Research Article

Synthesis, Characterization and Biological Evaluation of Chitosan epoxy n-methyl piperazine as Antimicrobial Agent

Richa Chauhan*, Sangeeta Loonker
Department of Chemistry, Jai Narain Vyas University, Jodhpur (Rajasthan), India.
***Corresponding author's E-mail:** richaachauhan10@gmail.com

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ABSTRACT

A naturally occurring linear polysaccharide matrix of chitosan was cross linked with epichlorohydrin and then n-methyl piperazine was incorporated in it for the synthesis of novel CE-MP. The newly synthesized CE-MP was characterized by XRD, Mass spectra, FT-IR, 1HNMR and elemental analysis. Other physicochemical characteristics like moisture content, water regain, bulk density and bulk volume were also studied. The antimicrobial activity was carried out by well diffusion method. The antimicrobial activity of the newly synthesized against some pathogenic bacteria such as *E.coli, Pseudomonas aeruginosa*, *Klebseilla pnemoniae, Staphylococcus aureus* and some strains of fungi such as *C. auris, Geotricum candidum, C. kefyr, C. tropicalis, C. glabrata, Rhodoturula, C. parapsilosis, C. albicans and C.krucei* were evaluated.

Keywords: CE-MP, antimicrobial activity, antibacterial activity, antifungal activity and inhibition zone.

INTRODUCTION

Chitosan is a linear polysaccharide composed of β-(1→4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance.[1] Chitosan is the naturally occurring and renewable polymer which have properties of biodegradability, bio-compatibility, non-toxicity, and adsorption.[2] Most of the naturally occurring polysaccharides are acidic in nature whereas chitosan is a basic polysaccharide. Chitosan has a good ability of forming complexes with metal ions and hence has its application in various fields like in industrial waste water treatment,[3] cell immobilization process for biotechnology and food industry,[4,5] as an antimicrobial agent,[6] in cosmetic and pharmaceutical industries,[7] it also acts as an agent in forming fibres, films and membranes etc.[8-10]

In present society microbial infections are a great concern because they are one of the primary causes of death worldwide, especially in healthcare institutions as here patients are more prone to diseases.[11-13] The persistence of potentially pathogenic microbes (bacteria, viruses and fungi) are in several locations such as textiles, healthcare products, medical devices, water purification systems, sanitation facilities etc[14] and it may be lethal to health. Chemically modified chitosan showed greater antimicrobial activity than crude chitosan.[15] The silver nanoparticles of chitosan also act as an antimicrobial agent against different bacteria and fungi.[16,17]

The aim of the present investigation is to synthesize CE-MP and to study the antimicrobial activity of the newly synthesized against some pathogenic bacteria such as *E.coli, Pseudomonas aeruginosa*, *Klebseilla pnemoniae, Staphylococcus aureus* and some strains of fungi such as *C. auris, Geotricum candidum, C. kefyr, C. tropicalis, C. glabrata, Rhodoturula, C. parapsilosis, C. albicans* and *C.krucei.* The novel CE-MP was characterized by XRD, Mass spectra, FT-IR, ^{1}HNMR and elemental analysis. Other physicochemical characteristics such as moisture content, water regain, bulk density and bulk volume were also studied.

MATERIALS AND METHODS

Chitosan (100 mesh size) was obtained from local industry. All AR grade chemicals used were procured from Sigma, Loba Chemicals and Ases Chemical Works. The microbial strains were procured from Dr. S. N Medical College, Jodhpur (Rajasthan), India. The newly synthesized CE-MP was characterized using XRD (Bruker D8 advance with Cu K α radiation {λ=1.54Å} as x-ray source), Mass spectrometry (Thermo Scientific TSQ 8000 Gas Chromatograph), FTIR (ALPHA EX Bruker spectrophotometer in range of 400 cm^{-1}) and 1HNMR (Bruker Advance-500MHz).

Synthesis of Chitosan epoxy n-methyl piperazine (CE-MP)

The synthesis of CE-MP is carried out in two steps:

A. Preparation of epoxy ether of chitosan:

Chitosan (0.02 moles) was soaked in dioxane in a round bottom flask for one and a half hour then epichlorohydrin (0.04 moles) and sodium hydroxide(0.04 moles) were added in it, with continuous stirring. The round bottom flask was then sealed and kept in an oven for 5 hours at 50°C. Then the product formed was filtered on a vacuum pump and was washed with aqueous methanol containing nitric acid. And finally after washing with solvent ether, it was oven dried at 50°C for 2 hours. The crosslinked chitosan thus formed was used for further derivatization.

