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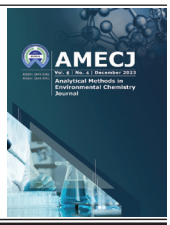

Rapid extraction and separation of mercury in water and food samples based on micelles and azo-thiazoles complexation before determination by UV-Vis spectrophotometry

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ABSTRACT
A simple and sensitive procedure has been established for analyzing mercury (II) ions spectrophotometrically in the presence of micellar medium using three azo-thiazoles complexing reagents: 2-amino-6-(thiazole-2-yldiazenyl)-3-pyridinol ($C_8H_7N_5OS$), 8-hydroxy-7-(thiazole-2-yldiazenyl) quinoline-5-sulfonic acid ($C_{12}H_8N_4O_4S_2$), and 1-hydroxy-4-(thiazole-2-yldiazenyl)-2-naphthoic acid ($C_{14}H_9N_3O_3S$). H1 NMR spectra validated the three azo thiazoles synthesized material. Tween 80 (polysorbate 80) and cetyltrimethylammonium bromide ($C_{19}H_{42}BrN$ as molecular biology) are micellar mediums to enhance sensitivity. Absorbances were measured for Hg (II) complexation with R_1, R_2, and R_3 at λ_{max} of 617, 633, and 554 nm, respectively. The UV-Vis spectrophotometer showed calibration curves in the 0.2-15 mg L^{-1}. The molar absorptivity, Sandell's sensitivity, detection, and quantification limits (LOD, LOQ) were determined. The interferences of various ions were investigated, and a statistical assessment of the results was performed. The methods have been applied for trace determination of mercury (II) in food and environmental water samples. For food samples, all samples were digested before complexation with the azo-thiazoles material at optimized pH before determination by UV-Vis spectrophotometry.

1. Introduction

Environmental monitoring is a subject that requires the development of novel analytical methods. The speciation of potentially hazardous metal ions is essential for comprehending their eco-toxicological and biological properties, which depend on the chemical species. Extensive research has been devoted to developing sensitive, relatively simple, accurate, rapid, and cost-effective methods for determining industrially pertinent metals that may harm human health [1]. Mercury is a problematic natural pollutant because it can hurt almost all living things [2]. As a result of human environmental achievements, mercury compounds can exist in various settings [3]. They frequently exist in trace amounts in natural water types [4]. Mercury pollution is a significant problem in the lakes and rivers near industrial zones. Therefore, it is essential to develop new, selective, efficient, and cost-effective monitoring procedures for mercury ions [5]. Low Hg (II) concentrations in the target species pose a significant challenge in mercury determination. In naturally occurring water samples, the predominant forms of mercury are inorganic

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mercury (mercurous and mercuric) and organic mercury (CH_3Hg^+). Modern records indicate that the total (Hg^{2+}, MHg^+) concentrations range from 0.2-100 ng L^{-1} and the organic mercury (CH_3Hg^+) has a a concentration of 0.05 ng L^{-1} in water [6]. Many analytical techniques, such as inductively coupled plasma (ICP) [7], cold vapour atomic absorption spectrometry (CV-AAS) [8], neutron activation analysis (NAA) [9], x-ray fluorescence [10], atomic fluorescence spectrometry (AFS) [11], and spectrophotometric technique [12] had been advanced to monitor mercury ion at a micro level. Each of the above methods has some advantages; however, they may have disadvantages, such as low reproducibility and limited sample flexibility. Without pre-concentration, the inductively coupled plasma method was suitable for determining trace amounts of Hg (II). However, this instrument is quite expensive to purchase and maintain. Additionally, this technique has some significant interference [11]. Due to its simplicity, atomic absorption spectrometry was a suitable and widely utilised technique for accurate Hg(II) determination. In the meantime, its application is limited due to its limited linear range and significant spectral interference from volatile substances [13]. Due to the low Hg (II) concentration, these practises are not directly applicable to environmental and biological models and/or frequently require pre-concentration steps to enhance selectivity. Several photometric reagents have been used for spectrophotometric mercury ion determination. Dithizone was the most common reagent used for this purpose [14]. Before photometric analysis, the Hg(II)-dithizone complex is taken out with either CCl_4 or $CHCl_3$ [15]. Based on the absorbance measurements of the formed complexes in the presence of surfactants, we report for the first time the direct spectrophotometric determination of the Hg(II) ion with three novel azo dyes. Also, many methods based on nanotechnology were used for mercury extraction/removal from different water, air, human and food (vegetable and nut) samples. Mousavi et al used Tetraethyl thiuram disulfide [$(C_2H_5)_2$-$NCSS_2CSN$-$(C_2H_5)_2$; TET] mixed with ionic liquids for extraction/speciation mercury in human samples by dispersive liquid-liquid microextraction (DLLME) coupled to CV-AAS [16]. Osanloo et al used silver nanoparticles coating on micro glassy balls for removal mercury from air and Rouhollahi et al can be determined mercury in air and human samples [17-19]. Golbabaei, Bagheri and hassani showed that mercury can be determined in biological human samples based on adsorbent by the CV-AAS. In addition, they used the nano-palladium functionalized on the silica nanoparticles for mercury removal from air by the GFSC method [20-22]. Moreover, some methods based on adsorbents such as MWCNTs, pyrrolic and pyridinic nitrogen doped porous graphene nanostructure(N-D-PNG) extracted mercury from water, food, and air samples [23-25].

In this study, we prepared the azo dyes based on new synthesis include, 2-amino-6-(thiazole-2-yldiazenyl)-3-pyridinol (C8H7N5OS) [R_1], 8-hydroxy-7-(thiazole-2-yldiazenyl) quinoline-5-sulfonic acid ($C_{12}H_8N_4O_4S_2$) [R_2], and 1-hydroxy-4-(thiazole-2-yldiazenyl)-2-naphthoic acid ($C_{14}H_9N_3O_3S$) [R_3] (Fig. 1). The H1 NMR spectra of all synthesized azo dyes. Herein, Hg(II) was successfully measured at the micro level in different water and food samples using the proposed methodologies by UV-Vis spectrometry after complexation with azo dyes. The food samples were digested before the complexation and determination procedure. The approach has several advantages, including its low cost, ability to be applied to real samples, and broad linear range.

2. Experimental

2.1. Instrumentation

All absorption measurements are taken with a Jasco UV-Vis spectrophotometer (model V530, Jasco, Tokyo, Japan) with a scanning speed of 400 nm/min, a bandwidth of 2.0 nm, and 1.0 cm pair-matched quartz cells. A pH meter (HI 8014, HANNA Instruments, Woonsocket, RI, USA) was used to adjust the pH of all solutions. We used a Fluoromax-4 (Horbiba Scientific, Kyoto, Japan) for the spectrofluorimetric observations. Both the excitation and emission slit widths were 9 nm.

