Spectrophotometric Method for the Determination and Biological Activity of Trace Amount of Zn(II) and Hg (II) with Reagent 2 – (6 –Methoxy – 2 – Benzothiazolyl) azo – 4 , 5 – diphenyl imidazole

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Abstract:

A direct method has been developed for the spectrophotometeric determination of Zn(II) and Hg(II) using 2–(6 –methoxy – 2 – benzothiazolyl) azo] – 4 , 5 – diphenyl imidazole (6-MBTADI) as a complexing reagent . Beer's law was obeyed over the range from 2- 25µg/mL for Zn(II) and 0.5-13µg/mL for Hg(II). Sandell's sensitivity and Molar absorptivity have been found to be 0.0582µg.cm^{-2} and 0.1123x10^{4} L.mol^{-1}.cm^{-1} for Zn(II), 0.0262µg.cm^{-2} and 0.7640x10^{4} L.mol^{-1}.cm^{-1} for Hg(II). Each metals complex were stable for more than 24 hrs under optimized conditions. The limit of detection , relative standard deviations, relative errors and recovery for Zn(II) and Hg(II) complexes were found to be LOD=0.8, 0.45; R.S.D%=3.94 ,1.892; Erel%=2.58, 3.5 and Re%=97.42, 96,5 respectively.

Introduction

Heterocyclic azo dyes are an important class of organic complexing reagents used in spectrophotometric analysis as they have attracted the interest of many research groups. They also are found in a variety of industrial applications mainly in the fields of textiles, papers, leather, laser materials xerography, laser printing, materials for organic solar cells and chemosensors.[1,2]

The 2-aminothiazole derivatives, called thiazolylazo is one important class of heterocyclic compounds particularly because they can form different types of coordination compounds with transition metals due to the several electron rich donor centers with unusual structural and chemical properties. The main applications of thiazolylazo dyes in chemical operations include spectrophotometry , solid phase extraction, liquid chromatography, electrochemistry and liquid and cloud point extraction. Heterocyclie ring containing sulphur, nitrogen and oxygen impart specialbiological activity to these Schiff bases and their metal complexes . A number of benzothiadiazoles showed selective antiproliferative activity , imidazo benzothiazoles,as well as, polymerized benzothiazoles and other substituted benzothiazoles showed remarkable antitumor activity against malignant cell lines[3,4]. The application of thiazolylazo dyes in spectrophotometry is based on the coloured compounds resulting from their reaction with most metals, especially some transition metals. Usually, stable chelates are produced [5-7].

Heavy metals are priority toxic pollutants that severely limit the beneficial use of water for domestic and industrial application1.There are over fifty elements that can be classified as heavy metals, but only seventeen that are considered to be both very toxic and relatively accessible. Mercury, lead, arsenic, cadmium, selenium, copper, zinc, nickel, and chromium should be given particular attention in terms of water pollution[8]. Heavy metals are present in the soil, natural water and air, in various forms and may contaminant food and drinking water[9].

For the quantative determination of Zn(II) and Hg(II) in trace amount, there are several frequently adopted methods such as atomic absorption spectrophotometery, X-ray fluorescence spectroscopy, spectroflurimetry, spectrophotometry etc. Among these, spectrophotometric methods are preferred as they are economical, easy to handle, with a comparable sensitivity and accuracy and good precision. It is one of the most commonly used techniques for routine analysis of metals. So, there is need to develop a simple, sensitive and selective method for determination of these metal ions in various samples. Different chelating agents have been proposed , such as 5-(2-Benzoic acid azo)-8-hydroxy quinoline form colord complexes with Hg(II), Zn(II) and Cd(II) having general formula [ML_2] [10]. Many thiazolylazo reagents have been reported for spectrophotometeric determination of zinc[11-17] and mercury[18,19] such as 1-(2-Thiazolylazo)-2-naphthol(TAN) .

This paper reports, 2–(6 –nitro – 2 – benzothiazolyl) azo] – 4 , 5 – diphenyl imidazole , which was prepared by Al – adely [20] ,as an analytical reagent for the micro determination of zinc(II) and Hg(II) , whereas, a very limited number of heterocyclic azo dyes find their uses for the determination of zinc. Comparatively this reagent has been found to have fair sensitivity and high selectivity for zinc (II). Thus the reagent was utilized to determine zinc in biological samples and food stuffs. The method has been found to be simple, rapid and sensitive for the determination these metal ions.

