

## RESEARCH ARTICLE

## A PRELIMINARY STUDY ON DETECTION OF CADMIUM (II) ION USING BENZOYL PHENYLTHIOUREA

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## ARTICLE DETAILS

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## ABSTRACT

Two benzoyl phenylthiourea derivatives namely: 1-benzoyl-3-(4-bromophenyl)-2-thiourea (**M**) and 1-(4-chlorobenzoyl)-3-(4-methylphenyl)-2-thiourea (**MI**) were successfully synthesized and characterized by using Carbon, Hydrogen and Nitrogen (CHN) micro elemental analysis, Fourier Transform Infra-Red (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy methods. Elemental analysis for **M** (C<sub>14</sub>H<sub>11</sub>N<sub>2</sub>SOBr) and **MI** (C<sub>15</sub>H<sub>13</sub>N<sub>2</sub>SOCl) were compatible with the expected theoretical calculation. The FTIR spectrum obtained for **M** and **MI** compounds show the presence of four important functional groups;  $\nu(\text{N-H})$ ,  $\nu(\text{C=O})$ ,  $\nu(\text{C-N})$  and  $\nu(\text{C=S})$  that appeared at 3380-3367 cm<sup>-1</sup>, 1666-1681 cm<sup>-1</sup>, 1342-1268 cm<sup>-1</sup> and 1146-1152 cm<sup>-1</sup>, respectively. The NMR spectrum data for **MI** shows the chemical shift at 6.63-7.85 ppm for Ar-H group, 2.26-2.40 ppm for methyl group, 7.44 ppm for N<sub>1</sub>H and 3.09 ppm for N<sub>2</sub>H. For the application, **M** and **MI** were used as probes in detecting Cd<sup>2+</sup> in methanol solution. UV-Visible spectrophotometer was used to analyze the absorbance values of the compound before and after Cd<sup>2+</sup> was added. It is found that compound **M** displayed the behavior of 'turn off' sensors while compound **MI** possessed the behavior of 'turn on' when detecting 0.5x10<sup>-5</sup> M and 1x10<sup>-5</sup> M of Cd<sup>2+</sup> ion. The behavior of 'turn on' and 'turn off' is due to the increasing or decreasing of absorbance when Cd<sup>2+</sup> was added to the compounds.

## KEYWORDS

Synthesized, Thiourea, Cd<sup>2+</sup> ion, Detection.

## 1. INTRODUCTION

Recently, environmental pollution by heavy metals become more serious. This due to anthropogenic activities and the waste are released into the environment. The released metals (lead, cadmium, copper, and zinc) are not ecological that can cause environmental pollution, public health, and economic impacts. These released metals have several applications in common consumer products and basic engineering works, paper and pulp industries, leather tanning, plastic stabilizers, and batteries (Halawa and Zabin, 2015).

In recent years, numerous analytical technologies have been developed to detect metal ion in such environmental, medical and cellular analytes. For instance, graphite furnace atomic absorption spectrometry (AAS), ion selective membrane, inductively coupled plasma atomic emission spectrometry (ICP-MS), voltammetry and liquid chromatography-mass spectrometry. However, their operations involve expensive and complex instrumentations (Zhang et al., 2013). Hence, a new method for detection of metal was discovered which is chemosensor. It is rapid method, economical and easy to handle.

Chemosensor is the most convenient way to detect the presence of heavy metal in a water system. This application uses ligands of different moieties,

such as thiocarbamate, naphthalimides, anthraquinone, and benzothiazole as metal sensors or receptors (Misra et al., 2010). A chemical sensor is a piece of equipment that converts chemical data (existence of an element) into an analytically good signal. These chemical data may derive from a chemical reaction of the studied sample or from a physical property of the system inspected. This equipment is used in various fields for example in medicine, environmental deterioration and many others (Wen, 2016).

In fact, chemical sensor commonly contains two primary items joined in array which are first, a chemical admission system and second a physiochemical transducer. The necessary part is the receptor which part that will interact with chemical species in sample. The behavior of the receptor is satisfied in a lot of issues by a thin layer which can reach out with the substance in sample, catalyze a reaction selectivity, or aid in a chemical stability with the component in sample. The receptor layer can react to some appropriate substances. In connection action, the most important for chemical sensors are adsorption, ion exchange etc. Mainly these phenomena act at the interface between chemical species in sample and receptor surface (Stetter et al., 2003).

