



Research Article

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Formulation and *in vivo* Evaluation of Acyclovir Loaded Chitosan Nanoparticles for Ocular Delivery

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ABSTRACT

The present study was aimed to formulate and evaluate chitosan nanoparticles containing acyclovir as potential ophthalmic drug delivery system. 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. The acyclovir loaded chitosan nanoparticles were prepared by ionic gelation of chitosan. Differential scanning calorimetry and fourier transform infra red spectroscopy measurements were carried out on the prepared nanoparticles, pure acyclovir and chitosan polymer. Fifteen different formulations were prepared and evaluated for particle size, Zeta potential, scanning electron microscopy, entrapment and loading capacity and *in-vitro* drug release profile. All the prepared formulations resulted in nano size in 377.9 to 720.6 nm and displayed spherical shape with zeta potential of +33.2 to +42.8 mV. The encapsulation efficiency and loading capacity were 70.7% - 90.9% and 25% - 50.8% 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 hours and fit best with Higuchi model with zero order and non-Fickian diffusion was superior phenomenon. The *in vivo* results reveal that ocular viral infections can be inhibited by the nanoparticles more significantly than the drug in conventional dosage forms. No appreciable difference was observed during 90 days in which nanoparticles were stored at various temperatures. Thus the results suggest that acyclovir loaded chitosan nanoparticle suspension appears promising for effective management of ocular viral infections.

Keywords: Acyclovir, Chitosan nanoparticles, Ocular delivery, Ionic gelation method.

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INTRODUCTION

Herpes simplex virus's infection is very common in humans. It has been estimated that one third of the world population have recurrent infection. Keratitis caused by herpes simplex viruses is the most common cause of cornea-derived blindness in developed nations. [1-2] 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. [3-6] The drug possess 10-30% of bioavailability with short plasma half-life of 2-3 hours, therefore necessitates 4-5 times application when administered as an ophthalmic ointment. These conventional preparations are ill-accepted on account of their short pre-corneal retention time, greasiness and vision-blurring effects. [7]

Many attempts have been made to improve the ocular bioavailability and the therapeutic effectiveness of acyclovir, e.g., chemical modification of the drug and its incorporation into colloidal systems such as liposomes or nanoparticles. [8-9] Nanoparticles have been used as ophthalmic delivery systems because they are able to penetrate into the corneal or conjunctival tissue by an endocytotic mechanism. [10] 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. [11] Chitosan (Poly [-(1, 4)-2-amino-2-deoxy-D-glucopyranose]) is a natural carbohydrate polymer prepared by the partial N-deacetylation of chitin. 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. [12] Besides, the cornea and conjunctiva have a negative charge; use of the cationic polymer chitosan will interact intimately with these extra ocular 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. 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. 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%; intrinsic viscosity, 1390 ml/g in 0.30 M acetic acid/0.2 M sodium acetate solution; and viscometric molecular weight, 4.08×10^5 Da) was obtained as gift sample from Central Institute of Fisheries Technology (Cochin, India). Sodium tripolyphosphate (TPP) 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

Before formulation of drug substances into a dosage form, it is essential that the drug and polymer should be chemically and physically characterized. Compatibility studies give the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipient in the fabrication of a dosage form. [13-14]

Fourier Transform Infra Red Spectroscopy

Compatibility study of pure acyclovir, chitosan polymer, acyclovir with chitosan polymer and acyclovir loaded chitosan nanoparticles (F-9) were determined by FTIR Spectroscopy using Perkin Elmer RX1. The pellets were prepared by gently mixing sample with potassium bromide at high pressure. The scanning range used is 450 to 4000 cm^{-1} . The pellets thus prepared were examined and the spectra of drug and the polymer in the formulations were compared with that of pure drug and polymer spectra.