EXPERIMENTAL

Apparatus

Absorption spectra in absolute ethanol were recorded using shimadzu, UV-visible 1650 spectrophotometer double beam using 1cm quartz cells while absorrtion measurements were obtained with RSP-721 UV-visible spectrometer. Functional groups of reagent and its complexes were identificated with a FT-IR spectrometer shimadzu 8400, in range(4000-400) cm^{-1} using KBr disc. pH measurements were carried out using a microprocessor 211 pH meter (pH± 0.001).

Reagents and Solutions

All the chemicals used were of analytical reagent grade, and were used without further purification. Ethanol were purchased from (GCC, England) . A 1.0 x 10^{-3} mol L^{-1} (6-MBTADI) was prepared by dissolving 0.1028 g of 2–(6 –methoxy – 2 – benzothiazolyl) azo] – 4 , 5 – diphenyl imidazole in 250 mL ethanol. Stock solutions of Zn (II) and Hg (II) ions (1000 mg L-1) were prepared by dissolving (0.1598 g) of $ZnCl_2$(BDH) and (0.1353 g) of $HgCl_2$ (Merck) in 100 mL distilled water, respectively. Working standard solutions of each metal ion were freshly prepared by appropriate dilutions of the stock standard solutions. The pH of the medium (3-9) were adjusted with ammonium acetate (0.01 mol L^{-1}) –ammonia – glacial acetic acid buffer solution .

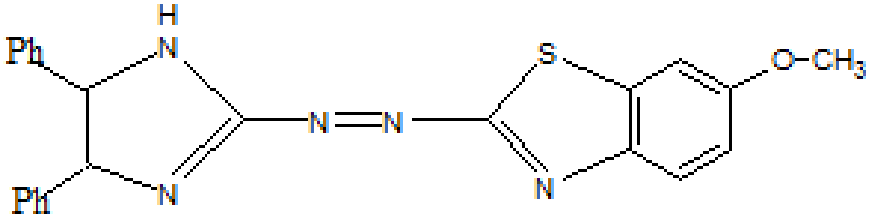


Fig.(1): Structure of the reagent (6 – MBTADI)

General procedure

In to a 10 mL calibrated flask , transfer 1 mL of sample solution containing less than 100 μg.ml^{-1} of zinc(II) and adjust the pH to 6 with ammonium acetate buffer, add 3 ml 1.75×10^{-4}M ethanolic (6-MBTADI) solution and diluted to the mark with deionized water. The absorbance of the resultant solution was measured after 10 min at 538 nm at 25 C° against the corresponding reagent blank prepared under identical conditions but without zinc. The same procedure for mercury (II) at pH 5 and the absorbance of the result solution after 15 min at 522 nm.

Results and Discussion:

Infrared spectra

The IR spectra of the reagent (6 – MBTADI) and their complexes are complicated due to the extensive overlap of a number of bands arising from OH μ, C=N and N=N groups in addition other bands originated from phenyl and thiazole rings appeared in the region 4000-400 cm^{-1}. The shifts in the position of these bands compared with those absorption bands due to the free reagent suggest the probable modes of bonding in the complexes. Some of these main shifts and conclusion are given bellow.

A new broad medium band absorbed at 3393-3346 cm^{-1} in the spectra of the latter complexes which may indicates the presence of water molecule in them. The weak bands observed at 3120 cm^{-1} and 2910 cm^{-1}in the spectrum of the ligand are due to υ (C – H) aromatic and aliphatic respectively . These bands are stable in position as well as in intensity for both ligand and metal chelates .

The spectrum of ligand shows absorption bands at 1580 cm^{-1} and 1595 cm^{-1} due to υ (C = N) of imidazole and thiazole rings respectively [21]. These bands υ (C = N) of imidazol ring shifts to lower wave number 1540 – 1535 cm^{-1} in the metal complexes, these shifts suggest the linkage of metal ion with nitrogen . Two absorption bands are absorbed at 1500 cm^{-1} and 1430 in the reagent spectrum. which are due to the azo υ (N = N) [22,23]The positions of these bands in the spectra of complexes are shifted to a lower wave numbers (1435 – 1410) cm^{-1} with decreased in intensity . Both bands are shifted and reduced intensities this may indicate that azo group is coordinated to the metal ions [24].

Some other new bands appeared in the (410 – 580) cm^{-1} region these bands did not present in the spectrum of the reagent which the may be attributed υ(M-N)[25,26] and (M , Cl) . Fig (2,3 and 4) show the spectra of reagent (6 – MBTADI) and (6 – MBTADI – M (II) complex .

