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Determination of mercury levels in environmental samples using 2-acetylfuran isonicotinoyl hydrazone by spectrophotometric method

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ABSTRACT

2-acetylfuran isonicotinoyl hydrazone (AFINH) has been synthesized and proposed as a new chromogenic reagent for a rapid, simple, selective, direct and non-extractive spectrophotometric method is developed for the determination of mercury (II) in aqueous dimethyl formamide (DMF). The reagent gives deep yellow coloured, 1:1 (M: L) complex with mercury (II) in sodium acetate-acetic acid buffer medium of pH 5.5 at λ_{max} 365 nm. The colour reaction is instantaneous and the absorbance remains constant for about 12 hrs. The molar absorptivity of mercury complex is 2.3×10^3 $Lmol^{-1}$ cm^{-1} and sandell's sensitivity are found to be 0.0888 $\mu g cm^{-2}$. The method was successfully applied to a number of environmental, biological and soil samples. The results were comparable with those obtained by dithizone method. The proposed system produced satisfactory results for the determination of Hg (II). The results are quite encouraging and can bring awareness among the public.

KEYWORDS

Biological;
Soil;
Environmental samples;
Mercury (II);
Substituted hydrazones;
Spectrophotometry.

INTRODUCTION

The analytical monitoring of mercury in environmental, biological, industrial and food samples is extremely important because of the high toxicity of this metal both in its inorganic and organic compounds[1]. An example for acute mercury poisoning is "Mina-mata diseases" which causes mental disturbance, a loss of balance, speech, sight and hearing, difficulty in swallowing and finally coma and death[2]. The toxicity of mercury depends on its chemical nature. Inorganic mercury has a very high affinity for protein sulf-hydryl groups, accumulates in kidneys, whereas organic mercury is more toxic since it is soluble in fat, the lipid fractions of membranes, and brain tissue. Mercury toxicity is caused mainly by the fact that it enters the living organism and reacts with different enzymes inhibiting the catalysis of basic metabolic reactions[3]. The ability of living organisms to convert inorganic mercury to organic mercury compounds which are more toxic and accumulate to a greater extent in sensitive tissues, is also a considerable fact. The main sources of mercury from which it accumulates in the environment are synthetic adhesives, air conditioner filters, amalgams, auto exhausts, anatomic preservatives, antifouling paints, dental amalgams, batteries, cathode tubes, blood bank saline, cinnabar etc.

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Mercury causes serious damages to human organism, mainly of neurological disorder, which can even lead to the death of the exposed individuals[4]. The other toxic effects of mercury are allergy, anxiety, bad temper, dizziness, emotional disruption, blood pressure disorder, cancer, cardiac disorder, chronic inflammation, constipation, intestinal disturbance, irregular heartbeat, joint pain, less muscular capacity, memory loss, muscle pain, paralysis etc. The severity of these damages depends on the quantity acquired, the duration of the exposure and the chemical species of mercury[3]. However, people who eat a lot of fish may consume much more; for instance, a level of 0.6 mg Hg Kg^{-1}. Fish could provide 0.15 mg of methyl mercury in one meal. All these findings cause great concern regarding public health, demanding an accurate determination of this metal ion at trace and ultra-trace levels in water, biological and soil samples.

This paper describes the non-extractive spectrophotometric determination of mercury (II) as its AFINH complex in aqueous medium. A close survey reveals that AFINH has so far not been employed for the analytical determination of Hg (II). The present method does not require a solvent extraction step, hence the use of carbon tetra chloride or chloroform as solvents is avoided which are reported as toxic, environmental pollutants and carcinogens. Compared to some recently published methods the present method here offers several distinct advantag.

MATERIALS AND METHODS

The reagent solution (0.01 M) was prepared by dissolving 50 mg of the compound in dimethyformamide (DMF) in 25 ml standard flask. The reagent solution is stable for atleast 12 h.

Hydrochloric acid (1 M)- sodium acetate (I M) pH (0.5-3.5); 0.2M NaOAc-0.2M AcOH (pH 4-6) and 2 M NH_4Cl-2M NH_4OH (pH 7-10) solution were used.