One of the receptor layer is thiourea. Thiourea is a good ligand for metal particles because it contains nitrogen and sulphur molecules which are the empty donor destination for the transitions metals coordination (Jumal et

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al., 2012). Other than that, thiourea and its derivatives are also skilled ligands, which able to coordinate covalent bonds which dipolar bonds to metal centers either as neutral ligands, a single negative charge (monoanions), or two negative charges (dianions) (Mallick et al., 2010). Thiourea are necessary organic compounds acquire high biological activity, act as antioxidant, and are polymer components. Meanwhile, thiourea and urea derivatives also show the same role as thiourea but much better as antibacterial, anti-HIV, analgesic properties etc (Struga et al., 2007).

## 2. MATERIALS AND METHOD

The melting points were determined on Electro Thermal Digital Melting Point apparatus. The IR spectra were run on Perkin Elmer Spectrum GX with the range 800-3400  $\text{cm}^{-1}$  while NMR spectra were recorded on a NMR Bruker 400MHz instrument and chemical shifts are given in  $\delta$  ppm $^{-1}$  with TMS as internal references. The elemental analysis (C, H and N) of the compounds were performed using Thermo-Finnigan EA1112 Series Flash Elemental Analyzer.

### 2.1 Preparation of 1-benzoyl-3-(4-bromophenyl)-2-thiourea (M)

A mixture of 0.005 mol 4-bromoaniline, in acetone (5 mL) was added to an acetone solution (20 mL) containing of benzoyl chloride (0.01 mol) and ammonium thiocyanate (0.01 mol) was heated under reflux for one and half hour, then cooled and poured into a beaker containing some ice cubes. The white precipitate was filtered off and washed with distilled water, dried and purified by recrystallization from cold ethanol to give M as black crystals, yield 70%, m.p. 110.5 °C. IR.

### 2.2 Preparation of 1-(4-chlorobenzoyl)-3-(4-methylphenyl)-2-thiourea (MI)

A compound of 1-(4-chlorobenzoyl)-3-(4-methylphenyl)-2-thiourea was synthesized by using the same method as M, except benzoyl chloride and 4-bromoaniline were replaced by 4-chlorobenzoylchloride and 4-methylaniline, respectively

### 2.3 Preparation of Cd<sup>2+</sup> Ion and Probe Solution.

The method used for analysis on the application was based on a study with some modifications (Hamedan et al., 2017). The concentration for compounds M and MI in this step were fixed at  $1 \times 10^{-5}$  M while the concentrations for Cd<sup>2+</sup> ion were  $0.5 \times 10^{-5}$  M and  $1 \times 10^{-5}$  M. 0.00508 g of MI and 0.00560 g of M were dissolved with saturated methanol in 25 mL volumetric flask. Then 0.15 mL from each solution was transferred into two 10 mL volumetric flask. Saturated methanol was added into both volumetric flask until calibration mark. The solutions were left aside for 30-60 minutes to allow them to dissolve completely in methanol solution.

Cd<sup>2+</sup> ion (0.05 M) was prepared by transferring 0.9166 g of cadmium (II) chloride (CdCl<sub>2</sub>) to 0.1 L or 100 mL volumetric flask and dissolved in saturated methanol. Then from the stock  $0.5 \times 10^{-5}$  M and  $1 \times 10^{-5}$  M of Cd<sup>2+</sup> ions were prepared by transferring 0.5  $\mu\text{L}$ , 10  $\mu\text{L}$  and 0.1 mL into three separated 10 mL volumetric flask and saturated methanol was added until calibration mark. All solutions were left aside for 30-60 minutes to allow them to dissolve completely in methanol solution (Hamedan et al., 2017).

