Levofloxacin: formulation and *in-vitro* evaluation of alginate and chitosan nanospheres

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Background and objectives

Levofloxacin, the active L-isomer of ofloxacin, is a widely used fluoroquinolone, with activity against bacteria that causes respiratory, skin, and genitourinary tract infections, (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido benzoxazine-6-carboxylic acid hemihydrates. It is a new quinolone antimicrobial agent that exhibits broad-spectrum *in-vitro* bactericidal activities against gram-positive and gram-negative aerobes. The aim of this study was to formulate sodium alginate nanospheres containing levofloxacin and evaluate its physiochemical properties, exploring alternative routes of administration, such as nanoparticle to develop a targeted drug delivery system and to act locally on the organ of infection with enriched therapeutic efficacy.

Materials and methods

Sodium alginate and calcium chloride solutions were prepared. A constant volume (20 μl) of levofloxacin solution was incorporated into the sodium alginate solution, and then the same method was followed for the preparation of hybrid chitosan–alginate nanoparticles. *In-vitro* release study was carried out by dialysis membrane for 7 h in the physiological fluid (pH 7.4 phosphate buffer solution). Morphology and structure characterization of nanoparticles were investigated by field emission scanning electron microscope and Fourier transform infrared spectra, zeta potential, X-ray diffraction, particle size analysis, respectively.

Results and conclusion

This paper reports the possibility to entrap lipophilic levofloxacin within chitosan/alginate (CS/ALG) nanoparticles using a very simple ionotropic pregelation technique; strong electrostatic interactions exist in the nanoparticles. The nanoparticles with a diameter of 25-55 nm were obtained at the optimal mass range of sodium alginate: calcium chloride:chitosan in the meta acid environment. The delivery behavior of levofloxacin from nanoparticles was studied. Levofloxacin released from chitosan–alginate nanoparticles was 71% at pH 7.4 within 7 h. The release profile was characterized by an initial burst effect in phosphate buffer solution, followed by a continuous and controlled release phase. The drug release mechanism from polymer also offers an interesting potential for the delivery of lipophilic compound.

Keywords:
calcium chloride, chitosan, ionotropic pregelation, levofloxacin, nanoformulations, sodium alginate

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Introduction

The use of natural biopolymers specifically polysaccharides in drug delivery has attracted particular interest because of their desirable biocompatible, biodegradable, hydrophilic, and protective properties [1]. The interaction between biodegradable cationic and anionic biopolymers leads to the formation of polyionic hydrogels, which have demonstrated favorable characteristics for drug entrapment and delivery [2]. Chitosan and alginate are two biopolymers that have received much attention and have been shown to maintain their structure and activity and protect them from enzymatic degradation [3]. Moreover, many of these polymers, particularly hydrogels, are naturally hydrophilic, which is advantageous, as this property is thought to contribute to longer in-vivo circulation time and allow the highest encapsulation of drug [4]. Chitosan is a natural cationic polysaccharide obtained by the *N*-deacetylation of chitin, a product found in the shells of crustaceans [5]. Alginate is an anionic polysaccharide consisting of linear copolymers of a-l-guluronate and b-d-mannuronate residues. Alginates, which are a group of hemocompatible polymers, have not been found to accumulate in any major organs and have shown evidence of *in-vivo* degradation [6]. In the presence of calcium ions, ionic interactions between the divalent calcium ions and the guluronic acid residues cause alginates to form gels. The properties of calcium alginate gel beads make them one of the most widely used carriers for controlled release systems [7]. Coating of these beads with other polymers including chitosan has been shown to improve their stability during (shelf-life) storage and their half-life in biological fluids. Alginate–chitosan polyionic complexes form through ionic gelation by

interactions between the carboxyl groups of alginate and the amine groups of chitosan. The complex protects the encapsulant, has biocompatible and biodegradable characteristics, and limits the release of encapsulated materials more effectively than either alginate or chitosan alone [8]. A further advantage of this delivery system is its nontoxicity, which permits its administration to be repeated as a therapeutic agent.

