

## CHITOSAN LOADED MICROSPHERES AS AN OCULAR DELIVERY SYSTEM FOR ACYCLOVIR

S.SELVARAJ\*, J. KARTHIKEYAN., N. SARAVANAKUMAR.

Department of Pharmaceutics, Cherraan's College of Pharmacy, Coimbatore, Tamilnadu, India. 641039. Email: selvasangari@gmail.com

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## ABSTRACT

The aim of the present investigation was to evaluate the potential use of chitosan microspheres for ocular delivery of acyclovir. 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 chitosan microspheres containing acyclovir as potential ophthalmic drug delivery system. The acyclovir loaded chitosan microspheres were prepared by ionic gelation of chitosan with tripolyphosphate anions. The microspheres were characterised by particle size, Zeta potential, surface morphology, Differential Scanning Calorimetry and Fourier Transform Infra Red Spectroscopy. All the prepared formulations resulted in micron size particles (2 to 12  $\mu\text{m}$ ) and displayed spherical smooth morphology with Zeta potential (+36.1 to +43.6 mV). The encapsulation efficiency and loading capacity were 52% - 78% and 12% - 26% respectively. The acyclovir loaded chitosan microspheres displayed crystallinity than acyclovir. The *in vitro* diffusion profile of acyclovir from the microspheres showed a sustained release of the drug over a period of 24 hrs. Kinetic release profiles of acyclovir from microspheres appeared to fit best with Higuchi model with first order and the non-Fickian diffusion was superior phenomenon. Thus the results suggest that acyclovir loaded chitosan microparticle suspension appears promising for effective management of ocular viral infections.

**Keywords:** Acyclovir, Chitosan, Microspheres, Ocular delivery, Ionic gelation method.

## INTRODUCTION

Eye is most interesting organ due to its drug disposition characteristics. For ailments of the eye, topical administration is usually preferred over systemic administration, before reaching the anatomical barrier of the cornea, any drug molecule administered by the ocular route has to cross the precorneal barriers. These are the first barriers that slow the penetration of an active ingredient into the eye and consist of the tear film and the conjunctiva. Actual corneal permeability of the drug is quite low and very small corneal contact time of the about 1-2 mins in humans for instilled solution commonly less than 10%<sup>1,2,3</sup>. Consequently only small amount actually penetrates the cornea and reaches intraocular tissue. Controlled drug delivery to the eye is restricted due to these limitations imposed by the efficient protective mechanism<sup>4,5</sup>.

The conventional ocular drug delivery systems like solutions, suspensions and ointments are no longer sufficient to fulfill the present day requirements of providing a constant rate delivery and for a prolonged time. The major deficiencies of this conventional dosage form include poor ocular drug bioavailability, pulse drug-entry, systemic exposure to the nasolacrimal duct drainage and poor entrance to the posterior segments of the eye due to the lens-iris diaphragm<sup>6</sup>.

Many attempts have been made to improve the ocular bioavailability and the therapeutic effectiveness of drug, e.g., chemical modification of the drug and its incorporation into colloidal systems such as liposomes or nanoparticles<sup>7,8</sup>. Previous studies showed that both poly (alkylcyanoacrylate)<sup>9,10</sup> and poly- $\epsilon$ -caprolactone<sup>11,12,13</sup> colloidal systems are able to improve the intraocular penetration of drugs. However, the duration of these systems at the ocular surface is limited to a few hours. In contrast, mucoadhesive polymers can increase the residence time of drugs on the ocular surface<sup>14</sup>. One of these, the cationic polymer chitosan, possesses some favorable biological characteristics such as biodegradability, nontoxicity and biocompatibility<sup>15,16,17</sup> that make it a promising candidate for ocular drug delivery<sup>18</sup>.