Differential Scanning Calorimetry

Differential scanning calorimetric curve of pure acyclovir, chitosan polymer, acyclovir with chitosan polymer and acyclovir loaded chitosan nanoparticles (F-9) were carried out by using thermal analysis instrument equipped with liquid nitrogen sub ambient accessory; 2-6mg samples were accurately weighed in aluminum pans hermetically sealed and heated at a rate of 10°C per min^{-1} under nitrogen flow of 40 mL/min.

Preparation of acyclovir loaded chitosan nanoparticles

Chitosan nanoparticles were prepared based on the ionic gelation of chitosan with sodium tripolyphosphate anions. [15] Chitosan nanoparticles were prepared by ionic gelation of chitosan solution with sodium tripolyphosphate prepared in the presence of Tween 80 as a re-suspending agent to prevent aggregation, at ambient temperature. 350 mg acyclovir and various concentrations of chitosan dissolved in acetic acid in aqueous solution under magnetic stirring at room temperature for 45 min in the presence of Tween 80; 10 mL of sodium tripolyphosphate aqueous solution was added into 10

mL chitosan-acyclovir solution and the mixture was subjected to different sonication times. The nanosuspensions were cold centrifuged at 12000 g in a glucose bed for 30 min using Hitachi centrifuge. The supernatant liquid was analyzed by spectrophotometer to calculate the drug loading and percentage drug entrapment. The final suspensions were frozen and lyophilized by using freeze drier (Lab Conico, USA) at 0.4 mbar and -40°C for 5 hours using glucose and lactose. The lyophilized nanoparticles were stored in a desiccator at 4°C. The concentrations and amounts applied are summarized in Table 1.

Table 1: Composition of Acyclovir Loaded Chitosan Nanoparticles

Formulation Code	Drug (mg)	Polymer (mg)	Tween 80 (0.5%)	STPP (0.25%)	Sonication Time (min)
F-1	350	150	0.5	0.25	0
F-2	350	150	0.5	0.25	5
F-3	350	150	0.5	0.25	10
F-4	350	250	0.5	0.25	0
F-5	350	250	0.5	0.25	5
F-6	350	250	0.5	0.25	10
F-7	350	350	0.5	0.25	0
F-8	350	350	0.5	0.25	5
F-9	350	350	0.5	0.25	10
F-10	350	450	0.5	0.25	0
F-11	350	450	0.5	0.25	5
F-12	350	450	0.5	0.25	10
F-13	350	550	0.5	0.25	0
F-14	350	550	0.5	0.25	5
F-15	350	550	0.5	0.25	10

Evaluation of acyclovir loaded chitosan nanoparticles

Evaluation 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 (Hanna instruments, Italy H198).

Particle size and Zeta potential

The prepared acyclovir nanoparticles were evaluated for their particle size and zeta potential by zeta potential analyzer Zetasizer 3000 HS (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 degrees.

Surface morphology by Scanning Electron Microscopy

The morphology of the acyclovir nanoparticles were 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.

Acyclovir Encapsulation efficiency and loading capacity

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 g for 30 minutes. The amount of free acyclovir in the supernatant was measured by UV method at 253 nm. [11] The acyclovir encapsulation efficiency (EE) and loading capacity (LC) of the nanoparticles was calculated as follows:

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

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

In-vitro release studies

The acyclovir loaded chitosan nanoparticles were separated from the aqueous suspension medium through ultracentrifugation. Nanoparticles of acyclovir were redispersed in 10 mL of (pH 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. The bag was tied and placed into 10 mL of phosphate buffer solution in a beaker which acts as a receptor compartment. The entire system was kept at 37°C with continuous magnetic stirring; at appropriate time intervals 1 mL of the release medium was removed and 1mL fresh 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.

