

New Analytical Technique For The Determination Of Mercury (II) By Synergistic Extractive Spectrophotometric Method With N'',N'''-Bis[(E)-(4-fluorophenyl)methylidene] Thiocarbonohydrazide

Rekha A. Nalawade^{a,b}, Avinash M. Nalawade^b, Arjun.N. Kokare^a, Sunil B. Zanje^a, Mohan D. Jamdar^a, Anita Ghare^a, Mansing A. Anuse^{a*}

^aAnalytical Chemistry Laboratory, Department of Chemistry, Shivaji University, Kolhapur 416004, Maharashtra, India

^bLal Bahadur Shastri College of Arts, Science and Commerce, Satara, 415002, Maharashtra, India.

Abstract: A new technique was developed for the extractive spectrophotometric determination of mercury (II) by using newly synthesized chromogenic reagent N'',N'''-bis[(E)-(4-fluorophenyl)methylidene] thiocarbonohydrazide bis-(4-fluoroPM)TCH. It forms yellow colored ternary complex with mercury(II) in presence pyridine having composition 1:1:1 (M:Reagent:Py) in acidic pH range 1.7-3.7. The reagent is highly sensitive and selective towards mercury(II). So spectrophotometric method of mercury(II) is found to be very rapid, reliable and show synergistic effect. Absorption of colored organic layer in iso amyl acetate is measured with reagent blank at λ_{max} 375 nm. Pyridine showed synergistic effect with reagent by the adduct formation in organic phase. Beer's law was obeyed in the concentration range 0.25 to 3.5 $\mu\text{g mL}^{-1}$ for mercury (II). Molar absorptivity and sandell's sensitivity values of mercury(II)-bis-(4-fluoroPM)TCH-Py complex are $0.50127 \times 10^5 \text{ lit mol}^{-1} \text{ cm}^{-1}$ and $0.004 \mu\text{g cm}^{-2}$, respectively. The selectivity of the method was checked by using various foreign ions. The composition of the complex was determined by slope ratio method, mole ratio method and Job's method of continuous variation. The colour of ternary complex was stable for more than 12 h. Various factors influencing on degree of complexation are the effect of pH, reagent concentration, synergent concentration, equilibrium time, solvent were determined. The method was applicable for determination of mercury(II) in binary mixture, ternary mixture, ayurvedic sample, homoeopathic sample, industrial waste water, spiked water and dental unit waste water.

Keywords: Synergistic extraction, Mercury (II), bis-(4-fluoroPM) TCH, Pyridine, ternary mixture

I. Introduction

The abundance of mercury in the earth crust is 0.067 ppm. Mercury is so volatile that it could be exposed easily to human environment. It would cause a neurological damage and even result in death [1]. Mercury is also believed to be the most dangerous of all the metal contaminants which may be present in our daily foods[2]. Compounds of mercury consumed in fish, cereals and other food stuffs have resulted in numerous poisoning[3]. The acute mercury poisoning is "Minamata disease" which causes mental disturbance; a loss of balance, speech, sight and hearing, difficult in swallowing and finally coma and death[4]. The analysis and monitoring of mercury in environmental, biological, industrial and food samples is extremely important because of its high toxicity both in its organic and inorganic compounds[5]. The toxicity of mercury depends on its chemical state[4]. Inorganic mercury has a very high affinity for protein sulfhydryl groups, and accumulated in the kidneys, whereas organic mercury has a greater affinity for brain. Elemental and inorganic mercury are found in scientific instruments, electrical equipment, dental amalgams, felt making, disinfectants and enters the environment as metallic, inorganic and organic mercury compounds through various industries like pulp and paper industry, chlor-alkali plants, gold and silver mining, electrical energy, paints, fungicides and pharmaceuticals[6]. Hence there has been a growing interest to develop the analytical procedures for the determination of trace amounts of mercury. Many sensitive instrumental techniques, such as spectrofluorimetry, X-ray fluorescence spectrometry, neutron activation analysis, atomic absorption spectrometry, chemiluminescence, electrochemical analysis, and other have been widely applied to the determination of mercury[7-11]. However, the spectrophotometric method has still the advantage of its simplicity and accessibility, not needing expensive or complicated equipments. For this reason, a wide variety of spectrophotometric methods for the determination of mercury have been reported [12-17]. Each chromogenic system has its advantages and disadvantages with respect to sensitivity, selectivity and analysis speed. Therefore, we have proposed synergistic, highly sensitive, simple and rapid method for extraction and spectrophotometric determination of mercury(II) when compared with other spectrophotometric methods[18-30](Table 1).

II. Materials And Methods

2.1. Apparatus

All chemicals used were of analytical grade, from S. d. Fine. Lab Junction digital spectrophotometer model LJ-1306 using 1 cm quartz cell was used to obtain optical density. A model LI-120 Elico digital pH meter with combined glass electrode was used for measurement of pH. Glasswares were washed with chromic acid followed by double distilled water.

2.2. Standard mercury(II) solution and solution of other cations and anions

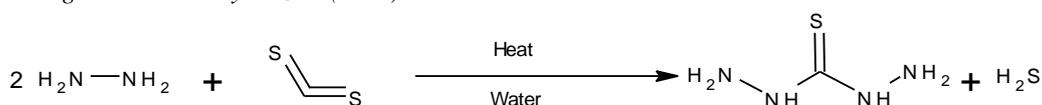
A 1.353 g of mercuric chloride, (HgCl₂ Merck) was dissolved in water and diluted to 1 lit to make stock solution of mercury(II) (1 mg mL⁻¹). It was standardized by a complexometric titration [31]. Double distilled water was used throughout the experiment. Salts of different cations and anions were dissolved in water or dil. HCl/H₂SO₄/HNO₃ to make their solutions [32]. Different synthetic mixtures containing mercury (II) were prepared by combining with commonly associated metal ions in definite composition [33].

2.3. Synthesis of N',N'''-bis[(E)-(4-fluorophenyl)methylidene]thiocarbohydrazide [bis-(4-fluoroPM)TCH]

It is two step synthesis process.

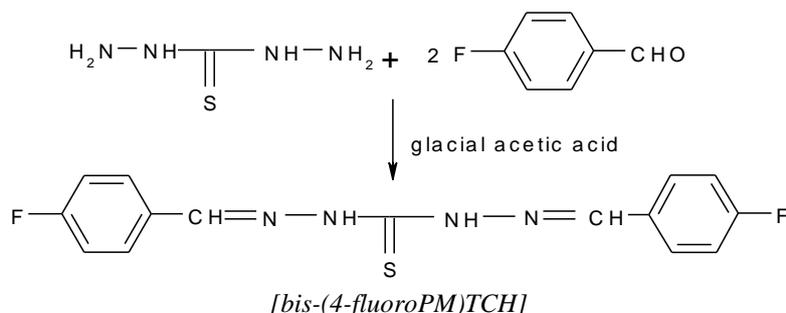
Preparation of thiocarbohydrazide (TCH) – It is synthesized by various methods [34-37]. We have followed the method of Li. Zheng., X. Feng, Y. Zhao.

Two moles of hydrazine hydrate were refluxed for two hours in an aqueous medium with one mole of carbon disulphide to give thiocarbohydrazide (TCH).



TCH

b. One mole of TCH was refluxed with two moles of *p*-fluoro benzaldehyde in alcohol as a solvent and glacial acetic acid as a catalyst to give N',N'''-bis[(E)-(4-fluorophenyl)methylidene] thiocarbohydrazide [bis-(4-fluoroPM)TCH] [38].



