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“Sciences for Sustainable Development”

Horison Ultima Hotel Palembang, South Sumatra - Indonesia
November 6-7th, 2018

PREFACE

The 1st SRIWIJAYA INTERNATIONAL CONFERENCE ON BASIC AND APPLIED SCIENCES (SICBAS 2018) with the theme “Sciences for Sustainable Development”. The conference held on November 6-7th, 2018 at the Horison Ultima Hotel Palembang, South Sumatra, Indonesia. This event is launched so as to provide a chance and venue for researchers to come together for research presentations and dissemination’s. The conference is biennial important event to publish communicate they research results in basic and applied sciences.

Hopefully this international proceeding will be able to encourage students, teachers, lecturers, practitioners, researchers, and higher education community in developing scientific and academic atmosphere in Indonesia.

On behalf of the committee of the SICBAS 2018 we would like to thank to all parties for their participation in supporting this publication and we would like to invite all of researchers and scientists to take a part in the 2nd SICBAS which will be held in 2020 by The Faculty of Mathematics and Natural Sciences, Sriwijaya University.

Palembang, November 7th 2018
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Synthesis and characterization schiff base and complexes with Copper (II) and Iron (II) and their application as antibacterial agents

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Abstract. The Schiff base N-(2-hydroxybenzylidene)chitosan and its complexes with copper(II) and iron(II) were synthesized and applied as antibacterial agent. The Schiff base and its complexes characterized by UV-Vis, FTIR spectrophotometer and XRD diffractometer. Antibacterial activity of the Schiff base and its complexes toward *Escherichia coli* and *Staphylococcus aureus* bacteria were assessed by paper disc diffusion method. Electronic transition indicated the absorption of azomethine group from Schiff Base and absorption of d-d transition from metal ion. FTIR characterization of Schiff base indicated the azomethine group have been formed. Absorption Schiff base shifted indicating coordinated to metal ion. X-ray diffractometer studies show the formation of complexes decreased of cristalinity of chitosan. The complexes of copper(II) and iron(II) with N-(2-hydroxybenzylidene)chitosan have antibacterial activities bigger than the Schiff Base or pure chitosan attributed to the increased lipophilic nature of the complexes arising due to chelation.

1. Introduction

During last few years Schiff base compound become one of hot interests because their interesting or useful properties. Chitosan Schiff bases have binding ability to heavy metal ion formed complex compound by chelation. These complexes uses for a large activities such as catalyst, antimicrobial agent, antioxidant and antibacterial agent therefore studies on complex Schiff base with numerous metal ion has been reported [1-4]. Interestingly, most of research about Schiff base modified chitosan-metal ion only focus on their application as catalyst. Copper, cobalt and nickel complexes of Schiff modified chitosan was reported have catalitic activity in cyclohexane oxidation. Research about application complexes of Schiff modified chitosan as antibacterial agent only few available.

Chitosan (poly- β -(1 \rightarrow 4)-glucosamine) is a abundance non toxic natural biopolymer. It is derived from deacetylation of chitin, natural polysaccharide that is usually obtained from shell of crustaceans such as crab, shrimps, crawfish. Chitosan is a biocompatible, biodegradable and non toxic natural biopolymer which its applications ranging from cosmetics, artificial skins, photography, food and nutrition, ophthalmology and wastewater treatment [5-9].

Chitosan contains amine and hydroxy group which under mild reaction condition can be used to chemically alter its properties. Amino group of chitosan can be reacted with aldehyde or ketone form Schiff Base. Schiff base compounds containing an azomethine or imine group (-RC=N-), are synthesized by nucleophilic addition or condensation of primary amine and carbonyl group. Schiff



bases which containing various donor atoms can act as chelating ligand and react with metal ion [1,8,9].

The present work describes the preparation of Schiff Base through reaction between chitosan with 2-hydroxybenzaldehyde. The Schiff Base then reacted with Cu(II) and Fe(II) to form complex compound. The characterization of products have been formed by spectroscopy UV-Vis, FTIR and XRD. Antibacterial activities of the product against *E.coli* and *S.aureus*.