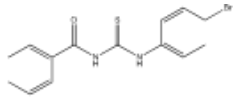
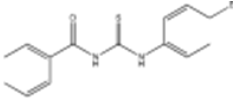
## 3. RESULTS AND DISCUSSIONS

### 3.1 Preparation of 1-benzoyl-3-(4-bromophenyl)-2-thiourea (M) and 1-(4-chlorobenzoyl)-3-(4-bromophenyl)-2-thiourea (MI)

A cloudy solution was produced by the reaction of benzoyl chloride with equimolar amount of ammonium thiocyanate. White precipitated formed was the byproduct (NH<sub>4</sub>Cl) and the clear yellow filtrate was benzoyl isothiocyanate. Compound M was formed when benzoyl isothiocyanate was added with 4-bromoaniline in acetone. Then, solid black crystal was formed with 70% yield. The same method was used to synthesize compound MI. However, benzoyl chloride and 4-bromoaniline were replaced by 4-chlorobenzoyl chloride and 4-methyl aniline respectively. Compound MI

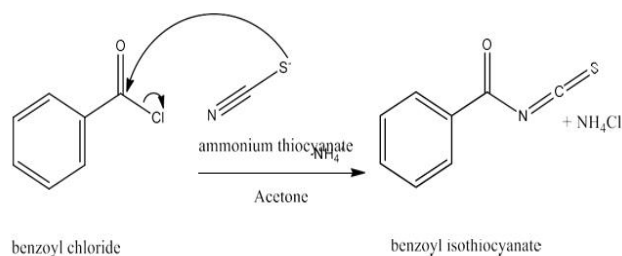
formed brown solid with 75% yield. The melting point for each compound M and MI were determined and it was found that M and MI melted at 110.5°C and 126.7°C, respectively. Table 1 shows the physical data of compounds M and MI.

**Table 1:** The physical data of compounds

Compounds	Color	Melting Point (°C)	Percentage Yield (%)
C <sub>14</sub> H <sub>11</sub> N <sub>2</sub> SOBr (M) 	Black	110.5	70
C <sub>15</sub> H <sub>13</sub> N <sub>2</sub> SOCl (MI) 	Brown	126.7	75

### 3.2 Mechanism Reaction of Compound M and MI

Formation of the products involved two major steps in established mechanism. Resulting from the reaction between benzoyl chloride and ammonium thiocyanate, the formation of benzoyl isothiocyanate is obtained. White precipitate of ammonium chloride (NH<sub>4</sub>Cl) is formed as byproduct in this reaction. Scheme 1 shows the reaction mechanism involved. Strong nucleophile of SCN<sup>-</sup> is having more favor to attack C=O, where consequently breaks the bond of C-Cl and ends up with the formation of benzoyl isothiocyanate and NH<sub>4</sub>Cl.



**Scheme 1:** The reaction mechanism of benzoyl isothiocyanate formation

The formation of benzoyl isothiocyanate is the second step and the product formed is shown in Scheme 2 below. It shows the reaction with 4-bromoaniline where the nitrogen amine as the nucleophile, attacks the carbon atom that bond with N and S of benzoyl isothiocyanate. Intermediate species is formed, and the rearrangement occurs to form the final product of benzoyl thiourea derivatives. The same mechanism is applied to the formation of 1-(4-chlorobenzoyl)-3-(4-methylphenyl)-2-thiourea.

### 3.3 Characterization Compounds M and MI

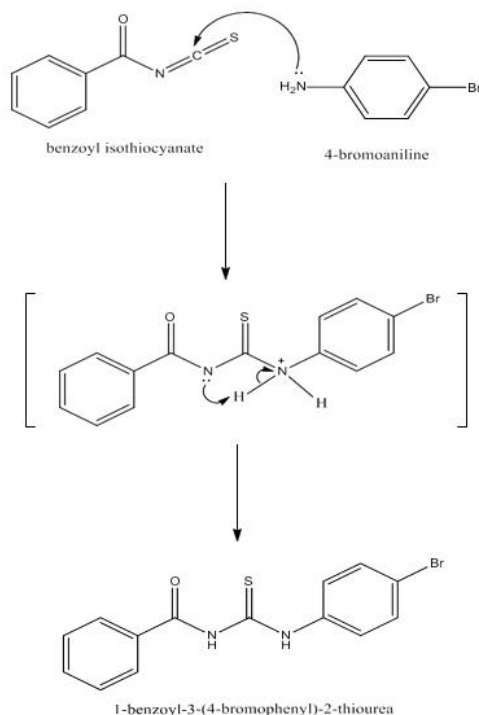
#### 3.3.1 CHN Elemental Analysis

The synthesized M and MI compounds were characterized by CHN micro-elemental analysis (Table 2). The results from the table shows that the percentage of the carbon, hydrogen and nitrogen of the synthesized compounds are not much different compared to the theoretical values. This shows that the synthesized compounds are in agreement with the expected result.

