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Antibacterial, antioxidative, biological activity, etc.

CHARACTERISATION OF PREPARED CEFIXIME AND METRONIDAZOLE WITH CHITOSAN NANOPARTICLE FOR PHARMACEUTICAL APPLICATION

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ABSTRACT

Naturally existing polymer compounds, particularly polysaccharide polymers, have been employed in pharmaceuticals to deliver a wide range of therapeutic medicines. Next to cellulose-β-(1,4) linked N-acetyl-D-glucosamine units, chitosan is the second most prevalent naturally occurring polysaccharide. It is a mucoadhesive polymer that is biocompatible and biodegradable and has been widely used in the creation of both micro- and nanoparticles. Natural polymers made of randomly arranged N-acetyl-D-glucosamine (acetylated unit) and deacetyl-D-glucosamine (deacetylated unit) are chitin and its deacetylated derivative chitosan. Due to the free protonable amino groups found in the D-glucosamine units, chitosan is soluble in acidic environments whereas chitin is insoluble in aqueous media. Chitosan is a particularly promising source for pharmaceutical application. Applications of chitosan as a wound dressing material include development of antimicrobial agents, chitosan bandage for wound healing, suitable for starting material for wound dressings, its hemostatic effect -ability to inhibit microbial growth. Chitosan is biodegradable non-toxic natural polymer that promotes wound healing and can be used as antibiotics for bacterial control by chitosan-based wound dressings. Due to its inherent antibacterial qualities and capacity to transfer extrinsic antimicrobial agents to wounds and burns, chitosan is used to treat wound and burn infections.

Keywords: chitin, biocompatibility, biodegradable, wound healing.

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AIMS AND BACKGROUND

Nanotechnology, dealing with materials at the nanoscale (one hundredth of a millimeter or smaller), has gained widespread application in various fields, including electronics, biotechnology, and drug delivery[1]. Due to its wide range of uses, it has a considerable social influence. Richard P. Feynman introduced the idea of nanomaterials in 1959 and stressed the advantages of operating at the nanoscale[2]. Due to the high surface-to-volume ratio of nanomaterials, unique nanostructures with distinctive features evolved as nanotechnology advanced[3,4]. The importance of nanoscale processes in life makes them interesting for biomedical applications. Nanotechnology has the potential to transform technology and improve many facets of our life. Along with cellulose, chitin is the most prevalent biological macromolecule[5,6].

Chitin is an insoluble linear polymer made up of -(1,4) connected N-acetyl-D-glucosamine units that is present in coral, bryozoans, algae, fish scales, mollusk shells, sponges, and the exoskeletons of insects and crustaceans[7]. Three distinct chitin polymers that occur in nature distinguish them. Natural chitin is recognised as a suitable functional material with great features such as biocompatibility, biodegradability, non-toxicity, and adsorption that can be used in medicine, cosmetics, textiles, wastewater treatment, and agriculture[8,9].

Chitosan nanoparticles (CNPs), which are flexible and have a high safety profile, are becoming more and more common in a variety of industries, including healthcare, pharmaceuticals, agriculture, and the environment[10–13]. Because of their biocompatibility and biodegradability, CNPs are preferred for drug delivery because they are suitable for non-parenteral drug delivery in treating a variety of diseases[14–17]. Chitosan is a natural polymer having these characteristics, making it a good contender for a variety of potential purposes[18,19]. It is regarded as a safe and promising chemical in nanomedicine applications[20].

EXPERIMENTAL

The *Pila mizoramensis* shells were gathered in Villupuram from the lakeshore and agricultural areas. They were cleaned, dried, and ground into powder. Chitosan nanoparticles are made by combining acetic acid (2%), sodium tripolyphosphate (0.25%). To prepare the chitosan bandage, antibiotics Metronidazole (10 mg/ml) and Cefixime (10 mg/ml) were employed.