Kinetics of drug release

In order to understand the mechanism and kinetic of drug release, the drug release data of the *in-vitro* diffusion study were analyzed with various kinetic models like zero order, first order, Higuchi's and Peppas. Coefficient of correlation (r) values were calculated for the linear curves by regression analysis. [16-17]

In-vivo drug release studies

Six healthy albino rabbits (1.5–2.2 kg) were selected for the study with no signs of ocular inflammation. As per CPCSEA guidelines the procedures were followed. The experimental protocols have been approved by the Institutional Animal Ethics Committee (Nandha College of Pharmacy, 688/2/C-CPCSEA dated 21-02-2015). Out of 15 formulations F-9 was taken for *in-vivo* study on the basis of *in-vitro* drug release study. The marketed formulation of acyclovir (Acivir Eye 3% w/w ointment) was used as control. The left eyes of rabbits were used for control preparation and the right eye of the rabbits for prepared nano suspension. Aqueous humor acyclovir levels were monitored at 1, 4, 8, 16, 20 and 24 hours after instillation of nanosuspension and control into the conjunctival sac. During the time interval the rabbits were anaesthetized by i.v. injection of ketamine (25 mg/kg) and 150µL aqueous humor aqueous was withdrawn from the limbus region of

rabbits with a heparin-rinsed glass syringe connected to a 27-gauge needle. Samples were denatured by the addition of an equivalent volume of 2% zinc sulphate solution centrifuged and the supernatant liquid was filtered through a 0.2- μ m millipore membrane. A similar experiment was carried out on the second group of rabbits with marketed formulation of acyclovir. Samples were analyzed by high performance liquid chromatography at a wavelength of 253 nm [18-20]. The HPLC system was operated in a binary mode with a photodiode array detector, auto injector and column oven. The analysis was performed at 253 nm on a reversed phase chemisorb C-18 cartridge column (250 mm \times 4.6 mm, 5 μ m) maintained at 25°C. The mobile phase comprised of 0.02M potassium dihydrogen phosphate - acetonitrile in the ratio of 99:1 with a flow rate of 1 mL/ min. Acyclovir concentration in aqueous humor was estimated using linearity chart. [21-22]

$$\text{Concentration in } \mu\text{g/mL} = \frac{\text{Sample area} \times \text{Standard weight} \times \text{Dilution factor}}{\text{Standard area} \times \text{Dilution factor} \times \text{Sample weight}} \times \text{Purity}$$

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Stability Studies

The short term stability study was carried out as per ICH guidelines using the optimized formulation F-9. Formulation F-9 was divided into 3 sets of samples and stored at 4°C in refrigerator, room temperature 37°C and at ambient temperature. *In vitro* release study of formulation F-9 was carried out after 90 days of storage. [23]

RESULTS AND DISCUSSION

The results of the present investigation demonstrated the potential use of chitosan nanoparticles for effective delivery of acyclovir for treating various ocular viral 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. [24] Among them chitosan based systems are acknowledged more suitable for ocular pathway, based on the favorable biological characteristics of chitosan. [25-26] Several studies have shown that nanoparticles can transport across epithelia more readily than microparticles. [27] Moreover chitosan nanoparticles can be easily prepared under mild conditions, and can be incorporated in macromolecular bioactive compounds. This characteristic feature is extremely beneficial for drugs, proteins, genes or hydrophobic molecules that are poorly transported across epithelia. [25-26]

Compatibility studies

Fourier Transform Infra Red Spectroscopy (FTIR)

There was no appearance or disappearance of any characteristic peaks of pure drug or of polymer in the physical mixture and acyclovir loaded nanoparticles, thus indicating absence of any physical interaction between the drug and polymer. The results show that the incorporation of the drug in to the polymer did not change the characteristics of the drug. Observations of compatibility studies of infrared spectral data for the

pure drug, the mixture of acyclovir with chitosan and acyclovir loaded chitosan nanoparticle (F-9) are shown in Table 2.

Differential Scanning Calorimetry (DSC)

The results of the DSC study of acyclovir, chitosan, acyclovir with chitosan and acyclovir loaded chitosan nanoparticle (F-9) are shown in Figs. 1A, 1B, 1C and 1D. The DSC curve of acyclovir showed characteristic peaks at 120.61°C, 150.48°C and 254.07°C. The DSC curve of chitosan showed characteristic broader peak at 102.81°C. The thermogram of acyclovir chitosan mixture exhibited same characteristic peaks of acyclovir at 121.06°C, 150.48°C and 254.07°C. The results of the thermogram suggested that there was no chemical interaction between acyclovir and chitosan.