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<sup>19, 20, 21, 22</sup>. Acyclovir is currently marketed as capsules (200 mg), tablets (200, 400 and 800 mg) and suspensions for oral administration, intravenous injection and topical ointment<sup>23</sup>. Acyclovir is available as 3% w/w eye ointment to be applied 5 times a day in the eye<sup>24</sup>. The topical application of acyclovir is limited by the low corneal penetration of the drug, 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. The microparticulate drug-carrier seems a promising means of topical administration of acyclovir to the eye<sup>25</sup>. So development of acyclovir loaded chitosan microspheres was undertaken for effective ocular delivery of the drug with improved bioavailability.

Chitosan obtained by deacetylation of chitin (a naturally occurring polymer) has been shown to possess mucoadhesive properties owing to the molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces<sup>26</sup>. Chitosan has 1 primary amino and 2 free hydroxyl groups for each C building unit. Due to the easy availability of free amino groups in chitosan, it carries a positive charge and thus, in turn, reacts with many negatively charged surfaces/polymers<sup>27</sup>.

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<sup>28</sup>, biodegradability and ability to enhance the paracellular transport of drugs<sup>29</sup>. 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.

However, literature research indicates that the role of acyclovir concentration on microspheres 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 microspheres.

## MATERIALS AND METHODS

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,  $4.08 \times 10^5$  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.

### Preparation of Acyclovir loaded chitosan microspheres

The chitosan microspheres were prepared by the method described by Bodmeier and Paeratakul<sup>30</sup>. Shiraishi et al and Shu and Zhu have also reported the interaction of chitosan with tripolyphosphate<sup>31,32</sup>. Chitosan microspheres 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. 250mg chitosan and acyclovir at various concentrations (25,50,75,100 and 125mg)

were dissolved in acetic acid solution under magnetic stirring at room temperature for 45 mins. The microspheres were formed by dropping the bubble-free dispersion of drug chitosan solution through a disposable syringe onto a gently agitated 0.25% w/v sodium tri polyphosphate solution. The batches were coded as F1, F2, F3, F4 and F5 respectively. The chitosan microspheres were separated after 2 hours by filtration and rinsed with distilled water; then they were freezing dried (Labconico, USA). Table1. shows the composition of acyclovir loaded chitosan microspheres.

**Table 1: Composition of acyclovir loaded chitosan microspheres formulation**

Formulation Code	Drug(mg)	Polymer(mg)	Drug Polymer Ratio(mg)
F1	25	250	1:10
F2	50	250	2:10
F3	75	250	3:10
F4	100	250	4:10
F5	125	250	5:10

### Evaluation of Acyclovir Loaded Chitosan Microspheres

#### Fourier Transform Infra Red Spectroscopy

Drug polymer interactions were studied by FTIR spectroscopy. The FTIR spectra of acyclovir, chitosan and acyclovir loaded chitosan microspheres were determined by using Perkin Elmer RX1 model. The pellets were prepared by gently mixing of 2mg sample with 200mg potassium bromide with a hydrostatic press at a force of 40psi for 4 min. The scanning range was 450 to 4400 cm<sup>-1</sup> and the resolution was 4 cm<sup>-1</sup>.

#### Differential Scanning Calorimetry

The thermal behavior of pure acyclovir, chitosan and acyclovir loaded chitosan microspheres were carried out by using a thermal analysis instrument (DSC DA 60 Shimadzu, Japan) 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 60 to 300°C under nitrogen flow rate of 35cc / min.

#### Particle size and Zeta potential of microspheres

The particle size and zeta potential of microspheres were measured by Photon Correlated Spectroscopy and laser doppler anemometry using a Zetasizer, 3000 HS (Malvern Instruments, UK) using dynamic light scattering principles. The samples were diluted with pH 7.4 phosphate buffer and placed in eletrophoretic cell and measured in the automatic mode.

#### Morphological study of microspheres

The scanning electron microscopy (Jeol Model JSM 6400, Tokyo) was used to characterize the surface morphology of microspheres. The microspheres were mounted directly on the scanning electron microscopy 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 of the microspheres

An accurately weighed quantity of the microparticles was extracted with pH 7.4 phosphate buffer for 24h, centrifuged at 6000 rpm for 30 min and filtered (Hitachi Centrifuge USA). The dispersion was filtered and the absorbance of the filtrate was measured at 253 nm after appropriate dilution in a UV-visible spectrophotometer (Perkin Elmer Lambda 25). The drug content was estimated in triplicate using a calibration curve constructed in the same solvent. Polymers did not interfere with the assay at this wavelength. The amount of acyclovir encapsulated in to the microspheres is determined by using the formula.