2. Experimental Section

2.1. Materials

Chitosan was purchased from CV. Bio Chitosan Indonesia with the degree of deacetylation was 87.5%. Glacial acetic acid, 2-hydroxybenzaldehyde, dimethylsulfoxide (DMSO), methanol, ethyl alcohol (96%), CuSO₄.5H₂O and Fe(SO₄).7H₂O all in analytical grade were purchased from Merck. *E.coli* and *S.aureus* were supplied by Microbiology Laboratory, Department of Biology, Faculty of Mathematic and the Natural Sciences University of Sriwijaya.

2.2. Instrumentation

The instruments used for characterization included UV-Vis Spectrophotometer (Shimadzu 1420 Mini) for identify electronic transition. Fourier Transform Infrared (FTIR) Spectrometer (Shimadzu 8201) was used for identify the presence of functional groups. X-ray powder diffraction determinations were accomplished using X-ray diffractometer (Shimadzu XRD-6000).

2.3. Procedure

2.3.1. Synthesis The Schiff Base N-(2-hydroxybenzylidene)chitosan. An 800 mg chitosan dissolved in 50 mL acetic acid 0.1 mgL⁻¹ and stirred for 30 min at room temperature. A 4 mL 2-hydroxybenzaldehyde dissolved in 20 mL ethanol previously and then added to chitosan solution. The mixture was refluxed with stirring at 60°C for 12 h. After the cooling product was filtered and the unreacted aldehydes were washed using ethanol. The product was dried at 60°C for 24 h.

2.3.2. Synthesis Complexes N-(2-hydroxybenzylidene)chitosan with Cu(II) and Fe(II). Each a 400 mg N-(2-hydroxybenzylidene)chitosan dissolved in 10 mL DMSO. The solution then added 10 mL Cu(II) 0.1 mgL⁻¹ and 10 mL Fe(II) 0.1 mgL⁻¹ respectively then refluxed at 60°C for 12 h. After cooling, the mixture was filtered. The complexes compound yielded was washed with ethanol and dried at 60°C.

2.4. Characterization of N-(2-hydroxybenzylidene)chitosan and its Cu(II),Fe(II) complexes.

2.4.1. FTIR spectroscopy. The infrared spectra were recorded on a Shimadzu 8201 FTIR spectrophotometer using KBr pellet at room temperature. The spectrum was recorded within the wave number range 400-4000 cm⁻¹.

2.4.2. UV-Vis Spectroscopy. UV-Vis absorption spectra of the N-(2-hydroxybenzylidene)chitosan and its Cu(II),Fe(II) complexes were recorded on spectrofotometer with a scan range 200-800 nm. All samples were diluted 0.1 mgL⁻¹ acetic acid as solvent.

2.4.3. XRD Diffraction. XRD analysis of N-(2-hydroxybenzylidene)chitosan and their complexes with Cu(II) and Fe(II) were performed in the 2θ range from 5° to 80°.

2.5. Antibacterial assay

Antibacterial activities were investigated using paper disc method. The activity of tested samples was studied against the *S.aureus* (as gram-negative bacteria) and *E.coli* (as gram positive bacteria). The bacteria were cultured in 12 mL nutrient agar medium in sterilized petri dish (12 mm diameter). The complex in various concentration poured over discs (6 mm diameter) and placed over on the top of

agar layer. The discs were incubated at 37°C for 24 hours. The zone of inhibition of growth was measured, which indicated the inhibitory activities of the compound. The average of three diameter was calculated for each sample. The antibacterial activities also investigated for chitosan and N-(2-hydroxybenzylidene)chitosan.

3. Results and Discussion

3.1. Identification Electronic transition by UV Vis Spectrophotometer

A schematic illustration of the complexation between Schiff Base and ion metal is depicted in figure 1. Electronic spectra of Schiff Base N-(2-hydroxybenzylidene) chitosan (abbreviated as CSB) and their complex compounds namely CSB-Cu(II) and CSB-Fe(II) obtained by spectrofotometer at wavelength range 200-800 nm. Figure 2 represented electronic spectra of CSB, CSB-Cu(II) and CSB-Fe(II). Absorptions at around 320 nm were an electronic transition from $n \rightarrow \pi^*$ of an azomethine group of Schiff base derived from the reaction between chitosan and 2-hydroxybenzaldehyde [10,11].