Purity of analytical reagent was tested by melting point, TLC, IR and NMR. A 0.01 M stock solution of bis-(4-fluoroPM)TCH was prepared by dissolving 0.299 g in a 100 cm³ of amyl acetate.

2.4. Recommended procedure

Mercury(II) (1ml of 25 μg mL⁻¹) solution was taken in 25 mL calibrated flasks and pH adjusted to 3.0 with dil hydrochloric acid or sodium hydroxide was used. The solution was transferred into 125 mL separatory funnel, followed by addition of 1ml of 0.002M bis-(4-fluoroPM)TCH, 4 ml amyl acetate, 5.0 mL of 0.5 M pyridine in amyl acetate. The two phases were equilibrated to 5 min. The yellow coloured organic extract was collected over anhydrous Na₂SO₄ to remove the traces of water. Then solution was transferred into 10 mL standard volumetric flask and made up to the mark with amyl acetate if necessary. The absorbance of the complex was measured at λ_{max} 375 nm against a reagent blank prepared in the same way, without addition of mercury(II). Unknown amount of mercury(II) was determined from the calibration graph prepared in the same manner.

III. Results And Discussion

3.1. Absorption spectra and spectral characteristics of coloured complex

Faint yellow coloured ternary complex was formed by mercury(II) with bis-(4-fluoroPM)TCH having proportion 1:1:1 (M : Reagent : Py), in the presence of pyridine as an auxiliary ligand. It was then extracted into amyl acetate at pH 3.0. The absorption spectra of mercury(II)-bis-(4-fluoroPM)TCH-Py complex in amyl acetate was studied over the wavelength range 300–800 nm. The coloured mercury(II)-bis-(4-fluoroPM)TCH-

Py complex was showed maxima at λ_{\max} 375 nm in amyl acetate, while reagent showed maxima at λ_{\max} 365 nm. [Fig. 1] Complex was stable for more than 12 h. The optimum conditions for the effective extraction of mercury(II) was established by studying the effect of pH, reagent concentration, pyridine concentration, choice of solvent, equilibrium time and interference of various diverse ions.

3.2. Effect of pH

The absorbance of mercury(II)-bis-(4-fluoroPM)TCH-Py complex was studied for wide range of pH from 1 to 10 by using 0.1N NaOH and 0.1N HCl solution. The extraction of mercury(II) was carried out with the bis-(4-fluoroPM)TCH-Py in presence and absence of pyridine. The study showed that the maximum absorbance was shown in the pH range 2.5-4.5 in the absence of pyridine for mercury(II)-bis-(4-fluoroPM)TCH-Py complex. However, in the presence of pyridine there was increase in the absorbance and shifting in the pH range to more acidic medium 1.7-3.7, showed synergistic effect on absorption mercury(II)-bis-(4-fluoroPM)TCH-Py complex [Fig. 2]. Further extraction was carried out at pH 3.

3.3. Influence of solvents on determination of mercury(II)

Mercury-bis-(4-fluoroPM)TCH-py complex was found to be insoluble in aqueous medium. However it is very soluble in non-polar solvents. The percentage efficiency of the complex in various solvent is shown in decreasing order as iso amyl acetate (99.9) > methyl iso butylketone (95.33) > xylene (91.61) > benzene (89.15) > dichloromethane (72.0) > toluene (65.4) > chloroform (53.0) > amyl alcohol (45.7) > butyl alcohol (28.0) > 1,2-dichloro ethane (4.67) > carbon tetrachloride (2.8).

As percentage efficiency of the mercury(II)-bis-(4-fluoroPM)TCH-Py complex in iso amyl acetate is maximum among these solvents, iso amyl acetate was use for further extraction.

3.4. Effect of [bis(4-fluoroPM)TCH]concentration

Extraction and spectrophotometric determination of mercury(II)-bis-(4-fluoroPM)TCH-Py complex was effected by concentration of chelating reagent bis-(4-fluoroPM)TCH. The concentrations of bis-(4-fluoroPM)TCH in amyl acetate were varied from the range 1×10^{-5} to 10×10^{-4} mol L⁻¹ and were added to a 1 ml of 25 $\mu\text{g mL}^{-1}$ mercury(II) ion solution in the absence of pyridine and absorbance was measured according to the recommended procedure. For maximum absorbance of complex 1.5 mL of 0.001 mol L⁻¹ reagent was used. However in presence of pyridine only 0.3 ml of 0.002 mol L⁻¹ reagent was sufficient for full color development. In order to ensure the complete complexation, 1.0 mL of 0.002 mol L⁻¹ reagent was recommended. In the absence of pyridine reagent concentration was 1.5×10^{-4} M and in presence of pyridine reagent concentration was 6×10^{-5} M. Requirement of less concentration of reagent in presence of pyridine showed synergistic effect on the extraction. Excess of reagent does not affect absorbance of the complex upto, 2.0 mL of 0.002 mol L⁻¹ concentration of reagent. Then there was decrease in absorbance with more increase in concentration [Fig.3].

3.5. Effect of equilibrium time

The equilibrium time was varied from 0.25 to 60 min in the absence and presence of pyridine. The minimum shaking time of 7 min of mercury(II)-bis-(4-fluoroPM)TCH-Py complex was investigated for full color development in the absence of pyridine. In the presence of pyridine absorbance was increased and required minimum of 3 min shaking time for full color development. Prolonged shaking time has no adverse effect on the absorbance of mercury(II) in the presence of pyridine. Hence, a shaking time of 5 min was recommended for further study.

3.6. Effect of concentration of pyridine (Auxillary ligand)

Mercury(II)-bis-(4-fluoroPM)TCH complex was affected by pyridine concentration. Solution of Hg(II) (1 ml of 25 $\mu\text{g mL}^{-1}$) and 1 ml of 0.002 mol L⁻¹ of bis-(4-fluoroPM)TCH was equilibrated with 0 to 5.0 ml of 0.5 mol L⁻¹ concentration range of pyridine. The mercury(II)-bis-(4-fluoroPM)TCH complex was decomposed after 4 h in the absence of pyridine. However, absorbance of mercury(II)-bis-(4-fluoroPM)TCH complex increases with pyridine concentration and remains constant above 3.0 mL of 0.5 mol L⁻¹ pyridine for 12 h. This indicates that the absorbance of mercury(II)-bis-(4-fluoroPM)TCH complex was increased by the formation of an adduct with pyridine. Therefore, 5.0 mL of 0.5 mol L⁻¹ pyridine was used in experimental study.

3.7. Effect of stability of complex

Mercury-bis-(4-fluoroPM)TCH-Py complex showed stable absorbance for more than 12 h in presence of pyridine in amyl acetate solution, while in the absence of pyridine absorbance was found to be low and decreased after 4 h. Therefore it was recommended that study of mercury(II)-bis-(4-fluoroPM)TCH-Py complex was carried out in presence of pyridine.

