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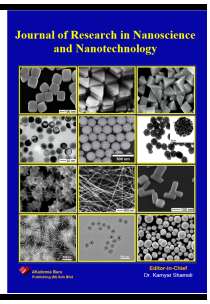

Synthesis and Characterization of Ionically Cross-Linked Chitosan Nanoparticles

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ABSTRACT

Chitosan is an amino polysaccharide with exciting scientific uses because of its distinct structure and several various functions. High biocompatibility, strong biodegradability, and low toxicity are some of the chitosan's most notable characteristics. Chitosan holds great promise for biomedical uses including targeted delivery of drugs. Therefore, this research suggests tripolyphosphate (TPP)-based ionically cross-linked chitosan nanoparticles. XRD and FTIR analysis methods were used to characterize the acquired samples. The outcomes proved that chitosan nanoparticles have an XRD pattern similar to an amorphous polymer. Additionally, FTIR verified that the nanoparticles included chitosan ammonium groups linked to tripolyphosphoric groups of TPP.

Keywords: Chitosan nanoparticles, tripolyphosphate, Biopolymer, Biodegradable compound, ionic-crosslinking.

1. Introduction

Nanotechnology is an emerging science that deals with materials of the nanometer scale [1, 2]. Nanostructures and nanophases have been applied across all scientific disciplines, but particularly in nanomedicine, as a result of the growing advances in nanotechnology. Nanoparticles are of great interest because of their distinctive structural, chemical, mechanical, magnetic, electrical, and biological features. Numerous natural and synthetic polymers, such as chitosan and polyethyleneimine (PEI), have been used to manufacture nanoparticles that can transport DNA/siRNA or anticancer drugs [3]. With an annual production of more than 100 million tonnes [4], chitosan ranks as the second most prevalent polysaccharide on the planet [5]. It is a copolymer that contains ⍟-(1,4)-2-acetamido-D-glucose and ⍟-(1,4)-2-amino-D-glucose unit (Figure 1), chitosan is prepared by removing an acetate moiety from chitin through hydration in concentrated alkali [6]. Due to its favorable physicochemical characteristics, including its excellent biocompatibility, low

toxicity, biodegradability, and good solubility in water, this polymer has received a great deal of attention [7, 8]. Additionally, the abundance of hydroxyl and amino groups in chitosan makes it possible to create nanoparticles through both chemical and physical crosslinking [9]. The pharmaceutical industry has shown a particular interest in chitosan and chitosan nanoparticles (Cs-NPs), notably for application in drug delivery systems that target specific delivery sites because they possess mucoadhesive properties [10]. The majority of diluted acids can dissolve chitosan. Chitosan could be dissolved in water and subjected to acidic conditions to impart positive charges, gelation, and membrane-forming features. Chitosan's molecular weight and level of deacetylation are the major factors that determine its chemical and physical properties [6]. Chitosan is a renewable pharmaceutical adjuvant that is a natural substance with a good biocompatibility [6]. The covalent cross-linking of polymer chains or the use of physical interactions between the polymer chains, like hydrogen bonds, electrostatic forces, or hydrophobic associations, can be used to manufacture polymer-based nanoparticles [11].

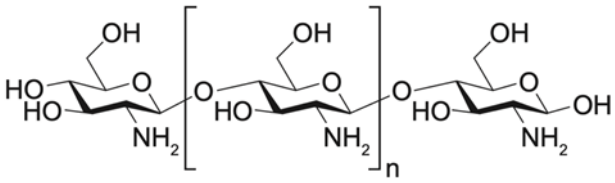

Fig. 1: Structure of chitosan.

