RESEARCH ARTICLE

# Formulation and characterization of levofloxacin-loaded biodegradable nanoparticles

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Effective management of various ocular infective diseases using levofloxacin eye drops remains challenging owing to poor ocular drug bioavailability. Hence, this study aimed to develop and evaluate nanosphere colloidal suspension-containing levofloxacin as potential ophthalmic drug delivery system. The levofloxacin-loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions. The nanoparticles were characterized by scanning electron microscopy, zeta potential analyzer, differential scanning calorimetry, and fourier transform infrared spectroscopy. All the prepared formulations resulted in nano-range size particles (317–501 nm) and displayed spherical smooth morphology with zeta potential (+37.2 to +43.5 mV). The encapsulation efficiency and loading capacity were 65–83% and 15–25%, respectively. The levofloxacin-loaded chitosan nanoparticles displayed more crystallinity than levofloxacin. The *in vitro* diffusion profile of levofloxacin from the nanoparticles showed a sustained release of the drug over a period of 20 h. Kinetic release profiles of levofloxacin from nanoparticles appeared to fit best with Higuchi model with zero order and the non-Fickian diffusion was superior phenomenon. Thus, the results suggest that levofloxacin-loaded chitosan nanoparticle suspension appears to be promising enough for effective management of ocular infections.

***Key words:*** *Chitosan, ionic gelation method, levofloxacin, nanoparticles*

## INTRODUCTION

Levofloxacin is a broad-spectrum antibacterial with a half-life of 6–8 h, frequently used in ocular infections, and is sparingly soluble in water. Many attempts have been made to improve the ocular bioavailability and the therapeutic effectiveness of levofloxacin. Continuous delivery of drugs to the eye offers major advantages over conventional therapies that involve administration of drug solutions or suspensions as eye drops. Eye drop administration often results in poor bioavailability and therapeutic response due to rapid precorneal elimination of the drug and is also associated with patient compliance problems.[1,2]

Among the mucoadhesive polymers investigated until now, the cationic polymer chitosan has attracted a great deal of attention because of its unique properties, such as acceptable biocompatibility,[1] biodegradability, and ability to enhance the paracellular transport of drugs.[2] Besides, the cornea and conjunctiva have a negative charge; use of the cationic polymer chitosan will interact intimately with these extraocular structures, which would increase the concentration and residence time of the associated drug. Moreover, chitosan has recently been proposed as a material with a good potential for ocular drug delivery.

The potential use of chitosan nanoparticles for ocular drug delivery and their interactions with ocular mucosa *in vivo* and also toxicity in conjunctival cell cultures was studied, and it was reported that the chitosan nanoparticles are able to interact and remain associated to the ocular mucosa for extended periods of time, thus being promising carriers for enhancing and controlling the release of drugs to the ocular surface.[3] Similar conclusion has been proposed that chitosan nanoparticles readily penetrate conjunctival epithelial cells and were well tolerated by the ocular surface tissues of the rabbits and further stated that chitosan nanoparticles hold promise as a drug delivery system for the ocular mucosa.[4] A recent study on the effect

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of levofloxacin-loaded chitosan nanoparticles in rabbit's eye indicated that chitosan nanoparticles facilitated absorption of levofloxacin compared with market preparations.[5] However, literature research indicates that the role of sodium tripolyphosphate (STPP) concentration on nanoparticles has not been studied in detail, and hence this study was attempted to demonstrate the influence of STPP and chitosan concentration on the physicochemical characteristics and release profile of the chitosan nanoparticles.

## MATERIALS AND METHODS

Levofloxacin was obtained as a gift sample from Alkem labs (Mumbai, India). Chitosan (degree of deacetylation of 75–80%; viscometric molecular weight, $4.06 \times 10^5$ Da) was purchased from Purex Labs. Pvt. Ltd. (Bangalore, India). STPP was purchased from S.D. Fine Chemicals Ltd. (Mumbai, India) and Tween-20 was supplied by Loba Chemical Pvt. Ltd. (Mumbai, India). Ultra pure water was purchased from Himedia Ltd. (Mumbai, India). All other reagents and solvents used were of analytical grade.