Electronic spectra of Schiff base complexes with Cu(II) and Fe(II) showed an absorption maximum above 400 nm due to d-d transitions derived from metal ions. Absorption at 458 nm and 455 nm corresponds to the d-d electron excitation of Cu(II) and Fe(II). This band observed to be quite weak because transitions would theoretically be forbidden by Laporte rule. The copper (II) ion and iron (II) ion in aqueous solution have absorption maximum at 810 and 1020 nm respectively [12]. This result indicated that absorption maximum of Cu(II) and Fe(II) in shifting to a lower wavelength due to complexation. Chelating formation between Schiff base and Cu(II) or Fe(II) resulting a more stonger binding therefore splitting parameter (Δ_0) of d electron became larger. The more larger splitting parameter more bigger the energy of absorbed light and more lower the wavelength.

Table 1. electronic transition of CSB, CSB-Cu(II) and CSB-Fe(II)

Compound	Absorption (λ_{maks}) (nm)	
	d-d	$n \rightarrow \pi^*$ azomethine
CSB	-	323
CSB-Fe(II)	458	320
CSB-Cu(II)	455	325

3.2. Identification of functional group by FT-IR

A broad absorption band which corresponds to stretching vibrations of O-H and N-H functional group from chitosan observed at approximately 3400 cm^{-1} . Stretching bands due to skeletal vibration of C-O occur at 1037 cm^{-1} . Absorption of β -(1 \rightarrow 4) glycoside bridge which characterized chitosan saccharide structure is noted at 1158 cm^{-1} and 892 cm^{-1} [2]. These significant bands which characterized chitosan are expected presence in CSB and its complexes.

The FT-IR spectra of CSB, CSB-Cu(II) and CSB-Fe(II) are shown at Figure 3. The broad absorption band which corresponds to stretching vibration O-H and N-H which derived from CSB can be observed at wave number 3448.7 cm^{-1} . Absorption band observed at 2885.5 cm^{-1} and 2924.1 cm^{-1} due to the C-H vibration stretch of $-\text{CH}_2$ group. Stretching vibration of C-O-C from CSB can be observed at 1033.85 whereas for CSB-Fe(II) and CSB-Cu(II) at 1018.4 cm^{-1} [2,13, 14, 15]. The functional group of O-H stretching vibration of CSB appeared at wavenumber 3448.7 cm^{-1} . This band has been shifted to shorter wavenumber at 3433.3 cm^{-1} for CSB-Cu(II) and 3425.6 cm^{-1} for CSB-Fe(II) respectively indicating coordination between the metal ion and Schiff base through hydroxy functional group. The formation of coordination bond between M and azomethine is confirmed by absorption at 393.48 cm^{-1} due to vibration of M-N [16]. Absorption at wavenumber 300-600 cm^{-1} indicating that complex compound has formed.

3.3. X-ray diffraction analysis

The Crystallinity of chitosan, chitosan Schiff base (CSB) and their complexes with Cu(II) and Cu(II) were investigated by XRD. The x-ray diffraction pattern of chitosan and its derivative compound had characteristic peak around 20° [2]. Figure 4 shows diffraction pattern of chitosan, CSB and their complexes. The characteristic peak of CSB, CSB-Fe(II) and CSB-Cu(II) at $2\theta \approx 20^\circ$ are much wider and weaker than chitosan. This result indicates that crystallinity of CSB, CSB-Fe(II) and CSB-Cu(II) decreased compared to chitosan due to deformation of strong intramolecular hydrogen bonding in the chitosan backbone was caused by azomethine formation.

