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Acyclovir Loaded Chitosan Nanoparticles for Ocular Delivery

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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 develop and evaluate nanosphere colloidal suspension containing acyclovir as potential ophthalmic drug delivery system. The acyclovir loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). The nanoparticles were characterised by Scanning Electron Microcopy (SEM), Zeta potential analyser, Differential Scanning Calorimetry (DSC) and Fourier Transform InfraRed (FTIR) Spectroscopy. All the prepared formulations resulted in nano range size partricles (200 - 495 nm) and displayed spherical smooth morphology with Zeta potential (+36.7 - +42.3 mV). The encapsulation efficiency and loading capacity were 56% - 80% and 10% - 25% respectively. The acyclovir loaded chitosan nanoparticles displayed crystallinity than acyclovir. The invitro diffusion profile of acyclovir from the nanoparticles showed a sustained release of the drug over a 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 was superior phenomenon. Thus the results suggest that acyclovir loaded chitosan nanoparticle suspension appears promising for effective management of ocular viral infections.

Keyword: 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 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 [9], biodegradability and ability to enhance the paracelluar transport of drugs [10]. 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. 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 [11]. Among them chitosan based systems are acknowledged more suitable for ocular pathway, based on the favorable biological characteristics of chitosan [12] [13]. Several studies have shown that nanoparticles can transport across epithelia more readily than micro particles [14]. Moreover Chitosan nanoparticles can be easily prepared under mild conditions, besides can incorporate 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. Moreover, chitosan has recently been proposed as a material with a good potential for ocular drug delivery.

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 [15]. 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 [16]. 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 [17]. However, literature search indicates that the role of acyclovir concentration on nanoparticles has not been studied in detail and hence the present study was attempted to demonstrate the influence of acyclovir 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

Preparation of Acyclovir loaded chitosan nanoparticles

Chitosan nanoparticles were prepared according to the procedure first reported by Calvo et al [18] based on the ionic gelation of chitosan with sodium tri polyphosphate (TPP) anions. Chitosan nanoparticles were prepared by ionic gelation of chitosan solution with sodium tri polyphosphate (0.25%) prepared in the presence of Tween-80 (0.5%) as a resuspending agent to prevent aggregation, at ambient temperature while stirring.. The final suspension was then frozen and lyophilized at 0.4 mbar and -40ºC for 5 hrs using glucose and lactose (1:2). The lyophilized nanoparticles were resuspended in P^H 7.4 phosphate buffer and submitted to characterization experiments. The concentrations and amounts applied are summarized in Table No.1.

Table No.1 Composition of Acyclovir loaded chitosan nanoparticles

Formulation Code	Drug(mg)	Polymer(mg)	Tween-80 (%)	STPP (%)	Sonication Time (min)
F1	25	250	0.5	0.25	3
F2	50	250	0.5	0.25	3
F3	75	250	0.5	0.25	3
F4	100	250	0.5	0.25	3
F5	125	250	0.5	0.25	3

Acyclovir Encapsulation efficiency and Loading capacity of the nanoparticles

The amount of free acyclovir in the supernatant was measured by UV method at 253 nm. The acyclovir encapsulation efficiency (EE) and loading capacity (LC) of the nanoparticles was calculated as follows.

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

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

Particle size and Zeta potential of nanoparticles

The size and zeta potential of nanoparticles were analyzed by using a Zetasizer, 3000 HS (Malvern instrument). The samples were diluted with PH 7.4 phosphate buffer and placed in eletrophoretic cell and measured in the automatic mode.

Scanning Electron Microscopy (SEM)

The scanning electron microscopy (JEOL MODEL JSM 6400, Tokyo) was used to characterize the surface morphology of nanoparticles. The nanoparticles were mounted directly on the scanning electron microscopy (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.

Fourier Transform Infra Red Spectroscopy (FTIR)

The FTIR spectra of acyclovir, chitosan and acyclovir loaded chitosan nanoparticles were determined by using Perkin Elmer RX1 model. The pellets were prepared by gently mixing of 1mg sample with 200mg 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.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetric curve of pure acyclovir, chitosan and acyclovir loaded chitosan nanoparticles measurement were carried out by using a thermal analysis instrument (DSC DA 60 Shimadzu Japan) equipped with a liquid nitrogen sub ambient accessory.

Drug Release Study

Diffusion studies

Nanoparticles 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 10 ml 7.4 phosphate buffer solution which acts as a receptor compartment, under agitation, at 37ºC, in order to assess sink conditions during the release studies. At different time intervals, samples were centrifuced and the acyclovir released determined by by UV-Visible Spectrophotometer at 253 nm.