### Preparation of levofloxacin-loaded chitosan nanoparticles

Chitosan nanoparticles were prepared according to the procedure first reported by Calvo *et al*,[6] based on the ionic gelation of chitosan with STPP anions [Table 1]. Chitosan nanoparticles were prepared by ionic gelation of chitosan solution with STPP (0.25%) prepared in the presence of Tween-20 (0.5%) as a resuspending agent to prevent aggregation, at ambient temperature while stirring. Chitosan at various concentrations [Table 1] and drug (10 mg) were dissolved in acetic acid in aqueous solution under magnetic stirring at room temperature for 45 mins in the presence of Tween-20 (0.5%); 10 ml STPP aqueous solution was added to 10 ml chitosan solution and the mixture was sonicated for 3 min. The nanosuspensions were cold centrifuged at 12,000 *g* in a glucose bed for 30 mins using Hitachi centrifuge (Hitachi koki USA Ltd., USA). The supernatant liquid was analyzed by spectrophotometer to calculate the percent drug entrapment and drug loading. Chitosan nanoparticles separated from suspension were dried by a freeze dryer (Labconco, Kansas city, USA) and lyophilized at 0.4 mbar and −40°C for 5 h using glucose and mannitol (1:2).The lyophilized nanoparticles were stored in a desiccator at 4°C. The lyophilized nanoparticles were resuspended in pH 7.4 phosphate buffer and submitted to characterization experiments.

### Evaluation of levofloxacin-loaded chitosan nanoparticles

#### *Fourier transform infrared spectroscopy*

The Fourier transform infrared (FTIR) spectra of levofloxacin and levofloxacin-loaded chitosan nanoparticles were determined by using Perkin Elmer RX1 model. The pellets were prepared by gently mixing of 1 mg sample with 200 mg potassium bromide at high compaction pressure. The pellets thus prepared were scanned at a resolution of 4 $cm^{-1}$ from 450 to 4000 $cm^{-1}$.

**Table 1: Formulation of levofloxacin-chitosan nanoparticles**

| Batch no | L1 | L2 | L3 | L4 | L5 | L6 | L7 | L8 | L9 |
|---|---|---|---|---|---|---|---|---|---|
| Conc. of chitosan (%w/v) | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 |
| Conc. of STPP (%w/v) | 0.2 | 0.4 | 0.6 | 0.2 | 0.4 | 0.6 | 0.2 | 0.4 | 0.6 |

#### *Differential scanning calorimetry*

Differential scanning calorimetric (DSC) curve of pure levofloxacin and levofloxacin-loaded chitosan nanoparticles measurement was carried out by using a thermal analysis instrument (DSC DA 60 Shimadzu, Japan) equipped with a liquid nitrogen subambient accessory; 2–6 mg samples were accurately weighed in aluminum pans thematically sealed and heated at a rate of 10°C/mins in a 30–300°C under nitrogen flow of 40 ml/min.

#### *Particle size, polydispersity, and zeta potential of nanoparticles*

The particle size, polydispersity, and zeta potential of nanoparticles were measured by Photon Correlated Spectroscopy (BI MAS, Multiangle sizing option on Zetaplus, Brookhaven Instruments, Holtsville, NY, USA) using dynamic light scattering principles. The samples were diluted with pH 7.4 phosphate buffer and placed in eletrophoretic cell and measured in the automatic mode.

#### *Scanning electron microscopy*

The scanning electron microscopy (SEM) (JEOL MODEL JSM 6400, Tokyo) was used to characterize the surface morphology of nanoparticles. The nanoparticles were mounted directly on the SEM stub, using double-sided, sticking tape and coated with platinum and scanned in a high vacuum chamber with a focused electron beam. Secondary electrons, emitted from the samples, were detected and the image formed.

#### *Levofloxacin encapsulation efficiency and loading capacity of the nanoparticles*

The encapsulation efficiency (EE) and loading capacity (LC) of the nanoparticles were determined by the separation of nanoparticles from the aqueous medium containing nonassociated levofloxacin by cold centrifugation (Hitachi Centrifuge) at 12,000 *g* for 30 mins. The amount of free levofloxacin in the supernatant was measured by UV-Visible Spectrophotometer (Perkin Elmer Lambda 25) at 290 nm.

