

Surfactant assisted spectrophotometric determination of copper(II), and mercury(II) in real samples using 2- amino-4-((4-nitrophenyl)diazenyl)pyridine-3-ol.

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ABSTRACT: Using 2-amino-4-(4-nitrophenyl) diazenyl pyridine-3-ol (ANPDP) as a reagent, a sensitive and selective surfactant-assisted spectrophotometric method for the rapid determination of copper (II) and mercury (II) was proposed. When the ANPDP reagent reacts with both Cu(II) and Hg(II) ions in a universal buffer solution containing sodium dodecyl sulfate, it produces a violet color. Cu(II) and Hg(II) had the highest absorbance at 565 and 605 nm, respectively. The complexes of Cu(II) and Hg(II) have a mole ratio of 1:2 [M:L]. Cu(II) and Hg(II) have linear ranges of 10-180 and 10-160 g mL⁻¹, respectively, with detection limits of 1.36 and 1.74 g mL⁻¹ and quantification limits of 4.1 and 5.26 g mL⁻¹. The complex's correlation coefficients of 0.994 and 0.995, respectively, show high linearity. Cu(II) and Hg(II) relative standard deviations for six replication measurements were 1.01% and 1.2%, respectively. The analytical parameters for the procedure were optimized. Most other metals and rather high concentrations of co-existing elements can be tolerated. This method has the benefits of being quick, easy, sensitive, and selective, and it was successfully used to determine the presence of Cu(II) and Hg(II) ions in real samples, and recovery percentage was found to be 95.0-98.0 %.

Keywords: Copper; Mercury; ANPDP; Surfactant assisted spectrophotometry; Environmental samples.

Date of Submission: 05-12-2022

Date of acceptance 21-12-2022

I. INTRODUCTION

The heterocyclic azo dyes have been produced and proposed as a very sensitive chromogenic reagent for measuring a variety of metal ions [1]. Heavy metal has long been regarded as a highly hazardous form of pollution that is poisonous to humans even at low doses [2]. Heavy metals are defined as elements with atomic weights ranging from 63.5 to 200.6 and a density of more than 5 g/cm³ [3]. The concentrations of heavy metals in aquatic systems, as well as their accumulation through the food chain, may pose a health risk and cause environmental problems [4]. Toxic metals in trace amounts cause cellular dysfunction by inhibiting enzymes, causing oxidative stress, and impairing antioxidant processes. Reactive oxygen species (ROS) are formed as a result of these pathways, which can induce DNA damage, lipid peroxidation, biosynthesis pathway arrest, and protein sulfhydryl depletion [5]. Direct identification of trace and hazardous heavy metals such as copper (Cu(II)) and mercury (Hg(II)) has proven critical in some samples [6]. In recent years, many researchers have been interested in the extraction and identification of trace metal ions or species from diverse materials [7]. increased atmospheric emission of hazardous heavy metals, primarily due to the industrial revolution, resulting in major pollution [8]. Heavy metals were particularly damaging among the numerous organic and inorganic contaminants because of their solubility in water, poor

biodegradation, and significant environmental contamination [9]. Although copper was harmful at greater concentrations and severe oral poisoning mostly affected the blood and kidneys [10], it was required for the regular metabolism of many living species [11]. Copper is a vital element in plants [12] that can be used in alloy manufacture [13–14]. Chemically [15] or physically with foreign sources [16], it can be reached. Excess copper in the human body causes neuritis and cirrhosis by damaging the nervous system and organs, particularly the liver and gallbladder [17]. Copper reduces zinc toxicity in biological systems, implying a Cu/Zn antagonistic relationship [18]. Some lesser organisms are more poisonous than humans to its salts. Because of its deposition in cell membranes, it becomes toxic at higher concentrations and inhibits transport across the cell wall [19]. Wilson's illness is a hereditary disease caused by excessive copper levels in the human body. It has potentially catastrophic implications, such as liver damage. Males develop violent tendencies as a result of high copper levels, which can cause depression and schizophrenia [20]. The determination of an ultra-trace amount of copper in biological samples is particularly difficult because of the complex matrix and the usually low concentration of copper, which requires sensitive instrumental techniques and frequently a preconcentration step [21–24]. Excessive Cu(II) uptake, on the other hand, can result in major health issues such as coronary heart disease, kidney disease, neurological disease, anemia, and bone abnormalities [25]. As a result, ultra-trace amounts of Cu(II) pollutants in water, food, and the environment must be monitored. Mercury is among the most poisonous heavy metals on the earth, and it exists in three valence states in nature at trace and ultra-trace levels [1]. Mercury (0, I, II) species can form complexes with a variety of inorganic and organic ligands, including HgX_4-2 (where $X=Cl, Br,$ and I) and methyl mercury [1]. Mercury may accumulate in plants and animals, as well as enter the human body via the food chain, causing disease of the central nervous system [26]. The determination of Hg(II) or organo mercury (II) has seen a spike of attention in recent years due to the hazardous effects and probable accumulation of mercury on human bodies and water organisms [27]. Mercury toxicity is determined by its chemical state [28]. Some types of mercury are relatively non-toxic and have been used to cure syphilis [29].