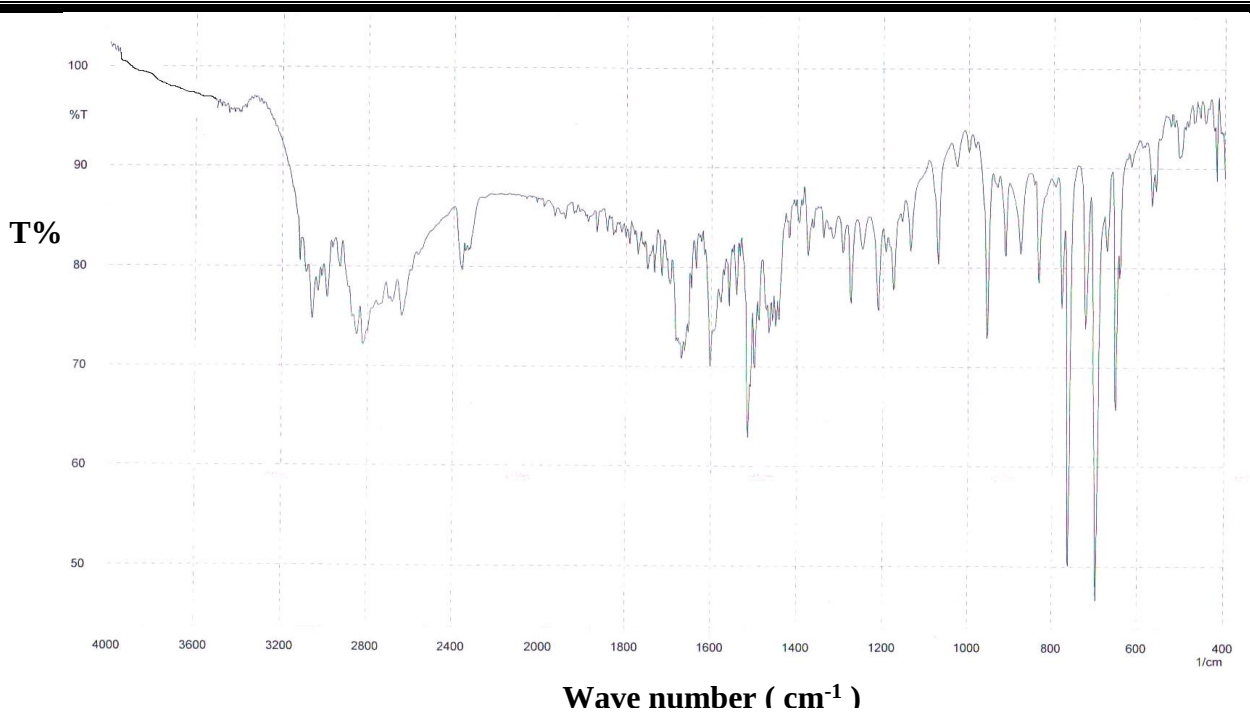


Fig.(2) : IR spectrum of the ligand (6 – M BTADI)

