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Determination of divalent Mercury in environmental samples using 1, 5-diphenyl-3-thiocarbazone: with modified, ultrasensitive, direct Spectrophotometric method

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Abstract

The present research investigated that, the analytical reagent,1,5-diphenyl-3 thiocarbazone dissolves in acetone in strongly acidic and 1,4 dioxane media which reacts with mercury (II) to produce orange-red coloured complex at pH 2 (0.2N sulphuric acid). The complex was showing maximum absorbance at 488 nm. Therefore, further analytical parameters were performed at 488nm. The reaction occurs within a minute and absorbance remains unchanged for 24 hrs. The analytical parameters like, effect of metal concentration, reagent concentration, acidity, interference by other metal ions, were investigated. The Lambert-Beer's law followed within a range of 0.1-25 µgml^{-1}of Hg(II). The stoichiometric ratio of a reagent with the reacting metal is 1:2 (Mercury:Dithiazone). The molar absorptivity was found to be 2.4×10^4 lmol^{-1}cm^{-1} and that of specific absorptivity was 0.015µg of mercury (II)/cm^2. The selected method is modified, ultrasensitive and is successfully applied for estimation of divalent mercury from polluted water samples.

Keywords: Divalent mercury, Dithizone, Complex, Absorbance, Concentration, Polluted water.

Introduction

Heavy metal pollution is a serious environmental concern as it causes lethal effects on living organisms. Mercury is well known as a toxic metallic pollutant in the environment for several decades[1]. It was reported that global release of mercury into the surroundings may cause lethal effects on human well-being and other living organisms. These early investigations shows that, due to human activities mercury has been released in the environment in such extent, that it rises up to the toxicological concern of fishes and aquatic ecosystem[1]. Accumulation of mercury in humans is primarily occurring through consumption of these fishes[1].

Humans are mostly exposed to these metals by inhalation (breathing) or ingestion (drinking or eating)[2]. The risk of mercury exposure to human health arises when their work places are near the toxic metal disposal site. Mercury is generally categorizes into elemental, organic and inorganic form, out of which methyl mercury can be extremely toxic[2]. Hence, bioaccumulation of such toxic metals occurs through food chain. All forms of mercury adversely affects human nervous system[2]. Long time exposure to increased level of mercury is known to damage the vital organ like brain, kidneys and unborn developing fetuses.

Problems associated with brain functioning may include, memory problems, bashfulness, shakiness, alteration in vision or audibility and irritability[2]. Less exposure to mercury vapors may cause nausea, pulmonary diseases, diarrhea, hypertension, eye irritation and skin rashes[2]. Metallic mercury is used in various instruments like thermometers, barometers and sphygmomanometer[3]. Mercury pollution is chiefly done through, electrochemical process of manufacturing chlorine, where mercury is used as an electrode and in chlor-alkali industry. Some discharge of mercury is also contributed by paper, plastic, electrical, paint industries and pharmaceutical industries. Also, increased use of agricultural pesticides, fungicides may add mercury in the environment[4].

Mercury analysis from various environmental water (potable and polluted) samples can be done by different analytical techniques as before, but it requires preliminary sample preparation, separation and concentration, which is time consuming[6-7]. Though determination of trace level of mercury by sensitive techniques like atomic absorption spectrophotometry and FT-IR analysis is available still because of the factors such as cost effectiveness, easy operating system, less consumables and rapid analysis have made spectrophotometric methods remain convenient, for the students, especially in laboratories of economically developing

nations with inadequate resources and facilities[7-8]. Though the existing methods were researched out in different areas, yet the proposed method is modified and ultrasensitive. The present study provides the knowledge required to overcome environmental problems in industrial sites, as metal contamination may severely affects human health and adjoining biota. The result of environmental sample analysis of mercury by spectrophotometric method is compared with those of result obtained by atomic absorption spectrophotometer were found within equivalent limit, which proves the sensitivity of the selected method.

Material and methods

Instrumentation: The absorbance measurements were performed using a Shimadzu (UV-1700) double beam UV-visible spectrophotometer instrument and pH of the solution was measured by Equip-tronics digital pH-meter (EQ-610) with combination electrodes. Comparison of the results was done with atomic absorption spectrophotometer (Shelton, CT, USA).