Some extractive spectrophotometric determinations of the investigated metals, such as naphthazarin (5,8-dihydroxy-1,4-naphthoquinone; Naph) [30], 1-[2-(allylamino)-1-methylethyl] NPAS [31], chloro-(phenyl) glyoxime [33], benzildithiosemi carbazone [34], thiomichlersketone [35], and 4,5,6-trihydroxyhexane-1,2-diylidene)bis(N-phenylhydrazinecarbothioamide) [36]. To detect Hg(II), 1,5-diphenylthiocarbazon (dithizone) [37], azathia-crown ether dye [38], Thio-Michler's Ketone [39], 2-[(2-sulfanylphenyl) ethanimidoyl]phenol [40], 6-hydroxy-3-(2-oxoindolin-3-ylideneamino)-2-thioxo-2H-1,3-thiazin-4(3H)-one [41] and 2-Acetylpyridine thiosemicarbazone [42]. Some preconcentration and separation approaches were utilized for determination of the studied metals [43- 46].

The current study aims to develop rapid, simple, selective, and sensitive spectrophotometric methods for quantifying low levels of Cu (II) and Hg (II) ions in various samples. The principle of the method is based on allowing the above metals to react with 2-amino-4-((4-nitrophenyl) diazenyl)pyridine-3-ol as a newly prepared azo dye reagent at selected pH values in the presence of a cheap anionic surfactant such as sodium dodecyl sulfate. The present work aims to investigate the optimum conditions for reactions like pH, time, temperature, reagent concentration, surfactants, and the sequence of additions. We have also encouraged this research to produce microscopic amounts of the investigated metals Cu(II) and Hg(II) ions in real samples.

II. Materials and Methods

2.1. Instrumentation:

At Benha University's chemistry department, a JASCO V-670 double beam UV–VIS spectrophotometer with wave length 200-800 nm was used to detect absorbance, using quartz cells, 1 cm. Infrared spectra were taken in the range of 4000-400 cm^{-1} using a Nicolette IS10 infrared spectrometer from Thermo Fisher Scientific in the United States. At the Chemistry Department, Faculty of Science, Mansoura University, this was done with conventional KBr pellets. The 1H NMR

spectra were obtained in DMSO-d₆ solvent on a JEOL (500 MHz) spectrometer at Mansoura University's Chemistry Department.

2.2. Chemicals and reagents

Both the chemicals and solvents utilized were analytical grade reagents that were used without additional purification. Throughout the tests, bidistilled water was used. Cu(II) and Hg(II) (1×10^{-3} M) stock solutions were prepared by dissolving 0.0249 and 0.0271g of CuSO₄.5H₂O and HgCl₂ (BDH), respectively in 100 ml bidistilled water. Standardization of their solutions is obtained by using EDTA titration [47]. The stock solutions were then diluted with bidistilled water to make working solutions, and were kept in bottles made of low-density polyethylene.

A series of buffer solutions (citrate, borate, phosphate, and universal) covering the range of 2.5-11.5 is prepared according to *Britton* [48]. The pH of each solution is adjusted to the required value using pH meter.

A series of surfactants, including cetyltrimethylammonium bromide (CTAB), Triton X-100, Sodium lauryl sulphate (SLS), and Tween 80 at concentration (5% v/v), were prepared by dissolving an exact volume of each surfactant in 100 mL of bidistilled water and sodium dodecyl sulphate (SDS) (0.6 M) was prepared by dissolving 17.3 g of pure SDS in 100 mL bidistilled water.

For studying the interference, different standard solutions of metal ions (cations and anions) were prepared by dissolving specified volumes of their respective salts in water or diluted hydrochloric acid and diluting them to the mark with bidistilled water in a 100 mL measuring flask.

2.3. Synthesis of ANPDP reagent

The p-nitroaniline (0.01 mole) in the aromatic amines was converted to the hydrochloride form by adding the least amount of concentrated hydrochloric acid, which was then diluted with bidistilled water and cooled at -5°C. The amine salt is progressively mixed with a cooled solution of NaNO₂ (0.01 mole) while being continuously flipped. The resultant diazonium salt solution is added gradually to a solution of 2-amino-3-hydroxypyridine (0.01 mole) dissolved in 10% NaOH and chilled at -5°C after being let stand for 15 minutes in an ice bath with continual flipping. To obtain brown crystals, the ained azo compound is filtered out, dried, and then recrystallized in alcohol/water to produce (88%) with a melting point of 230 °C. The synthesised reagent's (ANPDP) chemical composition was identified (Figure 1) [49].

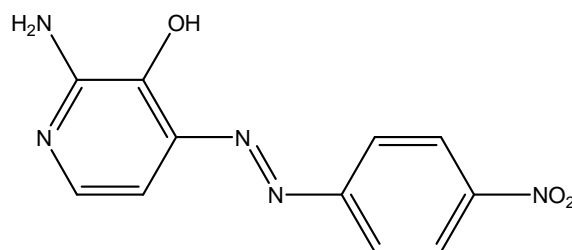


Figure 1: The structural formula of 2-amino-4-(4-nitrophenyl)diazenylpyridine-3-ol (ANPDP).

By dissolving an adequate weight (0.0026 g) of the ANPDP reagent in 10 mL of ethanol and then adding the same solvent to the mark in a 100 mL calibrated flask, the desired concentration of the reagent (1.0×10^{-4} mol L⁻¹) was achieved.

