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Effect of Different Grades of Chitosan on Physicochemical and *In-vitro* Release Characteristics of Acyclovir Nanoparticles for Ocular Delivery

K Karthik, NN Rajendran*, R Natarajan, R Subashini, P Rangapriya

Dept. of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal-637205, Tamilnadu, India.

ABSTRACT

The present study was to investigate the effect of different grades of chitosan on physicochemical and release characteristics of acyclovir nanoparticles for delivery to ocular surface. The acyclovir loaded nanoparticle suspensions were prepared by ionic gelation of chitosan of low (150KDa), medium (400 KDa) and high molecular weight (600KDa) with the tri polyphosphate anions (TPP). The nanoparticles were characterized by fourier transform infra red spectroscopy (FTIR), scanning electron microcopy (SEM), zeta sizer, differential scanning calorimetry (DSC), and in-vitro release. The nanoparticles were spherical solid dense with size ranging between 300-700 nm showing that ionic gelation method has yielded good results. Particle size decreased as the molecular weight of chitosan increased. The zeta potential of the formulations was +40±2.8 mV to +43±1.8 mV indicating formation of stable nanoparticles. The encapsulation efficiency and loading capacity were 58.54 % to 72.83 % and 32.70 % to 39.36 % respectively and increased as the molecular weight of chitosan increased. The nanoparticles showed slow release of the drug during 24 hours and the release rate was further retarded as the molecular weight of chitosan increased. The drug release rate of each formulation was independent of time, showing case II transport corresponding with zero-order kinetics (exponent n >1). Molecular weight of chitosan influences physicochemical and release characteristics of acyclovir nanoparticles and appears to be an important factor for developing acyclovir nanoparticles for ocular delivery with desired release characteristics.

Keywords: Acyclovir, Chitosan, Nanoparticle, Ocular delivery.

Corresponding author
E-mail: rajendran_natham@yahoo.com

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INTRODUCTION

Acyclovir is an antiviral drug that acts on herpes viruses, such as the one that cause herpes simplex keratitis. However it's use in the cornea is limited by low corneal penetration and poor water solubility [1]. Currently it is used as a 3% ointment in the treatment of herpes simplex infection. It is 10 – 30 % bioavailable and has a mean plasma half life of 2.5 hours. Conventional preparations are ill accepted on account of their short pre-corneal retention time, greasiness, vision blurring effects etc.,

One of the largest challenges faced by ocular treatment is sustaining a sufficient amount of the drug where needed. These difficulties, coupled with the importance of ocular drug delivery and the fact that drugs are often administered pre-corneal, illustrate the necessity of developing delivery systems that rapidly interact with the cornea and conjunctiva. Nanoparticles provide an interesting solution of these difficulties. A variety of different nanoparticles has been tested for ocular use, and has been successful [2-4]. Nanoparticles present several advantages over traditional delivery systems, such as extended cellular uptake, reduced clearance, sustaining drug delivery and tissue specific targeting [1, 5]. Nanoparticles have been successfully used for delivery of fluribioprofen [1], carteolol [6], and acyclovir [7] in the anterior chamber and gancilclovir [8] in the posterior chamber of the eye.

Many different materials have been used to produce nanoparticles for ophthalmic use. Some basic examples are polysaccharides, hyaluron, chitosan, albumin and gelatin [3, 4]. Chitosan was selected as the polymer in the study as it not only enhances cornea contact time through its mucoadhesion mediated by electrostatic interaction between its positive charge and negatively charged mucin , its ability to transient opening tight junction is believed to improve drug bioavailability [9]. Different grades of chitosan have been reported for varying degree of influence on physicochemical and release characteristics of nanoparticles [10].

Among the methods for preparation of nanoparticles, the ionic gelation method is simple and easy to prepare nanoparticles containing chitosan as polymer. Chitosan being positively charged can interact with negative charged sodium tri polyphosphate to form coacervates with a size in the range of nanometer [11].

The aim of this study was to develop acyclovir nanoparticles by ionic gelation method using different grades of chitosan.The prepared nanoparticles were characterized in terms of morphology, particle size, zeta potential, entrapment efficiency and loading capacity and release.

MATERIALS AND METHODS

MATERIALS

Acyclovir was supplied by Micro Labs Private Limited, Hosur. Tamilnadu, India. Chitosan (85% deacetylation, molecular weight 150 KDa, 400 KDa, 600 KDa) were obtained from Central

Institute of Fisheries Technology, Cochin, India. The other materials were of pharmaceutical and analytical grade and were used as received.

