ISSN: 0975-5772
Yadav.S et al / Journal of Pharmaceutical Science and Technology Vol. 3 (1), 2011,441-447

Formulation and In Vitro and In Vivo Characterization of Acyclovir Loaded Mucoadhesive Microspheres

Yadav. S., Jain S.,Prajapati S., Motwani M., Kumar S.
Institute of Pharmacy, Bundelkhand University, Jhansi (U.P.), 284001 INDIA

Abstract
The purpose of this study was to develop sustained release mucoadhesive microspheres of Acyclovir. Sodiumcarboxymethylcellulose and hydrxypropylmethylcellulose were used as mucoadhesive polymers. The microspheres were prepared using solvent evaporation technique. The effect of variable concentration of polymers on the characteristics of the microspheres was studied. The use of higher amounts of polymer significantly increased the median size of the microspheres. The efficiency of encapsulation increased when the concentration of polymers was increased. The poor bioavailability of acyclovir is attributed to short retention of its dosage form at the absorption sites. The results of mucoadhesion study showed better retention of Sodium CMC microspheres (8.0±0.8 h) in duodenal and jejunum regions of intestine. Pharmacokinetic study revealed that administration of mucoadhesive microspheres could maintain measurable plasma concentration of acyclovir through 24 h, as compared to 5 h after its administration in solution form. Formulation MS4 showed superiority over the other formulations. Nearly three times higher AUC_{0-24} value of acyclovir for these microspheres (382.02±23 ng/ml*h) as compared to drug solution (156.92±15 ng/ml*h) was observed. Overall, the result indicated prolonged delivery with significant improvement in oral bioavailability of acyclovir from mucoadhesive microspheres due to enhanced retention in the upper GI tract.

Keywords: Acyclovir; in vivo evaluation; mucoadhesive microsphere; Sodium carboxymethylcellulose, hydroxypropylmethylcellulose.

1. INTRODUCTION

Acyclovir, the first agent to be licensed for the treatment of herpes simplex virus infections, is most widely used drug for infections such as cutaneous herpes, genital herpes, chicken pox, varicella zoster infections and herpes keratitis. Acyclovir is currently marketed as capsules (200 mg), tablets (200, 400 and 800 mg) and suspension for oral administration, intravenous injection and topical ointment. Oral acyclovir is mostly used as 200 mg tablets, five times a day (Wagstaff et al., 1994). In addition, long term administration of acyclovir (6 month or longer) is required in immunocompetent patient with relapsing herpes simplex infection (Ruhnese et al., 1985). The presently available conventional therapy is associated with a number of drawbacks such as highly variable absorption and low bioavailability (10–20%) after oral administration (O'Brien et al., 1989). Furthermore, with increase in dose, there was decrease in bioavailability. Moreover, because the mean plasma half life of the drug is 2.5 h, five times a day administration is required. In order to make oral therapy of acyclovir more patient compliant there is need to develop controlled drug delivery dosage form. Researchers have investigated formulating acyclovir in delivery systems using different approaches like matrix tablets (Fuertes et al., 2006), microspheres (JalonDe et al., 2003) and polymeric films (Rossi et al., 2003). The main problem with the therapeutic effectiveness of acyclovir is its absorption that is highly variable and dose dependent thus reducing the bioavailability to 10–20%. Acyclovir is soluble in acidic pH and is predominantly absorbed from upper gastro intestinal tract (GIT) to duodenum to jejunum regions (Meadows et. al., 1990). There are indications of its active absorption from the duodenum and jejunum regions of GIT (Park et al., 1992). In commercially available dosages forms, the amount of drug absorbed is very low (10–20%) due to short residence time of the dosage forms at the absorption site. As a result, most of the drug is excreted in the faeces (50–60%) in unabsorbed form. Hence, it can be envisaged that increasing the residence time at the absorption site can enhance the absorption and bioavailability of acyclovir. The present investigation, therefore aimed at formulating mucoadhesive microspheres of acyclovir. Sodiumcarboxymethylcellulose and

hydroxypropylmethylcellulose were selected as mucoadhesive polymers in present study (Chowdary et al., 2000).

