



## UV-Vis Spectroscopic Study on 4-Hydroxy-N-[(E)-(2-hydroxyphenyl)methylidene]benzohydrazide as Calorimetric Reagent For Mg<sup>2+</sup> Ions

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### Keywords:

Cation.  
Chromogenic  
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Organic receptor

### ABSTRACT

This work is concerned about the study of the chromogenic properties of hydrazone compound, i.e. 4-hydroxy-N-[(E)-(2-hydroxyphenyl)-methylidene]benzo-hydrazide towards magnesium ion recognition. The compound was synthesized and characterized by FT-IR and NMR spectroscopy. The complexation ability of the studied ligand towards Mg<sup>2+</sup> was investigated using UV-Vis spectroscopy. Experimental parameters that affect the ligand-metal ion complexation such as effect of solvent, standing time, metal concentration and ligand concentration were investigated. Results show that HNHMB is to bind preferentially with Mg<sup>2+</sup> ions in ethanol. The addition of Mg<sup>2+</sup> to the ligand in ethanol gives rise to a large hyperchromic shift resulting in a colour darkness. From the linear regression analysis, Beer's law was obeyed in at least 0.1–1.4 × 10<sup>-4</sup> M. The complex showed maximum absorptions (λ<sub>max</sub>) at 396 nm.

## دراسة طيفية للأشعة المرئية-فوق البنفسجية حول المركب (4-Hydroxy-N-[(E)-(2-hydroxyphenyl)methylidene]benzohydrazide) ككاشف انتقائي لأيون المغنيسيوم

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### الكلمات المفتاحية:

Cation  
Chromogenic  
Hydrazone  
Magnesium  
Organic receptor

### المخلص

يهتم هذا البحث بدراسة الخصائص الصبغية لمركب الهيدرازون (4-Hydroxy-N-[(E)-(2-hydroxyphenyl)methylidene]benzohydrazide) (HNHMB) الذي تم تحضيره ودراسة خصائصه بواسطة التحليل الطيفي (FTIR) و (NMR) للكشف عن أيون المغنيسيوم. تم فحص هذا قدرة المركب على تكوين معقدات مع أيون المغنيسيوم باستخدام التحليل الطيفي للأشعة المرئية-فوق البنفسجية (UV-Vis). وقد تم دراسة جميع العوامل المؤثرة على تكوين المعقد في محلول الكحول الايثيلي.

### Introduction:

Hydrazones are organic compounds containing azomethine (–NH–N=CH–) group in their molecules are important class of ligands with interesting ligation properties because of the presence of several coordination sites. Owing to the relative easiness of preparation, these compounds possessed an attractive feature worth considering in the design of novel chromogenic compounds, which incorporate this fragment in their structure. These compounds have been widely used as photometric [1–5] and fluorimetric [6–11] as well as analytical reagents for the determination of metal ions. They act as multidentate ligands with metal ions, forming colored chelates [12,13].

Development of this kind of receptors have attracted considerable attention, since visual detection can give immediate qualitative

information without resort to any expensive instruments because of a specific color change of solution upon complexation. Over the past few decades, recognition and sensing of metal ions have been a key research area of many scientific research groups.

Magnesium has been shown to be the most important and common element in an organism and present in several regions of the body. It is one of the most important control and regulatory ions in physiological systems inside and outside cells. However, it is necessary to differentiate magnesium because of the role that it plays. Several methods are available to determine the concentration of total magnesium and other essential metal ions in blood, urine and other body fluids [14]. These include colorimetric determination based on the formation of colored magnesium complexes using the most

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common complexing agents. Other methods include flame spectrophotometric or AAS methods [15-17], ISE [18,19], ion chromatography [20], and FIA [21,22]. These techniques are sensitive and sophisticated, and they involve tedious and cumbersome extraction or preparation steps to enhance analytical utility.

In a previous work [23-25], the author synthesized a series of hydrazone ligands that showed high complexation abilities towards cations and anions. The present method describes the colorimetric determination of magnesium using 4-hydroxy-N-[(E)-(2-hydroxyphenyl)-methylidene]benzohydrazide (HNHMB).