METHODS

Preparation of Acyclovir Loaded Chitosan Nanoparticles

The formulation of acyclovir loaded chitosan nanoparticles is shown in Table 1. Nanoparticles were prepared according to the procedure described by Calvo et al (1997 b) based on the ionic gelation of chitosan with sodium tri polyphosphate (STPP) anions [10]. In this study, three different grades of chitosan (molecular weight 150 KDa, 400 KDa, 600 KDa) were used. 100 mg of chitosan and 100 mg of acyclovir (1:1) were dissolved in 10 ml of acetic acid in aqueous solution under magnetic stirring at room temperature for 45 min in the presence of Tween 80 (0.5%). 10 ml of sodium tri polyphosphate aqueous solution was added to chitosan-acyclovir solution and the mixture was sonicated for 3 mins. The nanosuspensions were cold centrifuged at 12000 g in a glucose bed for 30 mins using Hitachi centrifuge. The supernatant liquid was analyzed by spectrophotometer (Perkin Elmer Lambda 25) at 253 nm to calculate the percentage drug entrapment and drug loading. The final suspensions were then frozen and lyophilized at 0.4 mbar and -40°c for 5 hrs using glucose and lactose (1:2) as cryoprotective agents. The lyophilized nanoparticles were reconstituted into suspension with pH 7.4 phosphate and stored at 4°c.

Characterization of Nanoparticles

Fourier transforms infrared spectroscopy (FT-IR)

The compatibility between drug and polymer was determined by FTIR spectroscopy using Perkin Elmer RX 1. The pellets were prepared by gently mixing 1 mg sample with 200 mg potassium bromide at high compaction pressure and scanned at 450 to 4000 cm^{-1} with a revolution of 4 cm^{-1}. The spectra of the nanoparticles were compared with that of parent drug, polymer and physical mixture of drug and polymer [11, 12].

Scanning electron microscopy (SEM)

The morphology of nanoparticles was analyzed by SEM (JEOL MODEL JSM 6400). The nanoparticles were mounted directly in 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 sample were detected and the image formed [13].

Particle Size and Surface Characterization

The particle size and zeta potential of nanoparticles were measured with a Malvern Zeta Sizer, (Malvern Instruments, UK) Worcestershire. The samples were placed in the analyzer chamber and readings were performed at 25°C with a detected angel of 90 degree. Each

sample was measured three times and values were presented as mean ± standard deviation (SD). The particle size distribution is reported as a polydispersity index (PDI).The range for PDI is from 0 to 1. Values close to zero indicate a homogenous dispersion and those greater than 0.5 indicate high heterogenecity [14].

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on pure drug, physical mixture of drug and polymer and nanoparticles (F3) by using the thermal analysis instrument (DSC DA 60, Shimadzu, Japan) equipped with liquid nitrogen sub ambient accessory. 2-6 mg samples were accurately weighed in aluminium pans sealed and heated at a rate of 10°C min $^{-1}$ at 30 to 300°C under nitrogen flow of 40 ml / min [14].

Encapsulation efficiency and loading capacity of 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 12000g for 30 minutes .The amount of free acyclovir in the supernatant was measured by spectrophotometry at 253 nm [15].

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

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

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

Invitro release of nanoparticles

Nanoparticulate suspension equivalent to 2 mg of acyclovir was dispersed in 10 ml PH 7.4 phosphate buffer and placed in a dialysis membrane bag (molecular weight cut off 5 KDa) which acts as a donor compartment, tied and placed into 10 ml PH 7.4 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 (1-24 hrs), 1 ml of the release medium was removed and 1 ml fresh pH 7.4 phosphate buffer solution was added into the system. The amount of acyclovir in the release medium was determined spectrophotometrically at 253 nm. The experiment was repeated three times and the values recorded as mean ± standard deviation (SD) [15].

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Kinetics of drug release

In order to understand the mechanism and kinetics of drug release, the drug release data of in-vitro diffusion study were analyzed with various kinetics models and coefficient of correlation (r) values were calculated for the linear curves by regression analysis [15, 16].

Statistical Analysis

The in-vitro release data were statistically analyzed by ANOVA with Tukey-Kramer multiple comparison tests.

