

1.22_ Adsorbent of chitosan linked by methylene bridge and schiff base of 4,4-diaminodiphenyl ether-vanillin - synthesis, characterization and its application after reacted by Zn(II) ion as antibacterial agent

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Adsorbent of chitosan linked by methylene bridge and schiff base of 4,4-diaminodiphenyl ether-vanillin : synthesis, characterization and its application after reacted by Zn(II) ion as antibacterial agent

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Abstract

The synthesis chitosan linked by methylene bridge-Schiff base of 4,4-diaminodiphenyl ether-vanillin (modified chitosan adsorbent) has been studied. The aims of this research were modification of chitosan with Schiff base of 4,4-diaminodiphenyl ether-vanillin and formaldehyde and its application as antibacterial agent after used as an adsorbent of Zn(II) ion. Characterization of modified chitosan adsorbent was conducted using FTIR spectroscopy. Process of adsorption were conducted at 0.15 g of modified chitosan adsorbent reacted by concentration of Zn(II) ion solution (pH 6, 150 mg/L, 50 mL), contact time at 150 min. and at room temperature (28 °C). Modified chitosan adsorbent after reacted by Zn(II) ion solution as antibacterial agent with paper disk method. The result showed that chitosan can be modified with Schiff base of 4,4-diaminodiphenyl ether-vanillin and formaldehyde to form chitosan linked methylene bridge and Schiff base of 4,4-diaminodiphenyl ether-vanillin. Antibacterial of chitosan and modified chitosan adsorbent were higher than modified chitosan adsorbent after reacted by Zn(II) ion.

Keywords

modified chitosan, antibacterial agent

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1. INTRODUCTION

Chitosan is a natural biopolymer composed of a linear polysaccharide of α (1-4)-linked 2-amino 2-deoxy β -D glucopyranose. Chitosan was synthesized by deacetylation of chitin (Mohanasrinivasan et al., 2013). Chitosan and modified chitosan can be used as biosorbent (Krishnapriya and Kandaswamy, 2010; Du et al., 2009; Kocak et al., 2012; Monier, 2012; Mujeeb et al., 2014; Kandile and Nasr, 2014; Li et al., 2014).

Vanillin can be modified by organic compound which have the functional group of primary amine and metal to form vanillin schiff base and its complex compound respectively (Wu et al., 2014; Gulcan and Sonmez, 2011; Wang et al., 2011; Kaya et al., 2008). Vanillin schiff base also can act as adsorbent (Kumar et al., 2005). Vanillin Schiff bases and their complexes have been found as antibacterial activities (Zhong and Zhong, 2014; Anupama et al., 2014; Sirajuddin et al., 2014; Cheng et al., 2009; Joshi et al., 2014).

In this paper, we synthesized modified chitosan from the reaction between chitosan (with assumption : MW 161 g/mol (Jin et al., 2009), formaldehyde as linkages (Fajardo et al., 2012; Li et al., 2014; Monier, 2012) and schiff base of 4,4-

diaminodiphenyl ether-vanillin to form a new adsorbent of chitosan linked by methylene bridge and Schiff base of 4,4-diaminodiphenyl ether-vanillin (Fatoni et al., 2018). Characterization of this adsorbent analyzed by FTIR. Active site of this adsorbent can bind of Zn(II) ion after reacted by Zn(II) ion in aqueous solution. After reacted by Zn(II) ion, this adsorbent can act as antibacterial agent of *E.coli* and *S. aureus*.

2. EXPERIMENTAL SECTION

2.1 Materials

The stock solution of Zn(II) ion (1000 mg/L from Zn(Ac)₂ · 2H₂O). Chitosan (DD 87%) obtained from CV. Ocean Fresh Bandung, West Java, Indonesia. The pH of the Zn(II) ion solution was adjusted using HCl 0.1 M. Acetic acid glacial, methanol, vanillin, dimethyl sulfoxide (DMSO), formaldehyde (37% , v/v), hydrochloric acid (37%), sodium nitrate, zinc acetate dihydrate, sulfuric acid 97% (v/v) and nutrient agar. All in analytical grade were used without further purification and purchased from Merck except 4,4-diaminodiphenyl ether 97% from Sigma Aldrich. *E.coli* (ATCC 25922) and *S. aureus* (ATCC 25923) from Indonesia University.