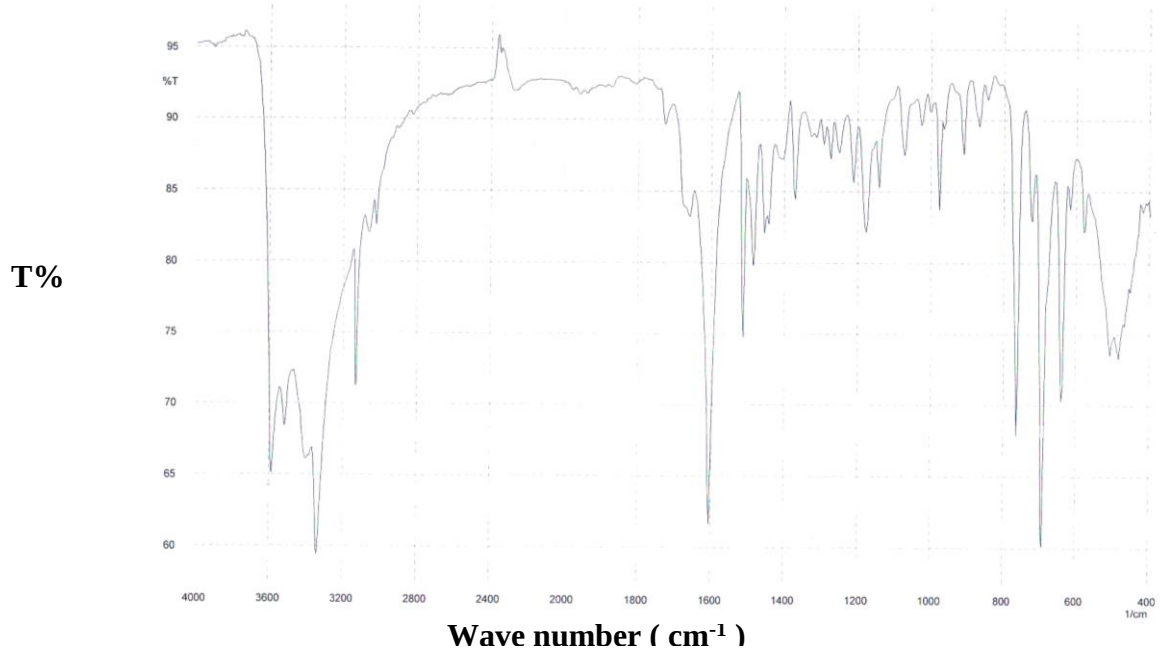


Fig.(3) : IR spectrum of (6 – M BTADI – Zn(II))

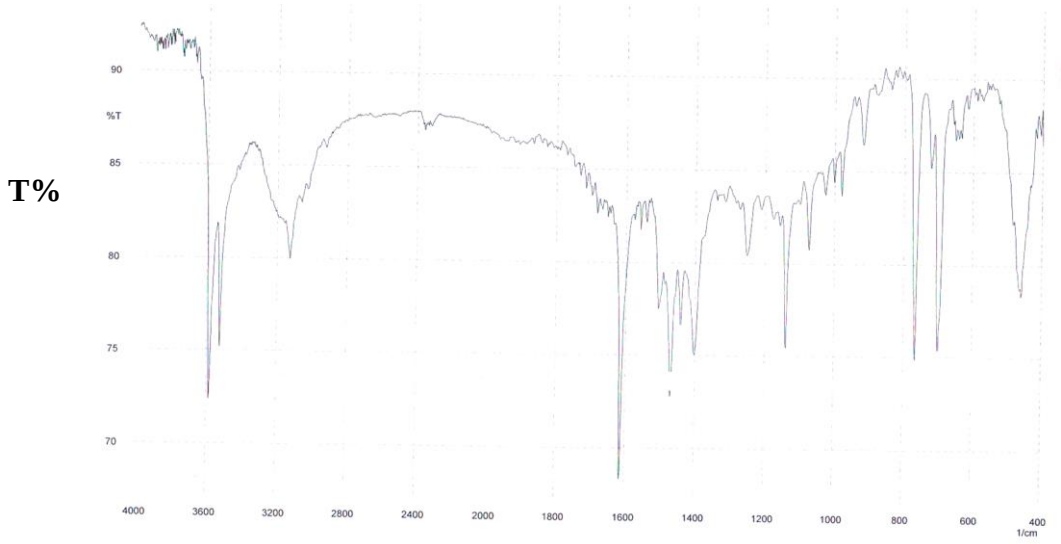


Fig.(4) : IR spectrum of (6 – MBTADI – Hg (II))

Absorption spectra and Characteristics of the Complex

The UV- Vis spectrum of an ethanolic solution of the reagent (6 – MBTADI) ($1.75x10^{-4}$ M) shown three peaks, the first and second peaks were observed at (224 nm) and (263 nm) were assigned to the moderate energy π- π* transition of the aromatic rings. The third peak (λ max) was observed at the (434 nm) due to the (π-π*) from aromatic ring through the azo group (charge transfer) was referred to the n- π* transition of intermolecular charge- transfer taken place from benzene through the azo group(-N=N) [27].

Interaction of the metal ions Zn(II) and Hg(II) with the reagent has been studied in aqueous ethanolic solution . A bathchromic shift of Zn(II) and Hg(II) complexes show the absorption maxima of 538 and 522 nm with molar absorptivities (ε) of 1123.2 L mol^{-1} cm^{-1} and 7640.9 L mol^{-1} cm^{-1} obtained respectively while the reagent gave the absorption maxima of 434 nm as depicted in Fig. 5. The wave length different(Δ λmax) is (104-88 nm) ,a great bathochromic shift in the visible region has been detected in the complex solutions spectra with respect to that of the free reagent. The high shift in the (λmax) gave a good indication for complex formation.