2. MATERIALS AND METHODS

Acyclovir sodium was a gift sample from Arochem industries, Thane, Mumbai India. Sodium carboxymethylcellulose and hydroxypropylmethylcellulose were obtained as gift sample from Colorcon Ltd., Mumbai India. Liquid paraffin were procured from E. Merck Ltd., Mumbai, India. Span 80 and n-hexane procured from central drug house Delhi, India.

2.1. Experimental design

Four samples were prepared where the Sodium CMC amount was varied at 400mg, 600mg, 800mg and 1000mg. An another four samples were prepared where the HPMC amount was varied at 400mg, 600mg, 800mg and 1000mg.

2.2. Method of Preparation of mucoadhesive microspheres

Controlled release mucoadhesive microspheres of acyclovir were prepared using Sodium CMC, HPMC and the solvent evaporation technique (Morishita et al., 1993). Eight preparations, labeled MS1–MS8, were prepared by dissolving a specific amount drug in distilled water and then it was mixed with aqueous polymer solution. A vortex homogenizer was used for make a homogenous mixer of drug and polymer. This solution was added drop wise to light liquid paraffin containing 0.5% span 80 as an emulsifying agent. The beaker and its content were heated at 40°c with constant stirring 1000 rpm for 1.5 hours using using a three blade propeller stirrer to form a w/o emulsion. After complete evaporation of aqueous phase the liquid paraffin was decanted and collected microspheres were washed three times with n-hexane to remove liquid paraffin. The microspheres were dried and stored in vacuum desiccators.

2.3 In vitro characterization of Microspheres

2.3.1 Morphological Examination

The morphology of microspheres was examined by Scanning Electron Microscopy. The outer surface was observed using Scanning Electron Microscope (LEO-430, UK). The microspheres were mounted on metal stubs using double-sided tape and coated with a 150 Å layer of gold under vacuum. Stubs were visualized under scanning electron microscope

2.3.2. Production yield

The percentage of production yield was calculated from the weight of dried Microspheres (W1) and the sum of initial dry weight of starting materials (W2) as the following equation*:* % Production Yield = *W1/W2 X 100*

Type of Polymer	Ratio (Drug:Polymer)	Formulation Code	Formulation Composition	
			Drug(mg)	Polymer(mg)
Sodium C.M.C.	1:1	MS1	400	400
	1:1.5	MS2	400	600
	1:2	MS3	400	800
	1:2.5	MS4	400	1000
H.P.M.C.	1:1	MS5	400	400
	1:1.5	MS6	400	600
	1:2	MS7	400	800
	1:2.5	MS8	400	1000

