Original Article

Spectrophotometric Determination of Hg (II) Using (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-4-yl) diazenyl) benzoic acid as analytical reagent

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DOI: 10.47750/pnr.2022.13.S01.65

Abstract

A simple and low cost spectrophotometric method was proposed for the determination of Hg (II) in trace amounts using azo ligand derivative from 4,5-bis(4-methoxyphenyl) imidazole as spectrophotometer reagent. The proposed method is based upon the chelation of Hg (II) with the novel azo ligand, the formed violet complex has a maximum absorption at 546 nm. The mole ratio of the complex was 1:2 (metal:ligand). The reaction obeys Beer`s-Lambert law in the concentration range of (0.5-10) µg mL-1. The limit of detection LOD and the limit of quantification LOQ were 0.190 and 0.634 µg mL-1. The molar absorptivity and sandell's sensitivity are 7.963 ×10+3 L mol-1 cm-1 and 0.025 µg cm-2, respectively. The relative standard deviation RSD% for (n=10) was 1.607%. The interfering effect of cations and anions was studied. The azo compound and its Hg (II) complex were investigated for their biological activity.

Keywords: Spectrophotometric determination, Azo ligand, Hg (II) – azo ligand complex, biological activity.

1- INTRODUCTION

Azo compounds are an important group of organic chemicals that contain at least one azo chromophore as (-N=N-) which gives the color to this famous type of compounds [1]. These compounds can be used as analytical reagents for the determination of micro quantities of elements in multiple specimens [2]. The azo compounds have biological importance as an antibacterial [3], antioxidant and antifungal [4], anticancer [5, 6] as well as to their ability to be as an inhibitor agent of COVID-19 [7]. The Imidazole azo compounds can coordinate with many low oxidation state metallic ions to form highly stable complexes, this is because of the ability of the imidazole π* orbitals to receive back the electrons that were donated from the metallic ions [8, 9].

The mercury element is one of the most common poisonous elements in ecosystems, and even low quantities of it can affect human health [10-12]. Its poisoning effect is due to its accumulation in the body tissue because of taking the food. Its presence in the human body can cause dangerous damage to the central nervous system, brain, kidney, lungs, and fetus development [13-17]. Mercury element may present in various forms [18], all of which are significantly poisonous. Various analytical methods were used for the selective determination of Hg (II) such as inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma-atomic emission spectrometry (ICP-AES) [19, 20], cold-vapor atomic absorption spectroscopy (CVAAS) [21]. Currently, some methods are employed as inexpensive and fast techniques for Hg (II) detection such as colorimetry [22, 23], fluorimetry [24] and voltammetric methods [25]. In this work, a rapid and sensitive spectrophotometric method was proposed for the determination of Hg (II) using azo ligand derivative from 4,5-bis(4-methoxyphenyl) imidazole as an analytical reagent, in addition to studying the biological effect for the reagent and his Hg (II)-complex.