2.2 Methods

2.2.1 Synthesis Schiff base of 4,4-diaminodiphenyl ether-vanillin

Schiff base of 4,4-diaminodiphenyl ether-vanillin was synthesized according to Cucos et al. (2014) procedure with mol ratio is 1 : 1. In beaker glass 250 mL, 4 g of 4,4-diaminodiphenyl ether (0.02 mol) was dissolved in methanol (20 mL). In this beaker glass, added of 3.04 g of vanillin solution (0.02 mol) was dissolved in 10 mL methanol. The mixture was stirred continuously at temperature 75 °C for 1 h. After 1 h, orange solution was appeared and stored at room temperature until yellow powder formed. The yellow powder washed with 10 mL of methanol and dried in an oven at 55 °C until constant weight.

2.2.2 Synthesis of chitosan - Schiff base of 4,4 diaminodiphenyl eter-vanilin

Chitosan-Schiff base of 4,4-diaminodiphenyl ether-vanillin was synthesized by mol ratio between chitosan and Schiff base of 4,4-diaminodiphenyl ether-vanillin : 1 : 2. Chitosan (0.805 g, 0.005 mol) was dissolved with 20 mL of acetic acid solution 3% (v/v) in beaker glass 250 mL and stirred continuously at room temperature for 30 min until clear of chitosan solution formed. Schiff base of 4,4-diaminodiphenyl ether-vanillin solution (3.340 g, 0.01 mol, 5 mL of DMSO) was added in this beaker glass. The mixture was stirred continuously at temperature 70 °C for 1 h. After 1 h, the mixture was transferred to the oven at temperature 70 °C until a brown gel formed. A brown gel washed with 10 mL absolute ethanol and stored for 15 min at room temperature. A brown gel dried in an oven at temperature 50 °C until constant weight.

2.2.3 Synthesis and characterization of chitosan linked methylene bridge and Schiff base of 4,4 diaminodi-phenyl eter-vanilin (modified chitosan adsorbent)

Chitosan-Schiff base of 4,4-diaminodiphenyl ether-vanillin (5 g) was soaked in a mixture of 5 mL of formaldehyde solution 37% (v/v) and 0.5 mL sulfuric acid solution 10% (v/v) in beaker glass 250 mL (Fajardo et al., 2012). The product was stored at room temperature for 30 min. After 30 min., the product was washed by a dist several times until the filtrate has pH 6-7. The residue dried in oven at 50 °C until constant weight and the product was named modified chitosan adsorbent.

2.2.4 Adsorption study

Interaction of modified chitosan adsorbent with Zn(II) ion in aqueous solution were obtained by batch operations. Adsorption experiments were carried out by shaking 0.15 g of modified chitosan adsorbent with concentration of Zn(II) ion solution (pH 6, 150 mg/L, 50 mL) at room temperature (28 °C). Modified chitosan adsorbent was placed in Erlenmeyer flask. The erlenmeyer flask was shaken mechanically for 150 min. The mixture was filtered and residue (metal complexes compound) was dried in room temperature until constant weight.

2.2.5 Characterization

FTIR Spectrophotometer (Shimadzu Prestige-21) used for characterization of functional group of chitosan, schiff base of 4,4-diaminodiphenyl ether-vanillin and modified chitosan adsorbent.

2.2.6 Antibacterial Study

The antibacterial activity was studied against Staphylococcus aureus (Gram-positive bacteria) and Escherichia coli (Gram negative bacteria). The solutions of modified chitosan adsorbent after reacted by Zn(II) ion (Zn(II) ion 150 mg/L (B) at pH 6 (C) and interaction time of 150 min. (A) were prepared in DMSO at a concentration of 10.000 µg/ml respectively. Modified chitosan adsorbent (D, negative control) was prepared in DMSO at a concentration of 10.000 µg/ml. Paper disks were sterilized in an autoclave and saturated with solution of modified chitosan adsorbent-Zn(II) ion, modified chitosan, chitosan (E, 10.000 µg/ml), acetic acid (F, 3% v/v) and DMSO (4) as a negative control respectively. All samples were placed aseptically in the petridishes containing nutrient agar media inoculated with the above mentioned two bacteria separately. The petridishes were incubated at 37 °C and the inhibition (clear) zones were recorded after 24 h of incubation period (Anupama et al., 2014).

3. RESULTS AND DISCUSSION

3.1 Synthesis Schiff base of 4,4-diaminodiphenyl ether-vanillin

The spectra FT-IR of synthesis Schiff base of 4,4-diaminodiphenyl ether-vanillin and its of interpretation data were reported by Fatoni et al. (2018).

3.2 Synthesis of chitosan - Schiff base of 4,4-diaminodiphenyl eter-vanilin and chitosan linked by methylene bridge and - Schiff base of 4,4-diaminodiphenyl eter-vanilin (modified chitosan)

The FTIR spectra chitosan, chitosan-Schiff base of 4,4-diaminodiphenyl eter-vanilin (1:2) and chitosan linked by methylene bridge and Schiff base of 4,4 diaminodi-phenyl eter-vanilin (modified chitosan adsorbent) were shown in Fig. 1.