The levofloxacin EE and LC of the nanoparticles was calculated as follows.

$$\% \text{ Encapsulation efficiency} = \frac{\text{Total amount of levofloxacin} - \text{Free levofloxacin}}{\text{Total amount of levofloxacin}} \times 100 \quad (1)$$

$$\% \text{ Loading capacity} = \frac{\text{Total amount of levofloxacin} - \text{Free levofloxacin}}{\text{Weight on nanoparticles}} \times 100 \quad (2)$$

### *In vitro release of levofloxacin from the nanoparticles*

The *in vitro* release profile of levofloxacin from nanoparticles was performed on the optimized formulation that showed the least size of the nanoparticles with high entrapment efficiency (L6). Nanoparticles equivalent to 2 mg of levofloxacin were redispersed in 10 ml of pH 7.4 phosphate buffer solution and placed in a dialysis membrane bag with a molecular cutoff of 5 kDa which acts as a donor compartment, tied and placed into 10 ml of pH 7.4 phosphate buffer solution which acts as a receptor compartment. The entire system was kept at 37°C with continuous magnetic stirring. At appropriate time intervals (1, 2, 3, 4…20 h), 1 ml of the release medium was removed and 1 ml fresh pH 7.4 phosphate buffer solution was added into the system. The amount of levofloxacin in the release medium was evaluated by UV-visible spectrophotometer at 290 nm.

### *Release kinetics*

In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study were fitted to various kinetics equations like zero order (cumulative % drug release vs time), first order (log cumulative % drug remaining vs time), and Higuchi matrix (cumulative % drug release vs square root of time).[7] In order to define a model which will represent a better fit for the formulation, drug release data were further analyzed by Peppas equation, $Mt/M\infty = ktn$, where $Mt$ is the amount of drug released at time $t$ and $M\infty$ is the amount released at $\infty$, $Mt/M\infty$ is the fraction of drug released at time $t$, $k$ is the kinetic constant, and $n$ is the diffusional exponent, a measure of the primary mechanism of drug release. $R^2$ values were calculated for the linear curves obtained by regression analysis of the above plots.

## RESULTS

### FTIR spectroscopy

There are three characterization peaks of levofloxacin at 1724.81 $cm^{-1}$ of carbonyl C=O, 2935.62 $cm^{-1}$ of aromatic C-H and 3265.81 $cm^{-1}$ of O-H group of carboxyl group. The comparison of the spectra of drug with the spectra of drug loaded nanoparticle reveals no drug–excipients interaction [Figures 1 and 2].

### DSC

Levofloxacin showed characteristic endothermic peaks at 86.2°C and 223.8°C. Levofloxacin-loaded chitosan nanoparticles showed characteristic peaks at 50.2°C, 85.8°C, and 224.1°C. The thermogram of levofloxacin-loaded chitosan nanoparticles exhibited all characteristic peaks of levofloxacin, thus indicating that there was no change in the crystallinity of levofloxacin [Figure 3].

### Particle size, polydispersity, and zeta potential of levofloxacin-loaded chitosan nanoparticles

The particle size, polydispersity, and zeta potential of levofloxacin-loaded chitosan nanoparticles (L1–L9) are shown in Table 2. The maximum size of nanoparticles was observed in L7 (501±15 nm) when compared with other formulations and the least size was seen in L6 (317±06 nm). The size of the nanoparticles varied with the chitosan and STPP

