Chitosan Loaded Nanoparticles as an Ocular Delivery System for Acyclovir

S.Selvaraj[1*] and V. Niraimathi[2]

1Department of Pharmaceutics, JKK Munirajah Institute of Health Sciences College of Pharmacy T.N.Palayam, Gobichettipalayam, Tamilnadu, India-638506.

2 Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai, Tamilnadu, India-625020.

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ABSTRACT

The topical application of acyclovir as eye ointment remains a concern for effective management of various ocular viral diseases owing to poor ocular drug bioavailability. Hence the present study was aimed to formulate and evaluate chitosan nanoparticles containing acyclovir as potential ophthalmic drug delivery system. The acyclovir-loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with sodium tripolyphosphate anions. Five different formulations were prepared and evaluated for compatibility studies, pH of nanosuspensions, particle size, zeta potential, scanning electron microscopy, entrapment and loading capacity, *in-vitro* drug release profile and stability studies. All the prepared formulations resulted in nano size in 100 - 350 nm and displayed spherical smooth morphology with zeta potential of +33.52 to +42.8 mV. The encapsulation efficiency and loading capacity were found to be 70% to 90% and 25% to 50% respectively. The acyclovir-loaded chitosan nanoparticles displayed crystallinity than acyclovir. The *in-vitro* release profile of acyclovir from the nanoparticles showed a sustained release of the drug over a prolonged period of 24 h. Kinetic release profiles of acyclovir from nanoparticles appeared to fit best with Higuchi model with zero order and the Non- Fickian diffusion was superior phenomenon. Stability studies showed that the nanoparticles could be stored safely at study storage conditions. Thus the results suggest that acyclovir-loaded chitosan nanoparticle suspension appears promising for effective management of ocular viral infections and capable of releasing the drug for a prolonged period of time and increased bioavailability.

INTRODUCTION

Acyclovir is an antiviral drug with a significant and highly specific activity against herpes viruses and is widely used in the treatment of various ocular viral diseases [1-4]. The topical application of acyclovir as eye ointment is limited by poor ocular drug bioavailability, pulse drug entry, systemic exposure due to the nasolacrimal duct drainage and poor entrance to the posterior segments of the eye due to the lens-iris diaphragm. Many attempts have been made to improve the ocular bioavailability and the therapeutic effectiveness of acyclovir, e.g., chemical modification of the drug [5] and its incorporation into colloidal systems such as liposomes or nanoparticles [6]. Nanoparticles have been used as ophthalmic delivery systems because they are able to penetrate into the corneal or conjunctival tissue by an endocytotic mechanism [7]. Further nanoparticles owing to their polymeric nature present some important advantages such as high storage stability, controlled release of the encapsulated drug, and a prolonged residence time in the precorneal area, particularly in the case of ocular inflammation and /or infection [8].

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, biodegradability and ability to enhance the paracellular transport of drugs [9]. 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.

Previous study on acyclovir-loaded poly d, l lactic acid (PLA) nanosphere for ocular drug delivery indicated that both types of PLA nanospheres were able to increase the aqueous levels of acyclovir and improve the pharmacokinetics profile, but the efficacy of the PEG-coated nanosphere was significantly higher than that of the simple PLA ones[10]. The potential 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 [11]. A similar conclusion has been proposed that chitosan nanoparticles

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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 [12]. A recent study on the effect of acyclovir-loaded chitosan nanoparticles in rabbits eye indicated that chitosan nanoparticles facilitated absorption of acyclovir compared to market preparations [13]. However, literature search indicates that the role of chitosan concentration on nanoparticles has not been studied in detail and hence the present study was attempted to demonstrate the influence of chitosan concentration on the physicochemical characteristics and release profile of the chitosan nanoparticles.