From the IR spectral analysis and DSC study, it was found that IR spectrum and thermogram of pure acyclovir and combination of pure drug with polymer like chitosan and prepared nanoparticles showed all the characteristic peaks of acyclovir confirming the physical and chemical compatibility of the pure drug and polymer.

Table 2: FT-IR spectral data

Molecular Vibration	Wave number in cm ⁻¹		
	Pure acyclovir	Acyclovir with polymer	Acyclovir loaded nanoparticles
NH stretch in NH ₂	3184.14	3184.26	3232.77
C = N stretch	1633.12	1633.14	1694.84
C = O stretch	1716.69	1717.96	1694.84
CH ₂ stretch	2927.96	2928.61	2873.04
OH stretch	3442.06	3442.48	3444.97

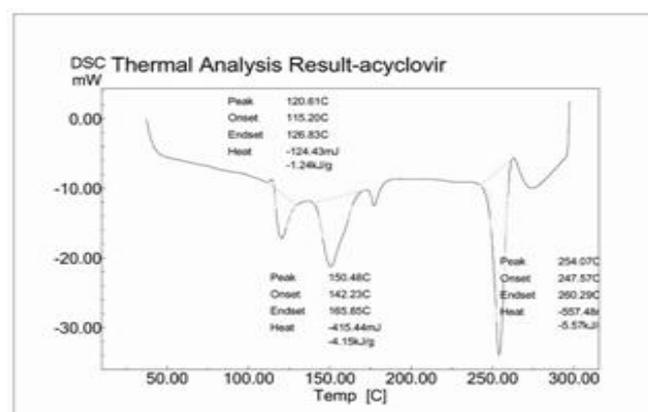


Fig. 1A: Thermogram of acyclovir

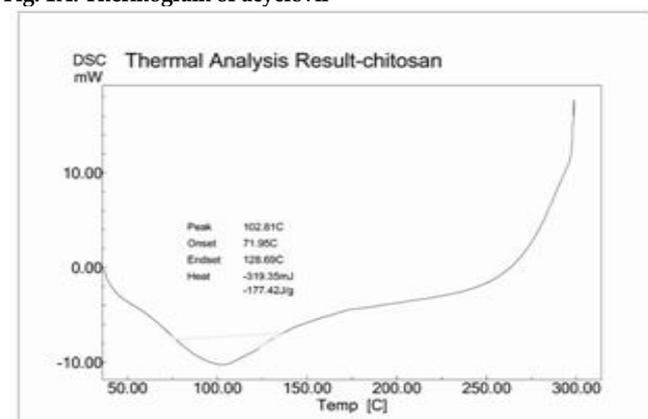


Fig. 1B: Thermogram of chitosan

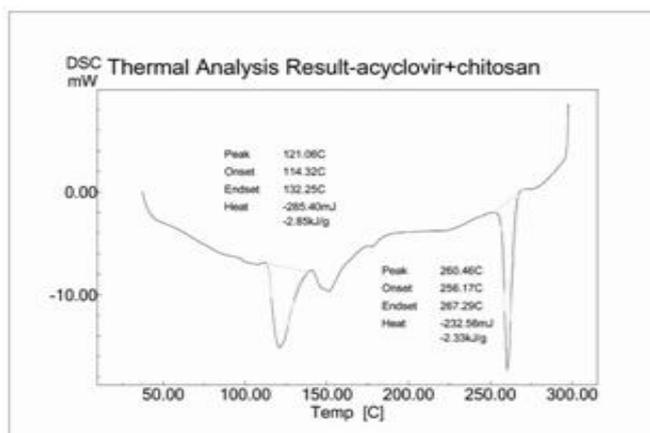


Fig. 1C: Thermogram of acyclovir and chitosan

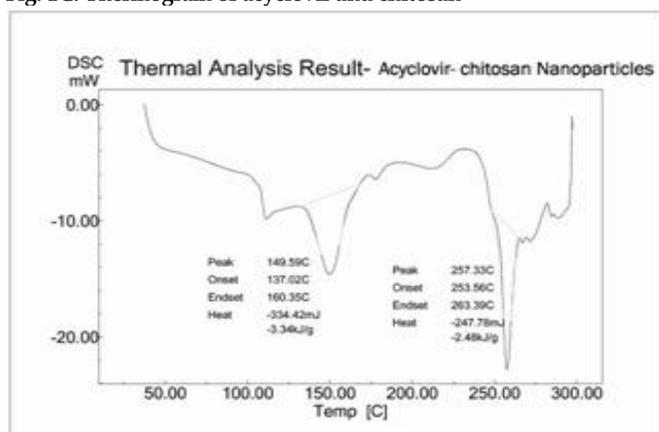


Fig. 1D: Thermogram of formulation F-9

Evaluation of acyclovir loaded chitosan nanoparticles pH

pH values for all the formulations are within acceptable range 6.6-7.4 and hence would not cause any irritation upon administration of the formulation. It was also observed that increase in chitosan polymer causes a slight increase in pH for formulations

Particle size and Zeta potential

The mean particle size and zeta potential of acyclovir loaded chitosan nanoparticles (F1- F15) are shown in Table 3 and Fig. 2. The mean particle size of the acyclovir loaded chitosan nanoparticles ranged between 377.9 to 720.6 nm. The sizes of nanoparticles of all the formulations are given in nanometer. The maximum size of nanoparticles was observed in F-15 as compared to other formulations and the least size was seen in F-1. Thus the results indicated that the size of the nanoparticles varied with the polymer concentration.

The presence of a nonionic surfactant is very important for the so-called "long-term" stability 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. Considering these factors the non-ionic surfactant Tween 80 (0.05%) was used to stabilize the formulation.