Chemicals and reagents: Chemicals and reagents were freshly prepared in distilled water during experiment. The chemicals and reagents used during experiment were of highest purityanalytical-reagent grade (e.g., Merck India, Ltd).

***Diphenylthiocarbazone (Dithizone*):** This reagent solution was prepared in dark brown colored bottle, by dissolving the requisite amount of 1, 5-diphenylthi-3thiocarbazone (dithizone) (Merck India, Ltd.) in a known volume of acetone LR (Merck India, Ltd), followed by distilled 1,4-dioxane. The reagent solutions were diluted as per requirement.

Mercury (II) standard solutions: The stock solution of divalent mercury was made by dissolving 135 mg of $HgCl_2$ (Merck) in 100 ml distilled water. This solution, by diluting was used for preparation of standard solutions required for experiment.

Mercury(I) stock solution: The 100 ml stock solution of mercury(I) was prepared by treating a 10 ml mercury(II) with hydroxylamine hydrochloride in dilute H_2SO_4 followed by boiling, for removal of the hydrochloride and made the volume 100 ml with distilled water.

Potassium permanganate solution: The potassium permanganate (Merck) 1% solution was prepared by dissolving it in distilled water. Sodium azide solution (2.5%) was also used.

Tartrate solution: The stock solution of tartrate (0.01%) was prepared by dissolving 10 mg of potassium sodium tartrate tetrahydrate in 100ml distilled water.

Procedure: It was preferred that, the standard samples of mercury were prepared in dilution series containing 1 to 100 µg of divalent mercury in volumetric flask was added with 1 ml of 1, 5-diphenyl-3-thiocarbazone (Dithizone) reagent solution followed by the addition of 0.5ml0.2N sulphuric acid (maintain pH of the solution within range 2.0-3.0).Acidity of the solution favor's rapid Hg (II)-Dithizone complex formation. Then 1 ml of 1, 4-dioxane solution was added. Finally, the mixture was diluted up to the mark with distilled water. Analytical reagent 1, 5-diphenyl-3-thiocarbazone (Dithizone) reacts with mercury (II) to produce orange-red coloured complex. The complex was showing maximum absorbance at 488 nm. Therefore, further analytical parameters were performed at 488nm. The reaction occurs within a minute and absorbance remains unaltered for 24 hrs. Mercury content from test sample was determined by plotting calibration graph[9].

Results and discussion

Factors affecting the absorbance: Absorption spectra: Using double beam UV-visible spectrophotometer, the absorption spectra of mercury (II)–dithizone complex were recorded in acidic medium (0.2N H_2SO_4). The mercury (II)-dithizone complex shows the symmetric curve with maximum absorbance at 488 nm wave length. The average value of molar absorption co-efficient was found to be 2.5×10^4 $lmol^{-1}$ cm^{-1}. The blank reagent shows very negligible absorbance at same wavelength[9]. The reaction mechanism of the present method is also previously done[11].

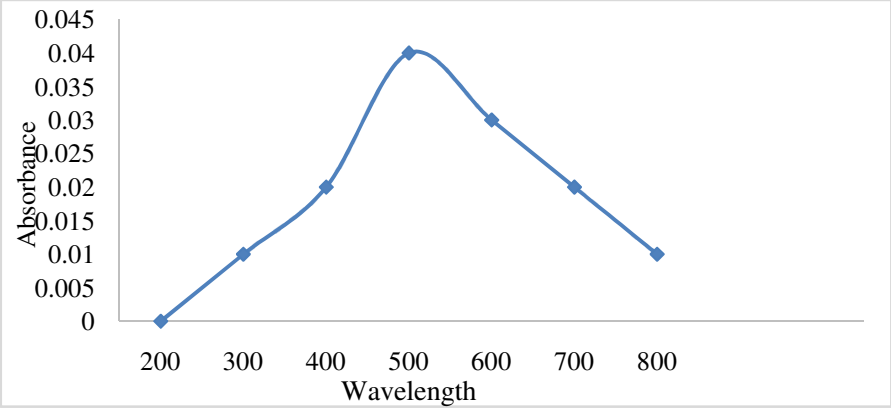

Figure-1: Spectrum of the Hg (II)-dithizone system formed, in the mercury (II) estimation. Wavelength in nm (λ max=488 nm).

