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Current Bioactive Compounds

ISSN (Print): 1573-4072
ISSN (Online): 1875-6646

Volume 19 , Issues 10, 2023

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Current Bioactive Compounds

ISSN 1573-4072 (Print)

The journal aims to provide updates to researchers about new bioactive compounds with proven activities in various biological screenings and pharmacological models. The journal will contain information about the structures, biological activities and sources of chemical entities discovered or designed by researchers and published in leading journals. The aim is to provide a valuable information source of bioactive compounds synthesized or isolated, which can be used for further development of pharmaceuticals by industry and academia.

The journal should prove to be essential reading for pharmacologists, pharmaceutical chemists and medicinal chemists who wish to be kept informed and up-to-date with the latest and most important developments about new bioactive compounds of natural or synthetic origin, including recent patents.

Publisher: Bentham Science Publishers

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Volume 14, Number 4, 2018

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REVIEW ARTICLE

Synthesis, Characterization and Antifungal Activity of N-Methyl Chitosan and Its Application on the Gauze

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ARTICLE HISTORY

Received: February 03, 2017
Revised: March 20, 2017
Accepted: March 29, 2017

DOI:
10.2174/1573407213666170420171005

Abstract: ***Background:*** Chitosan is a natural polymer which has limited solubility. Chitosan is capable of being modified using multiple alternatives for chitosan chemical modifications that provide different desirable properties.

Method: N-methyl chitosan was successfully prepared and characterized. N-methyl chitosan was characterized by the FTIR, XRD, SEM, TEM, solubility, water content, and specific surface area. Antifungal activity against *Candida albicans* of N-methyl chitosan and gauze coated N-methyl Chitosan were also determined in this study. The antifungal activity tests were performed using agar diffusion and broth microdilution methods. Gauze coating with N-methyl chitosan was performed with a pad-dry-cure method. The SEM photograph of N-methyl chitosan clearly showed the rugged nature of particles. The TEM image shows that N-methyl chitosan produced from synthesis possessed round shape, with an average diameter of 50 nm.

Results: The results of solubility in water, water content, and specific surface of N-methyl chitosan were 2.04 mg/mL, 18.12%, and 0.995 m^2/g, respectively.

Conclusion: N-methyl chitosan exhibited antifungal activity against *C. albicans* with MIC and MFC 0.75% and 1%, respectively. The maximum clear zone produced by gauze coated N-methyl chitosan occurred at 1% concentration of N-methyl chitosan, 60 seconds of dipping time, and 10 times of dipping frequency.

Keywords: Chitosan, N-methyl chitosan, gauzed, antifungal activity, *C. albicans*, respiratory tract.

1. INTRODUCTION

Chitosan is a non-toxic, biodegradable and biocompatible material that was reported to have several useful biological properties [1]. Chitosan is environmentally friendly and this property is the main reason for the development of chitosan applications in industry [2]. Since chitosan is insoluble in water, the use of chitosan in a basic environment is limited. Chemical modification of chitosan can give new functional properties for a variety of biological and biomedical applications of chitosan. N-alkylation was performed to change chitosan into a more water-soluble form and to give a better functional property [3]. Recently, increasing attention has been paid on water-soluble derivatives of chitosan and its applications [4].

Chitosan has attractive antimicrobial activity. The positive charges carried by the protonated amine groups of chitosan (in acidic conditions) that are the driving force for its solubilization are also associated with its antimicrobial activity [5]. Chitosan and quaternary chitosan derivatives are able to inhibit *Botrytis cinerea Pers* and *Colletotrichum lagenarium (Pass) Ell.et halst* [6] and *Candida albicans* [7, 8]. *C. albicans* is an opportunist pathogen fungus that can be found in digestion line, respiratory tract, urinary tract and bloodstream [9].

Chitosan is a weak base and soluble only in acidic solutions [10] due to the protonation of $-NH_2$ group at C2 position [11]. The limitation of chitosan use in various applications is due to its low solubility [12]. Therefore, its low solubility at physiological pH limits its biological activity [13]. Accordingly, chemical modifications of chitosan are required to enhance its solubility and broaden the spectrum of

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its applications, including as biocide. Chitosan amino groups on C2 can react with aldehydes and ketones, which give a product of aldimines and ketimines, respectively [11]. Thus, the addition of methyl on the amino groups resulted in the increase of the water solubility of the chitosan derivatives and the cationic polyelectrolyte chitosan derivatives [4].

N-methyl chitosan is one of chitosan derivatives that has better water solubility compared to chitosan since it possesses a bigger polycationic charge compared to chitosan, therefore, it may give better antifungal activity. N-methyl chitosan can be applied on fabric especially on gauze so that the fabric has antifungal activity against *C. albicans*. Therefore, the aim of this research is focused on the synthesis, characterization of N-methyl chitosan and its antifungal activity against *C. albicans* and its application on the gauze.

2. METHODS

2.1. Synthesis of N-methyl Chitosan

Chitosan solutions were made by dissolving 5 grams of chitosan in 1% acetic acid. A total of 3.4 mL of 10% formaldehyde was added to the chitosan solution at room temperature and stirring with a magnetic stirrer at 500 rpm. After one hour, the pH of the solution is conditioned at 4.5 by adding a solution of 1 M NaOH. Afterward to this solution, 2.6 mL $NaBH_4$ of 10% solution in water was added and the solution mixture was stirred for 1.5 hours. N-methyl chitosan product was then precipitated by conditioning the pH to 10. The precipitate was washed with distilled water to remove excess unreacted formaldehyde. The precipitate was dried at 35°C.