## Experimental

### Instruments

The infrared spectrum of the synthesized compound was recorded on a Perkin-Elmer 2000 FT-IR spectrophotometer, using potassium bromide (KBr) pellets in the frequency range from 4000-400  $\text{cm}^{-1}$ .  $^1\text{H}$ NMR spectrum was recorded on a Bruker 500 MHz NMR spectrometer. Chemical shift values were reported in ppm, using DMSO- $d_6$ . Elemental microanalyses (CHN) was carried out by CHN/O analyzer-Perkin Elmer Series II 2400. UV-Vis spectra were recorded using Perkin-Elmer-Lambda 25 UV/Vis spectrophotometer over the range 800-200 nm using a quartz cell of 1.0 cm path length.

### Chemicals and reagents

All chemicals and reagents used in this work were of analytical grade reagent and used without further purification. 4-Hydroxybenzohydrazide (HBH) and salicylaldehyde (Fluka).

### Synthesis of the ligand

#### 4-Hydroxy-N-[(E)-(2-hydroxyphenyl)-methylidene]benzohydrazide (HNHMB).

Accurately 1.5 g (0.01 moles) of HBH was weighed in a round bottom flask followed by the addition of 20 mL methanol. This mixture was heated to reflux with continuous stirring. Since HBH was not very soluble, concentrated HCl was slowly added drop wise to the mixture. After the addition of 3 mL HCl, complete dissolution occurred then 1.2 g (0.01 moles) of salicylaldehyde was dissolved in 20 mL of methanol. Slowly added into the refluxing solution. Finally rinsed with 10 mL of methanol. The reaction mixture was stirred for 2 hours. The resulting solution was condensed on a steam bath to 5 mL and then cooled to room temperature. Shiny yellow crystals was separated out and were filtered off, washed with 5 mL of cooled methanol and dried in air. Yield was 2.5 g (84%). m.p. 330°C.  $^1\text{H}$  NMR (500 MHz; DMSO),  $\delta\text{H}$  (ppm): 11.95 (1H, b, OH), 10.25 (H, vb, NH), 8.61 (H, s, NCH), 6.90-7.85 (6H, Ph). IR (KBr)  $\nu_{\text{max}}$  3486  $\text{cm}^{-1}$  (b, OH), 3320  $\text{cm}^{-1}$  (m, N-H), 1643  $\text{cm}^{-1}$  (s, C=N), 1508  $\text{cm}^{-1}$  (m, C=C), 1610  $\text{cm}^{-1}$  (vs, C=O) and 1280-1510  $\text{cm}^{-1}$  (m, NO<sub>2</sub>). Elemental analysis: Calc. for C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C, 65.62; H, 4.72; N, 10.93, found: C, 65.81; H, 4.31; N, 10.97. Figures 1 and 2 showed the IR and  $^1\text{H}$ NMR of the ligand.

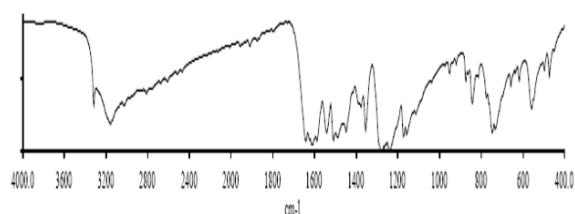


Figure 1: The FTIR spectrum of HNHMB

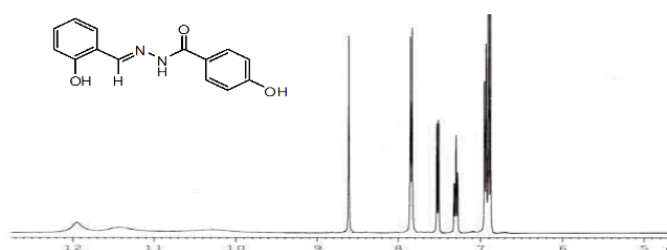


Figure 2: The  $^1\text{H}$ NMR of HNHMB

### Sample preparation for spectroscopic measurement

Stock solution of HNHMB ( $2.0 \times 10^{-3}$  M) was prepared by dissolving 0.77g of the ligand in the proper organic solvent. Stock solutions of metal ions ( $2.5 \times 10^{-3}$  M) were prepared by dissolving the appropriate amount of metal salts in the proper organic solvent. These solutions were directly used in the spectroscopic measurements.