Effect of organic solvent: The different organic solutions like, EDTA solution, carbon tetrachloride, chloroform, alcohol, benzene, acetone and 1, 4-dioxane solution, were used as solvents for the mercury (II)-dithizone system. There was no absorbance observed in the organic phase (exception of chloroform and carbon tetrachloride). Maximum absorbance was observed, in acetone with 1, 4-dioxane (50%) medium. Hence acetone with 1, 4-dioxane (50%) media was used in the present estimation method.

Effect of acidity: As sulphuric acid is most suitable acid for the detection of mercury. Hence in proposed method sulphuric acid is preferred for the entire experiment. For all subsequent measurements, a known volume of 0.2 N sulphuric acids was used. However, the effects of H_3PO_4, H_2SO_4 and HNO_3 on the absorbance of the mercury (II)-dithizone system were observed separately. Therefore, 0.2N H_2SO_4, was chosen to be an optimum acidity range for final dilution.

Effect of time: The reaction occurs within a minute and absorbance remains unchanged for 24 hrs.

Effect of Analytical reagent: If the reagent exceeds, it will not affect the tests with small concentration of Hg (II), as the mole ratio (Hg (II): reagent) was varied from 1:6 to 1:48. The mole ratios between 1:12 and 1:36 show constant maximum absorbance within the prescribed acidity.

The optimization of experiment was done for the selection of analytical parameters as summarized in Table-1.

Table-1: Selected values of analytical parameters obtained after optimization of experiment.

Analytical Parameters	Selected range	Selected value
λ max (nm) (max. Absorbance)	200-800 nm	488 nm
Acidity of solution (H_2SO_4)	0.1-0.3 N	0.2N
Time (hr.)	0-48 hrs.	24hrs.
Temperature (^OC)	1-50^{o}C	30^{o}C
Excess Reagent	1:6-1:50	1:12-1:36
Linear range (µg/ml)	0.01-100µg/ml	0.01-80 µg/ml
Detection limit	1-100	20
Reproducibility	0-10	0-2.0

Effect of metal concentration: Four different sets ranging from 0.01–0.1, 0.1–1, 1–10, 10–100 mgl^{-1}were made to study the effect of metal concentration. According to Beer's law, the linear absorbance was seen for 0.10–80 mgl^{-1} of mercury at 488 nm. The calibration graph of absorbance against divalent mercury concentration was linear and passed through the origin within the range of 0.10–80 mg/l mercury concentrations. The *molar absorptivity* was found to be 2.4×10^4 $lmol^{-1}cm^{-1}$ and *specific absorptivity* was found to be 0.015µg of Hg (II) cm^{-2}.