RESULTS

FT-IR spectra

FT-IR spectra of acyclovir, chitosan (low, medium and high molecular weight), physical mixture of acyclovir and chitosan, acyclovir unloaded chitosan nanoparticles and acyclovir loaded chitosan nanoparticles are shown in Figures 1A, 1B and 1C. Acyclovir showed absorption peaks at 3440, 1633, 1610,1388,1346,1307 and 1217 cm^{-1}. Peaks at 3440 may be due to –OH stretching and those at 1633 and 1610 cm^{-1}.may be assigned to C=O stretching. Peak observed at 1388 cm^{-1} is due to C-N stretching and peaks in the range of 1346 to 1217 may be due to N-H stretching. The IR spectra of physical mixture, acyclovir unloaded and loaded nanoparticles correspond to the superimposition of the spectra of acyclovir and chitosan with no significant shift in the major peaks corresponding to acyclovir. The spectral data indicates no possible interaction between acyclovir and chitosan.

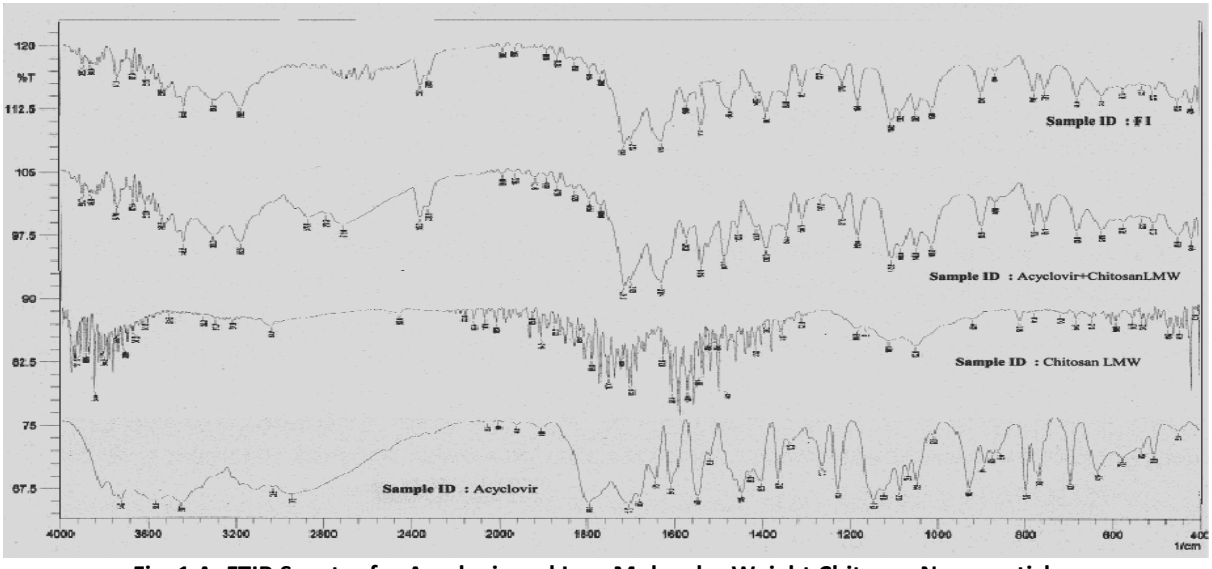

Fig: 1 A. FTIR Spectra for Acyclovir and Low Molecular Weight Chitosan Nanoparticles

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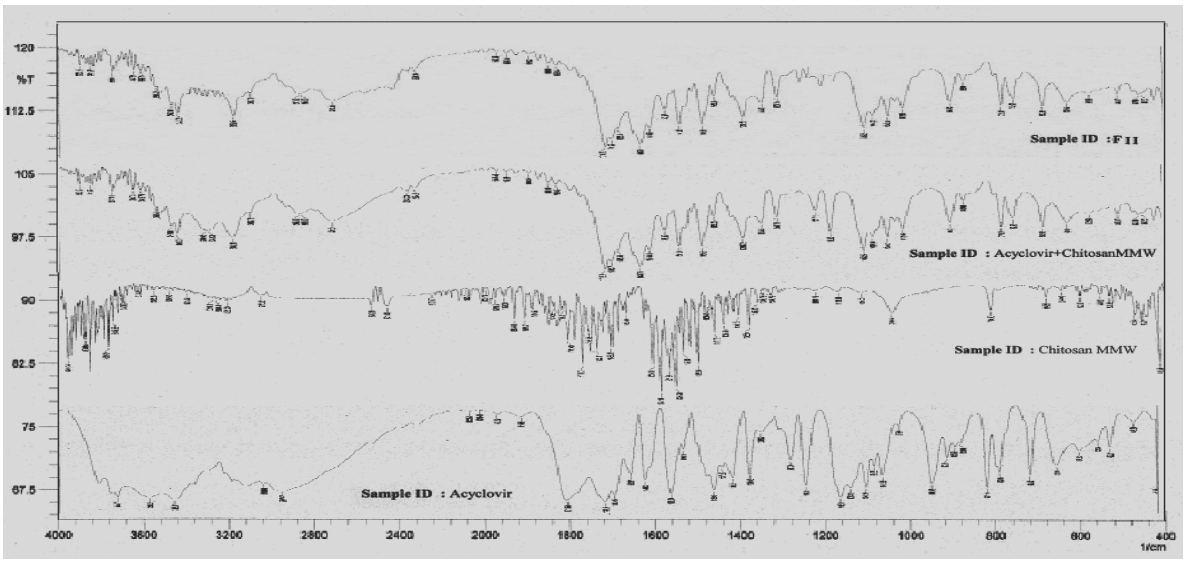