**Table 2: Elemental composition of M and MI**

Compounds	Elemental Composition (%)		
	C	H	N
<b>M</b>	48.90	2.01	7.42
	(50.16)	(3.28)	(8.36)
<b>MI</b>	57.98	3.01	8.09
	(59.12)	(4.27)	(9.20)

\* The calculated values are in bracket



**Scheme 2:** The reaction mechanism of 1-benzoyl-3-(4-bromophenyl)-2-thiourea

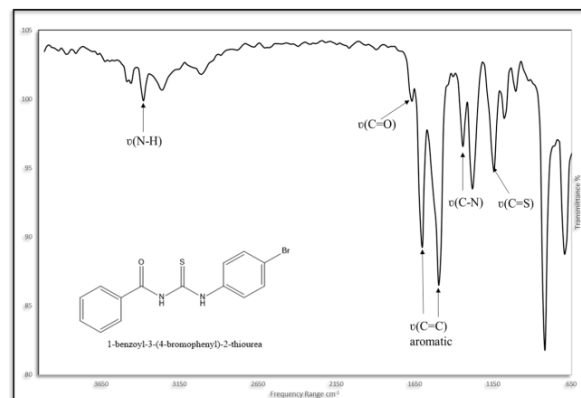
### 3.3.2 FTIR Spectroscopy

In this research work, the characteristic of thiourea derivatives were characterized using FTIR. The IR absorption band data and spectra of the synthesized compounds **M** and **MI** are shown in Table 3, Figure 3 and Figure 4. The IR spectra show four important stretching bands for  $\nu(\text{N-H})$ ,  $\nu(\text{C=O})$ ,  $\nu(\text{C-N})$  and  $\nu(\text{C=S})$  in both compounds. A medium intensity bands present at  $3380\text{ cm}^{-1}$  for compound **M** and  $3367\text{ cm}^{-1}$  for compound **MI** which corresponds to stretching  $\nu(\text{N-H})$  are in agreement with the work done (Devaraj et al., 2009). The N-H bands are often weaker and sharper than an O-H band. Amines may sometimes be differentiated from alcohols on that basis. Secondary amines determine give one spike in the N-H stretching region. Since both spectra show one spike appears at N-H stretching region, it indicates the presence of secondary amine for both compounds. The absorption bands around  $1666\text{ cm}^{-1}$  for compound **M** and  $1681\text{ cm}^{-1}$  for compound **MI** attributes to the stretching of  $\nu(\text{C=O})$ . Based on previous study (Zakaria et al., 2011), the typical frequency carbonyl absorption is approximately at  $1700\text{ cm}^{-1}$ . The decreasing in frequencies is caused by a conjugated resonance of aromatic ring and the formation of intramolecular hydrogen bonding with N-H (Mushtari and Yusof, 2009). Meanwhile, the medium stretching vibration of  $\nu(\text{C-N})$  bands are at  $1342\text{ cm}^{-1}$  (Zakaria et al., 2011) for compound **M** and  $1268\text{ cm}^{-1}$  (Abosaadiya et al., 2016) for compound **MI**. The medium absorption of compound **MI** is observed at the  $1357\text{ cm}^{-1}$  which can be assigned by C-H bending vibrations for methyl group (Bielienica et al., 2015). However, compound **M** does not show any absorption band at  $1357\text{ cm}^{-1}$  and it can be concluded that compound **M** does not have C-H bending vibrations in its compound. Based on previous research (Alfallous and Aburzeza, 2013) compound **M** and **MI** exhibit vibration of  $\nu(\text{C=S})$  at  $1146\text{ cm}^{-1}$  and  $1152\text{ cm}^{-1}$ . Also, the ring