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

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

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

#### In vitro release of acyclovir from the microspheres

Drug release was determined with the help of modified USP XXI dissolution rate model A 250 ml beaker was placed in the vessel. A plastic tube of diameter 17.5 mm opened from both the ends was tied at one end with treated cellophane membrane and dipped into the beaker containing dissolution media<sup>33</sup>. Paddle type stirrer was attached in the center of the beaker and the speed was maintained at 100 rpm. The beaker was filled with 90 ml phosphate buffer (pH 7.4) and temperature was maintained at 37±1°C. Acyclovir microspheres were suspended in 10ml of phosphate buffer. At appropriate time intervals (1, 2, 3, 4...12 hrs), 1 ml of the release medium was removed and 1 ml fresh 7.4 phosphate buffer solution was added in to the system. The amount of acyclovir in the release medium was evaluated 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). In order to define a model which will represent a better fit for the formulation, drug release data were further analyzed by Peppas equation,  $M_t/M_\infty = k t^n$ , where  $M_t$  is the amount of drug released at time  $t$  and  $M_\infty$  is the amount released at  $\infty$ ,  $M_t/M_\infty$  is the fraction of drug released at time  $t$ ,  $k$  is the kinetic constant and  $n$  is the diffusion 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<sup>34</sup>.

### RESULTS

#### FT-IR Spectroscopy

There are three characterization peaks of chitosan at 2883.87 cm<sup>-1</sup> of  $\nu$  (OH), 1095.41cm<sup>-1</sup> of  $\nu$ (C O C) and 1654.47 cm<sup>-1</sup> of  $\nu$  (NH<sub>2</sub>). The spectrum of chitosan-sodium tri polyphosphate is different from that of chitosan matrix. In chitosan- sodium tri polyphosphate the characteristic peak of chitosan at 3424.62 cm<sup>-1</sup> becomes wider, indicating that hydrogen bonding is enhanced. In chitosan-TPP, the 1654.47 cm<sup>-1</sup> peak of -NH<sub>2</sub> bending vibration shifts to 1085.82 cm<sup>-1</sup> and a new sharp peak at 2108.55 cm<sup>-1</sup> appears. These findings propose that there is linkage between phosphoric and ammonium ion. Compared with the spectrum of acyclovir, the spectrum of acyclovir loaded chitosan microspheres showed that the

absorption peak of  $3184.15\text{cm}^{-1}$  (amino group absorption peak) disappeared and a new sharp peak of  $2873.04\text{cm}^{-1}$  (linkage between hydroxyl group of acyclovir and amino group of chitosan) appeared. The results indicate electrostatic interactions between hydroxyl ethoxy methyl group of acyclovir and amino groups of chitosan.

#### Differential Scanning Calorimetry

The thermograms of acyclovir, chitosan and acyclovir loaded chitosan microspheres are shown in Fig.1A, 1B and 1C. Acyclovir showed characteristic endothermic peaks at  $121.06^\circ\text{C}$ ,  $150.48^\circ\text{C}$  and

$254.07^\circ\text{C}$ . Chitosan showed a broad peak at  $102.81^\circ\text{C}$ . The physical mixture of acyclovir and chitosan showed characteristic peaks at  $121.06^\circ\text{C}$ ,  $150.48^\circ\text{C}$  and  $254.07^\circ\text{C}$ . The thermogram of acyclovir loaded chitosan microspheres exhibited all characteristic peaks of acyclovir, thus indicating that there was no change in the crystallinity of acyclovir. However the broad peak of chitosan has disappeared in the thermogram of acyclovir microspheres. The absence of characteristic peak of chitosan at  $102.81^\circ\text{C}$  in acyclovir loaded chitosan microspheres may be due to an interaction between acyclovir and chitosan.