Fig: 1B. FTIR Spectra for Acyclovir and medium Molecular Weight Chitosan Nanoparticles

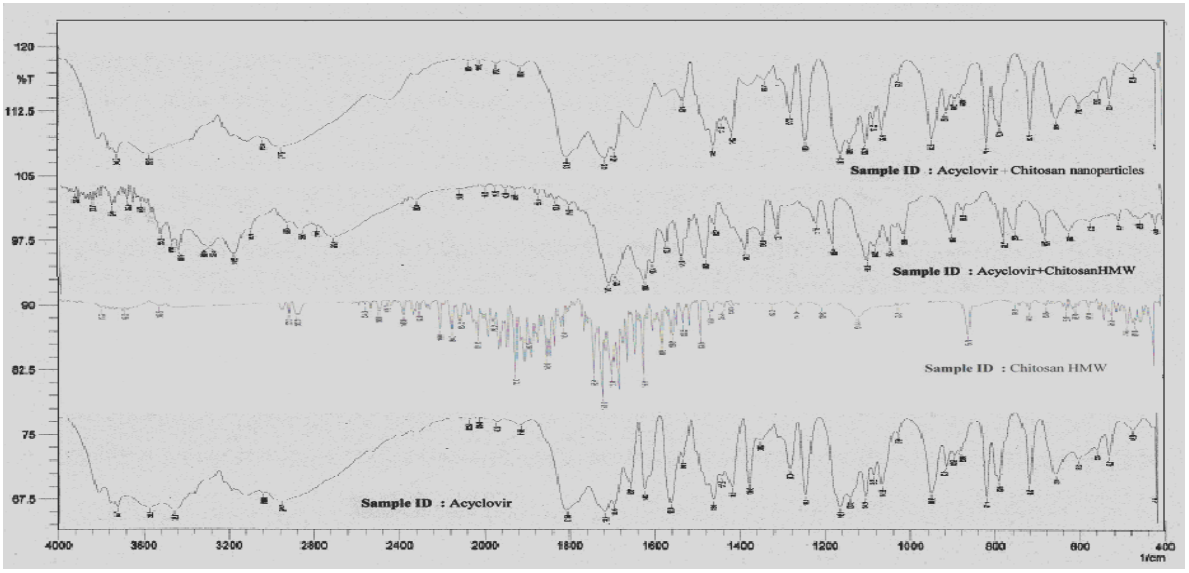

Fig: 1 C. FTIR Spectra for Acyclovir and high Molecular Weight Chitosan Nanoparticles

Scanning electron microscopy

The SEM of all the three formulations F1, F2 and F3 are shown in Figures 2A, 2B and 2C. Particles were solid dense and spherical with no aggregation and suggest that ionic gelation of chitosan with STPP has yielded good results. It was also observed that the particle size decreased as the molecular weight of chitosan increased.