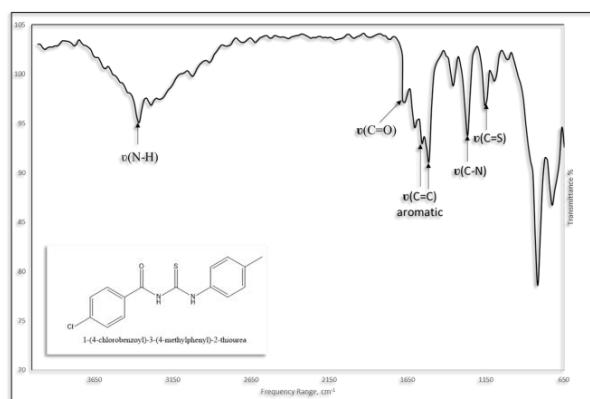
stretching vibrations tend to absorb near  $1450\text{--}1600\text{ cm}^{-1}$  in mono- and di-substituted benzene. There is  $\nu(\text{C=C})$  absorption band at  $1494\text{ cm}^{-1}$  and  $1495\text{ cm}^{-1}$  for compound **M**, and for compound **MI** at  $1527\text{ cm}^{-1}$  and  $1558\text{ cm}^{-1}$ .

**Table 3: IR absorption band data for compound M and MI**

Thiourea derivatives	Frequency range, $\text{cm}^{-1}$				
	$\nu(\text{N-H})$	$\nu(\text{C=O})$	$\nu(\text{C=S})$	$\nu(\text{C-N})$	$\nu(\text{C=C})$
<b>I</b>	3380	1666	1146	1342	1495, 1590
<b>II</b>	3367	1681	1152	1268	1527, 1558



**Figure 3:** IR spectrum of **M**



**Figure 4:** IR spectrum of **MI**

### 3.3.3 NMR Chemical Shift Spectroscopy

Compound **MI** was dissolved in deuterated chloroform ( $\text{CDCl}_3$ ). The  $^1\text{H-NMR}$  spectrum data of compound **MI** is shown in Table 4. The  $^1\text{H-NMR}$  spectrum of compound **MI** (Figure 5) shows the methyl resonance between  $2.26\text{--}2.40\text{ ppm}$ . In addition, there are multiplet signal at  $6.63\text{--}7.85\text{ ppm}$  which corresponds to the aromatic protons on phenyl ring in compound **MI**. These characteristic resonances are strongly influenced by the para- substituents positions at the phenyl rings. Also, the resonance of aromatics rings is observed as multiplet resonance due to *p*- substituent methyl groups and chlorine atom at the phenyl ring and overlapping of proton signals in the aromatic rings which is in agreement with the work reported (Rabiatun et al., 2011). The electronegative chlorine atom pulls the electron density toward itself, so there is less electron density around the protons, causing less shielding or downfield and higher chemical shift. The phenyl is more affected by the electronegative Cl because it is closer to it when compared to the methyl groups which is further away from Cl. Thus, proton of methyl moves upfield or shield and lower chemical shift. This phenomenon also might be related to the absence of secondary amide proton. Surprisingly, compound **MI** did not show the presence of the  $\text{N}_1\text{H}$  and  $\text{N}_2\text{H}$ . The predicted peak should appear at  $8.00\text{ ppm}$  ( $\text{N}_1\text{H}$ ) and  $4.00\text{ ppm}$  ( $\text{N}_2\text{H}$ ). However, there is a broad peak near at  $3.09\text{ ppm}$  indicates  $\text{N}_2\text{H}$  and a

small peak at 7.44 ppm indicates N<sub>1</sub>H. This is because N protons typically have wide ranges of expected chemical shifts; the actual  $\delta$  value depends on the solvent used, the concentration, and temperature. Also, this proton is acidic and, therefore, exchangeable, it appears broad peaks and usually do not couple with neighboring protons (typically they are broad singlets) (Jackman and Sternhell, 2013).

Table 4: <sup>1</sup> H-NMR data of compound MI	
	<sup>1</sup> H-NMR ( $\delta$ , ppm)
N <sub>1</sub> H	7.44
N <sub>2</sub> H	3.09
Methyl	2.26-2.40
Aromatic	6.63-7.85

