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Selective Spectrophotometric Method for the Determination of Mercury(II) in Water Samples

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Abstract: A simple spectrophotometric method is proposed for the rapid determination of mercury(II). The procedure is based on the hydrolysis of iodine in the presence of Hg(II), resulting in the formation of hypoiodous acid, which selectively oxidizes leucocrystal violet to crystal violet. The obtained violet dye shows maximum absorbance at 590 nm. Beer's law was valid in the concentration range of 0-1.0 µg mL^{-1}. The effects of different parameters, i.e., pH, time, temperature were studied. The molar absorptivity for the system was 1.49×10^{5} L mol^{-1} cm^{-1}. The detection limit and the quantification limit were 2.6×10^{-3} µg mL^{-1} and 0.8×10^{-2} µg mL^{-1}, respectively. The proposed method is simple, selective and was successfully applied for the determination of mercury(II) in several water samples.

Keywords: Mercury, iodine, leucocrystal violet, spectrophotometry.

Introduction

Mercury is a highly toxic element for biological systems and one of the most hazardous pollutants in the environment. It can be found in several organic and inorganic forms and has harmful consequences to human health [1]. It causes neurological damages, has teratogenic effects, and can be lethal. The concentration of mercury in water, soil, sediments, plants, fish and even human hair can be determined by monitoring Hg levels [2]. Mercury can exist as elemental or metallic forms, inorganic salts, and organic Hg compounds. The toxicity depends on the form of the chemical species, organomercurials being more toxic than inorganic mercury compounds. The mercurous (Hg^{+}) and mercuric (Hg^{2+}) ions are usually found in the solid state, as mercurous or mercuric salts and Hg compounds with chlorine, sulfur, or oxygen [3]. Major chronic diseases caused by Hg include acrodynia (pink disease), neurological disease, Mad hatter's disease, Hunter-Russell syndrome, leukemia and Minimata disease [4-8]. The maximum acceptable content of Hg(II) for human beings, in drinking water, is 1 µg L^{-1} [9], as recommended by the World Health Organisation (WHO).

Several methods have been reported for the determination of mercury(II), like spectrophotometric analysis [10], inductively coupled plasma mass spectrometry (ICP-MS) [11], inductively coupled plasma atomic emission spectrometry (ICP-AES) [2], gas chromatography (GC) coupled to atomic absorption spectrometry (AAS) [3], cold vapor atomic absorption spectroscopy (CV-AAS) [14], atomic fluorescence spectrometry (AFS) [15], high performance liquid chromatography (HPLC) [16],

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anodic stripping voltammetry (ASV) [17], neutron activation analysis (NAA) [18], etc. These methods suffer from one or more disadvantages: they require expensive experimental setup, high running costs and need professional operators. In general, the concentration of Hg(II) is very low in samples taken from the environment. Therefore, there is the need of sensitive, selective and cost effective analytical methods for the determination of Hg(II) in extremely low concentrations [3].

Spectrophotometric methods of analysis are excellent tools that can be extensively used in routine laboratories due to their simplicity, rapidity, cost effectiveness, high accuracy and precision, and are very useful and indispensable techniques for trace determinations. These methods have many applications and are widely used, as large number of chromogenic reagents with good sensitivities are available. Some have been reported for the determination of mercury(II), like diphenylthiocarbazone [19,20], phenanthroline and eosin [21], thiobenzoylacetone [22], variamine blue B [23], bromocresol green and thiacrown ethers [24], and methyl orange [25]. But most of them have limitations like stability of colours, interference from foreign species, etc.

The aim of this work is to develop a fast, low cost, sensitive and simple method for the analytical determination of mercury(II). The proposed procedure is based on the fact that Hg(II) can complex with iodide ion and can hence facilitate the hydrolysis of iodine. Thus, in the presence of Hg(II), hydrolysis of iodine, will result in the formation of more hypoiodous acid, which can selectively oxidize leucocrystal violet (LCV) to form crystal violet. The violet colored dye obtained has maximum absorbance at 590 nm. The proposed method, due to its simplicity, selectivity and low interference, has advantages over other reported spectrophotometric methods.