The chitosan/alginate system has been widely studied at the microscales and macroscales for drug delivery [9]. The drug levofloxacin selected for our study has become one of the most commonly prescribed fluoroquinolone antimicrobials in the USA during the past 5 years. The binding of metal ions contained in these preparations to the 4-keto-group and 3-carboxyl-group of quinolones to form nonabsorbable chelates has been suggested as the possible mechanism, responsible for the reduced absorption of quinolones. However, attempts to relate the magnitude of reduction in bioavailability by antacids to chemical structures of quinolones or to chelate formation constants have been generally unsuccessful, and the mechanism remains to be elucidated [10]. Guan *et al.* [11] had studied levofloxacin-loaded chitosan nanoparticles by ionotropic gelation using tripolyphosphate with particle size and polydispersity of 140 nm and 0.95, respectively. Thus, an objective of the present study was to develop an alginate-based oral drug delivery system for levofloxacin, to improve patient compliance and minimize potential toxicity. In this study, we investigated the entrapment efficiency (EE) and the polydispersity of levofloxacin-loaded nanoparticles as well as their physical characteristics and release behavior, which were not reported by Guan *et al.* [11].

Materials and methods

Bionanotechnology

Materials

Chitosan was acquired from Himedia Laboratories Pvt. Ltd. (23, Vadhani Industrial Estate, L B S Marg, Ghatkopar West, Mumbai, Maharashtra 400086, India). Sodium alginate was purchased from Himedia Laboratories Pvt. Ltd; calcium chloride dihydrate extra pure AR (molecular weight 147.02) was purchased from Sisco Research Laboratories (Prashanti Nagar, Kukatpally, Hyderabad - 500 072, India). Levofloxacin infusion IP drug was supplied by Cipla (2nd Floor, B-8, Parsn Commercial Complex, Kodambakkam High Rd, Nungambakkam, Chennai, Tamil Nadu 600006, India). All other chemicals and reagents used were of analytical grade.

Methods

Preparation of blank chitosan–alginate nanoparticles: Both sodium alginate and calcium chloride solutions were prepared by dissolving the chemicals in distilled water. The pH of the sodium alginate solution was adjusted to 5.1 using hydrochloric acid. Briefly, a known amount of chitosan was dissolved in 1% acetic acid solution and pH was modified to 5.4 using NaOH. The method used to prepare the nanoparticles is a two-step method adapted from the study by Rajaonarivony *et al.* [12], which is a method of preparing alginate-poly-l-lysine nanoparticles. Aqueous calcium chloride (4 ml of 3.35 mg/ml) was added dropwise to 20 ml aqueous sodium alginate (3.0 mg/ml) while stirring for 30 min (REMI–magnetic stirrer, 1200 rpm) and then 8 ml chitosan solution (0.8 mg/ml) was added into the resultant calcium alginate pregel and stirred for an additional 1 h. The resultant opalescent suspension was equilibrated overnight to allow nanoparticles to form uniform particle size [13,14].

Preparation of levofloxacin-loaded chitosan–alginate nanoparticles: After centrifugation, the supernatant was discarded and the pellet was collected, which contain hybrid nanoparticles. Thereafter, the substances were dried by heating using hot plate. A constant volume (20 µl) of levofloxacin solution was incorporated into the sodium alginate solution, and then the same was used for the preparation of blank chitosan–alginate nanoparticles.

Results and discussion

Chitosan–alginate nanoparticles are carried out at ambient temperature; preparation is simple, rapid, and reliable. CS/ALG nanoparticles are obtained spontaneously under very mild conditions. The preparation of levofloxacin-loaded CS/ALG nanoparticles is relatively difficult. As levofloxacin is lipophilic, these hydrophilic nanoparticles are used to encapsulate lipophilic drug. A number of experiments had to be performed to determine the appropriate conditions for the incorporation of the lipophilic levofloxacin into the CS/ALG nanoparticles. The incorporation of the CS to the calcium alginate pregel containing the levofloxacin solution and calcium alginate pregel in the form of discrete nanoparticles simultaneously entrap the levofloxacin solution suspended in the medium. Hence, the final product consists of a suspension of CS/ALG nanoparticles containing levofloxacin solution entrapped within the structure. It is proposed that nanoparticles can be formed by enveloping the negatively charged calcium alginate complex in pregel state with cationic polymer, and the pregel state is essential to enable the ionic interactions between ALG, calcium, and cationic polymer to form nanoparticles [15]. The optimal mass range of sodium alginate:CaCl sufficient cationic polymer was present to form nanoparticles.

