MATERIALS
AND
METHODS
5.1 **Materials / Equipment / Instruments**

5.1.1 **Materials**

Acyclovir was obtained as a gift sample from Ranbaxy Laboratories Limited, Gurgaon, India. Carbomer (Carbopol 974P) was a gift sample from Lubrizol Advanced Materials India Private Limited, Mumbai, India. Different grades of hypromellose (Methocel K100 MCR, K100 LVCR, Methocel K4 MCR and Methocel K15 MCR), polyethylene oxide (Polyox WSR 303) was purchased from Colorcon Asia Private Limited, Goa, India. Sodium alginate (Keltone HVCR) and microcrystalline cellulose (Avicel PH 101) were received as gift samples from Signet Chemical Corporation Private Limited, Mumbai, India. Povidone K 30 (Plasdone K 29/32) was purchased from International Specialty Products, Hyderabad, India. Sodium starch glycolate (Primojel) was purchased from DFE Pharma, Germany. Colloidal silicon dioxide (Aerosil 200) was purchased from Evonik Industries, Mumbai, India. Sodium bicarbonate was purchased from Mallinckrodt Baker, Inc., USA. Magnesium stearate (Hyqual, VegetableSource) was purchased from Mallincrodt Baker India, Mumbai, India. Isopropyl alcohol and citric acid were purchased from Avantor Performance Materials India Limited, India.

5.1.2 **Equipment / instruments**

Various equipment and instruments used in the current work are listed in Table 5.1.

**Table 5.1: Equipment / Instruments used**

<table>
<thead>
<tr>
<th>Equipment / Instrument</th>
<th>Make</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electronic weighing balance</td>
<td>Mettler-Toledo, USA</td>
<td>AG204</td>
</tr>
<tr>
<td></td>
<td>Sartorius, Germany</td>
<td>GP8201</td>
</tr>
<tr>
<td>UV-Vis Spectrophotometer</td>
<td>Varian, USA</td>
<td>Varian Cary 50</td>
</tr>
<tr>
<td>Dissolution Test Apparatus with auto sampler</td>
<td>Varian, USA</td>
<td>VK7010 with</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VK 8000 Auto</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sampler</td>
</tr>
<tr>
<td>Tablet Hardness Tester</td>
<td>VK200</td>
<td></td>
</tr>
<tr>
<td>Tablet compression machine</td>
<td>Cadmach, India</td>
<td>CMD3-16</td>
</tr>
<tr>
<td>Friability Test Apparatus</td>
<td>Electrolab, India</td>
<td>EF-1W</td>
</tr>
<tr>
<td>Tapped Density Tester</td>
<td>Electrolab, India</td>
<td>ETD-1020</td>
</tr>
</tbody>
</table>

Contd…
Table 5.1 Contd…: Equipment / Instruments used

<table>
<thead>
<tr>
<th>Equipment / Instrument</th>
<th>Make</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical Stirrer</td>
<td>Remi, India</td>
<td>RQ-124A</td>
</tr>
<tr>
<td>X-ray diffractometer</td>
<td>Bruker, Germany</td>
<td>BrukerD8-Advance</td>
</tr>
<tr>
<td>Differential scanning calorimeter</td>
<td>TA Instruments, USA</td>
<td>DSC Q20</td>
</tr>
<tr>
<td>Stability chambers</td>
<td>Newtronic, India</td>
<td>NEC2355</td>
</tr>
<tr>
<td>Texture analyzer</td>
<td>Stable Microsystems Ltd., UK</td>
<td>TA.XT.Plus</td>
</tr>
<tr>
<td>Particle size distribution analyzer</td>
<td>Sympatec GmbH, Germany</td>
<td>HELOS particle size analysis</td>
</tr>
<tr>
<td>HPLC</td>
<td>Waters</td>
<td>e2695/2489 with Empower Software</td>
</tr>
<tr>
<td>Blister packing machine</td>
<td>PAM-Pac, Mumbai, India</td>
<td>Minibliss</td>
</tr>
</tbody>
</table>

5.2 Pharmacokinetic Simulations

Pharmacokinetic simulations were performed for designing twice daily and once daily GR formulations. Twice daily and once daily GR formulations have been termed as SR and OD formulations, respectively. The objective was to replace dosage of 200 mg IR formulation given 5 times a day to SR or OD formulations. For SR formulation, dose as well as drug release profile were simulated. For OD formulation, dose was fixed as 1000 mg (total daily dose of IR formulation) and the drug release profile required was simulated. Detailed methodologies are given below.