MATERIALS AND METHODS

Materials

Acyclovir was obtained as a gift sample from Micro Labs (Hosur, India). Chitosan (degree of deacetylation of 85%) was obtained as gift sample from Central Institute of Fisheries Technology (Cochin, India). Sodium tripolyphosphate (STPP) was purchased from S.D. Fine Chemicals Ltd (Mumbai, India) and Tween-80 was supplied by Loba Chemie Pvt. Ltd. (Mumbai, India). Ultra pure water was purchased from Himedia Ltd (Mumbai, India). All other reagents and solvents used were of analytical grade.

METHODS

Compatibility Studies

Drug and polymer interactions were studied by FTIR spectroscopy using Perkin Elmer RX1 model. FT-IR spectral analysis of acyclovir, chitosan and combination of acyclovir with chitosan was carried out to investigate any changes in chemical composition of the drug after combining with the polymer. The pellets were prepared by gently mixing of 1mg sample with 200mg potassium bromide at high compaction pressure. The scanning range was 450 to 4000 cm^{-1} and the revolution was 4 cm^{-1}.

Formulation of Acyclovir Loaded Chitosan Nanoparticles

Chitosan nanoparticles were prepared according to the procedure first reported by Calvo *et al.* (1997b) based on the ionic gelation of chitosan with sodium tripolyphosphate (STPP) anions

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[14]. Chitosan nanoparticles were prepared by ionic gelation of chitosan solution with sodium tripolyphosphate (0.25%) prepared in the presence of Tween 80 (0.5%) as a re-suspending agent to prevent aggregation, at ambient temperature while stirring. 350 mg of acyclovir and various concentrations of chitosan (F1-F5) dissolved in acetic acid in aqueous solution under magnetic stirring at room temperature for 45 min in the presence of Tween 80. 10mL STPP aqueous solution was added into 10mL chitosan-acyclovir solution and the mixture at different sonication times. The nanosuspensions were cold centrifuged at 12000 rpm in a glucose bed for 30 min using Hitachi centrifuge. The supernatant liquid was analyzed by spectrophotometer to calculate the percentage drug entrapment and drug loading. The final suspensions were frozen and lyophilized at 0.4 mbar and -40ºC for 5 hrs using glucose and lactose (1:2). The lyophilized nanoparticles were stored in a desiccator at 4ºC. The concentrations and amounts applied are summarized in Table 1.

Table 1: Composition of Acyclovir Loaded Chitosan Nanoparticles

Formulation Code	Acyclovir (mg)	Chitosan (mg)	Tween 80(%)	STPP (%)	Sonication Time (min)
F1	350	150	0.5	0.25	5
F2	350	250	0.5	0.25	5
F3	350	350	0.5	0.25	5
F4	350	450	0.5	0.25	5
F5	350	550	0.5	0.25	5

Evaluation of Acyclovir Loaded Chitosan Nanoparticles

Determination of pH

pH is one of the most important factors involved in the formulation process. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. The pH of the prepared formulations was checked by using pH meter.

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Particle size and Zeta potential

The prepared acyclovir-loaded chitosan nanoparticles were evaluated for their particle size and zeta potential by Zetasizer 3000HS, Malvern instrument, UK. The formulations were diluted to 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per second. Analysis was carried out at 25°C with an angle of detection of 90 degree [15].

Surface Morphology by Scanning Electron Microscopy

The morphology of the acyclovir nanoparticles was analyzed by scanning electron microscope. The instrument used for this determination was JEOL MODEL JSM 6400 scanning electron microscope. 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 [15].

Acyclovir Encapsulation Efficiency and Loading Capacity of the Nanoparticles

The Encapsulation efficiency and loading capacity of the nanoparticles were determined by the separation of nanoparticles from the aqueous medium containing non associated acyclovir by cold centrifugation at 12000 rpm for 30 minutes. The amount of free acyclovir in the supernatant was measured by UV method at 253 nm [16].

