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Synthesis and Characterization of Chitosan with Incorporated Herb – A Novel Bionano Composite

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Abstract: In this study chitosan with incorporated herb bio nanocomposite was synthesized using multiple emulsion –solvent evoparation method. Mahavilvam (narangicrenulata) was selected based on its anti-bacterial activity. The synthesized bio nanocomposite was characterized by UV, FTIR and SEM studies. Anti-Bacterial study was done using well diffusion method. A comparative anti-bacterial study was carried out for chitosan ,mahavilvam, chitosan-mahavilvam composite and chitosan-mahavilvam nanocomposite .The mahavilvam nanocomposite showed an enhanced anti-microbial activity .The SEM analysis showed that nano composite was spherical in shape, the size of composite particle was in the range of 80-94nm.
Keywords: Chitosan, mahavilvam, nanocomposite, FTIR,UV,SEM, well diffusion method.

1. Introduction

Bio nanocomposite forms a vital part of environment friendly research studies[1]. Bio nanocomposite has emerged has a special class of research area that combines chemistry, physics, biology, engineering and nano technology. Bio nanocomposite have extraordinary properties such as ecofriendly, non toxic, non allergic, cost effective, bio degradable and bio active[2].

Bio polymers such as polysaccharides, proteins and nucleic acid were used in the preparation of bio nanocoomposites [3]. In the present investigation Chitosan, mahavilvam were chosen for the preparation of bio nanocomposite.

Chitin, a natural polymer, found in insects, crustaceans, fungi, worms, mushorroms etc.[4]. After cellulose, Chitin is the most abundant polymer and it functions as structural polysaccharide [5]

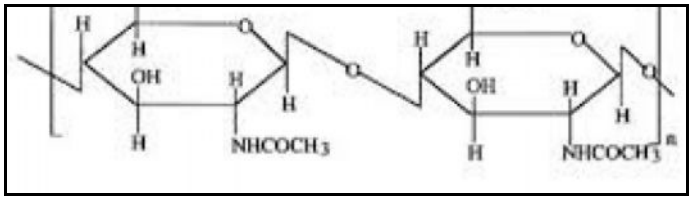


Figure 1

Chitin is a linear polysaccharide consisting of [1,4] linked 2-acetamido-2-deoxy-D gluco pyranose.

Chitosan is the derivative of Chitin which is obtained by the deacetylation of Chitin using NaOH [6]. The main difference between Chitin and Chitosan is the percentage of acetyl groups in the structure. Chitin is the linear polysaccharide consisting of 1, 4 linked 2-amino-2 deoxy-D-glucopyranose.

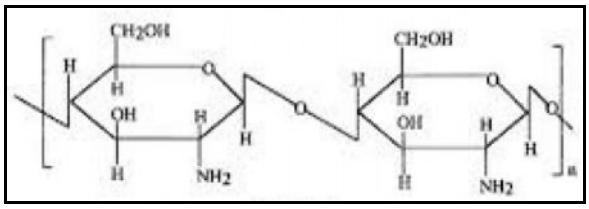


Figure 2

Chitosan has distinct properties such as non-toxic, bio degradable and bio compatible. These properties make Chitosan is the best polymer for various fields like textiles, food science, agriculture, biomedical and pharmaceuticals [8]. Chitosan can be made in the form of film which can be used in burns and wound dressing [9]. Usually, metals and metal oxides have been incorporated [10- 18] with chitosan to enhance the antibacterial activities in bio nanocomposite. Recently, chitosan bio nanocomposite with celluslose [19], rice straw [20], curcumin [21] and medicinal herbs [22 - 24] have been reported. Based on such reports, in this work, it has been aimed for the synthesis of industrially important chitosan herbal nanocomposite using medicinally important herbs. On the basis of importance an anti bacterial activity, mahavilvam has been selected for the synthesis of bio nanocomposite.

Narangicrenulata Nicolson belongs to Rutaceae family is also known as Mahavilvam in Tamil. It has been used as folk medicine [25]. Mahavivam leaves has anthelmintic property [26]. Considering the medicinal importance of this herb and unique properties of chitosan, bio nanocomposite was prepared.