5.2.1 Pharmacokinetic simulations for SR formulation

Intermittent first order release model described by Ritschel (1989) was used for all simulations, except for the simulation of plasma drug concentrations. The equation describing the time course of plasma drug concentrations from SR formulation was used to simulate the plasma drug concentrations (Gibaldi and Perrier, 1982). All calculations were carried out using Microsoft Excel. Simulation was carried out in three stages. In the first stage, steady state plasma concentration profile of the acyclovir IR formulation was simulated using the pharmacokinetic parameters reported in literature (Lewis et al., 1986; Zovirax®, 2005). The resultant steady state plasma concentrations were considered as the desired steady state plasma concentrations for GR formulations. In the second stage, the dose \( D \) and first order release rate constant \( k_1 \) required for SR formulation to achieve target plasma concentrations were
calculated. In the third stage, the target in-vitro release profile for the SR formulation was determined based on $k_1$.

**Stage 1: Simulation of plasma concentration profile of IR formulation**

Pharmacokinetic parameters listed in Table 5.2 were used for simulating the plasma concentration profile of IR formulation of acyclovir (Lewis et al., 1986). For ease of calculation, the unit of concentration was converted from µmol/l to µg/ml using the molecular weight of acyclovir (225 g/mol).

**Table 5.2: Pharmacokinetic parameters used for simulation of plasma concentration profiles of the IR formulation of acyclovir.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to maximum plasma concentration ($t_{max}$)</td>
<td>1.4 h</td>
</tr>
<tr>
<td>Apparent elimination half-life ($t_{1/2}$)</td>
<td>2.3 h</td>
</tr>
<tr>
<td>Area under plasma concentration profile ($AUC_{inf}$)</td>
<td>3.3 µg*h/ml</td>
</tr>
</tbody>
</table>

Absorption rate constant ($k_a$) was determined using Eq. 1, assuming that almost 99% of drug absorption is complete within the time of absorption (Ritschel and Kearns, 2007). To simplify calculations, time of absorption was replaced by $t_{max}$.

$$k_a = \frac{4.61}{t_{max}}$$  \hspace{1cm} (1)

Elimination rate constant ($k_{el}$) was determined from elimination half-life ($t_{1/2}$) using Eq. 2 (Gibaldi and Perrier, 1982).

$$k_{el} = \frac{0.693}{t_{1/2}}$$  \hspace{1cm} (2)

Volume of distribution based on area ($V_z$) was calculated from dose ($D$), bioavailability fraction ($f$), Area under the curve ($AUC_{inf}$) and $k_{el}$ using Eq. 3 (Ritschel and Kearns, 2007). Since the $AUC_{inf}$ represents only the bioavailable fraction of the IR formulation, $f$ was assumed to be 1 (100%) in Eq. 3.

$$V_z = \frac{D \times f}{AUC_{inf} \times k_{el}}$$  \hspace{1cm} (3)
Plasma concentrations of acyclovir from the IR formulation at various time intervals ($C_{t}^{IR}$), for a single dose of 400 mg were simulated using the Eq. 4 (Gibaldi and Perrier, 1982). Once again, $f$ was assumed to be 1 for the reason mentioned above.

$$C_{t}^{IR} = \frac{Dose \times f \times k_{u}}{V_{e}(k_{u} - k_{el})} \left( e^{-k_{el}t} - e^{-k_{el}t} \right)$$  \hspace{1cm} (4)$$

Plasma concentration profile of acyclovir for single dose of 200 mg IR formulation was extrapolated from the concentrations simulated for 400 mg dose. A factor of 0.69 was used instead of linear extrapolation (a factor of 0.5), since the pharmacokinetics of acyclovir is not dose proportional (non-linear pharmacokinetics) (Zovirax®, 2005).

Maximum and minimum steady state plasma concentrations of the IR formulation ($C_{max-IR}^{SS}$ and $C_{min-IR}^{SS}$) expected after repeated administration of 200 mg dose were simulated based on the principles of superposition. An accumulation table was constructed to determine the concentrations expected from five repeated doses at four hours interval on day 1, followed by similar dosing pattern on subsequent days (Ritschel, 1989). The desired maximum and minimum steady state concentrations for the GR formulations ($C_{max-des}^{SS}$ and $C_{min-des}^{SS}$) were fixed based on these simulations.