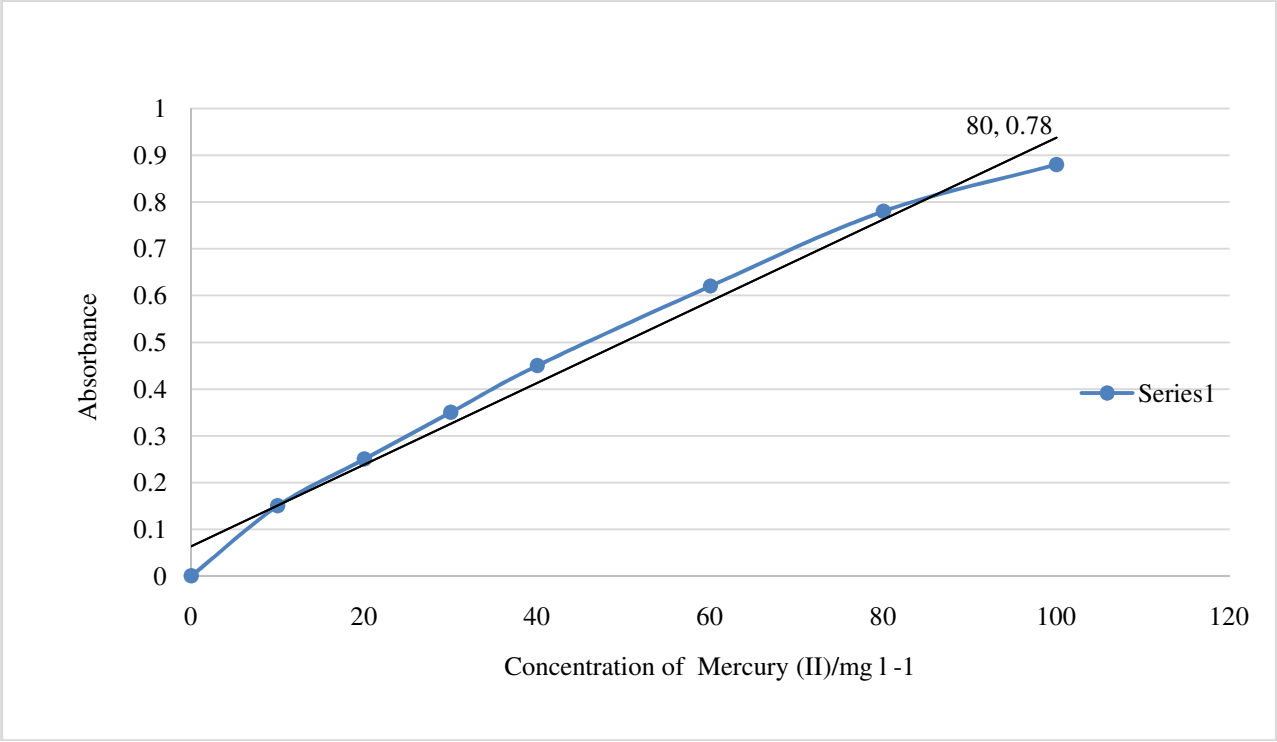

Figure-2: Calibration graph, 0.10-80mg/l of Hg (II)

Precision and accuracy: Precision describes the reproducibility of results where accuracy denotes the nearness of a measurement to its accepted value. In the present method, the different concentrations of mercury were evaluated. Under ideal conditions it was possible to achieve relative standard deviations, 0.0–2.0 for 1–100 µg of mercury (II) in 10.0ml.This enables the determination of mercury more accurate, precise and reproducible. The smallest concentration of mercury (II) that can be detected with 95% certainty was found to be 20ng/ml.

Effect of foreign ions: The study of different ions on the determination of mercury (II) was performed. As per the criterion for interference, an absorbance alter by more than 5% from the expected value is significant. There was no such interference from the ions like Br^-, I^-, EDTA, No_3, So_4^{--}, F^-, tartrate, citrate, oxalate, alkali metals, and different alkaline earth metals, ClO_4, PO^{-3}_4, Ni (II), Mg (II), Cu (II), Cr (III), Cd and Zn. In the presence of sodium tartrate or EDTA , negligible amount of Be, As(III), Sb(V), Fe(III), Cr(VI),Se(IV), Hg(I), Pd(II), Th, Zr, Cl^-, Sn(IV) or Co(III) and trace amount of CN, SCN and V(V) have been insignificant. By using H_2O_2 and boiling the solution interference from permanganate was removed[9].

Composition of the absorbent complex: The stoichiometric ratio of the Mercury: dithizone complex was determined by Job's method[19] and the molar-ratio method[21]. Mercury: dithizone complex was detected by both methods.

Environmental Applications: Determination of divalent mercury in polluted water samples: The industrial effluent water sample was collected and filtered for analysis .2 ml of 0.2N sulphuric acid was added to 100mlfiltered effluent water sample. The digestion of sample was carried out in presence of excess potassium permanganate solution as pert he method suggested by De[5]. The solution was then transferred into a 50-ml volumetric flask and diluted up to the mark with distilled water. An aliquot of this solution was taken into a 10-ml volumetric flask and the mercury content was estimated as per protocol[9]. The results of effluent water analysis by spectrophotometric method was found to be comparable with AAS.

Conclusion

The selected method is modified, ultrasensitive and is successfully applied for estimation of divalent mercury from polluted water samples. The method is very simple and convineninent to operate. This method not only enhances the sensitivity, selectivity and molar absorptivity but also avoids ridiculous solvent extraction steps and became a cost effective. The absorbance was found to be linear for 0.10–80 mgl^{-1} of mercury at 488 nm. Therefore, calibration linearity curve was generated using different levels of calibration standards. Mercury from polluted water samples has been determined by FT-IR, AAS. These methods are expensive and instruments are difficult to operate. AAS is often lacking in sensitivity and it requires control condition which limits the routine analysis. In proposed method, determination of mercury using dithizone is not only one of the most sensitive method but also is good in terms of easy operation and accuracy. Therefore, this method may be preferentially applied for the monitoring of traces of mercury in environmental samples.

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