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SYNTHESIS OF ANION-DEPENDED ZINC(II)-NIACINAMIDE COMPLEXES AND THEIR ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Zinc(II)-niacinamide complexes from various Zn(II) salts were prepared and their antibacterial activities toward the *E. coli* and *S. aureus* are reported here. The effect of the M: L molar ratio (1:2, 1:4, 1:6) on the yield was studied and a possible molecular formula of the products was also proposed. Sulfate, nitrate, chloride, and acetate were used as the Zn(II) salts. The solid products were characterized by SEM, FTIR, powder XRD, melting point test, and qualitative anion identification test. Experimental data shows that solid products were only yielded from the chloride and sulfate salts. The larger the molar ratio used, the higher the crystal yield obtained. The colorless crystals obtained from the sulfate and the chloride reactions are predicted to be complexes of $[Zn(L)_x(SO_4)]$ and $[Zn(L)_x]Cl_2$, respectively. Antibacterial test of both metal complexes toward *E. coli* and *S. aureus* shows that the complexes have better activity than that of the niacinamide.

Keywords: Anion, Antibacterial, Molar Ratio, Niacinamide, Zinc(II).

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INTRODUCTION

Many studies reported that transition metal complexes have been used as drugs to treat several human diseases caused by bacterial infections. The diversity of metals and ligands makes metal-based complexes very useful for drug development, especially for a new generation of antibacterial.[1,2] Bioactivity of a ligand may be altered due to the presence of transition metal ions, in which in some cases, the activity is mainly associated with certain metal ions.[3] Furthermore, according to Mittapaly (2018), antimicrobial effectiveness decreased in the order to the charge of the complex, i.e. cationic > neutral > anionic.[4] A promising antibacterial activity was shown by some of the nicotinamide metal complexes.[5] The niacinamide provides three possible donor sites as a ligand, namely pyridyl nitrogen, carbonyl oxygen, and amine nitrogen atoms.[6] The objective of this work is to prepare Zinc(II)-niacinamide complexes from various Zn(II) salts and to investigate their antibacterial activities toward *E. coli* and *S. aureus*. The antibacterial activities of Zn(II) complexes obtained in this work are also compared to that of the free niacinamide.

EXPERIMENTAL

Material and Methods

All chemicals were used as supplied, namely $Zn(CH_3COO)_2{\cdot}2H_2O$, $ZnCl_2{\cdot}2H_2O$, $ZnSO_4{\cdot}7H_2O$, $Zn(NO_3)_2{\cdot}4H_2O$, niacinamide, methanol, $AgNO_3$ solution, and distilled water. For characterization, Infrared Spectrophotometer (IRSpirit –T Shimadzu), digital melting point apparatus (InnoTech DMP800), powder-XRD (PANalytical X'pert3 Powder), and SEM-EDS (FEI Quanta FEG-650) were used.

Synthesis of Zn(II)-Niacinamide Complexes

The complexes were synthesized by a layering technique using a water-methanol system at room temperature for 21 days. Each metal salt was dissolved in 5 mL water as the bottom layer, while the niacinamide was dissolved in 5 mL methanol as the top layer. A water-methanol mixture (1:1) was used as the buffer layer to increase the possibility of gaining a better crystalline product. Each reaction is detailed in Table-1. After 21 days, the crystals were isolated and the solution was filtered off using filter paper. The

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products were then washed with water a few times, followed by air-drying for a few days. The solid products were saved in a desiccator equipped with silica gel beads as a drying agent.

Characterization of the Products

Infrared spectroscopy was conducted using a KBr pellet at a wavenumber of 4000-400 cm^{-1}. The melting point was measured in an open capillary tube at the increasing rate of 10 °C/minute from 25 to 400 °C. Powder-XRD was done at 25 °C with 2θ angles of 10-70° (Cu Kα = 1.54060 Å). SEM analysis was conducted at 0–200 kV using a gold coating system in various magnifications. Anion qualitative test was performed by dissolving the sample in a few mL of water and then added with aqueous $AgNO_3$ dropwise.

Antibacterial Activity

Zn(II) metal complexes and the niacinamide were both investigated for their activity against pathogens bacterial *in vitro*. In this study, one Gram-positive (*Staphylococcus aureus* strain CECT 239) and one Gram-negative (*Escherichia coli* strain CECT 516) strain of bacteria were used. These pathogens were grown in a nutrient broth with a load of 10^6 CFU/mL for 18-20 h. The antimicrobial assay includes the minimum inhibition concentration (MIC) and inhibition zones (IZ) were performed by a standard well diffusion assay and standard disk diffusion assay (Kirby-Bauer), respectively. Both were expressed as a mean of three replicates. Niacinamide and the solvent (DMSO 3%) were used as positive and negative controls, respectively.