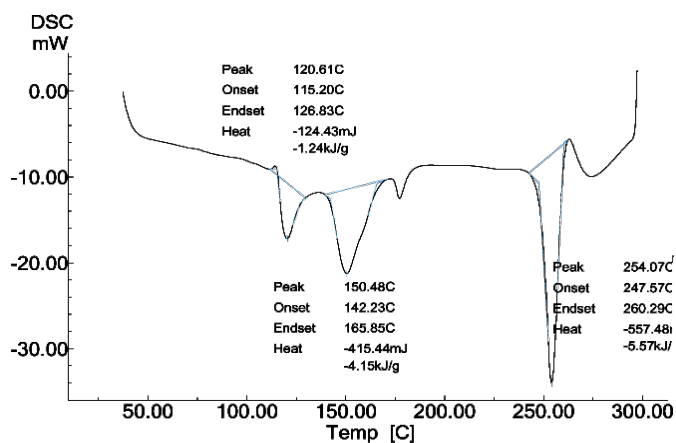


Fig. 1A: DSC thermogram of Acyclovir

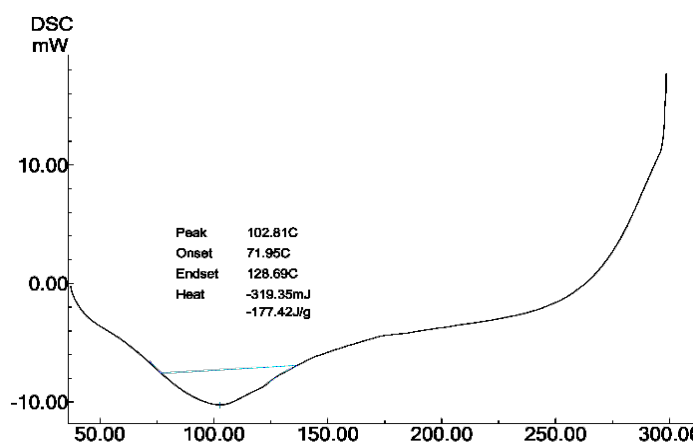


Fig. 1B: DSC thermogram of chitosan

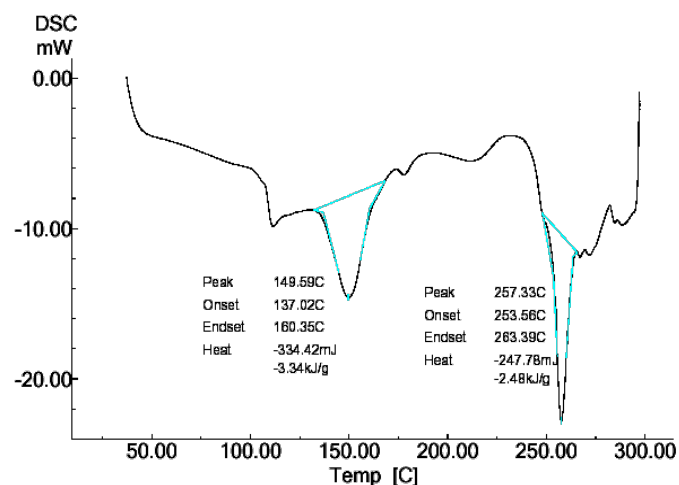


Fig. 1C: DSC thermogram of Acyclovir loaded chitosan microspheres

### Particle Size and Zeta potential of Acyclovir Loaded Chitosan microspheres

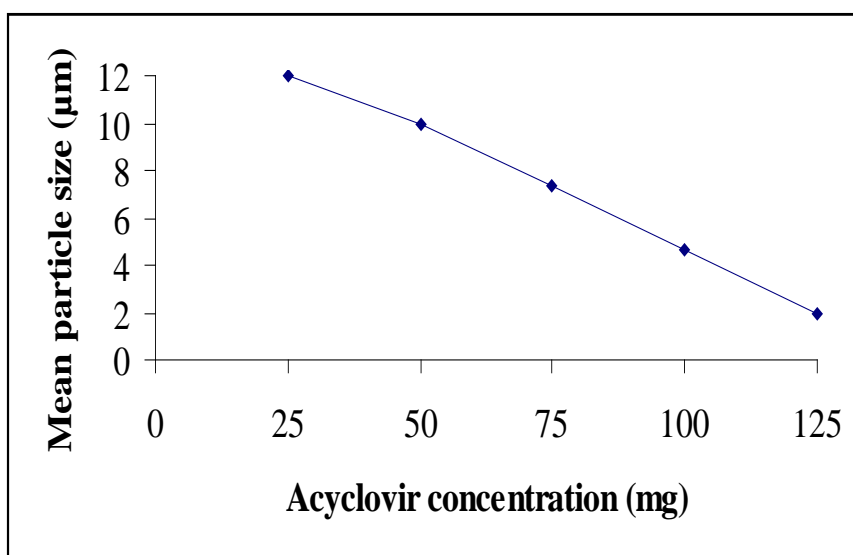
The particle size of acyclovir loaded chitosan microspheres (F1- F5) are shown in Table 2. The maximum size of microspheres was observed in F1 (12 $\mu$ m) as compared to other formulations and the least size was seen in F5 (2 $\mu$ m). The size of the microspheres varied with the acyclovir concentration (Fig.2A). The Zeta potential values of acyclovir loaded chitosan microspheres (F1-F5) are shown in Table No.2. The Zeta potential values ranged between +36.1 to +43.6

mV and the values decreased as the concentration of acyclovir increased (Fig.2B). Zeta potential above +30 mV indicating that the formulations are stable.

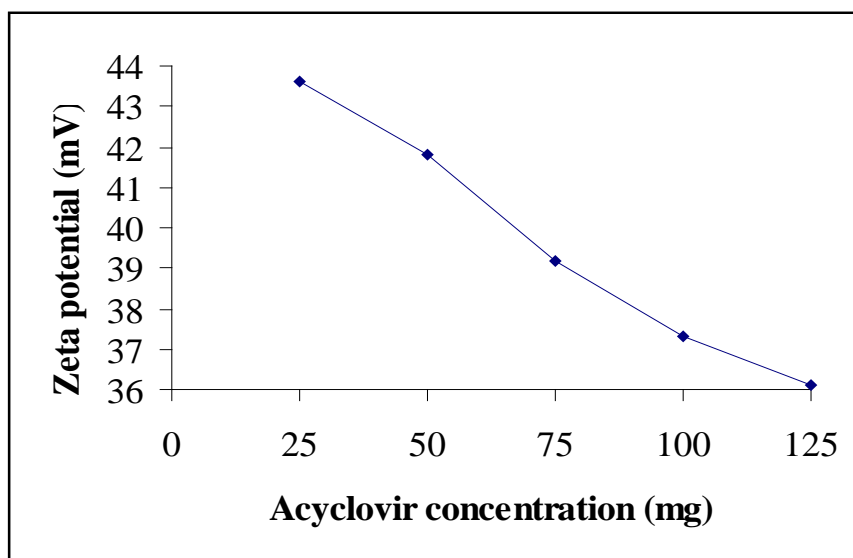