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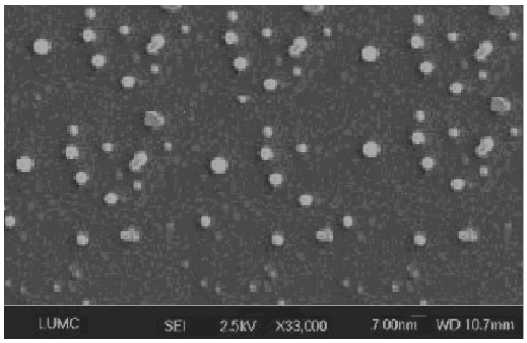

Fig: 2 A: SEM photography of low molecular weight chitosan nanoparticles (F1)

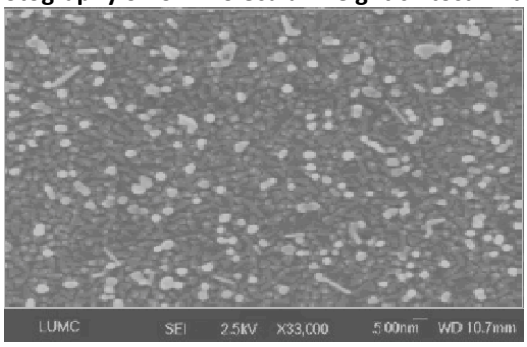

Fig: 2 B: SEM photography of medium molecular weight chitosan nanoparticles (F2)

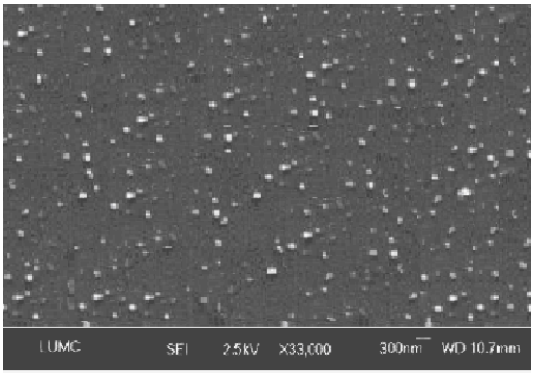

Fig: 2 C: SEM photography of high molecular weight chitosan nanoparticles (F3)

Particle size and surface characteristics

The mean particle size, zeta potential and polydispersity index of the formulations are shown in Table 2. The mean particle size of F1, F2 and F3 were 708, 503 and 298 nm respectively and when the molecular weight of chitosan increases the particle size decreases. The zeta potential of the formulations ranged from +40 ± 2.8 mV to +43 ±1.8 mV and greater than + or - 30 mV resulting formation of stable nanoparticles. The high zeta potential is due to the absorption of the anionic groups by the long amino groups of chitosan to keep the high value of electrical double- layer thickness, which in turn prevents the aggregation [17]. Polydispersity index (PDI) reveals the dispersion homogenecity of the formulations. As shown in Table 2, the PDI of all formulations was smaller than 0.5 indicating a relative homogeneous dispersion.

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Table: 2 Particle Size and Zeta Potential of Acyclovir loaded Chitosan Nanoparticles

Formulation code	Drug polymer ratio (mg)	Mean particle size (nm)	Poly dispersive index	Zeta potential (mV)
F1	1:1	708± 0.25	0.41	+41±1.2 mV
F2	1:1	503±0.12	0.39	+40±2.8mV
F3	1:1	298±0.52	0.32	+43±1.8mV

Differential scanning calorimetry

The DSC thermogram of acyclovir, physical mixture and nanoparticles are illustrated in figures 3A, 3B and 3C. The DSC curve of acyclovir shows endothermic peaks at 120.6^{0}C, 150.5 ^{0}C and 254.1^{0}C. The physical mixture of acyclovir with low molecular chitosan showed similar endothermic peaks of the drug; however the endothermic of the drug at 150.5^{0}C is broadened indicating partial amorphization of the drug in the physical mixture. In physical mixture with medium and high molecular weight chitosan, there was disappearance of endothermic peaks of acyclovir at 120.6^{0}C and 150.5^{0}C, but appearance of the endothermic peak at 248.8^{0}C and 254.9^{0}C for medium and high molecular weight chitosan respectively. These results show that degree of amorphization of the drug is more with medium and high molecular weight chitosan compared with low molecular weight chitosan. The DSC curve of nanoparticles F1, F2 and F3 showed similar thermogram pattern as observed with corresponding physical mixtures .These findings clearly demonstrate partial transformation of crystallinity of the drug to amorphous state in the nanoparticles that resulted in improved solubility of the drug in the polymer matrix of the nanoparticles.