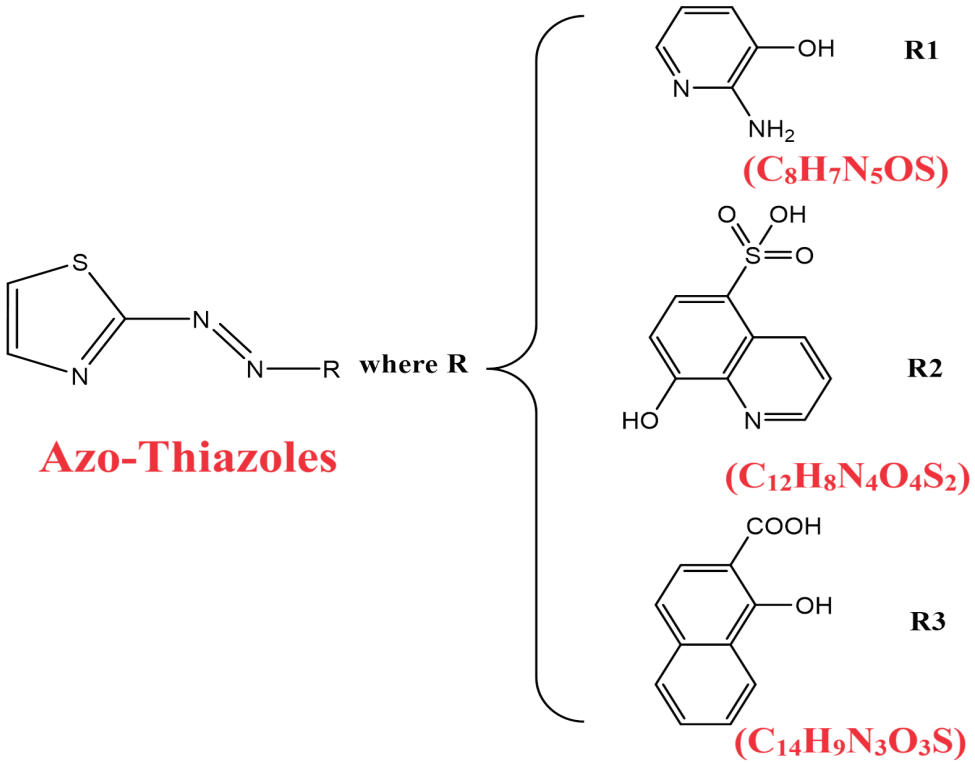


Fig.1. Synthesis of azo dyes include, 2-amino-6-(thiazole-2-yldiazenyl)-3-pyridinol (C8H7N5OS) [R_1], 8-hydroxy-7-(thiazole-2-yldiazenyl) quinoline-5-sulfonic acid ($C_{12}H_8N_4O_4S_2$) [R_2], and 1-hydroxy-4-(thiazole-2-yldiazenyl)-2-naphthoic acid ($C_{14}H_9N_3O_3S$) [R_3]

2.2. Reagents and Chemicals

All chemicals and reagents employed in this study were of analytical grade (Merck, Darmstadt, Germany), and the solutions were prepared using bi-distilled water. A stock solution of 1×10^{-2} M mercuric chloride was prepared by weighing out 0.679 g $HgCl_2.2H_2O$, dissolved in the least amount of bi-distilled water and completed in a 100-mL measuring flask to the mark with bi-distilled water. The stock solution was then standardized by EDTA [26]. All used solutions were carefully diluted from a stock solution. At room temperature, the solution held up for a whole month. The preceding method was used to prepare universal buffer solutions. As a result of the low Hg (II) concentration, these methods are either not directly applicable to environmental and biological models or necessitate additional pre-concentration processes to increase selectivity. Mercury ion concentrations have been measured spectrophotometrically using a variety of photometric reagents. The most commonly used reagent for this was dithizone [27]. Tween 80 [0.5% (v/v)], Triton X-100 [0.5% (v/v)], cetyltrimethylammonium bromide (CTAB) [0.5% (w/v)], and sodium dodecyl sulphate (SDS) [0.5% (w/v)] were used as surfactants due to their commercial availability in a highly purified form, low toxicity, and low charge. Tween 80 and Triton X-100 were prepared by adding 0.5 mL of each surfactant to 50 mL of bi-distilled water and then bringing the volume to 100 mL to achieve a 0.5% (v/v) solution. In the case of SDS and CTAB, a 0.5% (w/v) solution was intended by dissolving 0.5 g of the surfactant in 50 mL of bi-distilled water and then filling a 100 mL measuring flask with bi-distilled water to the desired volume. Nitric acid (HNO_3), 70 %, and hydrogen peroxide (H_2O_2), 30 % (w/w) in H_2O, were obtained from Aldrich.

2.3. Synthesis of reagents

A solution of 2-aminothiazole (10.014 g, 0.1 mole) dissolved in 1:1 (v/v) HCl aqueous solution was cooled in an ice bath at ca. – 5.0 °C. To this solution, while stirring vigorously, a cold aqueous solution of sodium nitrite (6.903 g, 0.1 mole) was added, and the reaction mixture was kept in an ice bath at a temperature range of 0-5.0°C for 30 min. The obtained cold diazonium salt was used for coupling with an equivalent quantity of cold solution of 2-amino-3-hydroxypyridine (11.01 g, 0.1 mole), dissolved in 10 % (w/v) NaOH. The formed azo dye (R_1) was kept for 40 min in an ice bath at ca. -5.0°C, filtered off, washed with bi-distilled water, and dried. The obtained azo compound was finally re-crystallized using absolute C_2H_5OH. For the preparation of the other azo compounds, 8-hydroxy-7-(thiazole-2-glaze) quinoline-5-sulfonic acid [R_2] and 1-hydroxy-4-(thiazole-2-ylazo)-2-naphthoic acid [R_3], a typical procedure was used using 8-hydroxyquinoline-5-sulphonic acid (26.125 g, 0.1 moles) and 1-hydroxy-2-naphthoic acid (18.81 g, 0.1 moles), respectively. The reaction yield was in the range of 75-85 %. The azo compounds showed a sharp melting point, indicating high purity. The compounds were characterized using ^{1}H-NMR spectroscopy (Fig. 2a-c). The reagent solutions were prepared by dissolving 0.110, 0.033 and 0.148 g of R_1, R_2 and R_3 in 100 mL ethanol to obtain 5×10^{-3}, 1×10^{-3} and 5×10^{-3} mol L^{-1} of R_1, R_2 and R_3, respectively.

2.4. General procedure

In a typical procedure, for the reagents R_1 and R_2, an appropriate volume of the sample containing 1×10^{-3} M of Hg(II) was placed in a 10 mL measuring flask. The universal buffer of pH 6.0 (5 mL) or pH

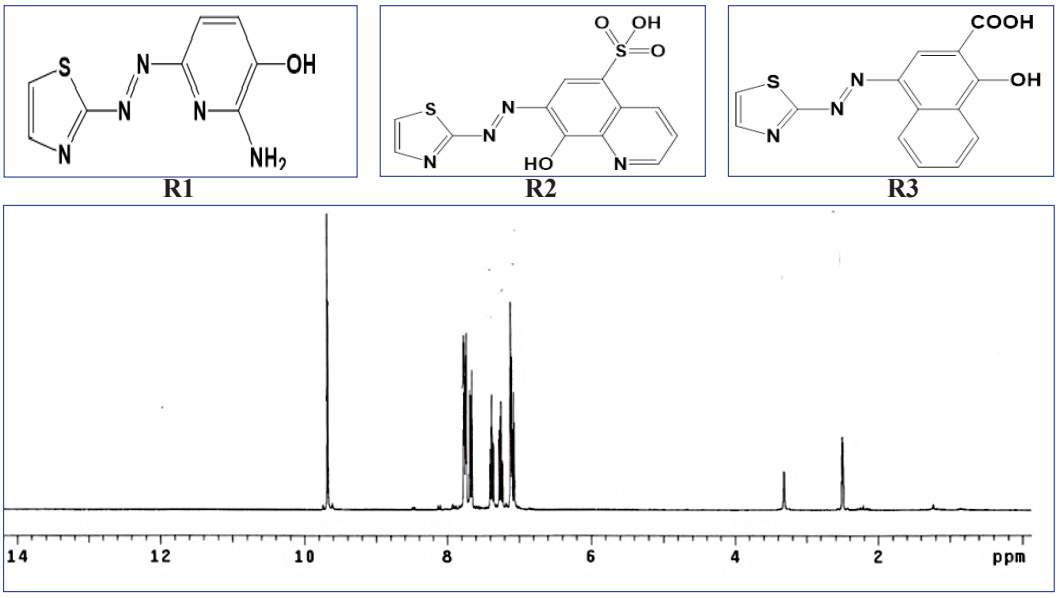


Fig. 2a. ^{1}H-NMR spectroscopy of 2-amino-6-(thiazole-2-yldiazenyl)-3-pyridinol (C8H7N5OS) [R_1]