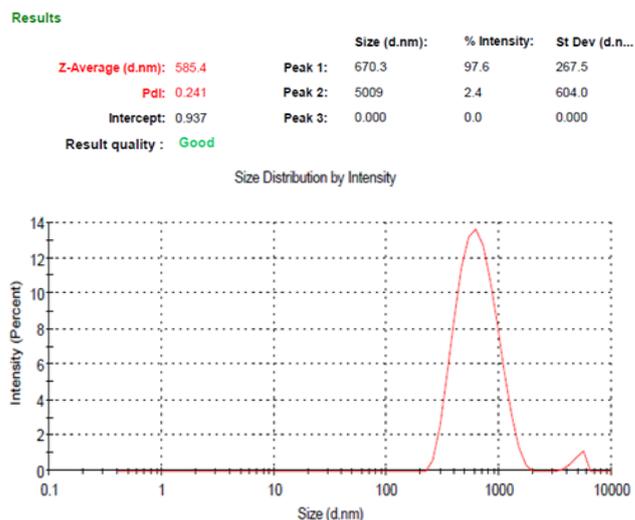


Fig. 2: Mean particle size of formulation F-9

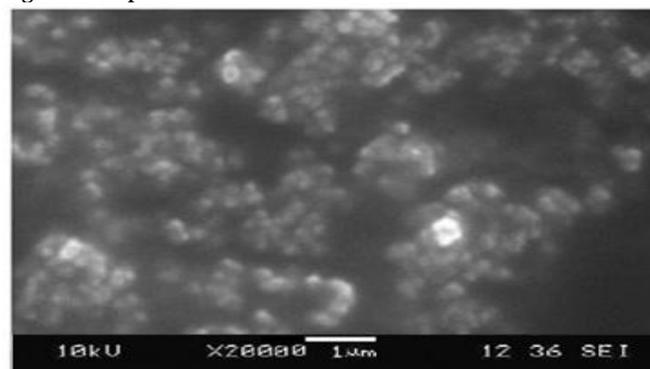


Fig. 3: SEM of formulation F-9

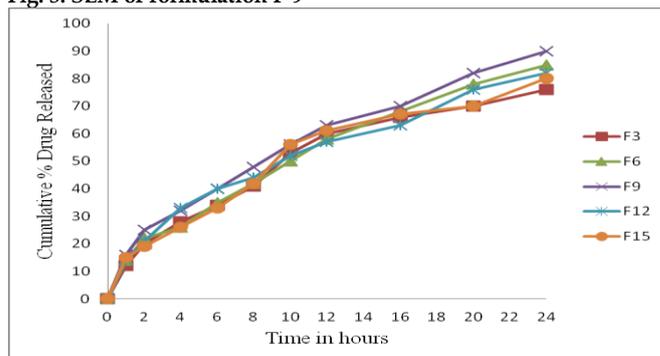


Fig. 4: Comparative *in-vitro* drug release profile of F-3, F-6, F-9, F-12 and F-15

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 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 are moderately stable.

Table 3: Mean Particle size, Zeta potential, Encapsulation efficiency and Loading capacity of acyclovir loaded chitosan nanoparticles

Formulation Code	Mean Particle Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	Loading Capacity (%)
F-1	411.7	+33.2	70.7	50.8
F-2	398.1	+33.4	71.5	50.6
F-3	377.9	+33.8	71.8	50.3
F-4	517.0	+35.7	74.3	40.58
F-5	492.3	+35.9	75.2	40.67
F-6	426.2	+35.8	74.8	40.54
F-7	596.3	+38.6	81.4	35.90
F-8	591.5	+37.3	80.8	34.89
F-9	585.4	+38.3	81.1	33.87
F-10	717.3	+40.5	86.3	30.90
F-11	681.5	+41.5	86.9	30.84
F-12	670.4	+40.9	87.0	29.76
F13	720.6	+42.8	90.5	25.4
F-14	710.3	+42.5	90.7	25.2
F-15	708.8	+42.1	90.9	25.0

Surface Morphology

According to morphological evaluation analysis by scanning electron microscopy all the prepared acyclovir loaded chitosan nanoparticles (F1-F15) seemed to have a similar spherical shape. The morphological characters of acyclovir loaded chitosan nanoparticles are shown in Fig. 3.

Acyclovir encapsulation efficiency and loading capacity

The encapsulation efficiency was increased by increasing the concentration of polymer. The encapsulation efficiency ranged between 70.7 to 90.9%. The maximum entrapment was found in F-15 (90.9%) and lowest entrapment in F1 (70.7%). Conversely the loading capacity of the nanoparticles decreased as the concentration of polymer increased. The loading capacity ranged between 25.00 