In this regard, the sodium tripolyphosphate (TPP)-aided CS-NPs were used as promising nanocarriers in the pharmaceutical industry. The effective delivery of drugs and proteins is made possible by the CS NPs' ability to enter tissues deeply through the fine capillaries [11]. Negatively charged functional groups that are a part of the cross-linker agent interact electrostatically to form an ionic gel. Tripolyphosphate (TPP) and native chitosan's positively charged amino groups are frequently utilized together [7]. Despite the fact that numerous harmful anionic crosslinkers, like glutaraldehyde, can be used, TPP's advantageous features, like its biocompatibility and biodegradability, make it a more acceptable crosslinker for medical applications. Since tripolyphosphate (TPP) is nontoxic, multivalent, and capable of forming gels through ionic interactions, it has frequently been utilized to produce chitosan nanoparticles. This process involves cationic chitosan reacting spontaneously with an anionic cross-linking agent, often tripolyphosphate (TPP), to create a polyelectrolyte complex known as TPP/Chitosan. This method relies on the electrostatic interaction of positively charged chitosan amino groups with negatively charged sodium tripolyphosphate (TPP) groups acting as a cross-linking agent [12]. In order to produce the material, TPP solution must be added to chitosan while it is being continuously stirred magnetically at room temperature [3]. As chitosan nanoparticles form, it gives off a milky look; these nanoparticles can be kept at ambient temperature. The created nanoparticles can be used both in vitro and in vivo in biomedical applications as a substantial drug/gene carrier system. Parenteral drug delivery, ocular drug delivery, mucosal drug delivery, oral drug delivery, gene delivery, vaccine delivery, cancer therapy, pulmonary drug delivery, and intranasal drug delivery are only a few possible applications for chitosan nanoparticles [13]. The goal of the current study was to synthesize Cs-NPs by ionic cross-linking utilizing low molecular weight chitosan (LMWC) and sodium tripolyphosphate (TPP), a cross-linker. X-ray powder diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) techniques were used to assess the physiochemical properties of the synthesized Cs-NPs.

2. Materials and Methods

2.1 Materials

Sigma Aldrich (St. Louis, MO, USA) supplied acetic acid glacial (CH_3COOH) (98 %), chitosan (low molecular weight, 190,000–310,000 degree of acetylation), TPP, and Tween-80. All of the chemicals used were analytical grade and were not purified further. Before usage, all glassware was cleansed in deionized water and dried.

2.2 Synthesis of Ionically Cross-linked Chitosan Nanoparticles (Cs-NPs)

The ionic cross-linking approach was used to make Cs-NPs. First, 0.250 g of chitosan powder was dissolved in 98 ml of deionized water with 1.0% of acetic acid to obtain a polymer solution. In 15 ml of deionized water, 0.5 g of TPP cross-linker was dissolved. The TPP solution was then added dropwise to the chitosan solution while the homogenizer was continuously vigorously stirred, resulting in TPP: Cs ratio of 2:1. 2 drops of Tween 80 were added to the solution, and gentle stirring at 9000 rpm was maintained for 1 hour. After that, the mixed solution was rinsed with distilled water and centrifuged three times for 15 minutes at 4000 rpm. Lastly, the nanoparticles were freeze-dried for 24 hours to obtain powdered nanoparticles for further analysis.

2.3 Characterization

2.3.1 X-Ray Diffraction (XRD) Spectroscopy

The X-ray diffraction (XRD) technique is recommended for determining the crystallinity of Cs-NPs. Cu K radiation (λ= 0.15406 nm) of A PANalytical X'Pert PRO XRD was used to examine the structural properties of the produced samples. In a 2θ (from 5° to 80°) with a scanning rate of 2θ/min, a 20 mA applied current and a 45 kV accelerating voltage were utilized. Cs-NPs are subjected to intense rays from the XRD machine during XRD examination, which penetrates through it and supplies valuable data about its structure. Due to the lack of a diffraction peak, this approach cannot be utilized to estimate the amorphous structure of nanoparticles. The nanoscale is shown by the widening of the XRD pattern.

2.3.2 Fourier Transform Infrared (FTIR) Spectroscopy

The existence of potential functional groups in produced Cs-NPs is determined using FTIR spectroscopy [14]. The spectral region between 4000 and 400 cm-1 was scanned and the KBr disc method was used for recording the spectra. At the point where infrared light is used to bombard samples of Cs-NPs, some of the radiation is absorbed. The molecular fingerprint of the unabsorbed radiation represents the Cs-NPs' identity.

3. Results and Discussion

Figure 2, suggest the possible intermolecular chemical interactions between functional groups in cross-linked Chitosan nanoparticles. Intermolecular interactions in the nanoparticles were triggered by the presence of Van der Waal and hydrogen forces [15]. Cationic groups of chitosan (NH_3^+) in the TPP solution, may react with $–OH^-$ and phosphoric ions during sample manufacturing to aid deprotonation of the Chitosan [15]. Moreover, the samples were potentially cross-linked well in the composites by Van der Waals interaction and hydrogen bonding [15]. The samples were freeze-

dried for the complete synthesis. The freeze-drying procedure, according to reports, helps to increase the number of –OH groups and forms fibrous white fluffy materials by causing gentle vaporization of the water molecules and sublimation of the ice crystals on the materials [15].