Release Kinetics

In order to understand the mechanism and kinetics of drug release, the results of the *invitro* 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)[19]. 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 ∞, $Mt/M\infty$ 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 AND DISCUSSION

FT-IR Spectroscop

The absorption peak of 3184.15cm−1 (amino group of chitosan) was absent in the spectrum of acyclovir-loaded chitosan nanoparticles as compared to the spectra of acyclovir and a new sharp peak of 2873.04 cm−1 (linkage between hydroxyl group of acyclovir and amino group of chitosan) appeared. The results indicate that there was an electrostatic interaction between hydroxyl ethoxy- methyl group of acyclovir and amino groups of chitosan.

Particle Size and Zeta potential of Acyclovir Loaded Chitosan nanoparticles

The particle size of acyclovir loaded chitosan nanoparticles (F1– F5) are shown in Table No.2. The maximum size of nanoparticles was observed in F1 (495 ± 05nm) as compared to other formulations and the least size was seen in F5 (200 ± 30nm). The size of the nanoparticles varied with the acyclovir concentration. Previously it has been reported that the particle size is dependent on the chitosan concentration, the minimum size corresponding to the lowest chitosan concentration [20].

The Zeta potential values of acyclovir loaded chitosan nanoparticles (F1-F5) are shown in Table No.2. The Zeta potential values ranged between +36.7 to +42.3 mV and the values decreased as the concentration of acyclovir increased.

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 centre of the *nanocapsule* or adsorbed onto the surface. The present study demonstrated decrease in Zeta potential as the concentration of acyclovir increased in consistent with earlier findings on the ammonium glycyrrhizinate loaded chitosan nanoparticles [21].

Encapsulation Efficiency and Loading Capacity of the nanoparticles

Table No.2 shows the results of encapsulation efficiency and loading capacity of the acyclovir loaded chitosan nanoparticles. The encapsulation efficiency was maximum with the lower drug concentration (F1) and minimum with the higher drug concentration (F5). The encapsulation efficiency ranged between 56 to 80%. Conversely the loading capacity of nanoparticles increased as the concentration of the drug increased. The loading capacity ranged between 10 to 25%.

The increase of acyclovir concentration leads to a decrease of encapsulation efficiency and an enhancement of loading capacity, 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 tripolyphosphate as observed in the previous study [21]. The failure to increase encapsulation efficiency proportionate with increase in acyclovir concentration may be due to shorter chains low molecular weight chitosan used in the present study.

Table No. 2 Mean Particle size, Polydispersity Index, Zeta potential, Encapsulation efficiency and Loading capacity

Formula code	Mean particle size (nm)	Polydispersity index (_μ2_/Γ 2)	Zeta potential (mV)	Encapsulation efficiency (%)	Loading capacity(%)
F1	495 ± 05	0.37	+ 42.3 ± 1.3	80.00	10.00
F2	450 ± 28	0.29	+40.2 ± 1.2	74.00	16.81
F3	368 ± 12	0.24	+38.1 ± 1.4	66.66	20.83
F4	298 ± 25	0.18	+37.5 ± 1.1	60.00	23.07
F5	200 ± 30	0.16	+36.7 ± 1.5	56.00	25.00

Scanning Electron Microscopy (SEM)

The morphological characters of acyclovir loaded chitosan nanoparticles (F5) are shown in Figure **1.** Acyclovir loaded c hitosan nanoparticles have shown spherical shape. The SEM of the acyclovir loaded chitosan nanoparticles showed that the nanoparticles have a solid dense structure with smooth spherical shape. In consistent with previous findings [21] a significant reduction of nanoparticle mean size was observed in the formulation (F5) with lowest concentration of chitosan relative to drug concentration (Table No.2). Previously it has been reported that the particle size of cyclosporin A loaded chitosan nanoparticles is dependent upon chitosan concentration, the minimum size corresponding to the lowest chitosan concentration [8].

Earlier it has also been demonstrated that the particle size of ammonium glycyrrhizinate loaded chitosan nanoparticles significantly increased as the concentration of ammonium glycyrrhizinate increased [21]. Interestingly such an observation was absent in our findings wherein the particle size of acyclovir loaded nanoparticles decreased as the concentration of acyclovir increased. Though the exact mechanism for the diagonally opposite effect with respect to nanoparticles size related to drug concentration is not clearly understood it can be considered that the size of the nanoparticles appears partly depend on the association between acyclovir and chitosan or on the increased solubility of low molecular weight chitosan that may aid in the colloidal stability of nanoparticles in solution [22].

