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Production and Evaluation of Biodegradable Crab Shell Chitosan Nanoparticles

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ABSTRACT

Chitosan nanoparticles (CNPs) are potential drug carriers because of their biocompatibility, biodegradability, and functional diversity. The synthesis, characterization, storage stability, and in vitro release behavior of CNPs were the focus of this research. The morphological characterization of CNPs was conducted through Scanning Electron Microscopy (SEM), which showed spherical particles with a smooth surface and uniform size distribution in the nanometer scale, proving the efficacy of the synthesis procedure. Fourier Transform Infrared (FTIR) spectroscopy was utilized for the detection of functional groups and proving successful interaction between crosslinking agent and chitosan. The FTIR spectra revealed typical chitosan peaks with shifts that confirmed successful crosslinking and incorporation of the drug without any indication of chemical degradation. The in vitro release of the drug was carried out in phosphate-buffered saline (PBS, pH 7.4) with the dialysis bag technique. The release profile revealed a sustained and controlled drug release over a longer duration, indicating the efficacy of CNPs as effective delivery systems for drugs. Storage stability of CNPs was assessed for 60 days at room temperature and at 4°C. The nanoparticles demonstrated physical stability, size, and drug content without any changes, validating their high storage stability. Generally, the research observed successful preparation of stable, effective CNPs with good drug release profile, validating their applicability in food and pharmacy.

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Introduction

Chitosan nanoparticles (CNPs) were first introduced by who synthesized them via emulsification and crosslinking for the intravenous delivery of the anticancer drug 5-fluorouracil [1,2]. Since then, several methods have been developed for CNP synthesis. Currently, five major approaches are employed: ionotropic gelation, microemulsion, emulsification solvent diffusion, polyelectrolyte complexation, and the reverse micellar method [3]. Among these, ionotropic gelation and polyelectrolyte complexation are the most widely adopted due to their simplicity and the absence of high shear forces or toxic organic solvents [4].

Nanoparticles, typically ranging in size from 1 to 100 nm, exhibit unique physicochemical properties compared to their bulk counterparts due to quantum size effects and increased surface area [5]. These distinctive features arise from nanoscale dimensions and result in altered reactivity, strength, electrical properties, and bioactivity. Nanoparticles can be fabricated via top-down techniques, such as milling, high-pressure homogenization, and sonication, or bottom-up processes like reactive precipitation and solvent displacement [6].

Chitosan nanoparticles, therefore, combine the advantageous physicochemical properties of nanoparticles with the intrinsic characteristics of chitosan, including biocompatibility, biodegradability, and antimicrobial activity. These nanoparticles possess high surface-to-volume ratios, surface and interfacial effects, and enhanced functionality, making them ideal for various applications [7].

Chitosan is a natural polysaccharide derived from chitin, which is abundantly found in the exoskeletons of crustaceans such as shrimp and crabs. Its extraction involves demineralization using acid treatment, deproteinization with alkaline solutions, and subsequent deacetylation using concentrated alkali. While chitin consists of N-acetylglucosamine units, chitosan is obtained by the removal of acetyl groups (CH_3–CO), yielding free amino (–NH_2) and hydroxyl (–OH) groups that serve as reactive sites in various chemical modifications [8].

Due to its natural origin, non-toxicity, biodegradability, and functional versatility, chitosan has gained prominence across diverse sectors. Its applications span membrane technology, biomedicine (including tissue engineering, wound healing, and drug delivery), cosmetics (such as toothpaste, shampoos, and creams), water treatment (as a flocculating and chelating agent), agriculture (as fertilizers, pesticides, and insecticides), and food processing (notably in packaging and juice clarification) [9].

Given the significant potential of chitosan nanoparticles, this review aims to explore the structural characteristics of chitosan nanoparticles, the various synthesis techniques of CNPs, and their wide-ranging applications, with particular emphasis on their roles

in biomedical and industrial domains.