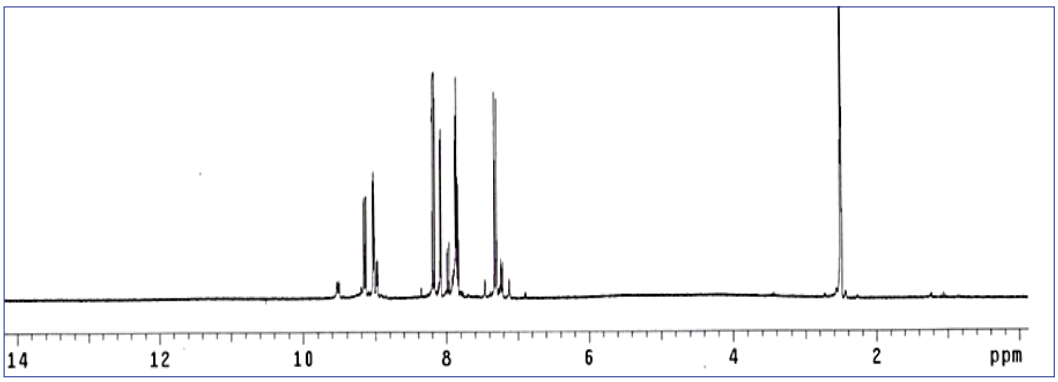


Fig. 2b. ^{1}H-NMR spectroscopy of 8-hydroxy-7-(thiazole-2-yldiazenyl) quinoline-5-sulfonic acid ($C_{12}H_8N_4O_4S_2$) [R_2]

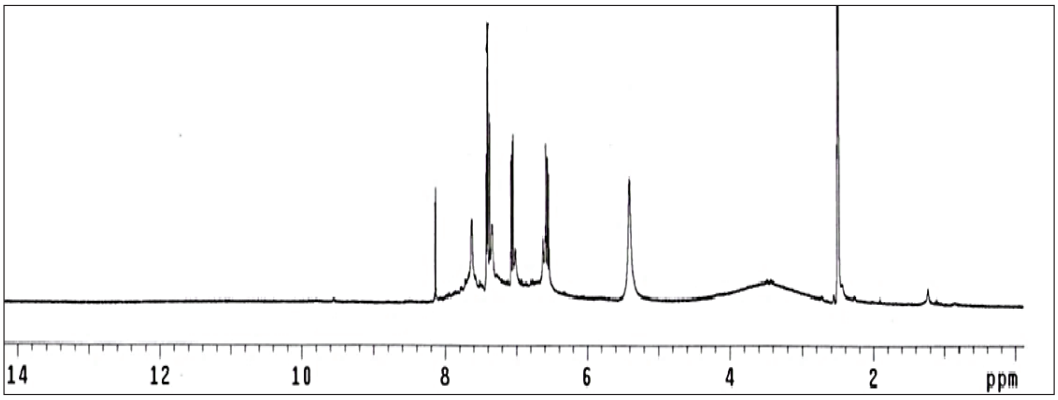
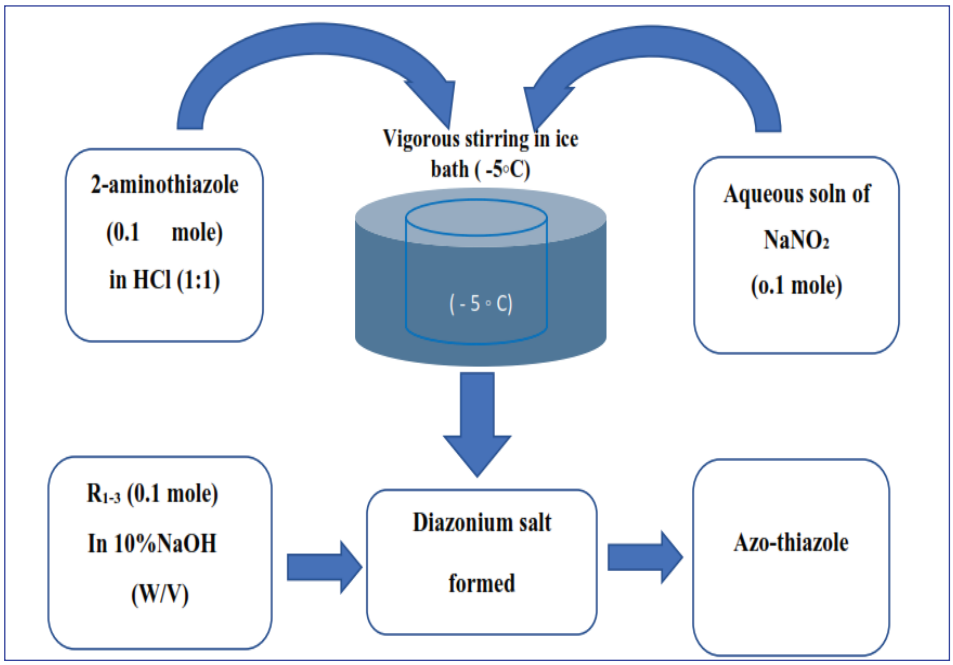


Fig. 2c. ^{1}H-NMR spectroscopy of 1-hydroxy-4-(thiazole-2-yldiazenyl)-2-naphthoic acid ($C_{14}H_9N_3O_3S$) [R_3]

7.13 (4 mL) was added to the sample for R_1 or R_2, respectively. Afterwards, 1.0 mL of (5×10^{-3} M) R_1 or 2.0 mL of (1×10^{-3} M) R_2 was added, then 1.0 mL of tween [0.5% (v/v)] or 1.5 mL of CTAB [0.5% (w/v)] was added, respectively. A blank solution with all the chemicals except Hg(II) was equipped and treated as the sample solution. The procedure was performed at room temperature (25°C). As outlined above, the difference in absorbance between the sample and its respective blank was measured at wavelengths of 617 or 633 nm for R_1 and R_2, respectively. On the other hand, a similar procedure was used when the R_3 reagent was used. The optimal order of addition was reagent R_1 (1.0 mL, 1×10^{-3} M), metal (1×10^{-3} M), buffer (pH 4.97, 4 mL), and surfactant (0.5 mL of tween [0.5% (v/v)]). In addition, at a wavelength of 554 nm, the difference in absorbance between the sample and its corresponding negative was measured (Scheme 1). After sample preparation, the optimal time was determined for each of the three reagents by measuring the absorbance at various intervals(times). Different experimental parameters, such as pH, buffer volume, reagent concentration, surfactant type, concentration, reaction time, and reaction temperature, have been investigated to determine the optimal conditions for the proposed spectrophotometric method.