to 50.8%. The results suggested that the encapsulation efficiency and loading capacity of the nanoparticles depend on the concentration of the polymer used in the preparation. The results suggested that the encapsulation efficiency and loading capacity of the nanoparticles depend on the concentration of the polymer used in the preparation. The mechanism of acyclovir association to chitosan nanoparticles was mediated by an ionic interaction between both chitosan and acyclovir. The encapsulation efficiency and loading capacity of acyclovir loaded chitosan nanoparticles are shown in Table 2.

In vitro drug release

The *in-vitro* diffusion of acyclovir from the nanoparticles was studied by monitoring the drug leakage for 24 hours. The acyclovir release profile from chitosan nanoparticles is characterized by an initial rapid release followed by a sustained release of the drug over a period of 24 hours (Fig. 4). The initial rapid release can be due to the burst effect resulting from the release of the drug encapsulated near the nanosphere surface and thereafter the slow release of acyclovir from the chitosan nanoparticles is possibly the consequence of the release of the drug fraction encapsulated in the core of the nanospheres. The cumulative percentage drug released for F3, F6, F9, F12

and F15 after 24 hours was 76.14%, 85.28%, 90.10%, 82.30% and 80.40% respectively. Maximum drug release was found in F-9 (90.10. %) and minimum was found in F-1 (70.2%). Among all the formulations F-9 was selected as an optimized formulation due to its desirable drug release during 24 hours. Maximum drug release was found in F-9 (90.10. %) and minimum was found in F-1 (70.2%). Out of all the formulations F-9 was selected as optimized formulation due to its desirable drug release in 24 hours.