Experimental

Equipment

A Systronics UV-visible spectrophotometer-117 (Carry 50 scan, Varian) with 1 cm quartz cell (1.0 mL) was used for the measurement of absorbance. The pH measurement was carried out using a digital pH meter (Systronics model-112). The temperature was kept constant with the help of the water bath.

Reagents

All solutions were prepared using chemicals of analytical reagent grade. A standard mercury(II) stock solution (1000 µg mL^{-1}) was prepared by dissolving 0.1354 g of $HgCl_2$ (LOBA) in 100 mL of water. LCV (Merck) (0.025 g) was dissolved in 20 mL of distilled water containing 0.3 mL of phosphoric acid (85 %). More distilled water was added to reach a volume of 100 mL. The obtained solution was stored in an amber coloured bottle. A solution was prepared by dissolving 0.004 g of solid iodine in 0.8 mL of acetic acid and diluting with distilled water to 20 mL. This solution was freshly prepared and stored in amber bottle. The working solutions were prepared by further dilution of the stock solution.

General procedure

Aliquots of 5 mL solution containing 0 to 10 µg of Hg(II) were placed in a series of 10 mL calibrated tubes. The pH was made up to 5 by 0.1 M HCl/0.1 M NaOH and 0.2 mL of I_2 solution was added. The contents were maintained at 30°C for 10 min with the help of a water bath. The mixture was then shaken and 2 mL of LCV was added, followed by distilled water to reach a final volume of 10 mL. The absorbance was measured at 590 nm against the blank. The final pH of solution was 3.5 ± 0.5. The blank experiment was performed without addition of Hg(II). A calibration curve was constructed by plotting absorbance against concentration.

Application to the real samples

To account for the applicability of the proposed procedure, the method was used for mercury(II) determination in four different water samples, collected from environmental matrices. 100 mL of each water samples were evaporated almost to dryness and 4 mL of concentrated HNO_3 were added to the residue and further diluted with 20 mL of distilled water. Then the contents were boiled and filtered through a Whatman filter paper (no. 40). The filter was repeatedly washed with deionized distilled water and the filtrates were

collected. An aliquot (5 mL) of filtrate was taken into a 10 mL calibration tube and the mercury content was determined by the proposed spectrophotometric method using a calibration curve.

Results and discussion

Plausible mechanism

Leucocrystal violet (LCV) gets oxidized to crystal violet (CV) in presence of several oxidizing agents but the reaction is generally slow and are not instantaneous [26, 27]. Hypoiodous acid (HOI) can selectively oxidize LCV to crystal violet, which occurs rapidly and in the proposed method, HOI is produced *in situ* by the hydrolysis of iodine (Scheme 1). The hydrolysis of iodine results in the formation of HOI and I^-. Hg(II) facilitates the oxidation of LCV. The oxidation of LCV by I_2 is slowed down due to production of iodide which opposes the hydrolysis of I_2 and, in turn, opposes the formation of HOI, as shown in Equation (1). Hg(II) removes iodide ion by complexing with it, thus enabling HOI to instantaneously oxidise LCV to CV [28], which gives a maximum absorbance at 590 nm.

$$I_2 + H_2O \leftrightarrows HOI + H^+ + I^- \quad (1)$$

$$Hg^{2+} + 4I^- \leftrightarrows HgI_4^{2-} \quad (2)$$

$$HOI + LCV \rightarrow CV \quad (3)$$

The overall reaction can be written as:

$$Hg^{2+} + 4I_2 + 4H_2O \rightarrow HgI_4^{2-} + 4HOI + 4H^+ \quad (4)$$

Spectral characteristics

The absorption spectra of CV dye showed maximum absorbance at 590 nm, compared to the blank (Figure 1). For the colored system, the values for molar absorptivity and Sandell's sensitivity were found to be 1.49×10^5 L mol^{-1}cm^{-1} and 1.34×10^{-3} µg cm^{-2}, respectively. Spectral characteristics are given in Table 1.