3.8. Determination molar extinction coefficient and sensitivity

Mercury-bis-(4-fluoroPM)TCH-Py complex was obeyed Beer's law over conc range 0.25 to 3.5 $\mu\text{g mL}^{-1}$. The precision and accuracy of the proposed method was ascertained from the absorbance values of the actual determinations of five replicates of fixed amounts of the mercury(II) samples. To determine the accuracy of the proposed method; different amount of samples containing mercury(II) were taken within the optimum Beer's range limits and analyzed by the recommended procedure. The molar extinction coefficient was evaluated from slope of curve was $5.0127 \times 10^4 \text{ lit mol}^{-1} \text{ cm}^{-1}$ and Sandell's sensitivity values of complex was $0.004 \mu\text{g} / \text{cm}^2$.

3.9 Ringbom's plot for complex

The optimum Beer's range was evaluated by Ringbom's plot. It is sigmoid shape with linear segment at intermediate concentration values in the range of 0.5- 3.0 $\mu\text{g mL}^{-1}$ for mercury(II).

3.10 Determination of metal:ligand ratio

The probable composition of the mercury(II)-bis-(4-fluoroPM)TCH-Py complex was ascertained by Job's plot, mole ratio method and slope ratio method.

3.10.1. Job's plot

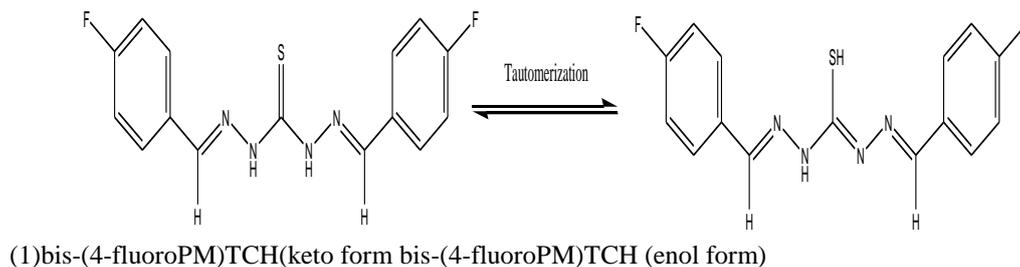
Mercury(II), bis-(4-fluoroPM)TCH and pyridine solutions were taken in equimolar ($2.494 \times 10^{-4} \text{ mol L}^{-1}$) proportions. Different aliquots(0.2-1.8) of equimolar mercury(II) solution was taken in 25 mL calibrated flask and pH was adjusted to 3.0. The solution was transferred into 125 mL separatory funnel followed by different aliquots of (1.8-2.0) equimolar reagent in each flask, keeping the total volume of metal and ligand solution constant at 2.0 mL. It was followed by 5.0mL of pyridine and amyl acetate, keeping the total volume of organic phase to be 10 mL. After equilibrium, the absorbances of the each organic phases were recorded at 375 nm, against the corresponding reagent blanks. The same procedure was described under the absence of pyridine. [Fig. 4]

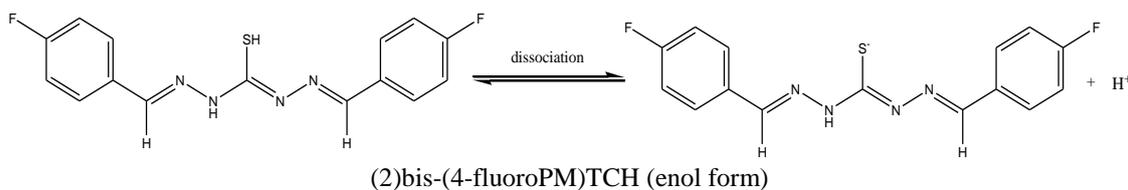
3.10.2. Mole ratio method:

In this method also mercury(II), bis-(4-fluoroPM)TCH and pyridine solutions were taken in equimolar ($2.494 \times 10^{-4} \text{ mol L}^{-1}$) proportions To a equimolar ($2.494 \times 10^{-4} \text{ mol L}^{-1}$) solutions of 1 ml of bis-(4-fluoroPM)TCH and 5.0 mL of pyridine different aliquots(0.2-1.8) of mercury(II) solution with pH 3.0 containing were added. Then 4.0 mL of iso amyl acetate was added to each of these solutions to keep the final volume of organic phase 10 mL. The absorbances of the organic phases were recorded at 375 nm. The similar procedure was followed for the absence of pyridine. A plot between the absorbance versus number of moles of the ligand per mole of the metal ion is shown in Fig. 5

3.10.3 Slope ratio method :

Mercury(II) (1ml of $25 \mu\text{g mL}^{-1}$) solution having pH 4.0 which was other than selected pH was taken in a 25ml volumetric flask. Different aliquots (0.1-1.7ml) of $0.002 \text{ mol} / \text{lit}$ of bis-(4-fluoroPM)TCH in iso amyl acetate and 5.0 mL of 0.5 mol L^{-1} pyridine and then required volumes of amyl acetate were added to keep final volumes of each organic phase 10.0 mL. The optical density of the organic phase was recorded at 375 nm against reagent blank. The composition of the mercury(II)-bis-(4-fluoroPM)TCH-Py complex was ascertained by plotting the graph of $\log D_{[\text{Hg}(\text{II})]}$ against $\log C_{[\text{bis}(4\text{-fluoroPM)TCH}]}$ at fixed 0.5 mol L^{-1} pyridine concentration and $\log D_{[\text{Hg}(\text{II})]}$ against $\log C_{[\text{Pyridine}]}$ at fixed reagent concentration at pH 4.0. Similar procedure was repeated at pH 5.0. The plots were linear having the slopes 0.95 and 0.93 at pH 4.0 and pH 5.0, respectively with fixed pyridine concentration (Fig. 4.11) and 0.59 and 0.62 at pH 4.0 and pH 5.0, respectively with fixed reagent concentration (4.12). The composition of extracted species (Metal:Reagent:Pyridine) was 1:1:1. The following chemical reactions are took place and probable coloured extracted species M:L:Py is