Table 1: Formulation Design

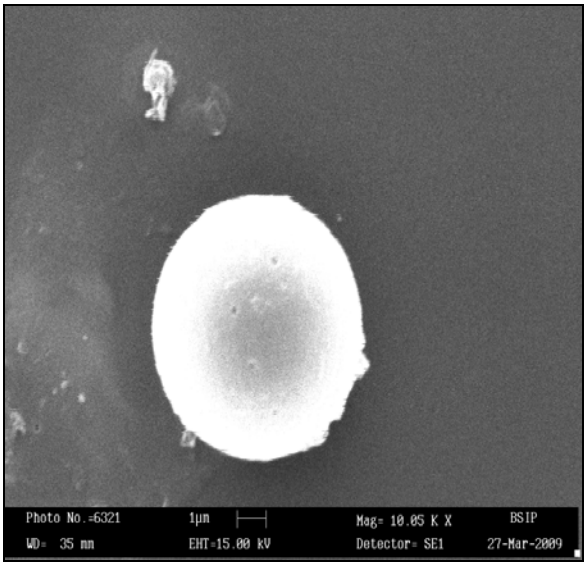

Fig.1: SEM Photograph of microspheres formulation MS3

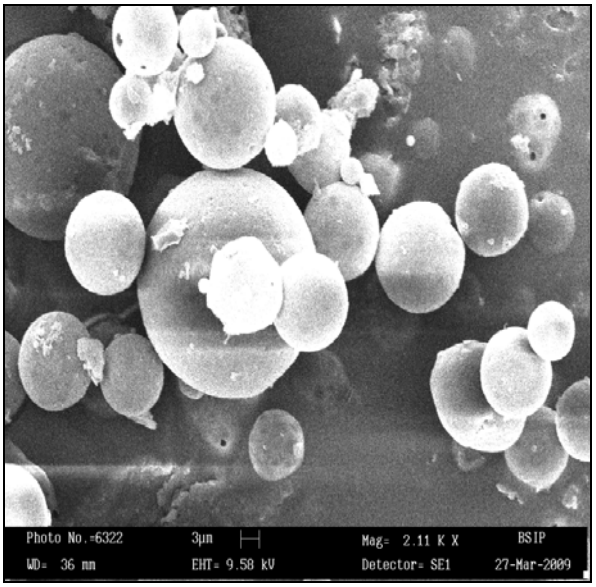

Fig. 2: SEM Photograph of microspheres formulation MS8

2.3.3. Particle Size Measurement

The particle size of the microspheres was measured using a stage micrometer scale. Dry microspheres (5 mg) were suspended in distilled water and ultrasonicated for 5 s. A drop of suspension was placed on a clean glass slide and microspheres were counted under stage ocular micrometer. A minimum of 200 microspheres was counted per batch.

2.3.4. Swelling study

The swelling of microspheres was conducted in phosphate buffer pH 6.8. The sizes of dried microsphere and those after incubation in phosphate buffer (pH 6.8) for 1.0, 3.0 and 5.0 h were measured by using microscopic method. The percentage of swelling at different time interval was determined by the difference between diameter of microspheres at time t (D_t) and initial time (t=0 [D_0]) as calculated from the following equation **:** Swelling % = D_t- D_0 / D_0 x 100

2.3.5. Bulk density and flow property

Accurate weight (W) of microspheres was transferred into a 100 ml graduated cylinder to obtain the apparent volume (V). The bulk density was calculated in gram per ml by the following formula: Bulk Density = Weight / Volume

The flow property of microspheres was evaluated using Carr's Index. The results were averaged from three determinations.

Carr's Index=Tapped density-Bulk density /Tapped density × 100

2.3.6. Drug entrapment efficiency

Acyclovir loaded microspheres (10 mg) were dispersed in 20 ml water and this mixture were vortexed for 5 minutes. This mixture was centrifuged for 10 minutes. The supernatant was collected and filtered. sThe filtrate was analyzed for the drug content spectrophotometrically after suitable dilutions using a digital UV-Vis spectrophotometer (Shimadzu). The determinations were done in triplicate. The Drug Entrapment Efficiency (DEE) was determined as: Drug Entrapment Efficiency = A1-A2/A1 × 100

A1 = Amount of drug added initially (Theoretical Drug Content)

A2 = Amount of drug determined in supernatant spectrophotometrically

A1-A2 = Amount of drug Entrapped in the formulation (Actual drug content)

2.3.7. In-vitro Wash off test for mucoadhesion

The mucoadhesion property of microsphere formulations was determined according to the method described by Vyas et al.,(1993). A

Formulation Code	Production yield (%)	Particle size (µm)	Entrapment efficiency (%)	Adhesion time (hour)
MS1	69.89 (±2.81)	29.59 µm	73.63% (±1.11)	6.0 (±1.60)
MS2	74.68 (±3.15)	30.67µm	77.27% (±2.64)	6.8 (±2.40)
MS3	72.83 (±3.41)	33.98 µm	83.33% (±2.49)	7.8 (±2.37)
MS4	80.66 (±3.81)	36.59 µm	85.75% (±2.47)	8.4 (±2.90)
MS5	66.45 (±3.63)	20.80 µm	59.69% (±3.66)	4.2 (±2.60)
MS6	69.54 (±2.25)	21.70 µm	65.75% (±2.63)	5.3 (±2.89)
MS7	70.45 (±2.45)	25.89 µm	68.78% (±2.45)	5.9 (±1.78)
MS9	73.89 (±2.78)	27.68 µm	74.84% (±2.96)	6.4 (±2.45)