RESULTS AND DISCUSSION

Synthesis of Zn(II)-Niacinamide Complexes

After 21 days, from four different anions, only chloride (code 1) and sulphate (code 2) reactions yielded a colorless crystalline product. The nitrate and the acetate reactions, in all molar ratios, did not give any precipitate although the solutions were slowly evaporated. In the case of nitrate, this is unexpected due to Zn(II)-niacinamide nitrate was reported previously.[7] The nitrate complex did not precipitate out in this works probably due to the use of different solvent and different temperature. Meanwhile, for the acetate complex, there is no published work reported on this complex yet. The chloride salt produces more yield compared to the sulphate salts of the same molar ratio. Within the same Zn(II) salt, the higher the molar ratio used; the more yield obtained. The result of all reactions is presented in Table-1.

Table-1: Reaction Series of Zn(II) Complexes

Code	Zn(II) salts	Molar ratio (mmol)	Yield (mg)	Melting point (°C)
1a	Chloride	1:2	137	222
1b		1:4	214	190
1c		1:6	479	187
2a	Sulphate	1:2	-	-
2b		1:4	150	324
2c		1:6	239	329
3a	Nitrate	1:2	-	-
3b		1:4	-	-
3c		1:6	-	-
4a	Acetate	1:2	-	-
4b		1:4	-	-
4c		1:6	-	-

The melting point of each complex was presented in Table-1 and compared to that of niacinamide and Zn(II) salts. Niacinamide melt at 127 °C, Zn(II) chloride dihydrates melts at 290 °C, while Zn(II) sulphate heptahydrates melt at 100 °C. Complexes 1 and 2 were all having different melting points from that of their precursors, thus it is confirmed that the product is not a free ligand or simple Zn(II) salt. In general, complex 2 has a significantly higher melting point compared to complex 1. Moreover, complex 1 shows different results between code a and code b-c, which indicates that the molar ratio may influence the thermal properties of the product. In the case of complex 2, both codes b and c show relatively close melting points. In other words, complex 1b and 1c (as well as complex 2b and 2c) were in the same form. To support this, the crystals were examined by SEM. SEM images of complex 1a, 1b, and 2c are presented in Fig.-1.

Complex 1a and 1b exhibit the same long block of colorless crystals, whereas complex 2c forms stacked plates of colorless crystals. However, the uniformity of bulk complex 1b is lower than that of complex 1a, in which complex 1a forms the better and regular shape of long block crystals.

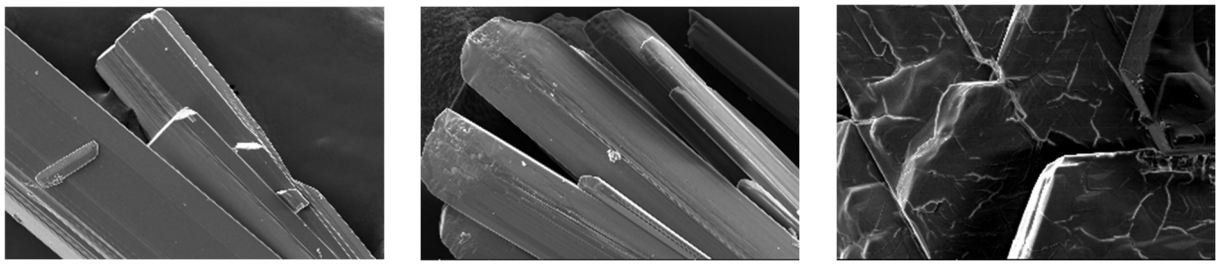


Fig.-1: SEM images of 1a (left), 1b (middle), and 2c (right).

Characterization of the Products

Infrared analysis were performed only on the solid product (codes 1 and 2) and compared to that of free niacinamide (Fig.-2). Infrared spectra of all metal complexes were identical to that of niacinamide, which shows characteristic functional groups of niacinamide, namely ν(N-H) around 3300-3200 cm^{-1}, ν(C=O) around 1700 cm^{-1}, and ν(C-N) around 1400 cm^{-1}. However, peak frequencies of Zn(II) complexes were shifted compared to that of the niacinamide, particularly the N-H and C-N groups of pyridines. The shift is slightly higher which indicates the coordination bond occurs through the nitrogen atom of the pyridyl ring, in which the nitrogen provides a potent electron donor to the metal center.[8] Moreover, there is no typical absorption peak of the chloride group, indicating complex formation in the 1a-1c. The absorption peak of the chloride group may occur in the fingerprint region, which needs to be further analyzed by other characterizations method to ensure the presence of a chloride group in the complex compound. Particularly for the sulphate complex, the presence of the sulphate group is confirmed from a new broad and intense peak around 1100 cm^{-1}. A new peak around 600-500 cm^{-1} was observed and is predicted to correspond to the Zn(II)–N between the metal center and the pyridyl group of the niacinamide. In detail, infrared spectra are presented in Table-2.