Fluctuation index of the IR formulation ($FI_{IR}$) over a 24 h period at steady state was calculated using Eq. 5 (Chung et al., 1999). Here, $AUC_{ss}$ and $\tau$ represent the area under the plasma concentration-time curve at steady state and dosing interval, respectively.

$$FI_{IR} = \frac{(C_{max-IR}^{ss} - C_{min-IR}^{ss}) \times 100}{AUC / \tau}$$  \hspace{1cm} (5)$$

Stage 2: Determination of the dose and target release rate for the SR formulation

Time for elimination ($t_{elim}$), the time from termination of release to the time of next dosing, was calculated using Eq. 6 (Ritschel, 1989). It was assumed that the terminal phase starts at a concentration of about 90% of the peak plasma concentration. Hence a factor of 0.9 was included in Eq. 6.

$$t_{elim} = \frac{\ln(C_{max-des}^{SS} \times 0.9) - \ln C_{min-des}^{SS}}{k_{el}}$$  \hspace{1cm} (6)$$
Chapter 5.0 – Materials and Methods

Duration of delivery \( (t_{\text{del}}) \), was determined using Eq. 7 (Ritschel, 1989). The dosing interval \( (\tau) \), was fixed as 12 h, since the formulation is for twice daily administration.

\[
t_{\text{del}} = \tau - t_{\text{elim}} \tag{7}
\]

Preliminary maintenance dose of the SR formulation \( (DM_{\text{pre}}) \) was estimated from the dose of IR formulation \( (DM_{\text{conv}}, 200 \text{ mg}) \), using Eq. 8 (Ritschel, 1989).

\[
DM_{\text{test}} = \frac{DM_{\text{conv}} \times 0.693 \times \tau}{t_{1/2}} \tag{8}
\]

First order release rate constant \( (k_1^l) \) was estimated using Eq. 9 (Ritschel, 1989), which considers that 10% of the drug is unreleased at \( t_{\text{del}} \). It was assumed that the intrinsic absorption rate of the drug is much higher than its release rate. Hence, drug release becomes the rate limiting step and \( k_1^l \) can be considered as the apparent absorption rate.

\[
k_1^l = \frac{\ln 100 - \ln 10}{t_{\text{del}}} \tag{9}
\]

Theoretical plasma concentrations expected from the SR formulation after administration of first dose of \( DM_{\text{pre}} \) \( (C_{\text{Er}}^{\text{ER}}) \) were simulated using Eq. 10 (Gibaldi and Perrier, 1982).

\[
C_{\text{i}}^{\text{ER}} = \frac{DM_{\text{pre}} \times k_1^l}{V_e \times (k_1^l - k_{\text{el}})} \left( e^{-k_1^l \tau} - e^{-k_{\text{el}} \tau} \right) \tag{10}
\]

The single dose concentration data simulated using Eq. 10 was used to estimate steady state plasma concentrations using the same method described for the IR formulation. Maximum and minimum steady state plasma concentrations of the SR formulation \( (C_{\text{SS max-ER}}^{\text{SS}} \text{ and } C_{\text{SS min-ER}}^{\text{SS}}) \) were read directly from the accumulation table.

Fluctuation index of the SR formulation \( (FI_{\text{ER}}) \) over a 24 h period at steady state was calculated using Eq. 11 (Chung et al., 1999).

\[
FI_{\text{ER}} = \frac{(C_{\text{SS max-ER}}^{\text{SS}} - C_{\text{SS min-ER}}^{\text{SS}}) \times 100}{AUC / \tau} \tag{11}
\]
**Stage 3: Simulation of the target in-vitro drug release profile of the SR formulation**

In the case of extended release formulations, the intrinsic absorption rate of dissolved drug is of minor importance as the apparent absorption rate will be determined by the release rate of the drug from the formulation. Hence, $k^1_r$ was considered to be the in-vitro drug release rate, assuming a one to one correlation between drug release and absorption rates. Since $k^1_r$ is the first order rate constant, a plot of logarithm of % drug remaining to be released versus time results in a straight line with a slope and intercept of $-k^1_r$ and 4.605, respectively (see Eq. 12).

$$\ln(100-%R) = -k^1_rt + 4.605 \quad (12)$$

% R represents the % drug released at time t. Eq. 12 was rearranged to obtain Eq. 13 and the latter was used to determine the target in-vitro drug release profile of the SR formulation.

$$%R = 100 - e^{(-k^1_rt+4.605)} \quad (13)$$

**5.2.2 Pharmacokinetic simulations for OD formulation**

The methodology used for simulating plasma concentrations of IR formulation remained same as mentioned in stage I in section 3.2.1. Dose was fixed as 1000 mg. The dosing interval ($\tau$), was fixed as 24 h, since the intent was to design an OD formulation. First order release rate constants ($k^i_r$) corresponding to arbitrarily chosen $t_{delt}$, were determined using Eq. 9.