Table 2: In Vitro Characterizations of Mucoadhesive Microspheres

piece of freshly cut pig intestine was obtained from a local abattoir within one hour of killing of animal, and was cleaned by washing with isotonic saline solution. Pieces of intestinal mucosa (3cm×2cm) were mounted onto glass slides using cyanoacrylate glue. An accurate weight of microspheres was spread onto each wet rinsed tissue specimen and immediately thereafter the support was hung onto the arm of USP Disintegration Apparatus. By operating the Disintegration Test machine, the tissue specimen was given a regular up and down movement in PBS pH 6.8 at 37°C taken in a 1 liter vessel of the machine. The time required for detaching all the microspheres from mucosal surface of intestine was recorded by visual inspection.

2.3.8. In Vitro Drug Release Study

In vitro release of acyclovir from microspheres was determined by carrying out dissolution test using USP paddle method at a stirring rate of 50±5 rpm at temperature 37±0.5°C. Nine hundred milliliters of HCl buffer (pH 1.2) was used as dissolution medium for first hour and phosphate buffered saline (PBS, pH 6.8) was used for next 11 h. The dried microspheres were filled in hard gelatin capsules and were placed in dissolution vessels. A 5 ml sample was withdrawn at various time intervals and the volume of the media was replenished with an equal amount of dissolution media. The samples were then analyzed spectro photometrically.

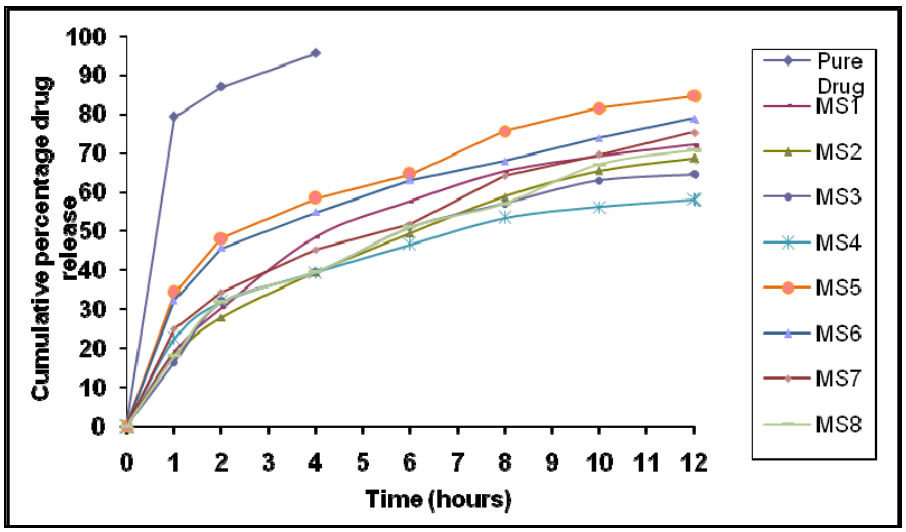

Fig. 3: Percentage in vitro drug release of acyclovir from microspheres formulations. Data are presented as mean ± SD (n=3)

