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Synthesis and Characterization of Chitosan with Silica (CS) Nanocomposite with Enhanced Antibacterial Activity

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ABSTRACT

A mixture of nanocomposite material has been obtained by in situ formations of an inorganic network in the presence of a preformed organic polymer. Chitosan biopolymer is the most common and then silica is used in composite materials, which were used for the sol-gel reaction. The average particles size of nanocomposite powder is 63.33nm as measured by XRD. Chitosan-silica nanocomposite (CS) has a rough surface texture which appears as agglomerates of varied sizes and shapes of microfibrils and separate binding crystalline structure measured by SEM. The agar well diffusion assay is used to confirm the inhibition zone of bacteria. The nanocomposite showed antibacterial activity when tested against gram-positive bacteria and gram-negative bacteria. Further, the synthesized nanocomposite was characterized by using X-ray powder diffraction (XRD), scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX), Particle size analysis (PSA), UV–vis absorption spectroscopy (UV) and Fourier transform infrared spectroscopy (FTIR).

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INTRODUCTION

Many researchers and industries have shown increased interest in developed progressive materials by merging macromolecules through inorganics to scheme organic-inorganic fusion materials [1]. Nanocomposite materials present an excellent platform of engineered nanomaterials due to the presentation of welfares such as simplicity in synthesis, reduced size, weight, and multifunctionality [2]. Additionally, they simultaneously are accomplishing improvements in mechanical, morphology, thermal, and electrical properties comparative to conservative composite materials [3]. Biopolymers are macromolecules created by living organisms; these include chitin [4], gelatin, cellulose [5] and a plethora of extra varied chemical objects [6]. Chitosan, the deacetylated derivative of chitin [7], is a linear cationic biopolymer of glucosamine residues [8], definitely, N-acetyl-D-glucosamine and N-D-glucosamine [9], linked through - (1–4)-glycosidic bonds [10]. Chitosan and its derivatives are low cost and plentiful biopolymer adsorbents and have important attention in eliminating organic and inorganic substances from wastewater due to their outstanding chelating performance [11]. Chitosan as biomass consumes a high biodegradability [12], high chemical stability [13], non-toxicity, and environmental benignity

is functional as a capable material in numerous fields for some years. Likewise, the excessive deal to the chitosan as an adsorbent is due mainly to the being of hydroxyl and abundant amino groups in its construction [14].

Additional improvement of chitosan to increase the vigorous places through compositing the chitosan with each organic or inorganic nanomaterial interact much research interest in a recent year. Chitosan nanoparticles are synthesized as drug carriers, as described in earlier studies. The single appeal of nanoparticles for their small size and quantum size effect might make chitosan nanoparticles demonstrate greater activities [15]. Silica is one of the most stimulating synthetic materials in the industry, which is being widely used for different applications such as coatings, adhesives, construction, fibers, and foam because of its high flexibility, good processability [16], excellent water resistance, good resistance to acids and solvents, better alkaline resistance than furthermost of other polymers, good abrasion resistance, and good motorized properties [17]. Its synthesis, morphology [18], chemical, and motorized properties have been the topic of a great deal of attention in current studies [19]. Because of their ease and simplicity in the sol-gel process, high purity and homogeneity [20], size uniformity [21], and small size [22], silica-based nano-particles or nanocomposite prepared using the sol-gel process have piqued the interest of many researchers in the development of various engineering materials and drug delivery systems [23]. This work aims to synthesize chitosan with silica nanocomposite. We have studied the interaction of chitosan with silica gel. The measurements were carried out using Fourier transform infrared spectroscopy (FTIR), Ultraviolet spectroscopy (UV), Scanning electron microscopy (SEM-EDX), X-ray diffraction (XRD), Particle size analyzer (PSA). The antibacterial activity of the chitosan with Silica gel nanocomposites was evaluated.

MATERIALS AND METHODS

All chemicals used in this study were of analytical grade and purchased from Merck, India, and used as obtained without additional purification. Chitosan with 80% deacetylation was purchased from Sigma–Aldrich, Acetic acid, hydrochloric acid; ethanol absolute was used as the starting materials (Sigma–Aldrich). All preparation procedures were carried out with distilled water. All chemicals and reagents were of analytical grade and were used without further purification.

Preparation of Chitosan Nanoparticle

1 gram of chitosan was weighed out and dissolved in 100ml of 1 % acetic acid solution followed by continuous stirring at room temperature for about 1 hour by using a magnetic stirrer, and then 2g of sodium tripolyphosphate was dissolved in 200ml of distilled water and then added dropwise and freeze this solution at 40° C for 24hrs and then precipitate was settled at the bottom and collected the precipitate and dried hot air oven at 50° C for 24hrs. Finally, the prepared chitosan nanoparticle was investigated.