B. Synthesis of Chitosan epoxy n-methyl piperazine:

The epoxy derivative of chitosan was slurried in dioxane and then 50% aqueous sodium hydroxide (NaOH) was added to adjust the pH to 9-10, with continuous magnetic stirring at 50°C under reflux. Afterwards 0.01 moles of n-methyl piperazine was added to the reaction mixture with continuous magnetic stirring at 55°C. Finally the whole reaction mixture was filtered and washed with absolute methanol and a few drops of nitric acid. Then the resultant formed was dried under vacuum. (Figure 1)

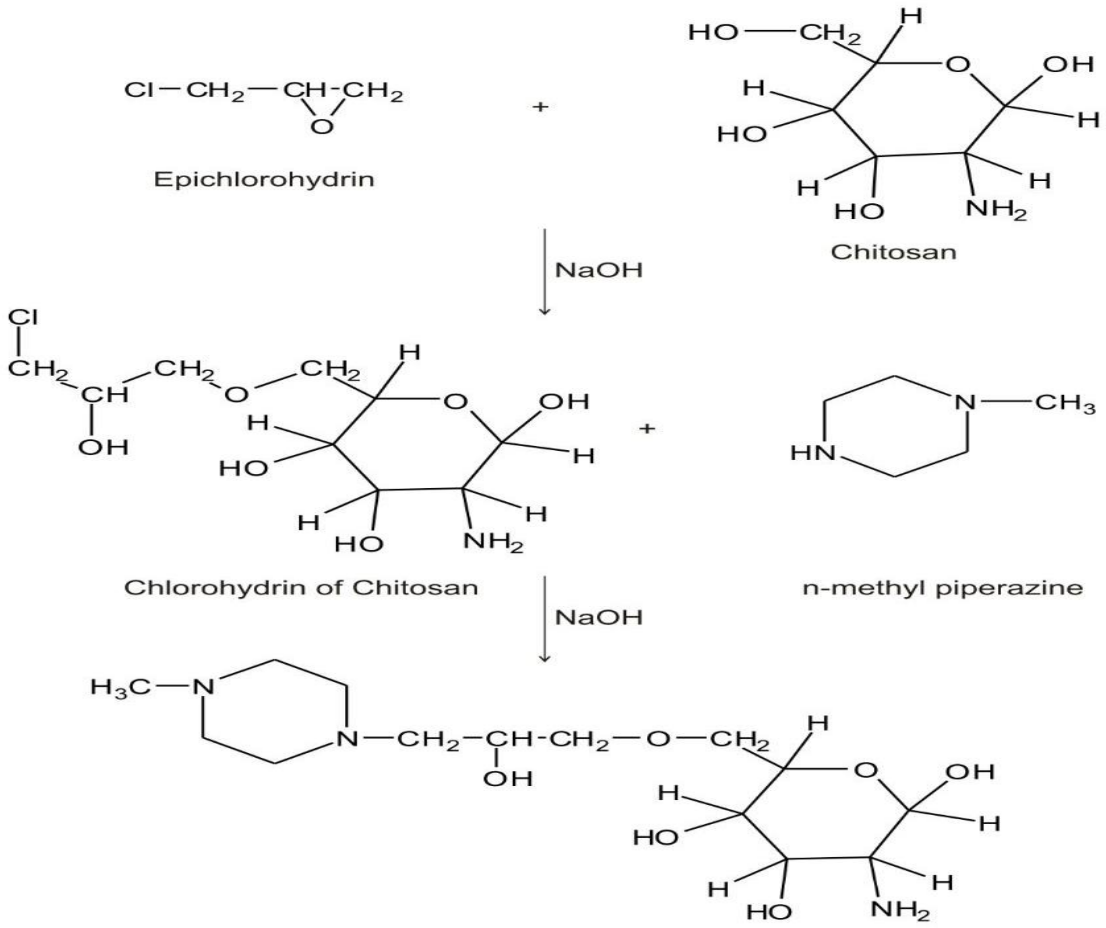

Figure 1: Synthesis of CE-MP

Characterization of newly synthesized CE-MP

The novel CE-MP formed was characterized using XRD (Figure 2), Mass spectra (Figure 3), 1HNMR (Figure 4) and FT-IR (Figure 5). Other physicochemical characteristics like swelling and water regain, moisture content, elemental analysis, bulk density and bulk volume were also studied. (Table 1)