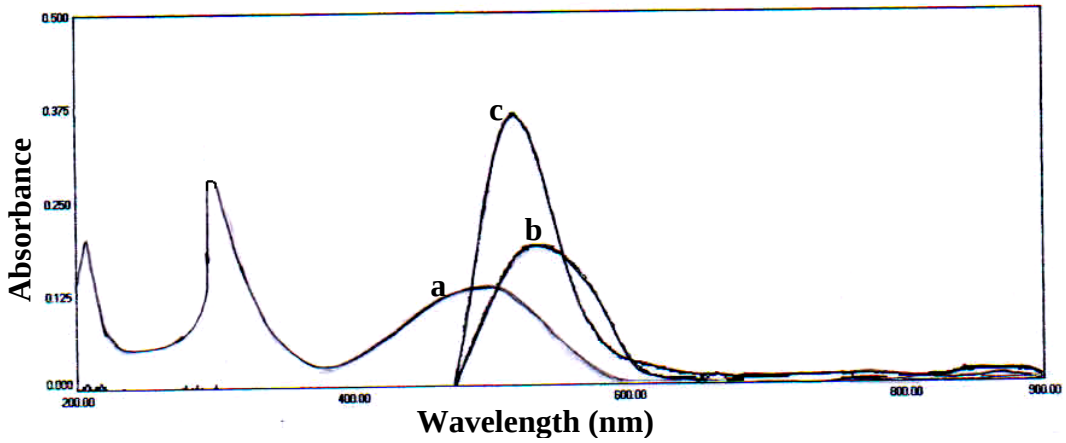


Fig.(5): Absorption spectra (a) Reagent (6 – MBTADI) = 1.75 x 10^{-4} M (b) Zn(II)-(6 – MBTADI) complex , Zn(II) = 25 µg mL^{-1} , 3 ml of (6 – MBTADI) = 1.75 x 10^{-4} M , pH= 6 . (c) Hg(II))- (6 – MBTADI)complex , Hg(II) = 13 µg mL^{-1} , 1.5 ml of (6 – MBTADI) = 1.75 x 10^{-4} M , pH= 5

Method Validation

Under the optimized conditions, the calibration graphs were constructed by plotting the absorbance signal against the concentrations of each analyte subjected according to the general procedure. The solutions were transferred into the optical cell of 10-mm for the measurement of each metal ion spectrophotometrically at the respective absorption maxima against a reagent blank prepared under similar conditions .The calibration data are summarized in Table (1).

Table (1): Method validation of the spectrophotometric determination of Zn(II) and Hg(II) .

Parameter	Zn (II)	Hg (II)
λ_{max} (nm)	538	522
Regression equation	A=0.0082C-0.0122	A= 0.0281 C - 0.0015
Correlation coefficient(r)	0.9991	0.9990
C.L. for the slope (b± tsb) at 95%	0.0082±0.00023	0.0281± 0.00085
C.L. for the intercept (a± tsb) at 95%	0.0122±0.0034	0.0015± 0.006117
Concentration range (µg mL^{-1})	2-25	0.5-13
Limit of Detection (µg mL^{-1})	0.8	0.45
Limit of Quantitation (µg mL^{-1})	2.926	1.530
Sandell's sensitivity (µg.cm^{-2})	0.0582	0.02625
Molar absorptivity (L.mol^{-1}.cm^{-1})	1123.2	7640.9
Composition of complex (M: L)	1:2	1:2
RSD% (n=7) at12 µg Zn (II) mL^{-1} and 6 µg Hg (II) mL^{-1}	3.94%	1.89
Recovery%	97.42	96.50

Optimization of Procedure

Effect of pH

The effect of pH on formation of the- M (II) (6- MBTADI) complexes was determined by recording their absorbance signals at λ_{max} ,over the range of 3-9 ,using different pH acetate buffer solutions. The results are shown in Fig.6.