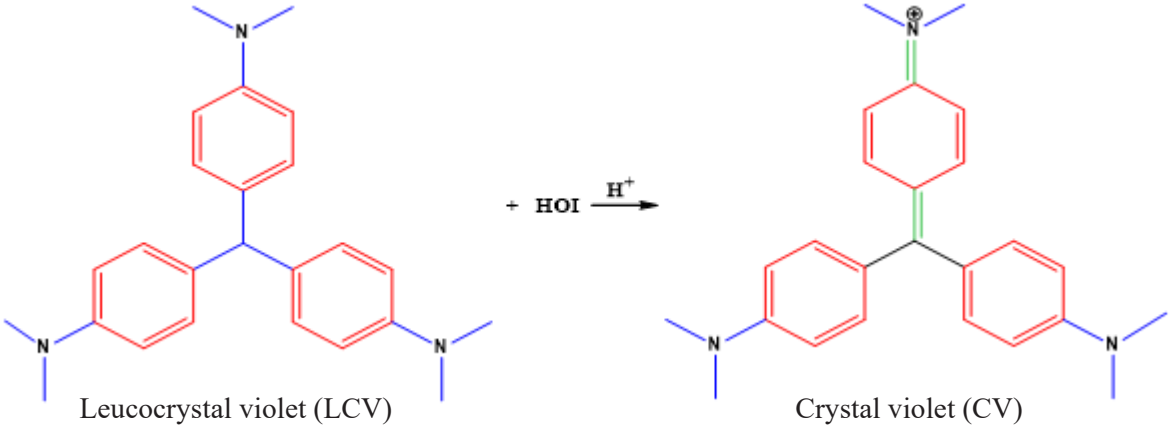
Method validation

Linearity

Beer's law was obeyed in the concentration range 0-1.0 µg mL^{-1} Hg(II), with a good correlation coefficient (0.9978) and an intercept of 0.0031 (Figure 2). The detection limit was calculated as DL = 3.3 r/S, where *r* is the standard deviation of the blank (n = 6) and S is the slope of calibration curve, was 2.6×10^{-3}. The quantification limit of Hg(II), defined as QL = 10 r/S was 0.8×10^{-2} (Table 1).

Accuracy and precision

The validity and reproducibility of the method was assessed in terms of accuracy and precision (Table 2). The accuracy was evaluated by comparing the measured amount with the actual amount added and the results indicated that the recommended procedure can be used with accuracy. The precision of the method was examined by assaying standard solutions of mercury(II), in five replicates, at three concentration levels (0.4, 0.8 and 1.0 µg mL^{-1}). Measurements were performed on the same day for intra-day precision and for five consecutive days for inter-day precision. These results indicated that the proposed method has within-day and between-day precision, with 1.95-2.15 % and 2.16-2.76 % relative standard deviation, respectively, as shown in Table 2.



Leucocrystal violet (LCV) Crystal violet (CV)

Scheme 1. Proposed mechanism for the determination of Hg(II)