3.4. Biological studies

The antimicrobial activities of CSB, CSB-Fe(II) and CSB- Cu(II) were screened against *E.coli* and *S.aureus* bacteria. Result of bacterial screening are summarized at Table 2. The bacterial screening results showed that complex compound of CSB have higher activities than chitosan and CSB. This attributed to the increased lipophilic nature of the complexes arising due to chelation. The enhanced activity of the metal complexes is probably due to faster diffusion of the chelates as a whole through the cell membrane or due to the chelation theory [6]. Metal ion-CBS chelates will increase the positive charge density of chitosan and in turn enhance inhibition activities

The antibacterial activities of CSB-Cu(II) were higher than that of CSB-Fe(II). Interaction between Copper (II) with CSB is stronger because Cu (II) has smaller radius and more extra nuclear electron than Fe(II) based on Irving-William Series. Cu (II) will possess stronger interaction with CSB so complex CSB-Cu(II) is easy to bind with cell surface of bacteria and antibacterial activities will increase. [17]

Table 2. Diameter of zone of inhibition of N-(2-hydroxybenzylidene)chitosan and their complex compounds

Compound	concentration (ppm)	Diameter of zone of inhibition (mm)	
		<i>E.coli</i>	<i>S.aureus</i>
Chitosan	0	0	0
	125	6	6
	250	6	6
	500	7	8
	1000	8.75	9.25
CSB	0	0	0
	125	6	6
	250	6	6
	500	7.6	6
	1000	9.5	9.33
CSB- Cu(II)	0	0	0
	125	6	6
	250	7	7
	500	8	8
	1000	10.67	11.58
CSB-Fe(II)	0	0	0
	125	6	6
	250	6	6
	500	7	7
	1000	9.86	10.16

4. Conclusions

Schiff base derived from chitosan and 2-hydroxybenzaldehyde and its Fe(II) and Cu(II) complexes were synthesized. The formation Schiff base and complexes were confirmed by spectral studies of

UV Vis, FT-IR and XRD. X-ray diffractometer studies showed the presence chitosan characteristic peak in all complexes. The formation complexes of chitosan Schiff base result in the decreased of crystallinity of chitosan. Chitosan Schiff base complexes exhibit antibacterial activity higher than chitosan and chitosan Schiff base.

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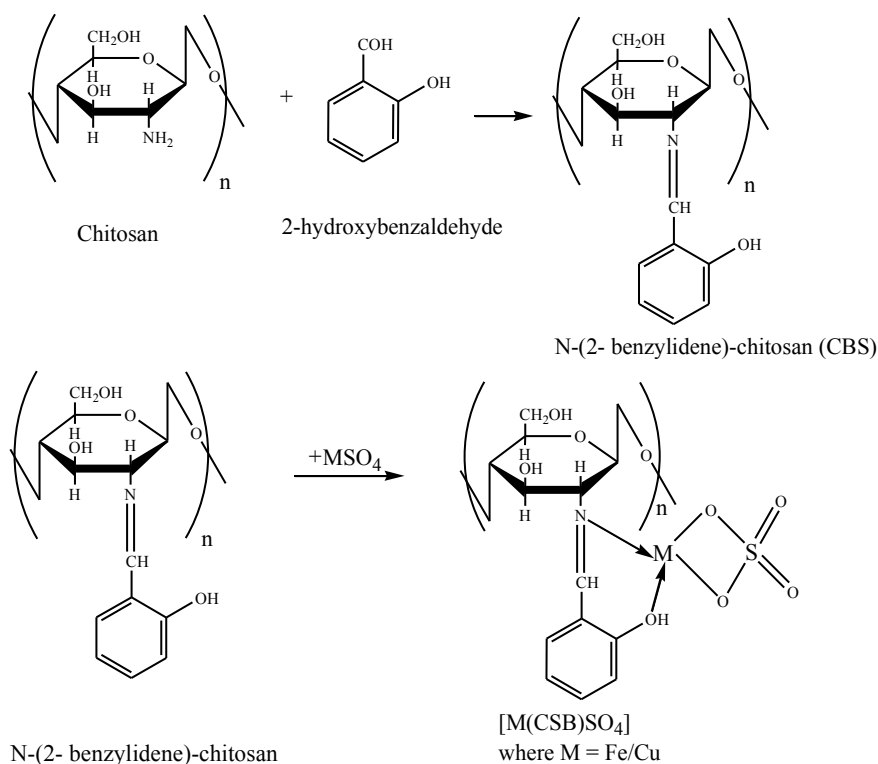


Figure 1. The schematic illustration formation of N-(2-benzylidene)chitosan and its complexes.

