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# Synthesis, Characterization, and Preliminary Study of [Co(2- aminopyridine)$_2$(NCS)$_2$] or bis(2-aminopyridine)dithiocyanato cobalt(II) as An Antibacterial

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**Abstract**

This study aims to synthesize complex compounds from the Co(II) ion with mixed ligands of 2-aminopyridine and thiocyanate ions. The complexes obtained have Co(II) : 2-aminopyridine: thiocyanate ratio of 1:2:2 based on preliminary analysis by SEM-EDX, DHL, and FTIR. The complex in the form of a blue needle crystal is stable at room temperature and melts at 169 °C. Characterization shows that the complex formed is neutral. The antibacterial activity test was carried out using the diffusion method and show that the resulting complex compounds could inhibit the growth of *S. typhi* and *S. aureus* bacteria better than 2-aminopyridine.

**Keywords:** Antibacterial, Blue needle crystal, Co(II) complexes.

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## 1. INTRODUCTION

The synthesis of complex compounds with SCN- ion, and aminopyridine as ligands has become an important field in inorganic chemistry (Yufanyi et al., 2021). The 2-aminopyridine and $SCN^-$ ion as ligands can bind to most transition metals (Akyuz, 1999; Akyuz, 1998; Swiatkowski et al., 2019). Generally, the complexes with $SCN^-$ ion or aminopyridine as ligands have antibacterial properties (Brađan et al., 2016; Chimaine et al., 2016; Colette et al., 2015; Mohammed, 2020). Its antibacterial and antiviral properties are fascinating to study and develop in the current pandemic conditions. Based on this potential, the compound can be used as an ingredient to make hand sanitizer, antibacterial paint, antibacterial cloth, and other materials to suppress an infection from bacteria or viruses and improve health.

Some of the Co(II) complexes with aminopyridine and $SCN^-$ ion as ligands that have been synthesized were [Co(3-aminopyridine)$_2$(NCS)$_2$] (Mautner et al., 2020), [Co(NCS)$_2$(THF). )] (Shurdha et al., 2013), [Co(acetate)$_2$(2-aminopyridine)$_2$] (Dojer et al., 2010), [Co(2,5-Bis(pyridine-2-yl)-1,3,4-oxadiazole)$_2$(SCN)$_2$]H$_2$O (Rhoufal et al., 2019), and [Co(Py)$_4$(NCS)$_2$] (Baer & Pike, 2010; Schutte, 1963). Generally, Co(II) complexes with aminopyridine and $SCN^-$ ligand have octahedral geometry. However, several Co(II) complexes can form tetrahedral geometry, such as [Co(3-aminopyridine)$_2$(NCS)$_2$]. The structure of [Co(3-aminopyridine)$_2$(NCS)$_2$] has been studied, and it is known that the coordination bonds are formed between the central Co(II) ion and the N donor atom from the thiocyanate ion and the pyridine ring of 3-aminopyridine


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ligand, respectively. The ortho position of $NH_2$ in the 2-aminopyridine ligand allows its role as a bidentate ligand (Colette et al., 2015; Liu et al., 2015) and forms stronger bonds with the central ion compared to the 3-aminopyridine ligands (Wang & Yang, 2009). However, it is known that 2-aminopyridine in [Co(acetate)$_2$(2-aminopyridine)$_2$] and [Co(2-aminopyridine)$_2$(dicyanamide)$_2$] acts as a monodentate ligand that binds to Co(II) by the N-pyridine donor. Meanwhile, the thiocyanate ion is an ambidentate ligand with the N or S donor atom. Based on the "hard and soft acid-base" (HSAB) theory, the thiocyanate ion coordinates to the Co(II) through the N donor atom (Małecki et al., 2011).

In addition, it is known that 2-aminopyridine ligand and $SCN^-$ ion have antibacterial activity. The 2-aminopyridine ligands are widely known to produce drugs, especially antihistamines, anti-inflammatory, and piroxicam (Colette et al., 2015; Trivedi et al., 2017; Zhang et al., 2017). Previous studies showed that complex compounds with aminopyridine ligands or $SCN^-$ ions, such as [Co(diacetylpyridine bis(trimethylammonium acetohydrazone))$(NCS)_2$]$(SCN)_2$ (Brađan et al., 2016), $[Cu(py)_2(SCN)_2]_n$ (Chimaine et al., 2016), [Co(phen)$(caf)_2(SCN)_2$] (Hamdani et al., 2016), and [Co(2-aminopyridine)$_2$(dca)$_2$] (Colette et al., 2015), have antibacterial, antiviral, and antifungal activities. The presence of N atoms also increases lipophilicity, making the complex quickly enters the bacterial cells (Fathima et al., 2020). Furthermore, the azomethine group in the complex can interact with the active sites in cells and interfere with the respiration process by curtailing protein synthesis. Thus, it can inhibit microbial growth (El-sherif & Eldebss, 2011; Fathima et al., 2020; Ommenya et al., 2020). Because of those essential applications of the complexes with x-aminopyridine and SCN- ligands, we synthesized a complex compound with those ligands and Co(II) as a central ion. Here we report the synthesis results and its antibacterial test against S. aureus and *S. typhi*.