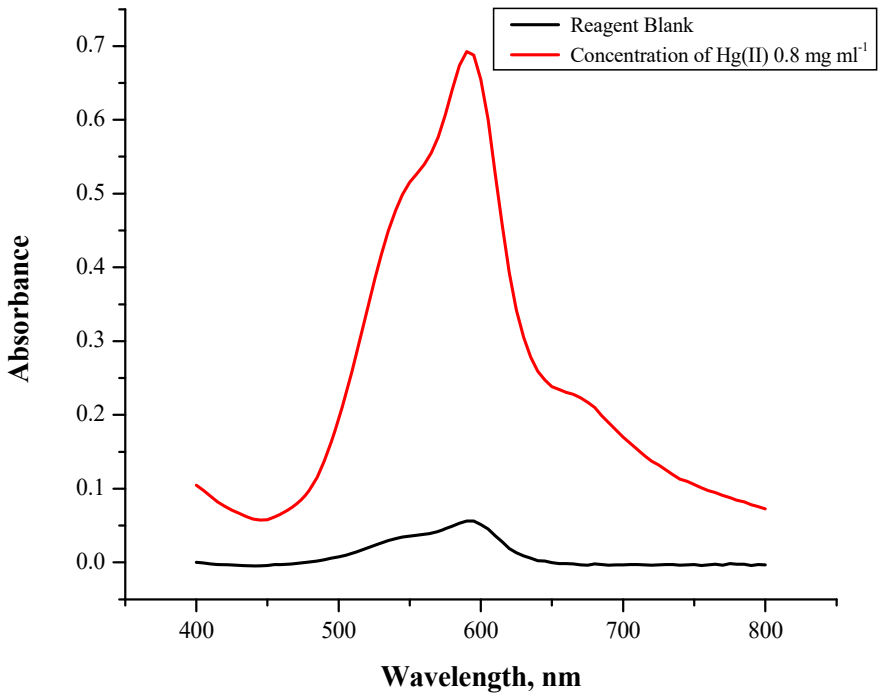


Figure 1. Absorption spectra of crystal violet dye and blank

Table 1. Spectral characteristics and precision data

Parameters	Results
λ_{max} (nm)	590
Beer's law limit (µg mL^{-1})	0 - 1.0
Molar absorptivity (L $mol^{-1}cm^{-1}$)	1.49×10^{5}
Sandell's sensitivity (µg cm^{-2})	1.34×10^{-3}
Limit of detection (µg mL^{-1})	2.6×10^{-3}
Limit of quantification (µg mL^{-1})	0.8×10^{-2}
Relative standard deviation (RSD) %	1.9338
Standard deviation (SD)	$\pm 0.354 \times 10^{-3}$
Correlation coefficient (R)	0.9978

Table 2. Evaluation of the accuracy, intra-day precision and inter-day precision of the proposed method for the determination of Hg(II)

Hg(II) added (µg mL^{-1})	Accuracy and intra-day precision			Inter-day precision		
	Hg(II) found* (µg mL^{-1})	SD	% RSD	Hg(II) found* (µg mL^{-1})	SD	% RSD
0.4	0.412	±0.008	1.95	0.397	±0.011	2.16
0.8	0.813	±0.017	2.11	0.782	±0.018	2.52
1.0	0.992	±0.021	2.15	0.807	±0.022	2.76

* Mean of five replicate analysis

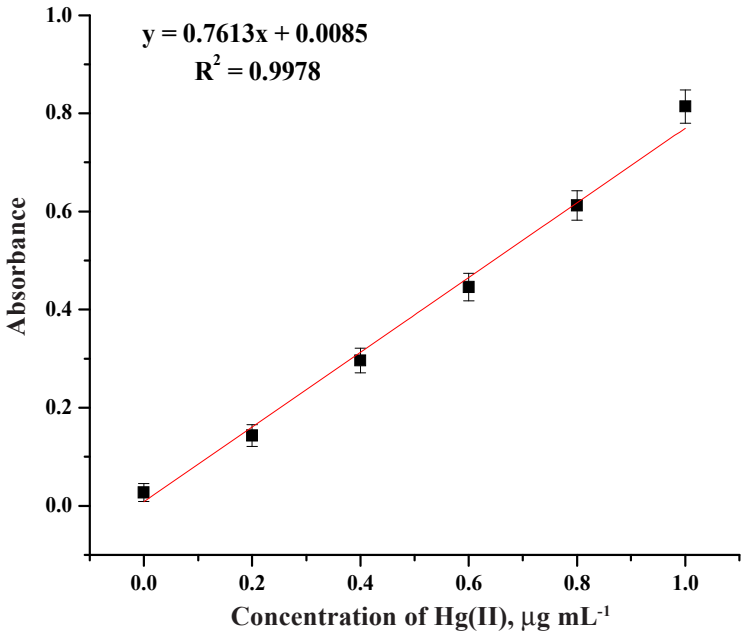


Figure 2. Calibration curve for determination of Hg(II)
Error bars indicate the relative deviation for six measurements

Optimization of reaction conditions

Effect of concentration of iodine

The effect of iodine concentration was studied. It was observed that for determination of Hg(II) at very low concentrations, a iodine solution of 0.002 % was sufficient. Furthermore, to optimize the amount of iodine, effect of variation of different volumes (0.1 - 0.5 mL) of 0.002 % iodine solution was studied. The results showed that 0.2 mL of 0.002 % iodine solution gave maximum absorbance. Above and below this amount the absorbance decreased, hence this optimized amount was chosen for further studies (Figure 3 A).