METHODS

Isolation of chitin from snail shell. The samples were collected, washed with freshwater, dried, and ground into powder. Demineralisation and deproteinisation were used to extract the chitin from *Pila mizoramensis* snail shell waste. The shell powder was demineralised by combining it with 1 M HCl at 60°C for 30 min, washing it with water to a pH of 7, and then drying it. The demineralised shell powder then underwent

120 min of deproteinisation using 3 M NaOH at 80°C, followed by filtration, rinsing, and drying to the required chitin level[21].

Conversion of chitin to chitosan. In the beginning, chitin was precisely deacetylated into chitosan using chitin powder and 50% NaOH solution[22]. Then, sodium TPP was added, 1% chitosan was dissolved in 2% acetic acid, and the mixture was allowed to go through an ionic gelation process to create chitosan nanoparticles[23]. With the use of UV-Vis. spectrophotometry, FTIR analysis, and X-ray diffractometry, these nanoparticles were thoroughly characterised, and scanning electron microscopy was used to look at their surface characteristics and morphology[24]. Next, by mixing, agitating, and adding Sodium Tripolyphosphate (STPP), chitosan was combined with antibiotics (Cefixime and Metronidazole) to form antibiotic-loaded chitosan nanoparticles[25]. In the end, a chitosan bandage was created by coating bandage fabric with a chitosan and antibiotic solution and letting it air dry for 15 min.

Antimicrobial activity of chitosan antibiotic bandage. The efficiency of chitosan antibiotic bandages against bacteria and fungi was evaluated in the study. By putting these bandages on nutrient agar plates with bacterial cultures and looking at inhibitory zones after incubation at 37°C, we were able to assess their antibacterial effectiveness against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *E. coli*. Cotton bandage discs without nanoparticles were used as a control. Using a similar method and overnight fungal cultures, the bandages were also tested for antifungal activity against *Candida albicans* and *Aspergillus niger*. Following incubation, inhibitory zones were seen once more, and cotton bandage discs devoid of nanoparticles served as a control[26].

In vitro cytotoxicity of chitosan antibiotic bandage. Using the MTT technique, sample cytotoxicity against Vero cells was evaluated. Vero cells were grown, incubated for 48 h, and then exposed for 24 h to various sample concentrations. Under a microscope, the modifications to the cell's morphology were noted before the addition and incubation of the MTT solution. To assess cytotoxicity, formazan crystals were dissolved, and 570 nm absorbance was measured[27].

Percentage of cell viability was calculated using the formula:

$$\text{Cell viability (\%)} = (\text{Absorbance of sample}/\text{Absorbance of control}) \times 100.$$

In vitro wound healing of chitosan antibiotic bandage. Vero cells were cultivated in accordance with a set methodology. In ideal circumstances, they were allowed to reach 90–95% confluence after being sown in 6-well plates. The cell monolayer was mimicked wounded and cleaned of contaminants. A sample (50 g/ml) was applied to the wound for 24–48 h at 37°C with 5% CO_2. Cells acting as negative controls were not treated. Two methods were used to determine if the wound had healed: assessing the difference in wound widths between time points using ImageJ software to determine the scratch closure rate (SCR), and taking digital photos at various time intervals using a digital inverted microscope[28].

RESULTS AND DISCUSSION

ISOLATION OF CHITIN FROM SNAIL SHELL

The samples of snail shells were collected, washed and dried (Figs 1–3). Then the shells were made into powder. The isolation of chitin is a two-step process:

1. Demineralisation
2. Deproteinisation

Figures 4 and 5 show the deproteinised protein powder and Chitosan powder, respectively.