The main bands and their assignments in chitosan (Fig. 1a) are as shows : stretching vibrations of O-H, ν(O-H) and overlap with ν(N-H) at 3441 cm⁻¹. Stretching vibration of C-H, ν(C-H) at 2924 cm⁻¹ (Kumari et al., 2015). Bending vibration of N-H, δ(N-H) at 1604 cm⁻¹ (Mohammed et al., 2013; Kumari et al., 2015) and deformation vibration in primary amine can be found at 1427 cm⁻¹ (Li and Bai, 2005). Bending vibration of C-H, δ(C-H) at 1381 cm⁻¹. Stretching vibration of C-O group, ν(C-O) at 1095 cm⁻¹ (Kandile and Nasr, 2014).

The main band and its of interpretation data in chitosan-Schiff base of 4,4-diaminodiphenyl eter-vanilin (Fig. 1b) are as follows : stretching vibration -OH group, ν(OH) and overlap with ν(N-H) at 3387 cm⁻¹ (Coates, 2006). The bending vibration of -NH group, δ(N-H) at 1627 cm⁻¹. Bending vibration OH group, δ(O-H) at 1226-1311 cm⁻¹. Stretching vibrations

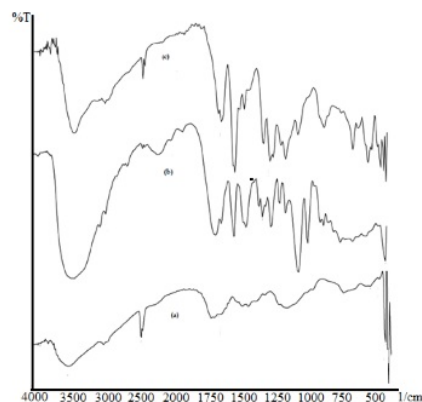


Figure 1. FTIR spectra of: (a) chitosan, (b) chitosan-Schiff base of 4,4-diaminodiphenyl ether-vanillin and (c) adsorbent of chitosan linked by methylene bridge and Schiff base of 4,4-diaminodiphenyl ether-vanillin

of $-\text{CH}_2$, $\nu(\text{C}-\text{H})$ at 2916cm^{-1} . Stretching vibrations of $(\text{C}=\text{C}-\text{C})$, $\nu(\text{C}=\text{C}-\text{C})$ appeared at 1411 and 1504cm^{-1} . Stretching vibrations of $\text{C}-\text{O}-\text{C}$ group, $\nu(\text{C}-\text{O}-\text{C})$ at 1118cm^{-1} . The stretching vibration of $-\text{OCH}_3$ vanillin, $\nu(-\text{OCH}_3)$ appeared at 1018cm^{-1} (Balachandran and Parimala, 2012). A peak observed at 1597cm^{-1} is due to the vibration stretch of Schiff Base $(-\text{N}=\text{CH}-)$ group (Coates, 2006; Li and Bai, 2005; Zhou et al., 2014).

In other hand, the main band and its of interpretation data in chitosan linked methylene bridge and Schiff base of 4,4-diaminodi-phenyl eter-vanillin (Fig. 1c, modified chitosan adsorbent) are as follows : Stretching vibration $-\text{OH}$, $\nu(\text{O}-\text{H})$ at $3324-3363\text{cm}^{-1}$ and bending vibration OH group, $\delta(\text{O}-\text{H})$ observed at $1211-1234\text{cm}^{-1}$ (Coates, 2006). A peak at 3363cm^{-1} is the functional group $-\text{NH}$ secondary (Kandile and Nasr, 2014). Stretching vibrations $\text{C}-\text{O}-\text{C}$, $\nu(\text{C}-\text{O}-\text{C})$ at 1118cm^{-1} (Coates, 2006). Stretching vibration of $-\text{OCH}_3$, $\nu(-\text{OCH}_3)$ at 1026cm^{-1} (Balachandran and Parimala, 2012). After added formaldehyde, a new peak at 1388 and 1496 were observed and this peak is a form of methylene bridge $(-\text{NH}-\text{H}_2\text{C}-\text{NH}-)$ (Du et al., 2009; Monier, 2012) and obscured of $\text{C}=\text{C}-\text{C}$ group in benzene ring (Can et al., 2012; Poljansek and Krajnc, 2005). Stretching vibrations $-\text{N}=\text{CH}-$ group, $\nu(-\text{N}=\text{CH}-)$ at 1597cm^{-1} was constant and this peak was almost not affected by adding formaldehyde.

