Synthesis And Characterization of Vancomycin-Loaded Chitosan Nanoparticles For Drug Delivery

Tajuddin Hafizi

Amirkabir University of Technology

Mehrnoosh Hasan Shahriari

Amirkabir University of Technology

Majid Abdouss (✉ phdabdouss44@aut.ac.ir)

Amirkabir University of Technology

Shafiq Ahmad Kahdestani

Amirkabir University of Technology

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Abstract

Chitosan is a linear polysaccharide with prominent physicochemical and biological properties such as biocompatibility, biodegradability, nontoxicity, non-immunogenicity, bioadhesion, antibacterial, antifungal and hemostatic activity. Due to these properties, it has found many applications in cosmetic, textile, and food industries; agriculture; biotechnology; pharmaceutical industry and medicine; specially in biomedical applications. The special chemical structure of chitosan allows some specific modifications and by reducing the size of chitosan particles to Nano size, it becomes an excellent drug nanocarrier. Vancomycin is a typical antibiotic used for bacterial infections caused by gram positive bacteria. In this work, chitosan nanoparticles (CSNPs) were prepared via ionotropic gelation using tripolyphosphate (TPP) as crosslinker. The effect of chitosan and TPP concentration on the size of chitosan nanoparticles was studied and CS:TPP ratio of 1:1 with average size of nanoparticle about 100 nm were selected. The prepared samples were characterized using DLS, FTIR, TGA, DSC, and SEM techniques. The results confirmed that vancomycin has been loaded successfully on chitosan nanoparticles and there was not any interaction between vancomycin and chitosan. Also, it is observed that 40% of vancomycin is released burstly in the first 9 h and after that the drug release is continued gradually to receive 90% at 100 h.

Introduction

Chitosan is a linear polysaccharide which is obtained by partial deacetylation of chitin in alkali environment [1–4]. Chitosan with its physicochemical and biological properties such as biocompatibility, biodegradability, nontoxicity, non-immunogenicity, bioadhesion, antibacterial, antifungal and hemostatic activity has attracted the attention of many researchers [2, 5–7]. Due to these properties, chitosan has found many applications in cosmetic, textile, and food industries; agriculture; biotechnology; pharmaceutical industry and medicine; specially in biomedical applications [2–4, 8–11].

Chitosan has a special chemical structure which makes it unique over other polysaccharides. The structure of chitosan allows some specific modifications via amine or hydroxyl functional groups, which can be loaded with drug directly or through linker [6].

It is found that nanostructures have real potential as effective drug delivery systems, especially in the treatment of microbial infections, due to their advantages over conventional antibiotic formulations [12–14]. Nanoparticles (NPs) due to their small size (1–100 nm) can carry high drug loads as nanovehicles. Also, NPs are used to deliver proteins, peptides, enzymes, genes as well as vaccines [5]. Chitosan nanoparticles (CSNPs) are widely studied in the field of drug, protein, and gene delivery [4, 6]. Small particle size, compactness and high surface area are the unique physical and chemical properties of chitosan nanoparticles which are associated with many biomedical applications such as drug release and mucosal remodeling [15]. CSNPs are prepared by different methods like ionotropic gelation, polyelectrolyte complexation, reverse micellar, emulsion solvent diffusion and electrospraying techniques [1, 6].

One of the most studied method for preparing chitosan nanoparticles is ionotropic gelation, because it is simple and easy and the aqueous medium used in this method eliminates the hazards of using organic solvent. Ionotropic gelation is based on crosslinking of cationic chitosan amino groups to a polyanionic crosslinker. Tripolyphosphate (TPP) is mostly used as negatively-charged polyanion to crosslink with chitosan. The obtained complex is used as drug nanocarrier [1, 4, 5].

Vancomycin is a glycopeptide antibiotic (Scheme 1) that blocked the synthesis of peptidoglycan in bacterial cell wall. It has strong bactericidal activity against Gram positive resistant pathogen. Vancomycin is used only parenterally in clinical therapies, because the continuous oral dosing increases vancomycin concentration and may leads to toxicity and development of resistance [16–19].