Stock solution (1mg L^{-1}) was prepared by dissolving 0.2715 g of mercuric chloride ($HgCl_2$) (ARBDH pre analysis) in 100-ml doubly distilled water. Hydrolysis of mercury was prevented by adding 2 ml of conc. HCl. Dilute standard solutions were prepared from this stock solution as and when required.

Recommended procedure

An aliquot of the solution containing mercury in Beer's law validity range, 10 ml of NaOAc - AcOH buffer solution (pH 5.5) and 1.0 ml of 0.01 M AFINH were mixed in a 25-ml volumetric flask and resulting solution was diluted to the mark with distilled water. The absorbance of this solution was measured at 365 nm against reagent blank. The measured absorbance is used to compute the amount of mercury present in the samples using pre-determined calibration plot[11].

Shimadzu 160A UV-Visible spectrophotometer equipped with 1.0 pm quartz cell and an Elico model LI-610 pH meter were used in the present study.

Preparation of AFINH

The reagent (AFINH) was prepared by simple condensation of 2-acetylfuran and isoniazid. In a 250-ml Erlenmeyer flask, a hot methonolic solution (5 ml) of 2-aecetylfuran (0.05 mol), and isoniazid (0.05 mol, 6.65g), dissolved in 10 ml of hot distilled water were taken. Suitable quantity (~ 2 ml) of dilute hydrochloric acid was added to the reaction mixture and refluxed for 4 hours. On cooling the reaction mixture, pale yellow coloured product (AFINH) was separated out. It was collected by filtration and washed several times with hot water and 50% cold methanol. This compound was re-crystallized from ethanol and dried in *vacuuo*. Yield 81%; M.P. 188^0 C.

Characterization of AFINH

The compound was characterized by IR and ^{1}H-NMR, Mass and U.V-Visible spectral data. Infrared spectrum of AFINH shows bands at 3244(s), 3157(m), 3042(m), 2945(s), 1665(v,s), 1599(m), 1571(m), 1528(s), 1477(m), 1284(s), 754 (s), 703(s) and 662(m) cm^{-1} respectively corresponding to u(NH) secondary, u (C-H) aromatic stretch (Isoniazid), u (C-H) aromatic stretch (Furanyl), u (C-H) aliphatic stretching, u(C=O) hydrazine, u(C=N) azomethine, u(C-C) aromatic ring, u(C-C) aromatic ring, u(C-C) aromatic ring, u(C-N) stretch, u(C-H) oop bend (furan), u(N-N) stretch, u(C-H) oop bend (isoniazid) aromatic ring vibrations.

^{1}H-NMR spectrum of AFINH ($CDCl_3$+DMSO-d_6) showed signals at 2.31 (3H, s), 6.56-8.76(8H, m) and 10.96(1H, s) due to CH_3 and isonicotine + furan

proton, -NH (imino) groups of hydrazone respectively.

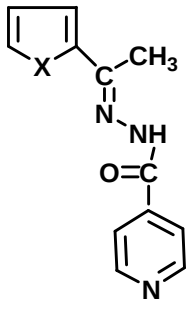
Mass spectrum of AFINH shows molecular ion peak at m/z 252 corresponding to the molecular ion peak associated with Na (Base peak). Other peaks due to loss of methyl radical and furanyl radical are also observed in mass spectrum.

The pKa values of AFINH

The pKa values of AFINH were determined by recording the UV-Visible spectra of micro molar (4 x 10^{-6} M) solution of the reagent at various pH values and by taking the arithmetic means of the values obtained from the measurements at different wavelengths determined spectrophotometrically using Phillip and Merritt method[7]. The values of deprotonation of AFINH are 3.6 (pK_1) and 6.3 (pK_2) corresponding to the formation of enol form and conjugated mono anion form respectively.

RESULTS AND DISCUSSION

The reagent 2-acetylfuran isonicotinoylhydrazone is easily prepared under reflux conditions. A 0.001M solution of AFINH is stable for more than two hours. In buffer medium (pH 5.5), the ligand presumably exists in enolic form and coordinates the divalent metal ion as mono anion. The reagent gives intense colour reaction only with mercury and show maximum absorbance at 355nm. The reagent (AFINH) is considered as potential reagent for selective spectrophotometric determination of mercury (II).