Fig. 1. Shells

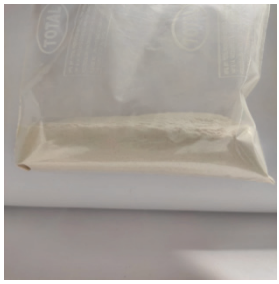

Fig. 2. Shell powder

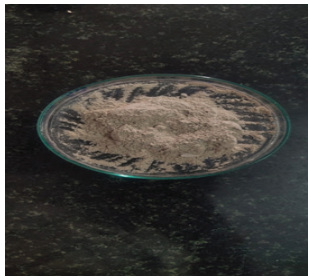

Fig. 3. Decalcified powder

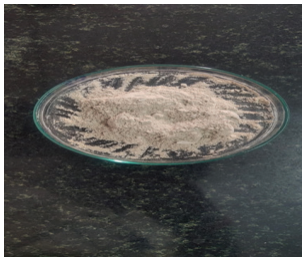

Fig. 4. Deproteinised powder

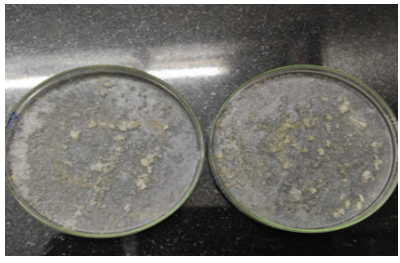

Fig. 5. Chitosan

SYNTHESIS OF CHITOSAN NANOPARTICLES

The chitosan nanoparticles were synthesised by the Ionic gelation method (Fig. 6).

Fig. 6. Chitosan nanoparticles

UV analysis. The NPs were first examined using the UV-Vis. spectrophotometric method (Fig. 7). The formation of CNPs was monitored using visual and UV visible spectroscopy. The wavelength was scanned from 200 to 800 nm, and peaks at 225 nm were obtained from CNPs (Fig. 8) indicating the presence of chitosan nanoparticles.

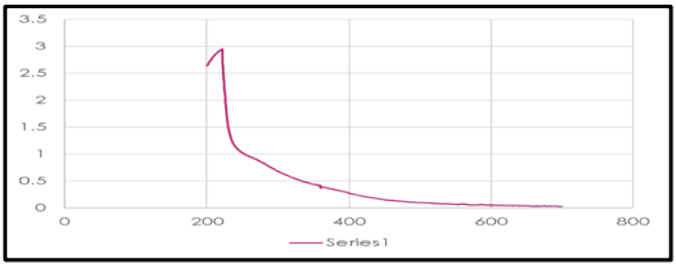

Fig. 7. UV graph

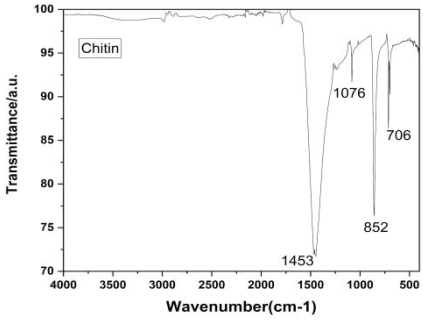

Fig. 8. FTIR for chitin

FTIR analysis. The FTIR spectroscopy was seen in the range 400–4000 cm^{-1}.

The sample's spectrum analysis offers important information. There is a weak NH bending signal at 1453 cm^{-1}, which points to the existence of particular molecular bonds. The bonding within the Pyranose ring is represented by the medium intensity peak at 1076 cm^{-1}. Furthermore, the different molecular interactions are highlighted by the prominent peaks at 852 and 706 cm^{-1} (Fig. 9), which signify significant C–O–C stretching.

Numerous molecular interactions are found in the sample according to the spectrum analysis. H-bonded OH stretching (3387 cm^{-1}), CH stretching (2625 cm^{-1}), aliphatic CH stretching (1704 cm^{-1}), NH stretching (1641 and 1383 cm^{-1}), C–O stretching (1013 cm^{-1}), and NH bending (1264 cm^{-1}) are among the important characteristics. The combination of these results offers a thorough insight of the molecular structure and make-up of the material.