Table 1: Physicochemical characteristics of newly synthesized derivative of Chitosan (CE-MP)

S.No.	Characteristic property	CE-MP
1	Water regain	0.32
2	Moisture content	1.4%
3	Bulk volume	4.0 cc/g
4	Nitrogen content (%)	25.5%
5	Oxygen content (%)	23.6%

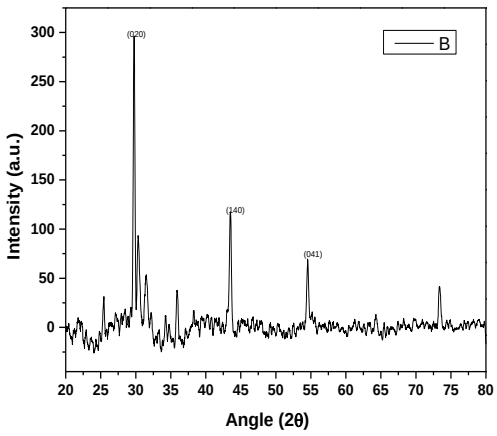

Figure 2: XRD spectra of CE-MP

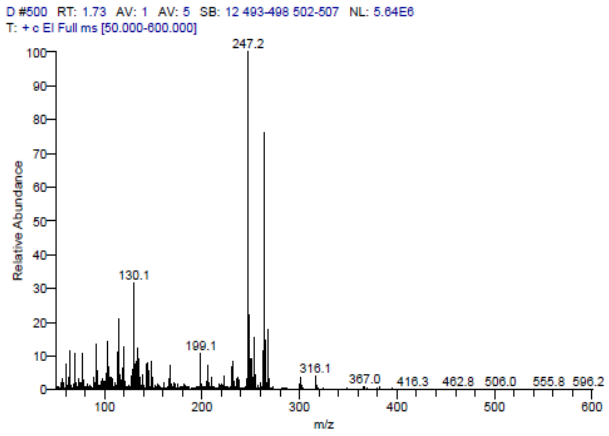

Figure 3: Mass spectra of CE-MP

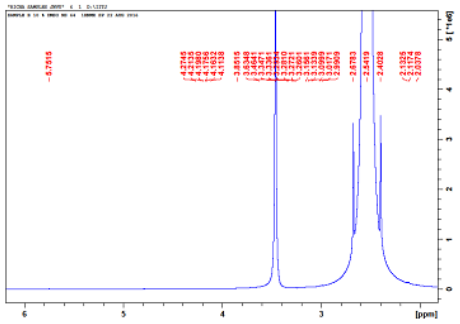

Figure 4: 1HNMR spectra of CE-MP

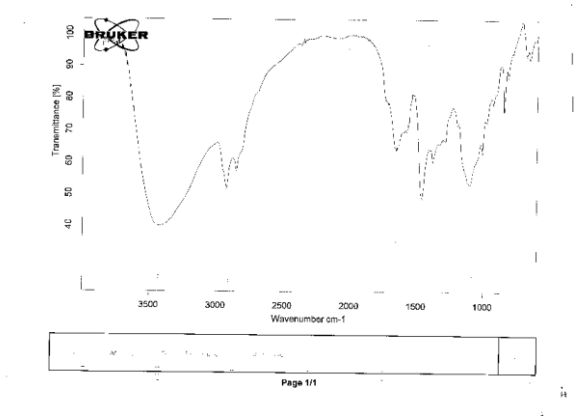

Figure 5: FT-IR spectra of CE-MP

Antimicrobial activity

The pathogenic bacterial and fungal strains used, were isolated from Dr. S.N Medical College, Jodhpur (Raj), India. They were maintained as pure culture in specific agar medium and were preserved by sub culturing every 6 month. The present study includes the study on pathogenic bacteria and fungi and its susceptibility to the newly synthesized derivative. If certain bacteria and fungi are resisted by any antimicrobial agent then it stops the growth of that particular microbe and a zone is created where the growth of bacteria or fungi is not visible, this zone is called as zone of inhibition and it depicts the ability of an antimicrobial agent to inhibit the growth of microorganisms. The pathogenic strains of bacteria used in the present study are *E.coli, Pseudomonas aeruginosa, Klebseilla pnemoniae*, *Staphylococcus aureus* and the strains of fungi used are *C. auris, Geotricum candidum , C. kefyr, C. tropicalis, C. glabrata, Rhodoturula, C. parapsilosis, C. albicans* and *C.krucei.*

Preparation of inoculum

Pure isolate of each fungi and bacterium was firstly sub cultured in nutrient broth at 37°C for 24h. Approximately 100 microlitres of standard inoculums of each strain of bacterium and fungi was spread evenly over a nutrient agar plate using a sterile swab.