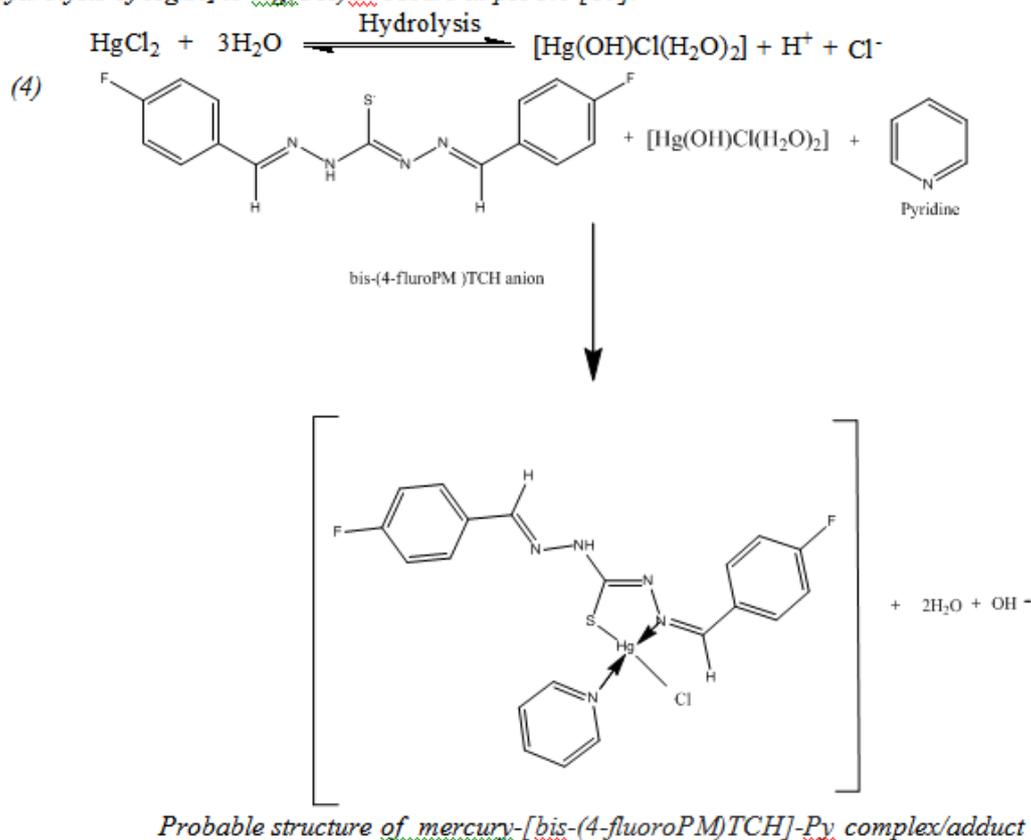




Anionic form of bis-(4-fluoroPM)TCH

Probable structure of mercury-[bis-(4-fluoroPM)TCH]-Py complex/adduct

(3) Hydrolysis of HgCl_2 to $\text{Hg}(\text{OH})\text{Cl}$ occurs at pH 3.0 [39].



3.11 Interference study

Under the optimum conditions cited above with 25 $\mu\text{g}/\text{ml}$ a systematic study of the effect of foreign cations and anions ions was undertaken (Table 4.4). The selectivity of the method was eliminated by using suitable masking agents for cations.

IV. Applications

4.1. Separation of mercury (II) from gold(III)

When gold comes into contact with mercury, the two substances mix to form a compound called amalgam. Gold amalgam is simply an alloy of gold and mercury. Bis-(4-fluoroPM)TCH reagent is used for the separation and determination of mercury(II) from gold(III) by its extraction given in recommended procedure. Mercury(II) was separated from gold(III) by its extraction with recommended procedure without using masking agent. Under optimized conditions gold(III) remained quantitatively in the aqueous phase. The aqueous phase was evaporated to moist dryness by treating with 2.0 mL conc HNO_3 followed by 2 to 3 ml of conc HCl . The residue was dissolved in dil HCl and diluted to appropriate volume. Gold(III) ions from aqueous phase were determined by standard method [40]. The absorbance of mercury(II)-bis-(4-fluoroPM)TCH-Py complex was determined spectrophotometrically at 375 nm against reagent blank.

4.2. Separation of mercury(II) from associated metal ions

By following the recommended procedure and using bis-(4-fluoroPM)TCH mercury(II) was also separated from Fe(III), Ni(II), Bi(III), Cr(III), Al(III), Zn(II), Cd(II), Pb(II), Sn(II), Sn(IV), Se(IV), Te(IV). But Fe(III), Ni(II), Bi(III), Cr(III), Al(III), Zn(II), Cd(II), Pb(II), Sn(II), Sn(IV) ions were interfered with extraction of mercury(II). So Ni(II), Cd(II) masked with 1.0 mg thiocyanate; Fe(III), Pb(II), Zn(II) and Cr(III) masked with 5 mg tartarate; each Bi(III), Al(III) masked with 0.03 mg citrate; Sn(II) and Sn(IV) masked with 5 mg oxalate. The masked metal ions as an anionic complex were remained in the aqueous phase quantitatively and were subsequently demasked by treating with 2.0 mL of conc HClO₄ and followed by 1 ml conc HCl. The solution was evaporated to moist dryness. Obtained residue was dissolved in water, diluted to appropriate volume and metal ions were determined spectrophotometrically by standard methods [32,33,41]. Mercury(II) was separated from Se(IV) and Te(IV) by using recommended procedure. Under optimum conditions Se(IV) and Te(IV) were remained in the aqueous phase quantitatively. The aqueous phase was evaporated to moist dryness by treating with 5.0 mL conc HNO₃ followed by 1ml conc HCl. The residue was dissolved in water and diluted to appropriate volume. The added metal ions were determined spectrophotometrically by standard methods [42,43]. The absorbance of mercury(II)-[bis(4-fluoroPM)TCH]-Py complex from organic phase was measured at 375 nm against reagent blank (Table 3).

4.3. Determination of mercury(II) from synthetic mixtures

By using 25 µg mL⁻¹ of mercury(II) and known concentration of associated metal ions several synthetic mixtures of varying compositions were prepared. It was followed by suitable masking agent if required. The extracted mercury(II) was analyzed by employing the bis-(4-fluoroPM)TCH by recommended procedure. The results obtained were same as the amount added (Table 4).

4.4 Analysis of mercury(II) from ayurvedic samples

The developed method was applied for the determination of mercury(II) in ayurvedic samples such as Echabhedi and Ekangveer Rasa. 1 tablet of a known weight of sample was dissolved in required volume of aqua regia followed by treatment with 2.0mL of conc HClO₄. The solution was evaporated to moist dryness. Obtained residue was dissolved in hot dil HCl and filtered through Whatmann filter paper No. 1. The filtrate was diluted to required volume with water to obtain results within optimum Beer's range. An aliquot of mercury(II) content in solution was determined by the recommended procedure. The results were in good agreement with those obtained by atomic absorption spectroscopy (Table 5).

4.5 Analysis of mercury (II) from homeopathic pharmaceuticals

An aliquot (5.0 mL) of each homeopathic pharmaceutical sample was used for determination of Hg(II) and followed by the recommended procedure. The results of analysis was found to be in excellent agreement with those obtained by AAS (Table 6).

4.6 Analysis of mercury(II) from industrial waste water samples

Each waste water sample (100.0 mL) was evaporated almost to dryness. The residue was dissolved in 5.0 mL of conc nitric acid and 20.0 mL of water, boiled and filtered through Whatmann filter paper No. 1. The filtrate was diluted to required volume with water. An aliquot (5ml) of sample was taken into 25 ml calibration flask and mercury content was determined by recommended spectrophotometric method. The results of analysis were confirmed by AAS (Table 7).

4.7 Determination of mercury (II) in spiked water samples

The water samples were spiked with several known amounts of mercury(II). The analytical determination of mercury was carried out by recommended spectrophotometric method. The results of analysis were checked by AAS (Table 8)

4.8 Determination of mercury (II) in dental waste water samples

Mercury(II) in dental-unit waste water samples were collected from dental clinics at Kolhapur city. Each sample (100.0 mL) was evaporated almost to dryness. The residue was dissolved in 5.0 mL of conc nitric acid and 20.0 mL of water, boiled and filtered through Whatmann filter paper No. 1. The filtrate was diluted to required volume with water. An aliquot (5ml) of sample was transferred into 25 ml calibration flask and mercury(II) content was determined by recommended method. The results of analysis were compared with AAS (Table 9).

V. Conclusion

A simple, synergistic, rapid, sensitive spectrophotometric method was proposed for determination of trace amount of mercury(II) in binary, synthetic mixtures, and various real samples with satisfactory results.

The newly synthesized reagent bis-(4-fluoroPM)TCH has been proved to be highly sensitive for solvent extraction and spectrophotometric determination of mercury(II) when compared to other spectrophotometric methods (Table I). Therefore very less concentration of mercury(II) [25 µg mL⁻¹] can be determined by using this reagent with very low reagent concentration in a single stage extraction procedure. The extracted complex is stable for more than 12 h in presence of pyridine. The selectivity was enhanced by the use of suitable masking agents.