2 Materials and Methods

2.1 Materials

All the chemicals used in the synthesis were Analytical grade. Chemicals used for the synthesis such as Chitosan, Tween 80, Span 80, Tripolyphosphate were purchased from HIMEDIA are used as such and distilled water is used for the syntheisis.

2.2 Collection, Processing and Extraction of Herb:-

The herb selected for study was Naringicrenulata which are collected in and around Chennai. The collected leaves were shade dried at room temperature to reduce the moisture content. The leaves were then powdered and sieved. 20 grams of the ground herbal powder was suspended in 100 ml methanol and incubated overnight. The supernatant liquid was filtered twice using whatmann no. 1 filter paper.

2.3 Preparation of Herb-Chitosan Composite:-

About 1 gram of the chitosan was dissolved in 1% acetic acid(1ml of acetic acid in 99 ml of distilled water). About 10 ml of the herbal extract was added drop wise to the prepared chitosan solution under constant stirring.

2.4 Preparation of Mahavilvam Chitosan Nanocomposites:

Multiple emulsion\solvent evaporation procedure was adopted for the preparation of Mahavilvam Chitosan nanocomposite. Chitosan solution (3%) was added to Tween 80 (5%(w\v) and kept it in magnetic stirrer for 1hr. To this emulsion, Mahavilvam extract was added and stirred for 5 minutes. Parallely, Span 80 (5%) was prepared with palm oil & stirred for 10 minutes. Both the solutions were mixed in 9:1 ratio & stirred well for 5 minutes. To this, Tripolyphosphate (0.01g) was added and stirred for 5 minutes. The above suspension was then incubated for 1 hour in a water bath at 50^0C & then cooled. By repeated washing with petroleum ether, nanocomposite was segregated from the palm oil.

3 Results and Discussion:

3.1 Ultraviolet Spectroscopy

The chitosan - herb nanocomposites, and chitosan were analysed using UV-Visible Spectrophotometer.

The UV-Visible spectrum of chitosan - herb composite is shown in fig. (3)

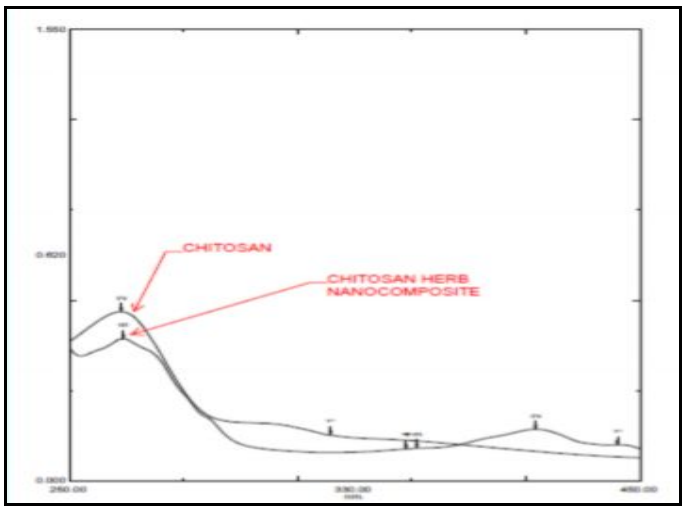


Figure – 3 UV Spectoscopy of Chitosan, Chtosan Herbal Nanocomposite

Both chitosan and the composite has absorption peak at 268nm^{-1}.Composite has absorption at 413 nm (visible region) which indicates the week electronic transition between chitosan and the herb.

