Original Article

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Optimization Parameters to Prepare Chitosan Nanoparticles Containing Sulfacetamide Sodium

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Abstract

Management of ocular disease can be improved by prolonging the contact time of ophthalmic antibiotics with the ocular surface, with bioadhesive polymers such as chitosan. Additionally, this polymer with antifungal and antibacterial activities could increase the antimicrobial effects of the antibiotics. In the present study, chitosan (CS) nanoparticles were investigated as a vehicle for ophthalmic delivery of antibiotic, sulfacetamide sodium. Ionotropic gelation method was used to fabricate chitosan -sulfacetamide sodium nanoparticles. The effects of various factors including concentration of CS, concentration of tripolyphosphate (TPP), and stirrer rate on the size of nanoparticles were studied. Different weight ratio of CS to sulfacetamide sodium on the encapsulation efficiencies of nanoparticles was assessed. The particles were prepared under optimal condition of 0.45% CS concentration, 0.45% TPP concentration, stirrer rate at 6000 rpm. Their particle size was 72nm. In these particles with 1:2 weight ratio of CS to sulfacetamide sodium the encapsulation efficiency was 42%. In vitro release profile showed that sulfacetamide sodium could not be released sufficiently during 24h. Future studies should focus on in vitro and in vivoantibacterial properties to evaluate their potential as an ocular delivery system.

Keywords: Sulfacetamide Sodium, Chitosan , Tripolyphosphate, Nanoparticles

1. Introduction

Continuous tear flow in the eye is the most important protective factor against the microorganismsattack. Tears also contain some antimicrobial agents including immunoglobulins, lymphocytes, lysozyme and lactoferrin which specifically inhibit bacterial proliferation on the ocular surface (1).

However, when the eye exposes to risk factors such as injury, allergic hypersensitivity, and systemic diseases, its defense mechanisms may be damaged. These conditions provide the media for bacterial growth (2). Contact time of antibiotics in the eye is limited by the tear flow and reflex blinking. Therefore a few percent of administered drug remain in the site of action. Using mucoadhesive drug delivery systems can prolong the contact time of antibiotics to the infectious site (2, 3). Many natural and synthetic polymers have proper characteristics for this aim (4, 5). Chitosan is a good choice in this regard (3).

Chitosan as a natural mucoadhesive polymer has antibacterial activity against Gram positive and Gram negative bacteria (6-9). Binding to the negatively charged bacterial cell wall, with consequent destabilization of the cell envelope and changed permeability, followed by attachment to DNA, is the proposed mechanism for chitosan antimicrobial action (10-12).

Particulate systems such as microparticles

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or nanoparticles of chitosan is desirable for delivery of eye antibiotics (6). There are various methods for chitosan nanoparticles preparation. Among them ionic gelation method is a simpler and a safer one since nanoparticles are formed through simple ionic interaction between polycationic chitosan and a poly anionic agent like tripolyphosphate (TPP)(13).

Sulfacetamide is a sulfonamide antibiotic with bacteriostatic actions and broad-spectrum activity against most gram-positive and many gram-negative organisms. It inhibits multiplication of bacteria by acting as competitive inhibitors of p-aminobenzoic acid in the folic acid metabolism cycle (14). Eye drops containing 10-30% sulfacetamide sodium and eye ointments containing up to 10% are used for the treatment of ocular infections(14).In this studychitosan nanoparticles loaded by sulfacetamide sodium were prepared using ionic gelation method. The effects of various factors including concentration of chitosan, concentration of tripolyphosphate (TPP), and stirrer rate on the size of nanoparticles were investigated. Drugencapsulation efficiency and *in vitro* drug release were studied.

2. Material and methods

Sulfacetamide sodium was purchased from Exir pharmaceutical Co. (Iran), low molecular weight chitosan (CS) and tripolyphosphate (TPP) from Sigma Aldrich Co. (USA). All other reagents were of analytical grade and used as received.