Effect of concentration of leucocrystal violet (LCV)

The effect of concentration of LCV on the reaction was studied in the range of 0.1 - 2 mL. It was found that optimum absorbance was obtained with 2 mL 0.125 % of LCV. There was not much change above this range, hence, the above concentration was selected for the assay (Figure 3B).

Effect of pH

The effect of various acids on proposed reaction was studied. The results showed that 0.1 M HCl gave the best effects. The effect of initial pH was varied by dropwise addition of 0.1 M HCl and 0.1 M NaOH solutions from 2.0-8.0 pH range. The maximum colour development was observed when the initial pH was maintained at pH 4.5±0.5 (Figure 4). Hence, this pH value was chosen for further studies. The pH of final solution for colour development was 3.5±0.5.

Effect of temperature and time

The effect of temperature on the colour reaction was studied in the range of 25°-55°C (Figure 5), with the concentration of reagents optimized. It was found that the optimum temperature for the reaction was 30°C with a decrease at higher temperatures. As the reaction between HOI and LCV is an instantaneous reaction, colour appears immediately, 10 min being sufficient for maximum colour development.

Effect of interfering ions

The effects of various interfering ions were studied at different concentrations. The tests were carried out using 10 µg of Hg(II), in a total volume of 10 mL solution. As can be seen, the method is affected by the presence of some ions, namely Fe^{3+} and Zn^{2+}. Concerning other ions, K^{+}, Cu^{2+}, CO_3^{-}, Pb^{2+}, Ag^{+}, Sn^{2+}, SO_4^{2-}, Cr^{3+}, Cd^{2+} and Mg^{2+} almost did not interfere up to 200 µg mL^{-1}. Results are summarised in Table 3.

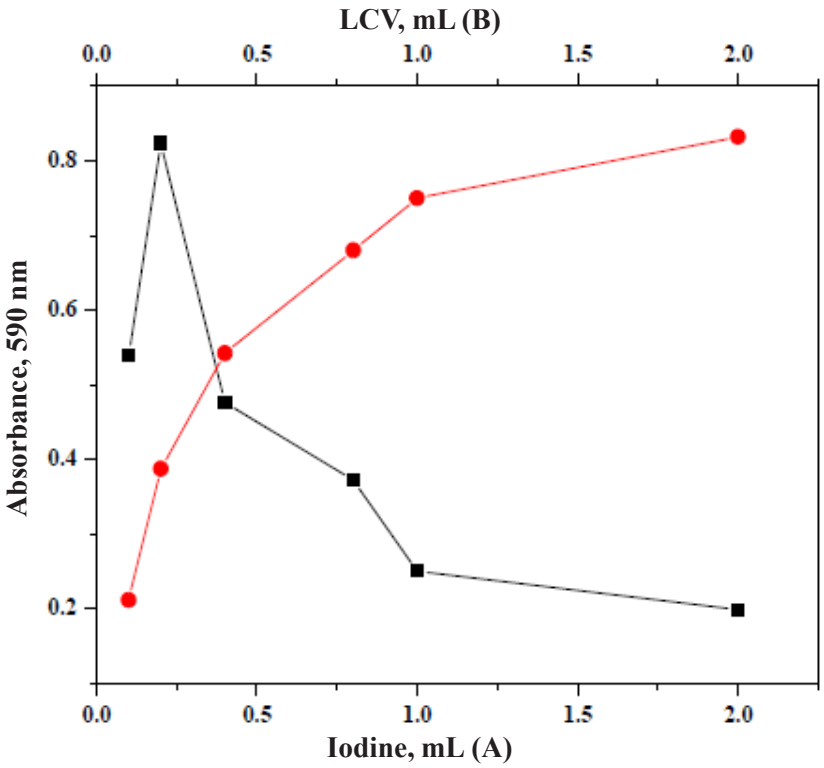


Figure 3. Effect of Iodine (A) and LCV (B) on oxidation of LCV to CV [Hg(II) (0.8 µg mL $^{-1}$)]