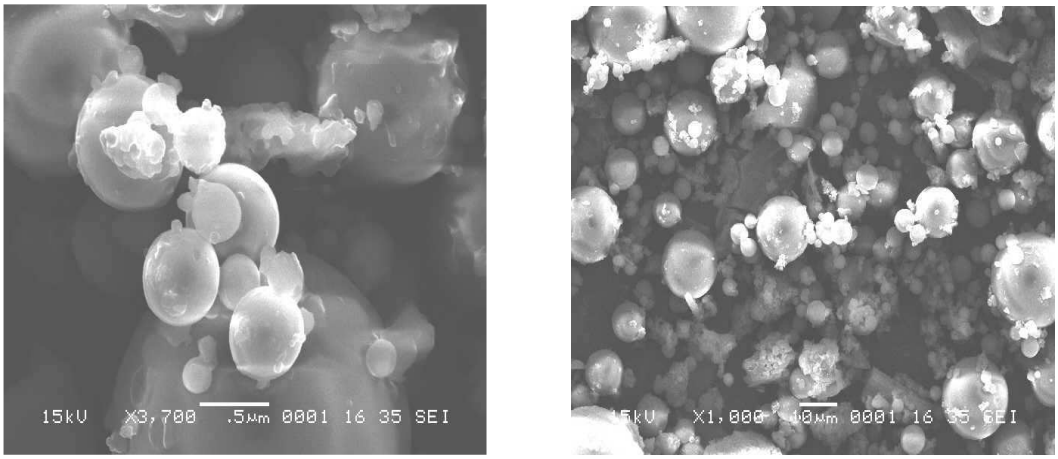

Figure.1 SEM Photograph of acyclovir loaded chitosan nanoparticles (F5)

Differential Scanning Calorimetry (DSC)

The thermograms of acyclovir, chitosan and acyclovir loaded chitosan nanoparticles are shown in Figures. 2A, 2B and 2C. Acyclovir showed characteristic endothermic peaks at 121.06ºC, 150.48ºC and 254.07ºC. Chitosan showed a broad peak at 102.81ºC. The thermogram of acyclovir loaded chitosan nanoparticles exhibited all characteristic peaks of acyclovir, thus indicating that there was no change in the crystallinty of acyclovir.