Spectrophotometric study

In-vitro release study was carried out by dialysis membrane for 7 h in the physiological fluid (pH 7.4 phosphate buffer solution). Samples were withdrawn at appropriate intervals to measure the optical density using UV spectrophotometer (UV-1800; Shimadzu Corporation, Kyoto, Japan) at 287 nm. The drug content was calculated using the equation generated from standard calibration curve. The concentration of drug was analyzed by UV spectrophotometer (UV-1800; Shimadzu Corporation) at 287 nm [13]. Percentage of drug release was calculated using the formula:

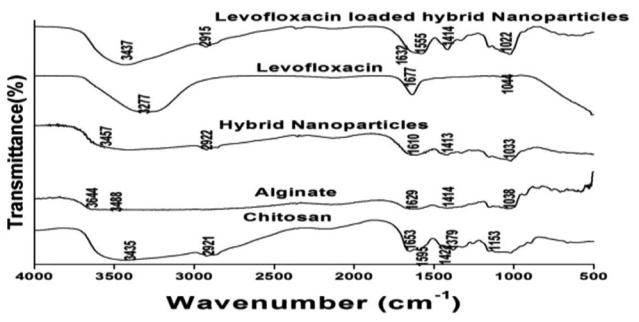
$$\text{Percentage of drug release} = \frac{\text{OD value for release of drug after 330 min}}{\text{OD value of loaded drug}} \times 100$$

where OD is the optical density.

FTIR study

Fourier transform infrared spectra (FTIR) was adopted to characterize the potential interactions in the nanoparticles. FTIR spectra of alginate, chitosan, levofloxacin, hybrid, and levofloxacin-loaded chitosan/alginate nanoparticles are shown in Fig. 1. In the spectra of CS, the broad band at 3435 cm^{-1} was caused by –OH stretching; the absorption band of the carbonyl (C=O) stretching of the secondary amide (amide I band) at 1653 cm^{-1} and the bending vibrations of the N–H (*N*-acetylated) residues (amide II band) at 1595 cm^{-1} belong to the N–H stretching of the amide and ether bonds and N–H stretching (amide III band), respectively. The band around 1038 cm^{-1} (C–O–C stretching) presenting in the IR spectrum of sodium alginate is attributed to its saccharide structure. The bands at 1629 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups. In the IR spectrum of levofloxacin of loaded chitosan/alginate nanoparticles, we can observe the asymmetrical stretching of –COO. In addition, the absorption band at 1595 cm^{-1} after the reaction with alginate, the stretching vibration of –OH and –NH, at 3435 cm^{-1} and becomes broad. Pure levofloxacin displays a peak characteristic of the N–H stretching vibration at 3274 cm^{-1} indicative of the C=O stretch of the esteric group. The characteristic absorption band of levofloxacin-loaded chitosan/alginate nanoparticles probably indicated that the levofloxacin molecule was filled in the polymeric network. These results indicate that the carboxylic group of alginate associates with ammonium groups of chitosan through electrostatic interactions to form the polyelectrolyte complex.

Figure 1



The FTIR spectra of chitosan, sodium alginate nanoparticles, levofloxacin, levofloxacin-loaded nanoparticles. FTIR, Fourier transform infrared spectroscopy.

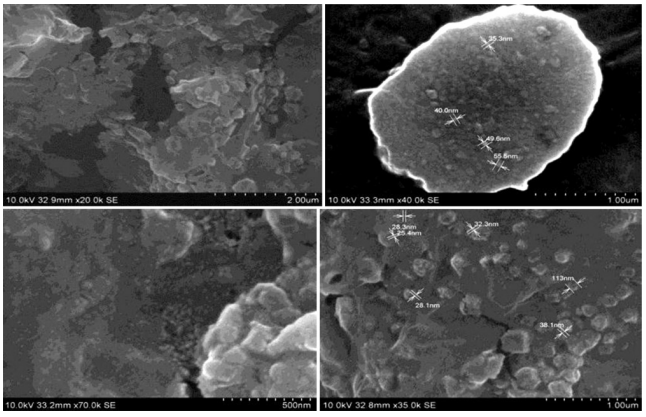
Morphology and structure of nanoparticles

Electron microscopy analysis confirmed the presence of nanoparticles and provided morphological information of the typical levofloxacin-loaded chitosan/alginate nanoparticles. Field emission scanning electron microscopy analysis confirmed that particles with target size and narrower size distributions could be prepared using an ionotropic pregelation method. Fig. 2 shows that levofloxacin–sodium alginate–chitosan nanoparticles had spherical shape with size ranging from 25 to 55 nm. This was achieved by adapting the optimized parameters for the preparation of nanoparticles.