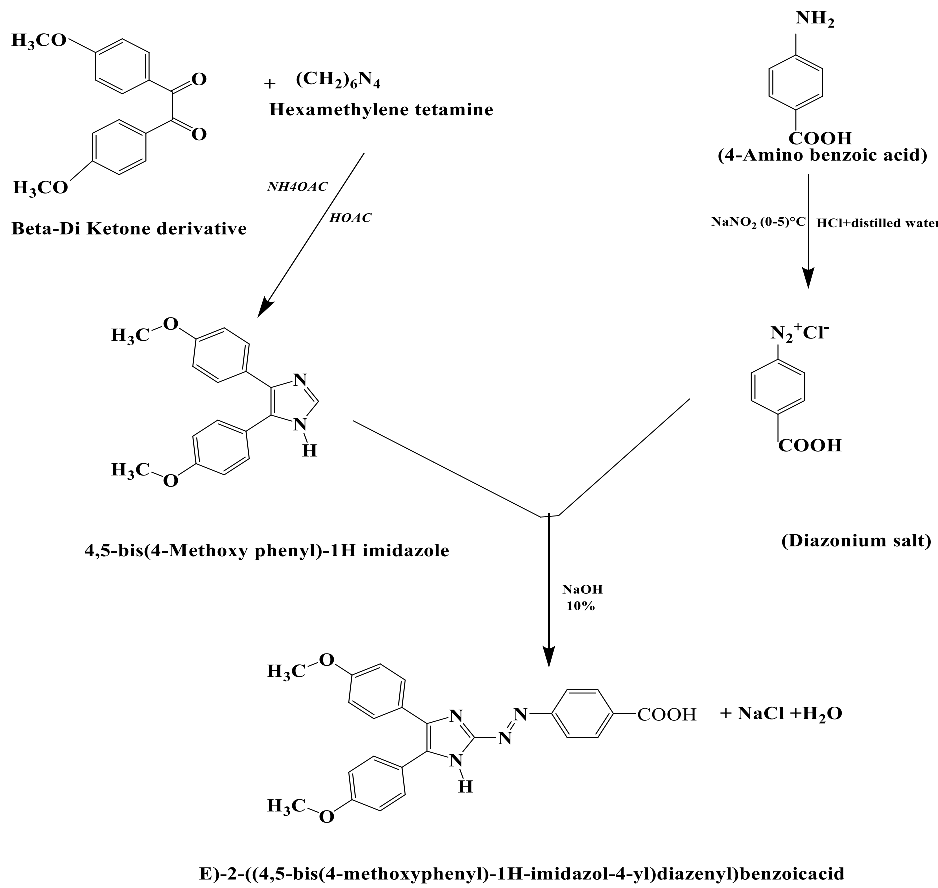
2- The Chemicals and Instruments

All the chemical compounds that are used are of analytical reagent grade (AR) except for 4,5-bis(4-methoxy phenyl) imidazole and (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-4-yl) diazenyl) benzoic acid, that were synthesized as mentioned earlier in [26] and [27], respectively. Distilled water has been used throughout the present work. The UV- Vis spectrum was recorded using Shimadzu (UV-1700) spectrophotometer using 1 cm glass cells, pH measurements was implemented using Oakton 2100 Series pH Meter.

3- Synthesis of (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-4-yl) diazenyl) benzoic acid Ligand

The imidazol derivative 4,5-bis(4-methoxy phenyl) imidazole [26] was prepared by mixing (2.70 g, 0.01 mol) of (4,4-dimethoxy benzyl), (0.28 g, 0.002 mol) of (hexamethylene tetramine), (6.0 g, 0.077 mol) of (ammonium acetate) and (150 mL) of acetic acid and escalate the mixture for 90 min, then cooled in an ice path. The resulting precipitate was filtered and washed with distilled water to reduce the impurities then dried. Recrystallization was performed from ethanol to obtain a pure crystalline precipitate as 4,5-bis(4-methoxy phenyl) imidazole.

The azo-imidazole ligand [27] was prepared by dissolving (0.428g, 0.001mol) of 4-Amino benzoic acid in a mixture solution of (3 mL concentrated HCl + 20 mL distilled water). Keeping the mixture in an ice bath (0 – 5) oC then add (10 mL) of the aqueous solution of (0.70 g, 0.01 mol) of NaNO2 drop by drop with stirring, leave the solution for (30 minutes) to settle. This diazonium solution has been mixed with (2.80 g, 0.01 mol) of 5,4-di (4-methoxyphenyl) imidazole solution dissolved in a mixture of (150 mL methyl alcohol + 5 mL of NaOH (10%)). The color of the solution will convert to a dark orange color. Let the solution for 24 hours, and then adjust the pH value to (7) by adding drops of (0.1N) of HCl. Leave the solution to settle, then filter and wash with distilled water to remove any inorganic salts, dried and re-crystallized from ethanol and characterized. Scheme. 1 illustrate the preparation steps.



Scheme. 1: Preparation steps of the azo ligand

4- Preparation of standard and work solutions

In a volumetric flask of 100 mL, the stock solution of the azo ligand (1000 µg mL-1) was prepared by dissolving an accurate weight (0.1 g) of the reagent in 96% ethanol, followed by dilution with the same solvent to the mark. Further dilution was performed with the same solvent to prepare the working solutions. The stock solution of Hg (II) (100 µg mL-1) was prepared by dissolving an accurate weight (0.01 g) of HgCl2 in the distilled water and completing the volume to the mark (100 mL). The working solutions were prepared by further dilution with the same solvent.