2.1. Analysis method of Sulfacetamide sodium

Stock solution of sulfacetamide sodium in phosphate buffer (pH=7.4) at concentration of 100µg/mL was made. Then the stock solution was diluted into series of standard solutions with sulfacetamide sodium concentrations of 20 µg/mL, 10 µg/mL, 5 µg/mL, 2 µg/mL, 1 µg/mL, and 0.5 µg/mL respectively. The absorbance of each solution at 256nm was determined by UV-Vis spectrophotometer (PG instruments, Model T80+, England). Then sulfacetamide sodium standard curve was drawn and the corresponding data was regressed as standard equation.

2.2. Preparation of Sulfacetamide sodium -loaded chitosan nanoparticles

Chitosan nanoparticles were prepared using ionic gelation method based on previous study (15). CS was dissolved in aqueous solution of 1% v/v acetic acid and TPP solution was added to it drop wise under stirring at room temperature. Nanoparticles were formed spontaneously and faint turbidity was appeared. Orthogonal experiment was used for evaluation of the effect of CS concentration, TPP concentration, and stirring speed on the particle size. According to Minitab (V16.2.4) software, three variable factors and their three levels were defined and particle size was considered as desirable feature. These factors were shown in Table 1. According to these factors, 6 formulations were designed.

The formulation with the lowest particle size was selected for preparation of sulfacetamide sodium- loaded chitosan nanoparticles. Sulfacetamide sodium was added into CS solution at different polymer: drug ratio (W:W) prior to the addition of TPP solution.

2.3. Characterizations of nanoparticles

2.3.1. Particle size

Particle size distribution of nanoparticles was determined using a laser diffraction particle size analyzer (Shimadzu, Model SALD-2101, Japan). Span index for determining the polydispersity of size distributionwas calculated:

Table 1. Variable factors and their levels for fabrication of chitosan nanoparticles.

LEVELS	VARIABLE FACTORS		
	CS Conc.(W/V)%	TPP Conc.(W/V)%	Stirrer speed (rpm)
1	0.2	0.3	6000
2	0.3	0.45	9000
3	0.45	0.675	13500

Table 2. Particle size distribution of chitosan nanoparticles in different formulations.

Formulation Code	CS Conc. (W/V)%	TPP Conc. (W/V)%	Stirrer speed (rpm)	Particle Size (nm)	Span
F1	0.2	0.3	6000	5.77±117.33	1.29±0.208
F2	0.2	0.45	9000	2.64±112	1.110±0.056
F3	0.2	0.675	13500	45.13±160	1.49±0.225
F4	0.3	0.3	9000	15.00±94	0.785±0.016
F5	0.3	0.45	13500	92.66±623.33	0.71±0.027
F6	0.3	0.675	6000	2.89±111.67	1.103±0.058
F7	0.45	0.3	13500	29.51±338	0.717±0.059
F8	0.45	0.45	6000	13.32±71.67	0.705±0.057
F9	0.45	0.675	9000	83.74±159.33	1.001±0.169

Data are presented as mean± STD (n=3).

$$Span=(D_{90}-D_{10})/D_{50} \quad \text{Eq.1}$$

Where D_{90}, D_{50} and D_{10} represents the particle size for which 90%, 50% and 10% of the particles are smaller than these volumes, respectively.

2.3.2. Drug Loading and Encapsulation efficiency

The encapsulation efficiency was analyzed as indirect method. After preparation of sulfacetamide sodium- loaded chitosan nanoparticles, unloaded drug was separated from the nano suspension by ultracentrifugation (Hettich, Model Mikro220R, Germany) at 15500 rpm and 4 °C for 30 min. The amount of free sulfacetamide in the supernatant was measured at 256 nm. The encapsulation efficiency (LE) was calculated by the equation 2:

$$EE\%= ((T-F)/T)*100 \quad \text{Eq. 2}$$

And drug loading amount (LA) was calculated using equation 3:

$$LA\%= ((T-F)/W)*100 \quad \text{Eq.3}$$

Where T is the total amount of sulfacetamide added into CS solution, F is the free amount of drug in the supernatant and W is the weight of sulfacetamide sodium-loaded chitosan nanoparticles.All analyses were carried out in triplicate.