Theoretical plasma concentrations expected from the OD formulation after administration of 1000 mg at $k^i_r$ were simulated using Eq. 10. The single dose concentration data was used to estimate steady state plasma concentrations using superposition method. Maximum and minimum steady state plasma concentrations of the OD formulation were read directly from the accumulation table. Based on the steady state plasma concentrations obtained, optimal $k^i_r$ was chosen. To simulate in-vitro drug release Eq. 13 was used.
5.3 Preformulation Studies

5.3.1 Identification and characterization of acyclovir

Acyclovir drug substance was analyzed for description and solubility as per the procedure specified in British Pharmacopoeia (BP, 2008). The infrared absorption spectrum was also recorded and compared with reference IR spectrum of Japanese Pharmacopoeia (JP, 2011).

5.3.2 UV absorption spectrum of acyclovir

Accurately weighed quantity of acyclovir was dissolved in 0.1 N hydrochloric acid (HCl) and suitability diluted to get a concentration of about 20.4 µg/ml. UV absorption spectrum of this solution was recorded using a UV spectrophotometer (Varian Cary 50, Varian Inc., USA) at a scanning speed of 1 nm/sec.

5.3.3 Standard curve of acyclovir by UV spectrophotometry

Standard curve acyclovir was prepared to determine the linearity of UV absorbance of acyclovir as per Beer-Lambert’s law. Standard curves were prepared in 0.1N HCl, 0.01N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer. About 170 mg of acyclovir was accurately weighed and dissolved in respective media in a 100 ml volumetric flask and the volume was made up (stock solution 1). 20 ml of this solution was then diluted to 200 ml with respective media (stock solution 2). Stock solution 2 was suitably diluted to get solutions of concentrations of 1.7 µg/ml, 3.4 µg/ml, 6.8 µg/ml, 10.2 µg/ml, 13.6 µg/ml, 17.0 µg/ml and 20.4 µg/ml. Absorbance of these solutions were measured at 255 nm (λmax) using a UV spectrophotometer (Varian Cary 50, Varian Inc., USA). Absorbance values were plotted against the concentrations using Microsoft® Excel and the data points were linearly regressed to get the regression equation. Linearity was determined through the coefficient of determination (R²) of the regression line.

5.3.4 pH solubility profile

Excess amount of acyclovir was added to aqueous media of different pH in conical flasks. The flasks were placed in shaker water bath (SW22, Julabo GmbH, Germany) and shaken for 24 h. The temperature was maintained at 37°C throughout the experiment. After 24 h the saturated solutions were filtered through 0.45 µm
membrane filters and the drug concentrations were analyzed using HPLC method (USP, 2008a).

5.3.5 Powder X-ray diffraction (pXRD)

pXRD pattern was determined to evaluate the crystallinity of acyclovir. The drug substance was passed through mesh #40 ASTM and the pXRD pattern was recorded from 3° 2θ to 50° 2θ, using X-ray diffractometer (Bruker D8-Advance, Bruker, Germany). The step time was 30.8 sec with a step size of 0.2°.

5.3.6 Differential scanning calorimetry (DSC)

The study was performed to determine the polymorphic form of acyclovir and its solid state transitions under heat. 4.1 mg of acyclovir was evenly spread in a hermetic aluminum pan and sealed with aluminum lid with pin hole. The sample was heated at a heating rate of 10°C / min, under a nitrogen flow of 50 mL/min (DSC Q20, TA Instruments, USA).

5.3.7 Thermogravimetric analysis (TGA)

TGA was performed to complement the findings of DSC. TGA thermogram of 14.2 mg acyclovir was recorded using thermo gravimetric analyzer (Q500, TA Instruments, USA) under the same experimental conditions of DSC.