Purification of drug-encapsulated chitosan–alginate nanoparticles

Drug-encapsulated chitosan–alginate nanoparticle solution was purified using centrifugation at a rate of 1000 rpm for 10 min. The pellet was removed and the

Figure 2



FESEM of levofloxacin-loaded nanoparticles magnification (3 × 70 000); (4 × 40 000). FESEM, field emission scanning electron microscopy.

supernatant was taken. The supernatant was taken in a cuvette. The absorbance of the supernatant and the pellet of the drug-loaded chitosan–alginate nanoparticle was measured at 287 nm. The drug-loading efficiency was calculated using the following formulae:

$$\text{Drug loading efficiency} = \frac{\text{Total drug} - \text{free drug}}{\text{Total drug}} \times 100$$

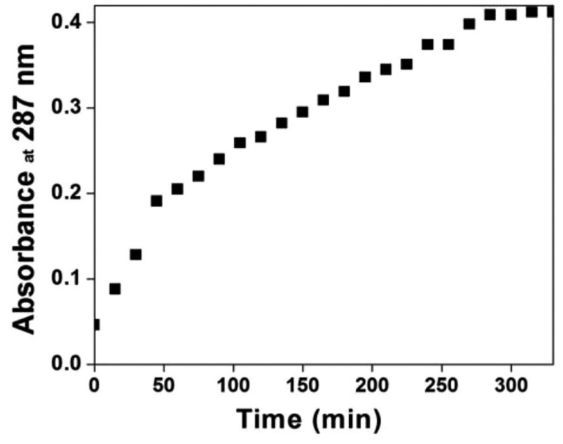
$$\text{Total drug} = \frac{0.581 - 0.535}{0.581} \times 100$$

which is equal to 79% of the drug that is loaded in the chitosan–alginate nanoparticles.

In-vitro release properties of levofloxacin

Figure 3 shows the controlled release curves of levofloxacin from CS/ALG nanoparticles at particular pH 7.4 as a function of time. It can be seen that levofloxacin released from CS/ALG nanoparticles was 71% at pH 7.4 within 7 h. This suggests that the drug release properties of CS/ALG nanoparticles are pH sensitive. The release profile was characterized by an initial burst effect in one media, followed by a continuous and controlled release phase within 7 h. The release of levofloxacin from CS/ALG nanoparticles is incubated in phosphate buffer solution (pH 7.4). A total of 71% of levofloxacin was eluted out in PBs; the burst release results seem to indicate that a significant amount of levofloxacin initially associated with nanoparticles remained on their surfaces by weak interactions forces between polyelectrolytes. The acceleration in the pH media is more likely owed to the reduced electrostatic interactions between the polysaccharide-based polyion complexes and the nanoparticles at this pH:

Figure 3



In-vitro drug release profile of drug-loaded nanoparticles.

$$\text{Percentage of drug release} = \frac{\text{OD value for release of drug after 330 min}}{\text{OD value of loaded drug}} \times 100$$

$$= \frac{0.412}{0.58} \times 100$$

Percentage of drug release is equal to 71%.

Alginate-based nanospheres were prepared using chitosan. The relative mass ratios of sodium alginate, calcium chloride, and chitosan are critical to form nanospheres rather than microspheres. Specifically, a calcium chloride to sodium alginate mass ratio less than 0.2 was necessary to maintain the pregel state essential for the preparation of nanospheres, as was the addition of either cationic chitosan, and to prepare chitosan/alginate nanoparticles based on the formation of a polyionic complex between the two biopolymers. This system may have some interesting features:

(a) Chitosan/alginate nanoparticles are obtained spontaneously under very mild conditions;
(b) Carried out at ambient temperature, preparation is simple, rapid, and reliable;
(c) The release results noted that the nanoparticles have pH-responsive release pattern;
(d) The out-layer of chitosan cannot only endow nanoparticles positive surface charge, but also prolongs the time that the active ingredients contact with the intestinal epithelia and enhances absorption by the paracellular transport pathway through the tight junctions at neutral and alkaline pH environments;
(e) The complex protects the encapsulant, has biocompatible and biodegradable characteristics, and limits the release of encapsulated materials more effectively than either alginate or chitosan alone; and
(f) On the basis of *in-vitro* drug release, a new nanoparticulate dosage form can be designed.

Measurement of zeta potential

Zeta potential of nanoparticles is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (±) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential can also be used to determine whether a charged active material is encapsulated within the center of the nanoparticles or adsorbed onto the surface. The zeta potential of

levofloxacin-loaded chitosan nanoparticles ranged from –22.2 mV as shown in Fig. 4. These reduce zeta potential between the particles, thus minimizing the undesirable rapid elimination of nanoparticles.