Release kinetics

In order to investigate the release mechanism of present drug delivery system, the release data of prepared acyclovir loaded chitosan nanoparticles in phosphate buffer (pH 7.4) were compared to classic drug release kinetics models. The release rates were analyzed by least square linear regression method. Release models such as zero order, first order model, Higuchi model and Ritger-Peppas empirical model (Table 4) were applied to the release data. [28-29] The coefficient of determination (R^2) of equation for release of acyclovir from acyclovir loaded chitosan nanoparticles (F-3, F-6, F-9, F-12 and F-15) in phosphate buffer were 0.9653, 0.9698, 0.970, 0.9776 and 0.9980 signifying zero order release pattern and the value of coefficient of determination (R^2) in Higuchi equation were found to be 0.9941, 0.9976, 0.9867, 0.9952 and 0.9978 which indicates the integrity of chitosan nanoparticles and diffusion-controlled release. Substituting the release values in Ritger-Peppas equation, the value of coefficient of determination was found to be 0.9956, 0.9980, 0.9875, 0.9867 and 0.9976.

The value of n for formulations F-3, F-6, F-9, F12 and F-15 were found to be 0.9981, 0.7801, 0.9864, 0.9899 and 0.9734 indicating Non-Fickian release; $n = 0.43$ indicates Fickian release (case I), less than 1 or greater than 0.5 for Non-Fickian (anomalous) release and greater than 1 indicates super case II type of release. Non-Fickian refers to a combination of both diffusion and erosion controlled drug release. [30] This result was attributable to the sustained release of drug signifying mixed type of release pattern. These results are consistent with those obtained by earlier study of release pattern for

tetracycline; potent antibiotic from nanoparticles prepared by using chitosan for maximum bioadhesivity and controlled drug release. Similar Non-Fickian release for nifedipine loaded chitosan microspheres prepared by emulsification solvent evaporation method was also reported. [31-32] From the release kinetics data the diffusion profile of the drug from the nanoparticles showed fitted with Higuchi plot of zero order release kinetics and indicated Non-Fickian diffusion mechanism for the release of the drug from the nanoparticles.

Table 4: Kinetic analysis of acyclovir released from different formulation

Formulation Code	Zero order	First order	Higuchi's	Peppas's	'n'values
F3	0.9653	0.9172	0.9941	0.9956	0.9981
F6	0.9698	0.8956	0.9976	0.9980	0.7801
F9	0.9701	0.8881	0.9867	0.9875	0.9864
F12	0.9776	0.8265	0.9952	0.9867	0.9899
F15	0.9980	0.9508	0.9978	0.9976	0.9734

Table 5: *In-vivo* release of acyclovir after topical administration (RP-HPLC)

Time in hours	Peak area	Control ($\mu\text{g/mL}$)	Peak area	Formulation F-9 ($\mu\text{g/mL}$)
1	131651 \pm 1.56	18.231 \pm 1.70	169088 \pm 1.07	25.49 \pm 1.54
4	149782 \pm 2.61	22.10 \pm 2.78	229876 \pm 2.90	35.71 \pm 2.80
8	221456 \pm 1.28	33.65 \pm 2.45	308764 \pm 1.98	48.36 \pm 2.50
16	267352 \pm 2.98	40.12 \pm 2.87	514520 \pm 1.54	78.59 \pm 3.67
20	187003 \pm 1.21	28.18 \pm 1.58	348976 \pm 2.62	54.92 \pm 2.01
24	138786 \pm 1.78	20.13 \pm 1.34	270752 \pm 1.38	40.54 \pm 2.16