The zeta potential of microspheres is commonly used to characterize the surface charge property of microspheres. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Microspheres with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles.

**Table 2: Mean Particle size, Zeta potential, Encapsulation efficiency and Loading capacity of Acyclovir loaded chitosan microspheres**

Formula code	Mean particle size ( $\mu$ m)	Zeta potential (mV)	Encapsulation efficiency (%)	Loading capacity(%)
F1	12 $\pm$ 0.5	+ 43.6 $\pm$ 1.3	78.00	12.00
F2	10 $\pm$ 2.8	+42.8 $\pm$ 1.2	74.00	17.81
F3	7.4 $\pm$ 1.2	+39.2 $\pm$ 1.4	67.69	21.83
F4	4.7 $\pm$ 2.5	+37.3 $\pm$ 1.1	57.00	24.07
F5	2.0 $\pm$ 3.0	+36.1 $\pm$ 1.5	52.00	26.00



**Fig. 2A: Mean Particle Size of Acyclovir loaded chitosan microspheres**



**Fig. 2B: Zeta potential of acyclovir loaded chitosan microspheres**

### Surface morphology of microspheres

The morphological characters of acyclovir loaded chitosan microspheres (F5) are shown in Fig.3. Acyclovir loaded chitosan

microspheres have shown spherical shape. The SEM of the acyclovir loaded chitosan microspheres showed that the microspheres have a solid dense structure with smooth spherical shape.