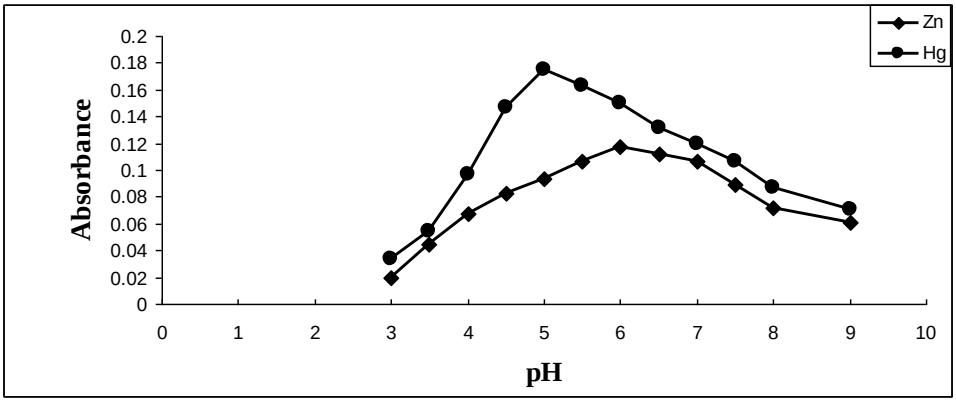


Fig.(6) Effect of pH on the formation of(6- MBTADI)- M(II) complexes formed with Zn (II) and Hg (II)). Conditions : Zn(II) = 15 µg mL^{-1} , 3 ml of (6- MBTADI) =1.75x10^{-4} M .and Hg(II) = 6 µg mL^{-1} , 1.5 ml of (6- MBTADI) =1.75x10^{-4} M

As can be seen in Fig.(6), the absorbance first increased with increasing pH and reached a maximum at pH 6.0 and 5.0 for Zn(II) and Hg(II) complexes ,respectively. The absorbance gradually decreased because of partial dissociation of the complexes at higher pH, which may result in incomplete extraction of both complexes. Therefore, pH 6.0 and 5.0 were selected as the optimum pH's for complete formation of for Zn(II) and Hg(II) complexes respectively .

Effect of Temperature and Time

The effects of the temperature and the time were examined due to their importance for the reaction completion . Consequently, a study was carried out to choose the range of temperature that enhances higher absorbance signals for Zn (II) and Hg (II) ions. The temperature was varied from 10 ^{O}C to 80 ^{O}C in a search of optimum value. It can be seen from Fig. 7 that the highest absorbance signals were achieved when the temperature at 40 ^{O}C.

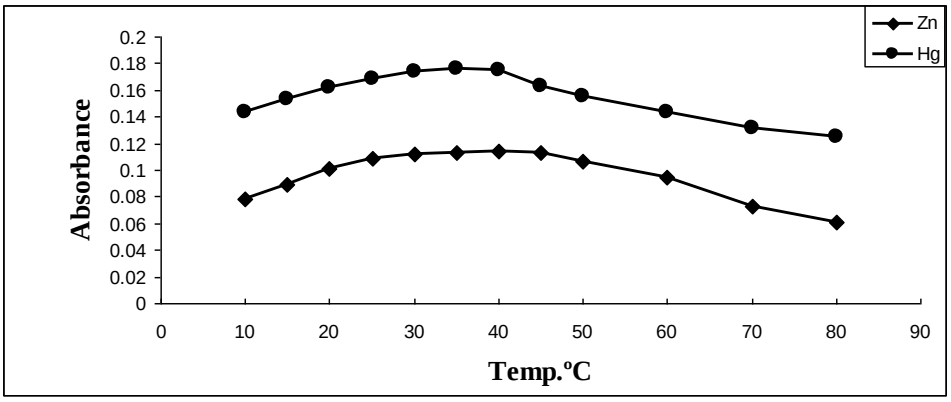


Fig.(7)Effect of the temperature on the absorbance for (6- MBTADI)- M(II) complexes formed with Zn (II) and Hg (II)). Conditions : Zn(II) = 15 µg mL^{-1} , 3 ml of (6- MBTADI) =1.75x10^{-4} M .and Hg(II) = 6 µg mL^{-1} , 1.5 ml of (6- MBTADI) =1.75x10^{-4} M