2.2. Characterization of the Product

Characterization was performed by FTIR for the identification of its structure, X-RD to test crystallization, SEM, and TEM for the identification of morphology, analysis by SAA to determine the specific surface area, solubility test [14], water content, ash content [15].

2.2.1. Solubility Test

A 500 mg N-methyl chitosan was inserted into a glass beaker, and 1% acetic acid was added to it slowly to dissolve N-methyl chitosan completely. The solubility of N-methyl chitosan is calculated as below:

$$\text{N-methyl chitosan solubility (w/v)} = \frac{m}{v}$$

Description: m = weight of N-methyl chitosan (mg)
v = volume of solvent (mL)

2.2.2. Water Content

500 mg of N-methyl chitosan was weighted and the sample was then heated in an oven to a temperature of 105°C for 3 hours. The dried sample was put into desiccators, allowed to cool and reweighed. The heating process was repeated until a constant weight was obtained.

$$\text{Water content (\%)} = \frac{W2 - W3}{W2 - W1} x100\%$$

Description: W1 = Initial weight of empty dish
W2 = Weight of dish + undried sample
W3 = Weight of dish + dried sample

2.2.3. Ash Content

An accurately weighted 500 mg of N-methyl chitosan was heated in a moisture extraction oven for 3h at 100°C before being transferred into a muffle furnace at 550°C until it turned white and free of carbon. The sample was then removed from the furnace, cooled in a desiccator to a room temperature and reweighed immediately. The weight of the residual ash was then calculated as ash content.

$$\text{Ash content (\%)} = \frac{weight\ of\ ash}{weight\ of\ original\ of\ sample} x100\%$$

2.3. Antifungal Activity

The antifungal activity tests were performed using agar diffusion and broth microdilution methods. The micro agar dilution assay was used to determine Minimal Inhibitory Concentrations (MIC) and the Minimal Fungicidal Concentration (MFC). *C. albicans* was a clinical isolate randomly selected from a group of clinical isolates of the Candida species collected in our laboratory.

2.3.1. Agar Diffusion

The agar diffusion method was used to determine the inhibition of the antifungal activity of the test samples. The inoculum was prepared using 24-hour plate cultures of *C. albicans*. The colonies were suspended in PDB to produce a yeast suspension. The antifungal activity was performed using sterilized of 10 mL PDA. PDA media was poured into the petri dish and then was cooled to room temperature to solidify. As much as 200 µl prepared suspension was inoculated on the dried surface of agar plate using spread plate method over the entire sterile agar surface. The suspension was spread using sterile rod while rotating petri dish once in a while so that the spread is homogenous. Furthermore, holes were bored in the center agar plate, and the samples were added to a final volume of 20 µl. The plates were incubated at room temperature for 24h and then the inhibition zone form was observed. Ketoconazole at a concentration of 1% was used as a reference drug.

2.3.2. Minimal Inhibitory Concentrations (MIC)

The broth microdilution was implemented as explained in M27-A2 (CLSI) [16] with modifications. The medium used was PDB. N-Methyl chitosan samples were prepared in 0.5% acetic acid. The correct volume of the compound solution was placed in the microplate well with PDB medium. The compound was prepared with 100µl suspension cultures of *C. albicans* with each varying concentrations sample of 0.375%; 0.75%; 1.5%; 3% each well. The cell suspension was prepared in PDB. This suspension was inoculated in each well of a microdilution plate previously prepared with the sample. The plates were incubated at room temperature for 24 h. The Minimum Inhibitory Concentration (MIC) was determined by broth microdilution, The MIC for the sample was defined as the lowest concentration able to inhibit any visible fungal growth. Results were analyzed visually. The experiments were performed in triplicate.

2.3.3. The Minimal Fungicidal Concentration (MFC)

The MFC is tested to estimate fungicidal activity. It was defined as the lowest concentration of antimicrobial agent

needed to kill 99.9% of the initial inoculums after incubation [17, 18]. The MFC was defined as the lowest concentration at which no turbidity was visible after 24 h incubation at room temperature [19]. In this experiment, The MFC was determined by swabbing onto PDB of broth from each clear well. It is based on the result of MIC determination. Various concentration of each sample was prepared in 2%; 1.5%; 1%. MFC of ketoconazole was performed in the same procedure as the test compound. The experiments were performed in triplicate.

2.4. Application on The Gauze

2.4.1. Optimizing Dip Coating Techniques

Optimizing dip coating a technique was performed on around shaped sterilized gauze with 1.5 cm diameter and known weight. The optimizing process covers optimizing concentration, coating time and frequency. Five sheets of gauze were cut into round shape, then dipped each gauze into N-methyl chitosan solution with a various concentration of 0.2, 0.4, 0.6, 0.8 and 1% over 60 seconds for 10 times. After dipping the gauze, air-dried it until it is completely dry then placed in an oven at 100°C for 3 minutes and cured 150°C for 3 minutes.

Continue by dipping four sheets of round shaped gauze into optimum concentration sample solution with dipping time variation of 40, 50, 60, and 70 seconds. Dipping frequency is 10 times. After gauze was dipped then air dried then dried at 100°C for 3 minutes and cured at 150°C for 3 minutes. Store the coated gauze inside desiccators.

Get four sheets of round shaped gauzes and dip it individually into optimum N-methyl chitosan with concentration and time based on the previously optimized result. Dipping frequency for individual gauze is varied for 8, 9, 10, and 11 times in sequences. After being dipped, air dries the gauze until dry then dried at 100°C for 3 minutes and cured at 150°C for three minutes.