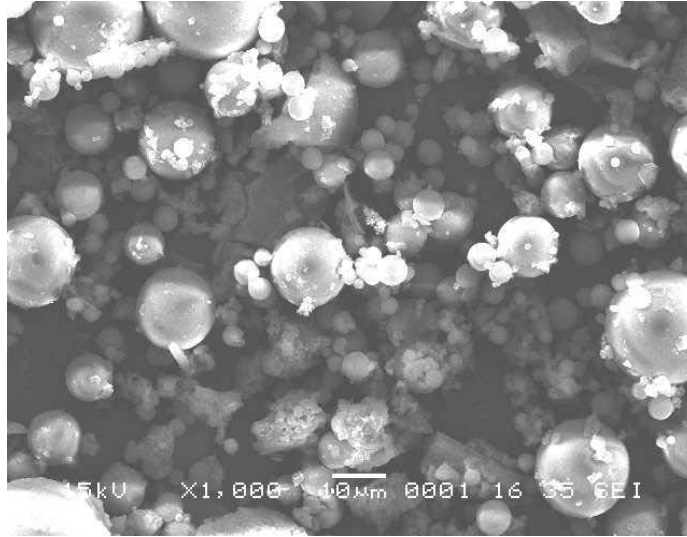


Fig. 3A: SEM Photograph of acyclovir loaded chitosan microspheres (F5)

### Encapsulation Efficiency and Loading Capacity of the microspheres

Table 2 shows the results of encapsulation efficiency and loading capacity of the acyclovir loaded microspheres. The encapsulation efficiency was maximum with the lower drug concentration and

minimum with the higher drug concentration (Fig.4A). The encapsulation efficiency ranged between 52 to 78%.

Conversely the loading capacity of microspheres increased as the concentration of the drug increased (Fig.4B). The loading capacity ranged between 12 to 26 %.