Table-2: Infrared Spectra Data of the Zn(II) Complexes and Niacinamide

Compound	FT-IR, cm^{-1}							
	ν(N-H) asymmetric	ν(N-H) symmetric	ν(C-H)	ν(C=O)	ν(C-C)	ν(C-N)	$\nu(SO_4)$	ν(M-N)
Niacinamide	3368	3161	2783	1682	1593	1397	-	621
1a	3377	3198	2772	1681	1605	1440	-	610
1b	3371	3174	2782	1704	1623	1437	-	607
1c	3370	3173	2779	1704	1623	1437	-	603
2b	3382	3200	2768	1681	1605	1440	1118	-
2c	3382	3200	2768	1681	1605	1440	1118	-

Powder-XRD analysis (Fig.-2) shows different diffraction patterns, in which complex 1a differs significantly from complex 1b, although both resulted from the same chloride reactions but in different M: L mol ratio. This result is also in accordance with the melting point data of complex 1, in which complex 1a has a higher melting point than complex 1b. This information indicates that although both complexes were yielded from the same chloride reaction and formed long block colorless crystals, the crystal system and, most likely, the molecular formula of both complexes may be different. Different from complex 1, complex 2c has resulted from a sulphate reaction, hence the product would not be similar to complex 1a or 1b. In addition, to predict the molecular formula of the products, a qualitative anion identification test using silver nitrate solution was also performed. This test confirms the role of chloride or sulphate in the complex, whether as a coordinated ligand or as a lattice anion. White precipitation of AgCl resulted from an aqueous solution of complex 1a and 1b, whereas a clear solution resulted from an aqueous solution of complex 2b and 2c. In other words, the chlorides in complexes 1a and 1b most likely acted as free ions, while the sulfates in 2b and 2c most likely acted as ligands.

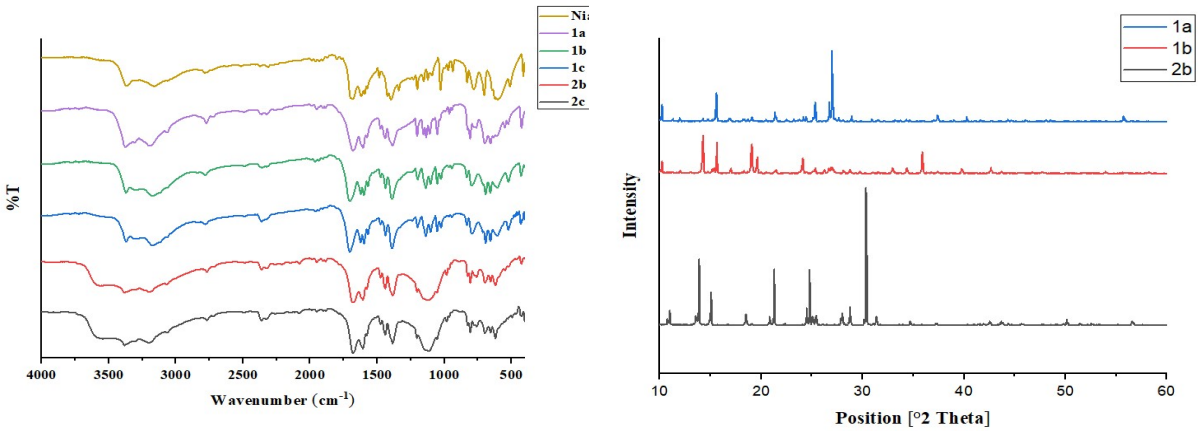


Fig.-2: Infrared Spectra of Niacinamide and Zn(II) Complexes (left) and X-ray Powder Diffraction Pattern of Zn(II) Complexes (right)

The test results for complex 1 are different from the results of a previous study on Zn(II)-niacinamide complex compounds by Dziewulska *et al*. in 2009.[9] In addition, anion-depended metal complexes are frequently observed in metal complexes that use neutral ligands, for example, pyrazinamide ligand as reported by Khunur and Prananto (2019).[8] In summary, based on powder-XRD, melting point, and infrared analysis, complex 1a and 1b were predicted to be $[Zn(niacinamide)_x]Cl_2$, whereas complex 2b and 2c were predicted to be $[Zn(niacinamide)_x(SO_4)]$. The amount of *x* in complex 1a may differ from the amount of *x* in complex 1b, as indicated by different melting points and different X-ray diffraction patterns.