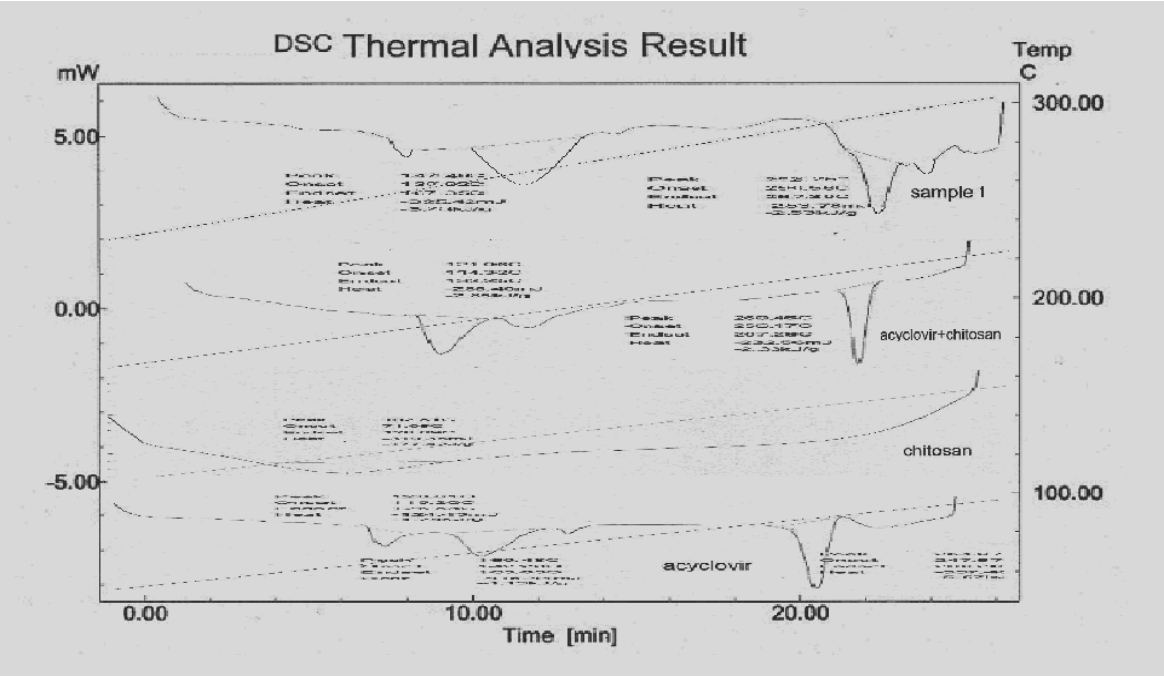

Fig: 3A. DSC of acyclovir and low molecular weight chitosan nanoparticles

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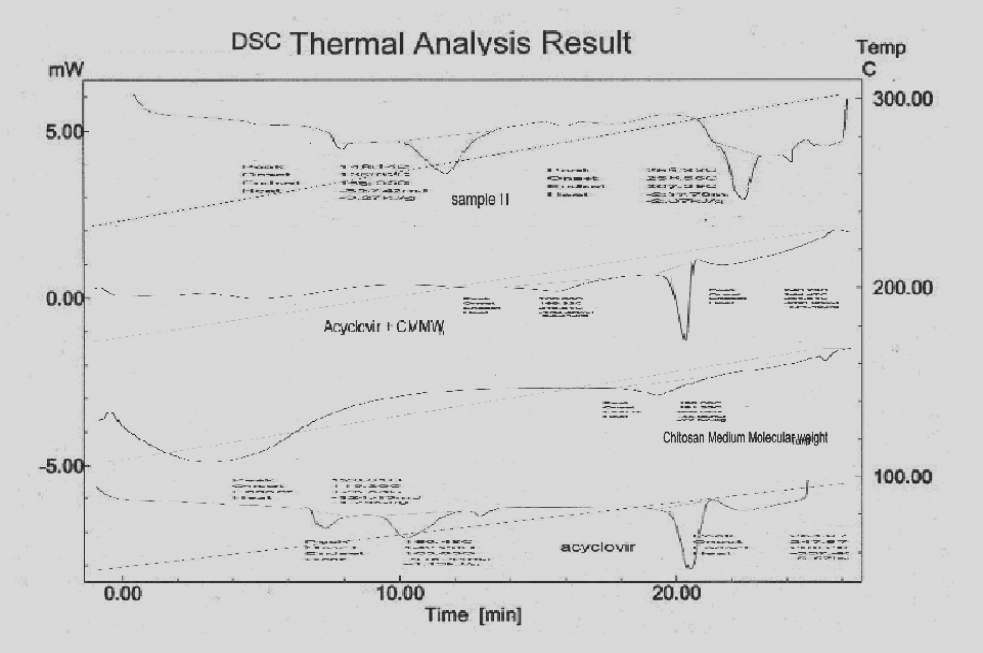