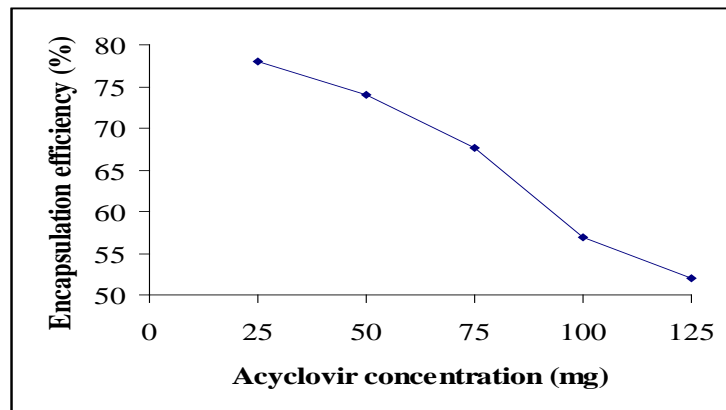


Fig. 4A: Encapsulation efficiency of Acyclovir loaded chitosan microspheres

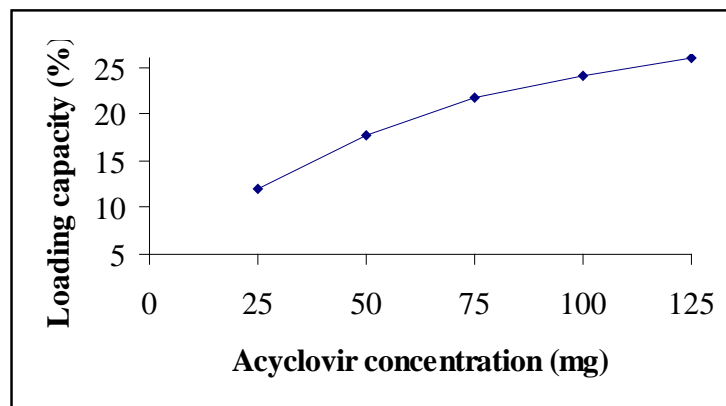


Fig. 4B: loading capacity of Acyclovir loaded chitosan microspheres

### **In vitro release of acyclovir from the microspheres**

In vitro release behavior of microspheres exhibiting least particle size (F5) and marketed formulation of acyclovir was investigated in phosphate buffer (pH7.4) for 24hrs (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 microspheres containing 2mg of acyclovir for once daily. At the end of 24hrs the drug release was 89.23% for F3 and 27.2% for marketed product. The release pattern demonstrated a very slow release of drug at each point of time from microspheres. There was an initial phase of rapid release of acyclovir followed by a more gradual release over a period of 24hrs.

### **DISCUSSION**

The results of the present investigation demonstrated the potential use of chitosan microspheres 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<sup>35</sup>. Among them chitosan based systems are acknowledged more suitable for ocular pathway, based on the favorable biological characteristics of chitosan<sup>36,37</sup>. Moreover chitosan microspheres 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 microspheres, 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.

The maximum size of microspheres was observed in F1 (12 $\mu$ m) as compared to other formulations and the least size was seen in F5 (2 $\mu$ m). The size of the microspheres varied with the acyclovir concentration (Table 2 and Fig.2A). The particle size of microspheres increased with increase in chitosan which might be due to the fact that increase in the concentration of polymer increases the cross-linking, and hence the matrix density of the microspheres resulting in increased particle size of the microspheres<sup>38</sup>. Increase in size might be because of the increase in viscosity of the droplets present in the internal phase caused by the increase in drug concentration as explained by Denkbas et al<sup>39</sup>.

The presence of a nonionic surfactant is very important for the so-called "long-term" stability of the colloidal suspension, which is determined by the adsorption of hydrophilic macromolecules on the microsphere surface, thus increasing the steric repulsion between particles<sup>40</sup>. The presence of hydrophilic macromolecules on the surface of microsphere leads to a change of the surface properties (zeta potential) of the colloidal carrier. In particular, the zeta potential of colloidal microspheres is significantly reduced by coating with nonionic surfactants. Considering these factors the non-ionic surfactant Tween 80 (0.5%) was used to stabilize the formulation<sup>41</sup>.

The zeta potential is commonly used to characterize the surface charge property of microspheres. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. The zeta potential of acyclovir loaded chitosan microspheres ranged from +36.1 to +43.6 mV. Microspheres 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 microspheres or adsorbed onto the surface. Morphology and surface characteristics of the microspheres were determined using scanning electron microscopy. Tri poly phosphate is a non-toxic and multivalent anion that can form cross-links by ionic interaction between positively charged amino groups of chitosan and multivalent negatively charged tri poly phosphate molecules<sup>32, 42</sup>. The tri poly phosphate was selected because of its non toxic and stability improving nature. The SEM of the acyclovir

loaded chitosan microspheres showed that the microspheres have a solid dense structure with smooth spherical shape (Fig.3).

As shown in Table. 2 the encapsulation efficiency and loading capacity of the acyclovir loaded chitosan microspheres were affected by initial acyclovir concentration in the chitosan solution and the amount of acyclovir incorporated. The encapsulation efficiency of the microspheres ranged from 52 to 78%. The loading capacity of the microspheres ranged from 12 to 26 % and the loading capacity of microspheres increased as the concentration of drug increased (Fig.4B). The increase of acyclovir concentration leads to a decrease of encapsulation efficiency (Fig.4A) 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 gelled with tri poly phosphate as observed in the previous study<sup>43</sup>. 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.