2.3.3. Differential scanning calorimetric (DSC) analysis

Differential scanning calorimetric method was used to characterize the thermal behavior of the sulfacetamide sodium, unloaded drug chitosan nanoparticles, and drug-loaded nanoparticles using differential scanning calorimeter (TA Instruments, Model 302, Germany). Samples in sealed standard aluminum pan were run at a heating rate of 10 °C/min over a temperature range of 25-300°C under nitrogen atmosphere.

2.3.4. In vitro drug release

Drug release phenomena was carried out in three different pH values of 5.0, 6.8 and 7.4 in phosphate buffer solution (PBS). Dialysis bag (cutoff: 12kDa, Sigma Aldrich, USA, supplier: Kimia Teb Tajhiz, Shiraz, Iran) containing of suspended drug loaded nanoparticles in PBS was immersed in the 100 ml of related buffer. The cell was put into shaker incubator (Farazma, Iran) set at 37 °C and 25 rpm. To determine the amount of drug released, at scheduled time points samples (5 ml) were withdrawn from the cell and replaced by the same volume of fresh pre-warmed PBS solution to maintain the sink condition. Samples were analyzed at 256 nm. All measurements were performed in triplicate. The drug released percent at each time was calculated and cumulative released curves were drawn.

2.3.5. Statistical analysis

The comparison between different formu-

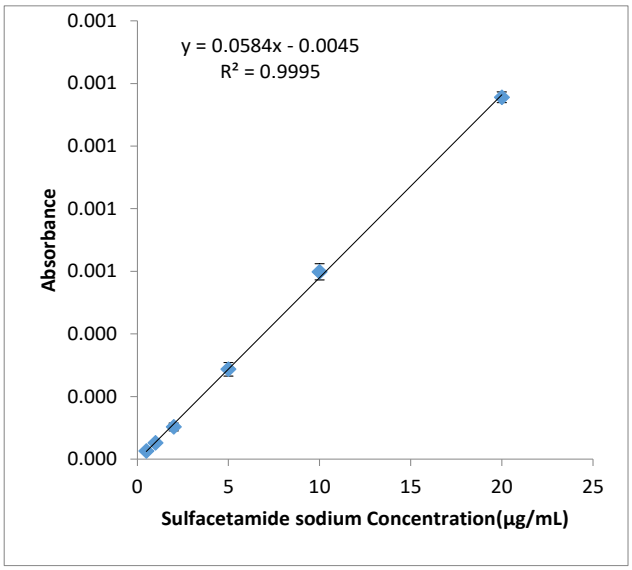

Figure 1. Standard curve of sulfacetamide sodium.

lations to determine the optimum EE% and between drug release profiles at different pH values, was performed using the ANOVA test. Differences were considered significant at $P<0.05$.

3. Results

3.1. Analysis method

The standard curve of sulfacetamide sodium was shown in Figure 1. The analysis method validation showed the linearity of method ($r^2=0.9995$) in the range of 0.5-20 µg/mL. The CV% was less than 9% and the accuracy was more than 92% within this range of concentrations. The specificity was assessed in the presence of the nanoparticles components.

3.2. Particle size

The mean particle size and span index of CS nanoparticles in aqueous medium for all designed formulations were presented in Table 2 Formulation F4 with the lowest particle size, 72 nm, was selected for continue experiments.