3.2 FT-IR SPECTROSCOPY

FTIR spectroscopy was measured using IR affinity I model of SHIMADZU UV 1650PC. FTIR spectra of chitosan and chitosan herb composites were shown in **fig(4) & (5)**

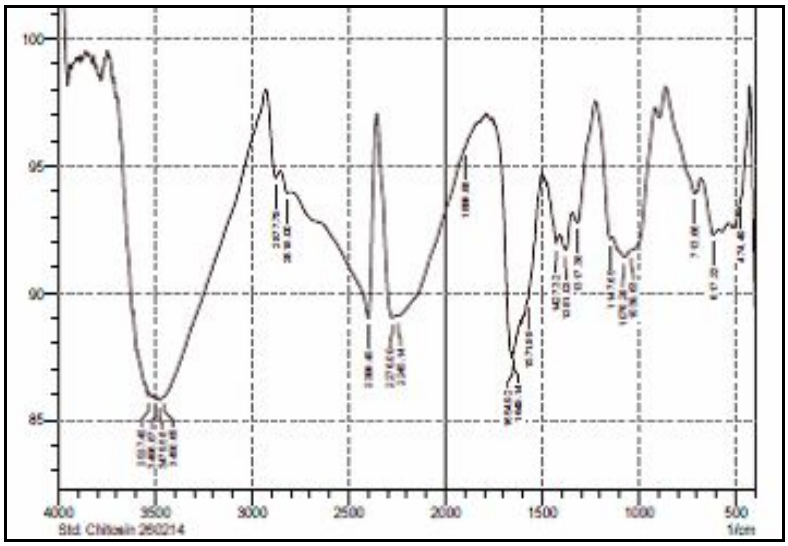


Figure 4 – FTIR of Chitosan

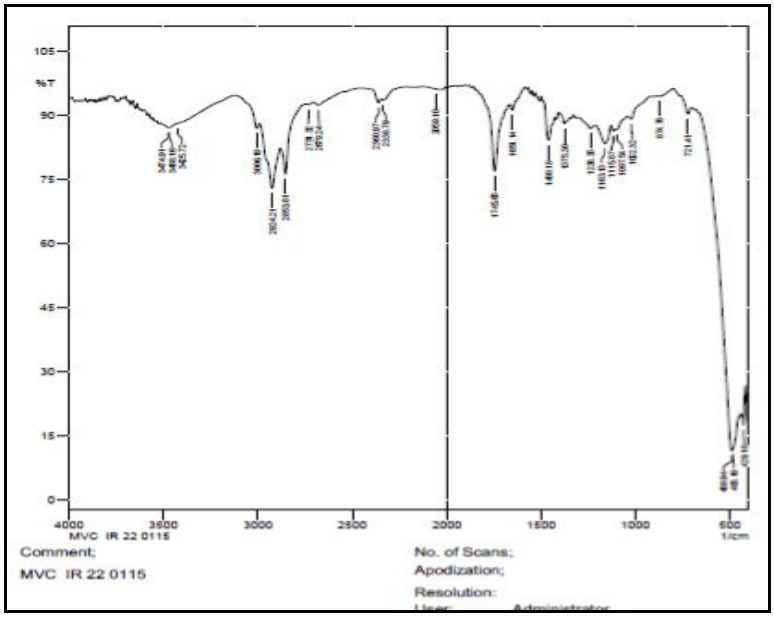


Fig 5 – FTIR of Chitosan herb nanocomposite

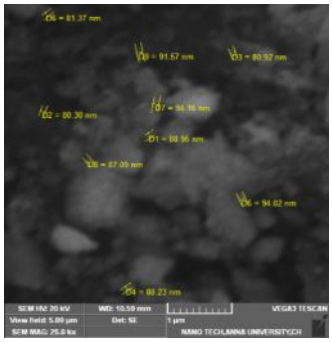
The Fourier transform infrared spectrum of chitosan shows vibration bands at 3498 and 3537cm^{-1} due to overlapping of OH and amine NH stretching bands; a peak at 2877 cm^{-1} indicated aliphatic C-H stretching; 1649 and 1571cm^{-1} for C=O stretching and NH bending; 1427,1381,1317cm^{-1} for C-H bending. The OH & NH stretching bands of chitosan shifted from 3498-3539cm^{-1} to 3468 and 3474 cm^{-1} and amide peaks were shifted from 1649-1571cm^{-1} to 1745-1651cm^{-1}.The spectra of the chitosan-herb nanocomposites were characterised by the presence of the absorption bands typical of the pure chitosan. All characteristic peaks of the chitosan were observed in the spectra of the chitosan herb nanocomposite. The intensity of the absorption peak at 3468 and 3474cm^{-1};1745 & 1654cm^{-1} for the nanocomposite were lower than that of chitosan, possibly because of the result of formation of intermolecular hydrogen bonds between chitosan & herb. Moreover due the presence of coo^- of herb and NH_3^+ of chitosan, electrostatic interaction between the herb & chitosan molecules might also occur.