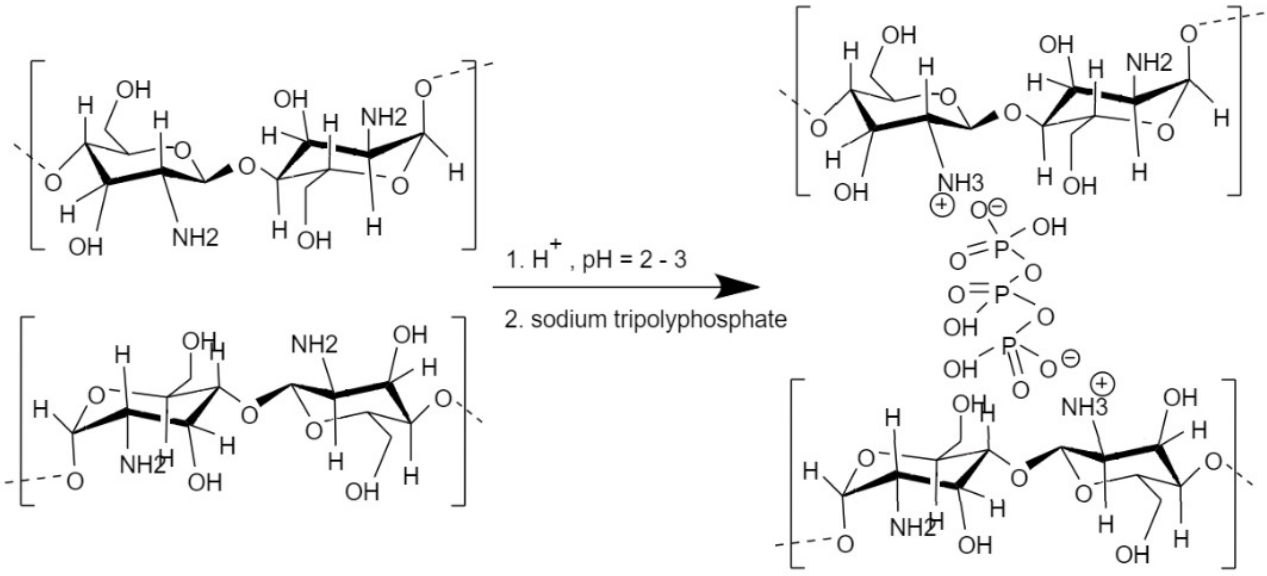

Fig. 2: The possible intermolecular chemical interactions in cross-linked chitosan nanoparticles.

3.1 X-Ray Diffraction Analysis of Cross-linked Cs-NPs

Figure 3 displays the XRD analysis of cross-linked chitosan nanoparticles (Cs-NPs) at the weight ratio of TPP: Cs is 2:1. X-ray diffraction studies of native chitosan exhibit very broad peaks at 2θ = 10.82° and 2θ = 20.19° that correspond to the plane of (020) and (110), respectively [16]. After crosslinking with TPP during the fabrication of chitosan NPs, the crystalline structure of native chitosan was destroyed, where the peak intensity of (020) and (110) was observed to disappear and shifting of small peak near to 2θ = 18.89° in the pattern of cross-linked Cs-NPs respectively, this is consistent with earlier findings and reveals the amorphous nature [17-19]. The decrease in crystallinity could be related to the fact that chitosan NPs are formed of a dense network structure of interpenetrating counter ions of TPP, which crosslink the chains of polymer. As a result, chitosan nanoparticles have an amorphous polymer-like XRD pattern.