## Results and discussion

### Optimization of the colorimetric method

#### Effect of solvent

HNHMB is not soluble in water but soluble in most common organic solvents such as methanol, ethanol, acetone, acetonitrile, tetrahydrofuran, dimethylsulfoxide, and chloroform. Table 1 presented the chromogenic complexation behavior of the free ligand and its complexes with magnesium ion. The main absorption band was located in the visible region within a range of 382-397 nm which could be assigned to  $\pi-\pi^*$  transition in the large conjugated system. This transition is associated with intramolecular charge transfer character. Hyperchromic change was observed (Figure 3) in the spectrum of the ligand ( $1 \times 10^{-5}$  M) in ethanol upon addition of an equivalent amount of  $\text{Mg}^{2+}$  ions. Higher absorption (at  $\lambda_{\text{max}}$ , 396 nm) for the ligand complex was observed, indicating that the complex formation has taken place. No change in the solution color was observed in other solvents.

Table 1: UV-Vis characteristics of the reagent and its complex with  $\text{Mg}^{2+}$  in different solvents.

Solvent	$\epsilon$	HNHMB		HNHMB- $\text{Mg}^{2+}$	
		$\lambda_{\text{max}}$ (nm)	A	$\lambda_{\text{max}}$ (nm)	A
Methanol	33.0	397	0.039	394	0.054
Ethanol	24.5	392	0.037	396	0.123
Acetone	21.0	---	0.00	---	0.00
DMSO	18.4	382	0.053	---	0.00
Acetonitrile	37.5	397	0.049	395*	0.129

\*= Not stable complex, A=Absorbance, Dielectric constant

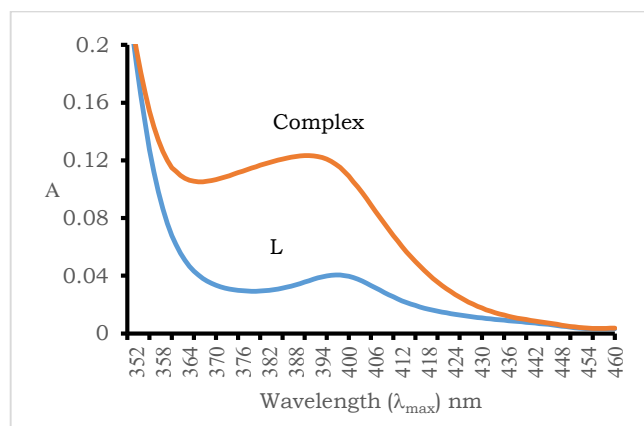
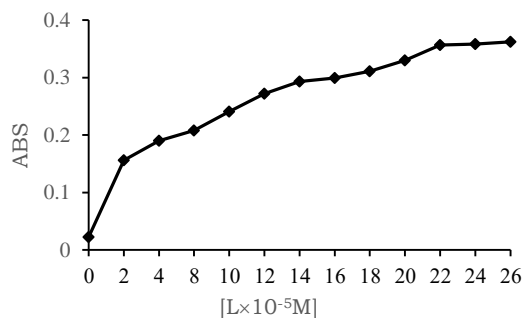


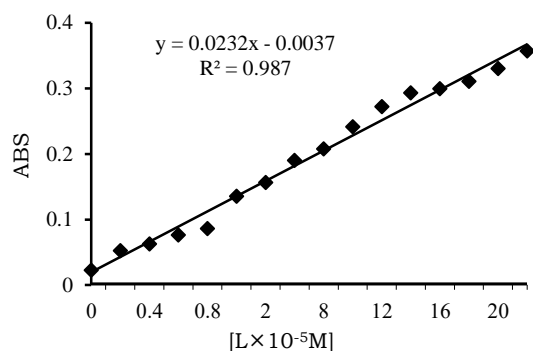
Figure 3: The absorption spectra of the free ligand (L) and its complex with  $\text{Mg}^{2+}$  in ethanol