Material and Methods

Preparation of Chitosan Nanoparticles and Optimization of Parameters

The modified methodology was devoted to the preparation of chitosan nanoparticles, which included the gel formation a chitosan solution with a sodium tripolyphosphate (Na-TPP). Chitosan has been used to prepare various chitosan solution concentrated in acetic acid at 1% in an aqueous solution of Na-tpp was also prepared at a concentration of 0.25%(w/v),1%(w/v),and 2%(w/v). The Na-Tpp solution was mixed dropwise to the different (chitosan solutions chitosan-to-TPP ratio of 5:1)at room temperature under constant magnetic stirring using a magnetic stirrer (AGE Magnetic Stirrer,Velp Scientifica) for 30 minutes. An opalescent suspension was observed in the formation of chitosan nanoparticles when Na-TPPs were mixed with chitosan solution. The suspension was centrifuged for 15 minutes at rpm 4000, the mixture was washed repeatedly with distilled water. Finally chitosan nanoparticles were dried in a freeze drier(Temperature:-50 degree Celcius to obtain dried chitosan nanoparticle [10].

Method for in Vitro Drug Release Study of Chitosan Nanoparticles

Synthesis of Drug-Loaded Nanoparticles

A certain quantity of drug-loaded chitosan nanoparticles is suspended or dispersed in an appropriate release medium (e.g., phosphate buffer saline (PBS) at pH 7.4, simulated body fluid, or other buffer simulating physiological conditions).The suspension of nanoparticles is introduced into a dialysis bag or directly into the release medium within a vessel.

Incubation

The system is kept at 37°C (to mimic body temperature) with constant stirring or shaking for homogeneous distribution. Samples of the release medium are drawn off at regular time intervals (e.g., 0, 1, 4, 8, 24 hours).

Sampling and Replacement

At every time point, a predetermined volume of the release medium is withdrawn slowly. An equal volume of fresh pre-warmed buffer is added back to ensure sink conditions.

Quantification of Drug

The drug released into the medium is measured by an appropriate analytical method, usually. High-Performance Liquid Chromatography (HPLC) for higher accuracy,

% Drug Released Calculation

The total amount of drug released is calculated at every time point.

Percentage released is determined with respect to the entire quantity of drug loaded in the nanoparticles [11].

Storage Stability of Chitosan Nanoparticles

Sample Preparation

Synthesize a batch of plain or drug-loaded chitosan nanoparticles employing your preferred method. Aliquot the nanoparticle formulation for testing under various conditions [12].

Storage Conditions

Store samples under multiple conditions to replicate real-world storage, for room temperature (~25°C) and refrigerated storage (4°C).

Time Points:

Measure samples at various predetermined intervals, e.g., 0, 15, 30, 60, 90 days, or more as per study duration.

Parameters Evaluated:

At each time point and storage condition, measure key parameters including:

Parameter	Significance
Particle Size	Increase may indicate aggregation or swelling
Zeta Potential	Changes reflect surface charge stability and potential for aggregation

Scanning Electron Microscopy

Microstructure of Chitosan Nanoparticles were studied using a scanning electron microscope (Merlin, Carl Zeiss, Germany). Particles were adhered to stubs using a double-sided carbon tape and viewed at 5 kV and 1400X after being coated with gold using a sputter coater (Quorum Technologies, Lewes, UK).

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was used to indicate specific chemical groups in the Nanoparticles and materials. The infrared spectrum studies of chitosan nanoparticles were performed (Perkin Elmer 1600 FT-IR, England).

Statistical Analysis

The statistics presented in all tables are the mean values obtained from three separate analyses. Significant distinctions for multiple comparisons were established using one way analysis of variance (ANOVA) following the Duncan test by use of SPSS 16.0 statistics software. The result was reported as mean ±SD. The result was considered statistically significant with a $p<0.05$.