5.3.8 Water content estimation

Water content of acyclovir was determined by Karl-Fischer titration method (Karl-Fischer titrator model: 716 DMS Titrino, Metrohm, Switzerland). The measurement was performed in triplicate.

5.3.9 Drug-excipient compatibility study

Suitability of excipients with respect to their influence on stability of acyclovir was evaluated through drug-excipient compatibility studies. Mixtures of acyclovir with individual excipients were prepared and these mixtures were packed in glass vials (Type I) as well as Low Density Polyethylene (LDPE) bags. These samples were stored in stability chambers (NEC2355, Newtronic, India). Samples packed in glass vials were stored at 60°C and 40°C/75% RH and those packed in LDPE bags were stored in 40°C/75% RH. Samples were withdrawn after predefined time intervals and analyzed for content of guanine, the major impurity of acyclovir, using HPLC method (USP,
2008b). Filtered (0.2 µm) and degassed solution of 0.02M acetic acid was used as mobile phase. Quantity of sample equivalent to about 10 mg of acyclovir was accurately weighed and dissolved in 10 ml of 0.1 N sodium hydroxide in 100 ml volumetric flask and then the volume was made up with water. This solution was filtered through 0.2 µm nylon membrane filter. 20 µl of the filtered solution was eluted in C-18 column (250 x 4.6 mm, HyperSil) at 40°C. The flow rate was 1.5 ml/min and the peak areas were recorded at 254 nm using diode array UV detector (1260 Series, Agilent, USA). Guanine content was determined as the ratio of peak area of guanine peak to acyclovir peak. Samples were also observed for physical/morphological changes in terms of color change or lump formation.

5.3.10 Determination of bulk properties

5.3.10.1 Angle of repose

Acyclovir was passed through a funnel kept at a height of 3 cm from the base. The powder was passed till it formed a heap and touched the tip of the funnel. The radius of the base of the conical pile, and the height of pile were measured and the angle of repose was calculated using Eq. 14. The experiment was performed in triplicate.

\[ \theta = \tan^{-1} \left( \frac{h}{r} \right) \]  

(14)

Where \( \theta \) = angle of repose

\( h \) = height of the pile

\( r \) = radius of the base of the pile

5.3.10.2 Bulk density and tapped density

Acyclovir was passed through mesh # 18 ASTM to break agglomerates. Weighed quantity of the powder was then transferred into 100 mL measuring cylinder without significant mechanical stresses during transfer. The surface of the powder was leveled carefully without compacting and the volume occupied was measured (initial volume). The cylinder was subjected to 1250 taps in tap density tester (ETD1020, Electrolab, India) and the final volume was measured (tapped volume). Bulk and tapped density were calculated using Eq. 15 and 16, respectively. The experiment was performed in triplicate.
### 5.3.10.3 Compressibility index

The compressibility index (CI) was expressed in percentage and calculated using Eq. 17 or 18.

\[
CI = \frac{TD - BD}{TD} \times 100
\]  

\[
CI = \left( \frac{v_i - v_t}{v_t} \right) \times 100
\]

### 5.3.10.4 Hausner’s ratio

Hausner’s ratio was determined by the ratio of tapped density and bulk density based on Eq. 19.

\[
\text{Hausner’s ratio} = \left( \frac{v_t}{v_i} \right) \frac{TD}{BD}
\]

### 5.3.11 Particle size distribution

Particle size distribution of acyclovir was determined by laser diffraction technique (HELOS particle size analysis, Sympatec GmbH, Germany). Dry dispersion module was used for the analysis with an air jet pressure of 3.2 bars and trigger time base of 100 ms.

### 5.4 Preparation of Gastroretentive Formulations

#### 5.4.1 Preparation of formulations using wet granulation method

Acyclovir and other intragranular ingredients were weighed (electronic weighing balance: AG204, Mettler-Toledo International Inc., USA / GP8201, Sartorius
Chapter 5.0 – Materials and Methods

AG, Germany), sifted together through sieve #40 ASTM and blended in a polybag for 5 min. This blend was then granulated manually using binder solution prepared by dissolving povidone K 30 in isopropyl alcohol. The wet mass was dried in a tray dryer for 60 min at 50° C to get loss on drying less than 2% w/w (IR moisture analyzer-MA 100, Sartorius AG, Germany). Dried mass was passed through sieve # 20 ASTM to form granules. The resultant granules were lubricated by blending with magnesium stearate (previously sifted through sieve # 60 ASTM) for 2 min in a polybag. Wherever applicable based on the composition, granules were blended with extragranular ingredients (previously sifted through sieve # 40 ASTM) for 5 min before blending with magnesium stearate. The lubricated granules were compressed into tablets (Tablet press model: CMD3-16, Cadmach, India). Tablets of SR formulations were compressed using 19 mm x 9 mm capsule shaped punches with beveled edges and of OD formulations were compressed using 21.3 mm x 10.5 mm capsule shaped punches.

