

Synthesis And Optimization of Streptomycin Loaded Chitosan-Alginate Nanoparticles

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Abstract- Nanoformulation consisting of Streptomycin loaded chitosan-alginate nanoparticles were prepared using ionotropic-pregelation method and optimization was done in terms of polymer concentration, crosslinker concentration and stirring time. The optimal parameters were found to be Chitosan 0.75mg/ml, calcium chloride 1% (w/v) and stirring time 90 min. Polymer (chitosan and sodium-alginate) and crosslinker (calcium-chloride) at these concentrations had significant synergistic effect on particle size and % encapsulation efficiency. Increase in polymer and crosslinker concentration resulted in an increase in particle size. Encapsulation efficiency, first showed an increase followed by a decrease, on increasing the polymer concentration whereas it increased with an increase in cross linker concentration. The nanoformulation so formed showed particle size 328.4 nm & drug encapsulation 93.32%.

Keywords: Streptomycin, Chitosan, Sodium alginate, nanoformulations, calcium chloride, ionotropic pregelation

1 INTRODUCTION

THE efficacy of many drugs is often limited by their potential to reach the site of therapeutic action due to various problems such as - poor bioavailability, in vivo stability, solubility, intestinal absorption, sustained and targeted delivery to site of action, therapeutic effectiveness; side effects, and fluctuations of drug concentration in plasma which either fall below the minimum effective concentrations or exceed the safe therapeutic concentrations. In most cases only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biological properties [1]. Streptomycin is bactericidal antibiotic drug under aminoglycosides category and derived from *Streptomyces griseus* [2]. It is also used to control bacteria, fungi, and algae in crops [3]. It is known to have toxic effects causing nephrotoxicity and neuroparalysis [4]. Application of nanotechnology in drug delivery system has opened up new possibilities in sustained and targeted release of drugs [5]. Specifically designed tiny nanoparticles can reach less accessible sites in the body by escaping phagocytosis and entering tiny capillaries. Controlled release of the drug from the nanoformulations could maintain steadier levels of drug in bloodstream for longer durations. Sustained release of drug can be achieved by encapsulating the active ingredient in a polymer matrix such that drug find its way through the restrictive cavities in the matrix. Thus, the dose and frequency of administration would be reduced [6-7]. Sodium-alginate and chitosan both are extensively used in encapsulation of drug for the purpose of sustained release. These are polysaccharide polymers, formed of repeating units (either mono- or disaccharides) joined together by glycosidic bond [8]. Both polymers have the properties of an ideal carrier for drug delivery, such as biocompatibility, biodegradability, non-toxicity, and low cost [9].

Chitosan is a polycation component, which has amino groups [10] and sodium alginate has many anionic or cationic groups in the structure; therefore, they exhibit unique physical property by electrostatic interaction [11-12]. Many other antibiotic nanoformulations are reported such as Oral administration of ciprofloxacin containing sodium-alginate nanoparticles [13]; Sepia nanoparticles as a potential drug carrier for amoxicillin trihydrate. Sepia nanoparticles system has also been used as a model for carrying out *in vitro* drug release and stability studies in response to different drug and polymer ratios [14]. Sodium-alginate nanosphere containing ofloxacin were also formulated by using controlled gelation method in which the prepared nanoparticles were evaluated to assess the various parameters such as drug polymer ratio, drug content analysis, particle size analysis (SEM analysis), and *in vitro* drug release[15]. In the present work we encapsulated streptomycin into polymers to form nanoformulations and optimized its various parameters to enhance its encapsulation efficiency and to reduce the particle size which may be helpful in reducing its toxicity.

2 MATERIALS AND METHODS

Chitosan and Streptomycin sulphate were procured from Hi media laboratories (P) Ltd. (Mumbai, India). Sodium-alginate and calcium chloride were procured from S.D. Fine Chemicals Ltd. (Mumbai, India).

2.1 Synthesis of chitosan-alginate nanoparticles

Chitosan/alginate nanoparticle formulation is two step process based on ionotropic pre-gelation previously described [16] but modified according to ideal preparation. Calcium chloride solution (7.5ml) was added dropwise to sodium alginate solution (117.5ml, 0.0063% w/v) to induce gelation. It was stirred for 60 minutes and then 25 ml of chitosan solution was added dropwise along with constant stirring for 90 minutes. Drug was incorporated at the rate 1 mg/ml to sodium alginate solution in step one itself, before adding to calcium chloride for gelation. Nanoparticles were concentrated by centrifugation at 11,500 rpm for 40 min. Nanoparticles thus formed were analyzed for particle size. The optimized nanoparticles were frozen at -80 °C for 4 h followed by Lyophilization on freeze dryer (Alpha 2-4 LD plus, Martin Christ, Germany) for 24 h at -90 °C at 0.0010 mbar using mannitol (1% w/v) as cryoprotectant.