The acyclovir encapsulation efficiency (EE) and loading capacity (LC) of the nanoparticles were calculated as using following formulae.

$$\text{Encapsulation efficiency} = \frac{\text{Total amount of acyclovir} - \text{Free acyclovir}}{\text{Weight of nanoparticles}} \times 100$$

$$\text{Loading capacity} = \frac{\text{Total amount of acyclovir} - \text{Free acyclovir}}{\text{Total amount of acyclovir}} \times 100$$

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In-vitro Drug Release

The acyclovir-loaded chitosan nanoparticles (F1-F5) were separated from the aqueous suspension medium through ultracentrifugation. Nanoparticles equivalent to 2mg of acyclovir were re-dispersed in 10mL 7.4 phosphate buffer solution and placed in a dialysis membrane bag with a molecular cut-off of 5 kDa which acts as a donor compartment, tied and placed into 10mL 7.4 phosphate buffer solutions in a beaker which act as a receptor compartment. The entire system was kept at 37ºC with continuous magnetic stirring. At appropriate time intervals, 1mL of the release medium was removed and 1mL fresh 7.4 phosphate buffer solution was added into the system. The amount of acyclovir in the release medium was estimated by UV-Visible Spectrophotometer at 253 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), Higuchi matrix (% cumulative drug release Vs. square root of time). In order to define a model which will represent a better fit for the formulation, drug release data were further analyzed by Peppas equation, $Mt/M\infty = kt^n$, where Mt is the amount of drug released at time t and $M\infty$ is the amount released at ∞, $Mt/M\infty$ 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[17].

Stability Studies

The stability study was carried out using the formulation F-3 and was divided into 3 sets of samples and stored at 4^0C in refrigerator, Room temperature (29°C), 45 ± 2°C/75% RH in humidity control ovens. *In-vitro* release study of formulation F-3 was a carried out after 90 days of storage [18].

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RESULTS AND DISCUSSION

Compatibility Studies

From the FTIR spectral analysis, it was found that IR spectrum of pure drug acyclovir and combination of pure drug with polymers like chitosan showed that all characteristic peaks of acyclovir confirming the compatibility of the pure drug and polymer.

pH

pH of all the formulations was found to be in the acceptable range between 6.4-7.2 and hence would not cause any irritation upon administration of the formulation. It was also observed that increase in polymer concentration causes a slight increase in pH of the formulations.

Particle Size and Zeta potential of Acyclovir Loaded Chitosan Nanoparticles

Acyclovir loaded chitosan nanoparticles have shown spherical shape. The average particle size of acyclovir-loaded chitosan nanoparticles (F1– F5) was found to be 140-300nm, 125-260 nm, 100-200 nm, 110-300 nm and 150-350 nm respectively. The maximum size of nanoparticles was observed in F5 as compared to other formulations and the least size was seen in F3. The size of the nanoparticles varied with the polymer concentration.

The zeta potential values of all the acyclovir-loaded chitosan nanoparticles displayed a positive surface charge ranging from +33.2 to +42.8 mV. The zeta potential values increased as the concentration of polymer increased. All formulations showed zeta potential above +30 mV indicating that the formulations were found to be stable.