Abbreviations

bis-(4-fluoroPM)TCH	N'',N'''-bis[(E)-(4-fluorophenyl)methylidene] thiocarbonohydrazide
4'-chloro PTPT	1-(4'-chlorophenyl)-4,4,6-trimethyl(1H,4H)-2-pyrimidine thiol
2',4'-dinitro APTPT	1-(2',4'-dinitro aminophenyl)-4,4,6-trimethyl-1,4-dihydropyrimidine-2-thiol
DMG	Dimethylglyoxime
PAR	4-(2-Pyridylazo) resorcinol
Py	Pyridine

Table I Comparison of proposed method with reported solvent extraction and spectrophotometric methods for determination of mercury(II).

Reagent	λ _{max} nm	Acidity/pH	Beer's law	Molar absorptivity	Remarks	Ref. No.
N'',N'''-bis[(E)-(4-fluorophenyl)methylidene] thiocarbonohydrazide [bis-(4-fluoroPM)TCH]	375	1.7-3.7	2.5-30	0.501x10 ⁵	*Simple *Highly sensitive *Rapid	P.M.
2-mercaptobenzothiazole (MBT)	320	10.00	1.0x 10 ⁻⁷ 1.0x 10 ⁻⁵	-	*Use of nonionic surfactant Triton X 100 *Buffer solution is required to adjust pH	18
Isonitriso p-isopropyl Acetophenone Phenyl Hydrazone (HIPAPH)	395	7-11	1.0 to 20	2.67x10 ⁻³	*Stoichiometry 1:2 *Hg(II) was determined from real samples	19
6-hydroxy-3-(2-oxoindolin-3-ylideneamino)-2-thioxo-2H-1,3-thiazin-4(3H)-one,	366, 505	4.0-6.0	0.2-2.0	4x10 ⁴	*Excess of chromogenic reagent *Hg(II) was determined from real samples	20
o-carboxy phenyl diazoamino p-azobenzene	540		.08-8.0	2.22x10 ⁵	*Excess of chromogenic reagent *Surfactant used was Triton X 100	21
4,4'-(dimethylamino)thiobenzophenone	575	1-6	12-140	1.7x10 ⁴	*Sensitive *Hg(II) was determined from water samples * High reagent concentration required	22
6-mercaptopurine	315	5-8	002-.048	0.26x10 ⁻⁶	*Wide Beer's range. *Buffer solution was required to adjust pH. *surfactants are used	23
Thiacrown ether and Bromocresol Green	420	-	-	-	*Less sensitivity *Interference from. Cu ⁺² , Cd ²⁺ and Ag ⁺ . *Time-consuming	24
Hydentoin,5- amino1,3,4- thiadizole - 2- thiol (HTT)	490	6.0-8.0	2.2	6.45x 10 ⁴	*Reagent was sensitive * Narrow Beer's range.	25
2-(2-thiazolylazo)-p-cresol)	500	9.5	10	5.7x10 ⁴	Surfactants are used	26
Anthrone phenylhydrazone (APH)	367	7	0.81-8.14	1.267x10 ⁴	*Less stability of complex. *Buffer solution was	27

					required to adjust pH.	
Diphenylthiocarbazon (dithizone)	488	0.18–1.8	0.1-25	2.5×10^4	*Use of surfactants was sensitive.	28
2-Acetylpyridine thiosemicarbazone (APT)	351	6	0.24-2.40	5.4×10^4	*Less stability of complex solution was required to adjust pH.	29
4-Hydroxy 3, 5-dimethoxy benzaldehyde 4-hydroxy benzoylhydrazone (HDMBHBH)	420	4	0.30-3.09	4.56×10^4	*Complex was stable in micellar solution.	30

Table 2 Effect of diverse ions for the determination of $25 \mu\text{g mL}^{-1}$ mercury(II) with bis(4-fluoroPM)TCH and pyridine complex. (relative error $\pm 2\%$)

Amount of foreign ion added mg	Tolerance limit, mg
NO_3^- , NO_2^-	25
Oxalate, salicylate, F^-	10
Tartarate	5
SCN^-	1
Citrate, Ag(I)	0.03
^c Zn(II)	0.8
Ga(III) , Y(III) , ^c Pb(II) , ^b $\text{Fe}^+(III)$, ^b Ni(II)	0.5
^b Cr(III) , ^b Cr(VI) , Te(IV) , ^d Bi(III) , ^d Pd(II) , ^b Cd(II)	0.1
^c Sn(II) , ^c Sn(IV) , Au(III) , ^a Pt(IV) , ^b Co(II)	0.05
^d Sb(III) , ^b Mo(VI)	0.08
^d Al(III)	0.3
^b Mn(II) , ^b Mn(VII)	0.01
Se(IV)	0.4

^a 0.01 mg of $\text{S}_2\text{O}_3^{2-}$

^b 1.0 mg of SCN^-

^d 0.03 mg of citrate

^c 10 mg of oxalate

^c 5 mg of tartarate

Table 3. Separation of mercury(II) from associated metal ions

Metal ion	Amount taken in μg	Average recovery* %	R. S. D. %	Chromogenic ligand	Ref. No.
Hg(II)	25	98.2	1.7	2',4' Dimitro APTPT	40
Au(III)	100	98.5	0.9		
Hg(II)	25	98.1	1.9	4' Chloro PTPT	42
Se(IV)	400	97.9	2.0		
Hg(II)	25	97.2	2.4	4' Chloro PTPT	43
Te(IV)	100	98.1	1.9		
Hg(II)	25	98.8	1.3	DMG Method	41
Ni(II)^b	200	98.8	1.2		
Hg(II)	25	97.2	2.4	Thiocyanate Method	33
Fe(III)^b	400	98.2	1.5		
Hg(II)	25	98.4	2.0	Iodide method	33
Sb(II)^d	100	97.8	1.8		
Hg(II)	25	99.9	0.8	Diphenyl carbazide	33
Cr(III)^c	100	99.2	0.7		
Hg(II)	25	98.8	1.3	Iodide method	33
Bi(III)^d	100	99.2	0.7		
Hg(II)	25	98.9	1.2	PAR	32
Al(III)^d	100	97.2	2.3		
Hg(II)	25	98.2	1.6	PAR	32
Zn(II)^c	100	98.5	0.9		
Hg(II)	25	98.9	1.2	PAR	32
Pb(II)^c	100	97.2	2.3		
Hg(II)	25	98.1	1.9	PAR	32
Cd(II)^b	100	97.9	1.9		
Hg(II)	25	98.1	1.9	Pyro catechol violet	33
Sn(II)^a	50	97.6	2.3		
Hg(II)	25	97.6	2.0	Pyro catechol violet	33
Sn(IV)^a	50	98.0	1.8		