X = O

Figure 1 : Structure of AFINH

Determination of mercury (II)

Mercury (II) reacts with AFINH in acidic pH (5.5) to give coloured complexes. The colour reaction is instantaneous even at room temperature. The order of addition of reagent, metal ion, buffer, 2.5 ml of DMF. The absorbance of the coloured complex remains constant for more than 2 hours. A 10-fold molar excess of the reagent is adequate for full colour development. Addition of excess of reagent has no adverse effect on the absorbance of the complexes. The system obeys Beer's law in the concentration range 3.21-32.1 μg/ml of mercury. The molar absorptivity and Sandell's sensitivity of the methods for Hg (II) are found to be 0.41 x 10^4 $Lmol^{-1}$ cm^{-1} and 0.0875 $\mu g/cm^2$ respectively. The specific absorptivity of the system is 0.01142 $ml/g^{-1}cm^{-1}$ Hg (II). The relative standard deviation for ten replicate analysis of Hg (II) is 0.082 percent. Job's and Molar ratio methods gave the composition of the Hg (II) complexes as 1: 2 (M: L). the stability constants of Hg (II) complex calculated by Job's method is found to be 1.5 x 10^{10}.

Determination of mercury in various water, soil and biological samples

The present method is applied for determination of mercury in (i) Water samples, (ii) Soil samples, and (iii) Biological samples.

Water samples[4,9]

Each filtered (with whattman No. 40) water sample[4,9] (250 ml) was mixed with 10 ml of concentrated nitric acid in a 500 ml distillation flask. The sample was digested in the presence of an excess potassium permanganate solution according to the method recommended by Fifield *et al*[5]. The solution was cooled and neutralized with dilute NH_4OH solution. The digest was transferred into a 25-ml calibrated flask and diluted up to the mark with deionized water. The results were given in TABLE 2.

Soils samples[5,9] and Biological samples[6,9]

Dried fish samples[6,9] and various soil[5,9] samples 2 - 5 grams were taken in a 250 ml beaker. A 6 ml of concentrated nitric acid was added and gently heated for half-an-hour. After the disappearance of the froth, 6 ml of 1:1 nitric acid and perchloric acid were added[8-9]. The contents were digested for 1 hour and repeatedly treated with 6 ml portions of nitric acid and percholoric acid mixture until the solution becomes colourless. The acidic solution was evaporated to dryness and the resulting white residue was dissolved in minimum volume

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of 1M nitric acid and made up to the volume in a 50-ml volumetric flask. Aliquots of these solutions were taken for analysis following the recommended procedure. The results were given in TABLE 3 & 4.

Determination of mercury (II)

Mercury (II) reacts with AFINH in acidic pH (5.5) to give coloured complexes. The colour reaction is instantaneous even at room temperature. The order of addition of reagent, metal ion, buffer, 2.5 ml of DMF. The absorbance of the coloured complex remains constant for more than 2 hours. A 10-fold molar excess of the reagent is adequate for full colour development. Addition of excess of reagent has no adverse effect on the absorbance of the complexes. The system obeys Beer's law in the concentration range 3.21-32.1 μg/ml of mercury. The molar absorptivity and Sandell's sensitivity of the methods for Hg (II) are found to be 0.41 x 10^4 $Lmol^{-1}$ cm^{-1} and 0.0875 μg/cm^2 respectively. The specific absorptivity of the system is 0.01142 ml/$g^{-1}cm^{-1}$ Hg (II). The relative standard deviations for ten replicate analysis of Hg (II) are 0.082 percent. Physicochemical and anyitical properties of the complex is given in TABLE 1

Job's and Molar ratio methods gave the composition of the Hg (II) complexes as 1 : 2 (M : L). The stability constants of Hg (II) complex calculated by Job's method is found to be 1.5 x 10^{10}. The structure of Hg

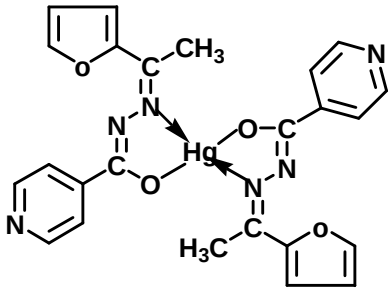

Figure 2 : Structure of Hg (II) complex with AFINH

$(AFINH)_2$ complex is given in Figure 2.