2.4. General procedures

A 2.0 mL of the ANPDP solution ($2.0 \times 10^4 \text{ mol L}^{-1}$) was added to an aliquot of Cu(II) and Hg(II) standard solution ($10\text{-}180 \text{ }\mu\text{g mL}^{-1}$ and $10\text{-}160 \text{ }\mu\text{g mL}^{-1}$), respectively in a set of 10 mL measuring flasks. About 3.0 mL solution of universal buffer with a pH of 8.0 and 8.5 for Cu(II) and Hg(II), respectively. Then, 1.0 mL of (0.6 mol L^{-1}) SDS solution was added, and the required amount of bidistilled water was added to finish the mixture. For two minutes, the mixture was left at room temperature. In comparison to a reagent blank prepared under the same experimental conditions but without metal ions, the absorbance was measured at 565 and 605 nm for Cu(II) and Hg(II), respectively. Calculating the concentration of Cu(II) and Hg(II) and performing the calibration graph.

2.5. Application for real samples

2.5.1. Water samples

Various water samples, including surface and industrial waste water samples, were subjected to the suggested methodology. The samples were gathered in polyethylene bottles from a natural pond in the city and vicinity of Benha, Egypt. To remove any suspended particles, the water samples were filtered through a Millipore cellulose membrane filter with a 0.45 μm pore size. Then, 100 mL of each filtered water sample was precisely transferred into a 250 mL round bottom flask, and 10 mL of a solution consisting of HNO_3 and H_2O_2 (1:9, v/v) were added. These samples were heated under reflux for two hours to facilitate digestion. The samples were placed in 100 mL volumetric flasks after cooling, filled to the prescribed level with deionized distilled water, mixed well, and then had the pH of the samples corrected to the optimum pH by buffer solution before being placed in a dark refrigerator. Water aliquots were tested using the general procedure in accordance with the basic protocol mentioned above to evaluate Cu(II) and Hg(II).

2.5.2. Vegetable samples

Various vegetable samples (carrot, potato, and chilli pepper) were collected from the markets in Benha, Egypt. The samples were dried in an oven at 100°C for 24 hours and homogenized in a Kenwood blender (model BL335, Watford, UK) and stored in clean, dry airtight polyethene bags. Then, an accurately weight of the samples (1.0 g) were weighed and treated with 10 mL of a mixture of concentrated $\text{HNO}_3\text{-H}_2\text{O}_2$ (2:1, v/v) in Teflon tubes and the tubes were sealed. Microwave digestion procedure has been applied for sample preparation. Then, the digested samples were subjected to the proposed procedures in order to evaluate Cu(II).

2.5.3. Urine sample

Urine samples (5.0 mL) were taken from Benha University Hospital, and 8.0 mL of mixed acid ($\text{HNO}_3\text{: HClO}_4\text{: H}_2\text{SO}_4 = 3\text{:}1\text{:}1$) was put into a beaker. The mixture was slowly heated until it was practically dried. The solution was boiled again with a little water until the residue was dissolved. After filtering the solution into a 25 mL test tube, the above-mentioned procedure was used to analyze Cu(II).

2.5.4. Mercurochrome sample

A definite volume of sample (10 mL) of mercurochrome (2%, Flower Misr Company) was dissolved in 4 mL nitric acid (1:1) and heated for a few minutes. The eluent was rinsed with deionized water after the solution was cooled to room temperature and filtered. Dilute the filtrate to 25 mL. According to the prior approach, known volumes (0.5-2 mL) from the above solution were taken and the general procedure was applied to evaluate the concentration of Hg(II).

2.5.5. Ammoniated mercury ointment sample

A weighed sample of ointment (5%, Delta Pharm Company, Egypt) (1.0 gm.) was dissolved in ether, then in a little volume of diluted hydrochloric acid, heated for a few minutes, and the aqueous layer separated. Deionized water was used to dilute the solution to 25 mL. Following the previous procedure, exact volumes (1-3 ml) of the above solution were drawn in a 10 ml measuring flask and the general procedure was applied to evaluate the concentration of Hg(II).

III. RESULTS AND DISCUSSIONS

3.1. Absorption spectra

The spectra of the Cu(II) or Hg(II)-ANPDP complex solution have a maximum absorbance at 565 and 605 nm for Cu(II) and Hg(II), respectively against reagent blank in an aqueous ethanolic and surfactant-rich phase using the proposed procedure, while the reagent has absorption maxima of 450 nm, as seen in (Figures 2 and 3). In comparison to the free reagent, the complex solution spectra show a significant bathochromic shift in the visible region with difference in wave length ($\Delta \lambda_{\max}$) is (115-155 nm). The large shift in the (λ_{\max}) indicated the formation of a complex.