^a Masked with 10 mg of oxalate

^b Masked with 1.0 mg of SCN^-

^d Masked with 0.03 mg of citrate

^c Masked with 5 mg of tartarate

* Average of five determinations

Table 4. Determination of mercury(II) in a synthetic mixtures

Composition, µg	Average % Recovery*	R.S.D. %
Hg(II), 25; Cr(III) ^b , 100; Cd(III) ^b , 100	97.2	1.82
Hg (II), 25; Cr(III) ^b , 100; Pb(II) ^c , 300	97.1	1.92
Hg (II), 25; Sn(II) ^a , 50; Fe(III) ^b , 100	97.7	0.70
Hg (II), 25; Sn(IV) ^a , 50; Fe(III) ^b , 100	97.3	1.40
Hg (II), 25; Zn(II) ^c , 100; Cd(II) ^b , 100	97.4	1.52
Hg (II), 25; Sb(III) ^d , 100; Pb(II) ^c , 100	97.2	1.82
Hg (II), 25; Bi(III) ^d , 400; Pb(II) ^c , 100	97.2	1.82
Hg (II), 25; Al(III) ^d , 100; Zn(II), 100	97.2	1.82
Hg (II), 25; Fe(III) ^b , 400; Al(III) ^d , 100	97.1	1.92
Hg (II), 25; Al(III) ^d , 100; Cd(III) ^b , 100	97.2	1.82
Hg(II),25; Se(IV), 400; Te(IV),100	97.1	1.92

^a Masked with 10 mg of oxalate

^c Masked with 5 mg of tartarate

^d Masked with 0.03 mg of citrate

^b Masked with 0.8 mg of SCN⁻

* Average of five determinations

Table 5. Determination of mercury(II) from ayurvedic samples

Name of sample	Certified Values of Hg(II) mg	Amount of Hg(II) found* (mg/250 gm)		R.S.D., %
		Proposed method	AAS method	
Echabhedi	25.0	25.1	25.0	1.36
Ekangveer Rasa ² Fe ^b , Sn ^a , Pb ^c	66	66.2	66.0	3.78

* Average of five determinations

^a Masked with 10 mg of oxalate

^c Masked with 5 mg of tartarate

^b Masked with 1.0 mg of SCN⁻

Table 6. Determination of mercury(II) from homoeopathic samples

Homoeopathic medicine	Amount Added (µg/ml)	Amount of Hg(II) found* (µg/ml)		R.S.D., %
		Proposed method	AAS method	
Mer cor Q	0.100	0.116	0.115	1.36
Mer sol Q	0.100	1.108	0.108	1.20

* Average of five determinations

Table 7. Determination of mercury(II) from Industrial Waste water samples

Water sample	Amount of Hg(II) found*(µg/ml)		R.S.D., %
	AAS method	Proposed method	
Sample 1	0.147	0.148	1.36
Sample 2	0.118	0.120	1.20

* Average of five determinations

Table 8. Determination of mercury(II) from Spiked water samples

Water samples	Hg(II) Added (µg/ml)	Amount of Hg(II) found*(µg/ml)		R.S.D., %
		Proposed method	AAS method	
Tap water	3.0	3.05	3.05	1.40
	5.0	5.06	5.08	1.66
	7.0	7.08	7.08	1.20
Lab water	0.0	20.1	20.1	1.10
	100	120.2	120.1	1.35
	500	500.2	500.2	1.36

* Average of five determinations

Table 9 Determination of mercury(II) from Dental waste water samples

Water sample	Hg(II) Added (µg/ml)	Amount of Hg(II) found*		R.S.D., %
		AAS method	Proposed method	
Dental waste water sample 1	- ^a	2.22	2.22	1.36
	2.0 ^a	4.22	4.24	1.45
	4.0 ^a	4.26	6.26	1.40
Dental waste water sample 2	- ^a	3.57	3.57	1.20
	1.00 ^a	4.60	4.60	1.08
	3.00 ^a	6.60	6.61	1.22

* Average of five determinations

^a Masked with 0.01 mg of S₂O₃⁻²

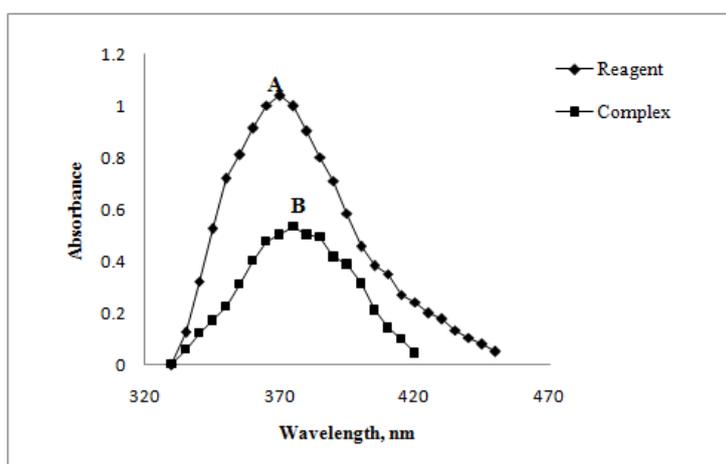


Fig. 1

(A) The spectra of bis-(4-fluoroPM)TCH in *iso*-amyl acetate :

(bis-(4-fluoroPM)TCH = 1.0 mL of 0.002 mol L⁻¹; λ_{max} = 365 nm

(B) The spectra of Hg(II)-bis-(4-fluoroPM)TCH-Py complex

Hg(II) = 25 µg mL⁻¹; bis-(4-fluoroPM)TCH = 1.0 mL of 0.002 mol L⁻¹; λ_{max} = 375 nm

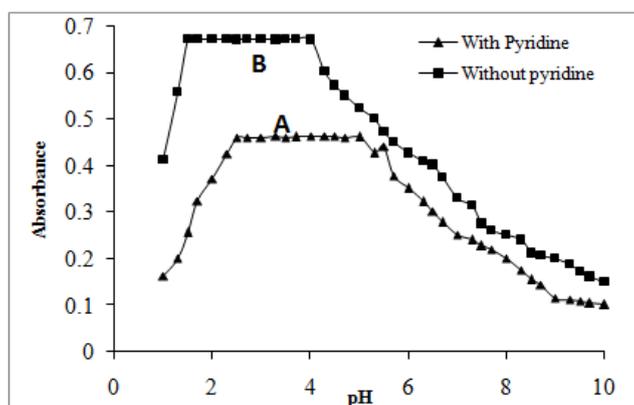


Fig. 2

A]Influence of H⁺ ions conc on the extraction Hg(II)-bis-(4-fluoroPM)TCH complex: (without pyridine)

Hg (II) = 25 µg mL⁻¹; [bis(4-fluoroPM)TCH]= 1.0 mL of 0.002 mol L⁻¹;

pH = 1 to 10; $\lambda_{\max} = 375 \text{ nm}$

B] Influence of H⁺ ions conc on the extraction mercury(II)-bis-(4-fluoroPM)TCH-Py complex: (with pyridine)

Hg (II) = 25 $\mu\text{g mL}^{-1}$; [bis(4-fluoroPM)TCH] = 2.0 mL of 0.002 mol L⁻¹; pyridine = 5.0 mL of 0.5 mol L⁻¹ pH = 1 to 10; $\lambda_{\max} = 375 \text{ nm}$

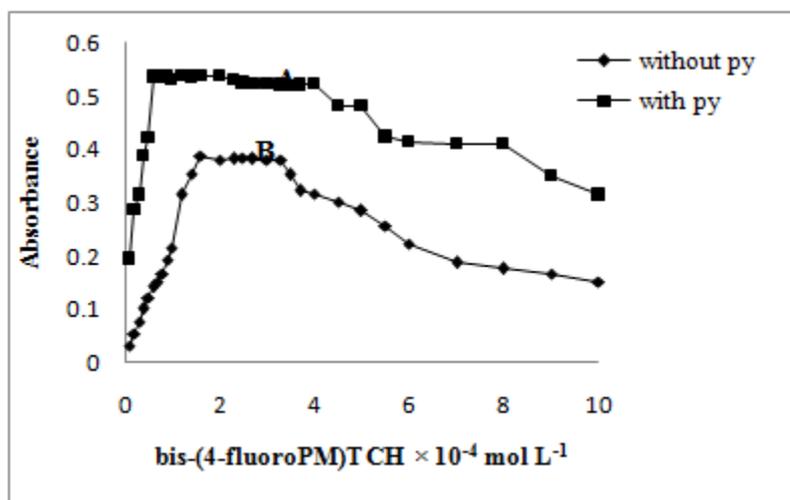


Fig.3.