Scheme 1. Procedure for extraction and separation of mercury in water and food samples

2.5. Determination of the molecular structures using the mole ratio method

In the mole ratio procedure illustrated in [28], the Hg(II) ion concentration was held constant at 1 mL of 1.0×10^{-4} M, while that of the ligand was systematically modulated between 0.2 and 2.4 mL of 1.0×10^{-4} M. The absorbance of the prepared solutions was measured at the optimal wavelength under the optimal conditions. The obtained absorbance was then plotted against the mole ratio of ligand, [ligand/metal]. The stoichiometric ratio of the formed stable complexes was extracted from the purified intersections.

2.6. Mercury ion determination in some real samples

The food samples (potatoes, beans, rice, soybeans, and legumes) were dried in an oven at 75°C until their weights stabilized and then ground into a fine powder. In a 100 mL vial, 2.5 g of powder was weighed, and 10 mL of concentrated nitric acid was added. The obtained mixture was digested for 20 minutes in a microwave oven. After allowing the flask to settle, an additional 10 mL of nitric acid and 1 mL of hydrogen peroxide were added, and the mixture was allowed to stand for approximately 25 minutes [29, 30]. The mélange was then re-heated in the microwave for 40 minutes. The digest was then permitted to chill. Finally, 1.0 mL of nitric acid was added and allowed to sit for 10 minutes. The obtained solution was adjusted to a pH range of 8.0 to 9.0 by adding sodium carbonate. The solutions were then processed by the general procedure. Standard addition was used to estimate the recovery percentage and verify the results' precision.

2.7. Determination of mercury (II) in water samples

Water samples were collected around the settlements of Benha and Qalyub in the Al-Qalyubia Governorate. After adding (1:2) (v/v) concentrated H_2SO_4 and concentrated HNO_3 to each filtered water sample in a fume chamber [29, 30], each sample was roughly evaporated to dryness. After chilling, the remaining solution was re-heated with 10.0 mL of double-distilled water. The solution was cooled and neutralized with diluted ammonium hydroxide. The solution was then filtered and transferred precisely into a 25 mL measuring vial. The solutions were then processed by the general procedure.

3. Results and Discussion

Under ideal conditions, mercury (II) creates pink complexes with R_1, R_2, and R_3. Figure 3 shows the absorption spectra of Hg(II)–R_1, Hg(II)–R_2, and Hg(II)–R_3 complexes at maximum values of 617 nm, 633 nm, and 554 nm, respectively. The mercury complexes exhibited a bathochromic shift in their absorption spectra compared to the free reagents, which absorbed at 557 nm, 563 nm, and 477 nm. Studies were conducted to determine the optimal conditions for obtaining high colour intensity and maximal colour development for micro quantification of mercury. Each of the following parameters' effects on colour development was investigated.

3.1. Influence of pH of the medium

Specifically, pH is a crucial factor influencing the chelation of the Hg ion by the proposed reagent molecules. A universal buffer system ranging from 2.7 to 12.0 was utilized to ascertain the optimal pH. As shown in Figure 4, the maximum absorption values for Hg (II) complexes with R_1, R_2, and R_3 were obtained at pH 6.0, 7.13, and 4.97, respectively. In addition, the effect of buffer volume on the analytical peak was investigated between 0.5 and 6 mL (in a total volume of 10 mL). With a buffer volume of 5.0 mL for R_1-Hg (II) and 4.0 mL for both R_2-Hg (II) and R_3-Hg (II) complexes, the highest analytical absorbance was found (Fig.4).

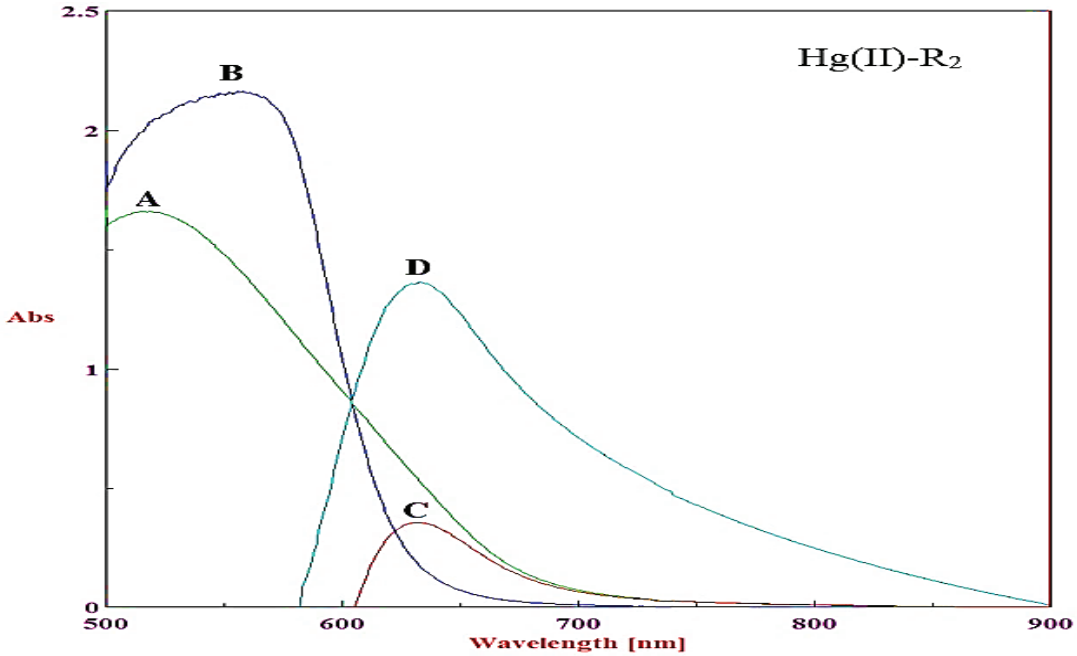


Fig. 3. The absorption spectra of the complex formed between reagents R_2 with Hg(II) where **A**: Spectrum of pure ligand at pH 7.13 against buffer solution as a blank, **B**: Spectrum of a complex solution containing ligand, metal ion and buffer using the same buffer as blank. **C**: Spectrum of solution (B) against ligand and buffer of the same pH as blank and **D**: spectrum of solution (C) in the presence of CTAB.