Surface Morphology

According to morphological evaluation analysis by SEM, all the prepared acyclovir-loaded chitosan nanoparticles (F1-F5) seemed to have a similar spherical shape. The sizes of all the formulations were found in nanometer. The morphological characters of acyclovir-loaded chitosan nanoparticles (F3) is shown in Figure 1.
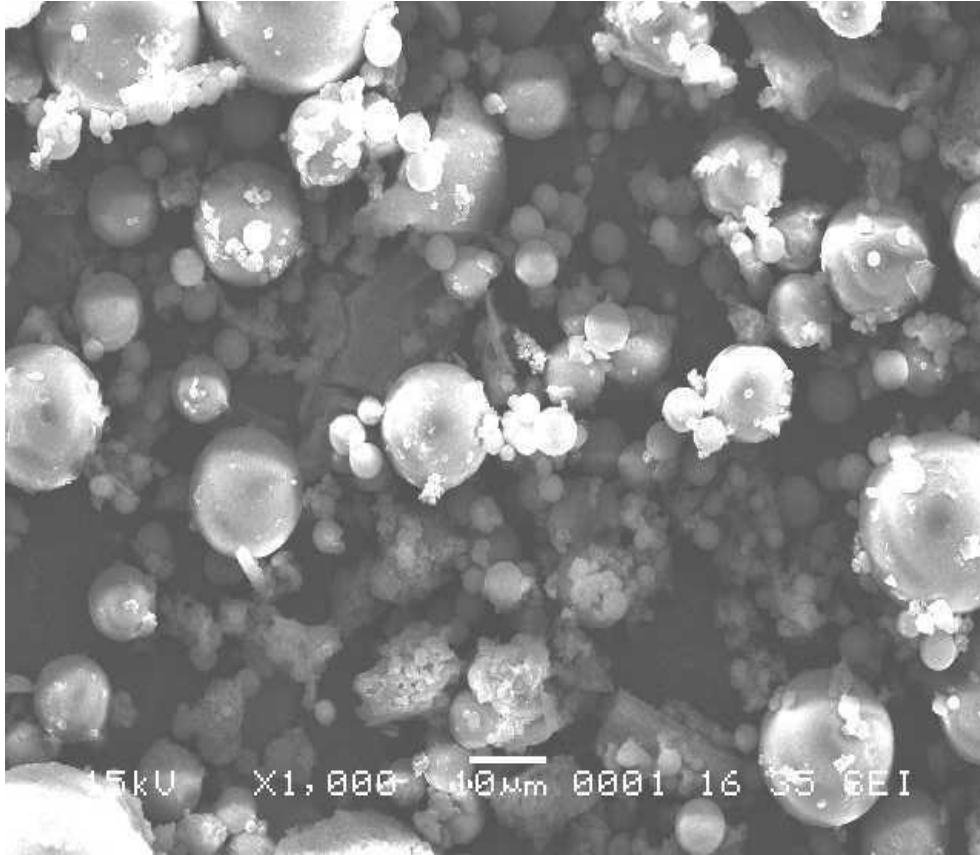
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Figure 1: SEM Photograph of acyclovir-loaded chitosan nanoparticles (F3)

Acyclovir Encapsulation Efficiency and Loading Capacity of the Nanoparticles

Results showed that the encapsulation efficiency was increased by increasing the concentration of polymer. The encapsulation efficiency ranged between 70 to 90%. The maximum entrapment was found in F-5 (90.0%) and lowest entrapment in F1 (70%). Conversely, the loading capacity of nanoparticles decreased as the concentration of polymer increased. The loading capacity ranged between 25 to 50%. The results suggested that the encapsulation efficiency and loading capacity of the nanoparticles depended on the concentration of the polymer used in the preparation.

In-vitro Drug Release Studies

From *in-vitro* drug release data for F1-F5, it was observed that increase in polymer concentration delays the drug release due to increased particle size and reduced surface area available for drug release. In the first hour, drug released was 12.0%, 14.71%, 16.62%, 14.56% and 15.85% for F-

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1, F-2, F-3, F-4 and F-5 respectively. The acyclovir release profile from chitosan nanoparticles was characterized by an initial rapid release followed by a sustained release of the drug over a period of 24 hrs. The initial rapid release was may be due to the burst effect resulting from the release of the drug encapsulated near the nanosphere surface. The burst release in the first hour can be attributed to the drug loaded on the surface of nanoparticles.

