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## Formulation and evaluation of acyclovir loaded chitosan nanoparticles

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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 with sodium tripolyphosphate anions. Differential Scanning Calorimetry (DSC) and Fourier Transform Infra-Red (FTIR) spectroscopy measurements were carried out on the nanoparticles and on the pure acyclovir and chitosan polymer. Five different formulations were prepared and evaluated for particle size, Zeta potential, scanning electron microscopy, entrapment and loading capacity, in vitro drug release profile. All the prepared formulations resulted in nano size in 150 - 250 nm and displayed spherical shape with Zeta potential of +33.2 to +42.8 mV. The encapsulation efficiency and loading capacity were 70% - 90% and 25% - 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 hrs. Kinetic release profiles of acyclovir from nanoparticles appeared to fit best with Higuchi model with zero order and the non-Fickian diffusion mechanism. The in-vitro results reveal that ocular viral infections can be inhibited by the nanoparticles more significantly than the drug in conventional dosage forms. Thus it can be conclusively stated that the acyclovir loaded chitosan nanoparticles suspension may be considered as an improved ophthalmic drug delivery system for the treatment of ocular viral infections.

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

### **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 bioavailability ocular and the therapeutic effectiveness of acyclovir, chemical e.g., 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 acceptable as biocompatibility, biodegradability and ability to enhance the paracelluar transport of drugs [9]. 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.

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]. 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 [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

#### **Preformulation studies** [14, 15]

Before formulation of drug substances into a dosage form, it is essential that the drug and polymer should be chemically and physically characterized. Preformulation 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.

# Fourier transform infra-red spectroscopy (FTIR)

Compatibility study of drug with the polymer was determined by FTIR Spectroscopy using Perkin Elmer RX1. 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 <sup>-</sup>1and the revolution was 4 cm <sup>-</sup>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 or polymer spectra.

#### **Differential Scanning Calorimetry (DSC)**

Differential scanning calorimetric curve of pure acyclovir, chitosan polymer and mixture of drug and polymer measurement were carried out by using a thermal analysis instrument equipped with a liquid nitrogen sub ambient accessory. 2-6mg samples were accurately weighed in aluminum pans thematically sealed and heated at a rate of 10°C per min <sup>-1</sup> in a 30 to 300 °C temperature under nitrogen flow of 40 ml / min.

#### **Estimation of pure acyclovir [16]**

Acyclovir can be estimated spectrophotometrically at 253 nm in the range of 2-18 mcg/ml as per Beer Lambert's law.

#### Preparation of Phosphate buffer ph 7.4 [17]

50 ml of potassium Dihydrogen phosphate was placed in a 200ml volumetric flask and added 39.1 ml of 0.2m sodium hydroxide and then distilled water to make up to 200ml.

# Preparation of 0.2m potassium Dihydrogen phosphate

27.218g of potassium Dihydrogen phosphate was dissolved in distilled water and made up to 1000 ml.

#### Preparation of 0.2m sodium hydroxide

8g of sodium hydroxide was dissolved in distilled water and made up to 1000 ml.

### Preparation of standard drug solution

#### **Stock Solution**

100mg of Acyclovir was dissolved in 100ml of Phosphate buffer saline pH 7.4 so as to get a stock solution of 1000  $\mu$ g/ml concentration.

#### **Standard solution**

2 ml of stock solution was diluted to 100ml with pН 7.4 phosphate buffer thus giving concentration of 20 µg/ml of the drug .Aliquot of standard drug solution ranging from 1ml to 9ml were transferred 10ml volumetric flask and were diluted up to the mark with pH 7.4 phosphate buffer. Thus the final concentration ranges from 2-18 µg/ml as per Beer Lambert's law .Absorbance of each solution was measured at 253.0 nm against phosphate buffer pH 7.4 as a blank and the concentrations of drug versus absorbance was plotted.

# 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 [18]. 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 resuspending agent to prevent aggregation, at ambient temperature while stirring. 350 mg acyclovir and various concentrations of chitosan (F1-F5) dissolved in acetic acid in aqueous solution under magnetic stirring at room temperature for 45 mins in the presence of Tween 80. 10mL STPP aqueous solution was added in to 10mL chitosan-acyclovir solution and the mixture at different sonication times. The nano suspensions were cold centrifuged at 12000g 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 -40°C for 5 hrs using glucose and lactose (1:2). The lyophilized nanoparticles were stored in a desiccator at 4°C. The concentrations and amounts applied summarized in Table No.1. are

Formulation	Acyclovir (mg)	Chitosan (mg)	Tween 80(%)	STPP (%)	Sonication Time (min)
Code					
F1	350	150	0.5	0.25	10
F2	350	250	0.5	0.25	10
F3	350	350	0.5	0.25	10
F4	350	450	0.5	0.25	10
F5	350	550	0.5	0.25	10

Table .1 Composition of Acyclovir Loaded Chitosan Nanoparticles

## EVALUATION OF ACYCLOVIR LOADED CHITOSAN NANAOPARTICLES

#### 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 [19].

### 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 [20].

#### 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 12000rpm for 30 minutes. The amount of free acyclovir in the supernatant was measured by UV method at 253 nm [21].