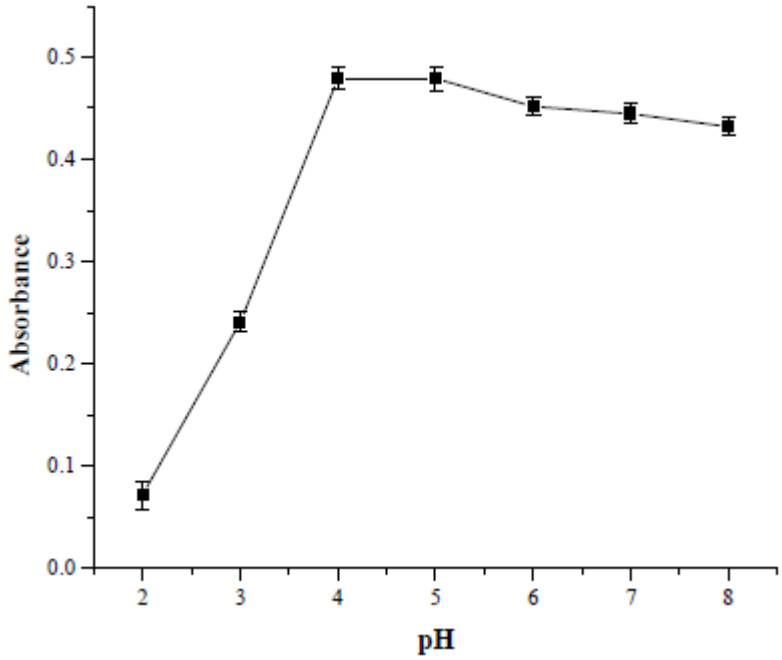


Figure 4. Effect of initial pH on absorbance [Hg(II) (0.4 µg mL^{-1})]
Error bars indicate the relative deviation for six measurements

Application

Determination in tap water and ground water

Tap water and ground water samples were collected from Bhilai (Chhattisgarh, India). The samples showed negative results. Therefore, known amounts of mercury were added and analyses were carried out as described in the procedure above. The determination was obtained from

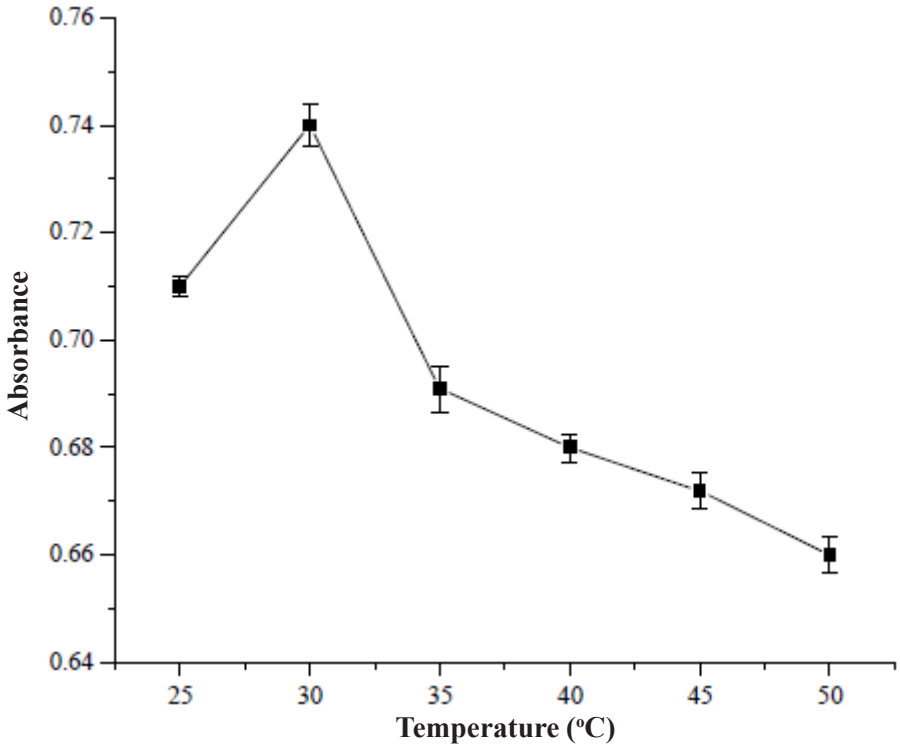


Figure 5. Effect of temperature on absorbance [Hg(II) (0.8 µg mL^{-1})]
Error bars indicate the relative deviation for six measurements