## 2. MATERIALS AND METHODS

### Materials

The materials used for the synthesis were distilled water, $H_3COH$ (Merck, p.a.), KSCN (Merck, p.a.), 2-$NH_2C_5H_4N$ (Merck, p.a.), and $CoCl_2 \cdot 6H_2O$ (Merck, p.a.). All chemicals were used directly without purification.

### Procedures

A total of 1 mmol of $CoCl_2 \cdot 6H_2O$ was dissolved in 5 mL of methanol, stirred at 700 rpm, and heated to boiling (57-59 °C). Methanolic solution of 2 mmol of 2-aminopyridine (5 mL) was added slowly into the previous solution and refluxed for 2 hours. Then, 5 mL of methanolic solution of 2 mmol of KSCN was slowly added into the previous mixture and refluxed for 3 hours. The molar ratio of Co(II), 2-aminopyridine, and KSCN was 1:2:2. Next, the dark blue solution was filtered and the filtrate was evaporated at room temperature. After 13 days, needle-shaped blue crystals were obtained.

The determination of the optimal composition of the ligand with the specified metal employed the continuous variation method. This method was done by making a series of fixed volume solutions with a variation of the composition of KSCN, $[CoL_2]^{2+}$ (L=2-aminopyridine), and $SCN^-$. This solution was tested utilizing Spektronic$^{@}$Genesys$^{TM}$LR45227.

### Characterization

The characterization of complex compounds was performed using the IR Prestige-21 Shimadzu FT-IR Spectrophotometer to know the functional groups of the ligands. Inspect-S50 FEI SEM-EDX was used to see the composition and crystal surface morphology. CyberScan CON 11/110 Conductivity/TDS/RS232 Meter was employed to measure the electrical conductivity of the synthesized material while Fisher-Johns melting point apparatus was utilized to determine the melting point. Moreover, we used the Kirby-Bauer method to test its antimicrobial activity.

## 3. RESULTS AND DISCUSSION

The synthesized crystals have a needle-like shape and are blue, as shown in Figure 1. The crystal melts at 169 ℃ different from the reactants' melting points, as displayed in Table 1. It indicates that the crystals obtained are different compounds from the reactants. We also observed color changes during the synthesis process. The first color change occurred when purple Co(II) solution was added to the colorless 2-aminopyridine

solution producing a milky blue solution. Subsequently, the milky blue solution turned to a dark blue solution when the colorless KSCN solution was added. The color changes indicate chemical reactions in the solution to give different products from the reactants.

**Figure 1.** The synthesized complex by reflux technique

**Table 1.** The melting points of [Co(2-aminopyridine)$_2$(NCS)$_2$] and its precursors

| Compound | Melting Point (℃) |
|---|---|
| $CoCl_2 \cdot 6H_2O$ | 56 |
| 2-$NH_2C_5H_4N$ | 55-58 |
| KSCN | 173 |
| [Co(2-aminopyridine)$_2$(NCS)$_2$] | 169 |

The morphology of the formed crystal from the SEM analysis can be seen in Figure 2. The crystal was formed in a rod-like shape. Furthermore, from Table 1, we can conclude that the average atomic ratio of Co: S: N atoms is 1:1.8:3.4. The ratio of S to Co atoms indicates that 2 SCN- molecules bonded to 1 Co atom in the tested crystal. This result follows the molar ratio of the reactants in the synthesis.

Meanwhile, the discrepancy between the N to Co atoms ratio with the reactants' molar ratio in the synthesis may be caused by the lack of sensitivity of the EDX instrument in detecting samples. As a result, not all atoms were taken into account in calculating the atomic ratio. It leads to a difference in the possible molecular formula of the compound based on the EDX and reactants' molar ratio in the experiment. The former gives [Co(SCN)$_2$(2-aminopyridine)] as the possible complex compound, whereas the latter suggests [Co(SCN)$_2$(2-aminopyridine)$_2$] as the possible complex compound.