5- General procedure

Into a series of 10 mL volumetric flasks, appropriate volumes of the mercury ion solution containing 5 - 100 µg were transferred, add 0.5 mL of 200 µg mL-1 azo reagent solution to each flask. Dilute the mixtures with distilled water up to the mark. Measure the absorbance at 546 nm against reagent blank absorbance. Draw the calibration curve between the absorbance values and the concentration values.

6- Absorption spectra of the azo ligand and its Hg (II) complex

The UV- Vis spectrum of the reagent (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-4-yl) diazenyl) benzoic acid showed a maximum absorption peak (λmax) at 470 nm. While the absorption spectra of the Hg (II)-complex showed a bathochromic shift in the reagent (λmax), this shift makes the maximum absorption peak for the complex solution at 546 nm as shown in Figure 1.

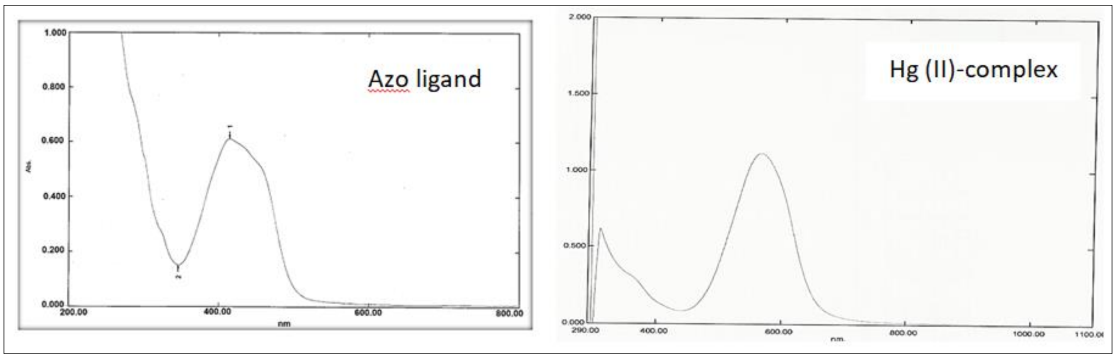


Figure 1: On the left, UV- Vis spectrum of the azo ligand, on the right, UV- Vis spectrum of the Hg (II)-complex

7- Results and discussion

7- 1- Effect of the azo ligand concentration

The concentration effect of 0.25 mL azo ligand on complex formation with Hg (II) (1 mL, 3 µg mL-1) in aqueous solution was studied in the range of (50-350) µg mL-1. The complex absorbance increases with the increase of the reagent concentration up to 200 µg mL-1, then decrease at the high concentrations, as shown in Figure 2.

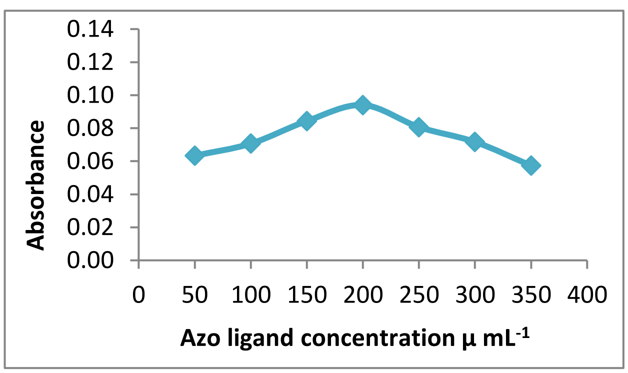


Figure 2: Effect of the azo ligand concentration

7- 2- Effect of time and temperature

The effect of the complexation time and the temperature on the Hg (II)-complex formation has been tested in the range of (0.5-45) minutes and (25-70) oC, respectively. The complex absorbance was noticed to be increased with complexation time up to 1 minute then decreased and at room temperature until reaching 30 oC then decreased.

7- 3- Effect of azo ligand solution volume

The azo volume effect on the complexation reaction of the Hg (II) (1 mL, 3 µg mL-1) and 200 µg mL-1 azo has been examined in the range of (0.2-0.8) mL. The results revealed that the complex maximum absorbance was noticed when using 0,5 mL of the azo ligand, then decrease when using greater volumes, as shown in Figure 3.