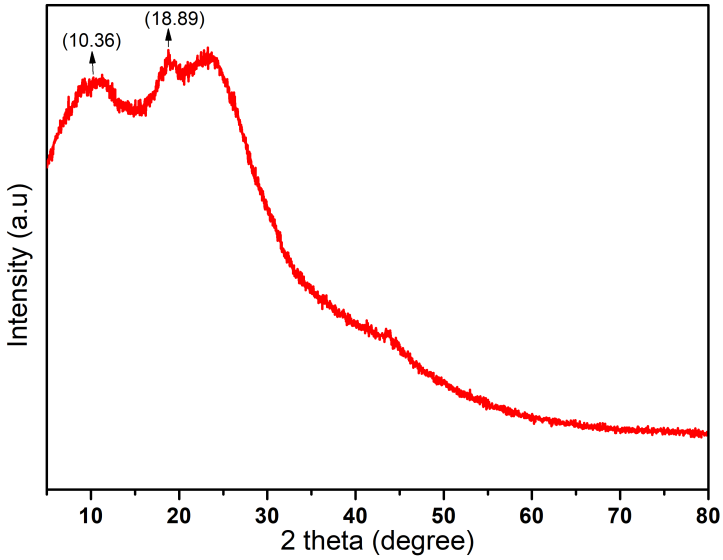

Fig. 3: XRD analyses of cross-linked chitosan nanoparticles.

3.2 Fourier Transform Infrared Spectroscopy analysis of Cross-linked Cs-NPs

By producing an infrared absorption spectrum, FTIR is used to determine changes in the chemical structure of a material. Figure 4 exhibits the FTIR analysis results of cross-linked Cs-NPs. The -NH2 and hydrogen-bonded O-H stretching vibrations are attributed to the strong and wide peak between 3300-3500 cm-1, while the asymmetric stretch of C-O-C and C-N stretching vibrations of type I amine are attributed to the peaks at 1150 cm-1 and 1317 cm-1 in the chitosan spectra, respectively [15, 20]. Also, a band for P=O appears at 1152 cm-1. The peaks 1535 cm-1 and 1635 cm-1 for N-O asymmetric stretching and N-H bending vibration of amine I and the amide II carbonyl stretch respectively confirm linkage between phosphoric and ammonium ions [20, 21]. In Cs-NPs, the broader peak 3428 cm-1 with higher relative intensity indicates that hydrogen bonding has been enhanced [22]. As a result of the FTIR data, the chemical structure related Cs-NPs is cross-linked with sodium tripolyphosphate.

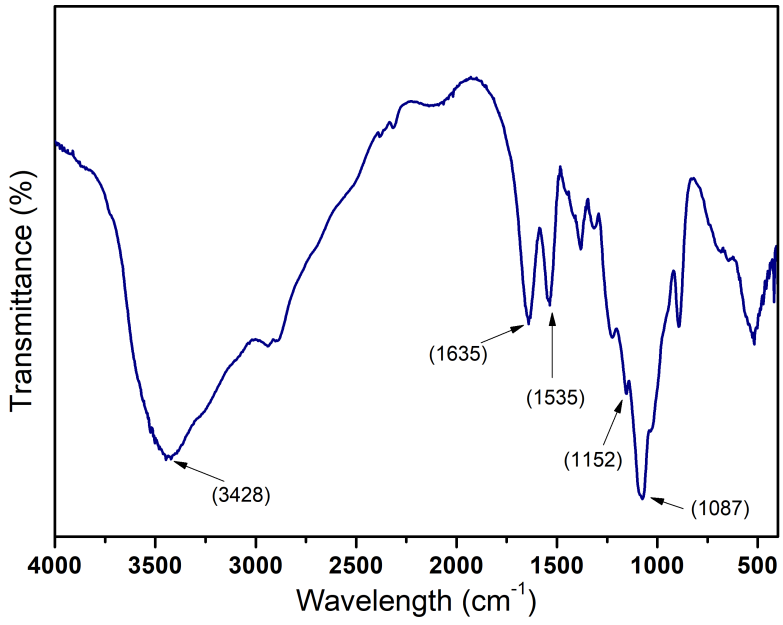

Fig. 4: FTIR analyses of cross-linked Cs-NPs.

Conclusion

The development in nanoscience has significantly increased the application of nano-polymers in medical science. Cs-NPs were effectively synthesized using the ionic gelation method, TPP as a cross-linking agent, Tween-80 as a stabilizer, and acetic acid as a catalyst for hydrogen ion formation. XRD analysis showed that the cross-linked Cs-NPs were amorphous due to TPP cross-linked chitosan. The FTIR results indicated the functional groups related to the formation of Cs-NPs. Therefore, this study indicated the successful fabrication and characteristics of Cs-NPs cross-linked with TPP.

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