2.4.2. Antifungal Activity Against N-Methyl Chitosan-Coated Gauze

The procedure for activity testing is as follows: *Candida albicans* fungus is grown in liquid PDB for 24 hours. 200 µL liquid *C. albicans* cultures are distributed evenly on solid PDA medium. Afterward, the sample coated gauze on optimum condition of 1.5 cm size placed on solid medium. Each culture was incubated for 24 hours at room temperature, with triple treatment. The resistor area (clear zone around the sample coated gauze) was measured from each *C. albicans* for every sample solution. The clear zone around the gauze indicating the sample coated gauze has antifungal activity.

3. RESULTS AND DISCUSSION

3.1. Synthesis of N-methyl Chitosan

Alkyl group can be easily incorporated into amine group of chitosan using Schiff base intermediates. The first step in the synthesis of N-methyl chitosan is to dissolve chitosan into dilute acetic acid so that the reaction proceeds homogeneously. Formaldehyde solution was added into the chitosan solution. Chitosan condensation with aldehyde functional groups gives a Schiff base intermediate called aldimine [11]. The formation of Schiff base was followed by the reduction of an amine bond using $NaBH_4$ solution to produce N-methyl chitosan [20]. The choice of the reducing agent is important in this particular reaction because the reducing reagent must reduce imine selectively [11].

The reaction proceeds in two steps which are the formation of Schiff base and the reduction of the Schiff base as shown in Fig. (**1**). The product was precipitated and basified to pH 10 using NaOH. The formed gelatin was washed using distilled water. To determine the unreacted aldehyde, a qualitative test was performed using Fehling A and B reagents. The product was then dried in an oven to obtain N-methyl chitosan as a white powder.

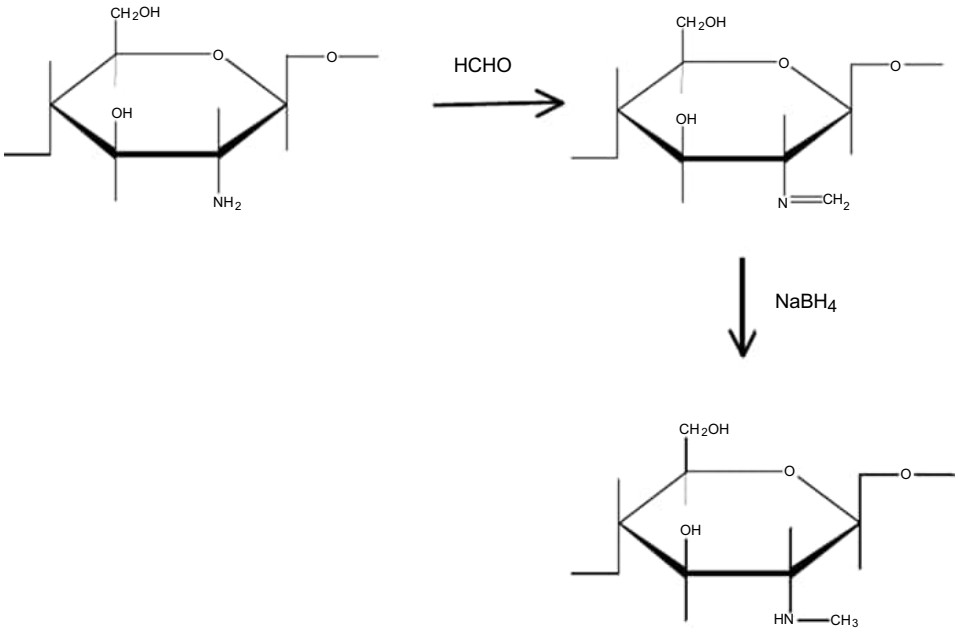


Fig. (1). The reaction of the formation and reduction of Schiff base in Chitosan.

Table 1. The assignment of the relevant bands of N-methyl chitosan and chitosan.

Assignments	Wave Number (cm^{-1})	
	Chitosan	N-methyl Chitosan
ν(O-H) overlapped to the ν_S(N-H)	3448	3425
ν(C-H)	2885	2877
ν(-C=O)	1597	1604
δ(C-H)	1419, 1381	1427,1381
ν_s(-CH3)	1327	1327
ν_{as}(C-O-C) andν_s(C-O-C)	1087, 1033	1072, 1033