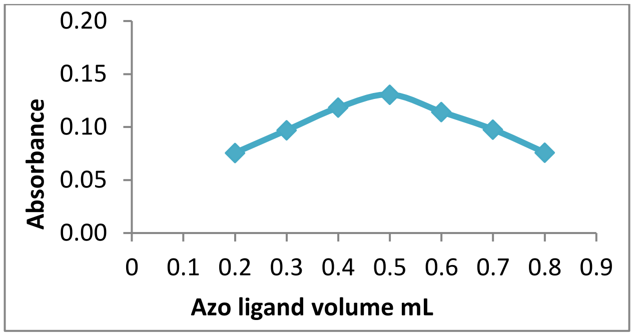


Figure 3: Effect of the azo ligand volume

7- 4- The calibration curve

The calibration curve was established at 546 nm according to the specified experimental conditions. By preparing a series of Hg (II) solutions with increasing concentrations, each as five replicates. The absorbance values have been recorded against the ligand blank, then drawing the absorbance values against the concentration values. The results showed that the Beer`s-Lambert low was obeyed in the range of (0.5-10) µg mL-1, as shown in Figure 4. The calibration curve data and some important analytical parameters are listed in the Table 1.

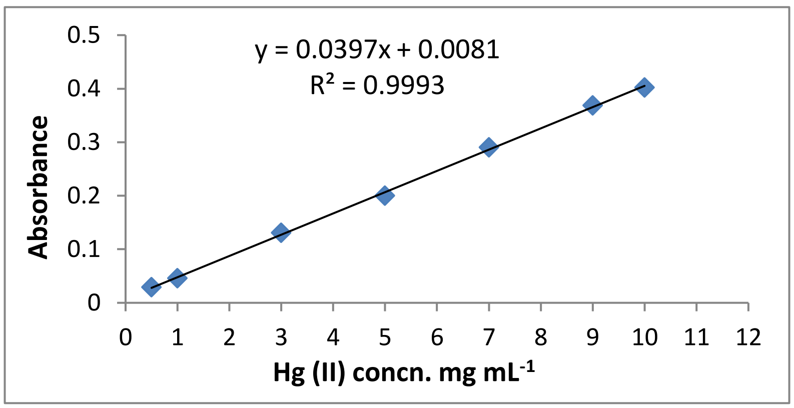


Figure 4: the calibration curve of the Hg (II)-azo ligand complex

Table 1: calibration data and analytical parameters of the proposed method for the determination of Hg (II)

Analytical parameter	**Hg (II)-complex**
λmax / nm	546
Regression equation	Y = 0.0397 + 0.0081
Specific absorption coefficient / L gm^{-1} cm^{-1}	39.7
Molar absorption coefficient / L mol^{-1} cm^{-1}	$7.963 \times 10^{+3}$
Sandells sensitivity / µg cm^{-2}	0.025
Correlation coefficient / r^2	0. 9993
Detection limit D. L / µg mL^{-1}	0.190
Quantitation limit Q. L / µg mL^{-1}	0.634
Linear range / µg mL^{-1}	0.5 - 10
Standard deviation	0.002
Relative standard deviation %	1.607
Composition of complex (M:L)	1:2

7- 5- Composition of the complex

The composition of the Hg (II)-complex was studied by the mole ratio method. As shown in Figure 5, the method indicated that the molar ratio of the mercury ion to the azo ligand is 1:2 (metal: ligand).

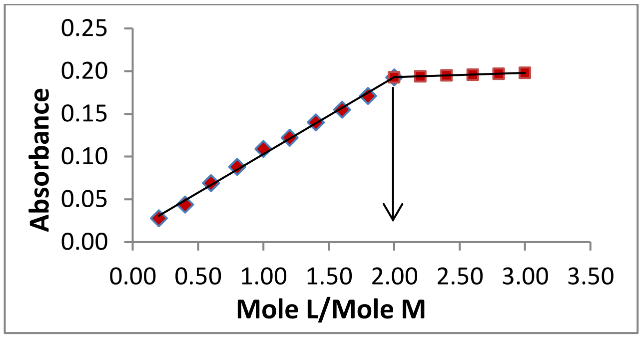
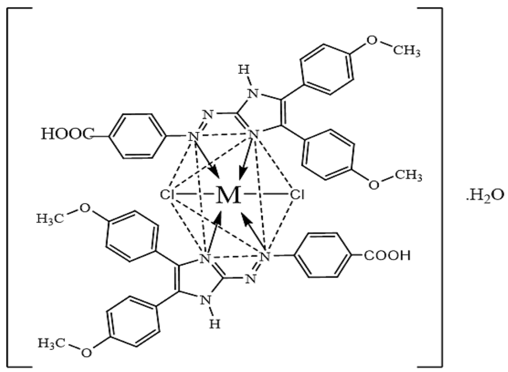