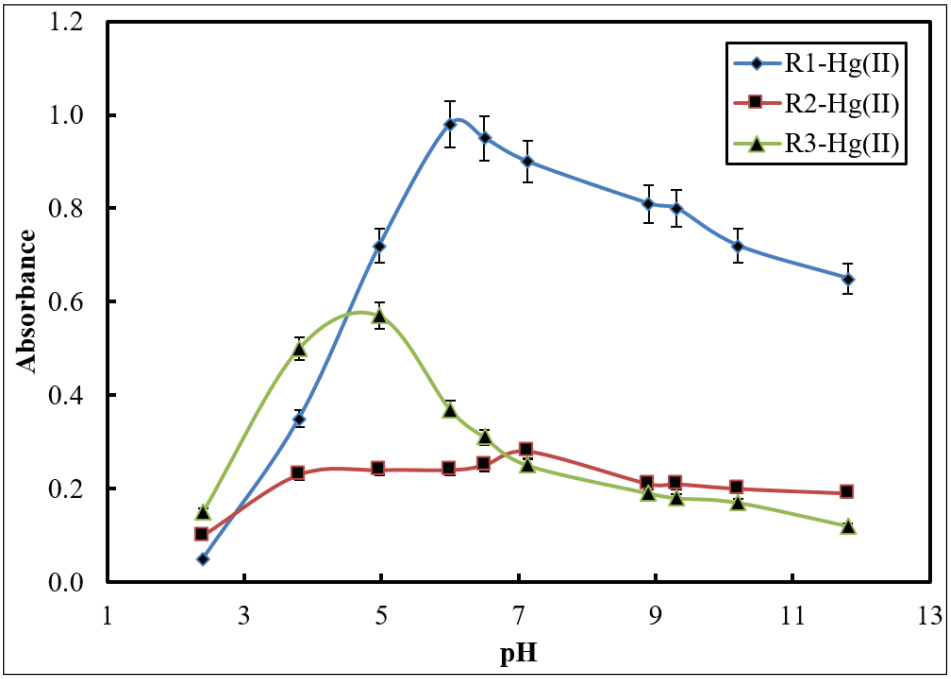


Fig. 4. Effect of pH on the absorption spectra of complexes formed between Hg(II) and the reagents under study.

3.2. Influence of the reagent concentration

The effect of R_1, R_2 and R_3 concentrations on the analytical signal (absorbance) of the formed complexes with Hg(II) is shown in Figure 5. It was clear that for R_1-Hg(II) and R_3-Hg(II) complexes, the absorbance increased with the reagent concentration up to 5×10^{-4} M and then suffered from some decrease by increasing the reagent concentration. Therefore, 5×10^{-4} M of the reagents R_1 and R_3 was selected as the optimum concentration. However, the absorbance enhanced with R_2 concentration and reached its maximum value at 2×10^{-4} M. Consequently, 2×10^{-4} M was chosen as the optimal concentration for R_2 reagent.

3.3. Effect of surfactant volume

Tween 80 was the most appropriate solvent for R_1 and R_3, while CTAB was the most suitable for the R_2 complex. The optimal volumes of 0.5% (v/v) Tween-80 were 1 and 0.5 mL for Hg(II) complexes with R_1 and R_3, respectively. For the Hg(II)-R_2 complex, however, the optimal volume for obtaining a high absorbance value was 1.5 mL of 0.5% (w/v) solution.

3.4. Effect of sequence of addition

The effect of the sequence of addition [reagent (R), metal (M), buffer (B), and surfactant (S)] on the formation of complexes was investigated by measuring the absorbance of sample solutions prepared using various addition sequences. The sequence [M.B.R.S.] was the best for Hg(II)-R_1 and Hg(II)-R_2 complexes, while [R.M.B.S.] was the best for reagent R_3. The optimal addition sequence and other optimal conditions for each metal complex are listed in Table 1.

3.5. Effect of temperature and time

The influence of temperature and time on the proposed methodologies was investigated over 25 - 60°C and 2 - 50 min, respectively. The optimal reaction temperature for R_1-Hg(II), R_2-Hg(II), and R_3-Hg(II) complexes was determined to be 25°C. The optimization of the reaction time revealed that all complexes instantly attained their maximum absorbance, except for R_1, which achieved its maximum absorbance 10 minutes after blending. Table 1 presents the optimal time and temperature for other complexes.

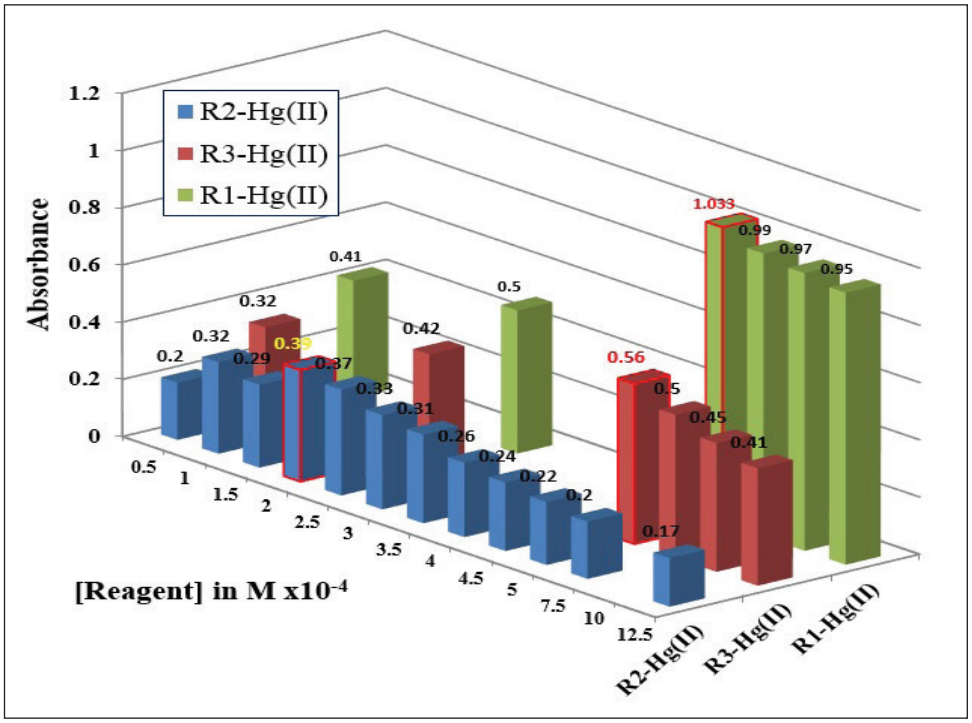


Fig. 5. Effect of reagent concentration on the absorption spectra of complexes formed between Hg(II) and the reagents under study.

Table 1. The optimum condition parameters of Hg(II) with the azo-dye reagents.

Parameters	Hg(II)		
	R_1	R_2	R_3
Type of buffer	Universal	Universal	Universal
Working pH	6.00	7.13	4.97
Volume of buffer, mL	5.00	4.00	4.00
λ_{max}, nm	617	633	554
Reagent's concentration, M	5×10^{-4}	2×10^{-4}	5×10^{-4}
Surfactant used	Tween 80	CTAB	Tween 80
Volume of surfactant, mL	1.0 [0.5% (v/v)]	1.5 [0.5% (w/v)]	0.5 [0.5% (v/v)]
Sequence of addition	M.B.R.S	M.B.R.S	R.M.B.S
Time, min	10	1	1
Stoichiometric ratio (M: L)	(1:1)	(1:1)	(1:1), (1:2)
Stability constant[a]	11.11	7.62	9.54

R: Reagent B: Buffer M: Metal S: surfactant
[a]Stability constant using the molar ratio method

3.6. Stoichiometric ratio

Utilizing a molar ratio routine, the stoichiometry of Hg (II)-R_{1-3} complexes was determined. The optimal absorbance wavelengths for each complex were 617 nm, 633 nm, and 554 nm for the R_1, R_2, and R_3 complexes, respectively. The results indicated that the absorbance curves for R_1 and R_2 complexes attained their maximum value at the same molar ratio (1:1). This demonstrated that a singular complex compound composed of (Hg-R_1) and (Hg-R_2) can be formed from these two systems. The results indicated that the R_3 complex's molar ratio curve attained two maximum values with molar ratios of 1:1 and 1:2 (M: L). This indicated that two complex compounds with the structures (Hg-R_3) and (Hg-$[R_3]_2$) may be present in the method. The calculated stability constants for the formed (1:1) complexes of Hg(II) with the reagents R_1, R_2, and R_3 were 11.11, 7.62, and 9.50, respectively. Due to the high chemical stability of its structure, the Hg(II)-R_1 complex has a high value for its stability constant. This is likely because reagent R_1 contains two electron-donating groups (-OH and -NH_2), which increase the electron cloud on the chelation centre and subsequently increase the stability of this complex relative to the other complexes under investigation.