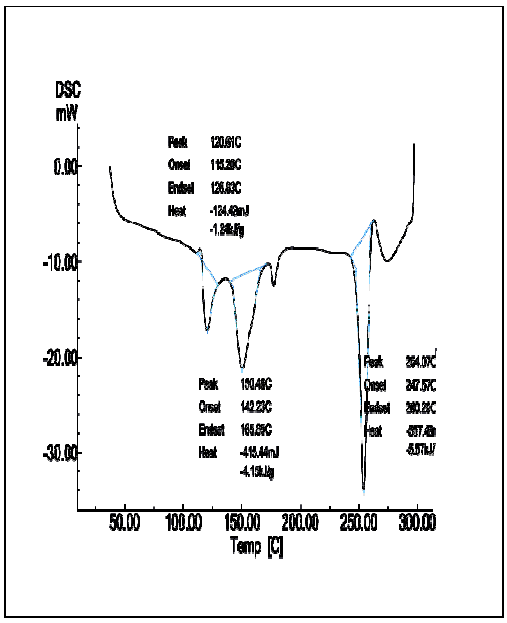

Figure.2A DSC thermogram of Acyclovir

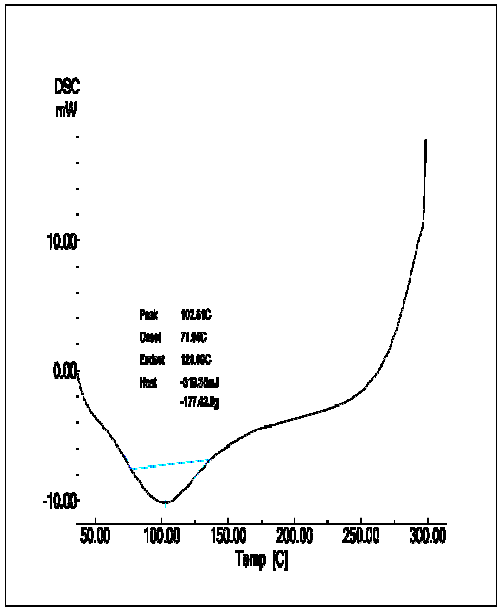

Figure.2B DSC thermogram of chitosan

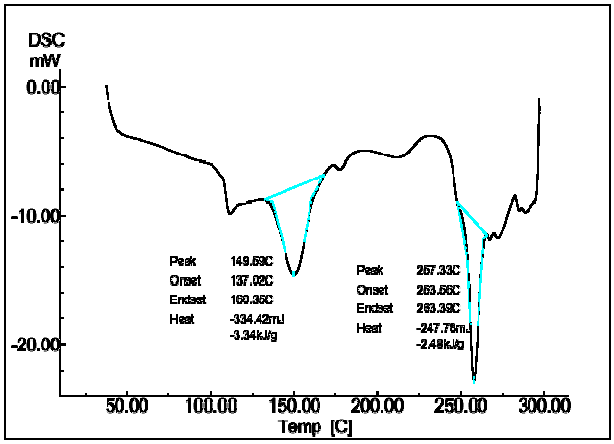

Figure.2C DSC thermogram of Acyclovir loaded chitosan nanoparticles

Drug Release Study

Diffusion studies

The *Invitro* release data for Acyclovir loaded chitosan nanoparticles (F5) are shown in Table No.3. The diffusion study was performed on the formulation (F5) that showed the least particle size (200 ± 30nm). The invitro drug release profiles of formulation (F5) are shown in Figure.3. The release pattern demonstrated a very slow release of drug at each point of time from nanoparticles. 52.46% release in 12hrs and 90.70% release in 24hrs. There was no initial rapid release of the drug.

Table No. 3 *Invitro* release data for Acyclovir loaded chitosan nanoparticles (F5)

Time In hrs	Absorbance (253nm)	Concentration	Amount released in mg	Cumulative Drug release	Mean Cumulative % drug release
1	0.008	0.122	0.006	0.061	3.04 ± 0.02
2	0.017	0.257	0.012	0.134	6.36 ± 0.53
3	0.023	0.348	0.017	0.193	9.24 ± 0.59
4	0.032	0.484	0.024	0.278	13.48 ± 0.64
5	0.04	0.606	0.030	0.363	17.69 ± 0.69
6	0.046	0.696	0.034	0.439	21.44 ± 0.75
7	0.054	0.818	0.040	0.534	26.17± 0.80
8	0.060	0.909	0.045	0.621	30.83 ± 0.32
9	0.069	1.045	0.052	0.734	36.13 ± 0.85
10	0.077	1.166	0.058	0.847	41.74 ± 0.91
11	0.083	1.257	0.0628	0.951	46.89 ± 0.96
12	0.090	1.363	0.0681	1.067	52.46 ± 1.28
14	0.097	1.469	0.073	1.188	58.65 ± 1.10
16	0.104	1.575	0.078	1.315	64.94 ± 1.15
18	0.110	1.666	0.083	1.439	71.11 ± 1.20
20	0.116	1.757	0.087	1.568	77.52 ± 1.26
22	0.122	1.848	0.092	1.701	84.14 ± 1.31
24	0.128	1.939	0.096	1.833	90.70 ± 1.36

