

Synthesis of Chitosan Nano Particle from chitin and its Industrial and Clinical Application

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ABSTRACT

In the last few decades Nanotechnology based approaches showed profound usage in industrial, clinical, biomedical and environmental Applications. Chitosan based Nano materials promise wide applications due to its Physiochemical Properties such as Polycationic linear structure, bio degradation, biocompatibility and Non toxicity. Current Article aims at Synthesising nanoparticles using bottom-up Ionic gelation Method. As an Industrial application Photocatalytic Dye degradation using chitosan nanoparticles against the commercial dye such as Methyl orange was tested. Chitosan nanoparticles showed significant better degradation for Methyl violet.

INTRODUCTION

Now-a-Days different types of dyes and their related compounds released from various industries are hazardous to human health and a major source of environmental pollution. These types of dyes are mainly employed in textile, pharmaceutical, paper, dyeing, printing and pulp industries. Particularly, the azodye such as Picric acids and nitrophenols are highly toxic, non-biodegradable, mutagenic and carcinogenic agents in various environments. The dyes commonly used as colorants in the industrial sector are crystal violet, congo red and Malachite green etc. the direct release of these dyes into the environment has caused severe health issue. Various techniques like filtration, adsorption, oxidation and biological treatments have been attempted. Nano particles have shown good impact in bioremediation.

Chitosan is Natural Polycationic linear polysaccharide found in Exoskeleton of insects, Crustaceans and Cell wall of Fungi and is second most abundant after cellulose. It is composed of β -(1-4)-Linked D-Glucosamine and N-acetyl-D-glucosamine randomly distributed. Due to its cationic nature it forms multiple layers with Electrostatic Interactions. The Characteristics such as Biocompatibility, Non toxicity, Low Allergenicity and Biodegradability lead chitosan to wide applications. Chitosan has been widely used for different biological and biomedical applications recently due to its unique properties. For instance, it can be used in water treatment, wound-healing materials, pharmaceutical excipient or drug carrier, obesity treatment and as a scaffold for tissue engineering. There is increased interest in pharmaceutical as well as biomedical applications. Chitosan Nanoparticles due its small size protrudes into bacterial cell wall with high efficiency against Gram Negative bacteria due to its cationic nature and also accounts for dye removal due to presence of Free N-modified reactive sites which exactly poses as a solution to above mentioned problems. Chitosan has to undergo modifications for specific applications and procedures exist accordingly. In this study, the chemical modification of chitosan, dye degradation against methyl orange dye and cytotoxicity activity on normal cell line was evaluated.

Material and methods

Deacetylation of chitin to chitosan: Deacetylation of chitin was achieved by reacting chitin with 12.5 M NaOH at a solid/liquid ratio of 1:15 (g/mL). The reaction mixture was cooled down and kept frozen at -83°C in an ultra-freezer for 24 h. Afterwards, the temperature of the

mixture was raised to 115 °C, and the reaction proceeded with agitation at 250 rpm for 4 or 6 h. The resulting chitosan was filtrated, washed with distilled water until neutral pH and dried in an oven at 70 °C.

Synthesis of chitosan nanoparticles: Chitosan solution is prepared as 0.5g is added in 50ml of water, followed by addition of 3-4 drops of Acetic acid. This positive ionic solution is allowed to react against negative ionic Tripolyphosphate (TPP) of 0.5g in the same. Solution is stirred for 3-4hours. Ionic gelation is noticed through thread formation. Drops of the nanoparticles were plated and dries for further characterisation.

Photocatalytic activity of chitosan Nanoparticles: The photocatalytic activity of chitosan Nano particles was determined using three different dyes namely Crystal violet, Methyl orange and Malachite Green as Model systems. To 25 ml of dye solutions of each, 3ml of chitosan nano particle is added in test sample and the ones without nanoparticle are control, solutions are Magnetic stirred for 30 minutes in the dark to maintain equilibrium. Then the dyes were kept under direct sunlight, after every 1hour ml aliquots of dye solution were taken, were centrifuged for 10min and the absorbance of the supernatant was measured using UV spectrophotometer.

Cytotoxicity effect

Vero (African green monkey kidney normal cell line) was obtained from the National Centre for Cell Sciences (NCCS), Pune, India. The cytotoxicity effect of the chitosan was tested against Vero cell line by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Mossman, 1983). The cells were seeded in 96-well microplates (1 x 10⁶ cells/well) and incubated at 37°C for 48 h in 5% CO₂ incubator and allowed to grow 70-80% confluence. Then the medium was replaced and the cells were treated with different concentrations (20, 40, 60, 80, 100 µg/mL) of sample and incubated for 24 h. The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (40X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20 µl of (MTT) solution (5 mg/mL in PBS) was added to each well. The plates were then stand at 37°C in the dark for 2 h. The formed crystals were dissolved in 100 µl DMSO and the absorbance was read spectrometrically at 570 nm. Percentage of cell viability was calculated using the formula,

Cell viability (%) = (Absorbance of sample/Absorbance of control) X 100.