#### Effect of Ligand concentration

The optimum ligand concentration is another important parameter, which affects the complexation of the ligand with metal ion. Therefore, the effect of HNHMB concentration on the absorbance of its complex with  $\text{Mg}^{2+}$  was studied by mixing fixed amounts ( $2.5 \times 10^{-5}$  M) of  $\text{Mg}^{2+}$  with different concentrations of the ligand, varied from  $2 \times 10^{-6}$  to  $2.6 \times 10^{-5}$  M in ethanol. It was observed that, the color intensity of the tested solution increased with an increase in the ligand concentration (Figure 4). The absorbance was maximized at  $2.2 \times 10^{-6}$  M, indicating optimal color development for the complex. The absorbance was found to be linearly proportional ( $R=0.9868$ ) to the ligand concentration (Figure 5).



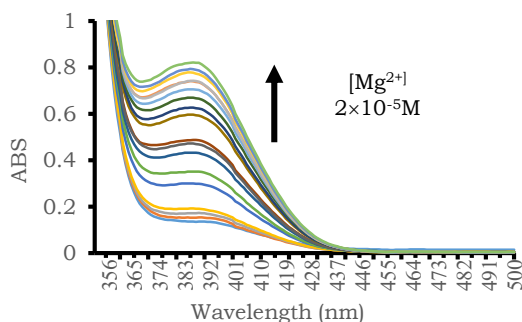
**Figure 4:** Effect of ligand concentration on the complexation of the ligand with  $Mg^{2+}$  in ethanol.  $[Mg^{2+}] = 2.5 \times 10^{-5} M$ .



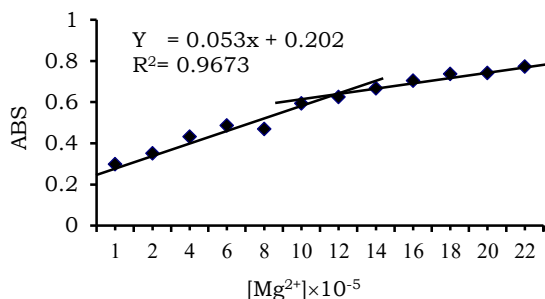
**Figure 5:** Plot of absorbance of the ligand- $Mg^{2+}$  complex in ethanol.

#### Effect of $Mg^{2+}$ concentration

At a fixed ligand concentration of  $2.5 \times 10^{-5} M$  for the ligand, the effect of  $Mg^{2+}$  ion concentration was investigated. The total amount of  $Mg^{2+}$  ions in the reaction mixture was varied within the range of  $1.0 \times 10^{-6}$  to  $2.6 \times 10^{-4} M$ . The maximum absorbance of ligand- $Mg^{2+}$  complex (Figure 6) was observed at  $1.4 \times 10^{-5} M$  of  $Mg^{2+}$  ions with a mole ratio of 1:2 ( $Mg^{2+}$ :L). The absorbance was found to be linearly proportional (Figure 7) to the concentration of  $Mg^{2+}$  in the concentration range from  $1.0 \times 10^{-5}$  to  $1.4 \times 10^{-4} M$ .



**Figure 6:** Effect of  $[Mg^{2+}]$  on the absorbance of complex

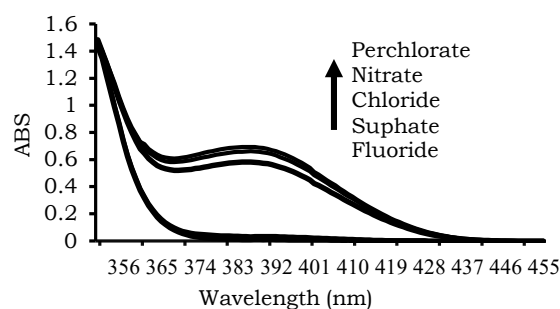


**Figure 7:** Plot of  $[Mg^{2+}]$  on the ligand- $Mg^{2+}$  complex

#### Effect of salt type

The effect of the anion on the HNHMB complexation with  $Mg^{2+}$  ions was also investigated using different magnesium salts, namely, chloride, fluoride, nitrate, perchlorate and sulphate. The ligand

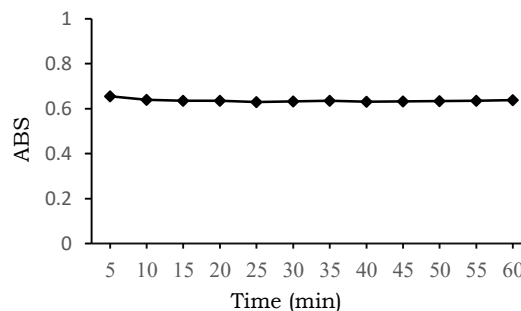
obtained a very good complexation towards  $Mg^{2+}$  upon addition of chloride, perchlorate and nitrate solutions (Figure 8), while no changes observed by other anions.