- All values are expressed as mean ±S.D, n =3

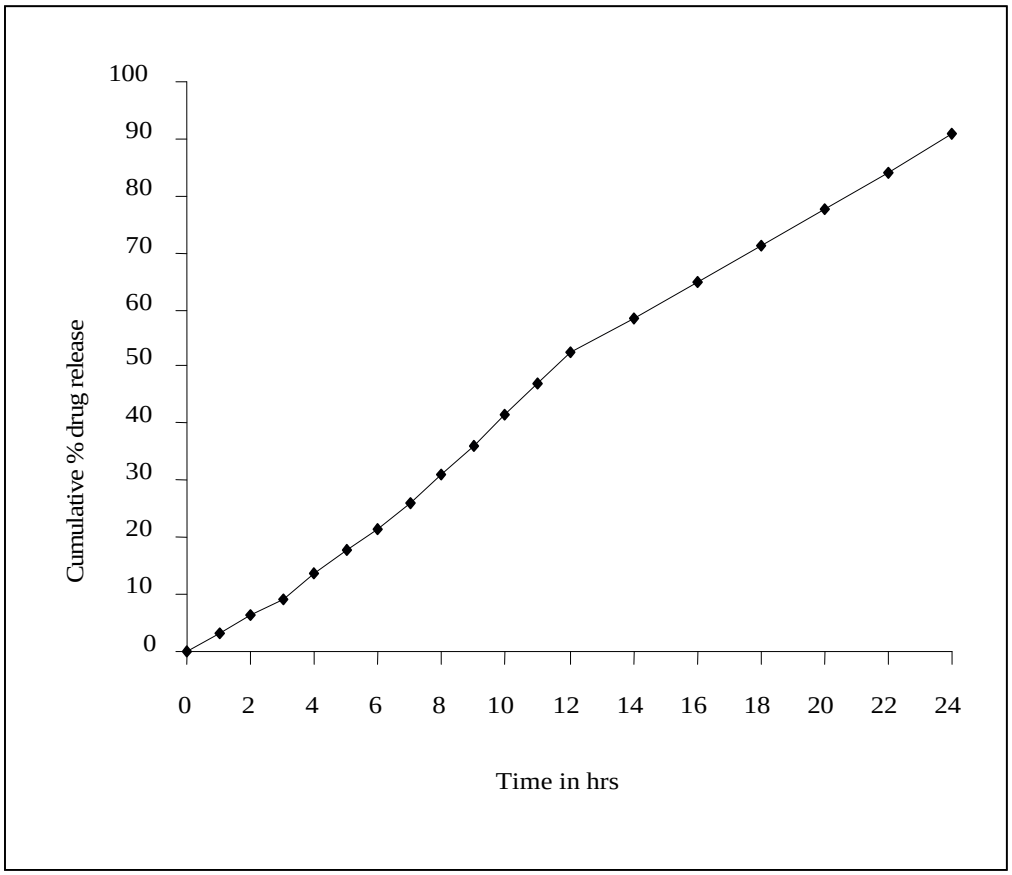

Figure.3 Invitro diffusion of acyclovir from acyclovir loaded chitosan nanoparticles (F5)

Release kinetics

The kinetic release data for Acyclovir loaded chitosan nanoparticles (F5) are shown in Table No 4. 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 No.4A. 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.

Table No. 4 Kinetic release data for Acyclovir loaded chitosan nanoparticles (F5)

Time in hrs	Square root of time	log time	Cumulative % drug release	log cumulative % drug release	Cumulative % drug remaining	Log Cumulative % drug remaining
0	0	0	0	0	100	2
1	1	0	3.04	0.532	96.59	1.984
2	1.414	0.150	6.36	0.806	93.59	1.971
3	1.732	0.238	9.24	0.967	90.71	1.957
4	2.00	0.301	13.48	1.131	86.47	1.936
5	2.236	0.349	17.69	1.248	82.27	1.915
6	2.449	0.389	21.44	1.331	78.52	1.894
7	2.645	0.422	26.17	1.418	73.78	1.867
8	2.828	0.451	30.83	1.494	68.75	1.837
9	3.000	0.477	36.13	1.558	63.78	1.804
10	3.162	0.5	41.74	1.621	58.18	1.764
11	3.316	0.520	46.89	1.671	53.03	1.724
12	3.464	0.539	52.46	1.722	47.27	1.674
14	3.741	0.573	58.65	1.769	41.25	1.615
16	4.000	0.602	64.94	1.813	34.96	1.543
18	4.242	0.627	71.11	1.852	28.78	1.459
20	4.472	0.650	77.52	1.889	22.38	1.3499
22	4.690	0.671	84.14	1.925	15.75	1.197
24	4.898	0.690	90.70	1.957	9.24	0.965

Table No. 4A Parameters of the model equation on the *in vitro* release kinetics

Formula code	Zero order		First order		Higuchi's	Korsmeyer-Peppas	
	K_0	R^2	K_1	r^2	r^2	n	R^2
F_5	3.9608	0.9927	-0.032	0.8988	0.9311	0.9184	0.9962

In the previous study, the particle size, zeta potential and encapsulation efficiency of nanoparticles were 253nm, + 43.9 mV, and 15.6% respectively. However, the present study showed smaller particle size (200nm) and higher encapsulation efficiency (56%) (F5) as compared to earlier findings [17]. The results thus demonstrate that the concentration of acyclovir in the chitosan nanoparticles did influence the physicochemical characteristics and the release profile of the nanoparticles.

The improved interaction of chitosan loaded nanoparticles with the cornea and the conjunctiva could be found in the mucoadhesive properties of chitosan [23] or it is due to the electrostatic interaction between the positively charged chitosan nanoparticles and the negatively charged corneal and conjunctival cells [24] 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 the present study we propose that chitosan nanoparticles may be beneficial in improving the corneal

permeation, contact time and bioavailability of acyclovir for the treatment of ocular viral infections.

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 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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