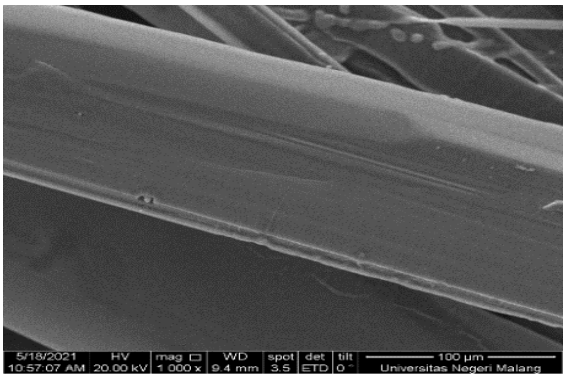


**Figure 2.** The micrograph of the synthesized material

**Table 2.** The EDX data of the synthesized material

| Elements | %Wt | %At |
|---|---|---|
| N | 17.73 | 23.07 |
| S | 21.65 | 12.31 |
| Co | 22.64 | 7.00 |

To determine which structure is more possible for the synthesized material, we determined the ratio of $[CoL_2]^{2+}$:SCN ratio by employing the continuous variation method using UV-Visible spectroscopy. According to the Lambert-Beer law, the visible absorbance of a solution is proportional to its concentration (Nurjayadi, Romundza, & Moersilah, 2021). The absorbance measurement in the sample solution was carried out at the maximum wavelength of the complex solution, $\lambda$max = 519 nm. According to Figure 3, the maximum absorption was obtained when the molar fraction of KSCN equals to 0.7 (3 mL of 0,1M $[CoL_2]^{2+}$ solution and 7 mL of 0,1M SCN- solution, L = 2-aminopyridine). This means that the molar ratio of $[CoL_2]^{2+}$:$SCN^-$ is 3:7 1:2. This result agrees with the Co: S ratio from the EDX analysis in Table 1, suggesting that Co:S is 1:1.78 1:2.

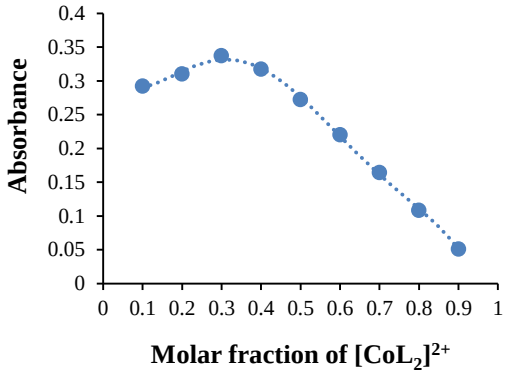


**Figure 3.** The visible absorbance of $[CoL_2]^{2+}$:$SCN^-$

The FTIR analysis was intended to determine the ligand's donor atom and functional group. The data from the analysis are in the form of a spectrum, which provides wave number information specific for certain functional groups. Separated spectrums of the ligand precursor and the complex are presented in Figure 4. The absorption bands at 1444, 1492, 1566, and 1631 $cm^{-1}$ in the complex evince the strain vibrations of aromatic C=C, C=N, and CN, which are typical bonds in the pyridine ring (Kartal & Sahin, 2021; Rhoufal et al., 2019; Schutte, 1963). Absorption band shift from 3452 $cm^{-1}$ in the 2-aminopyridine spectrum to 3429, 3469, and 3327 $cm^{-1}$ in the complex spectrum proves asymmetric and symmetrical vibrations of the free $–NH_2$ group attached to the pyridine ring (Colette et al., 2015; Dojer et al., 2010). It suggests that, in the complex, the Co(II) ion binds to the 2-aminopyridine ligand through the N atom in the pyridine ring. The CN vibration of thiocyanate is shown as the absorption band of 2077 $cm^{-1}$ and shifts to 2061 and 2073 $cm^{-1}$ in the complex. It indicates that the thiocyanate ion attaches to Co(II) through the N atom and acts as a terminal ligand (Neumann et al., 2018). The presence of thiocyanate ions in the complex is supported by a shift in the absorption band from 750 to 769 $cm^{-1}$, indicating C-S vibrations.