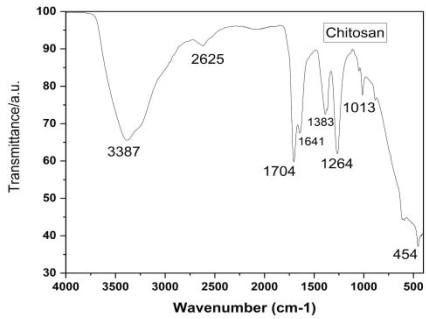

Fig. 9. FTIR for chitosan

XRD analysis. The XRD analysis for chitosan nanoparticles was done.The diffraction pattern of native chitosan indicates that the chitosan nanoparticles have an ordered crystalline structure as shown in Fig. 10.

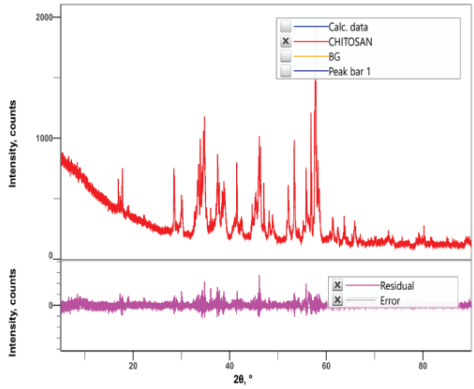

Fig. 10. XRD analysis

SEM analysis. The study of Chitosan nanoparticles using scanning electron microscopy produced important results. The nanoparticles' size range of 73 to 98 nm, showing their nanoscale dimension, was observed. The SEM scans also showed that their shape had a porous and layered character as shown in Fig. 11.

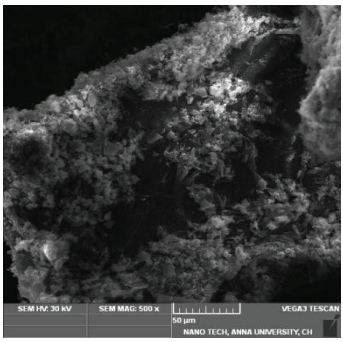

Fig. 11. SEM of chitosan nanoparticles

INCORPORATION OF CHITOSAN WITH CEFIXIME AND METRONIDAZOLE

Figures 12–15 show the incorporation of chitosan with cefixime and metronidazole.

Fig. 12. Metronidazole

Fig. 13. Cefixime

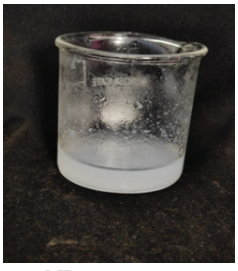

Fig. 14. Chitosan NPs

Fig. 15. Chitosan NPS conjugated with Cefixime and Metronidazole

PREPARATION OF CHITOSAN ANTIBIOTIC BANDAGE

The chitosan bandage was prepared and air dried for 15 min. Later it is used for various analysis (Figs 16 and 17).

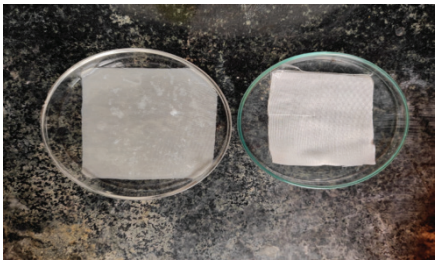

Fig. 16. Chitosan antibiotic bandage

Fig. 17. Chitosan antibiotic bandage

ANTIMICROBIAL ACTIVITY OF CHITOSAN ANTIBIOTIC BANDAGE

The chitosan nanoparticles showed effective antibacterial activity results (Figs 18–20) and zone of inhibition.