Results and Discussion

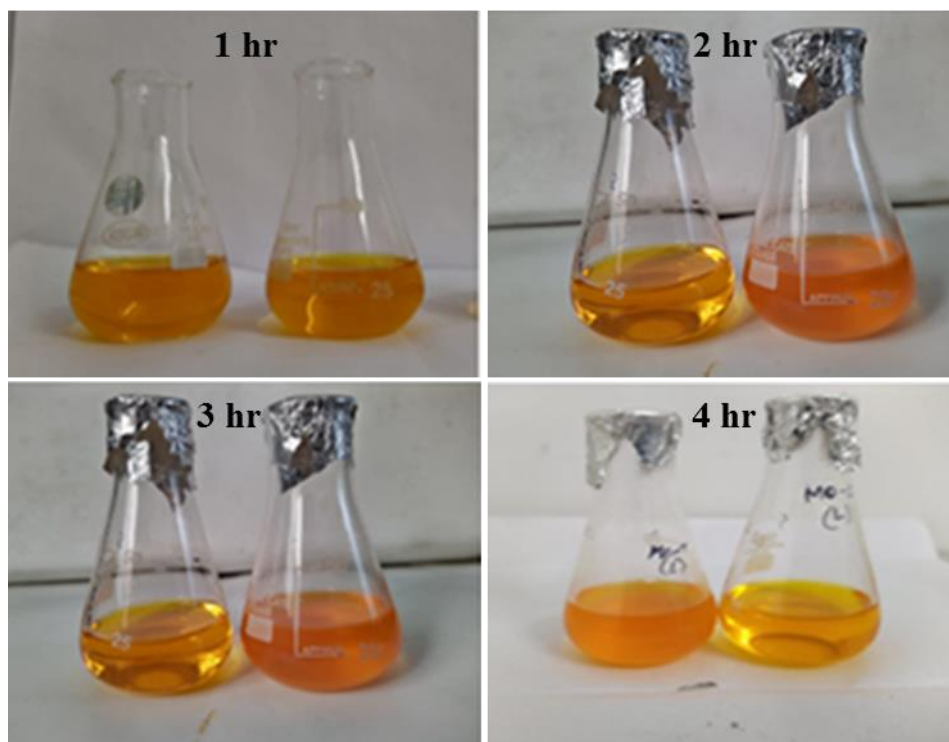
Chemical Deacetylation

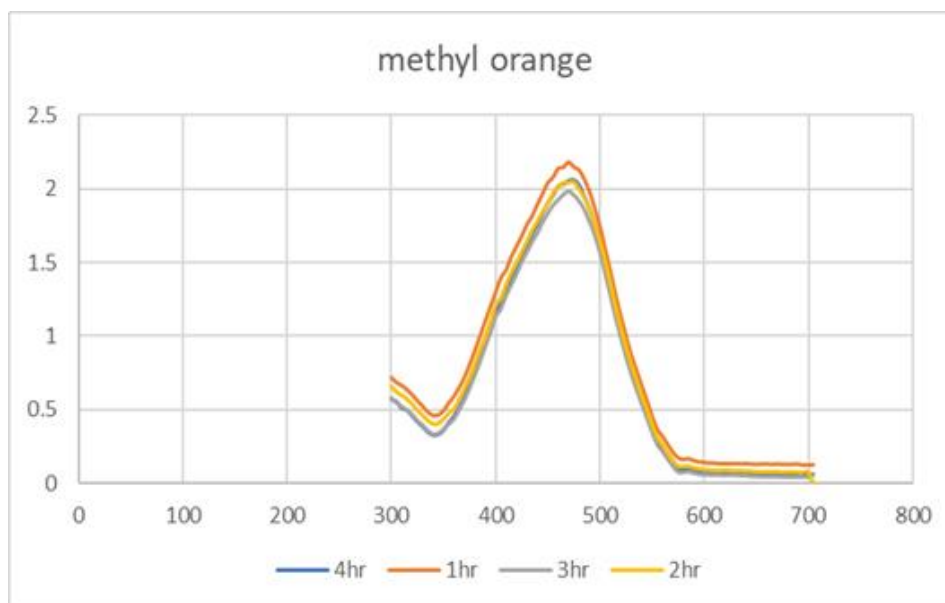
From a chemical point of view, either acids or alkalis can be used to deacetylate chitin. However, glycosidic bonds are very susceptible to acid; therefore, alkali deacetylation is used more frequently. The N-deacetylation of chitin is either performed heterogeneously [102], or homogeneously [103]. Commonly, in the heterogeneous method, chitin is treated with a hot concentrated solution of NaOH during few hours, and chitosan is produced as an insoluble residue deacetylated up to ~85%–99%. According to the homogeneous method, alkali chitin is prepared after dispersion of chitin in concentrated NaOH (30 g NaOH/45 g H₂O/ 3 g Chitin) at 25 °C for 3 h or more, followed by dissolution in crushed ice around 0 °C. This method results in a soluble chitosan with an average degree of acetylation of 48%–55%

Synthesis of nanoparticle

ChNPs are usually synthesized using a bottom-up ionic gelation method in which a solution of an anionic cross-linker, for example, sodium tripolyphosphate (TPP), and Ch is prepared where both reactants self-assemble into ChNPs because of the electrostatic interaction between the positively charged amine group of Ch and a negatively charged polyanion like TPP [14,37,39,40,41]. While several anionic cross-linkers like glutaraldehyde can be employed for ChNP synthesis, the use of TPP is more favourable because it is biocompatible and biodegradable [42]. NP size, hydrodynamic diameter, shape, and monodispersity were shown to be influenced by the Ch molecular weight (MW), initial concentrations of TPP and Ch, Ch: TPP mass ratio, the degree of acetylation of Ch, and pH of the reaction media. (29,30,31,32).