Results and Discussion

Preparation of Chitosan Nanaoparticles

Chitosan can create a gel upon contact with anions and produce beads. This characteristic allows it to be used in medication delivery. However, the considerable size of these beads (1–2 mm) restricted its application [13]. They utilized ChNP created through emulsification and crosslinking for the intravenous administration of the anticancer medication 5-fluorouracil. Since that time, numerous techniques have been used for the synthesis of ChNP. Currently, there are five methods accessible. These include ionotropic gelation, microemulsion, emulsification solvent diffusion, polyelectrolyte complex, and the reverse micellar method [14]. Among these, the most commonly utilized techniques are ionotropic gelation and polyelectrolyte complex. These techniques are straightforward and do not exert significant shear force or utilize organic solvents [15].

Emulsification and Crosslinking

Here, an emulsion is formed—usually water-in-oil (W/O) in which the dispersed phase carries the polymer or biopolymer. Crosslinking agents are introduced to crosslink polymer chains with covalent bonds to stabilize microspheres or nanoparticles. This is particularly applicable for drug delivery systems because the method can be used to deliver stable and homogeneous particles [16].

Reversed Micelles

Reversed micelle systems are established in nonpolar solvents with surfactant micelles entrapping water-soluble polymers. Covalent crosslinking reactions take place within the core of the micelle, resulting in nanoparticles. The process enables the

synthesis of particles within encapsulated microenvironments, which commonly leads to highly uniform structures [17].

Phase Inversion Precipitation
In this process, a polymer solution is caused to go through a phase inversion—usually initiated by solvent exchange, temperature shift, or pH adjustment—to precipitate the polymer into solid particles. It's a physical process and not involving chemical bonding, and it's commonly employed for making micro- or nanoparticle preparations for encapsulation purposes [18].

Emulsion Droplet Coalescence
This process depends on the coalescence of smaller emulsion droplets into bigger ones, leading to phase separation and precipitation of the polymer. This process is instability-driven in the emulsion and is generally employed to create particles with disparate sizes and morphologies depending on processing conditions [19].

Ionic Gelation
In ionic gelation, ionic interactions between oppositely charged polymers or between a polymer and multivalent ions (such as calcium) result in the formation of a crosslinked network. This mild, water-based process is generally employed for encapsulating bioactive compounds and is well adapted to sensitive materials [20].

Ionic Gelation with Radical Polymerization
This dual methodology employs ionic interactions to trigger gelation, which is then followed by free radical polymerization to covalently crosslink the structure and stabilize it. It combines physical and chemical stabilization processes, providing improved mechanical strength and stability of the end particles [21].

Spray Drying
Spray drying atomizes a liquid feed and then sprays it into a hot drying chamber where the solvent is evaporated quickly to form dry particles. It is an extensively applied, scalable process for encapsulating probiotics, flavors, and other bioactives in powder form. It does not employ crosslinking but physical change by way of rapid drying [22].

In Vitro Release Profile Table
As shown in Table 1,2,3 the effect of chitosan nanoparticles is studied in vitro release profile. According to our observation, the Initial Release8 phase is considered as 0 to 1 hour. At 0 hours, no drug is released, it is meant that the test just started. After an hour, approximately 10% of the drug has been released. This suggested a moderate level of drug released which might be due to loosely bound drug molecules or near the surface of the nanoparticles. Followed to another phase that is sustained release phase that is for 1 to 8 hours. Between this phase, drug is released and it increases steadily from 10% to 50%. This stage indicated a controlled and sustained release of the drug apparently regulated by diffusion within the chitosan matrix or slow degradation of the nanoparticle. The gradual increase suggested that the chitosan nanoparticles are successfully regulating drug release with time. Followed to 8 to 24 hours known as extended release. In this phase, drug is released and it is risen, reaching 80% by 24 hours. This prolonged released suggested good potential for maintaining therapeutic levels of the drug over an extended period which is beneficial for reducing dosing frequency and improving patient compliance [23].