Figure 5: Mole ratio method for Hg (II)-azo ligand complex

7- 6- Suggestion of structural formula of the complex

From the obtained results from the mole ratio method (metal to ligand ratio) and based on the properties of the Imidazol compounds, the complex structure can be suggested as:



7- 7- Effect of the interferences

The interference effect of different cations and anions, which can form complexes with the azo ligand or affect the complexation reaction was studied to estimate the method's utility. By applying the general procedure with adding a known quantity of interfering ions (3 and 30 µg mL-1) to a 3 µg mL-1 Hg (II) ion solution. To eliminate the effect of cations interferences, the selectivity of diverse masking agents is examined such as EDTA, NH3, Sodium acetate and NaNO2, the best masking agent for the cations was EDTA, Table 2. While the anions don't have any interfering effect on the complex formation in the two concentrations (3 and 30 µg mL-1).

Table 2: The interference effect of cations on 3 µg mL-1 Hg (II)

Interference cation type	**Interference conc. µg mL^{-1}**	**Masking agent EDTA 0.34×10^{-3} M, VmL**	**Error %**
Zn^{+2}	3	---	2.956
	30	1	2.463
Fe^{+3}	3	0.3	-1.923
	30	1	-2.404
Pb^{+2}	3	---	-0.481
	30	0.4	-1.923
Cd^{+2}	3	0.3	0
	30	1.2	6.557
Ni^{+2}	3	0.1	0
	30	1.5	2.941
Cu^{+2}	3	0.3	-0.476
	30	2	-2.381
Co^{+2}	3	0.4	-2.381
	30	1	0.476
Al^{+3}	3	0.5	-1.026
	30	0.8	0.513

Mn^{+2}	3	0.3	0
	30	1	0.476

8- The biological activity study

The biological activity of the azo ligand [27] and its Hg (II) complex was tested against two kinds of gram-positive bacteria (Staphylococcus aureous (S. aureus) and Enterococcus faecalis (E. faecalis)) and two kinds of gram-negative bacteria (Eschershia coli (E. coli) and Klebsiela pneumonia (K. pneumonia)) using the agar well diffusion method. The chosen bacteria have been swabbed on the solidified agar surface, then a 6 mm diameter wells in the agar plates were made and filled with (0.1 mL) of the azo ligand and Hg (II) complex at five increasing concentrations of (25, 50, 100, 200 and 400 µg mL-1). The plates were incubated at (37 oC) for a day, then recorded the biological activity by measuring the diameter (in mm unit) of the inhibition zone.

The results indicated in Table 3 that the azo ligand exhibited a higher inhibition activity against E. faecalis than E. coli, and a moderate inhibition activity against S. aureus and K. pneumoniae. While its Hg (II) complex showed higher inhibition activity against all the bacteria types than the ligand alone, with higher activity against K. pneumoniae than E. faecalis and S. aureus and lower activity against E. coli. There is no inhibition activity against any type of bacteria at the concentration of (25 µg mL-1) for both the azo ligand and its Hg (II) complex.

Table 3: The interference effect of anions

Compound	Concentration µg mL^{-1}	gram positive bacteria		gram negative bacteria	
		S. aureus	*E. faecalis*	*E. coli*	*K. pneumoniae*
L ligand	25	-	-	-	-
	50	11	11	10	-
	100	13	15	13	14
	200	15	17	16	15
	400	16	19	17	16
Hg (II) complex	25	-	-	-	-
	50	13	14	11	18
	100	14	17	14	21
	200	16	19	16	23
	400	20	20	18	24

9- Conclusion

This work describes the feasibility of using (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-4-yl)diazenyl) benzoic acid as an analytical reagent for rapid and efficient spectrophotometric determination of the Hg (II) without heating or extraction. The interfering effect of different ions was studied, and an appropriate masking agent was used to reduce the interfering effect. The biological activity of the azo ligand and its Hg (II) complex was investigated and compared.

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