Antibacterial activity

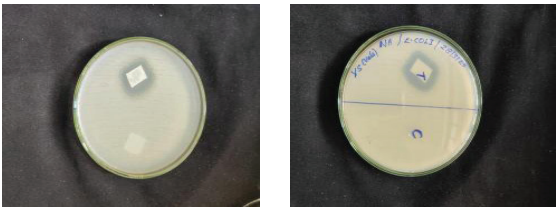

Fig. 18. *E. coli*

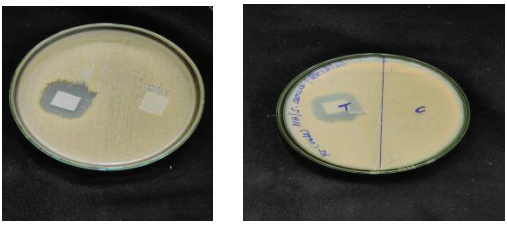

Fig. 19. *Staphylococcus aureus*

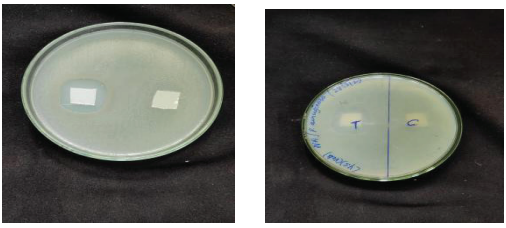

Fig. 20. *Pseudomonas aeruginosa*

RESULTS AND DISCUSSION

Table 1 details the substance's antibacterial effectiveness against various bacteria. A 25 mm zone of inhibition for *E. coli* indicates a potent antibacterial action and is indicated by the word "yes". A similar zone of inhibition of 22 mm is seen for *Staphylococcus aureus*, indicating a positive antibacterial response. The 25 mm zone of inhibition displayed by *Pseudomonas aeruginosa* suggests a potent antibacterial capacity. Based on the available evidence, it appears that the compound effectively combats the three microorganisms that were tested: *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Table 1. Antibacterial activity results

S. No	Bacteria	Zone of inhibition (mm)	Antibacterial activity
1	*Escherichia coli*	25	yes
2	*Staphylococcus aureus*	22	yes
3	*Pseudomonas aeruginosa*	25	yes

Antifungal activity. The chitosan nanoparticles showed effective antifungal activity results and zone of inhibition.

Table 2. Antifungal activity results

S. No	Fungi	Zone of inhibition (mm)	Antifungal activity
1	*Candida albicans*	29	yes
2	*Aspergillus niger*	23	yes

A significant zone of inhibition for *Candida albicans*, measuring 29 mm, is present, indicating a potent antifungal effect. The same 23 mm zone of inhibition exists for *Aspergillus niger*, indicating a positive antifungal response. These findings imply that the compound has a significant antifungal effect on both *Candida albicans* and *Aspergillus niger* (Figs 21 and 22).

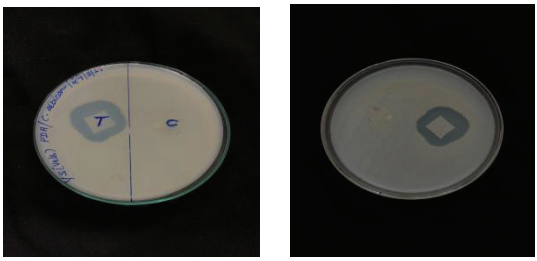

Fig. 21. *Candida albicans*

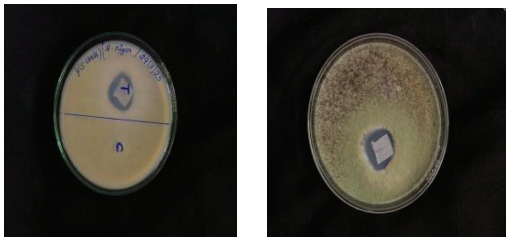

Fig. 22. *Aspergillus niger*

In vitro CYTOTOXICITY OF CHITOSAN ANTIBIOTIC BANDAGE

Cytotoxic effect. The bandage showed less cytotoxic effect. So the bandage does not cause harmful effects on skin when it is used.