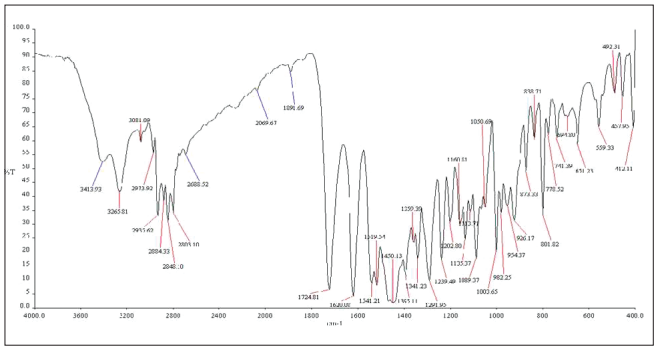

**Figure 1:** FTIR spectra of levofloxacin

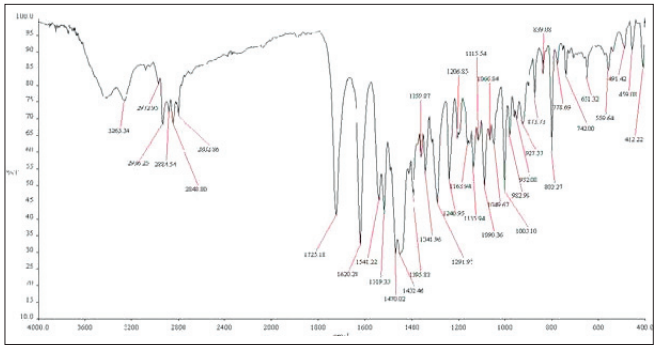

**Figure 2:** FTIR spectra of levofloxacin-chitosan nanoparticles (L6)

**Table 2: Evaluation of levofloxacin chitosan nanoparticles**

| Formula code | Mean particle size (nm) | Polydispersity index | Zeta potential (mV) | EE (%) | LC (%) |
|---|---|---|---|---|---|
| L1 | 473±13 | 0.27 | +42.3±1.4 | 65±2.1 | 15±0.53 |
| L2 | 420±08 | 0.25 | +41.2±0.8 | 68±2.5 | 17±1.13 |
| L3 | 319±11 | 0.23 | +38.2±1.1 | 66±1.9 | 16±0.62 |
| L4 | 475±07 | 0.32 | +43.4±1.2 | 75±2.2 | 18±0.85 |
| L5 | 435±17 | 0.28 | +41.4±1.2 | 81±3.1 | 21±1.32 |
| L6 | 317±06 | 0.16 | +37.2±0.6 | 83±0.7 | 23±0.42 |
| L7 | 501±15 | 0.29 | +43.5±1.2 | 80±1.9 | 21±0.85 |
| L8 | 450±11 | 0.21 | +40.3±0.6 | 76±1.1 | 23±1.05 |
| L9 | 349±08 | 0.20 | +38.8±1.1 | 82±0.6 | 25±1.32 |