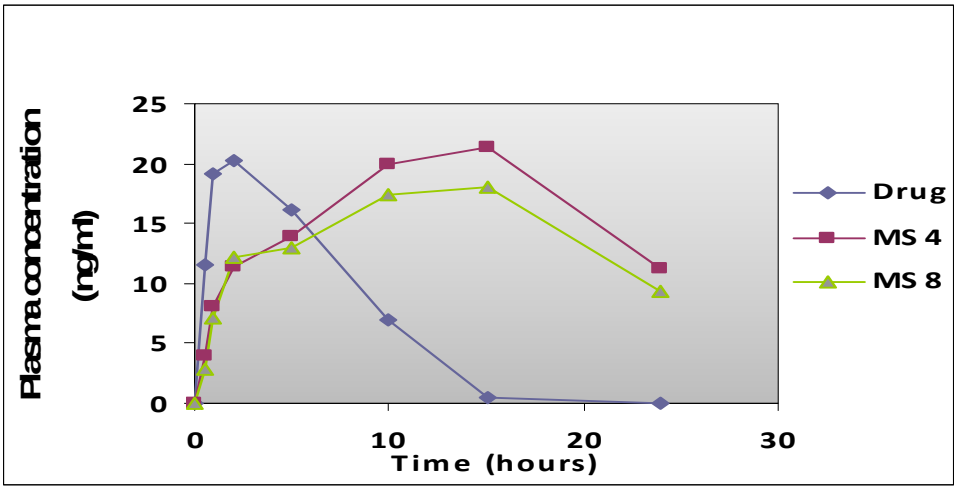

Fig. 4: Blood Plasma concentration of Acyclovir after administration of drug solution and microsphere formulations MS4 and MS8.

2.4. In vivo study

In vivo evaluation studies were performed using healthy rabbits. Rabbits, weighing 2.50-2.75 kg were divided in to three groups, each consisting of three animals. Rabbits were kept on fasting 12 h before drug administration and until 24 h post dosing. Water ad libitum was given throughout the study. The dose selected of acyclovir was 40 mg/kg. The first group received oral administration of 5.5 mg/ml drug solution in PBS (pH 7.4). The remaining two groups received oral administration of formulation MS4 and MS8 respectively. A 400 mg sample of microsphere corresponding to 110 mg of acyclovir were suspended in 15.0 ml saline and administered orally using a rubber tube under non-anesthetic condition.

Blood samples (1 ml) were collected through the marginal ear vein by insulin plastic syringe at 0, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0and 24.0 hours. Blood samples were collected in eppendrof tubes and centrifuged at 1800rpm for 15 minutes. Supernatant was collected and acetonitrile was added to precipitate the proteins. The precipitated proteins were settled by centrifugation at 1800rpm for 15 min. The supernatant was collected and drug concentration was determined by HPLC assay.

Statistical Analysis

Statistical Analysis Data are expressed as the mean ± standard deviation (SD). Statistical analysis was carried out employing ANOVA followed by studentized range test using the sigma stat2.03. A value of $p<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Preparation and In Vitro Characterization

Table 1 shows the composition of different microsphere formulations. The mucoadhesive microspheres appeared as fine powder. Fig. 1 and Fig. 2 depict the photomicrographs of microspheres prepared using Sodium CMC and HPMC polymers. All microsphere formulations were spherical in shape and possessed smooth surface as visualized under SEM. The production yield of microspheres formulations was found in the range of 66.45% to 80.66%. The production yield was increased with increasing the concentration of the polymer; and the reason behind this result is because of more amount of polymer which was added in the same volume of continuous phase. The drug entrapment efficiency of the microspheres was in the range of 59.69 -85.75% being highest for MS4 and lowest for MS5. Drug entrapment efficiency increased with increasing polymer concentration because higher viscosity of sodium carboxymethylcellulose solution reduces the diffusion of the drug in the surroundings which not allow entrapped particle to escape easily but for HPMC

microspheres has low entrapment efficiency due to its low viscosity which causes rapid diffusion of drug in the surroundings and results into decreased entrapment.

3.2. Mucoadhesive Measurement

Table 2 summarizes the results of mucoadhesive measurement of different microspheres formulation in chicken intestine. The adhesion time of microspheres followed the increasing order of Sodium CMC > HPMC microspheres because HPMC is a nonionic polymer which has only hydrogen with mucosal surface while sodium CMC forms both electrostatic and hydrogen bond due to its ionic nature. The molecular weight and viscosity also affects the mucoadhesive strength. Therefore, Sodium CMC with higher molecular weight and higher viscosity shows higher mucoadhesion than HPMC.