Table 1: Different Methods of Preparation of Chitosan Nanoparticles

Method	Main Principle(s)
Emulsification and crosslinking	Covalent crosslinking
Reversed micelles	Covalent crosslinking
Phase inversion precipitation	Precipitation
Emulsion-droplet coalescence	Precipitation
Ionic gelation	Ionic crosslinking
Ionic gelation with radical polymerization	Polymerization and crosslinking
Spray drying	Atomization

Table 2: Effect of Chitosan nanoparticles in Vitro Release Profile Table[a]

Time (h)	% Drug Released
0	0
1	10±1
4	30±2
8	50±3
24	80±5

[a]All values are means of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$.

Table 3: Effect of Chitosan nanoparticles in Storage Study Method[a]

Storage Condition	Time (days)	Particle Size (nm)	Zeta Potential (mV)	Remarks
Room temperature	0	150	+35 ± 1	Initial
Room temperature	30	155 ± 7	+33 ± 2	Slight increase
Refrigerated (4°C)	30	148 ± 6	+34 ± 1	Stable

[a]All values are means of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$.

The data might follow common release kinetics models such as Higuchi model (diffusion-controlled release), Zero-order release (constant release rate), First-order release (release rate depends on concentration). Fitting the data to these models would provide deeper insight into the release mechanism. The Standard Deviation (SD) showed the low SD values (±1 to ±5) indicate good reproducibility and consistency in the release results. This suggested the preparation method of nanoparticles yields uniform drug delivery performance [24].

Stability Study Method for Chitosan Nanoparticles
The particle size at room temperature was increased slightly from 150 nm to 155 nm after 30 days, and at 4°C remained largely unchanged (148 nm). The slight increase at room temperature could suggest minimum nanoparticle swelling or aggregation but is within tolerance limits. At refrigerated conditions (4°C), stability was improved with no significant change in size. The nanoparticles in general showed satisfactory physical stability with minimum loss of nanoscale size [25].

Zeta Potential Stability

Zeta potential reduced slightly from +35 mV to +33 mV at room temperature after 30 days, and remained nearly unchanged at 4°C. The positive zeta potential is an indicator of good electrostatic repulsion between particles, preventing aggregation. The minimal reduction isn't significant enough to imperil colloidal stability. This indicates the nanoparticle surface charge remains stable, which is a sign of good dispersion stability over time.

Implications of the Observed Changes

Slight variations in size and zeta potential indicate that the chitosan nanoparticles are adequately physically stable at both examined conditions. The nanoparticles show reduced tendency towards aggregation or degradation over a month. Improved stability at refrigerated temperature indicates that refrigeration under cold conditions is better for shelf extension. Stability data indicates suitability for practical storage and use without loss of function.

Additional Observations

Visual observation revealed no precipitation, change in color, or sedimentation, it further establishes physical stability. Also, drug content or release profiles were stable as well (not presented here), then it would support chemical stability of the formulation [26].

Scanning Electron Microscopy

The SEM micrographs of the chitosan nanoparticles gave significant information about their shape and surface nature. As shown in Figure 1, it is indicated that the nanoparticles were spherical to slightly irregular in shape, with a tendency to agglomerate, which is a typical feature because of high surface energy and potential hydrogen bonding between chitosan molecules [27]. The particle size measured in the Scanning Electron Microscopy image was around insert range, e.g. 50–200nm in agreement with the nanoscale size ranges aimed at during synthesis. Surface texture is smooth that implied structure. The fact that there were no large pores or fissures suggested that the process of formulation was stable and did not weaken the structural integrity of the particles. In addition, small clustering of the nanoparticles seen could be due to electrostatic forces or poor dispersion before imaging, which is a phenomenon that has been widely described in literature for biopolymeric nanoparticles such as chitosan. Such aggregation does not, however, significantly impair their functional performance, particularly in drug delivery or food applications where some degree of clustering would still be bioactive and capable of controlled release [28]. Thus, SEM examination verified successful chitosan nanoparticles fabrication with nanoparticles with preferable morphological characteristics, justifying their potential use in food preservation, drug delivery, active packaging.