Antibacterial Activity

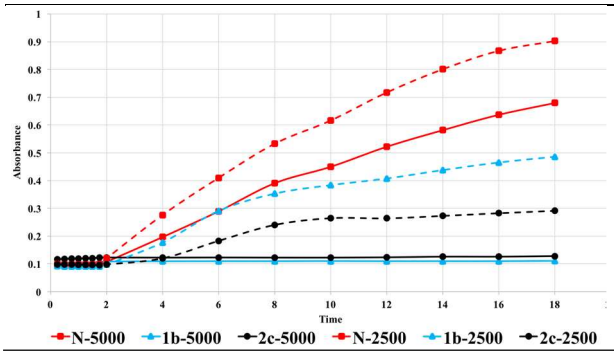
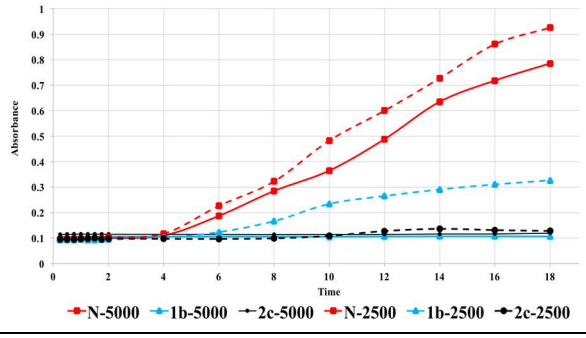
Based on MIC value, level of antibacterial activity is categorized into weak (>625 µg/mL), moderate (100 < MIC < 625 µg/mL), and active (<100 µg/mL).[10] Result of the antibacterial test shows that although the Zn(II) complexes show stronger activity than that of the free niacinamide, it remains classified at a weak level. Nevertheless, this result remains suggests that the antimicrobial potency of niacinamide increases upon metal complexation. The MIC values are summarized in Table-3.

Table-3: MIC Values of the Niacinamide and the Zn(II)-Niacinamide Complexes

Compounds	*S. aureus* CECT 239 (µg/mL)	*E. coli* CECT 516 (µg/mL)
	DMSO 3%	DMSO 3%
Niacinamide	nd*	nd*
$[Zn(L)_x]Cl_2$	5000	5000
$[Zn(L)_x(SO_4)]$	2500	5000

**nd = not detected*

Table-4: Growth Curve of *E. coli* and *S. aureus* in DMSO 3%

E. coli	*S. aureus*
	

The results of the inhibition zone showed that there was no significant clear zone in all test samples, both for *S. aureus* and *E. coli* bacteria (Table-3) with a test dose of 5000 ppm. The test also shows that the

inhibition zone diameters were observed to be less than 7 mm against the two tested bacteria. Furthermore, the growth curve of bacteria was also done to study the effect of the test compound on changes in the growth curve, which would provide additional information on the ability of a test compound to affect the growth curve of a test bacterium. On exposure to the niacinamide test compound with a concentration of 5000 ppm, there was a lengthening of the lag phase (adaptation phase) up to 4 hours, but the bacteria continued to experience log and stationary phases, with maximum growth that was not significantly different from that of no treatment (solvent only). At the highest test dose (5000 *µg/mL*), 1c and 2c compounds could inhibit bacterial growth hence the bacteria did not have a log and stationary phase. However, only complex 2c shows better inhibitory abilities. In the case of *E. coli*, the highest test concentrations of 1c and 2c can inhibit bacterial growth for up to 18 hours of testing.

CONCLUSION

The anion of Zn(II) salt affects the crystallization of the Zn(II)-niacinamide complex, in which sulfate and chloride anions produce colorless block crystalline solids, whereas acetate and nitrate anions did not produce any solid. In the Zn(II)-niacinamide complexes, the chloride is positioned in the crystal lattice as a free ion, while the sulphate is coordinated to the metal center as a ligand. The molar ratio of Zn(II): N influences the yield, in which the higher the molar ratio used, the higher yield obtained. Based on powder-XRD, melting point, and infrared analyses, the synthesized complexes were predicted to be $[Zn(niacinamide)_x]Cl_2$ and$[Zn(niacinamide)_x(SO_4)]$. Antibacterial test in DMSO 3% of both complexes shows that the Zn(II)-niacinamide complexes show stronger activity than that of the free niacinamide. The antimicrobial potency of niacinamide increases upon metal complexation. However, further development of Zn(II)-niacinamide metal complexes are needed to increase its antibacterial activity.

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