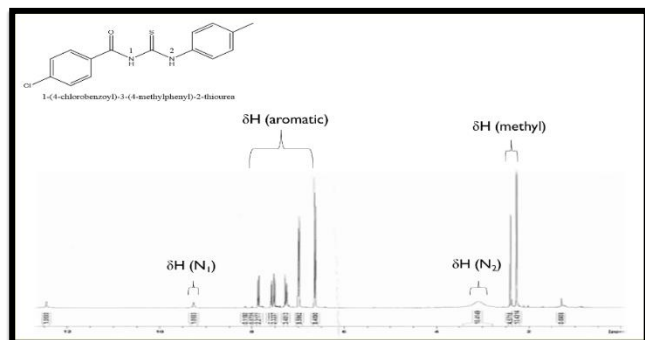


Figure 5: <sup>1</sup>H NMR spectrum of MI

### 3.4 Analysis on The Application -Detection of Cd<sup>2+</sup>

#### 3.4.1 UV-Visible Spectroscopy

An experiment was carried out to study compound **M** and **MI** absorption. The experimental maximum absorption bands of compound **M** and **MI** are shown in Figure 6 and Table 5. The experimental spectra were recorded in methanol solvent ( $5 \times 10^{-5}$  M), where the maximum absorption bands at 248 nm (**M**) and 237 nm (**MI**), correspond to  $\pi \rightarrow \pi^*$  transition of the aromatic system while the minimum absorption band of 312 nm and 307 nm. correspond to  $n \rightarrow \pi^*$  transition of thiols. According to Chetana and co-workers (2016), the electronic absorption spectra of compounds N, N'-disubstituted thiourea show two intense broad bands with maxima in the range of  $\sim 270$  and  $\sim 325$  nm; the high energy band can be attributed to ligand  $\pi \rightarrow \pi^*$  transitions on the benzene aromatic rings, whereas the lower energy band can be considered as a thione (C=S) bond.

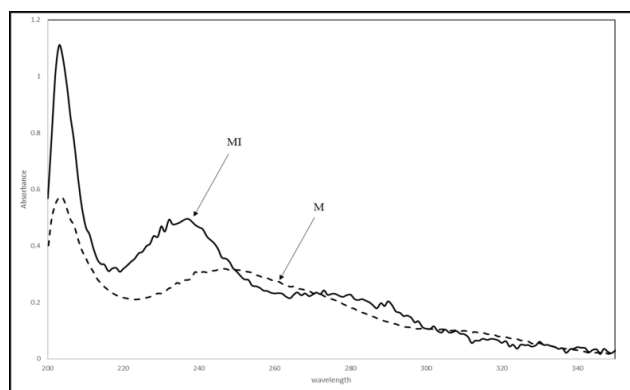


Figure 6: The UV spectra of the M and MI, C =  $5 \times 10^{-5}$  M in methanol

Table 5: Experimental  $\lambda_{\max}$  (nm) of  $\pi \rightarrow \pi^*$  transition of compound M and MI

Samples	Wavelength, nm
<b>M</b>	248
<b>MI</b>	237

The cation binding abilities of the compound **M** and **MI** were investigated by UV-vis spectroscopic. The abilities of compound **M** and **MI** as detection of Cd<sup>2+</sup> ion was primarily investigated by adding two different concentration of Cd<sup>2+</sup> ion ( $0.5 \times 10^{-5}$  M, and  $1 \times 10^{-5}$  M) into solutions of **M** and **MI**. Figure 7 and Figure 8 show the difference between compound **M** and compound **MI** before and after addition of  $0.5 \times 10^{-5}$  M of Cd<sup>2+</sup> ion. For compound **M**, before the addition of Cd<sup>2+</sup> ion the absorbance value is 0.314 Å at wavelength 248 nm. But after addition of Cd<sup>2+</sup> ion, the absorption peak was shifted to a new peak at 245 nm with decreasing in absorbance value to 0.231 Å. For compound **MI** the absorption peak is shifted from 232 nm to 230 nm with increasing absorbance value from 0.492 Å to 0.700 Å after addition of Cd<sup>2+</sup> ion for compound **MI**. This suggests that when Cd<sup>2+</sup> ion was added to **M**, the compound displays a 'turn off' sensor behavior due to the decrease in absorbance value of the compound. Meanwhile for compound **MI**, it displays 'turn on' behavior since the absorbance value of **MI** compound is increased after the addition of Cd<sup>2+</sup> ion.