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2.2 Encapsulation efficiency

$$\% \text{ encapsulation} = \frac{(\text{Total amount of drug added} - \text{Unbound drug}) \times 100}{\text{Total amount of drug}}$$

The amount of drug entrapped was determined by centrifuging the solution and supernatant was analyzed for drug content spectrophotometrically by measuring the absorbance at 195 nm in UV Spectrophotometer (Shimadzu UV 2450). Confirmation for loading of the drug was done by comparing optical absorbance of nanoformulations with and without drug loading at 195nm.

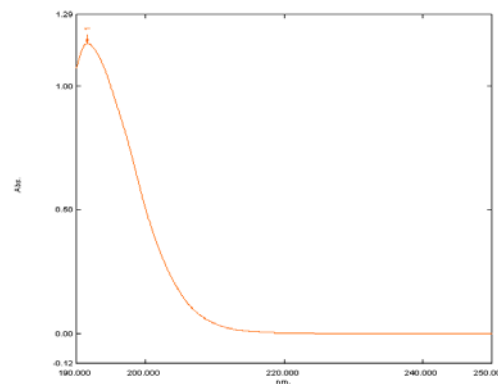


Fig. 1. UV Spectra of streptomycin-loaded chitosan-alginate nanoparticles taking dummy chitosan-alginate nanoparticles as baseline.

2.3 Optimization of process variables

The various parameters that were optimized for obtaining maximum encapsulation efficiency along with smaller size of the nanoparticles, included, polymer concentration, cross-linker concentration and stirring time. Chitosan concentration optimization – Three different concentrations of chitosan were studied for synthesis of nanoparticles i.e. 0.5%, 0.75% and 1% while other factors such as cross linker concentration and stirring time were kept constant. Cross linker concentration optimization - At the best chitosan concentration, three different concentrations of calcium chloride (0.5%, 0.75%, 1%) were tested, while keeping stirring rate constant. Stirring duration optimization - Effect of stirring time on particle size was also observed for three different durations i.e. 90, 120 and 180 min at best polymer and cross linker concentration.

2.4 Characterization - Particle size

Dynamic light scattering (DLS) was used to measure the average particle size and size distribution (polydispersity index) of formulated nanoparticles. The mean particle size of streptomycin loaded nanoparticles was determined at 25 °C using the Zetasizer nano ZS (Malvern instruments, Malvern, UK).

2.5 Characterization - Zeta potential

Stability of particles was studied by measuring zeta potential as high zeta potential (>|30| mV) can provide an electric repulsion to avoid the aggregation of particles [17]. The Zeta potential of the drug loaded nanoparticle dispersion was determined by the laser light scattering technique using Zetasizer nanoseries ZS90 (Model No. ZEN 3690). Measurements were obtained at an angle of 90°.

3 RESULTS AND DISCUSSION

3.1 Confirmation of drug loading by chitosan-alginate nanoparticles

Nanoformulation loaded with the drug, exhibited higher absorbance at 192 nm as compared to the not loaded nanoformulation (Fig. 1). Maximum absorbance of streptomycin has been reported at 195nm [18]. Peak of drug-loaded nanoparticles was at 192 nm by taking dummy nanoparticles as baseline in UV Spectrophotometer (Shimadzu UV 2450).

3.2 Optimization of process variables

Effect of polymer concentration was observed with the results that particle size and encapsulation efficiency varied from 304.2-416.3 and 53%-93%, respectively (Table 1). The best particle size along with maximum encapsulation efficiency was found at 0.75 mg/ml. Impact of different concentrations of cross linker was studied at this polymer concentration.

TABLE 1

EFFECT OF CHITOSAN CONCENTRATION ON PARTICLE SIZE AND ENCAPSULATION EFFICIENCY

| Sr. No. | Concentration mg/ml | Encapsulation efficiency (%) | Particle size (nm) |
|---------|---------------------|------------------------------|--------------------|
| 1. | 1 | 53.00 | 416.3 |
| 2. | 0.75 | 93.00 | 400.2 |
| 3. | 0.5 | 79.66 | 304.2 |

Cross linker with concentration of 1%, maximized the encapsulation as shown in table 2 giving a maximum encapsulation efficiency of 93% and particle size of 416.3 nm. Impact of different stirring durations was studied at these polymer and cross-linker concentrations.