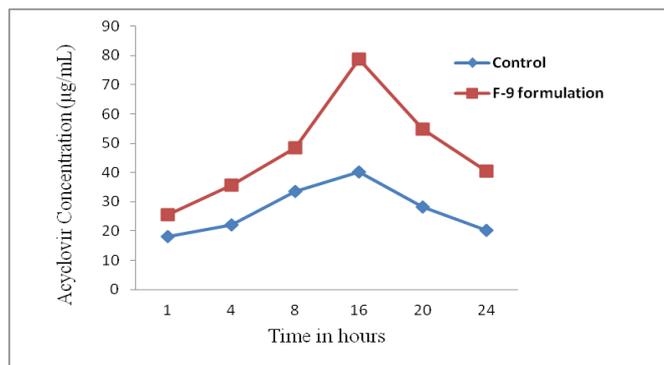


Fig. 5: Acyclovir concentration for formulation F-9 and control

In vivo studies

Formulation F-9 with satisfactory *in vitro* release was selected for *in vivo* drug studies. Drug concentration was determined by using HPLC method. The concentration of acyclovir in the cornea after topical administration of optimized formulation and controlled preparations are given in Table 4 and Fig. 5. The animals treated with acyclovir loaded chitosan nanoparticles had higher corneal drug level (< 0.05) than those treated with marketed acyclovir ointment. The maximum concentration was found to be in F-9 at eighth hour 78.59 $\mu\text{g/mL}$ where as controlled preparation showing 33.65 $\mu\text{g/mL}$. The concentrations of drug obtained from aqueous humors with formulation F-9 and controlled preparation were studied by one way ANOVA which showed a P value of 0.087. Earlier researchers suggested that the

improved interaction of cyclosporin A loaded chitosan nanoparticles with the cornea and the conjunctiva could be found in the mucoadhesive properties of chitosan. [25] It has also been proposed that it might not be the mucoadhesive character of chitosan molecules but the electrostatic interaction between the positively charged chitosan nanoparticles and the negatively charged corneal and conjunctival cells that is the major force responsible for the prolonged residence of cyclosporin A in these epithelia. [33] Such a mechanism for acyclovir from chitosan nanoparticles cannot be ruled out, since our study revealed the presence of positively charged nanoparticles as evident from the zeta potential. Further, the positively charged chitosan is able to increase the precorneal residence time of ophthalmic formulations containing active compounds when compared with simple aqueous solution. [34]

Stability studies

Based on the *in-vitro* and *in-vivo* performance, the formulation F-9 was selected for short-term stability studies. The *in-vitro* release profile of formulation F-9 after 90 days of stability testing at different storage conditions were compared with the previous data of F-9; it was observed that there were no significant changes in drug release. *In-vitro* release studies proved that the formulation F-9 stored at 4°C showed 91.23% release, the one which stored at ambient temperature and humidity showed 90.54% and formulation stored at room temperature (37°C) showed 92.15% release after 24 hours. These results indicate that the drug release from the formulation stored at room temperature (37°C) was highest followed by formulation stored at 4°C and at ambient temperature. On comparing this data with the previous release data of F-9 (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. Thus the results of the present study demonstrated that chitosan nanoparticles may be beneficial in improving the corneal permeation, contact time and bioavailability of acyclovir for the treatment of ocular viral infections.

Acyclovir loaded chitosan nanoparticles exhibited excellent capacity for the association of acyclovir. The mean particle size, morphological characteristics, surface property, encapsulation efficiency and loading capacity of the nanoparticles appear to depend on the concentration of polymer. The *in vitro* release profile of acyclovir from nanoparticles has shown a slow controlled release following zero order kinetic with Non-Fickian diffusion mechanism. The *in vivo* release profile indicated that polymeric system of acyclovir has achieved the objectives of increased contact time, prolonged drug release and decreased frequency of administration. 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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