One of the diseases in which vancomycin is used is osteomyelitis. Osteomyelitis is an inflammatory bone disease caused by pathogenic microorganisms, mostly *Staphylococcus aureus* and leads to progressive bone destruction and loss. The disease is diagnosed by the formation of bacterial plaque around the infected area. Depending on the duration of the infection, osteomyelitis is classified to acute or chronic. The pathogenic microorganisms can adhere to and even invade mammalian cells. Methicillin-resistant *S. aureus* and multidrug-resistant mycobacteria are drug-resistant pathogens, which can develop persistence in the intracellular locations after drug treatment. Many researches have been done on the vancomycin loaded nanoparticles, especially CSNPs [16–22].

In the present work, chitosan nanoparticles were prepared through ionotropic method and then loaded with vancomycin. The prepared samples were characterized using Fourier transform infrared spectrometry (FTIR), dynamic light scattering (DLS), scanning electron microscopy (SEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and UV-Vis spectrophotometry.

Experimental

Materials

Low molecular weight chitosan (80–85% deacetylation), sodium tripolyphosphate (TPP), and acetic acid were obtained from Sigma-Aldrich Chemicals Co., Germany. Vancomycin was purchased from Razavi Pharmaceutical Co. (Iran).

Preparation of chitosan nanoparticles

In order to prepare chitosan nanoparticles, low molecular weight chitosan was dissolved at 25°C by adding acetic acid (2 M) gradually and stirring at 9000 rpm (Heidolph, Germany). By using NaOH (2 M) solution, the solution pH was adjusted to 5.5. To remove any undissolved chitosan, the solution was filtered using 0.45 µm cellulose acetate filter.

TPP solution was prepared by dissolving 0.25 wt% TPP in double-distilled water and passed through 0.25 µm cellulose acetate filter.

Three different concentrations of chitosan (0.1, 0.2, and 0.3 wt%) were prepared to evaluate the effect of the chitosan to TPP ratio on nanoparticle size. Then 2 ml of TPP at a concentration of 0.2 wt% was added dropwise by burette to 4 ml of chitosan solution at a rate of 0.2 ml/min. To prepare the chitosan nanoparticles, TPP solution was added drop by drop in CS:TPP volumetric ratios (1:2, 1:3, 1:4) to the chitosan solution, and stirred at room temperature at 9000 rpm.

Some of the prepared samples were used to determine the nanoparticle size and the remaining was centrifuged (Sigma, Germany) for 20 min. Then the samples were washed with water and centrifuged again to remove unreacted gradients. Then, the nanoparticles were air dried at ambient temperature and analysed. Each of the reported results is an average of three repetitions of the test.

Loading of vancomycine

To prepare vancomycin-loaded CSNPs, vancomycin was added at a concentration of 0.5 µg/ml to chitosan solution at a concentration of 0.2 mg/ml. After that, TPP solution with concentration of 0.45 mg/ml was added to the chitosan solution containing vancomycin. The steps of CSNPs preparation and vancomycin loading are shown typically in Fig. 1.

Characterization

To evaluate the linking of functional groups in chitosan, chitosan nanoparticles, and drug to the nanoparticles, a Fourier Transform Infrared Spectrometer (Perkin Elmer, Germany) were used.

The particle size and the nanoparticle size distribution were determined using dynamic light scatter (Cordovan Tech, France). For this purpose, the solution was sonicated for 3 h after the formation of nanoparticles and immediately subjected to DLS test.

The morphology investigation of the prepared sample was performed using scanning electron microscope (SEM) (Seron Technology, South Korea). Determination of of the prepared nanoparticles crystallinity was carried out using X-ray diffractometer (XRD) (Inel Inc., France).

Melting point, glass transition temperature, and the weight loss of the prepared samples were determined by differential scanning calorimeter (DSC) and thermal gravimetric analyzer (TGA) (Sanaf, Iran). The test was done at heating rate of 10°C/min under nitrogen flow of 40 ml/min and the specimen was heated from room temperature to 300°C.

Drug release study

UV-vis spectroscopy (280-4 Alph, Germany) at 280 nm was used to measure vancomycin dispersion in the nanocrystalline membrane. For this purpose, a membrane containing nanoparticles (15 mg weight) was placed in 30 ml phosphate solution and incubated at 37°C with shaking at 60 rpm. 1 ml of the nanoparticles were recovered at predetermined intervals and the volume of fresh PBS equivalent was added to the suspension. The drug solution concentrations used were 20, 40, 80, 120, 160 and 200 ppm. The standard curve in Fig. 2 was used to calculate vancomycin concentration in each sample. The

released drug from the sample (1 ml) was measured at predicted times of 20, 40, 60, 100, 150 and 210 min as well as 5, 7, 10, 22, 29, 36, 48, 54, 75, and 100 h. All experiments were repeated three times.