Table 3. Effect of diverse ions on absorbance

Concentration of Mercury = 1 µg mL^{-1}	
Diverse ions added	**Tolerance limit (µg mL^{-1}) ± SD**
K^{+}	1600±50
Cu^{2+}, CO_3^{-}	1000±30
Pb^{2+}, Ag^{+}	800±20
Sn^{2+}, SO_4^{2-}	600±10
Cr^{3+}, Cd^{2+}, Mg^{2+}	200±5
Fe^{3+}, Zn^{2+}	100±2

the calibration curve. Results are given in Table 4, showing that the developed method is applicable for analysing Hg(II) in water samples with a percentage recovery between 98.7 and 99.1.

Determination in river water, pond water and steel plant water

Water samples from upstream Shivnath river (Durg, Chhattisgarh, India) were analysed for mercury, which gave negative results. Samples from pond water and steel plant water were also collected. They also tested almost negative. Known amounts of Hg(II) were added to the samples, which were analysed as described above. Analysed water samples had recovery values of 98.3, 95.6 and 98.9 %, for river, pond water and steel plant water respectively.

Comparison with other reported methods

The proposed method was compared with various techniques including spectrophotometric methods. The comparision of the present method with other spectrophotometric reagents showed that the proposed procedure (Table 5) has lower limits of detection (DL) and limit of quantification (QL), comparably larger molar absorptivity,

Table 4. Determination of Hg(II) in water samples

Samples	Hg(II) concentration (μg mL^{-1}) Added (μg)	Found[a] (Proposed method)	SD	RSD (%)	Relative recovery (%)
Tap water sample	10	9.87	±0.062	0.63	98.7
Ground water sample	10	9.91	±0.081	0.82	99.1
River water sample[b]	10	9.83	±0.105	1.07	98.3
Pond water sample	10	9.56	±0.155	1.62	95.6
Steel Plant water sample[c]	10	9.89	±0.081	0.82	98.9

[a] Average of five determinations
[b] Shivnath river (CG), India
[c] Steel plant discharge Bhilai (CG), India

Table 5. Analytical features of several spectrophotometric methods for Hg^{2+} determination

Reagents	λ_{max}(nm)	pH	Beer's law limit (μg mL^{-1})	Molar absorptivity (L mol^{-1} cm^{-1})	Remarks
6-hydroxy-3-(2-oxo-indolin-3-ylideneamino) -2-thioxo-2H-1,3-thiazin -4(3H)-one [29]	505	4-6	0.2-2	4×10^4	Complicated steps
hydentoin,5 amino1,3,4 thiadizole 2 thiol (HTT) [30]	490	6-8	2.2	6.45×10^4	Less sensitive and narrow Beer's law range
2-(2-thiazolylazo)-p-cresol [31]	500	9.5	10	5.7×10^4	Use of Surfactants
isonitriso p-isopropyl acetophenone phenyl hydrazone (HIPAPH) [32]	395	7-11	1.0-20	2.67×10^{-3}	Difficulty in maintaining Stiochiometry and reaction condition
2-acetylpyridine thiosemicarbazone (APT) [33]	351	6	0.24-2.407	5.4×10^4	Lesser stability of complex
4-hydroxy 3, 5-dimethoxy benzaldehyde 4-hydroxy benzoylhydrazone (HDMBHBH) [34]	420	4	0.601-2.708	4.56×10^4	Complex stable in micellar solution only
1,5-diphenylthiocarbazone [20]	520	-	0-2	-	Sensitive but tedious procedure - adsorption and desorption invoved. Possibility for human errors

table 5. (continued).