Preparation of chitosan with silica gel Nanocomposite

Chitosan Nanocomposite (CS) was prepared in 1:1 ratio. Firstly, 1g of chitosan nanoparticle and then 1g of silica gel was mixed with 100ml of distilled water and then the solution was shaken by using a shaker for 3 days. Finally, the particle was settled and the solution was filtered by using Whatman No: 1 filters paper and was dried in a hot air oven at 50° C for 24 hours and then calcinated at 500^0 C for 4hrs finally, Chitosan Nanocomposite was prepared.

Antibacterial activity

The antimicrobial potential of chitosan with silica nanocomposite (CS) was tested against human pathogenic bacterial strains such as the selected microorganisms were *Pseudomonas, Enterobacter, Escherichia coli*, represented as gram-negative bacteria, while *Bacillus subtilis, Staphylococcus* represented as gram-positive bacteria. The tested bacteria were refreshed by sub-culturing aseptically on the nutrient agar in agar plates, respectively. The Mueller Hinton agar (MHA) was prepared, autoclaved and the tested microorganisms were added to the cold melted agar medium at the time of pouring of the plates in triplicate. Once the plates were solidified, equal volumes and sample concentrations (25 μL, 50 μL, 75 μL, and 100 μL) of the tested colloidal solutions were prepared and added into punched holes (6 mm). Bacterial plates were incubated at 37 °C for 24 hrs, respectively. After the incubation period, the plates were examined and zones of inhibition (mm) of bacterial growths were observed [24].

Characterization

The molecular structure of the prepared hybrids were analyzed (Nicolet Magma 550 series II, Midac, USA) at wavelengths ranging from 4000 to 400 cm^{-1}. Dry film was ground with KBr powder and compressed into discs for FTIR examination. Samples morphology was examined under a JEOL (Japan) JSM-T300 scanning electron microscope (SEM). The sample was coated with gold using a JEOL JFC-110E Ion Sputter. XRD patterns were obtained on a Bruker D8 Advance diffractometer

using Cu Kα radiation (k = 1.540 A°), operating at 40 kV and 40 mA. Scans were performed with a detector step size of 0.02° over an angular range 2θ = 10 – 80°. The bioreduction of metal particles in the arrangement was observed utilizing a UV-Vis spectrophotometer (Model no. Cary arrangement), taking a spectrum from 200 to 800nm for each example against purified water as clear. Particle sizes were measured in a Zetasizer ZSP (Malvern Instrument Ltd., Worcestershire, UK) at 25°C based on laser doppler velocimetry and dynamic light scattering (DLS) techniques. Previously, the suspension was homogenized using an ultrasonication probe for 5 min.

RESULTS AND DISCUSSION

X-ray Diffraction

XRD curve for the organized chitosan with silica nanocomposite powder dried at room temperature at 50°C for 24h is screened in Figure 1. Certainly, no peaks are perceived in nanocomposite powder dried at room temperature except the huge wide-ranging hump at 2θ between 22° and 25.1° credited to crystalline silica by way of exposed in Figure 1. Accredited to orthorhombic stage conferring to JCPDS card number (39-1894) [25]. The characteristic peaks for silica gel are attributed to according to JCPDS card number (89-6267). Additionally, the weak peaks for Chitosan with silica nanocomposite (CS) recommended that the crystallinity be credited to the introduction of silica gel addicted to the chitosan polymeric group, which establish that the CS as a polymeric chain were mixed well at the molecular level [26].

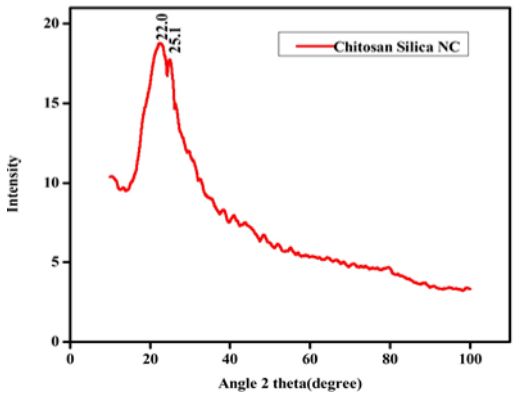


Figure 1: XRD image of Chitosan with silica nanocomposite

The crystalline structure was made up to be obtained as a result of the heat treatment of the sample at high calcination temperature (i.e. 500°C), which means crystal-like particles are having indiscriminate that means the random structure has been