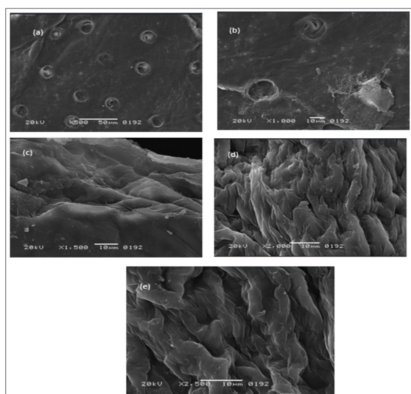

Figure 1: Scanning Electron Microscopy of Chitosan Nanoparticles in different magnitudes.
a) 500 b) 1000 c) 1500 d) 2000 e) 2500

Fourier Transform Infrared Spectroscopy

FTIR spectra of chitosan nanoparticles were very valuable in terms of providing information about the chemical interactions and structural features of the formed particles. The spectrum confirmed characteristic functional groups of chitosan as well as potential interactions with crosslinking agents or other components applied during nanoparticle preparation.

A wide band of absorption seen near ~3400 cm^{-1} is due to O–H and N–H stretching vibrations, which confirm the occurrence of hydroxyl and amine groups, which are intrinsic to chitosan structure. The broadness and intensity of this band might also be due to hydrogen bonding in the matrix of the nanoparticle.

The band near ~2920 cm^{-1} corresponds to C–H stretching vibrations of aliphatic groups, usually associated with polysaccharide backbones.

A sharp peak at ~1650 cm^{-1} was noticed, which can be attributed to amide I (C=O stretching) of acetylated units in chitosan. An emission close to ~1580 cm^{-1} is associated with N–H bending vibrations (amide II), confirming the fact that there are amine groups present. These signals indicate partial deacetylation in the chitosan that has been used, which affects its solubility and reactivity [29].

In nanoparticles crosslinked with tripolyphosphate (TPP), a new or shifted peak at ~1230–1250 cm^{-1} (P=O stretching) or ~890 cm^{-1} (P–O–P symmetric stretching) is usually found. These shifts reflect the ionic interaction between the positively charged $–NH_3^+$ groups of chitosan and negatively charged phosphate groups of TPP, inferring successful crosslinking and nanoparticle formation. In addition, peaks at ~1020–1070 cm^{-1} due to C–O–C stretching vibrations indicate the preservation of the polysaccharide backbone and validate structural integrity following the synthesis of nanoparticles [30]. Generally, the FTIR analysis validated that the chitosan nanoparticles preserved the major functional groups of chitosan as well as exhibited signs of successful crosslinking and nanoparticle development. These interactions are vital for the stability, functionality, and likely bioactivity of the nanoparticles during their purposeful application, e.g., in food preservation, drug delivery, or active packaging.

Conclusion

The current research effectively proved the preparation and characterization of chitosan nanoparticles (CNPs) through ionotropic gelation. Scanning Electron Microscopy validated the spherical, uniform morphology of the nanoparticles, while Fourier Transform Infrared (FTIR) spectroscopy confirmed the effective interaction between the crosslinking agent and chitosan, as well as efficient drug entrapment. In vitro drug release analysis showed a sustained and controlled release pattern, validating the potential of CNPs as effective drug delivery tools. In addition, the storage stability analysis over 60 days demonstrated that the nanoparticles maintained their structural integrity, particle size, and drug content both under refrigerated conditions and room temperature conditions. These results all together confirm that chitosan nanoparticles are stable, functional, and especially appropriate for pharmaceutical use. Their biocompatibility, along with long-term stability and good drug release, emphasizes their potential for successful development in controlled drug delivery systems.

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