Table 3. Cytotoxicity effect

Concentration (µg/ml)	Absorbance		Average	Cell viability (%)
	I	II		
Control	0.843	0.852	0.8475	100
20	0.838	0.843	0.8405	99.1740413
40	0.830	0.834	0.832	98.17109145
60	0.821	0.822	0.8215	96.93215339
80	0.809	0.813	0.811	95.69321534
100	0.802	0.808	0.805	94.98525074

The absorbance values at various drug concentrations (g/ml) are shown in Table 3 together with the related average values and cell viability percentages. Average absorbance for the control group was 0.8475 (100% cell viability). Cell health was not significantly affected by concentrations of 20 ng/ml (99.17%). There was a marginally stronger impact (98.17%) at 40 ng/ml. A noticeable effect was seen at 60 g/ml (96.93%), further cell health loss occurred at 80 g/ml (95.69%), and the greatest effect was seen at 100 g/ml (94.99%). Overall, the data show that the effects on cell viability are dose-dependent, with higher concentrations having a more profound impact.

In vitro WOUND HEALING OF CHITOSAN ANTIBIOTIC BANDAGE

Wound healing. Table 4 shows a healing percentage of 0% resulting from the absence of healing at the beginning, at 0 h. A considerable but incomplete improvement may be seen after 24 h, and the healing percentage, which was 19.23% on that first day, suggests some progress. Following that, at 48 h, wound healing continues to develop, with the healing percentage rising to 49.61%. This information shows how the control group's wounds gradually healed over the course of 48 h, with the greatest improvement occurring between 24 and 48 h.

Table 4. Wound healing for control

Time (h)	Healing (%)
0	0
24	19.23
48	49.61

Table 5. Wound healing for sample

Time (h)	Healing (%)
0	0
24	40.76
48	86.92

At the beginning, at 0 h, there is no notable healing. There is a noticeable improvement after 24 h, peaking at 40.76%, showing some healing within the first day (Table 5). With healing at 86.92% after 48 h, there has been a tremendous improvement and advancement. According to these data, wound healing significantly improved over the course of 48 h, with the most increase occurring between 24 and 48 h. Figures 23–28 show the 0, 24, and 48 h readings of control and sample, respectively.

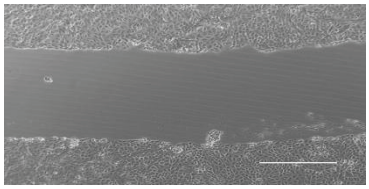

Fig. 23. Control 0 h

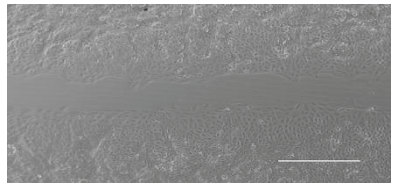

Fig. 24. Control 24 h

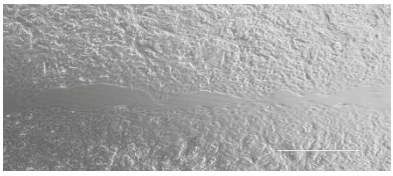

Fig. 25. Control 48 h

Fig. 26. Sample 0 h

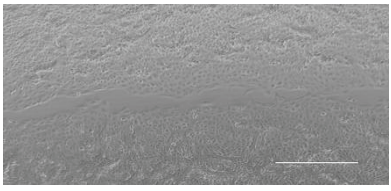

Fig. 27. Sample 24 h

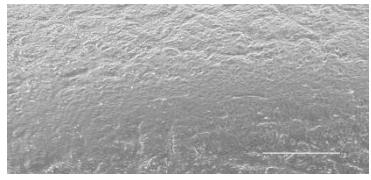

Fig. 28. Sample 48 h

CONCLUSIONS

Chitosan encourages the body's normal healing process and has no negative effects on mammalian cells. Given the wide variety of chitosan-based wound dressings on the market, we may consider chitosan to be a cost-effective therapeutic choice not only for the treatment of acute wounds but also in the event of severe chronic wounds, such as diabetic ulcers. Chitosan is one of the most important biopolymers that can be used to create wound dressings that hasten wound healing while also avoiding wound infection, an important stage in the wound healing process.The superior biological and medicinal qualities of chitosan make it a great material for wound dressings.

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