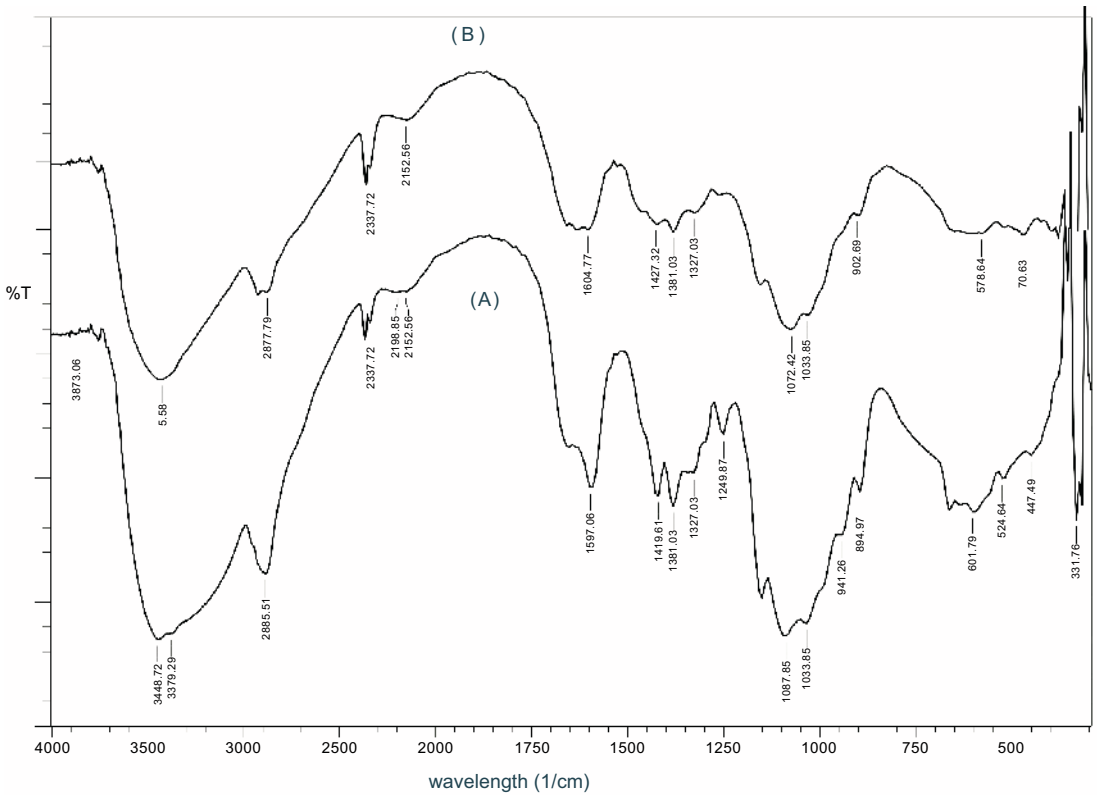


Fig. (2). IR spectra of (**A**) chitosan (**B**) N-methyl chitosan.

3.2. Characterization of the Synthesis Product

FTIR has often been used as a useful tool in determining specific functional groups or chemical bonds that exist in a material. The presence of a peak at a specific wave number would indicate the presence of a specific chemical bond [21]. IR studies were carried out for N-methyl chitosan and chitosan, and the spectra are given in Fig. (**2**). Table **1** showed the assignment of the relevant bands of N-methyl chitosan and chitosan.

Fig. (**3**) showed the diffraction patterns of chitosan and N-methyl chitosan. The diffraction pattern of chitosan showed characteristic peaks at 10.4º and 20.1º 2θ. In the spectrum of chitosan, it is obvious that chitosan is a crystal polymer to some degree. N-methyl chitosan showed similar diffraction patterns, where N-methyl chitosan peaks were displayed with less intensity. The spectrum of N-methyl chitosan showed the characteristic peaks at 8.9° and 19.2°, which suggest the formation of inter- and intra-molecular hydrogen bonds due to the presence of free amino groups in N-methyl chitosan.

Scanning Electron Microscopy (SEM) was carried out in order to study surface morphology and texture. The SEM photograph of N-methyl chitosan which clearly depicts the rugged nature of particles is depicted in Fig. (**4**). In this study, bio functionalized quantum dots were characterized using TEM for investigating the relevant morphological and structural features. The characterization of N-methyl chitosan using TEM is presented in Fig. (**5**). The TEM image shows that N-methyl chitosan produced from synthesis are round in shape.

Chitosan is a semi-crystalline polymer, a weak base, which is insoluble in water, alkali or aqueous solution above pH 7, and common organic solvents due to its stable and rigid crystalline structure. Chitosan is normally polydisper-

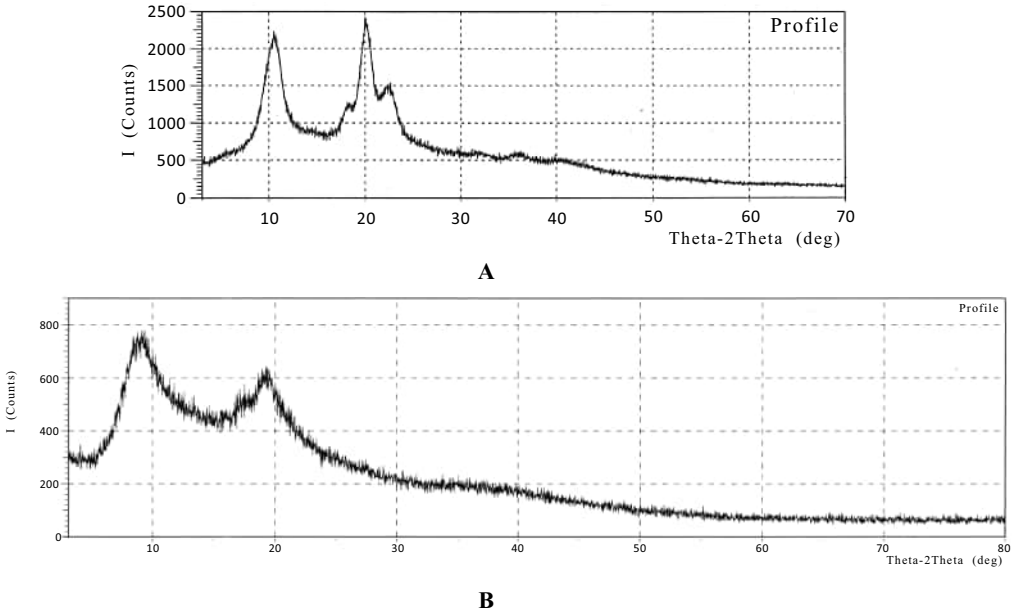


Fig. (3). XRD patterns of Chitosan (**A**) and N-methyl chitosan (**B**).