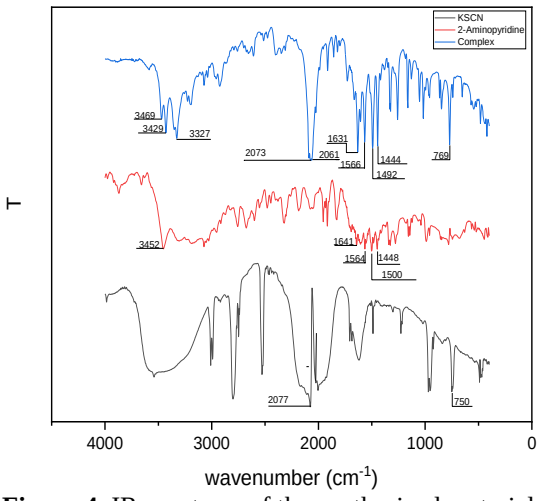


**Figure 4.** IR spectrum of the synthesized material

The electrical conductivity was tested to determine the complex compound formed, whether ionic or molecular. The DHL test in Table 2 shows that the resulting complex compound has an electrical conductivity of 21.3 S, which was closer to that of the solvent (5.29 S) than that of the central ion (86.2 S). It means that the resulting complex compound is molecular. We can predict that the synthesized complex compound has a tetrahedral geometry structure based on the SEM-EDX data (Tabel 1) and DHL data (Tabel 2). The prediction of the tetrahedral geometry of this structure is supported by the FTIR data, which shows that the SCN- ligand acts as a terminal ligand. It is comparable to the previous research, which proved that 2-aminopyridine acts as a monodentate ligand in complex compounds with Co(II) and Zn(II) ions (Dojer et al., 2010; Hafeez & Riaz, 2016; Swiatkowski et al., 2019; Yufanyi et al., 2021).

**Table 3.** The DHL test data of the synthesized complex compound, solvent, and the central ion salt

| No | Compound | DHL ($\mu S$) |
|---|---|---|
| 1 | $CoCl_2 \cdot 6H_2O$ | 86.2 |
| 2 | Methanol | 5.29 |
| 3 | $[Co(2\text{- aminopyridine})_2(NCS)_2]$ | 21.3 |

The antibacterial test was carried out with Millner-Tunner media using the Kirby-Bauer method (Bauer *et al*., 1966; Colette *et al*., 2015). The bacteria-contained media was given sample solution in 10% of DMSO and isolated for 24 hours. Chloramphenicol was used as a positive control for the test. The transparent zone in the media indicates the presence of antibacterial activity in the sample whilst its diameter measures the effectiveness of the complex compound as an antibacterial. The data is presented in Table 3. The as-synthesized complex had antibacterial activity in both gram-positive (*S. aureus*) and gram-negative (*S. typhi*). Based on the diameter of the inhibition zone, the complex had comparable potency to inhibit the growth of both bacteria.

Table 4 exhibits that the antibacterial activity of the complex against S. aureus is better than that of its precursors. Tweedy's theory can describe this phenomenon. The theory explains that complex formation increases the electron delocalization from the ligand to the central ion. Increasing the hydrophobic and lipophilic character of the complex (Malik et al., 2017; Chimaine et al., 2016). These characters arise because of the critical role of the azomethine group (-C=N-) in the ligand (Chohan et al., 2012).

**Table 4.** The Antibacterial Test Results

| No. | Sample | Inhibition Zone Diameter (mm) | |
|---|---|---|---|
| | | *S. aureus* (positif) | *S. typhi* (negative) |
| 1 | KSCN | 10.00 | 8.95 |
| 2 | 2-aminopyridine | 9.35 | 11.40 |
| 3 | $CoCl_2$ | 10.50 | 9.85 |
| 3 | [Co(2-aminopyridine)$_2$(SCN)$_2$] | 11.20 | 11.35 |
| 4 | Chloramphenicol | 31.50 | 34.60 |

Molecular lipophilicity is a crucial factor in determining antibacterial activity. The higher the lipophilic character of the complex compound, the easier it will be for the complex to pass through the lipid membrane of the cell wall. Thus, the growth of the bacteria is inhibited (Claudel et al., 2020; Malik et al., 2017; Chimaine et al., 2016). Meanwhile, the reverse phenomenon occurred in the bacterial strain of *S. typhi*. The free 2-aminopyridine ligand had higher antibacterial activity than that of the complex. This result is the same as Kadhum & Abduljleed (2014) (Kadhum & Abduljleel, 2014). They described it occurred due to the formation of coordination bonds in the central ion with the azomethine group (Kadhum & Abduljleel, 2014).

## 4. CONCLUSIONS

The complex compound of Co(II) with 2-aminopyridine and SCN- ligands have been successfully synthesized using the reflux techniques. This preliminary study of the complex structure indicated that the N-pyridine and the SCN- ion coordinated with the central cobalt(II) ion. The as-synthesized complex is an antibacterial agent against S. aureus and *S. typhi*.

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