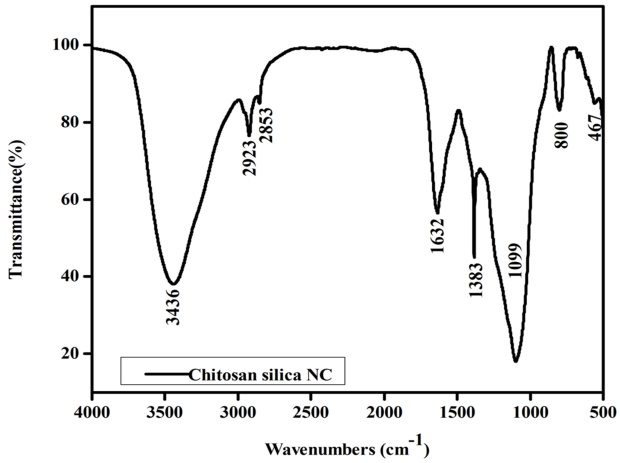


Figure 2: FTIR image of Chitosan with silica nanocomposite

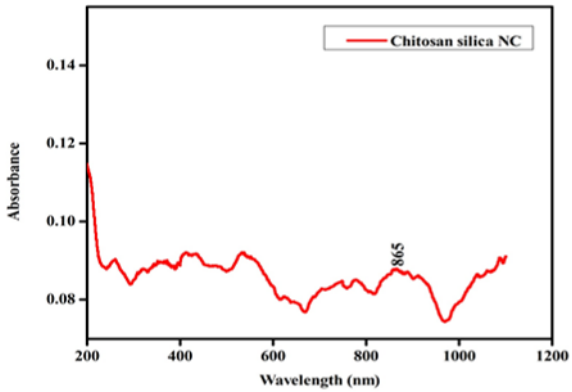


Figure 3: UV image of Chitosan with silica nanocomposite

achieved of the material. The average particles size of nanocomposite powder is 63.33nm as measured by the De-bye Scherrer equation [27].

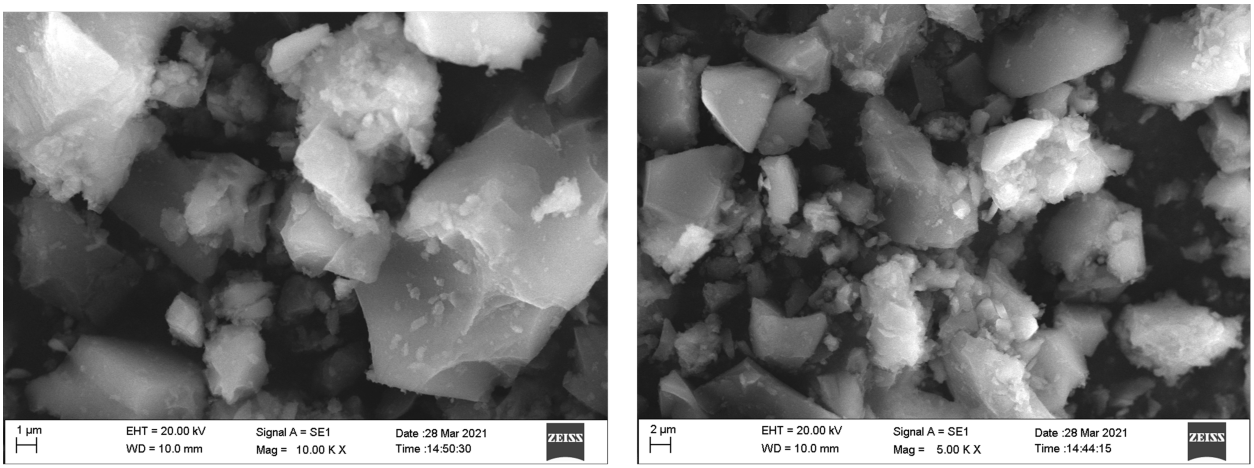
Fourier Transform Infra-Red Spectroscopy

Figure 2 shows that the FTIR spectrum of Chitosan with silica nanocomposite (CS) dried at 50°C. In

the FTIR spectrum [28], the characteristic absorptions peaks for Chitosan with silica nanocomposite were presented from 1383cm^{-1} to 1632 cm^{-1}attributable to amide bands I and II, respectively [29]. The attendance of a wide range and the strong band of about 3436 cm^{-1} is a suggestion for the attendance of hydroxyl groups (–OH) in the nanocomposite film [30].

The weak band was created from alcohol groups at 2923 cm^{-1}and 2853 cm^{-1}. The absorption bands at 1099 cm^{-1} came from the – C_2H_5 rocking mode of Si–O–Si asymmetric stretching mode, respectively [31]. This result designates larger communication between chitosan nanoparticles and silica gel.