3.7. Study of the interference

Taking into account the high selectivity provided by the spectrophotometric technique at the selected wavelengths of 617 nm, 633 nm, and 554 nm for Hg(II) chelated with R_1, R_2, and R_3, the tolerance limits for a maximum error of 3.0% were determined. Various interfering ions of varying concentrations were added to a solution containing 2 mg L^{-1} of Hg(II) for investigation. The results demonstrated that common coexisting ions had no appreciable effect on determining analyte ions. Co(II), Ni(II), and Mn(II) species were discovered to cause interference at high tolerance limits between 1 and 20. Therefore, they must be eliminated or disguised prior to mercury analysis. Sodium chloride, sodium borate, and sodium tungstate do not interfere with detecting mercury (II) ions. In addition, the concentrations of these ions are typically deficient in most water and food samples, so these procedures can be used to detect Hg(II) ions in actual water and food samples.

3.8. Spectrofluorometric measurements

As revealed in Figures 6-8, an aqueous solution of R_2 and R_3 with a concentration of 1.0×10^{-5} M displays a vigorous fluorescence intensity at $\lambda_{ex/em}$=507/614 and $\lambda_{ex/em}$=480/610 nm, respectively. Two phenomena were

observed for R_2 and R_3. The emission fluorescence peak of free reagents R_3 has suffered from quenching after adding 1.0 × 10-4 M mercury ion. This quenching increased seriously by increasing the concentration of Hg(II). The second observation was shown in the case of reagent R_2, where the absence of a new emission peak at λ_{em} 664 nm as shown in Fig. 6. These two observations gave evidence that the formation of nonluminous complexes between R_2, R_3 and Hg(II) [31]. Therefore, these observations can accurately estimate mercury ions using the prepared reagents R_2 and R_3 in this research. It turns out that mercury ion acts as a fluorescent quencher in the case of R_2 and R_3. Experimentally, it was found that the R_1 detector is not given fluorescence intensity, which may be attributed to the structure of R_1 compared to the other R_2 and R_3.

3.9. Analytical characteristics

The calibration graphs for Hg(II) complexes were linear in the concentration range 0.2-10.0 µg mL^{-1} with an excellent correlation coefficient (r^2) of 0.998 for R_1 complex while 0.6-13.0 µg mL^{-1} with (r^2) of 0.998 in case of R_2 complex and 0.9-5.0 µg mL^{-1} with (r^2) of 0.999 in case of R_3 complex. The regression equation may be expressed by

$A = 0.064\ C-0.024$ for Hg(II)-R_1

$A = 0.020\ C+0.001$ for Hg(II)-R_2

$A = 0.028\ C-0.013$ for Hg(II)-R_3

where C is the Hg(II) concentration in a sample solution in µg mL^{-1}, and A is the absorbance. The molar absorptivity and Sandell`s sensitivities of the formed complexes were calculated and exhibited a high sensitivity of the three reagents.

In addition, Table 2 tabulated the analytical parameters for the three proposed methods. The limits of detection and quantification were 51 µg L^{-1} and 156 µg L^{-1} for R_1-complex, 15 µg L^{-1} and 45 µg L^{-1} for R_2 complex, and 26 µg L^{-1} and 785 µg L^{-1} for R_3 complex. These concentration intervals are suitable for the measured [Hg(II)] in four real water and five food samples. The relative standard deviation (RSD) for six replicate measurements of 2 mg L^{-1} of Hg(II) with R_1, R_2 and R_3 was obtained at 2.88%, 3.42% and 3.32%, respectively.

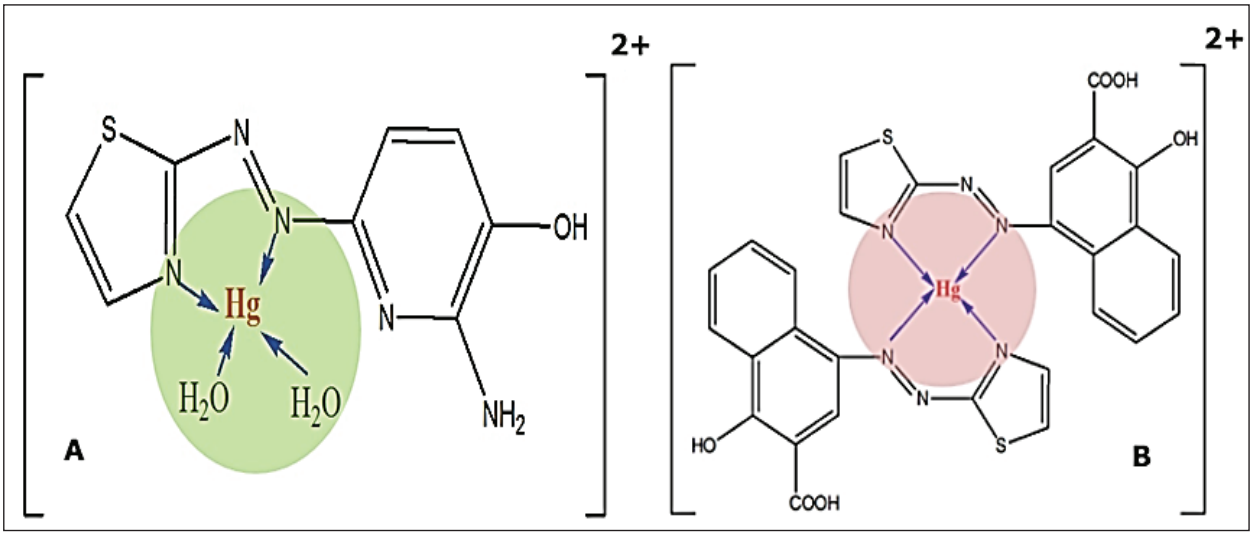


Fig. 6. The proposed structures of the complexes formed between Hg(II) and reagents where A) is (1:2)(Hg(II)-2R_3) and B) is (1:1)(Hg(II)-R_1)