3.2. Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiencies in different polymer: drug ratio (W:W) were shown in Figure 2. Sulfacetamide sodium was successfully entrapped into chitosan nanoparticles with the EE % and LA % up to 42 %. The EE %

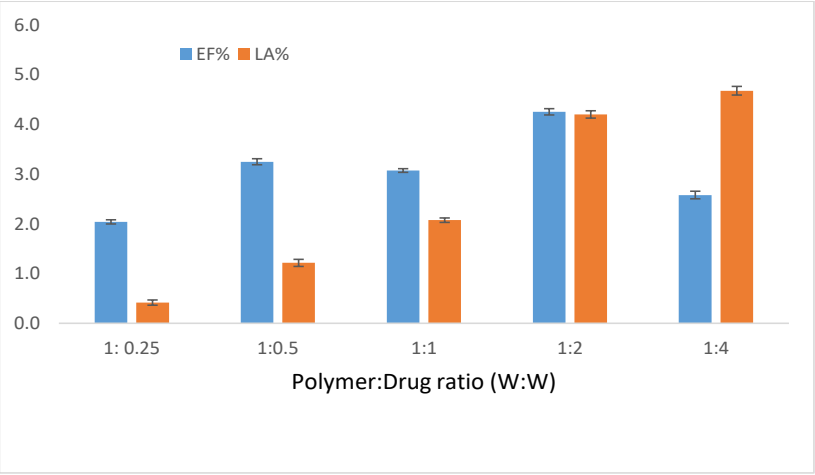

Figure 2. Sulfacetamide sodiumencapsulation efficiencies and loading amounts in different polymer: drug ratio (W: W). Data are presented as means ± standard deviations (n=3).

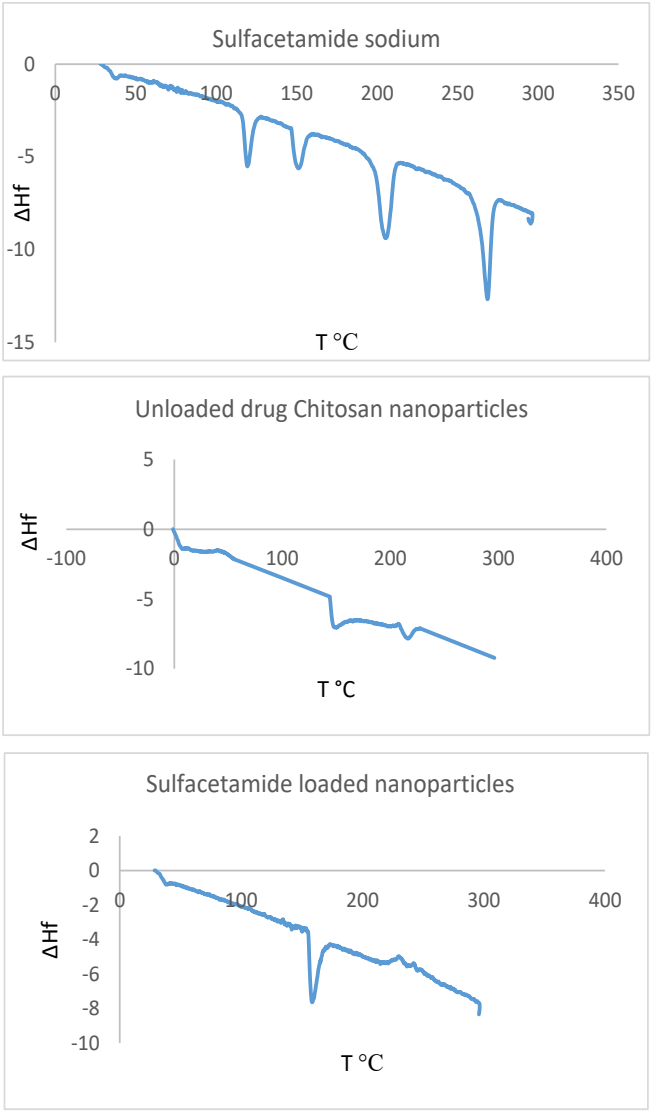

Figure 3. DSC thermograms of sulfacetamide sodium, blank chitosan nanoparticles, and drug-loaded nanoparticles

of F4 formulation was significantly different from other formulations (P<0.05). The LA% of F5 formulation was slightly higher than F4 (46.8% versus 42%, respectively) but its encapsulation efficiency was low (25%). Therefore, the F4 formulation was selected for further characterization tests.These particles had the particle size of 74±10.12 nm with the span index of 0.681± 0.508.