Next, the experiment was repeated by using  $1 \times 10^{-5}$  M of Cd<sup>2+</sup> ion. Figure 9 shows the spectrum for the compound **M** before and after addition of Cd<sup>2+</sup> ion. Before Cd<sup>2+</sup> ion was added, the absorbances value 0.314 Å was observed at wavelength 248 nm. After Cd<sup>2+</sup> ion was added, the absorbance value was increased to 0.228 Å at a wavelength 242 nm. At the same time, for compound **MI** (Figure 10) the absorbance value 0.493 Å was observed before addition of Cd<sup>2+</sup> ion at wavelength 232 nm. However, right after the Cd<sup>2+</sup> ion was added, the absorption peak was shifted to 230 nm and absorbance value 0.618 Å was observed. This indicates that compound **M** also exhibits the behavior of a 'turn off' sensor after  $1 \times 10^{-5}$  M of Cd<sup>2+</sup> ion was added, while compound **MI** still exhibits the behavior 'turn on' sensor when  $1 \times 10^{-5}$  M of Cd<sup>2+</sup> ion was added.

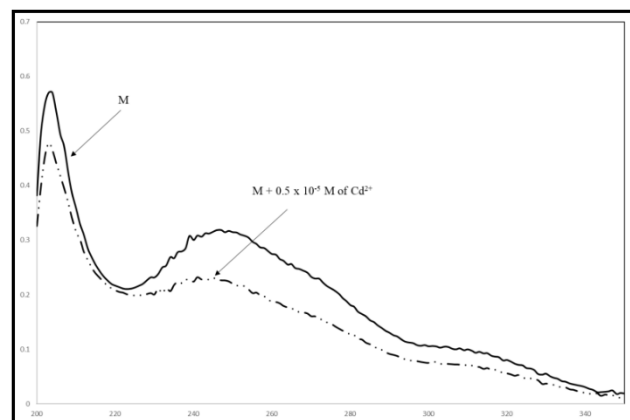


Figure 7: The UV spectra of compound M before and after addition  $0.5 \times 10^{-5}$  M of Cd<sup>2+</sup> ion

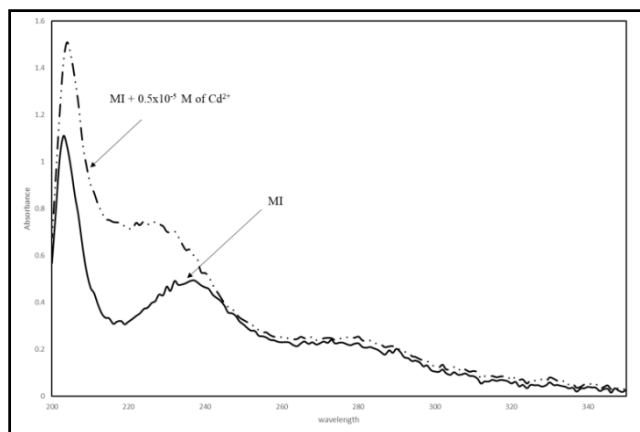
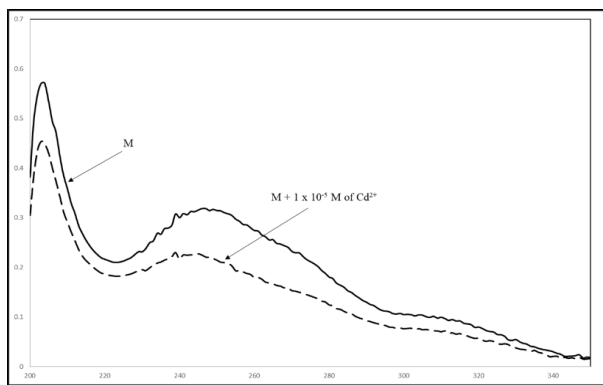
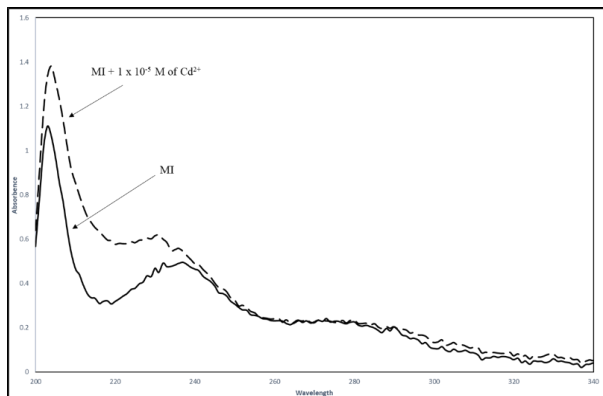


Figure 8: The UV spectra of compound MI before and after addition  $0.5 \times 10^{-5}$  M of Cd<sup>2+</sup> ion.

