	Wheat grain *(Triticum aesivum)*	0.012	0.01	0.001414
2	Cabbage *(Brassica oleracea)*	0.066	0.063	0.002121
3	Carrot *(Daucas carota)*	0.060	0.057	0.002121
4	Potato *(Solanum tuberosum)*	0.098	0.093	0.003536
5	Tomato *(Lycopersicon esculentum)*	0.13	0.11	0.014142

**Average of five readings*

Preparation of Synthetic mixtures

Metal ion solutions of Cu^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Hg^{2+}, Pd^{2+}, Cd^{2+} and Pb^{2+} are prepared from Merck - analytical grade stock standards of concentration 1000mg/l. The synthetic water solutions are then prepared by mixing the different metal ions as prescribed in the table.5. The aliquot is analyzed for Hg(II), using 2HAPINH adopting the recommended procedure.

Table. 5: Determination of Hg(II) in Synthetic mixtures

Samples	Metal 1 (2µg/mL)	Metal 2 (2µg/mL)	Metal 3 (2µg/mL)	Metal 4 (4µg/mL)	AAS (µg/mL	Present Method (µg/mL)	% of recovery
1	Fe	Co	Ni	Hg	3.98	3.94	98.5
2	Co	Ni	Mn	Hg	3.99	3.95	98.75
3	Fe	Zn	Ni	Hg	3.97	3.93	98.25
4	Cd	Pb	Ni	Hg	3.96	3.96	99.25
5	Cu	Pd	Mn	Hg	3.98	3.97	99.50

CONCLUSION

We demonstrated a simple and efficient spectrophotometric method for the determination of Hg (II) in water samples, food samples and synthetic mixtures using highly sensitive and selective N'-(1-(Pyridin-2-yl)ethylidene)Isonicotinohydrazide as an analytical reagent. It can be used as an alternative method for routine quality control analysis of Hg(II). The proposed method offers advantages like good sensitivity, selectivity, reliability, reproducibility, less interference and immediate color development. The developed method is found to be quantitative comparable to other standard methods. The molar absorptivity of the complex (5.48 x10^4 L $mol^{-1}cm^{-1}$) reveals that the ligand is highly sensitive for mercury (II) when compared with other hydrazones. A number of associated elements don't interfere in the determination of Mercury. Hence, 2ACPINH is an alternative ligand for the spectrophotometric determination of amount of Mercury (II) in low and trace levels from various natural water and food samples.

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