3.3. DSC analysis

The DSC thermograms of sulfacetamide sodium, blank chitosan nanoparticles, and drug-loaded nanoparticles were presented in Figure 3. Sulfacetamide sodium presented four endothermic peaks at 119.9 °C, 152 °C, 205 °C, and 216.5 °C. This can be related to the presence of different crystalline structure of the drug with different thermal stability. Blank nanoparicles without any drugs (unloaded particles) had two endothermic peaks at 147.9 °C and 216.5 °C. In the DSC curves of drug-loaded nanoparticles, characteristic peaks of CS nanoparticles could be seen.

3.4. Drug release studies

The release profile of sulfacetamide sodium from drug loaded nanoparticles at three dif-

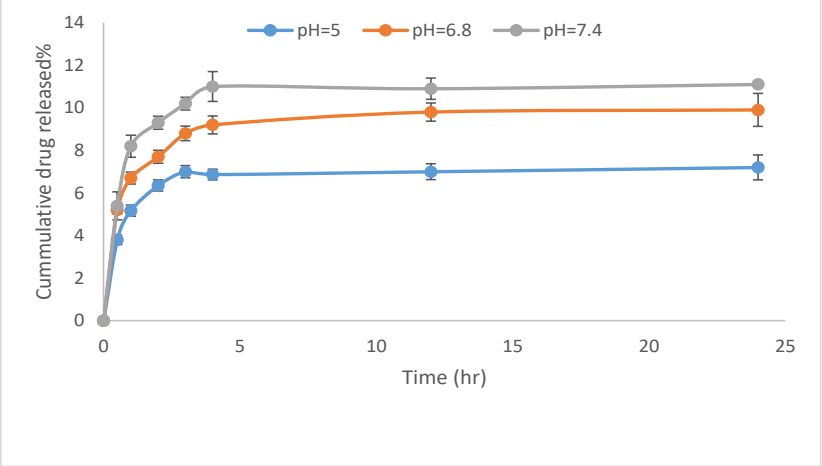

Figure 4. Sulfacetamide sodium release profile from chitosan nanoparticles at three different pH values. Data are presented as means ± standard deviations (n=3).

ferent pH values were presented in Figure 4.The amount of released drug from CS nanoparticles was very low and within 24 hours, less than 12% of drug could be released. By decreasing the pH, the percent of released drug was decreased. The release of free drug through dialysis bag was also evaluated and 100% of drug was permeated after 2 hours (data was not shown).