TABLE 2

EFFECT OF CROSS-LINKER CONCENTRATION ON PARTICLE SIZE AND ON ENCAPSULATION EFFICIENCY

| Sr. No. | Concentration | Encapsulation efficiency | Particle size (nm) |
|---------|---------------|--------------------------|--------------------|
| 1. | 1 % | 93% | 416.3 |
| 2. | 0.75 % | 79.66% | 400.2 |
| 3. | 0.5 % | 56% | 357.4 |

The particle size varied from 770 to 328.4 nm and encapsulation efficiency 62.33-93.32% in response to variation in duration of stirring. Highest encapsulation efficiency of 93.32% and particle size of 374 nm was obtained at stirring duration of 90 minutes (Table 3).

TABLE 3
EFFECT OF STIRRING RATE ON THE PARTICLE SIZE AND DRUG ENCAPSULATION.

| Sr. No. | Stirring time | Encapsulation efficiency | Particle size(nm) |
|---------|---------------|--------------------------|-------------------|
| 1. | 90 min | 93.32% | 328.4 |
| 2. | 120 min | 70% | 695.0 |
| 3. | 180 min | 62.33% | 770.0 |

With all the three parameters studied, best results were obtained at polymer concentration of 0.75mg/ml, crosslinker concentration of 1% and stirring duration of 90 min.

3.3 Zeta potential of streptomycin loaded chitosan-alginate nanoparticles

For the above nanoformulation, value of zeta potential was found to be 36.4 mV (Fig. 2). It indicates that the synthesized drug loaded nanoparticles could be expected to be stable for long time.

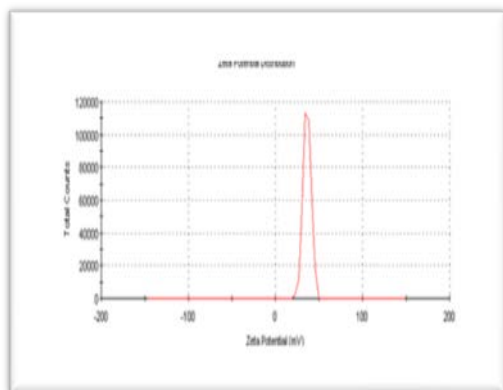


Fig. 2. Zeta potential of Streptomycin-loaded chitosan-alginate nanoparticles.

3.4 Particle Size

The particle diameter (z-average) for the streptomycin loaded chitosan-alginate nanoparticles was approximately 328.4 nm (Fig. 3). It is noteworthy that the hydrodynamic diameter of the particles measured by light scattering is higher than the size estimated from microscopy particularly because of high swelling capacity of chitosan-alginate nanoparticles. Hence, the actual diameter of these particles can be assumed to be significantly smaller than this.

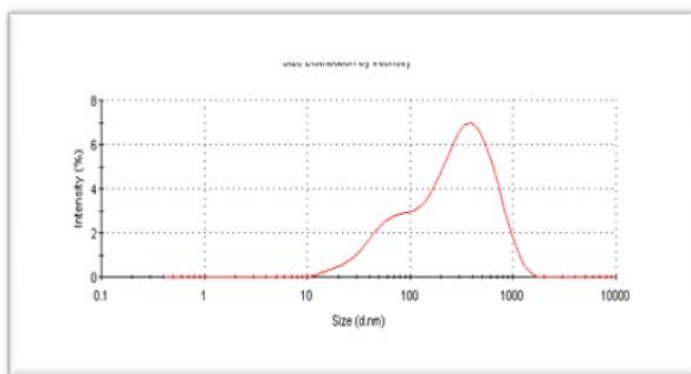


Fig. 3. Particle size of nanoformulation

4 CONCLUSION

Using ionotropic pregelation method, low average particle size and high entrapment efficiency could be produced and optimized. The results revealed that concentration of Chitosan-alginate and calcium chloride had significant synergistic effect on particle size and % encapsulation efficiency. The use of Nanotechnology in medicine and more specifically in drug delivery is set to spread rapidly, and a conceptual understanding of biological responses to nanomaterials shall be helpful in developing safe vehicles for drug delivery in the future.

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