Results And Discussion

Preparation of CSNPs

In this work, chitosan nanoparticles were prepared by ionotropic gelation. This method involves ionic interactions between the positive charge of CS and the negative charge of TPP. The chitosan molecules are gelled when they crosslinked ionically. Ionic crosslinking of chitosan is a noncovalent interaction which can occur in the presence of negatively charged multivalent ions like TPP. Noncovalent or physical crosslinking is more promising for pharmaceutical applications than covalent crosslinking, because it is reversible and may avoid toxicity of the reagents [23].

Previous studies showed that the particle size and surface loadings has been affected by the chitosan concentration, the ratio of CS:TPP, and pH of the solution. The primary aim was to determine the conditions that the nanoparticles can produced with optimal characteristics, such as proper nanoparticle size and minimum polydispersity index. The particle size affected the ability of the drug delivery system to penetrate the tissue and effective drug release [23, 24].

It was found that by increasing the CS concentration the size of the formed particles increase. This also occurs with increasing TPP concentration. In the present study, the suspension of CS-TPP nanoparticles with concentrations of 0.1, 0.2 and 0.3 CS as well as TPP with concentration of 0.45 mg/l was prepared. The effect of different concentrations of these two solutions on particle size and morphological properties was investigated.

Dynamic light scattering (DLS)

As was mentioned before, many factors affected the size and distribution of chitosan nanoparticle including molecular weight of chitosan, CS:TPP ratio, the addition conditions of TPP to chitosan, chitosan and TPP concentration, the pH of chitosan, ambient temperature, and agitation rate. DLS technique was used to measure the hydrodynamic diameter in the nanometer range. Table 1 shows the effect of CS:TPP ratio on the size of chitosan nanoparticle. According to the results the CSNPs sample with size of 99.73 nm and CS:TPP ratio of 1:1 was selected for further analysis.

Table 1
Effect of CS:TPP ratio on the nanoparticle size.

Chitosan conc. (mg/ml)	TPP conc. (mg/ml)	CS:TPP	Stirrer velocity (rpm)	Temperature (°C)	Nanoparticle diameter (nm)
0.1	0.45	1:1	6000	25	325.84
0.2	0.45	1:1	9000	25	680.93
0.3	0.45	1:2	9000	25	478.97
0.2	0.45	1:1	9000	25	99.73

Dynamic light scattering test was also performed after preparing vancomycin loaded chitosan nanoparticles. it was observed in Fig. 3 that the nanoparticles size increased due to drug loading and reached about 100 nm.

The nanoparticles size depends greatly on the ratio of CS:TPP, and with increasing this ratio from 1:1 to 2:1, the nanoparticle size has changed from 305.84 nm to 93.73 nm. The DLS test was also performed after loading vancomycin on the nanoparticles. It was observed that the size of the nanoparticles increased slightly due to the drug loading and the average nanoparticle size reached 100.88 nm (Fig. 3).

Scanning electron microscopy (SEM)

The SEM images of chitosan nanoparticles with and without vancomycin are shown in Fig. 4. As can see in Fig. 4a, the image of unloaded CSNPs revealed a smooth and homogenous surface. surface nanoparticles represent a very uniform and spherical shape morphology with average size of 86 nm. SEM image of vancomycin-loaded chitosan nanoparticles is shown in Fig. 4b. The image also revealed a smooth and homogenous surface without any crystallized vancomycin. On the other hand, the drug is distributed homogeneously at molecular level, which corresponds to the results obtained by some researchers [16, 27].