In vitro release behavior of microspheres exhibiting least particle size (F5) and marketed formulation of acyclovir was investigated in phosphate buffer (pH7.4) for 24 hrs. From the release studies, it was found that the drug release was about 52.68% and 25.23% for F3 and marketed product respectively after 12hrs. At the end of 24hrs the drug release was 89.23% for F3 and 27.2% for marketed product (Fig.5). The acyclovir release profile from chitosan microspheres was characterized by a rapid initial burst of 20.33% was observed in the first 1hour followed by a sustained release of the drug over a period of 24 hrs. The release involves two different mechanisms of drug molecules diffusion and polymer matrix degradation<sup>44</sup>. The burst release of drug is associated with those drug molecules dispersing close to the microsphere surface, which easily diffuse in the initial incubation time. The initial rapid release can be due to the burst effect resulting from the release of the drug encapsulated near the microspheres surface and thereafter the slow release of acyclovir from the chitosan microspheres is possibly the consequence of the release of the drug fraction encapsulated in the core of the microspheres and also due to strong association between the drug and polymer through electrostatic interaction between the hydroxyl ethoxy groups of acyclovir and the amino groups of chitosan as shown by FTIR spectra. Therefore, the rapid dissolution process suggests that the release medium penetrates into the particles due to the hydrophilic nature of chitosan, and dissolves the entrapped acyclovir. In addition, the microspheres with huge specific surface area can adsorb acyclovir, so the first burst release is also possibly due to the part of acyclovir desorbed from microparticle surface. Besides the crystallinity of acyclovir has not been affected as evident from DSC curve and this characteristic may also play a role in sustained release of drug from the microparticles.

In order to investigate the release mechanism of present drug delivery system, the release data of prepared acyclovir loaded chitosan microspheres in phosphate buffer (pH 7.4) were fitted 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 were applied to the release data<sup>45,46</sup>. The coefficient of determination ( $R^2$ ) of equation for release of acyclovir from acyclovir loaded chitosan microspheres (F5) in phosphate buffer was 0.9888 signifying first order release pattern. The value of coefficient of determination ( $R^2$ ) in Higuchi equation was found to be 0.9311 which indicates the integrity of chitosan gel and diffusion-controlled release. Substituting the release values in Ritger-Peppas equation, the value of coefficient of determination was about 0.9862. The value of  $n$  obtained was found to be 0.5184 indicating non-Fickian release as  $n = 0.43$  indicates Fickian (case I) release;  $> 0.43$  but  $< 0.85$  for non-Fickian (anomalous) release; and  $> 0.89$  indicates super case II type of release. Non-Fickian refers to a combination of both diffusion and erosion controlled drug release<sup>47</sup>. This result was attributable to the sustained release of drug signifying mixed type of release pattern. These results are consistent with those obtained by Govender et al. who studied the release pattern of tetracycline, potent antibiotic, from microspheres prepared using chitosan for maximum bioadhesivity and controlled drug release<sup>48</sup>. Dhawan and

Singla also reported the similar non Fickian release for nifedipine loaded chitosan microspheres prepared by emulsification solvent evaporation method<sup>49</sup>.

The improved interaction of chitosan loaded microspheres with the cornea and the conjunctiva could be found in the mucoadhesive properties of chitosan or it is due to the electrostatic interaction between the positively charged chitosan microspheres and the negatively charged corneal and conjunctival cells that is the major force responsible for the prolonged residence of the drug<sup>50,51</sup>.

## CONCLUSION

In attempt to prepare chitosan microspheres of acyclovir using ionic gelation method, the mean particle size, morphological characteristics and surface property of the microparticles appear to depend on concentration of acyclovir loaded in chitosan microspheres. Chitosan microspheres had shown an excellent capacity for the association of acyclovir. The release profile of acyclovir from microspheres has shown a sustained release following first order kinetic with non-Fickian diffusion mechanism. The results demonstrated the effective use of acyclovir loaded chitosan microspheres as a controlled release preparation for treatment of ocular viral infections. Further parameters for dosage form designing can be identified for optimum formulation in terms of desirable long-term stability and to study the therapeutic effects of these particles *in vivo*.

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