Ultra Violet Spectroscopy



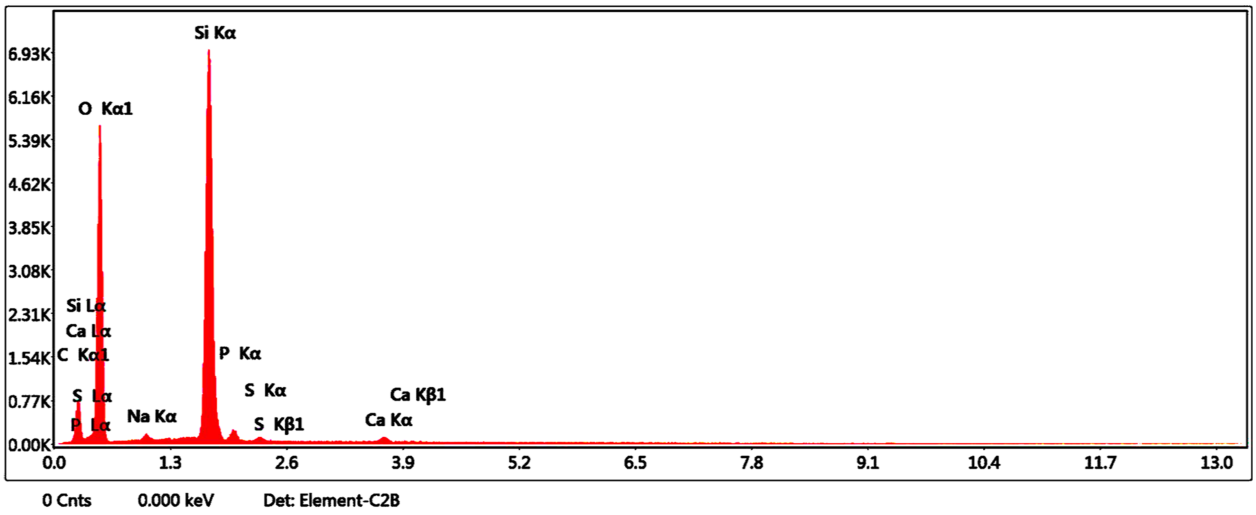


Figure 4: SEM-EDAX image of Chitosan with silica nanocomposite

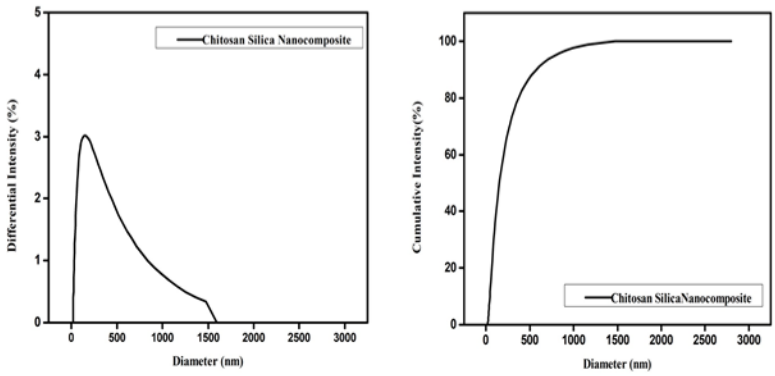


Figure 5: PSA image of Chitosan with silica nanocomposite

The optical properties of biopolymer materials were analyzed by UV-Visible spectroscopy and are shown in Figure 3. Figure 3 showed UV absorption bands of chitosan nanocomposite peak wavelengths were at 865 nm respectively [32]. Figure 3 indicated that the CS nanocomposite was formed due to absorbance bands after immobilization, reflecting the presence of chitosan with silica gel nanocomposite. This result shows that the formation of the sharp peak was present from Chitosan with silica gel nanocomposite.