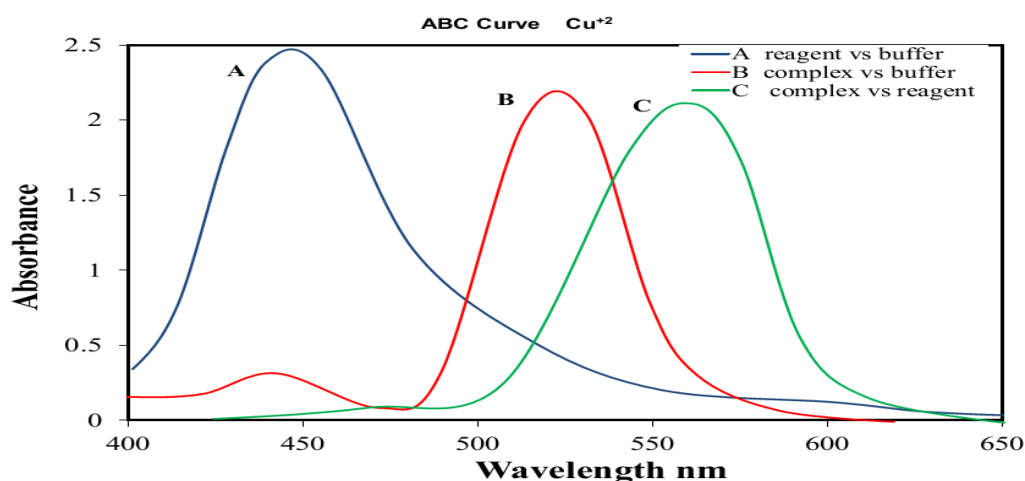


Figure 2: The absorption spectra of Cu(II) ($180 \mu\text{g mL}^{-1}$) complexed with ANPDP reagent ($2.0 \times 10^{-4} \text{ mol L}^{-1}$) at pH 8.0 using the proposed procedure against reagent blank.

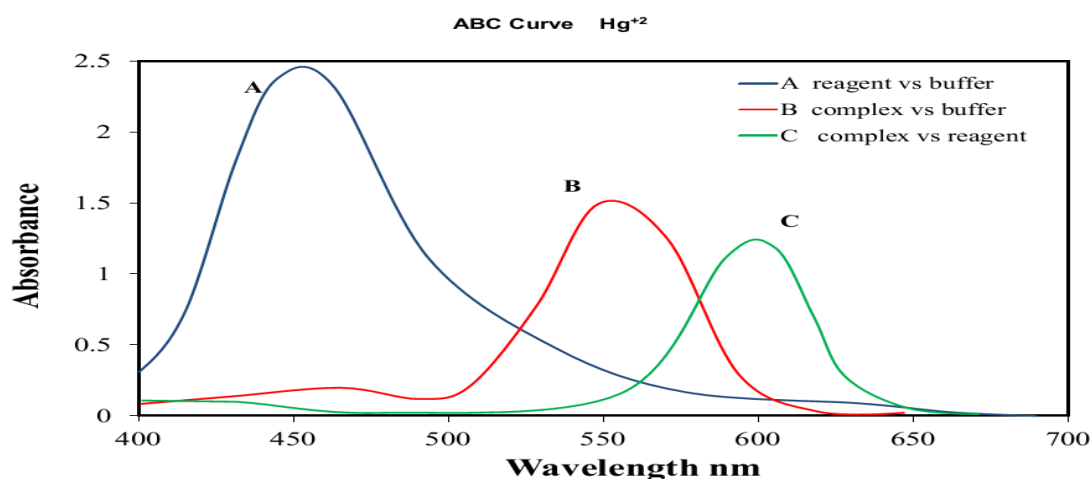


Figure 3: Absorption spectra for Hg(II) ($160 \mu\text{g mL}^{-1}$) complexed with ANPDP reagent ($2.0 \times 10^{-4} \text{ mol L}^{-1}$) at pH 8.5 using the proposed procedure against reagent blank..

3.2. Optimization of the cloud point extraction conditions

Using the proposed process outlined above, the analytical variables including pH, reagent and surfactant concentrations, and temperature, were optimized.

3.2.1. pH Effect

Due to its direct connection to the emergence of metal-ligand species, the pH of the aqueous solution plays a significant role in the determination of Cu(II) and Hg(II). Complexation was done with various buffer solution types. The optimal one, which provides the maximum absorbance value in addition to the stability of the colour complex, was maintained using the universal buffer solution. While maintaining the other conditions constant, the effect of pH on the method efficiency of analyte was investigated in the pH range 2.26-12.0, with the findings displayed in Figure 4. As can be seen, pH 8.0 and 8.5 for Cu(II) and Hg(II), respectively produced the highest absorption. The impact of the buffer volume was also evaluated. The outcomes indicated that 3.0 ml of the universal buffer solution was the best choice for achieving the highest level of sensitivity (Figure 5).

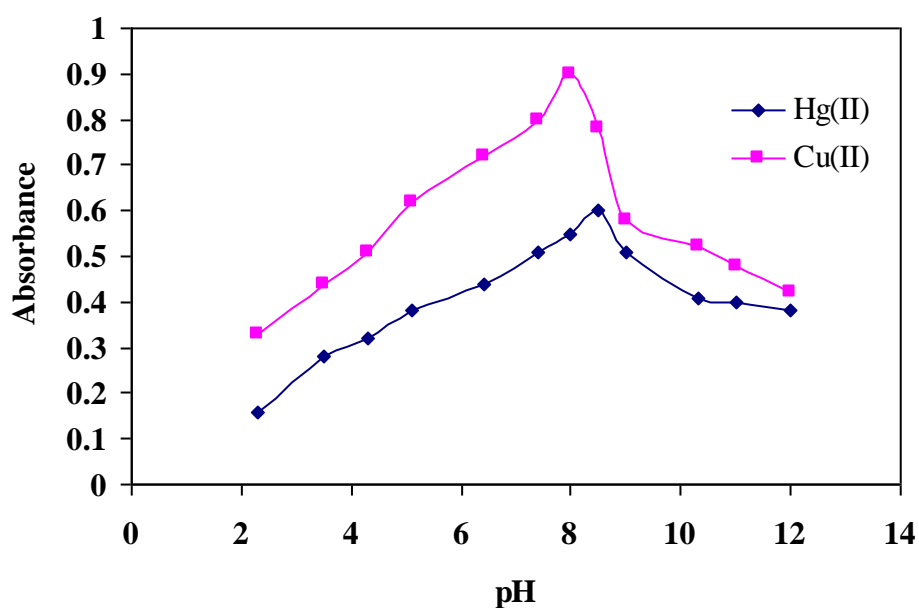


Figure 4: Effect of pH on the formation of Cu(II)-ANPDP and Hg(II)-ANPDP complexes.