Photocatalytic activity: Methyl violet, Malachite Green and Methyl orange were investigated for photocatalytic degradation potential of chitosan nanoparticle under sunlight irradiation. The absorbance peaks of degraded dyes were recorded at 480,355,470 for methyl violet, malachite green and methyl orange respectively. The degradation spectra were noted at different time intervals. The higher percentage of degradation of them were calculated as 72%. The absorbance peaks decreased gradually with an increase in duration of exposure indicating a higher photocatalytic breakdown. In the absence of chitosan nanoparticle there was no progress in the degradation process. Chitosan showed high degradation efficiency against Methyl violet and Malachite green and no significance degradation recorded against Methyl orange. The possible mechanism for the degradation of dyes is: The smaller size of Nano particles changes the properties, increase in concentration of chitosan nano particles shows an increase in dye degradation for first few minutes. The increase in degradation is probably due to increase in availability of Catalytic sites and adsorption site. This is due to fact that no of dye Molecules adsorbed and photons adsorbed increase with increase in photocatalyst concentration. The photodegradation of Contaminant in Water is usually due to Exhibition of semiconductor by uv light to produce free radicals, which aid the degradation of pollutant. The uv irradiation excites electrons from valence band to conduction band, generating high energy Electron-hole pairs. The smaller size effect of nanoparticles increases the energy band gap between Valence and conduction bands increase redox potential and allowing UV light to be used.



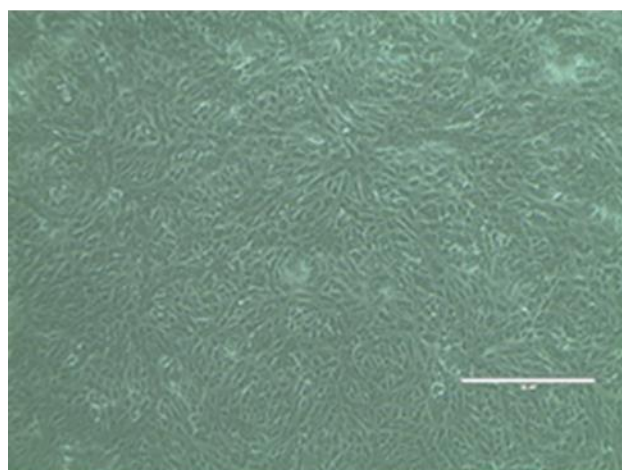


Cytotoxic effect

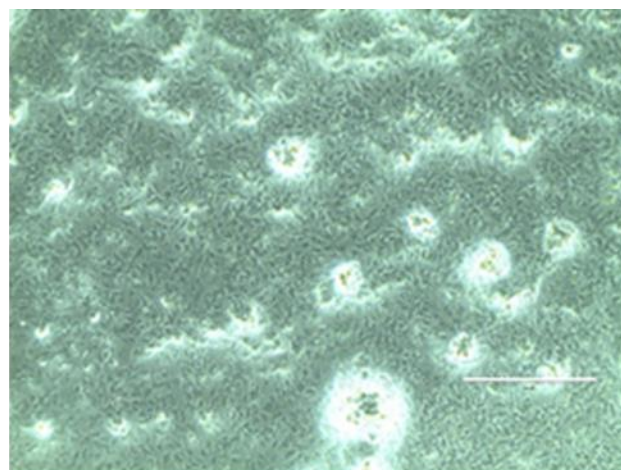
The chitosan nanoparticles revealed negligible toxic effect on Vero cells upto 80 $\mu\text{g/ml}$ of concentration (Table 1). The morphology of Vero cells was similar to that of control upto 80 $\mu\text{g/ml}$ and at higher concentration (100 $\mu\text{g/ml}$) only, there was shrinkage in cells observed. This confirms that the synthesised chitosan nanoparticles are safe to use in different applications.

Table 1. Cytotoxic effect of chitosan nanoparticles on Vero cell line

Concentrations ($\mu\text{g/ml}$)	Cell Viability (%)
Control	99.06
20	96.47
40	96.47
60	92.23
80	77.85
100	58.56



Control



100 µg/ml

Conclusion

The presented Article has reported potential use of chitosan nano particles in the fields of industry and medicine. Though it doesn't act as toxic on Vero cell line resulting in biocompatibility property. Photocatalytic activity performed using chitosan nano particles showed significant degradation of methyl orange dye.

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