Values are mean ± SD

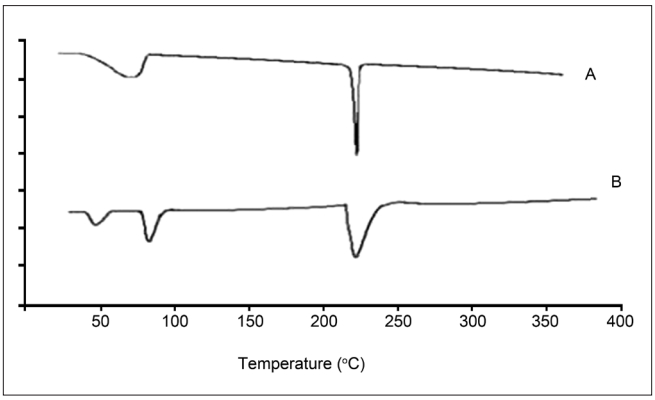


**Figure 3:** DSC thermogram of (a) levofloxacin (b) chitosan-levofloxacin nanoparticles (L6)

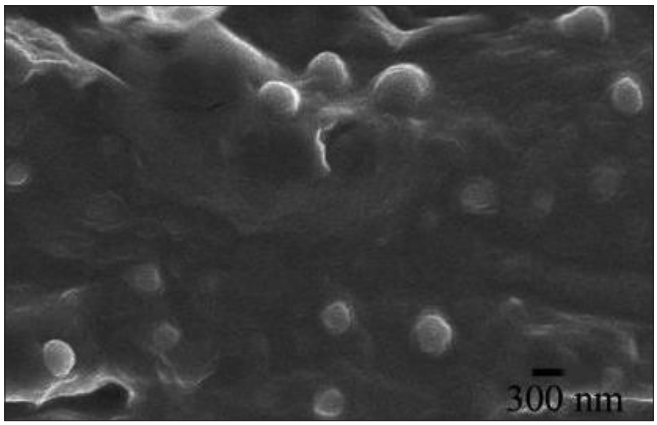


**Figure 4:** SEM image of L6 formulation

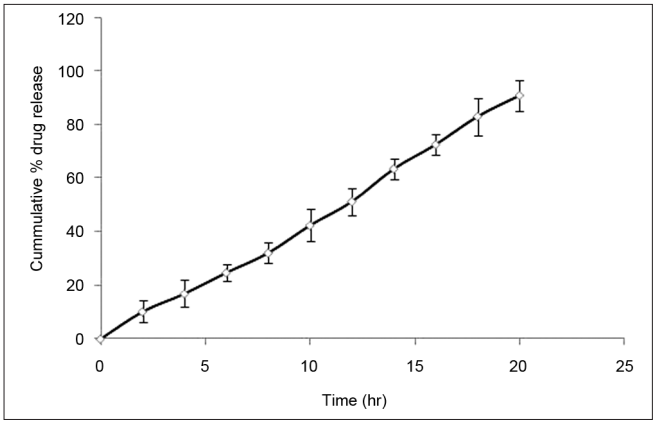


**Figure 5:** *In vitro* release profile of formulation L6. Data shown are mean ± SD (*n*=3)

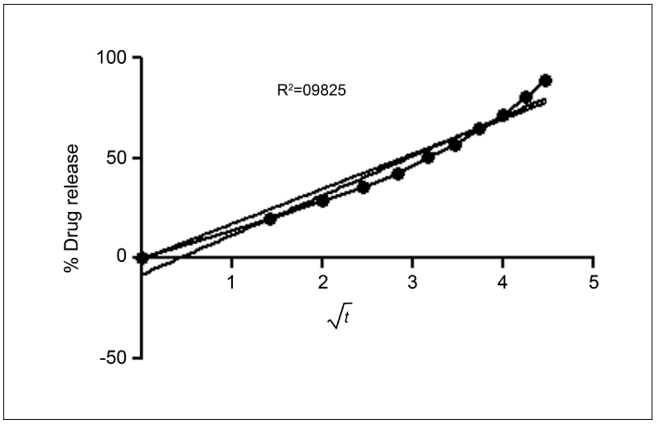


**Figure 6:** Higuchi plot of formulation L6 release profile

concentration. The range of polydispersity was 0.16–0.32. The zeta potential values ranged from +37.2 to +43.5 mV and the values decreased as the concentration of STPP increased. Zeta potential above +30 mV indicates that the formulations are stable.

### Scanning electron microscopy

The morphological characters of levofloxacin-loaded chitosan nanoparticles (L6) are shown in Figure 4. Levofloxacin-loaded chitosan nanoparticles have shown spherical shape.

### EE and LC of the nanoparticles

Table 2 shows the results of EE and LC of the levofloxacin-loaded chitosan nanoparticles. The EE was maximum with the formulation L6 and minimum with formulation L1. The EE ranged from 65 to 83%. Conversely, the LC of nanoparticles increased as the concentration of the drug increased. The LC ranged from 15 to 25%.

### *In vitro* release of levofloxacin from the nanoparticles

Figure 5 displays the release profile of levofloxacin from chitosan nanoparticles. The diffusion study was performed on the formulation (L6) that showed the least particle size (317±06 nm). The release pattern demonstrated a very slow release of drug at each point of time from nanoparticles. There was an initial phase of rapid release of levofloxacin followed by a more gradual release over a period of 20 h.

### Release kinetics

The *in vitro* release profile was analyzed by various kinetic models. The kinetic models used were zero order, first order, Higuchi, and Korsemeyer Peppas equation. The releases constant were calculated from the slope of the respective plots. Higher correlation was observed in the Higuchi equation. For planery geometry, the value of $n=0.5$ indicates a Fickian diffusion mechanism, for $0.5<n<1.0$, indicates anomalous (non-Fickian) and $n=1$ implies class II transport. Both dissolution and diffusion profile of the drug from the nanoparticles showed proper fitting to Higuchi plot [Figure 6] with zero-order release kinetics and indicated non-Fickian diffusion mechanism for the release of the drug from the nanoparticles.

## DISCUSSION

The results of the present investigation demonstrated the potential use of chitosan nanoparticles for effective delivery of levofloxacin for treating various ocular infectious

diseases. Drug delivery system for the ocular surface must overcome important physical barriers to reach the target cells. Different colloidal systems have been developed to solve these problems.[8] Among them chitosan-based systems are acknowledged to be more suitable for ocular pathway, based on the favorable biological characteristics of chitosan.[9,10] Several studies have shown that nanoparticles can transport across epithelia more readily than microparticles.[11] Moreover, chitosan nanoparticles can be easily prepared under mild conditions, besides incorporating macromolecular bioactive compounds. This characteristic is extremely beneficial for drugs, proteins, genes, or hydrophobic molecules that are poorly transported across epithelia.