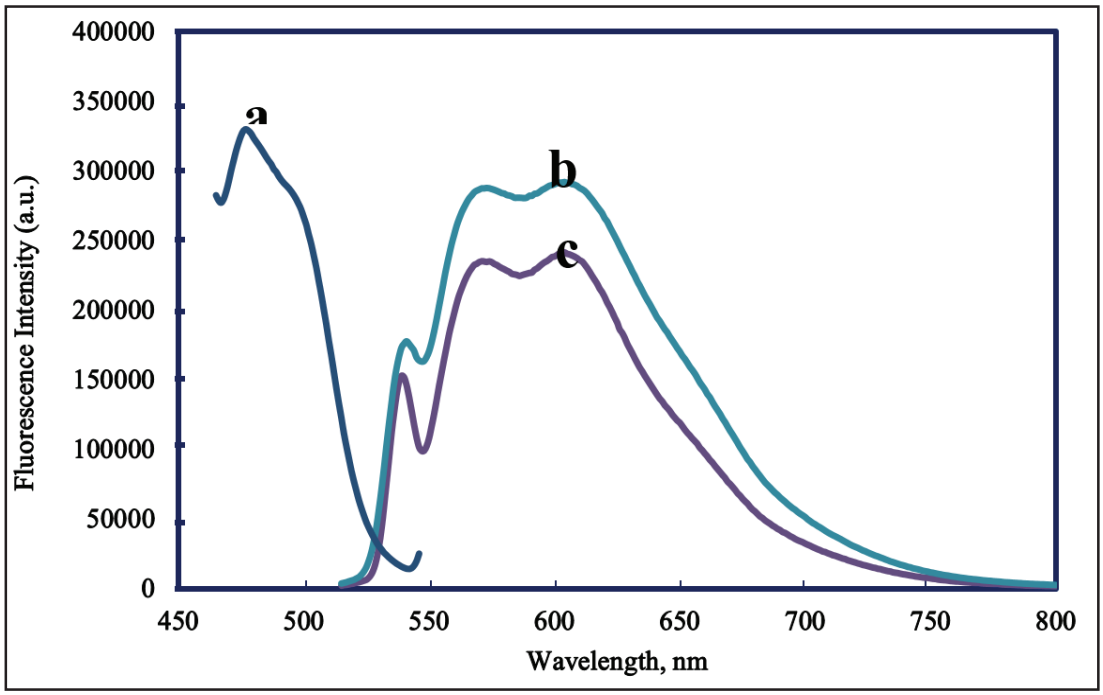


Fig. 7. Fluorescence excitation (a), emission (b) and after addition of 1.0×10^{-4} M Hg(II) (c) spectra for R_2 in a universal buffer of pH 7.13, λ_{ex}=507 nm and λ_{em}=614 nm.

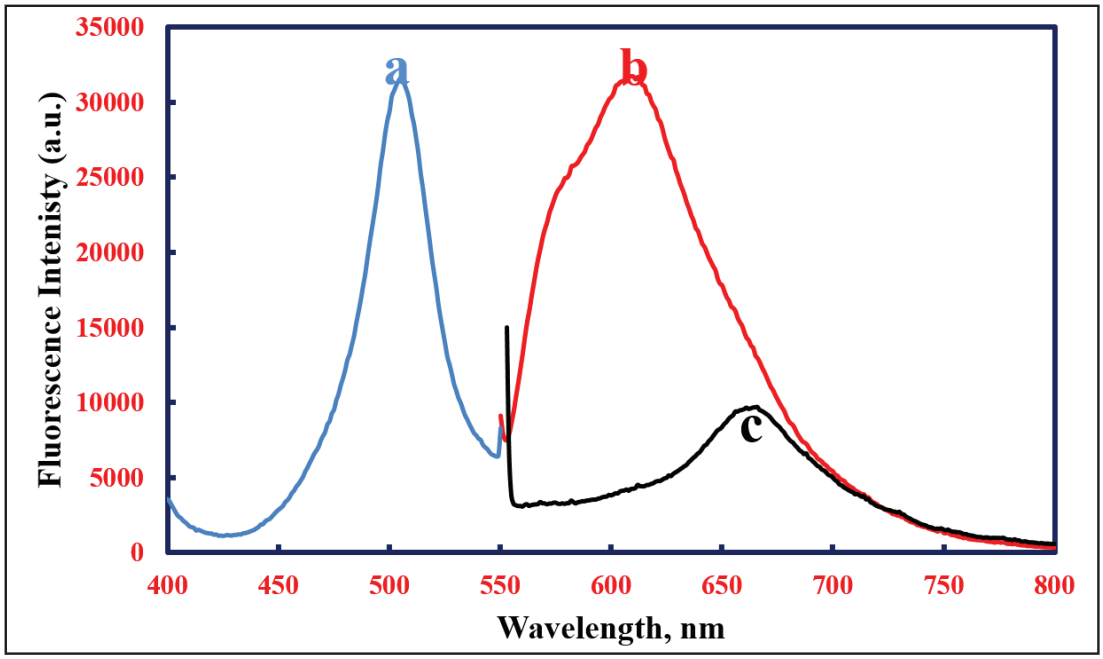


Fig. 8. Fluorescence excitation (a), emission (b) and after the addition of 1.0×10^{-4} M Hg(II) (c) spectra for R_3 in the universal buffer of pH 4.97, λ_{ex} = 480 nm and λ_{em} = 610 nm

Table 2. Cumulative data of the analytical conditions for spectrophotometric determination of Hg(II) with different reagents

Parameters	Hg(II)		
	R_1	R_2	R_3
Color	Violet	Violet	Violet
pH	6.00	7.13	4.97
λ_{max} (nm), A curve	557	563	477
λ_{max} (nm), B curve	567	521	496
λ_{max} (nm), C curve	624	632	572
λ_{max} (nm), D curve	617	633	554
Beer's law range (μg mL^{-1})	0.2-10.0	0.6-13.0	0.9-5.0
Ringbom range, (μg mL^{-1})	1.99-6.30	1.99-5	1.99-3.4
Detection limit, (μg mL^{-1})	0.051	0.150	0.26
Quantification limit, (ng mL^{-1})	0.156	0.450	0.785
Standard deviation (SD) (n=6)	0.022	0.007	0.015
RSD (%)	2.88	3.42	3.32
Variance x 10^{-4}	4.80	0.57	2.20
Error %	0.9%	0.31%	0.61%
Slope (b)	0.064	0.020	0.028
Intercept (a)	-0.024	0.001	-0.013
Correlation coefficient, r^2	0.998	0.998	0.999
Molar absorptivity (L mol^{-1} cm^{-1})	12838	4012	5617
Sandell's sensitivity (ng cm^{-2})	0.015	0.050	0.035
Confidence limit	5.2 ± 0.023	4.5 ± 0.007	4.8 ± 0.015

[a]Average of six consecutive measurements.

3.10. Analytical applications

For the availability and reliability of the proposed procedures, the methods were applied to four different real water samples and five different food samples with three azo dyes. The obtained results are similar based on the standard addition method. The accuracy was tested by the Student's *t*-test. According to this test, the calculated *t*-values ranging from 0.23 to 2.27 are less than that of the theoretical value (2.44) at a confidence level of 95 %. In addition, the statistical *F*-test was also applied to compare the precision of the studied methods with another reference method for the determination of trace mercury (UV-visible diffuse reflectance spectroscopy after complexation and membrane filtration enrichment) [32, 33]. The calculated $F_{4,2}$ test value at a 95% confidence level did not exceed the theoretical value (19.2) with a value ranging from (1.10 to 17.77), indicating no significant difference between the performance of the two methods. The results were validated for three dyes in food and water samples in Tables 3-5. Also, the proposed spectrophotometric methods are compared to other published analytical methods in Table 6 [34-40].