Fig: 3B. DSC for acyclovir and medium molecular weight chitosan nanoparticles

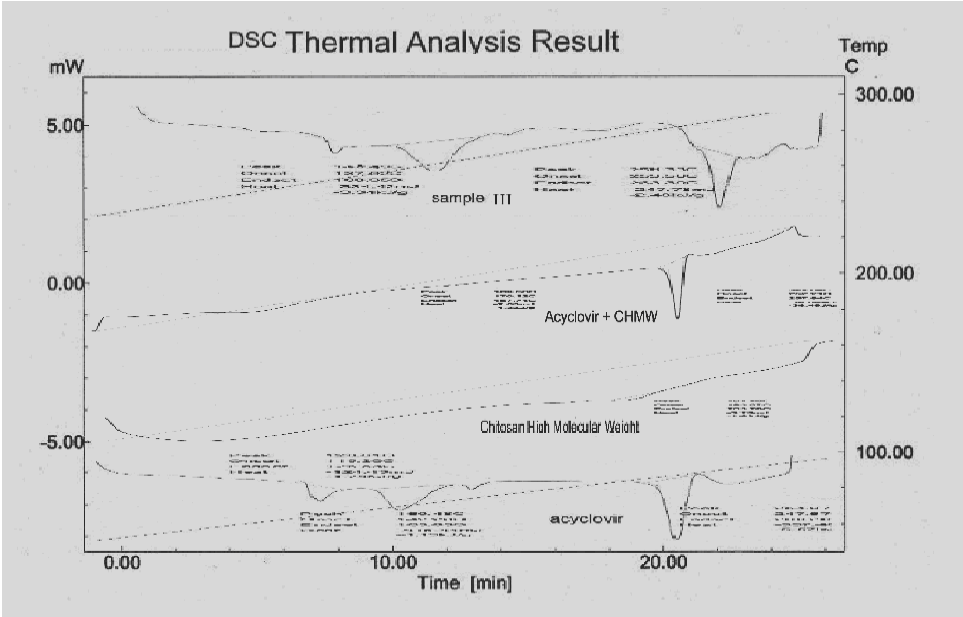

Fig: 3C. DSC for acyclovir and high molecular weight chitosan nanoparticles

Encapsulation efficiency and loading capacity

The encapsulation efficiency and loading capacity of nanoparticles are shown in Table 3.The encapsulation efficiency of F1, F2 and F3 were 58.5%, 65.6% and 72.8% respectively .and the loading capacity were 32.75, 36.6% and 39.5% respectively. As the molecular weight of polymer increased, both encapsulation efficiency and loading capacity increased possibly due to association between the drug and polymer that increased as the chain length of chitosan increased.

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Table: 3 Encapsulation Efficiency and Loading Capacity of Acyclovir loaded Chitosan Nanoparticles

Formulation code	Drug : polymer ratio (mg)	Encapsulation efficiency (%)	Loading capacity (%)
F1	1:1	58.54	32.70
F2	1:1	65.62	36.05
F3	1:1	72.83	39.36

In-vitro release

The in-vitro release data of F1, F2 and F3 are given in Table 4.The cumulative percent drug release of F, F2 and F3 in 24 hrs was 90.2%, 84.2% and 79.95 respectively. The values show the slow release pattern of acyclovir. The differences in drug release rate between formulations were statistically analyzed and found to be significant ($p < 0.001$). It seems that the interaction between chitosan and STPP could obtain a strong matrix polymer network that caused acyclovir entrapping in this matrix release and finally decreased acyclovir release in phosphate buffer solution at pH 7.4. It was also observed that as the molecular weight of chitosan increased the release rate of acyclovir further slowed. This finding suggests that the matrix polymer network caused by the interaction between chitosan and STPP might be stronger with high molecular weight chitosan as compared with medium or low molecular weight chitosan. In other words, the strength of the matrix polymer network appears to be dependent on the molecular weight of polymer.