(A) Effect of reagent conc. on extraction of mercury(II)-bis-(4-fluoroPM)TCH-Py complex:

Hg (II) = 25 $\mu\text{g/ mL}$ pH = 3.0;
 bis-(4-fluoroPM)TCH = 0.1-5.0 mL of 0.002 mol L⁻¹ Pyridine = 5.0 mL of 0.5 mol L⁻¹;
 Equilibrium time = 5 min; $\lambda_{\max} = 375 \text{ nm}$

(B) Effect of reagent conc. on extraction of mercury(II)-bis-(4-fluoroPM)TCH complex in absence of pyridine:

Hg (II) = 25 $\mu\text{g/ mL}$; pH = 3.0,
 bis-(4-fluoroPM)TCH = 0.1-10 mL of 0.001 mol L⁻¹ Equilibrium time = 7 min;
 $\lambda_{\max} = 375 \text{ nm}$

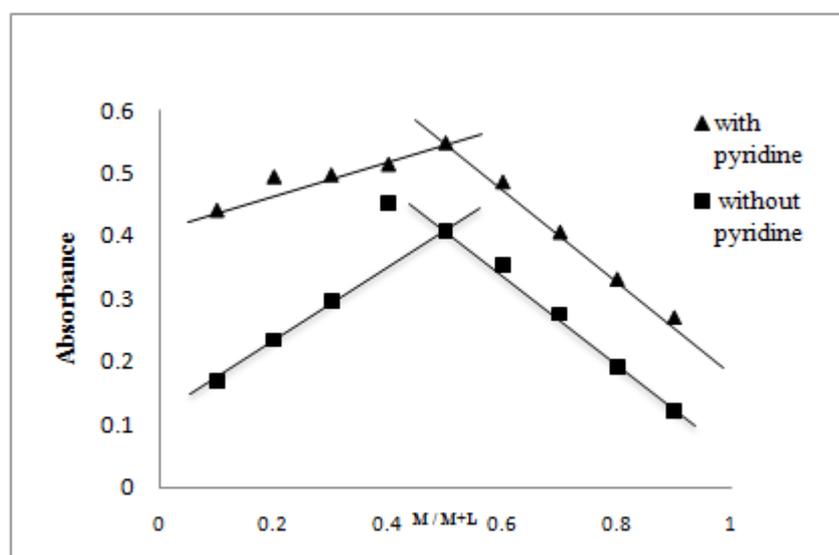


Fig 4.

Job's plot

Hg(II) = 0.2 to 1.8 mL of 2.494 × 10⁻⁴ mol L⁻¹,

bis-(4-fluoroPM)TCH = 1.8 to 0.2 mL of $2.494 \times 10^{-4} \text{ mol L}^{-1}$;
 pyridine = 5.0 mL of $2.494 \times 10^{-4} \text{ mol L}^{-1}$; pH = 3.0;
 Equilibrium time = 5 min; $\lambda_{\text{max}} = 375 \text{ nm}$

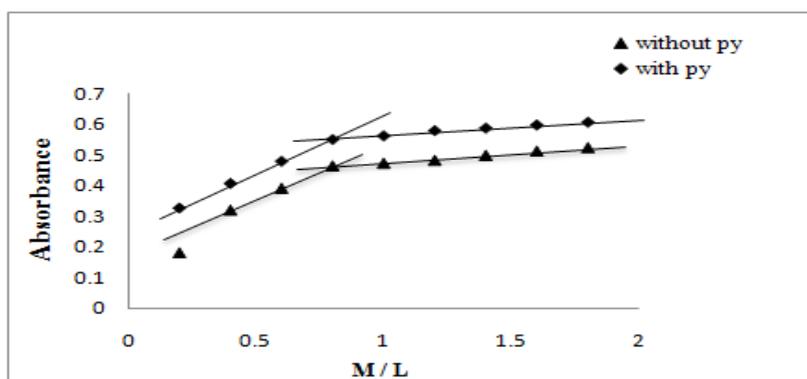


Fig. 5

Mole ratio method

Hg (II) = 0.2 to 1.8 mL of $2.494 \times 10^{-4} \text{ mol L}^{-1}$;
 bis-(4-fluoroPM)TCH = 1.0 mL of $2.494 \times 10^{-4} \text{ mol L}^{-1}$;
 pyridine = 5.0 ml of $2.494 \times 10^{-4} \text{ mol L}^{-1}$;
 pH = 3.0 ; shaking time = 5 min; $\lambda_{\text{max}} = 375 \text{ nm}$

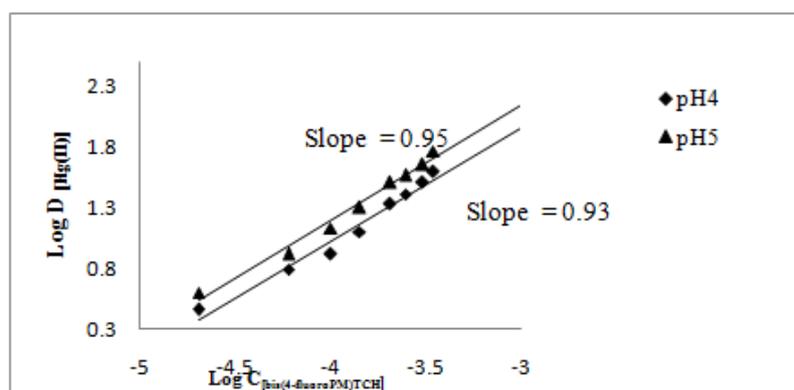


Fig. 6

Slope ratio method: [Fixed pyridine concentration]

Hg (II) = $25 \mu\text{g mL}^{-1}$; pyridine = 5.0 mL of 0.5 mol L^{-1} ; pH = 3.0 and 5.0;
 bis-(4-fluoroPM)TCH = 2×10^{-5} to $34 \times 10^{-5} \text{ mol L}^{-1}$;
 shaking time = 5 min; $\lambda_{\text{max}} = 375 \text{ nm}$

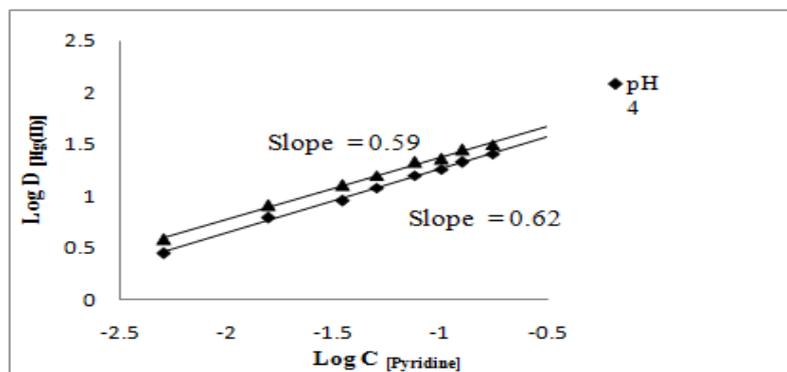


Fig. 7.