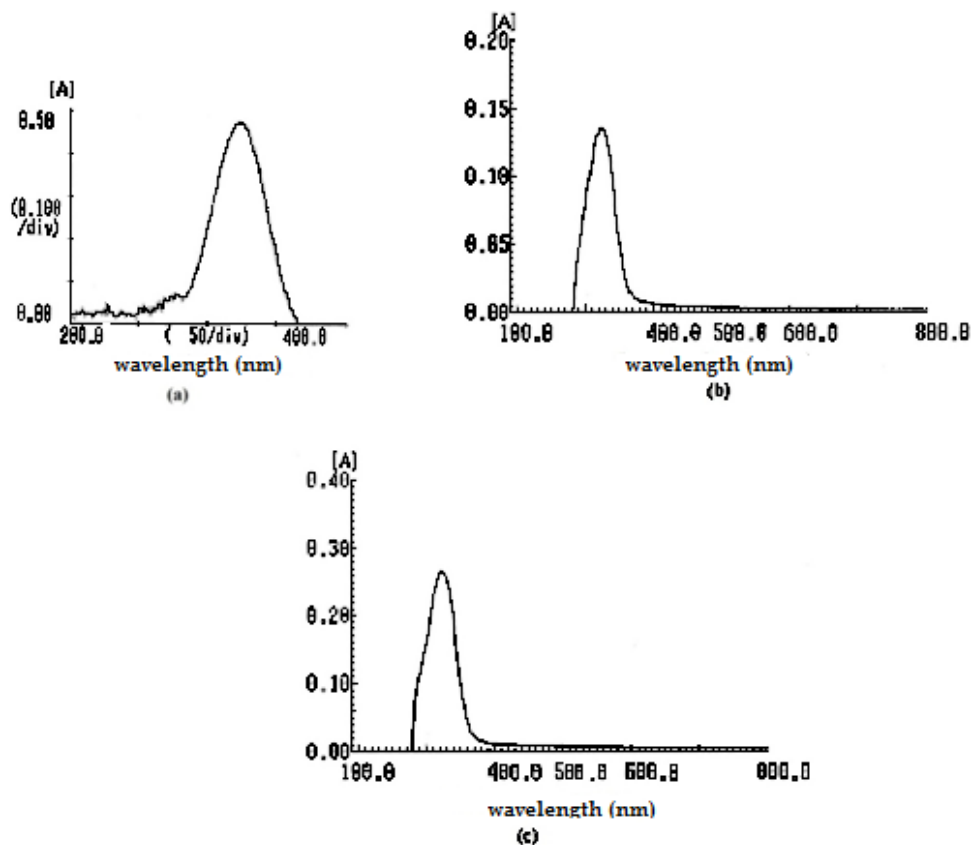


Figure 2. UV-Vis spectrum of CSB (a), CSB-Fe(II) (b) and CSB-Cu(II)(c)