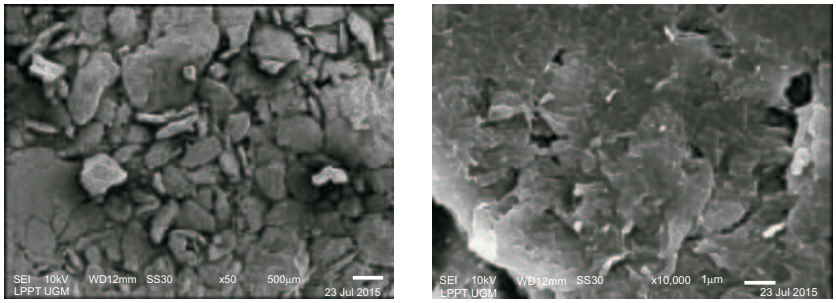


Fig. (4). SEM of N-methyl chitosan: (**A**) magnitude 50 (**B**) magnitude 10.000.

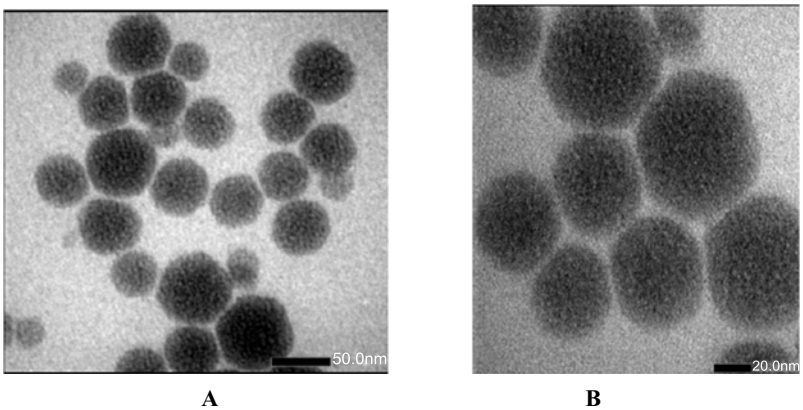


Fig. (5). TEM spectra of N-methyl chitosan: (**A**) view in 50 nm (**B**) view in 20 nm.

sed and has the ability to dissolve in certain organic acids such acetic acid at certain pH values after prolonged stirring. The investigation of chitosan dissolution characteristics revealed that its dissolution rate varies according to the type of acid used. Various studies were conducted to prepare chitosan derivatives with higher solubility properties by chemical modification techniques, such as N-methyl chitosan. The solubility for N-methyl chitosan was tested. The result shows that the solubility of N-methyl chitosan is greater than the solubility of chitosan. The solubility of N-methyl chitosan and chitosan in water was 2.04 mg/mL and 0.01 mg/mL, respectively. N-methyl chitosan has a solubility in the acetic acid is greater than chitosan. This is due to the CH_3 group which has positive inductive effect (driving toward N) so that the nitrogen atom in N-methyl chitosan become more basic than nitrogen atom on native chitosan, this renders N-methyl chitosan more soluble in acetic acid than chitosan.

The water content for N-methyl chitosan was measured and found to be up to 18.12%. This value is higher than the

water content of the chitosan which is up to 4.45%. The high water content suggested that N-methyl chitosan had stronger ability to bind with water than chitosan itself. This is due to the higher formation of hydrogen bonding between N-methyl chitosan with water molecules. It also can be due to residual water from the drying process in the synthesis.

N-Methyl chitosan was characterized using SAA to determine its specific surface area. The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms [22]. The result shows that the specific surface area of N-methyl chitosan is greater than the specific surface area of chitosan. The specific surface area of N-methyl chitosan and in chitosan was 0.995 m^2/g and 0.2234 m^2/g, respectively. The antibacterial properties are related to the total surface area of the nanoparticles. Smaller particles with larger surface to volume ratios have greater antibacterial activity [23]. The small size and the high surface to volume ratio *i.e*., a large surface area of the nanoparticles enhance their interaction with the microbes to carry out a broad range of probable antimicrobial activities [24].

3.3. Antifungal Activity

First antifungal activity test of N-methyl chitosan as anti-candidiasis was performed using agar diffusion method. Anti-candidiasis was also performed and compared 1% ketoconazole. The inhibition zone diameter of a clear zone was measured. Antifungal activity test of N-methyl chitosan was conducted in 3 concentrations namely: 0.5%, 1%, and 1.5%. The result of N-methyl chitosan anti-candidiasis test on *C. albicans* using agar diffusion method on PDA media is presented in Table **2**.

Based on the result on (Table **2**), N-methyl chitosan possessed an anti-candidiasis activity. This is shown by the clear zone around the hole. It shows that the growth of inoculated *C. albicans* was inhibited by N-methyl chitosan. The greater concentrations of N-methyl chitosan provide greater inhibition. N-methyl chitosan also gives greater inhibition zone when compared to the reference drug ketoconazole at the same concentration.

Derivatives of N-substituted chitosan through Schiff base reduction increased the microbial activity [25]. The structure of *C. albicans* consists of the cell wall and sterol membrane and cell membranes consisting double layer phospholipid [26]. The antifungal activity mechanism is believed to occur from the interaction between a cationic chain and negatively charged residue of macromolecules on the surface of the fungi cell, that leads to the leakage of intracellular electrolyte and other constituents [27], preventing nutrition transported to cell and further inhibit cell growth [6, 7].