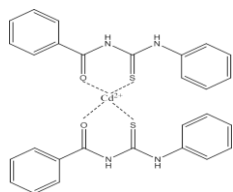


**Figure 9:** The UV spectra of compound **M** before and after addition  $1 \times 10^{-5}$  M of  $\text{Cd}^{2+}$  ion.

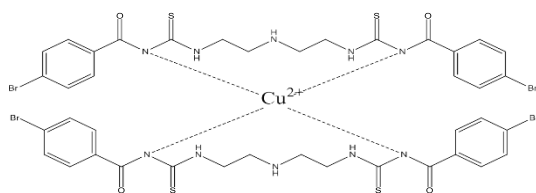


**Figure 10:** The UV spectra of compound **MI** before and after addition  $1 \times 10^{-5}$  M of  $\text{Cd}^{2+}$  ion.

The behavior of 'turn on' sensor can be explained when additional substance is added to the compound and the absorbance value is increase, while for 'turn off' sensor behavior, the absorbance is decrease. These two behaviors occur due to the presence of new bond formation between the compounds **M** and **MI** with  $\text{Cd}^{2+}$  ion. A group researcher revealed that the formation of new bond might be the binding of thiono group (C=S) and benzoyl group (C=O) from thiourea derivatives with metal (Scheme 3) (Alkherraz et al., 2014). In addition, Hamedan and his co-workers (2017) proposed the new bond formation is between the amide (N-H) group from thiourea derivatives with the metal (Scheme 4). Also, the presence of electron withdrawing group can affect the formation of new bond between the thiourea derivatives with the metal.



**Scheme 3:** The reaction mechanism of 1-phenyl-3-benzoyl-2-thiourea with  $\text{Cd}^{2+}$  ion



**Scheme 4:** The reaction mechanism of 1-(4-bromobenzoyl)-3-(2-{2-[3-(4-bromobenzoyl)-thioureido]-ethylamino)-ethyl}-thiourea with  $\text{Cu}^{2+}$  ion.

As for the appearance of the compounds when  $\text{Cd}^{2+}$  was added, there was no changes occurred to the color of solution or in other words there is no physical changes that can be observed by naked eyes. Table 6 summarizes the UV-Vis spectrum obtained for compound **M** and **MI**.

Table 6: UV-Vis spectrum result for application analysis		
Samples	Absorbance, Å	Wavelength, nm
<b>M</b>	0.314	248
<b>M</b> + $0.5 \times 10^{-5}$ M of $\text{Cd}^{2+}$	0.231	245
<b>M</b> + $1 \times 10^{-5}$ M of $\text{Cd}^{2+}$	0.228	242
<b>MI</b>	0.493	232
<b>MI</b> + $0.5 \times 10^{-5}$ M of $\text{Cd}^{2+}$	0.700	230
<b>MI</b> + $1 \times 10^{-5}$ M of $\text{Cd}^{2+}$	0.618	230

#### 4. CONCLUSION

As conclusion, 1-benzoyl-3-(4-bromophenyl)-2-thiourea (**M**) and 1-(4-chlorobenzoyl)-3-(4-methylphenyl)-2-thiourea (**MI**) were successfully synthesized based on FTIR, NMR and CHN results. The FTIR spectra show the presence of important functional groups with stretching bands at the certain wavenumber, and the structure of compound can be predicted with the aid from  $^1\text{H}$  NMR spectra. As for application part, **M** and **MI** were used as probes in detecting  $\text{Cd}^{2+}$  in methanol solution. UV-Visible spectrophotometer was used to analyze the absorbance values of the compound before and after  $\text{Cd}^{2+}$  was added. It is found that compound **M** displayed the behavior of 'turn off' sensors and compound **MI** displayed the behavior of 'turn on' when detecting  $0.5 \times 10^{-5}$  M and  $1 \times 10^{-5}$  M of  $\text{Cd}^{2+}$  ion. The behavior of 'turn on' is due to the increasing in absorbance value after a metal ion or substance is added, while 'turn off' is due to the decreasing of absorbance.

#### AUTHOR'S CONTRIBUTION

Ain carried out the research, wrote, revised the article, conceptualised the central research idea and provided the theoretical framework. Juliana anchored the review, revisions and approved the article submission.

#### CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

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