X-ray diffraction

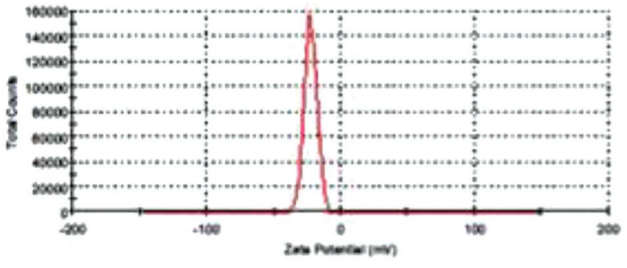
X-ray diffraction (XRD) patterns were determined for the drug levofloxacin, blank, and levofloxacin-loaded nanoparticles. Samples were exposed to a monochromatic nickel-filtered copper radiation (45 kV, 40 mA) in a wide-angle X-ray diffractometer with 2θ angle. XRD has been used for the study of molecular structure and polymorphism of polymeric nanoparticles [16]. XRD patterns of pure levofloxacin and levofloxacin–alginate–chitosan nanoparticles formulation are illustrated in Fig. 5. The XRD pattern of pure levofloxacin from 2θ showed distinctive peaks approximately at 5.5°, 10.8°, 15.9°, 18.8°, 20.7°, and 25.7°, which were comparable with XRD pattern of crystalline levofloxacin reported in the literature by Rojanarat *et al.* [17]. Blank nanoparticles do not show any high intensity peak revealing the amorphous nature of the polymer and stabilizer, respectively. The characteristic peaks of the levofloxacin were absent in levofloxacin–alginate–chitosan nanoparticle. This indicates that levofloxacin was molecular dispersed into the polymeric nanoparticles and there could be less or no free drug in crystalline form on the surface of the nanoparticles. From this, it is evident that an XRD signal of encapsulated drug is very difficult to detect, which showed that the drug is dispersed at a molecular level in the polymeric matrix [18].

Percentage entrapment efficiency

To determine entrapment, nanoparticles were analyzed for entrapment content using high-performance liquid chromatography and % EE was calculated using the following equation [17]:

$$\% \text{ EE} = \frac{1 - \text{Free drug}}{\text{Drug loaded}} \times 100$$

Figure 4



Zeta potential of levofloxacin–alginate–chitosan nanoparticles.

EE of drug polymer ratio 4 : 2 and 4 : 1 was found to be in the range of 80–99%. EE decreases with increase of rotation speed of stirrer, probably due to smaller microcapsules formed by increase of rotation speed of stirrer.

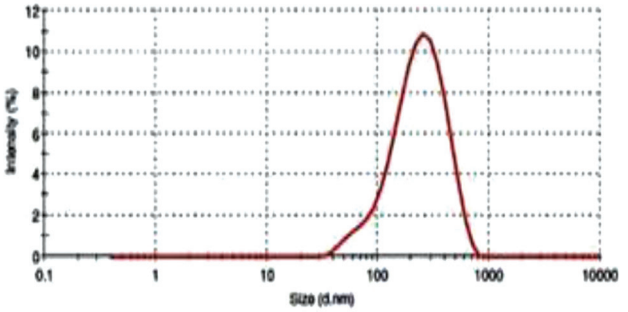
Particle size analysis

To analyze particle size, drug-loaded lyophilized nanoparticles were dispersed in deionized water, vortexed for 10 min, and sonicated for 5 min before sampling. Particle size was determined by laser scattering light using photon correlation spectroscopy (3000SH; Malvern Instruments Ltd, Malvern, UK). Nanosized particles of range between 150 and 225 nm were obtained. Particle size of the nanoparticles formulation was observed to be increased slightly with the increase in ET. Distribution of particles size range was observed narrow as shown in Fig. 5. The value of polydispersity was 0.32. Polydispersity indicates the degree of nonuniformity of the particle size. Obviously, a low polydispersity indicates more uniformity in size distribution.

Conclusion

The results of the present study revealed that levofloxacin-loaded nanoparticles were prepared by the ionotropic pregelation method. The FTIR, field emission scanning electron microscopy, UV, and XRD pattern study did not detect any crystalline drug material in the freshly prepared freeze-dried nanoparticles. The application of factorial design gave a statistically systematic approach for the formulation of nanoparticles with desired particle size and high EE and percentage drug release. Concentrations of drug and polymers were found to influence the particle size, EE, and percentage drug release of levofloxacin-loaded sodium alginate–chitosan nanoparticles. The release was found to follow non-Fickian diffusion

Figure 5



Particle size distributions of optimized levofloxacin–alginate–chitosan nanoparticles.

mechanism for optimized batch. These results indicate that levofloxacin-loaded sodium alginate–chitosan nanoparticles could be effective in controlled drug release and act as a good antibiotic.

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Conflicts of interest

None declared.

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