Table 3. Determination of mercury ion in environmental samples using reagent R_1

Sample	Added (µg mL^{-1})	Found (µg mL^{-1})	Recovery (%)	RSD (%)	The calculated student's t-test and F-values [b]
Potato	- 3 5	2.92±0.008 5.98±0.026 7.88±0.023	- 102.0 99.2	0.44 4.42 4.16	0.60, 4.00
Beans	- 3 5	0.70±0.001 3.66 ±0.008 5.90 ±0.001	- 98.6 104.0	4.87 0.33 0.13	0.41, 2.50
Rice	- 1 3	6.98±0.011 7.90±0.054 9.85±0.011	- 96.5 95.6	2.36 3.30 0.17	0.44, 1.56
Soybean	- 3 5	3.35±0.009 6.28±0.018 8.4±0.008	- 97.6 101.0	2.37 4.24 1.36	2.27, 4.93
Lentils	- 3 5	2.89±0.008 5.85±0.038 7.95±0.009	- 98.6 101.2	0.16 1.42 1.66	0.23, 1.10
Tap water	- 3 5	0.60±0.001 3.57±0.018 5.56±0.002	- 99.0 99.2	5.60 2.97 0.60	0.57, 4.93
Pump	- 3 5	1.08±0.057 4.15±0.017 6.02±0.001	- 102.4 98.7	1.11 3.25 0.27	2.11, 5.50
Surface water	- 3 5	0.70±0.001 3.48±0.001 5.59±0.003	- 98.5 97.8	4.70 0.55 0.90	0.54, 17.77
Well	- 3 5	0.68±0.001 3.72±0.005 5.61±0.005	- 101.3 98.6	5.50 1.90 1.20	1.80, 10.00

[a] The average values and their standard deviations for five replicate measurements.

[b] The tabulated student's *t*-and $F_{(4,2)}$ values are 2.44 and 19.2 for a 95% confidence level and four degrees of freedom

Table 4. Determination of mercury ion in environmental samples using reagent R2

Sample	Added (µg mL^{-1})	Found (µg mL^{-1})	Recovery (%)	RSD (%)	The calculated student's t-test and F-values [b]
Potato	-	2.97±0.002	-	2.66	2.10, 4.20
	3	6.15±0.003	106.0	1.77	
	5	8.12±0.001	103.0	0.12	
Beans	-	0.67±0.001	-	0.57	1.99, 2.20
	3	3.68±0.001	100.3	0.3	
	5	6.05±0.015	107.6	1.09	
Rice	-	6.94±0.018	-	1.10	0.87, 7.20
	1	7.85±0.016	97.0	2.86	
	3	9.70±0.005	96.0	2.05	
Soybean	-	3.33±0.001	-	0.44	1.97, 5.23
	3	6.35±0.006	100.6	3.90	
	5	8.29±0.001	99.2	0.36	
Lentils	-	2.91±0.001	-	0.67	0.54, 4.57
	3	5.96±0.086	101.6	2.23	
	5	7.92±0.006	100.1	3.14	
Tap water	-	0.57±0.001	-	1.77	1.66, 3.47
	3	3.70±0.003	104.3	4.00	
	5	5.06±0.011	100.6	2.67	
Pump water	-	1.10±0.003	-	1.56	0.89, 2.35
	3	4.25±0.002	105.0	2.25	
	5	6.20±0.009	102.0	2.94	
Surface water	-	0.85±0.003	-	1.66	0.68, 8.21
	3	3.65±0.002	97.6	2.16	
	5	5.62±0.001	95.4	0.13	
Well water	-	0.63±0.001	-	1.84	0.74, 7.89
	3	3.40±0.003	98.4	3.7	
	5	5.62±0.003	99.8	0.26	

[a] The average values and their standard deviations for five replicate measurements.

[b] The tabulated student's t-and $F_{(4,2)}$ values are 2.44 and 19.2 for 95% confidence level and four degrees of freedom

Table 5. Determination of mercury ion in environmental samples using reagent R3

Sample	Added (µg mL^{-1})	Found (µg mL^{-1})	Recovery (%)	RSD (%)	The calculated student's t-test and F-values [b]
Potato	-	2.69±0.009	-	2.65	0.69, 14.2
	1	3.62±0.003	96.5	2.27	
	2	4.65±0.004	98.0	2.50	
Beans	-	0.64±0.002	-	2.85	1.05, 7.21
	1	1.66±0.020	102.0	3.94	
	2	2.62±0.030	99.0	3.35	
Rice	-	1.50±0.004	-	4.20	2.11, 6.51
	1	2.54±0.003	104.0	3.40	
	2	3.48±0.001	99.0	1.06	
Soybean	-	2.66±0.004	-	5.00	1.68, 1.98
	1	3.67±0.007	101.0	3.55	
	2	4.60±0.006	97.0	3.47	
Lentils	-	2.84±0.016	-	1.66	1.58, 4.54
	1	3.87±0.002	103.0	1.05	
	2	4.70±0.007	94.7	4.16	
Tap water	-	0.73±0.002	-	1.43	1.58, 8.94
	1	1.67±0.009	94.5	1.82	
	2	2.71±0.004	99.0	2.84	
Pump	-	1.04±0.006	-	2.50	1.58, 4.54
	1	1.96±0.011	97.3	1.90	
	2	2.97±0.009	96.5	2.75	
Surface water	-	0.77±0.001	-	2.35	1.78, 4.69
	1	1.72±0.006	96.8	1.13	
	2	2.75±0.004	99.0	2.78	
Well	-	0.66±0.003	-	3.20	1.42, 4.57
	1	1.68±0.009	102.0	1.76	
	2	2.70±0.006	102.0	2.45	

[a] The average values and their standard deviations for five replicate measurements.

[b] The tabulated student's t-and $F_{(4,2)}$ values are 2.44 and 19.2 for a 95% confidence level and four degrees of freedom

Table 6. Comparison of the proposed spectrophotometric methods with other analytical methods

Reagent	λ_{max} (nm)	Range of Beer's law (µg mL^{-1})	Remark	Reference
TMK	570	5.0-80.0	Acetate buffer, Triton X-114	[34]
Iodide	330	10.0-400.0	H_2SO_4, Triton X-114	[35]
Rhodamine	556	10-100	pH5.0, Triton X-114	[36]
Dithizone	490	50-500	pH1-3, Triton X-100	[37]
PAN	554	10.0-1000.0	pH9.0, BR buffer, Triton X-114	[38]
TAR	389	500-2500.0	pH8.0, BR buffer, Triton X-114	[38]
Dithizone	490	0.2-4.0	Triton X-100	[39]
R1	617	0.2-10	Tween 80	This Work
R2	633	0.6-13	CTAB	
R3	554	0.9-5	Tween 80	

TMK: Thio-Michler's Ketone

PAN: 1-(2-pyridyl azo)-2-naphthol

TAR: 4-(2-thiazolylazo) resorcinol

4. Conclusions

The proposed paper established three new approaches for the micro-determination of mercury ions. Three prepared azo dyes, R_1, R_2 and R_3, were used as complex reagents. Absorbances of the formed complexes were measured at 617 nm, 633 nm and 554 nm, respectively. The mercury concentrations of 0.2-10.0 µg mL^{-1} for R_1, 0.6-13.0 µg mL^{-1} for R_2 and 0.9-5.0 µg mL^{-1} for R_3 were studied and determined. The detection limits are 50 µg L^{-1} for R_1, 150 µg L^{-1} for R_2 and 260 µg L^{-1} for R_3. The procedures were applied effectively to determine mercury in water and food matrices. The results were in excellent agreement relative to accuracy and precision. The developed methods can be used for the micro-determination of mercury in real samples by the UV-Vis-spectrophotometer without requiring any costly tool.

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