4. Discussion

Due tothe mucoadhesive and antimicrobial properties of chitosan, this polymer can be used as a delivery system for antimicrobial agents (9). Nanoparticles of chitosan have these suitable characteristics and also have the unique properties of nanoparticles in drug delivery. Therefore, chitosan nanoparticles containing antimicrobial agents can penetrate into infected tissues and deliver the related drug molecules at the site of action. Sulfacetamide sodium is one of the antibiotics used in the eye infections, like other traditional eye formulations (drop and ointment) the resistance time of drug in the eye is limited through tear flow and blinking reflex (4). The aim of this project wasp-reparation of chitosan nanoparticles containing sulfacetamide sodium for increasing the contact time of antibiotic in the infectious site. Perhaps these nanoparticles could increase the potency of the drug. These nanoparticles were fabricated through simple ionic gelation method. Positive charge of chitosan simply interacts with poly anionic agents like TPP. The effects of CS concentration, TPPconcentration, and stirrer rate on particle size distribution were analyzed. Nanoparticles with the lowest particle size were obtained under optimal conditions of 0.45% CS concentration, 0.45% TPP concentration and stirrer rate at 6000 rpm. Theirsize was 72 nm.Sulfacetamidesodium is a small molecule (MW: 236.23 g/mol) that can be easily loaded into chitosan nanoparticles. On the other hand, sulfacetamide sodium is a negatively charged molecule, and can interact with positively charged chitosan, spontaneously. Therefore, this drug can be loaded into CS nanoparticles with high affinity. Various polymer: drug weight ratios were evaluated to obtain the optimum drug encapsulationefficiency (Figure 2). By increasing the amount of drug in the polymer: drug ratio from 1:0.5 to 1:1, the average of EE% was not changed significantly (P>0.05), but through increasing the amount of drug (1: 2 polymer: drug ratio) this factor was increased to 42%. In this ratio the secondary mechanism may be involved for drug entrapment. Ionic interaction between polymer and drug is the most probable mechanism for drug loading. After increasing the drug amount in 1:4 polymer: drug ratio, the capacity of nanoparticles were saturated and the EE% was decreased. In similar studies which levofloxacin and ciprofloxacin loaded in chitosan nanoparticles, with particles size of 300-400nm, the LE% was 24% and 70%, respectively (13).

After loading sulfacetamide sodium into the chitosan nanoparticles, the DSC profile of

the resulting nanoparticles was different from the DSC related to the drug powder and drug-free nanoparticles (Figure3). It seems that by dissolving the drug powder in the chitosan solution for preparation of the chitosan nanoparticles, the crystalline structure of the drug changes to the amorphous state. So that the endothermic peaks of sulfacetamide sodium are not appeared in the DSC thermogram of drug loaded nanoparticles. Also after the drug was loaded into the nanoparticles, an ionic bond can be formed between the drug and the polymer, resulting in a new substance with different thermal stability.

In the infected tissues, the number of neutrophils, monocyts, and leucocytes increases and these cells release their acidic content into the extracellular environment. Dead microorganisms and particles may also subsequently decrease the pH of the infected tissues.Therefore, acidic pH values were selected for determination the drug release in the infectedsites (16). The release of sulfacetamide sodium from chitosan nanoparticles was generally low, although it occurs faster in the first hour (Figure 4). The low amount of drug released from nanoparticles could be due to the ionic interactions between drug molecules and the polymer. While in similar studies where ciprofloxacin or levofloxacin have been loaded and there is no ionic attraction between the drug and the polymer, release up to 100% can occur (13). In some studies, low present of antibacterial agents released from chitosan nanoparticles was reported but the antimicrobial activity of the loaded active agent was improved in the *in vitro* studies (17-19).

By decreasing the pH of the medium, the lower amount of drug was released. At the lower pH values, the positive charge of chitosan increases. Therefore, the ionic interaction between chitosan and TPP or sulfacetamide, which are both anionic,was increased, so the released drug was decreased.

5. Conclusion

Loading of sulfacetamide sodium in a mucoadhesive polymer for delivery of an eye antibiotic is a novelty of this study. In addition, preparation of chitosan nanoparticles with a particle size of <100 nm by simple ionic gelation method and high loading amount and encapsulation efficiencyiy are the remarkable features of this study. Sulfacetamide sodium-loaded chitosan nanoparticles can be fabricated by ionotropic gelation method. The optimal preparation parameters were 0.45% CS concentration, 0.45% TPP concentration, stirrer rate at 6000 rpm. Their particle size was 72 nm and encapsulation efficiency was 42% in 1:2 weight ratio of CS to sulfacetamide sodium nanoparticles. Antibacterial activity should be carried out to evaluate its potential as sustainable ocular delivery system.

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Conflict of Interest

None declared.

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