3.3 Antibacterial activity

The efficient antibacterial of chitosan (E), acetic acid (F), DMSO (G), modified chitosan after reacted by $\text{Zn}(\text{II})$ ion (A, B and C) and modified chitosan (D) were investigated in Fig.2 and Fig.3. Inhibition (clear) zone of *E. coli* and *S. aureus* was tabulated in table 1 and 2 respectively.

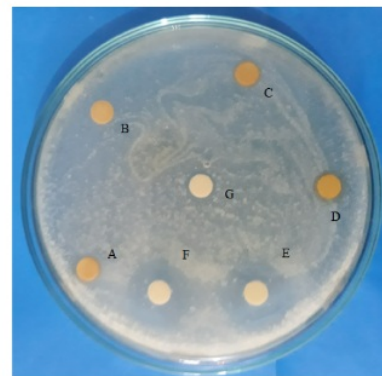


Figure 2. Study antibacterial of *E. coli* : modified chitosan after reacted by $\text{Zn}(\text{II})$ ion (A, B and C), modified chitosan (D), chitosan (E), acetic acid (F) and DMSO (G)

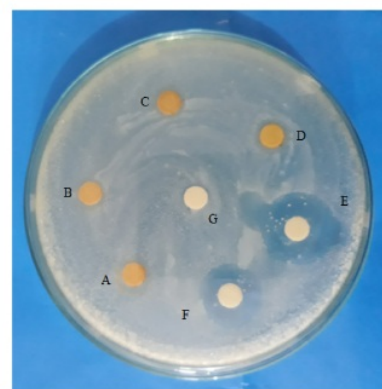


Figure 3. Study antibacterial of *S. aureus* : modified chitosan after reacted by $\text{Zn}(\text{II})$ ion (A, B and C), modified chitosan (D), chitosan (E), acetic acid (F) and DMSO (G)

Table 1. The result study as antibacterial of *E. coli*

No.	Compound	Inhibition (clear) zone (mm)
1	Modified chitosan- $\text{Zn}(\text{II})$ ion (A)	7.1
2	Modified chitosan- $\text{Zn}(\text{II})$ ion (B)	6.1
3	Modified chitosan- $\text{Zn}(\text{II})$ ion (C)	7.2
4	Modified chitosan (D)	7.8
5	Chitosan (E)	18.3
6	Acetic acid (F)	18
7	DMSO (G)	6.1

Table 2. The result study as antibacterial of *S. aureus*

No.	Compound	Inhibition (clear) zone (mm)
1	Modified chitosan-Zn(II) ion (A)	10.4
2	Modified chitosan-Zn(II) ion (B)	10.4
3	Modified chitosan-Zn(II) ion (C)	10.4
4	Modified chitosan (D)	11.1
5	Chitosan (E)	22.4
6	Acetic acid (F)	20.5
7	DMSO (G)	6.5

Table 1 and 2 showed that chitosan and acetic acid can act as antibacterial *E. coli* and *S. aureus* as reported by Liu et al. (2006); Ryssel et al. (2009); Jin et al. (2009). Its inhibition (clear) zone are greater than compound A, B, C and D. Its mechanism is the interaction between positively charged of chitosan molecules (NH_3^+ groups) and negatively charged microbial cell surface (Kumar et al. (2009)). The antimicrobial activities of acetic acid can be influenced by the characteristics of the environment, such as pH, matrix composition and cell density (In et al., 2013).

All Compounds (A, B, C and D) have antibacterial properties in *E. coli* and *S. aureus*. This properties can be explained that the mode of action of the compound D may involve formation of a hydrogen bond through the azomethine group ($>\text{C}=\text{N}-$) with the active centers of various cellular constituents, resulting in interference with normal cellular processes (Joseph and Nair, 2008). In other hand, the activity of the modified chitosan adsorbent after reacted by Zn(II) ion (A, B and C) can be explained on the basis of chelation theory (Tweedy, 1964). Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring. Such a chelation could enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane. The results indicate that the chitosan, modified chitosan and modified chitosan after reacted by Zn(II) ion have a significant inhibiting effect on *E. coli* and *S. aureus*.

4. CONCLUSIONS

Chitosan can be modified by Schiff base of 4,4-diaminodiphenyl ether-vanillin and formaldehyde to form adsorbent of chitosan linked methylene bridge and schiff base of 4,4-diaminodiphenyl ether-vanillin. This adsorbent can be reacted by Zn(II) ion and its result as antibacterial agent. Antibacterial agent of chitosan is higher than modified chitosan adsorbent before and after reacted by Zn(II) ion.

5. ACKNOWLEDGEMENT

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