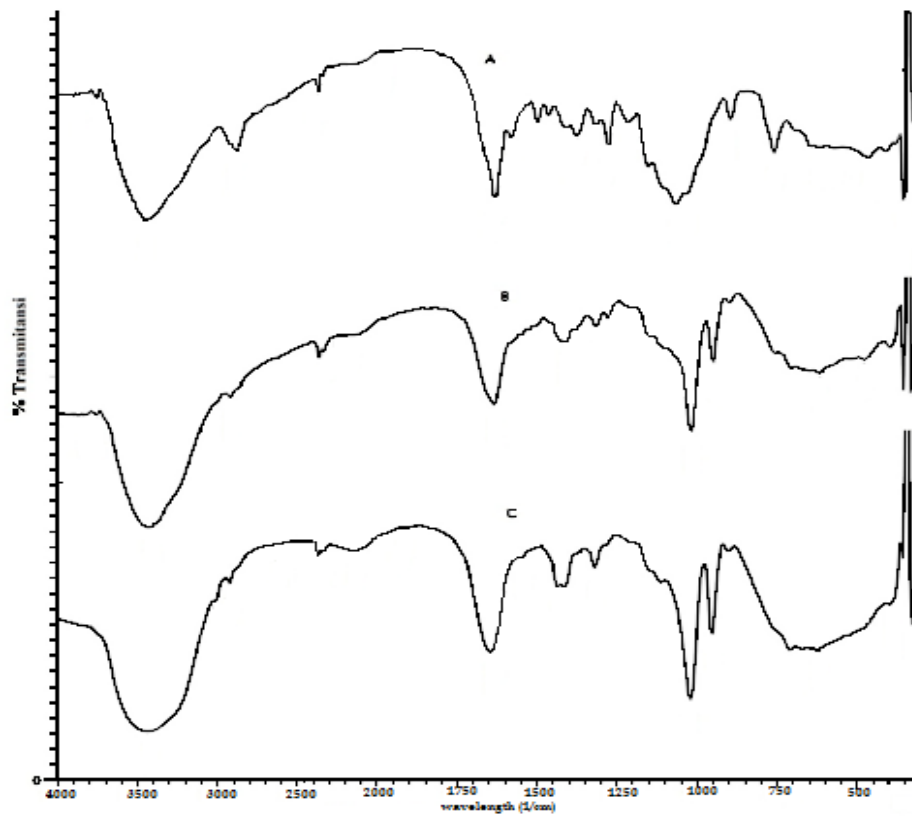


Figure 3. FT-IR Spectrum of CSB (a) CSB-Fe(II) (b) and CSB-Cu(II) (c)

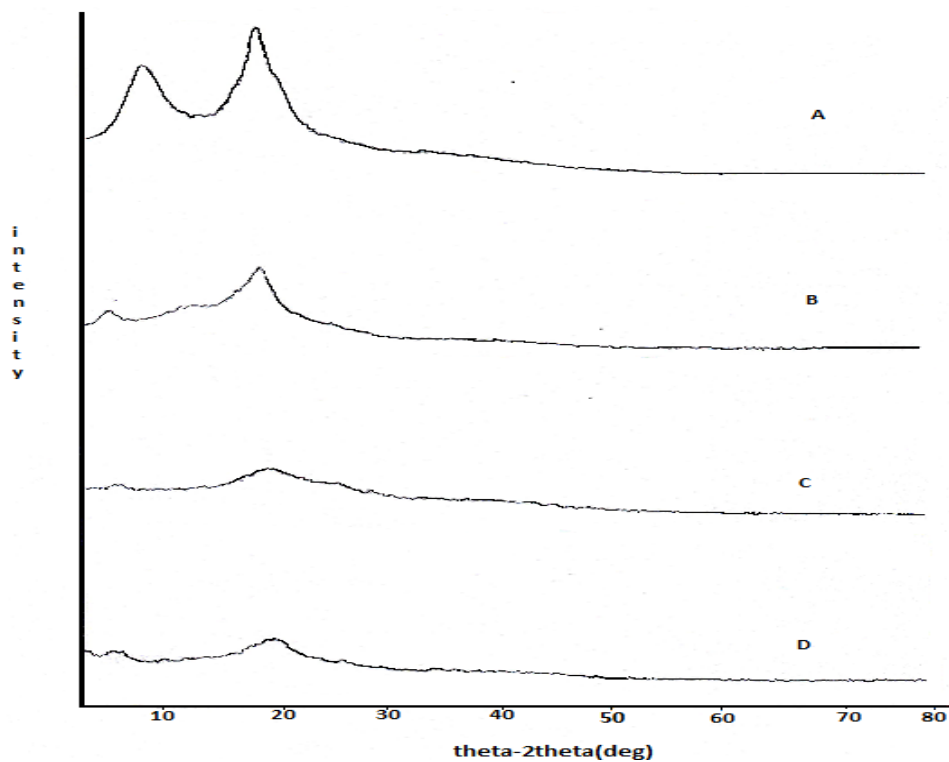


Figure 4. XRD pattern of chitosan (A), CSB (B), CSB-Cu(II) (C) and CSB-Fe(II) (D).