The antifungal activity of N-methyl chitosan was also determined by minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) procedure against *C. albicans*. The MIC and MFC were determined at the 24 h as shown in Table **3**. N-methyl chitosan exhibited antifungal activity against *C. albicans* with MIC and MFC 0.75% and 1%, respectively.

Table 2. Antifungal activity Test of N-methyl chitosan using agar diffusion method.

No.	Treatment	Average Clear Zone Diameter (cm)
1.	N-methyl Chitosan 0.5%	3.315
2.	N-methyl Chitosan 1%	3.76
3.	N-methyl Chitosan 1.5%	3.835
4.	Ketoconazole 1%	2.5

Table 3. Comparative results of antifungal activity against *C. albicans* of N-methyl Chitosan by microdilution methods.

C+	C	MIC (%)				MFC (%)		
		0.375	0.75	1.5	3	1	1.5	2
								

C: Control with ketoconazole
C+: Positive control (100% growth)

3.4. Application on the Gauze

Gauze has thin and loose knitting and ease in absorption of fluid. Gauze coating with N-methyl chitosan was performed with a *pad-dry-cure method*. The *pad-dry-cure* techniques have allowed the sample dispersion into fabric fiber.

The dry phase allowed water evaporation and further solvent until reach condensation and gel form. The curing phase aimed to stabilize the thin layer of fabric due to advance condensation that caused the formation of polymer texture on water and solution free fabric. The high temperature on cure phase caused fixation of hydroxyl (-OH) in sample cluster and cellulose hydroxyl cluster (-OH) on fabric through van der Waals interaction between the –OH in the sample cluster and –OH fabric cellulose cluster was stronger [28].

Sample coating on gauze was performed on optimum condition. The parameter of optimized dipping condition covers solution concentration, time of dipping and the frequency. Optimum condition of a coating was determined by the weight of the sample coated gauze and diameter of resistor zone formed against *C. albicans*.

Optimizing sample concentration using concentration of 0.2, 0.4, 0.6, 0.8 and 1%. The sample was diluted into 0.5% acetic acid, with dipping time of 60 seconds, for 10 times. The result on gauze with sample concentration variation is displayed in Fig. (**6**). Fig. **6** shows that at the higher concentration, the more samples that are coated in gauze. The most coated gauze is at 1% concentration. Each individual dipping condition on optimized dipping concentration was further tested with anti-fungal activity on *potato dextrose agar* (PDA) media against *C. albicans* fungus. Fig. (**6**) also indicates the amount of resistor zone formed around the sample coated gauzed. The maximum clear zone formed for N-methyl chitosan 1% coated gauze is 0.42 cm diameter. The maximum clear zone produced by both sample occurred on optimum dipping concentration, this is due to the high amount of sample coated on gauze. Therefore, it can be confirmed that based on the number of samples coating the gauze also the diameter of formed clear zone, the optimum dipping concentration is 1%.

Optimum dipping time was determined on optimum dipping concentration (1%) and 10 times frequency. The result of gauze coating to dipping time variation is displayed in Fig. (**7**) which reveals that the longer dipping time resulted in a higher number of the sample coated on gauze which reaches an optimum point, before finally decreased. Optimum dipping time is 60 seconds. After reaching an optimum point, the individual sample coated gauze will experience a decrease.

One of the interactions between sample molecules and cellulose on gauze was electrostatic interaction [29]. Electrostatic interaction between δ^+ and δ^- on N-H polar covalent bond and O-H on sample has allowed hydrogen bonding formation with O-H on cellulose. The hydrogen bond is easily disconnected bond [30]. After reaching the optimum point, sample molecules will be released because it cannot maintain their interaction with cellulose. Sample molecules will attach and retracted to its similar sample molecules in form of solution, which causes a decrease in a number of the sample coated in gauze.

Each dipping condition on dipping time optimization was then tested for its anti-fungal activity on PDA media on *C. albicans* fungus. The clear zone formed on dipping time variation is displayed in Fig. (**7**). Fig. **7** indicates the maximum clear zone produced by N-methyl chitosan was at 60 seconds dipping time. The diameter of the formed resistor zone was 0.42 cm. After reached maximum point, the resistor zone will decrease once more. This is due to the number of sample coating the gauze were also decreased. The last optimization was the dipping frequency on 1% concentration and dipping time of 60 seconds. The result of optimized dipping frequency is displayed in Fig. (**8**). The optimization

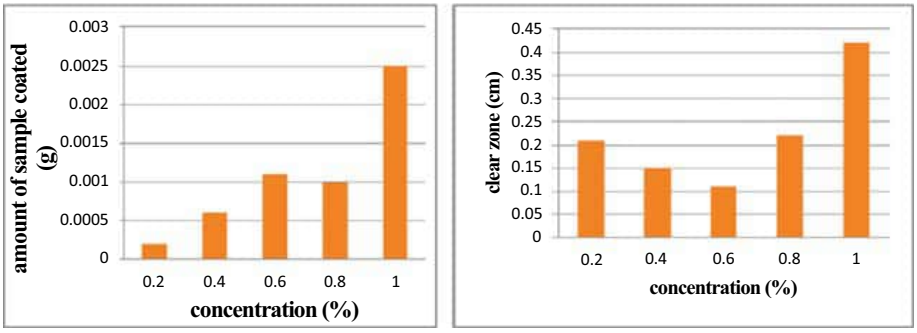


Fig. (6). Optimizing N-methyl chitosan concentration to gauze coating.