Reagents	λ_{max}(nm)	pH	Beer's law limit (μg mL^{-1})	Molar absorptivity (L mol^{-1} cm^{-1})	Remarks
1,5-diphenylthiocarbazone [35]	490	Acidic media	0.05-10	5.02×10^4	Sensitive but interference from Ce^{3+}, Ag^{+}, Au^{3+} and Cr^{5+} ions
Hexacyanoruthenate(II) [4]	370	4	0.2-6	4.2×10^3	Sensitive but λ_{max} in UV range
Present work	590	5	0-1.0	1.49×10^5	Sensitive, non-extractive, non toxic reagents, less time, easily available reagents

lesser interference, with no stringent conditions involved, uses easily available green reagents, is rapid, accurate, cost effective and can be used with ease in any routine laboratories. The sophisticated techniques, though more sensitive, have some disadvantages, as mentioned in Table 6.

Conclusions

Many techniques (like gas chromatography, atomic absorption spectrometry, voltammetry and high performance liquid chromatography) are available, particularly in advance laboratories. These sophiscated methods/techniques are useful in monitoring the amounts of mercury present in various environment samples, yet they are associated with several drawbacks. Spectrophotometric method can be an indispensable technique for trace determinations in routine laboratories. As compared to other reported spectrophotometric methods, the proposed method has lower detection limit and higher molar absorptivity.

The proposed procedure for mercury determination is highly sensitive, simple, has good selectivity and can be successfully applied to real environmental samples with good accuracy and precision. The easy availability and non-toxic nature of reagents, absence of interference from a large group of species and use of non-extractive procedures are other added advantages of the method. Hence, the process could be superior to other reported spectrophotometric methods and can be used in routine labs which, in turn, will be beneficial in the present day environmental protection and societal threats.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Table 6. Comparision with other techniques

Techniques	Method	DL (μg mL^{-1})	Remarks	Ref.
Spectrophotometer	Ionic liquid based dispersive liquid-liquid micro-extraction (IL-DLLME)	0.01	Sensitive but complicated steps and interference from Ag^{+} and Mn^{2+}	[1]
Cold Vapor Atomic Fluorescent Spectro-photometry (CV-AFS)	Multi-isocratic elution	0.11	Sensitive, selective, costly, high maintanence, trained technicians required	[36]
Spectrophotometry	Chemosensor	0.0804	Sensitive but interference from Ti^{4+}, Pb^{2+}, Ni^{2+}, Ag^{+} and UO_2^{2+}	[37]
ICP-MS	Microwave digester	0.03	Time taking and interference from Al^{3+}, Fe^{3+}, Mn^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+} and Fe^{2+}	[38]
Spectrophotometer	Complexation	0.01	Time consuming and interference from Cu^{2+}, Co^{2+} and Fe^{2+}.	[39]
Spectrophotometer	Oxidation	0.022	Sensitive but use of costly reagents.	[40]
Molecular fluorescence	Ion imprinting	0.004	Highly sensitive but not cost effective. Interference from Cu^{2+}, Ca^{2+} and Zn^{2+}	[41]
Fluorimetric	Chemosensor	0.151	Sensitive, high cost, time consuming and interference from Cd^{2+}, Mg^{2+} and Zn^{2+}	[42]
Reflect Spectrophotometer	Titration	0.1-30	Sensitive but more interference (Mn^{2+}, Mg^{2+}, Pb^{2+}, Cu^{2+}, Ag^{+}, Cl^{-} and $SO4^{2-}$)	[43]
Cold vapour generation atomic fluorescence spectrometry (CV-AFS)	Biomonitoring	0.004	Sensitive but high cost, and trained technician required.	[44]
photochemical vaporgeneration (PVG) coupled to cold vapor atomic absorption spectrometry (CVAAS)	Element extraction	0.0028	Highly sensitive but λ_{max} in UV range.	[45]
Spectrophotometer (Proposed work)	Simple oxidation mechanism	0.0026	Highly sensitive, selective, simple, lesser interference.	Present work

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