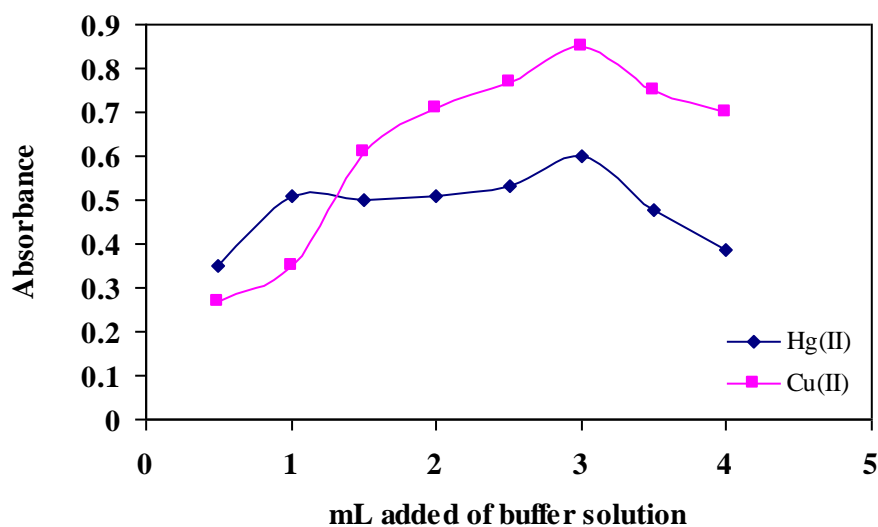


Figure 5: The influence of buffer volume on the formation of Cu(II)-ANPDP and Hg(II)-ANPDP complexes.

3.2.2. Effect of the reagent concentration

For Cu(II) and Hg(II) solutions and various concentrations of the reagent in the range of $5.0 \times 10^{-5} - 5.0 \times 10^{-4} \text{ mol L}^{-1}$, the impact of ANPDP concentration on analytical performance was examined. The complex's absorbance increased as the reagent concentration increased, and it was found to be constant between $1.5 \times 10^{-4} - 2.5 \times 10^{-4} \text{ mol L}^{-1}$ of ANPDP reagent (Figure 6). The complex's ideal absorbance in conjunction with the lowest blank measurement was discovered to be $2.0 \times 10^{-4} \text{ mol L}^{-1}$. When the reagent was used at a higher concentration, the absorbance remained

constant or decreased. In the subsequent studies, $2.0 \times 10^{-4} \text{ mol L}^{-1}$, was sufficient for the best outcomes. This concentration guarantees enough excess to offset the reagent use of other metals. Therefore, 1.0 mL of ANPDP ($2.0 \times 10^{-4} \text{ mol L}^{-1}$) solution was chosen as the ideal volume that gives the greatest complex formation yield for all subsequent investigations.

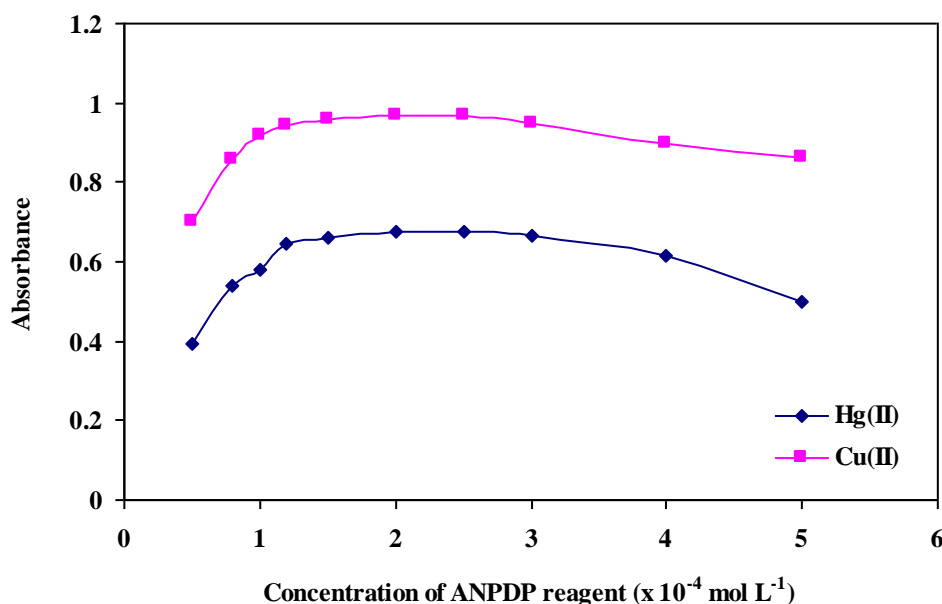


Figure 6: Effect of ANPDP reagent concentration on the absorbance of Cu(II)-ANPDP and Hg(II)-ANPDP complexes at pH 8.0 and 8.5, respectively.