Among the various methods developed for preparation of nanoparticles, ionic gelation method is simple to operate and also to optimize the required particle size of the drug that can penetrate the ocular surface and hence this method was followed in the study. Previously, it has been reported that the particle size is dependent on the chitosan concentration, the minimum size corresponding to the lowest chitosan concentration.[12] However, reports are scanty on the role of STPP concentration and hence this study attempted to demonstrate the influence of the chitosan and STPP concentration on the physicochemical characteristics and release profile of the levofloxacin-loaded chitosan nanoparticles.

The presence of a non-ionic surfactant is very important for the so-called "long-term" stability[13] of the nanosphere colloidal suspension, which is determined by the adsorption of hydrophilic macromolecules on the nanosphere surface, thus increasing the steric repulsion between particles. The presence of hydrophilic macromolecules on the surface of nanosphere leads to a change of the surface properties (zeta potential) of the colloidal carrier. In particular, the zeta potential of colloidal nanosphere is significantly reduced by coating with nonionic surfactants.[14] Considering these factors, the nonionic surfactant Tween-20 (0.5%) was used to stabilize the formulation. The SEM of the levofloxacin-loaded chitosan nanoparticles showed that the nanoparticles have a solid dense structure with smooth spherical shape [Figure 1]. In consistent with previous findings,[15] a significant reduction of nanoparticle mean size was observed in the formulation (L6) with optimum concentration of chitosan relative to drug concentration [Table 2]. Previously, it has been reported that the particle size of cyclosporine A-loaded chitosan nanoparticles is dependent upon chitosan concentration, the minimum size corresponding to the lowest chitosan concentration.[16]

The range of polydispersity was 0.16–0.32 and the values decreased as the concentration of STPP increased. Polydispersity indicates the degree of nonuniformity of the particle size. Obviously a low polydispersity indicates more uniformity in size distribution.

The 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 + 37.2 to + 43.5 mV [Table 2]. This study demonstrated decrease in zeta potential as the concentration of STPP increased.

As shown in Table 2, the EE and LC of the levofloxacin-loaded chitosan nanoparticles were affected by chitosan and STPP concentration. The LC of the nanoparticles ranged from 15 to 25% and the LC of nanoparticles increased as the concentration of both chitosan and STPP increased. The EE of the nanoparticles ranged from 65 to 83%. Enhancement of LC is possibly due to effect of the chain length of chitosan as longer chains of high-molecular weight chitosan can entrap greater amount of drug when gelated with more amount of STPP as observed in the previous study.[15]

With regard to the diffusion of levofloxacin from chitosan nanoparticles, the drug release was monitored for 20 h. The levofloxacin release profile from chitosan nanoparticles is characterized by a sustained release of the drug over a period of 24 h [Figure 2]. The release involves two different mechanisms of drug molecules diffusion and polymer matrix degradation.[17] Besides, the crystallinity of levofloxacin has not been affected as evident from DSC curve and this characteristic may also play a role in sustained release of drug from the nanoparticles. The profile of the drug from the nanoparticles showed fitting with Higuchi plot [Figure 6] with zero-order release kinetics and indicated non-Fickian diffusion mechanism for the release of the drug from the nanoparticles.

The improved interaction of chitosan-loaded nanoparticles with the cornea and the conjunctiva could be found in the mucoadhesive properties of chitosan[18] or it is due to the electrostatic interaction between the positively charged chitosan nanoparticles and the negatively charged corneal and conjunctival cells[19] that is the major force responsible for the prolonged residence of the drug. In consistent with these observations and also based on the results of this study, we propose that chitosan nanoparticles may be beneficial in improving the corneal permeation, contact time, and bioavailability of levofloxacin for the treatment of ocular viral infections.

## CONCLUSION

Chitosan nanoparticles have shown an excellent capacity for the association of levofloxacin. The mean particle size,

morphological characteristics, and surface property of the nanoparticles appear to depend on concentration of chitosan and STPP. The release profile of levofloxacin from nanoparticles has shown a sustained release following zero-order kinetic with non-Fickian diffusion mechanism. The results demonstrated the effective use of levofloxacin-loaded chitosan nanoparticles as a controlled release preparation for treatment of ocular infections. Further *in vivo* studies of this formulation may prove that these levofloxacin nanoparticles can be a promising once-a-day controlled release formulation.

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