The acyclovir encapsulation efficiency (EE) and loading capacity (LC) of the nanoparticles was calculated as follows.

	Total amount of acyclovir – Free acyclovir		
Encapsulation efficiency	=	x 100	
		Weight of nanoparticles	
		Total amount of acyclovir – Free acyclovir	
Loading capacity	=	×100	
		Total amount of acyclovir	

#### 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 redispersed 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 acts as a receptor compartment. The entire system was

kept at 37°C 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 in to the system. The amount of acyclovir in the release medium was estimated by UV-Visible Spectrophotometer at 253 nm.

# Comparative study of acyclovir nanoparticles with marketed formulation of acyclovir

The *in-vitro* release behavior of optimized acyclovir nanoparticles (F3) and marketed

formulation of acyclovir was investigated in phosphate buffer (pH7.4) for 24hrs.

#### **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,  $Mt/M\infty = ktn$ , 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 [22].

### **RESULTS AND DISCUSSION**

#### **Compatibility studies**

# Fourier transform infra-red spectroscopy (FTIR)

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

#### Drug and carrier interaction by Differential Scanning Calorimetry (DSC)

The results of the DSC study of acyclovir, chitosan and mixture of acyclovir and chitosan are shown in Figs. 1A, 1B and 1C. 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.



Fig. 1A DSC Thermogram of Acyclovir



Fig. 1B DSC Thermogram of Chitosan





#### 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) are shown in Table.2. 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 acyclovir loaded chitosan nanoparticles (F1-F5) are shown in Table.2. 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 stable.

Table.2 Average Particle size and Zeta	potential of acyclovir	loaded chitosan nand	oparticles
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Formulation Code	Average particle size (nm)	Zeta potential (mV)
F1	220	+33.2
F2	192	+35.7
F3	150	+38.3
F4	205	+40.5
F5	250	+42.8

#### **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 in nanometer. The morphological characters of acyclovir loaded chitosan nanoparticles (F3) was shown in Fig.2.



Fig.2: SEM Photograph of acyclovir loaded chitosan nanoparticles (F3)

### Acyclovir Encapsulation Efficiency and Loading Capacity of the Nanoparticles

The encapsulation efficiency and loading capacity of acyclovir loaded chitosan nanoparticles (F1– F5) are shown in Table.3. 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 nanopartcles was depend on the concentration of the polymer used in the preparation.

	Formulation	Encapsulation Efficiency	Loading Capacity (%)	
Code		(%)		
F1		70	25	
F2		74	34	
F3		81	41	
F4		86	46	
F5		90	50	

Table.3 Encapsulation efficiency and loading capacity of chitosan nanoparticles

### In-vitro Drug Release

From the *in-vitro* drug release data for F1-F5, it was observed that increase in the 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-1, F-2, F-3, F-4 and F-5 respectively. 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 hrs. The initial rapid release can 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. The cumulative percentage 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 hrs (Fig-3). 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 and 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 24hrs.



Fig.3 Comparative in vitro drug release profile for F1-F5

# Comparative study of acyclovir nanoparticles with marketed formulation of acyclovir

The *in-vitro* drug release behavior of optimized acyclovir loaded chitosan nanoparticles (F3) and marketed formulation of acyclovir was investigated in phosphate buffer (pH7.4) for 24hrs as shown in Fig.5. Acyclovir is available as a 3% w/w ointment to be placed in the eye five times a day as a 1cm ribbon each. The weight of such five ribbons approximately 66mg of the ointment that contains

around 2mg of acyclovir daily. Hence, it was decided to formulate ocular nanoparticles containing 2mg of acyclovir for once daily. At the end of 24hrs the drug release was 90.68% for F3 and 38.45% for marketed product. The release pattern demonstrated a very slow release of drug at each point of time from acyclovir nanoparticles. There was an initial phase of rapid release of acyclovir followed by a more gradual release over a period of 24hrs.



Fig.4 Comparative in-vitro drug release profile for F3 and Marketed formulation

#### **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.4). 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 conformed to Higuchi plot with zero order release kinetics and indicated non Fickian diffusion mechanism for the release of the drug from the nanoparticles.

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

Table: 4 Various Parameters of the Model Equation on the In Vitro Release Kinetics

### CONCLUSION

Chitosan nanoparticles had 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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