3.2.3. Impact of surfactant concentration

The impact of different types of surfactants (non-ionic, cationic, and anionic) was examined. In the method without CPE, the anionic surfactant (SDS) was the best one. The highest absorbance was obtained at concentration. Different volumes (0.25–3.0 mL) of SDS (0.6 mol L^{-1}) was tested, by increasing volume to 1.0 mL, the absorbance was increased. Therefore, 1.0 mL of SDS (0.6 mol L^{-1}) was chosen for the tests that followed (Figure 7).

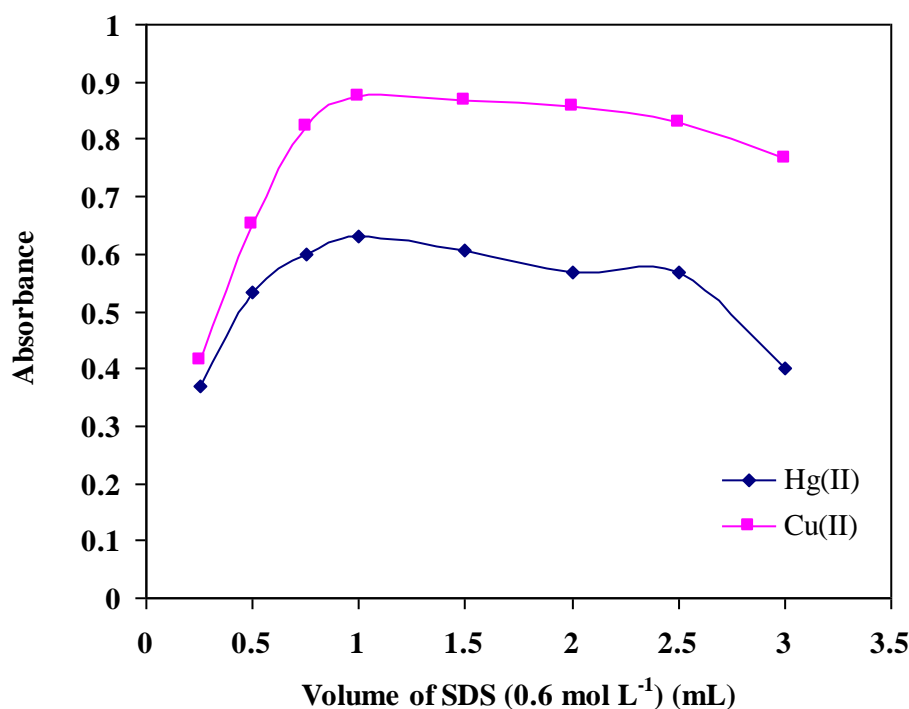


Figure 7: Effect of SDS (0.6 mol L^{-1}) volume on absorbance of Cu(II)-ANPDP and Hg(II)-ANPDP complexes at pH 8.0 and 8.5, respectively and ANPDP ($2.0 \times 10^{-4} \text{ mol L}^{-1}$).

3.2.4. Temperature and equilibration time effects

Optimizing the time and temperature is essential for achieving the efficiency of the proposed method. Investigations on the effects of time and temperature were conducted in the ranges of 1.0–10 min and $25\text{--}70^\circ\text{C}$. The outcomes show that room temperature of $25\pm 5^\circ\text{C}$ and an period of 2.0 min were selected for subsequent studies.

3.3. The complex's composition

The molar ratio (Figure 8) and continuous variation (Figure 9) approaches were used to determine the Cu(II)-ANPDP and Hg(II)-ANPDP complex's composition. Both methods' results indicated that molar ratios of (1: 2) (M:L) were present. For at least 10 hours, the chromogenic system could stabilize.

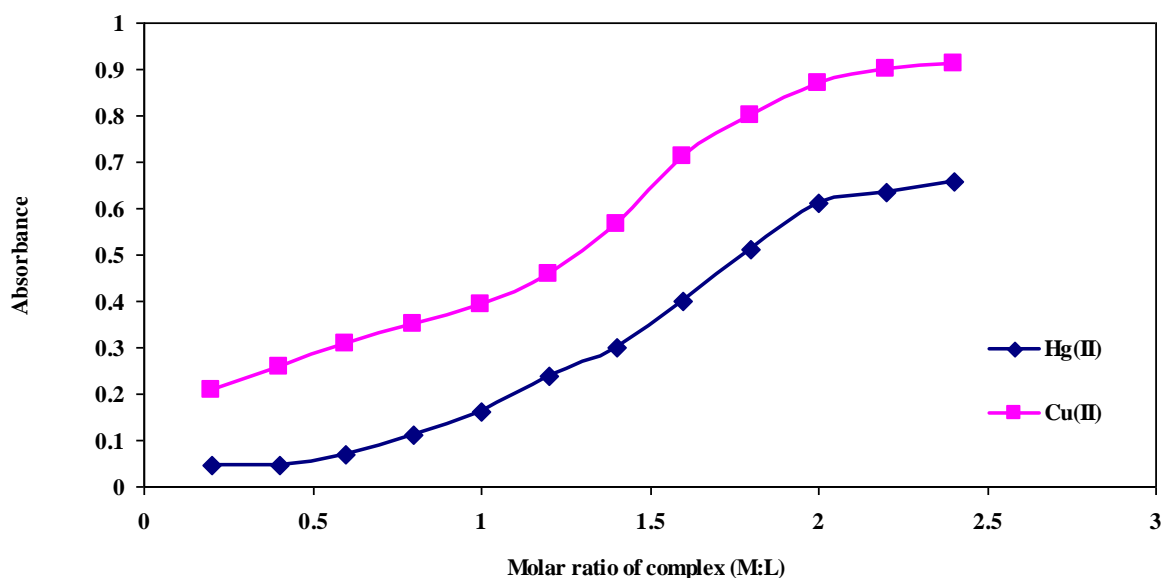


Figure 8: Molar ration method for M-ANPDP complex formation at optimum condition using (1×10^{-4} M) of metal ions and reagent.