Antimicrobial activity using well diffusion method

In well diffusion method, a hole of certain diameter was punched with a cork borer on the sterile nutrient agar plate. Further the extract of CE-MP was filled in the holes using micropipette, then the agar plates was incubated under suitable conditions depending on the test microorganism i.e. fungi or bacterium. The extract of CE-MP diffuses in the agar medium. If the bacterial or fungal strain is susceptible to the extract of CE-MP, then a zone of inhibition appears on the agar plate. The diameter of inhibition zone formed was measured in mm with a ruler and the results were recorded. (Table 2 and Table 3)

Table 2: Inhibition zone of CE-MP against pathogenic bacteria

S.no	Bacteria used	Inhibition Zone
1	*Escherichia coli*	-ve
2	*Psuedomonas aeruginosa*	2mm
3	*Klebseilla pnemoniae*	2mm
4	*Staphylococcus aureus*	2mm

Order of activity: 1<2=3=4

Table 3: Inhibition zone of CE-MP against some fungi

S.no	Fungi used	Inhibition Zone
1	*Candida auris*	-ve
2	*Geotricum candidum*	7mm
3	*Candida kefyr*	2mm
4	*Candida tropicalis*	-ve
5	*Candida glabrata*	-ve
6	*Rhodoturula*	4mm
7	*Candida parapsilosis*	8mm
8	*Candida albicans*	2mm
9	*Candida krucei*	4mm

Order of activity: 1=4=5<3=8<6=9<2<7

RESULTS AND DISCUSSION

The characteristic property of newly synthesized CE-MP is shown in tabular form (Table 1) which gives the information about its physicochemical property. Spectral analysis gives the idea about the structure and information of the functional group incorporated in the newly synthesized derivative of chitosan.

Spectral analysis

XRD interpretation *(figure 2):*

The XRD data indicates that n-methyl piperazine is loaded onto the surface of epoxy ether of chitosan. The spectra reveals that the structure of CE-MP resin is triclinic and its crystalline size is 84.1Å.

Mass interpretation *(figure 3):*

M+ (247.2)

^{1}HNMR interpretation *(figure 4):*

The signal at δ= 3.29 appears due to — (CH_2 — O — R) moiety. Signal at δ = 2.6 appears due to CH_3—N< moiety. Signals at δ = 2.40 and δ = 1.72 occurs due to the presence of

—CH_2—CH_2—N< & NH(—CH_2)(—CH_2—) moieties respectively.

FT-IR interpretation *(figure 5):*

FT-IR spectrum of CE-MP resin shows a strong peak at 3420.01 cm^{-1} which is characteristic peak for N-H stretching. The band at 2923.12 cm^{-1} is for C-H stretching vibrations. Peak at 1468.88 cm^{-1} corresponds to C-N stretching vibrations. Peaks of 1102.40 cm^{-1} and 1070.12 cm^{-1} attributes to C-O stretching in secondary alcohol and C-O stretching in ether respectively.

Antimicrobial Activity

The results of the antimicrobial activity of the newly synthesized CE-MP against bacterial and fungal strains are illustrated in Table 2 and Table 3. The present study shows that different micro-organisms have different ability to respond towards novel CE-MP, so zone of inhibition of different diameter were recorded for different micro-organisms. In the present study CE-MP shows significant antibacterial and antifungal activity. CE-MP showed small inhibition zone for *Pseudomonas aeruginosa*, *Klebseilla pnemoniae* and *S.aur*eus while *E.coli* showed no zone of inhibition. Some fungi like *C. keyfr, Rhodoturula, C. parapsilosis, Geotricum candidum*, *C. krucei* and *C. albicans* showed positive inhibition zone while *C. auris, C. tropicalis, C. glabrata* showed no inhibition zone. The results of the present study are quite encouraging as it exhibited antimicrobial activity against pathogens.

CONCLUSION

The approach of the present study was to synthesize a new derivative of chitosan, CE-MP which is environment friendly in nature and also to evaluate antibacterial and antifungal activities. From the results recorded, it can be concluded that CE-MP shows significant antimicrobial activity against bacteria and fungi and this application can be of great commercial use.

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