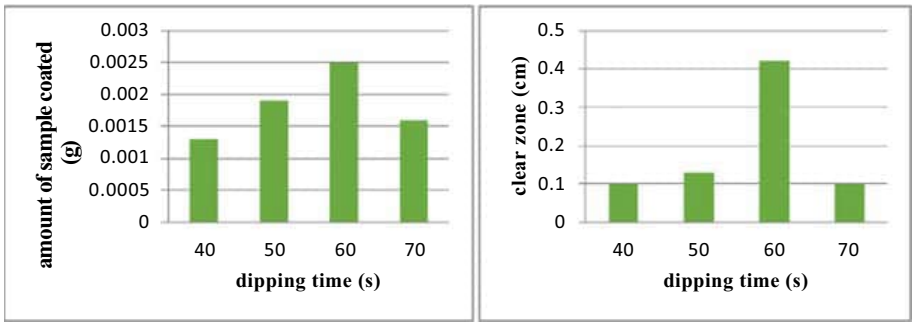


Fig. (7). Optimizing dipping time of N-methyl chitosan to gauze coating.

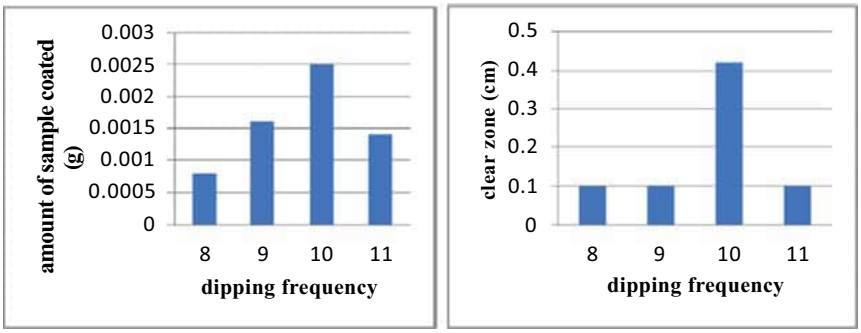


Fig. (8). Optimizing dipping frequency of N-methyl chitosan to gauze coating.

result indicating that the optimum dipping frequency was 10 times. The formed clear zone diameter was 0.42 cm.

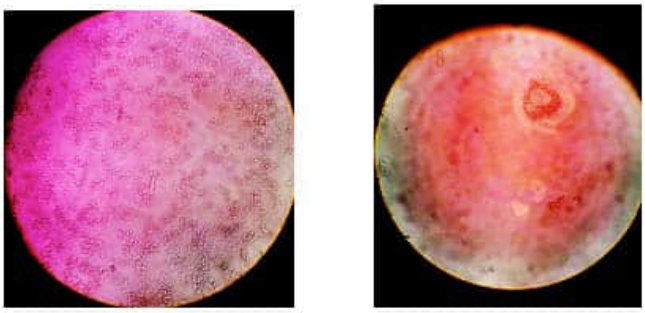
3.5. Antifungal Activity on N-Methyl Chitosan Coated Gauze

Antifungal activity testing for N-methyl Chitosan-coated gauze to *C. albicans* performed with agar diffusion method with PDA media. The antifungal activity testing was also performed on chitosan for comparison; acetic acid was used as a solvent control and on non-coating gauze as a blank.

The result obtained by the diffusion method showed a clear zone diameter that blocked the growth of *C. albicans* and was measured. The gauze clear zone coated with N-methyl chitosan was 0.36 cm while that of chitosan coated gauze was 0.15 cm. The greater the clear zone diameter, the higher antifungal activity [31]. Acetic acid coated gauze and non-coating gauze did not show antifungal activity.

Chitosan derivatives antifungal activity is due to the interaction between cationic N-methyl chitosan chain and the negative macromolecule residue on the fungal surface that led to intracellular leak and other constituents in a cell [27]. This interaction might also cause inhibition of function of the cell wall that causes disorder in metabolism, growth, and cell reproduction led to cell death. N-methyl chitosan has greater antifungal activity when compared with chitosan because of the presence of alkyl substituent on chitosan molecules. Antimicrobial N-Alkyl Chitosan activity will alongside the greater length of alkyl substituent chain on chitosan molecules as a contributing result of improvement in chitosan hydrophobic characteristic [32]. On condition during extremely low NH_2 protonated and causing NH_3^+ repulsion weaker, intermolecular will form micro-area hydrophobic on polymer chain that causes the formation of hydrophobic and hydrophilic parts that increase the structural affinity between the cell wall and chitosan derivatives [33]. The increase of affinity will lead to an increase in chitosan derivatives antimicrobial activity.

N-methyl chitosan was proven capable in a block the growth of *C. albicans* fungal growth marked by the clear zone around the sample coated gauze. The sample influence on *C. albicans* was visible through a microscope (400x magnification). The observation was performed by obtaining a small part of agar on the clear zone and non-clear zone for observation under a microscope. The observation result of *C. albicans* on PDA media is displayed in Fig. (**9**).



(A) **(B)**

Fig. (9). (**A**) *C. albicans* fungal on PDA media, (**B**) clear zone form by N-methyl chitosan.

Based on Fig. (**9**), N-methyl chitosan was proven capable of hampering the growth of *C. albicans*. *C. albicans* cell on PDA media without sample influence was in great number and seen on all lenses angle. While the *C. albicans* cell on clear zone formed by N-methyl chitosan was in smaller number and only appear in several parts. This might occur because of interaction formed between chitosan derivatives cationic chain with negative capacity on *C. albicans* cell surface (cell wall, sterol membrane, cell membrane) that cause inhibition in membrane permeability and *C. albicans* cell metabolism system led to cell death.