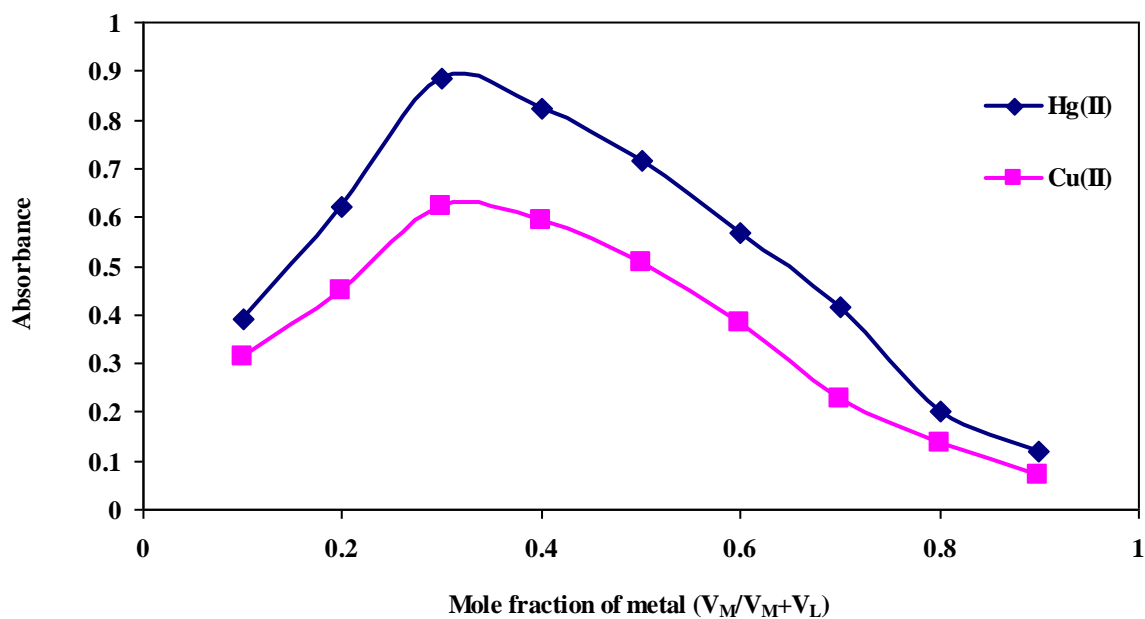


Figure 9: Continuous variation for M-ANPDP complex formation at optimum condition using (1×10^{-4} M) of metal ions and reagent.

3.4. Interference effect

Tests were conducted on the impacts of representative putative interfering species. These tests used synthetic solutions with 180 and 160 $\mu\text{g mL}^{-1}$ of Cu(II) and Hg(II), respectively and varying concentrations of other interfering ions, and they followed the suggested method for determining both metal ions. It was deemed acceptable for the absorbance reading to have a 5.0% inaccuracy. A smaller concentration of foreign ions was then generated for any metal ions that were discovered to interfere. Table 1 lists the tolerance limitations.

Table 1: Effect of interfering ions on recoveries of 180 and 160 $\mu\text{g mL}^{-1}$ Cu(II) and Hg(II), respectively for the proposed method (N=3).

Foreign ions	Cu(II)		Hg(II)	
	Maximum Tolerable amount ($\mu\text{g mL}^{-1}$)	Recovery (%) \pm SD ^a	Maximum Tolerable amount ($\mu\text{g mL}^{-1}$)	Recovery (%) \pm SD ^a
K ⁺	4200	96.0 \pm 3.0	3500	97.0 \pm 1.0
Na ⁺	5000	97.0 \pm 2.0	3000	96.0 \pm 3.0
Al ³⁺	2000	95.0 \pm 2.0	2000	96.0 \pm 2.0
Cr ³⁺	2500	96.0 \pm 2.0	2300	95.0 \pm 3.0
Ca ²⁺	32000	96.0 \pm 1.0	2800	97.0 \pm 3.0
Mg ²⁺	3800	96.0 \pm 2.0	3200	98.0 \pm 3.0
Ba ²⁺	3500	95.0 \pm 3.0	2600	96.0 \pm 1.0
Pb ²⁺	3000	96.0 \pm 4.0	3500	96.0 \pm 3.0
Ni ²⁺	4500	97.0 \pm 3.0	3800	98.0 \pm 2.0
Bi ³⁺	500	98.0 \pm 2.0	400	95.0 \pm 2.0
Co ²⁺	2600	96.0 \pm 1.0	1500	96.0 \pm 4.0
NO ₃ ⁻	2000	95.0 \pm 3.0	1500	96.0 \pm 2.0
SO ₄ ²⁻	3000	97.0 \pm 2.0	2500	95.0 \pm 3.0
Cl ⁻	1300	98.0 \pm 3.0	1000	97.0 \pm 2.0
F ⁻	1000	97.0 \pm 2.0	900	96.0 \pm 2.0

^a Mean \pm standard deviation.