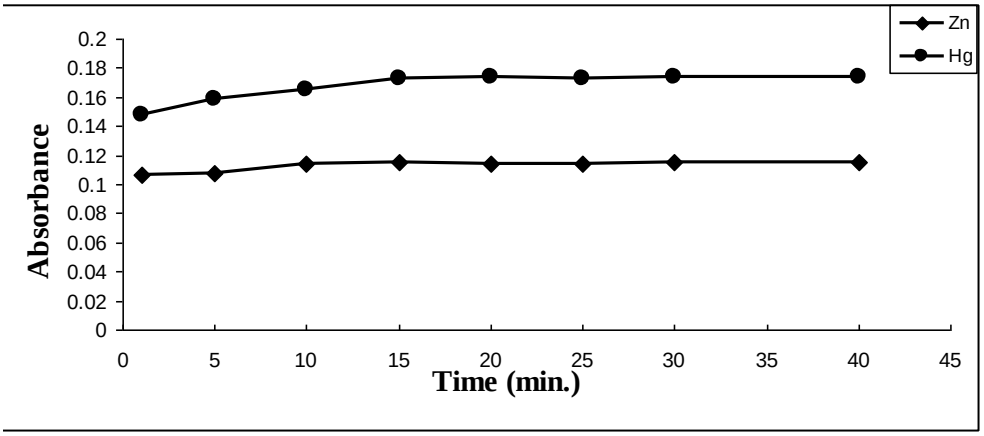
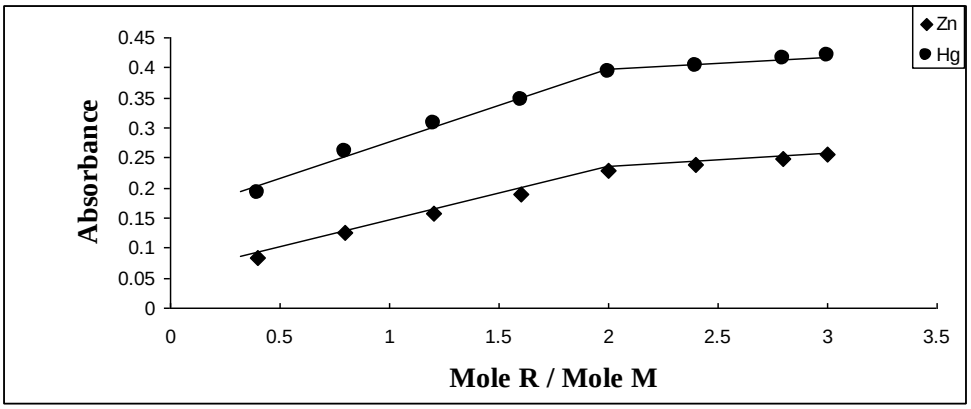


Fig. 8 Effect of time on the absorbance for (6- MBTADI)- M(II) complexes formed withZn (II) and Hg (II)). Conditions : Zn(II) = 15 µg mL^{-1} , 3 ml of (6- MBTADI) =1.75x10^{-4} M .and Hg(II) = 6 µg mL^{-1} , 1.5 ml of (6- MBTADI) =1.75x10^{-4} M

It was also observed that the incubation time of 10 min and 15 min are sufficient for the maximum absorbance of Zn(II) and Hg(II) ,respectively.

Composition and stability of complexes

The composition of the chelat complexes were determined by continuous variation and mole ratio method Fig. 9 and Fig.10. Both methods showed that the molar ratio of Zn(II) and Hg(II) ions to reagent (6- MBTADI) are 1:2 (M:L). The stability constant are found to be 0.6114 x$10^{8}L^{2}.mol^{-2}$ and 0.1640 x$10^{9}L^{2}.mol^{-2}$ for Zn(II) and Hg(II) respectively . The structural formula of prepared complexes can be suggested and showed in Fig.11.



Fig(9):- Mole ration method for Zn(II)-complex at pH=6 and Hg(II)-complex at pH=5