Slope ratio method: [Fixed reagent concentration]

Hg (II) = $25 \mu\text{g mL}^{-1}$; pyridine = 5.0×10^{-3} to $175 \times 10^{-3} \text{ mol L}^{-1}$;
 bis-(4-fluoroPM)TCH = 1 ml 0.002 mol L^{-1} (fixed concentration)

pH = 3.0 and 5.0; shaking time = 5 min; λ_{\max} = 375 nm;

References

- [1]. S. E. Manahan, Environmental Chemistry, 6th Ed., Lewis Publisher: Boca Raton, U.S.A., **1994**, 184.
- [2]. V.P.Kudesia, Toxicity of metals in Air Pollution, Pragati Prakashan ,Meerut, **1988**, 178- 199.
- [3]. H. M. Bowen, Trace elements in Bio-Chemistry, Academic Press, London,London, **1966**.
- [4]. C. D. Klaasen, Casarett and Doull's Toxicology, McGraw-Hill, New York, **2001**,834.
- [5]. Agency for Toxic Substances and Disease Registry(ATDSR), "Toxicological Profile for Mercury"(U.S. Department of Health and Human Service, Atlanta,GA,**2004**.)
- [6]. Lacerda L.D. & Salomons W, Mercury from gold and silver mining, a chemical time bomb, Springer, Berlin, Heidelberg, **1998**.
E. Wieteska, A. Ziolk, *Chem. Anal.*,**2000**, 45(3),325-339.
- [7]. J. Y. Lu, W.H. Schroder, *Water, Air, Soil Pollut.*,**1999**, Issue 3-4, 279-295.
- [8]. M. Morita, J. S. Edmonds, J. Yoshinaga, *Pure and appl. Chem.*,**1998**, 70(8),1585-1615.
- [9]. M. Rose, L. Owen, M. Baxter, M. A. Knaggs, *J. Anal. At. Spectrom.*, **2001**, 16(9),1101- 1106.
- [10]. G. Y. Yang, C. M. Zhang, Q. F. Hu, J. Y. Yin, *J. Chromatogr. Sci.*, **2003**, 4, 195-199.
- [11]. A. Z. Abu Zuhri, W. Voelter, *J. Anal. Chem.*, **1998**, 360, 1-9.
- [12]. A. Y. El-Sayed, *Anal. Lett.*, **1998**, 360, 1-9.
- [13]. M. P. Sang, H. S. Choi, *Anal. Chim. Acta* , **2002**, 459, 75–81.
- [14]. N. Samadi., R. Ansari, B.Khodavirdilo, *Am. J. of phytomedicine and clinical therapeutics*, **2005**, 3(5), 451-468.
- [15]. A R.G. Prasad, V. Seshagiri, L. K. Ravindranath, *Chem. Sciences J., CSJ-14*, **2010**,1-8.
- [16]. W. Yang, Q. Hu, J. Ma, L. Wang, G. Yang, G. Xie, *J. Braz. Chem. Soc.*, **2006**, 17(5),1039-1044.
- [17]. M.S. Jeoung and H. Seon, *Bull. Korean Chem. Soc.*, **2004**, 25(12), 1877-1880.
- [18]. B. S. Rao, S.S. Dubey, B.V. Kiran, *Int. J. Life Sci. and Pharma. Res.*,**2011**, 1, 75-79.
- [19]. A. Hamza, A.S. Bashammakh, A.A. Al-Sibaai, H.M. Al-Saidi, M.S. El-Shahawi, *J. Hazar. Mater.*, **2010**, 178, 287–292.
- [20]. S. Chatterjee, A. Pillai, V.K. Gupta, *Talanta* , **2002**, 57, 461–465.
- [21]. M.Gharehbaghi, F.Shemirani, M. Baghdadi, *Int. J. Environ. Anal. Chem.* **2009** , 1(89) , 21–33.
- [22]. M.S. Tehrani, H. H. Moghaddam, S. W. Husain and K. Zare, *Annal. Chim.*, **2007**, 97, 675-683.
- [23]. S. M. Sultan, *J. Flow Injection Anal.*, **2001**,18(1), 43-48.
- [24]. M. F. Ahmeda and Y. Lingappaa, *Intern. J. of Curr. Pharm. Res.*, 2011, 3, 102-104.
- [25]. R. Gurkan, T.C. Epken, H. I. Ulusoy, *Turk. J Chem.*, **2012**, 36, 159 – 177.
- [26]. V. Veeranna, A. R. G. Prasad and V. S. Rao, *Analele Universității din Bucuresti – Chimie (serie nouă)*, **2011**, 20 (1), 57 – 64.
- [27]. M.J.Ahmed, M.S.Alam, *Spectroscopy*, **2003**, 17, 45–52.
- [28]. S. V.Babu, K. H. Reddy, *Indian J. of Adv. in Chem. Sci.*, **2012**, 1, 65-72.
- [29]. D.G. Krishna and C.K. Devi, *Intern. J. Green Chem. and Bioprocess*, 1 (1), **2011**, 10-12.
- [30]. F.J.Welchar, The analytical uses of EDTA, D. Can Nostrand Company Inc., NewYork,**1958**.
- [31]. H. A. Flaschka, A. J. Jr. Barnard, Chelates in Analytical Chemistry, A collection of monographs, vol.4, Merce Dekkar Inc., New York, **1972**.
- [32]. Marczenko Z., Spectrophotometric Determination of Elements, Ellis Horwood Limited, Chichester, **1976**.
- [33]. Li.Zheng, X.Feng, Y., Zhao, *J. Heterocycl. Chem.*, **2008**, 45, 1489- 1492.
- [34]. Metwally M. A., halifa M. E, Koketsu M., *Am. J. of Chem.*, **2012**, 22, 38-51.
- [35]. Authenrith, Hefner, *Ber.*,58 , **1925**, 2151, US 4172092 A.
- [36]. Kurzer F., M. Wilkinson, *Chem. Rev.*, 113, **1969**, US 5376451 A.
- [37]. R. A. Nalawade, A. M. Nalawade, G. S. Kamble, M. A. Anuse, *Spectrochimica Acta Part A*: 146 (2015) 297–306
- [38]. F. A. Cotton, G.Wilkinson, C.A.Murillo, Bochmann M., *Advanced Inorganic Chemistry*, John Wiley and Sons, Newyork, London Sydney, Toronto, 6th edition,**1999**.
- [39]. G.S. Kamble, S.S. Kolekar, S.H. Han, M.A. Anuse, *Talanta*, **2010**, 81, 1088– 1095.
- [40]. E. B.Sandell, *Colorimetric Determination of Traces of Metals*, Interscience, New York, NY, USA, 3rd edition, **1965**.
- [41]. G. B. Kolekar and M. A. Anuse, *Res. J. Chem. Environ.*, **1998**, 2, 9-15.
- [42]. G. B. Kolekar and M. A. Anuse, *Bull. Chem. Soc. Jpn.*, **1998**, 71(4), 859–866.