Table: 4 Comparative percent release profile of Formulations in pH 7.4 medium

Time (hrs)	F1	F2	F3
0	0	0	0
1	3.656	2.55	2.07
2	5.116	4.76	4.1
3	8.89	8.02	7.78
4	13.586	14.22	11.68
5	17.476	18.07	14.77
6	21.623	20.71	18.71
7	26.273	25.53	22.42
8	31.966	30.18	25.72
9	38.26	34.59	29.61
10	43.6	39.99	34.65
11	48.7	45.28	40.05
12	53.536	49.74	44.33
14	59.376	55.45	49.48
16	64.743	61.23	55.13
18	70.61	65.79	61.51
20	76.03	71.21	66.84
22	82.96	78.04	72.81
24	90.21	84.18	79.86

Fickian and non-Fickian (anomalous) behaviors have been used for determining the mechanism of drug release from polymeric systems. However the drug release data in phosphate buffer solution at pH 7.4 were analyzed using the following general equation

$$M_t / M_\infty = kt^n$$

Where M_t is the amount of acyclovir released in a given time , M_∞ is the total amount of acyclovir within the nanoparticles, k and n are equation constants; k is a constant incorporating structural and geometric characteristics of the drug dosage form, and n is the release exponent indicating the drug- release mechanism [18]. Different values of n for cylindrical, spherical and slab geometrics are available in the literature. For spheres, values of 0.43.0.43 < n < 0.85 and 0.85 related to Fickian diffusion (case II transport), anomalous, case II transport respectively [19]. When the exponent n takes a value of 0.85, the drug release rate is independent of time, which corresponds with zero order kinetics.

Table: 5 Release Kinetics of Formulations

Formulation code	First order "R^2"value	Zero order "R^2"value	Higuchi plot "R^2"value	Peppas plot "R^2"value	n value	Best fit model
F1	0.9754	0.9865	0.9759	0.9813	1.0778	Zero order Case II transport
F2	0.9821	0.9890	0.9802	0.9886	1.0828	Zero order Case II transport
F3	0.9880	0.9946	0.9730	0.9925	1.0942	Zero order Case II transport

As shown in Table 5, the values of the exponent "n" for the formulations F1, F2 and F3 were > 0.85. This suggests that acyclovir release rate from nanoparticles is independent of time and follows zero- order kinetics, with case II transport mechanism.

DISCUSSION

Acyclovir is used in the treatment of various ocular viral diseases particularly herpes simplex infections. It is currently used as a 3% ointment and has poor bioavailability (10-30 %) with membrane plasma half life of 2.5 hrs following ocular application. Due to short pre corneal retention time, greasiness, vision –blurring effects and poor patient compliance with the ointment preparation, a drug delivery system that can over come the above problems is highly desirable to control the ocular viral diseases effectively.

Nanoparticles have been found to be potential carriers for ocular delivery following the observations that various types of nanoparticles tend to adhere to the ocular epithelial surface [9]. Chitosan was selected for the study owing to acceptable bio availability, biodegradability and ability to enhance the paracellular transport of drugs. Chitosan being cationic will adhere to

cornea and conjunctiva which have a negative charge and thus prolong the contact time [20, 21].

Chitosan is able to form nanoparticles using among other methods, ionotropic gelation. The method is based on the gelation of chitosan with positive charge when it comes in contact with specific poly anions due to the formation of intra molecular and inter molecular cross – linking mediated by these poly anions [10]. Ionic gelation of chitosan with STPP was used to prepare acyclovir – loaded nanoparticles.

In this study, the effect of chitosan of different molecular weights on nanoparticles characteristics was investigated. Results have shown small solid dense and spherical particles with no aggregation and the size of nanoparticles was dependent up on the molecular weight of chitosan. The zeta potential was in the higher range (> ±30 mV) indicating formation of more stable nanoparticles. The encapsulation efficiency and loading capacity of the nanoparticles increased as the molecular weight of chitosan increased suggesting a strong association between chitosan and acyclovir that is dependent on the molecular weight of the polymer. It has been reported that longer chain of high molecular weight chitosan can entrap greater amount of acyclovir when gelated with STPP [22]. It was observed that release of acyclovir from nanoparticles was better controlled with high molecular weight chitosan and the drug release followed case II transport mechanism that correspond to zero order kinetics. DSC curve has shown presence of acyclovir in amorphous state which results in increased solubility of drug in the polymer matrix and so the diffusion of the drug through polymer matrix appears better controlled by high molecular weight chitosan.

CONCLUSION

The present study demonstrated that molecular weight of chitosan can influence the particle size, entrapment efficiency, loading capacity and release characteristics of acyclovir loaded nanoparticles and thus appears critical to yield acyclovir nanoparticles of desired release characteristics.

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