3.3. Swelling Study

It is reported that adhesive properties and cohesiveness of mucoadhesive polymers are generally affected by their swelling behavior. The percentage swelling of different microspheres formulations at different time intervals are shown in Fig. 5 The results revealed that all microsphere formulations swelled rapidly when immersed in phosphate buffer (pH 6.8). Mucoadhesive microspheres show the swelling behavior due to the presence of certain hydrophilic groups such as carboxyl and hydroxyl which take up water from the underlying mucosal tissue by capillary action and thus maximize the number of adhesion sites.

The highest swelling observed in microspheres of Sodium CMC was due to its high ionization at pH 6.8, which was capable of absorbing a high amount of water.

3.4. In Vitro Drug Release

Fig. 3 shows the release of acyclovir from various mucoadhesive microspheres. Drug release from the microspheres was slow and dependent on the composition of the coat. HPMC microspheres gave relatively fast release as compared to Sodium CMC. The order of microspheres showing increasing release rate was MS4 <MS3 <MS2 <MS1 <MS8 <MS7 <MS6 <MS5. A significant difference in release pattern was observed between the formulation MS1, MS2, MS3 and MS4. MS1, MS2, MS3 and MS4 contain 400 mg, 600 mg, 800 mg and 1000 mg of Sodium CMC-1500cps and released 72.02%, 68.56%, 64.52% and 58.03% respectively after 12 hours of dissolution period in phosphate buffer solution (PBS). Whereas Formulation MS5, MS6, MS7 and MS8 contain 400 mg, 600 mg, 800 mg and 1000 mg of HPMC-50cps and released 84.56%, 78.77%, 75.22% and 70.65% respectively at the end of the dissolution period in PBS. Thus it is clearly evident that drug release decreases with the increase of percentage polymer loading of the drug formulation. It may be reasoned that the higher viscosity associated with the polymer Sodium CMC was responsible for the larger particle size which causes drug release to be slowed down due to increased particle size. The drug release from the microspheres was diffusion controlled, as plots (Fig. 6) of amount released versus the square root of time were found to be linear ($r > 0.90$).

3.5. In vivo study

The average serum concentration time curves in rabbits after a single oral dose of acyclovir as free solution and microsphere dispersion were shown in the Fig. 4. The pharmacokinetic parameters of acyclovir were calculated from the individual curves and the mean value was presented in Table 3. The microspheres formulation showed significantly ($p < 0.05$) higher value for AUC. Formulation MS4 microspheres showed superiority over the other formulations. Nearly three times higher AUC value of acyclovir for these microspheres (382.02 ± 23 ng/ml*h) as compared to drug solution (156.92 ±15 ng/ml*h) was observed. In addition, this formulation showed the ability to maintain the acyclovir plasma concentration through 24 hour as compared to the drug solution that could maintain this level of drug only for 5 hour. These results confirmed the sustained release potential of mucoadhesive microspheres of acyclovir prepared from sodium CMC. Hence, the overall better pharmacokinetic performance of sodium CMC microspheres in comparison to drug solution is

due to an intensified contact between the intestinal mucosa and microspheres as evident by mucoadhesion study and increased drug concentration at site of absorption as evident by in vitro drug release study.

4. CONCLUSION

The results of present study revealed that the retention time of acyclovir at its absorption site, i.e. the upper GIT, could be increased by formulating it into microspheres using Sodium CMC and HPMC. The microspheres prepared from Sodium CMC showed the highest mucoadhesiveness, fair entrapment efficiency and could prolong the release for a longer duration of time. These properties enabled sustained release of acyclovir from microspheres and plasma drug concentration in rabbits was maintained for 24 h. Hence, these microspheres of acyclovir may represent a useful approach for targeting its release at its site of absorption, sustaining its release and improving its oral availability.