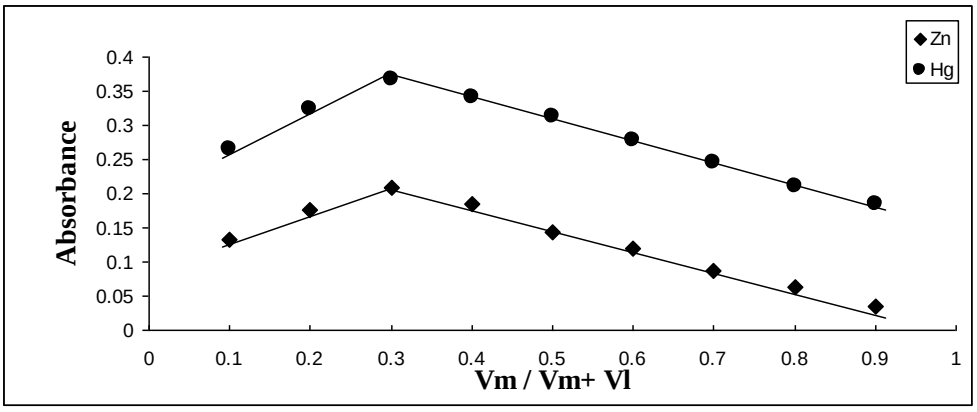
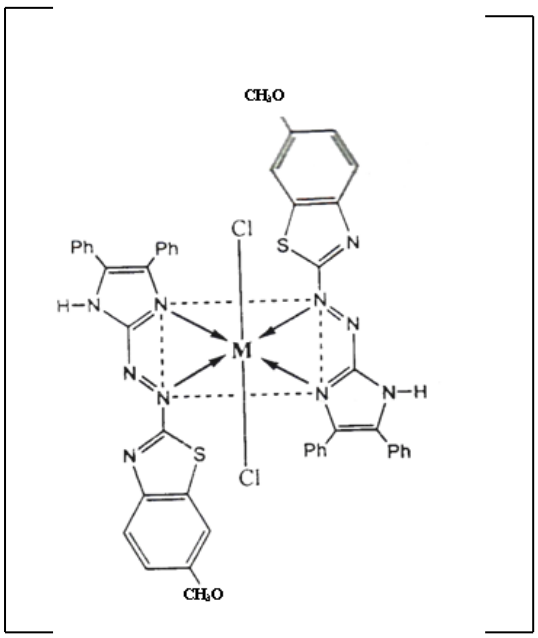


Fig.10 Continuous variation method for Zn(II)-complex at pH=6 and Hg(II)-complex at pH=5



n H_2O

where M= Hg n=1 , Zn n=1

Fig .(11) The suggested structural formula of Zn and Hg complex

Biological effect

The biological activity test [28] was a chivied for azo compounds through measurement the strength of this compounds to resistance the growth of a different species of bacteria . However Metal complexes are more active than their ligand because the metal complexes may serve as a vehicle for activation of ligand as the principle cytotoxic species[3]. It should motioned that the antimicrobial results were obtained concentration 1×10^{-4} M .

Table (2): Anti bacterial activities of tested synthesized compound

	Aspergillus	Fusarium	Staphelococcus	Psuedo monas
(6 –MBTADI)	+	+	-	+ +
(6 –M BTADI) Zn	++	+	+	-
(6 –MBTADI) Hg	++	-	+	+

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دراسة طيفية والفعالية البايولوجية لتقدير الكميات الضئيلة من Zn(II) وHg(II) مع الكاشف 2 – (6 ميثوكسي–2 – بنزوثيازوليل ازو) – 4 ، 5- ثنائي فينل اميدازول

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أزهار عبيس غالي أيفان فرحان جواد
جامعة القادسية –كلية التربية –قسم الكيمياء

الخلاصة :

تضمن البحث استخدام الكاشف العضوي(2 –[6-ميثوكسي 2 – بنزوثيازول ازو)] – 4 ، 5- ثنائي فينل اميدازول (6-MBTADI) في التقدير الطيفي لكميات مايكروغرامية قليلة من ايوني الزنك(II) والزئبق (II). بتفاعل هذا الكاشف مع ايون الزنك(II) عند pH=6 ويكون معقد عند الطول الموجي للامتصاص الاعظم 538 نانومتر ومع الزئبق يعطي معقد عند PH=5 عند الطول الموجي للامتصاص الاعظم 522 نانومتر. قيم معامل الامتصاص المولاري (∈) وجدت انها تساوي 0.1123×10^4 لتر.مول.سم$^{-1}$ 0.7640×10^4 لتر.مول.سم$^{-1}$ لكل من معقدي الزنك(II) والزئبق (II) على التوالي. مدى التراكيز التي تطاوع قانون بير (2-25)جزء بالمليون للزنك و (0.5-13) للزئبق. حد الكشف والانحراف القياسي والخطأ النسبي والاسترجاع بالمئة لهذه الطريقة الطيفية لمعقدي الزنك (II) والزئبق (II) هي 1.89 ,0.8 D.L=0.45, R.S.D%=3.94 ,Re%=97.42,96.5 Ere%=2.58,3.5 على التوالي. لقد بينت النتائج التحليلية ان نسبة الكاشف الى الفلز (طيفي النسبة المولية والتغيرات المستمرة) (M:L) هي (1:2) ولكلا المعقدين. لذلك تم اقتراح التراكيب المتوقعة للمعقدات. تم قياس ثوابت الاستقرارية للمعقدات ، اطياف الاشعة تحت الحمراء ، الاشعة فوق البنفسجية والفعالية البايولوجية للمعقدات.