Conclusively, both the intermolecular hydrogen bonding and electrostatic interactions contributed to the strong interactions between chitosan and herb.

3,3 Scanning Electon Microscopy (SEM)

An assessment on the morphological aspects of the synthesized Bionanocomposites was carried out with Field Emission Scanning Microscope (FESEM) DST-NanoEmission model. SEM image of chitosan herb composite is shown in **fig (6) & fig (7)**

SEM Images of Chitosan Mahavilvam



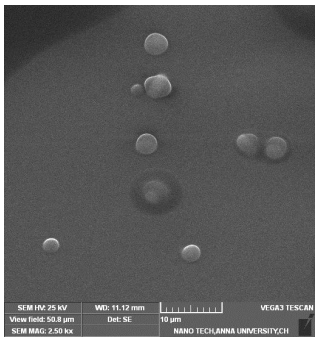


Fig (6) **Fig 7**

Fig (6) shows the SEM micrograph of the Chitosan herb nanocomposites.The average diameter of the composite particle was found to be in the range of **80-94 nm**, the particle size was also found to be uniform.

SEM was used to investigate the distribution of herbal nanoparticles in the Chitosan matrix. **Fig(7)** shows that SEM images of surface and cross sectional area of the films showed the nanoparticles were present in small aggregates near the surface as well as dispersed througth the Chitosan matrix.

3.2 Antibacterial Activity:

The extremely small size of the nanoparticles means they exhibit enhanced or different properties when compared with the bulk material. The extremely small size of nanoparticles results in the particles large surface area relative to their volume,which allows them to easily interact with the other particles and increases their antibacterial efficiency.

Antibacterial activity towards staphylococeus aureus and Escherichacoli was evaluated using agar plate method and it is shown in **fig (8) & fig (9).**Antibacterial activity were tabulated in **Table 1.**

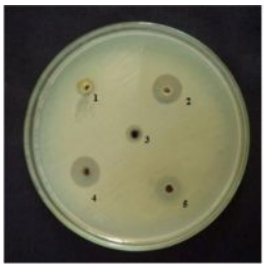


Fig (8)

Fig (9)

Inhibitory effect of Mahavilam (1) ChtosanMahavilvam composite (2) Chitosan (3) Chitosan Mahavilvamnanocomposite (4) and Streptomycin (5) against S. Aureus (Fig -8) and E.Coli (Fig 9)

Table- 1– Antibacterial activity of Chitosan, herb, Chitosan herb composite, Chitosan herb nanocomposite and streptomycin

Sample	Zone of Inhibition in mm	
	S. Aureus	E-Coli
Chitosan	10	9
Herb	9	8
Chitosan Herb Composite	11	12
Chitosan Herb Nanocomposite	14	13
Streptomycin	17	12

As shown in **fig(8) & (9)** an obvious zone of inhibition appeared around the chitosan. Herb, Chitosan composite and nanocomposite. For nanocomposite the inhibition zone was larger than that of composite, chitosan and herb which shows nanocomposite has enchanced antibacterial property.

4 Conclusion:

The present work integrates nanotechnology into bacteriology leading to possible advances in the formulation of new sites bactericides.

Bionanocomposites was synthesized by multiple emulsion solvent evaporation method and were characterized by UV, FTIR and SEM techniques. UV & FTIR confirmed the formation of composites.

The morphology of nanocomposite was examined by FESEM.The SEM results showed that the nanocomposites were spherical in the size range of **80-94 nm.**

The antibacterial effects of nanocomposites against S.Arus & E.Coli were examined by Augar diffusion method.The results revealed a nanoparticle formation within the chitosan matrix.It was proved that the nanoherb containing chitosan film had an excellent antibacterial performance.

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