ACKNOWLEDGEMENT

Authors are grateful to Birbal Sahani Institute of Paleobotany, Lucknow for providing SEM Facility. I would also like to thank the staff of NIPER, SAS Nagar for being kind enough to allow me to use their resources for literature survey.

REFERENCES

Asane, G. S. Nirmal, S. A. Rasal, K. B. Naik, A. A. And Mahadik, M. S. 2008 Polymers for Mucoadhesive Drug Delivery System: A Current Status.Informa health care. 34:1246–1266,

Chandler, S.G., Ilium, L. And Thomas, N.W., 1991Nasal absorption in rats. I1: Effect of enhancers on insulin absorption and nasal histology. Int. J. Pharm., 76, 61-70.

Chowdary KPR, Srinivas L. 2000 Mucoadhesive drug delivery systems: a status of current review. Indian Drugs;37:400-406.

Chowdary, K.P.R., Rao, Y.S., 2003.Preparation and Evaluation of Mucoadhesive Microcapsules of Indomethacin, Indian J. Pharm. Sci., 65(1): 49-52.

Chowdary, K.P.R., Rao, Y.S., 2004. Mucoadhesive Microspheres for Controlled Drug Delivery, Biol. Pham. Bull.; 27(11): 1717-1724.

Devarajan, P.V., Adani, M.H., In; Jain, N.K., Eds; 2002.Controlled and Novel Drug Delivery, CBS Publishers and Distributors; 52-81.

Fuertes, I., Miranda, A., Millan, M., Caraballo I., 2006, Estimation of the percolation thresholds in acyclovir hydrophilic matrix tablets. Eur. J. Pharm. Biopharm.64:336–342

Gavini, E., Rassu, G., Sama V., Cossu, M., Glunchedi, P., 2005. Mucoadhesive Microspheres for nasal administration of an antiemetic drug, metoclopramide: in vitro studies, J. Pharm. Pharmaco. 8: 287-294.

Grabovac, V., Guggi, D., Schnurch, A.B., 2005. Comparison of the mucoadhesive properties of various polymers, Adv. Drug Del. Rev; 57: 1713-1723.

Jalonde, E. G., Blanco-Prieto, M. J., Ygartua, P., Santoyo, S., 2003, Increasedefficacy of acyclovir-loaded microparticles against herpes simplex virus type 1 in cell culture. Eur. J. Pharm. Biopharm. 56:183–187.

Lehr CM, Bowstra JA, Tukker JJ, Junginer HE.1990 Intestinal transit of bioadhesive microspheres in an in situ loop in the rat. J Control Release.;13:51-62.

Meadows K. C. And Dressman J. B. 1990 Mechanism of acyclovir uptake in rat jejunum. Pharm. Res. 7(3):299–303 .

O'Brien J. J. And Campoli-Richards D. M. 1989 Acyclovir: an updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. Drugs. 37:233–309 .

Park, G. B. Shao, Z. And Mitra. A. K. 1992 Acyclovir permeation enhancement across intestinal and nasal mucosae by bile salt–acylcarnitine mixed micelles. Pharm. Res. 9:1262–1267

Rossi, S. , Sandri, G. . Ferrari, F, M. C. Bonferoni, and C. Caramella.2003 Buccal delivery of acyclovir from films based on chitosan and polyacrylic acid. Pharm. Dev. Technol. 8:199–208

Ruhnese, M., Sandstorm, F., Andersson. B., 1985, Treatment of recurrent genital herpes simplex infection with acyclovir. J. Antimicrob. Chemother. 16:621–628.

Vyas, S.P., Talwar, N., Karajgi, J. S., Jain, N. K., 1993. An erythrocyte based bioadhesive system for nasal delivery of propranolol. J. Control. Release. 23:231–237

Wagstaff A. G., Faulds D., and Goa K. L.. 1994 Aciclovir: a reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic efficacy Drugs.47:153–205 .