**Figure 8:** Effect of magnesium salt.

#### Effect of standing time on the ligand $Mg^{2+}$ complex

The stability of HNHMB- $Mg^{2+}$  complex in ethanol was examined at room temperature. The absorbance of the complex was measured at 396 nm in every 3 minutes within 2 hour (Figure 9). The absorbance of the complex remains almost constant after 36 hr. at room temperature. This indicates the high stability of the complex in ethanol.



**Figure 9:** Effect of standing time of the complex.

#### Interferences

The interferences of foreign ions on the complexation of  $Mg^{2+}$  with the ligand was investigated. A fixed amount ( $1.0 \times 10^{-5} M$ ) of  $Mg^{2+}$  ions was added to the solution of the ligand ( $1.0 \times 10^{-5} M$ ) in the presence of 1 equivalent of other cations. Table 2 showed the change (%I) in the absorbance of the ligand upon the addition of other cations. No remarkable interferences on the complexation of  $Mg^{2+}$  with HNHMB in the presence of  $Ag^+$ ,  $Ca^{2+}$ ,  $Li^+$ ,  $Na^+$  and  $Zn^{2+}$  was reported. Addition of  $Co^{2+}$ ,  $Cu^{2+}$ ,  $Fe^{3+}$ , and  $Ni^{2+}$  show the more interference in the detection of  $Mg^{2+}$  which enhance the absorbance of the complex, whereas, the absorbance of HNHMB- $Mg^{2+}$  complex at 396 nm was found increased significantly with addition of  $Li^+$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Hg^{2+}$  and  $Zn^{2+}$ . Among the metal ion studied,  $Li^+$ ,  $Co^{2+}$  and  $Ni^{2+}$  were found to be the most interfering species.

**Table 2:** Effect of interference on the complexation of  $Mg^{2+}$  with HNHMB

Metal ion	HNHMB		%I
	A	$\Delta A$	
$Mg^{2+}$	0.12839		
$Mg^{2+} + Ag^+$	0.10895	-0.01944	0.15141
$Mg^{2+} + Ca^{2+}$	0.11252	0.00357	0.03277
$Mg^{2+} + Co^{2+}$	0.39573	0.28321	2.51698
$Mg^{2+} + Cu^{2+}$	0.35701	-0.03872	0.09784
$Mg^{2+} + Fe^{3+}$	0.40492	0.04791	0.13420
$Mg^{2+} + Hg^{2+}$	0.05536	-0.34956	0.86328
$Mg + Li^+$	0.113	0.05764	1.04119
$Mg^{2+} + Na^+$	0.11522	0.00222	0.01965
$Mg^{2+} + Ni^{2+}$	0.38675	0.27153	2.35662
$Mg^{2+} + Zn^{2+}$	0.12233	-0.26442	0.68370

Where  $\Delta A = \{(Mg^{2+} + M^{n+}) - Mg^{2+}\}$ , and A= absorbance of  $Mg^{2+}$ . %I=  $(\Delta A/A) \times 100$

## Conclusion

In this work, the chromogenic complexation behaviour of the compound 4-hydroxy-N-[(E)-(2-hydroxyphenyl)methylidene]benzo-hydrazide (HNHMB) was studied spectrophotometrically. Among the metal cations and solvent medium investigated at room temperature, HNHMB is preferentially binds with  $Mg^{2+}$  in ethanol. An obvious colour intensity can be observed by the naked eyes almost immediately. Linear regression analysis shows that Beer's law is obeyed in the range of  $1.0 \times 10^{-5}$  to  $1.4 \times 10^{-4} M$ .

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