FTIR spectroscopy

Figure 5 shows the FTIR spectra of chitosan, CSNPs, vancomycin, and vancomycin-loaded CSNPs. In the chitosan spectrum (Fig. 5a), a peak at 3360 cm^{-1} is observed for O-H group overlapped with N-H group stretching vibrations. The peak appears at 2963 cm^{-1} is assigned to the C – H stretching vibration mode. The absorption peaks at 1633 and 1594 cm^{-1} are attributed to C = O bending vibration and N-H bending vibration of protonated amino ($-\text{NH}_2$) group, respectively. The C-N stretching vibration of the amine group was observed at 1230 cm^{-1} and the asymmetric C-O-C stretch was observed at about 1158 cm^{-1} . A weak peak observed at 1420 cm^{-1} is attributed to the bending vibration of O – H [26]. The 1580 cm^{-1} peak of – NH bending vibration shifts to 1630 cm^{-1} and a new peak appears at position 1530 cm^{-1} which is assigned to N-O-P stretching vibration. This indicates that the TPP anions were crosslinked with the

ammonium groups of CS to form CS-NPs as illustrated in Fig. 2. Similar results were also obtained in other works [11, 12].

The CSNPs spectrum (Fig. 5b), the peak at 3427 cm^{-1} is attributed to O-H group overlapped with N-H group stretching vibrations and indicates that hydrogen bonding is increased. The peak at 1594 cm^{-1} for bending vibration of N - H shifts to 1635 cm^{-1} and the new peak at 1553 cm^{-1} is attributed to N-O-P stretching vibration. which implies the crosslinking of TPP anions with ammonium groups of chitosan to form chitosan nanoparticles [28]. Figures 5c and 5d show the FTIR spectrum of vancomycin drug and drug-loaded CSNPs. FTIR spectra of vancomycin show its characteristic broader peak attributed to OH stretching vibration of $\text{R-CH}_2\text{-CH}_3$, COOH , R-NH-R around 3240 cm^{-1} , carbonyl stretching vibration of amide group at 1655 cm^{-1} , alkyl chain at 1492 cm^{-1} - 1122 cm^{-1} band, R-O-R stretching vibration at 1010 cm^{-1} and aromatic peak at 1552 cm^{-1} .

The spectra of drug-loaded CSNPs in Fig. 5d show the overlapped of N-H stretching vibration peak at 3431 cm^{-1} and shifting of carbonyl stretching to 1540 cm^{-1} - 1395 cm^{-1} , R-O-R stretching similar peaks observed at 1010 cm^{-1} and aromatic peak at 1637 cm^{-1} . Thus, the characteristic spectrum in Fig. 5d show shifting and overlapping of absorption peaks assigned the successful loading of vancomycin on CSNPs.

X-ray diffraction analysis (XRD)

The X-ray patterns of chitosan, chitosan nanoparticles, and vancomycin-loaded CSNPs are shown in Fig. 6. While chitosan has a strong peak at $2\theta = 22^\circ$ (Fig. 6a), CSNPs has a weak and broad peak (Fig. 6b). This is due the decreasing of intensity of the characteristic peak for chitosan nanoparticles which is attributed to the crosslinking. This pattern change can be assigned to the modification of molecules arrangement in the crystal lattice. This intensity reduction is more obvious in the vancomycin-loaded CSNPs pattern. It is clear in Fig. 6c that the pattern shows a stronger peak than that of CSPNs peak at $2\theta = 25^\circ$.

Thermogravimetric analysis (TGA)

The TGA thermogram of chitosan, chitosan nanoparticles, vancomycin, and vancomycin-loaded chitosan nanoparticles are shown in Fig. 7. It is clear that for chitosan, the first weight loss about 3.5% is occurred at 50°C to 100°C which is attributed to the loss of absorbed or bound water severe weight loss and related to the hydrophilic nature of chitosan [29]. The decomposition is initiated at 200°C and obvious weight loss occurred from 200°C to 450°C which probably corresponds to the dehydration of the anhydro glucosidic ring. The residue remained at the end of the experiment (at 800°C) is about 22%. As can be seen for chitosan nanoparticle, the thermal behavior is changed. The water elimination is occurred within 50°C to 100°C with 7.5% weight loss. It seems that more weight loss at the dehydration stage of CSNPs than CS due to its higher hydrophilicity that resulted in more bound water. CSNPs is decomposed from 150°C to 250°C with 60% remaining residue.