3.6. Analytical performance and calibration.

The produced complexes' calibration curves were derived from absorbance measurements carried out under the optimum conditions indicated above. As showed in (Figure 9), the calibration graphs were found to be linear over a concentration range of 10 -180 and 10-160 $\mu\text{g mL}^{-1}$ for Cu(II), and Hg(II), respectively. A ringbom plot was created to determine the optimum concentration range for a system that followed Beer's law. Cu(II), Hg(II), and Zn(II) were achieved at intermediate concentrations of 20-120 and 20-100 $\mu\text{g mL}^{-1}$ for Cu(II), and Hg(II), respectively, as indicated in Table 2. The molar absorptivity's, Sandell sensitivity, limit of detection and limit of quantification were calculated in Table 2.

Table 2: Analytical features of the proposal method.

Parameters	Cu(II)	Hg(II)
λ_{\max} (nm)	565	605
Beer's law range , ($\mu\text{g mL}^{-1}$)	10-180	10-160
Ringbom range , ($\mu\text{g mL}^{-1}$)	20-120	20-100
Regression equation ^a		
Slope (b)	0.0073	0.0057
Intercept (a)	0.24	0.011
Correlation coefficient	0.994	0.995
Relative standard deviation (RSD %)	1.01	1.2
limit of detection (LOD), ($\mu\text{g mL}^{-1}$)	1.36	1.74
limit of quantification (LOQ), ($\mu\text{g mL}^{-1}$)	4.1	5.26
Molar Absorptivity ($\epsilon \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$)	1.1	0.6
Sandell Sensitivity ($\mu\text{g cm}^{-2}$)	0.056	0.03

$$^a A = \text{Slope} \times C + \text{intercept}$$

3.5. Analytical applications

The application of the suggested procedure to the estimation of Cu(II) and Hg(II) from various samples was done to evaluate their dependability (water, urine, vegetable, and pharmaceutical preparations). To validate the devised procedure reliability and accuracy, certain amounts of Cu(II) and Hg(II) ions were spiked to the sample solutions utilizing addition/recovery test and the recoveries and RSD% were determined as shown in Tables 3 and 4. The developed method was tested for its ability to recognize Cu(II) ions in a variety of real water, urine and vegetable samples. The Cu(II) analyte had excellent quantitative recoveries, ranging from 95.0–98.0% with RSD% \leq 1.87% (Table 3). The Hg(II) analyte had excellent quantitative recoveries, ranging from 95.20–98.0% with RSD% \leq 1.55% (Table 4). As a result of these findings, the method may be used to analyse Cu(II) and Hg(II) in real samples.

Table 3: Addition/recovery test for determination of Cu(II) in real samples using the developed approaches (N=3).

Sample	Added ($\mu\text{g mL}^{-1}$)	Found ^a \pm SD ($\mu\text{g mL}^{-1}$)	Recovery \pm RSD(%)
Surface water	0	< LOD ^b	-
	50	48.0 \pm 0.76	96.0 \pm 1.58
	100	96.50 \pm 1.80	96.50 \pm 1.87
Urine	0	< LOD ^b	-
	50	47.70 \pm 0.38	95.40 \pm 0.80
	100	98.0 \pm 1.15	98.0 \pm 1.17
Carrot	0	12 \pm 0.12	-
	50	59.0 \pm 0.54	95.20 \pm 0.92
	100	109.0 \pm 1.40	97.0 \pm 1.28
Potato	0	15.0 \pm 0.15	-
	50	62.40 \pm 0.81	96.0 \pm 1.30
	100	111.60 \pm 1.60	97.0 \pm 1.43
Clove	0	11 \pm 0.10	-
	50	58.0 \pm 0.62	95.0 \pm 1.07
	100	106.60 \pm 1.20	96.0 \pm 1.13

^a Mean \pm standard deviation.

Table 4: Addition/recovery test for extraction of Hg(II) in various samples using the developed approaches (N=3).

Sample	Added ($\mu\text{g mL}^{-1}$)	Found ^a \pm SD ($\mu\text{g mL}^{-1}$)	Recovery \pm RSD(%)
Mercurchrome	0	25.0 \pm 0.21	-
	50	72.0 \pm 0.80	96.0 \pm 1.11
	100	122.50 \pm 1.90	98.0 \pm 1.55
Ammoniated mercury ointment (5%)	0	20.0 \pm 0.10	-
	50	67.0 \pm 0.40	95.70 \pm 0.60
	100	117.0 \pm 0.90	97.50 \pm 0.78
Industrial waste water	0	18.0 \pm 0.12	-
	50	65.80 \pm 0.36	96.80 \pm 0.55
	100	112.30 \pm 1.25	95.20 \pm 1.11

^a Mean \pm standard deviation.

IV. Conclusion

In the proposed work, surfactant assisted spectrophotometric method was developed for the determination of Cu(II), and Hg(II) in various water, vegetables, urine and pharmaceutical preparations samples. ANPDP is a very stable and fairly selective new complexing reagent with the aid of SDS surfactant as low cost, and is readily available in most laboratories. The proposed approach is convenient, safe, sensitive, simple, and economic, accurate, precise and has lower detection and quantification limits. It also offers the advantage of isolating the analyte from the sample matrix, allowing for interference-free analysis. The proposed method can be applied to the analysis of trace amounts of Cu(II), and Hg(II) in various real samples with good results.

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