Tolerance limits of foreign ions

The effect of various cations and anions which are generally associated with the metal ion in the determination of Hg (II) was studied by measuring the absorbance of the mercury complex containing 3.21 mg/ml of mercury (II) in solution. An error of ±2% in the absorbance reading was considered tolerable. The tolerance limit (TL) values in μg/ml (ppm) for various anions and cations in AFINH method are as follows Citrate (1632), tartrate (662), Iodide (757), thiourea (185), nitrate (372), phosphate (196), Sulphate (192), bicarbonate (120), bromide (160), chloride (126), acetate (94), fluoride (117); V^{5+} (95); W^{6+} (185); Cd^{2+} (58); Mn^{2+} (33); Zr^{2+} (28); Mg^{2+} (23); Co^{2+} (18); Cr^{6+} (5); Cu^{2+} (4); Zn^{2+} (3); Fe^{3+} (56); Pt^{4+} (2); Fe^{2+} (6); Au^{2+} (1); Tl^{1+} (1); Ag^{1+} (1); Pd^{2+} (0.3). Higher amounts of Fe^{3+} (56) do not interfere in the presence of 78 μg/ml of fluoride. Larger amounts of Cu^{2+} do not interfere in the presence of 200 μg/ml of iodide.

TABLE 1 : Physico-chemical and analytical properties of Hg-AFINH complex

S.No.	Characteristics	Hg – AFINH
1	λ_{max} (nm)	420
2	Mean absorbance	0.2062 ± 0.0032
3	pH range (optimum)	5.0 – 8.0
4.	Mole of reagent required per mole of metal ion for full colour development	15-fold
5	Time stability of the complex (in hours)	12
6	Beer's law validity range (μg/ml)	3.21 – 32.10
7	Molar absorptivity (lit $mol^{-1}cm^{-1}$)	0.41 x 10^4
8	Specific absorptivity (ml $g^{-1}cm^{-1}$)	0.01142
9	Sandell's sensitivity (μg of Hg (II) cm^{-2})	0.0875
10	Composition of the complex as obtained in Job's and molar ratio methods (M : L)	1 : 2
11	Stability constant of the complex	1.5 x 10^{10}
12	Standard deviation	0.0066 ‡
13	Relative Standard deviation (RSD), (coefficient of variation)	0.082 %

‡**In the determination of 3.21 μg/ml of mercury (II)**

TABLE 2 : Determination of mercury in some environmental water samples

Name of the samples	Amount of mercury[a] found (μg/ml)	
	AFINH method	Dithizone method
Sea[c] water	1.06	1.00
Waste water	3.29	3.29
Laboratory[d] water	1.93	1.85
Tap water	0.65	0.69
Well water	0.92	0.84
Drain[e] water	3.15	3.18
River[b]	0.62	0.61

[a]. Average of three determinations; [b]. Tungabhadra river water, (Kurnool); [c]. Bay of Bengal, (Chennai); [d]. Laboratory water, (Dept. of Chemistry, S.K.U. Anantapur); [e]. Anantapur town, Drain Water.

TABLE 3 : Determination of mercury in soil samples

Name of the samples	Amount of mercury[a] found (µg/ml)	
	AFINH method	Dithizone method
Urban soil	0.98	0.98
Agricultural soil	0.45	0.41
Road side soil	1.68	1.62

[a]**Average of three determinations.**

TABLE 4 : Determination of mercury in biological samples

Liver samples	Amount of mercury[a] found (µg/ml of dried liver)	
	AINHH method	Dithizone method
Fish liver	1.28	0.84
Sheep liver	0.83	0.86

[a]**Average of five determinations**

The present method (AFINH) was applied for the determination of Hg (II) when present alone and present in water, biological and soil samples and results were compared with dithizone method[10].

APPLICATIONS

Mercury was estimated in various water samples, biological and soil samples by employing the present method. The results are presented in TABLE 2

CONCLUSIONS

The present study clearly indicates that the mercury content can be determined in ultra trace level in water, biological and soil samples using present method. All these findings cause great concern regarding public health demanding an accurate determination of this metal ion and it may provide awareness among the public.

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