Characterization of gauze coated with N-methyl chitosan includes characterization with X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM). Based on Fig. (**10**), N-methyl chitosan showed similar diffraction patterns, where N-methyl chitosan peaks were displayed with less intensity. There is a possibility of N-methyl chitosan on gauze surface.

Based on the visual analysis and SEM photograph (Fig. **11**) difference appear between fabric without treatment and N-methyl chitosan coated fabric. Morphology of the fabric fiber before treatment was soft and well distributed. The distribution pattern of fabric surface was homogeneous. While for fabric with N-methyl chitosan was rough and not well distributed. This due to the coating process by N-methyl chitosan. The fabric looks more coarse compared to control fabric. The coarse surface was due to the interaction between the fabric fiber and N-methyl chitosan.

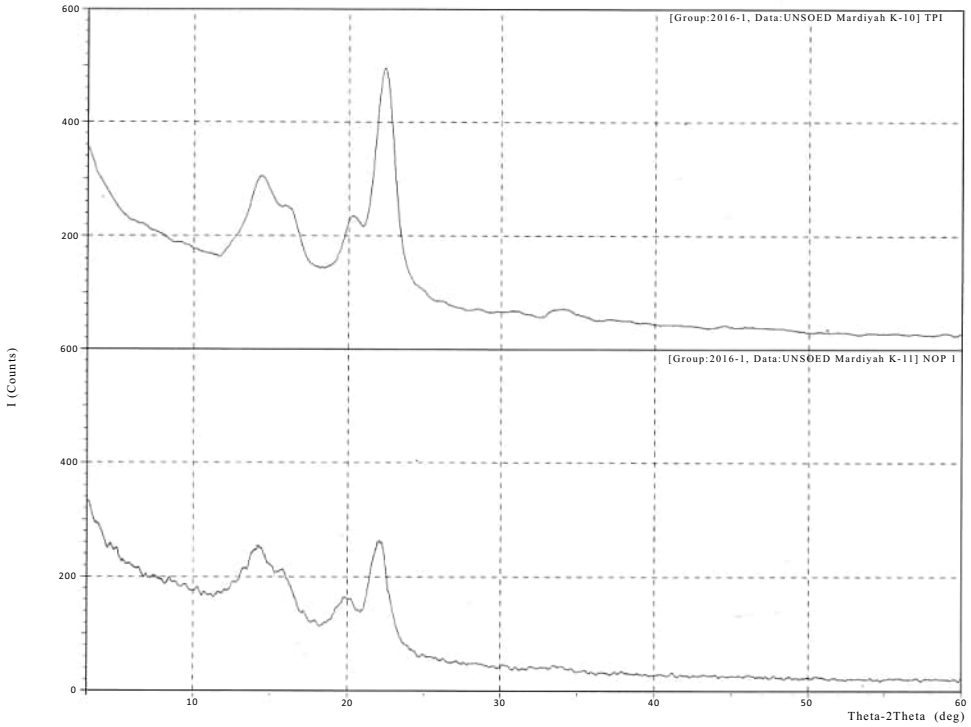
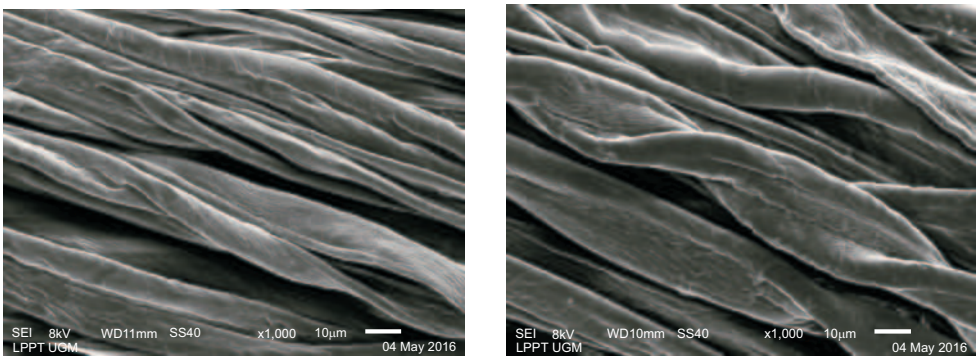


Fig. (10). XRD patterns of (**A**) gauze and (**B**) N-methyl chitosan coated gauze.



(A) **(B)**

Fig. (11). SEM of (**A**) gauze and (**B**) N-methyl chitosan coated gauze.

CONCLUSION

In this study, the synthesis of N-methyl chitosan and antifungal activity of *C. albican*s and its application on the gauze has been investigated. The results showed that the N-methyl chitosan has antifungal activity as well as gauze coated with N-methyl chitosan. However, further studies are needed to pursue the evaluation of the antimicrobial activity of other organisms.

LIST OF ABBREVIATION

PDA	=	Potato Dextrose Agar
PDB	=	Potato Dextrose Broth
FTIR	=	Fourier Transform Infra-Red
XRD	=	X-Ray Diffractometer
SAA	=	Surface Area Analyzer
TEM	=	Transmission Electron Microscopy
SEM	=	Scanning Electron Microscopy
MIC	=	Minimal Inhibitory Concentrations

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The present work was supported by the Ministry of Research, Technology, and Higher Education (RISTEKDIKTI) Indonesia through *Hibah Bersaing* Grant 2015-2016.

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