Scanning Electron Microscopy-EDX

Chitosan with silica nanocomposite was prepared by the sol-gel method, it has a rough and irregular surface when viewed from SEM images. This could portray a high specific surface area due to plenty of holes and canals on nanocomposite material such as Chitosan with silica nanocomposite. Figure 4

Table 1: Zone of inhibition of Chitosan with Silica Nanocomposite (CS)

S. No	Pathogens	TiO2 NPs			
		25μl	50μl	75μl	100μl
1	Bacillus	1.0	1.1	1.5	1.7
2.	*E.coli*	1.2	1.5	2.0	2.2
3.	Enterobacter	0.9	1.2	1.3	1.5
4	Pseudomonas	-	-	-	-
5	Staphylococcus	1.1	1.3	1.4	1.6

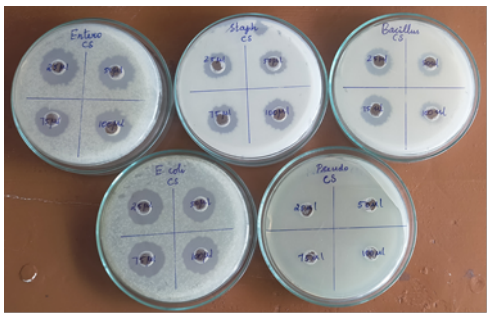


Figure 6: Chitosan with Silica nanocomposite

presents the scanning electron microscopy, which reveals the surface texture and morphology of the Chitosan with Silica nanocomposite produced. It is observed that the chitosan-silica nanocomposite has a rough surface texture which appears as agglomerates of varied sizes and shapes of microfibrils and separate binding crystalline structure [33].

SEM images of chitosan with silica nanocomposite samples reveal a particulate structure of original natural chitosan, which seems to be fused decorated by silica gel (white particles or beads), as shown in Figure 4. Some of the silica gel embedded within the structure of the chitosan matrix and other particles are dispersed into their surface [34]. The average particles size of nanocomposite powder is 63.33nm as measured by XRD from the particle size distribution. The analysis of the Chitosan with silica nanocomposite by EDAX showed the presence of C, O, Na, Si, P, S & Ca. Smart quant results show the weight of the oxygen element is 51.9 %. Similarly, so many peaks are present here, but the accurate weight of Silica is 26.1 %. Compared to Si (15.9%), element oxygen (55.7%) and Carbon (25.9%) becomes higher atomic percentages.

Particle Size Analyser

Particle size has reviewed the characterization of nanoparticles and nanocomposite by DLS has shown in (Figure 5), focusing on the case of nanoparticles and nanocomposite by DLS [35]. They show how different factors like suspension concentration, particle shape, colloidal stability, and surface coating of nanoparticles and nanocomposite influence the size value obtained by DLS measurements. DLS is an analysis of particle movement in solution caused by Brownian motion [36].

The size distribution and intensity correlation function normalized between one and zero of the Chitosan with Silica nanocomposite using dynamic light scattering (DLS) is shown in Figure 5. To be specific, from this study Chitosan with Silica nanocomposite size and polydispersity index were obtained [37]. The average size is measured based on the majority of particles sizes in a sample. There is no relation between the charge ratio and size reduction, while the polydispersity index describes the homogeneity of particles loaded. The versatility of this technique in terms of fast, easy, reproducible, and non-destructive results is its advantage. It was indicative of the apparent size adopted by the Chitosan with silica nanocomposite in the aqueous dispersion. Chitosan with silica nanocomposite exhibited a Gaussian peak located below 100 nm and then approximate particle size below 100 nm. The measurement carried out for the Chitosan with Silica nanocomposite material revealed a particle size outside the quantification limits of this technique. This indicates that the sample has a wide particle size distribution according to the results reported by [38].

Antibacterial activity

Chitosan with Silica nanocomposite (CS) is a positively charged macromolecule, which interacts with negatively charged microbial membrane, and results in the breakage of intracellular components (Figure 6). Chitosan acts as a chelating agent and limits toxin production and microbial growth [39].

Antibacterial screening of Chitosan with Silica nanocomposite against gram-positive bacteria such as *Staphylococcus, Bacillus* and gram-negative bacteria such as *E. Coli, Enterobacter* have been reported by [40]. Table 1 shows the antibacterial activity of synthesized Chitosan with silica nanocomposite, which shows the antibacterial zone of inhibition between 1 to 3.5mm at the concentration of 25, 50, 75, 100 μl against *Staphylococcus*

aureus, Escherichia coli, Enterobacter, Bacillus [41] .

CONCLUSIONS

A modified procedure for preparing chitosan with silica nanocomposite was developed through the sol-gel approach. The FTIR spectrum of the nanocomposite material is compatible with the main results described in the literature. SEM analysis revealed that the Chitosan with silica nanocomposite possessed a highly porous and nanostructure. Chitosan with silica nanocomposite sample is highly stable during many applications; this may be attributed to the good compositing between Silica gel and Chitosan. The good properties of chitosan with silica nanocomposite may make them valid and especially in antibacterial activity, because it is environmental friendly. Nanocomposite showed antibacterial activity when tested against gram-positive bacteria and gram-negative bacteria. This study also shows that Antibacterial activity with a high recombination rate to a suitable material will enhance its activity.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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