Vancomycin is decomposed at 155°C to 560°C with 20% final remaining residue. The thermal behaviour pattern of drug-loaded CSNPs is similar to CSNPs which the decomposition of CSNPs is occurred from 152°C to 280°C. In general, the results confirm that thermal stability of CSNPs is higher than CS, which can be corresponded to the interactions due to the crosslinking of CS molecules with TPP. Also, the crosslinker reduces the decomposition temperature. These results are consistent with the results of other researchers [28, 29].

Differential scanning calorimetry (DSC)

The DSC results of chitosan, chitosan nanoparticles, vancomycin, and vancomycin-loaded chitosan nanoparticles are shown in Fig. 8. It is obvious that vancomycin has melting point (T_m) of 240°C and a glass transition temperature (T_g) of 72 °C. The melting point and glass transition temperature for chitosan are 230°C and 67.5 °C and for and chitosan nanoparticles 240°C and 75.7 °C. These temperatures are 248°C and 70 °C, respectively for vancomycin-loaded chitosan nanoparticles. we had wider characteristics at melting temperature of 67.5 and glass °C 230. Thermography of chitosan nanoparticles The melting temperature of glass and its nanoparticles reached 75.7, 240 degrees Celsius, and the chitosan nanoparticles with drug showed marked signs of vancomycin at 70 degrees Celsius, 248 degrees Celsius.

As can be seen, teicoplanin thermogram shows a melting point (T_m) of 75.3 °C and a glass transition temperature (T_g) of 250.6 °C, while for chitosan these temperatures are 67.5 °C and 230 °C, respectively. Due to the nanoscale of chitosan nanoparticles, their T_m and T_g have risen to 75.5 °C and 240 °C, respectively. The thermogram of teicoplanin loaded chitosan nanoparticles exhibited all characteristic peaks of teicoplanin at 72°C and 250°C, therefore there is no interaction between teicoplanin and chitosan.

Drug release

The vancomycin release from chitosan nanoparticles was evaluated for 100 h. A burst release of vancomycin from chitosan nanoparticles was observed at the initial stage. 40% of vancomycin was released in the first 9 h. Then the release is continued gradually and finally 90 % of vancomycin was released in 100 h. It seems that chitosan nanoparticles reduce the drug release rate and vancomycin-loaded CSPNs are suitable for sustained drug release.

Conclusion

Chitosan nanoparticles are widely used in biomedical applications especially in drug delivery systems. Chitosan nanoparticles were prepared through ionotropic gelation using TPP as a crosslinker and vancomycin, which is a typical antibiotic used for bacterial infections caused by geram positive bacteria, was loaded. The effect of chitosan concentration and TPP concentration on the size of chitosan nanoparticles was studied and the CS:TPP ratio of 1:1 with average size of nanoparticle about 100 nm

were selected. The prepared samples were characterized using DLS, FTIR, TGA, DSC, and SEM techniques. The results confirmed that vancomycin has been loaded on chitosan nanoparticles and there was not any interaction between vancomycin and chitosan. Also, it is observed that 40% of vancomycin is released burstly in the first 9 h and after that the drug release is continued gradually to receive 90% at 100 h. Thus it seems that chitosan nanoparticles reduce the drug release rate and are suitable for sustained drug release.

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Figures

Figure 1

The steps of chitosan nanoparticle formation and vancomycin loading.

Figure 2

Standard curve for vancomycin uptake.

Figure 3

Dynamic light scattering of (a) Chitosan nanoparticles and (b) vancomycin-loaded chitosan nanoparticles.

Figure 4

SEM images of (a) chitosan nanoparticles and (b) vancomycin loaded nanoparticles.

Figure 5

FTIR Spectrum of (a) chitosan, (b) chitosan nanoparticles, (c) vancomycin, and (d) vancomycin- loaded CSNPs

Figure 6

X-ray diffraction pattern of (a) chitosan, (b) chitosan nanoparticles, and (c) vancomycin-loaded chitosan nanoparticles.

Figure 7

TGA thermogram of chitosan, chitosan nanoparticles, vancomycin, and vancomycin-loaded CSNPs.

Figure 8

DSC thermogram of (a) chitosan, (b) chitosan nanoparticles, (c) vancomycin and (d) vancomycin-loaded chitosan nanoparticles.

Figure 9

Diagram of drug release from chitosan nanoparticles as a nanocarriers.

Supplementary Files

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- [Scheme1.png](#)