Cumulative percent drug released for F1, F2, F3, F4 and F5 after 24 h was 76.14%, 85.28% , 90.10%, 82.30% and 80.40.78%, respectively. As regards diffusion of acyclovir from chitosan nanoparticles the drug leakage was monitored for 24 h (Figure 2). The slow release of acyclovir from the chitosan nanoparticles was possibly the consequence of the release of the drug fraction encapsulated in the core of the nanospheres and may also due to strong association between the drug and polymer through electrostatic interaction between acyclovir and the amino groups of chitosan. From all the formulations F3 was selected as optimized formulation due to its desirable drug release at 24h.
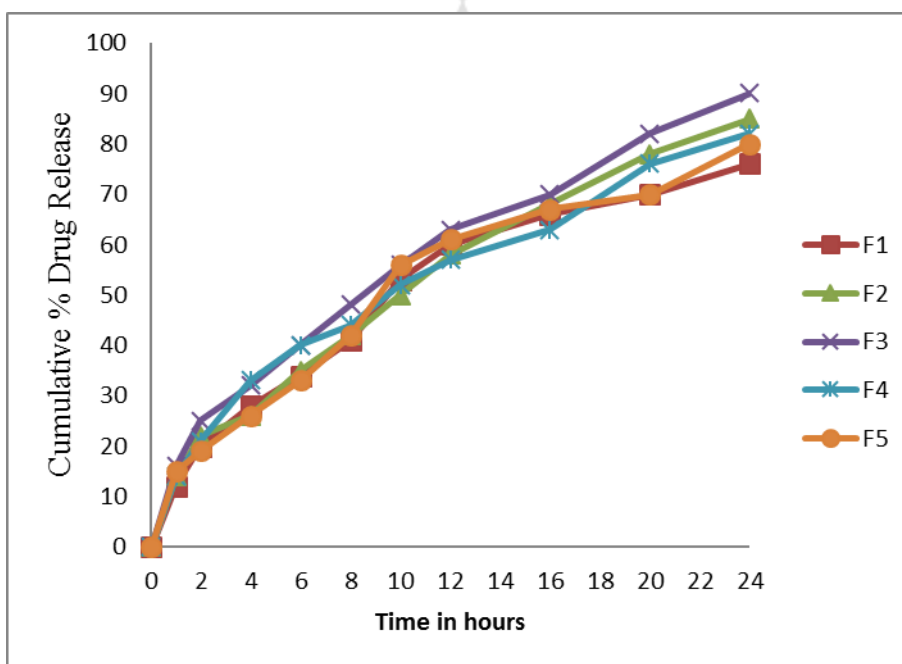

Figure 2: Comparative *in-vitro* drug release profile for F1-F5

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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 (Table 2). 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 fitting to Higuchi plot with zero order release kinetics and indicated Non Fickian diffusion mechanism for the release of the drug from the nanoparticles. The diffusion profile of the drug from the nanoparticles confirmed to Higuchi plot with zero order release kinetics and indicated Non Fickian diffusion mechanism for the release of the drug from the nanoparticles.

Table: 2 Various Parameters of the Model Equation on the *In-vitro* Release Kinetics

Formulation Code	Zero order	First order	Higuchi's	Peppa's	'n'values
F1	0.9653	0.9172	0.9941	0.9956	0.9981
F2	0.9698	0.8956	0.9976	0.9980	0.7801
F3	0.9701	0.8881	0.9867	0.9875	0.9864
F4	0.9776	0.8265	0.9952	0.9867	0.9899
F5	0.9980	0.9508	0.9978	0.9976	0.9734

Stability Studies

In-vitro release profiles of formulation F-3 after 90 days of stability study at different storage conditions were compared with the previous data of F-3, it was observed that there were no significant changes in drug release.

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In-vitro release studies proved that the formulation F-3 stored at 4°C showed 88.23 % release, the one which stored at ambient temperature and humidity showed 87.54% and formulation stored at room temperature (29^0C) showed 89.15% release after 24 h. These results indicated that the drug release from the formulation stored at room temperature (29^0C) was highest followed by formulation stored at ambient temperature and humidity and 4°C. By comparing this data with the previous release data of F-3 (90.10%), it was observed that there was an overall increase in the drug release. These results may be attributed to erosion of nanoparticles to some extent during storage.

CONCLUSION

Chitosan nanoparticles have shown an excellent capacity for the association of acyclovir. The mean particle size, morphological characteristics and surface property of the nanoparticles appear to depend on concentration of acyclovir-loaded in chitosan nanoparticles. The *in-vitro* release profile of acyclovir from nanoparticles has shown a sustained release following zero order kinetic with Non-Fickian diffusion mechanism. The results demonstrated the effective use of acyclovir-loaded chitosan nanoparticles as a controlled release preparation for treatment of ocular viral infections.

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