5.4.2 Preparation of formulations using direct compression method

Direct compression process was used in selected formulation trials. Acyclovir, polymer(s), microcrystalline cellulose, povidone K 30 and sodium bicarbonate were weighed (electronic weighing balance: AG204, Mettler-Toledo International Inc., USA / GP8201, Sartorius AG, Germany), sifted together through the sieve #40 ASTM and blended in a polybag for 15 min. This blend was lubricated by blending with magnesium stearate (previously sifted through sieve #60 ASTM) for 5 minutes. Tablet compression was performed manually (Model: CMD3-16, Cadmach, India), using 21.3 mm x 10.5 mm capsule shaped punches.

5.5 Characterization of Gastroretentive Formulations

5.5.1 In-process characterization

Properties of acyclovir gastroretentive tablets, such as hardness, friability, thickness and weight variation were determined as in-process characterization. Hardness was determined by using tablet hardness tester (VK200, Varian Inc., USA).

Friability was determined using Roche friability test apparatus (EF-1W, Electrolab, India) as per procedure described in Indian Pharmacopoeia (IP, 2010a). 10 tablets were dusted and accurately weighed. These tablets were subjected to 100 revolutions in the friability test apparatus and then reweighed after dusting. Friability
was calculated as the loss of mass and it is calculated as a percentage of the initial mass.

Weight variation was performed according to the procedure mentioned in Indian Pharmacopoeia (IP, 2010b). 20 tablets were selected at random and weighed individually and the average weight was calculated. According to the limits specified in IP, not more than two of the individual weights deviate from the average weight by more than 5% and none deviates by more than 10% (limits for tablets with average weight greater than 250 mg). Thickness of tablets was determined using digital vernier caliper (Mitutoyo, Japan).

5.5.2 Floating lag time

The time taken for the tablet to float and reach the surface of the drug release medium was measured as the floating lag time (Baumgartner et al., 2000). This parameter was noted under the same conditions used for the in-vitro drug release test (section 5.5.4) for formulations based on floating mechanism.

5.5.3 Duration of floating

The time for which the formulation constantly floated on the surface of the medium (duration of buoyancy) was measured as duration of floating (Baumgartner et al., 2000). This was also measured under the same conditions used for the in-vitro drug release test (section 5.5.4) for formulations based on floating mechanism. A longer floating time will ensure effective gastric retention of the tablets.

5.5.4 In-Vitro drug release studies

In vitro drug release studies were performed using USP type 2 dissolution apparatus (paddle) at 50 rpm (VK7010 with VK8000 Auto sampler, Varian Inc., USA). The drug release medium was 900 mL of 0.1N HCl at a temperature of 37°C ± 0.5°C. An aliquot (10 mL) was withdrawn at specific time intervals (1, 2, 4, 6, 8, 10 and 12 h) and drug content was determined by UV-visible spectrophotometer (Cary 50, Varian Inc., USA) at 255 nm. Immediately after withdrawal of the sample, 10 mL of 0.1N HCl maintained at 37 °C ± 0.5 °C was replaced to maintain the volume of dissolution medium constant. It was ensured that none of the ingredients used in the tablet formulations interfered with the analysis. When the formulations were subjected to in-
vitro drug release studies, their swelling behavior and other physical changes were also observed.

5.5.5 Similarity factor analysis

Similarity factor \( f_2 \) is simple and model independent approach to compare drug release profiles (Moore and Flanner, 1996). It is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent dissolution (drug release) between the two curves. Two drug release curves are said to be similar if similarity factor between 50 and 100. Similarity factor was determined using following Eq. 20.

\[
f_2 = 50 \cdot \log \left\{ \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right\}^{0.5} \cdot 100
\]

(20)

Where

\( R_t \) is the % drug release of reference at time \( t \)

\( T_t \) is the % drug release of test at time \( t \)

Similarity factors for all the formulations were determined. Target drug release profile obtained in pharmacokinetic simulations was used as reference and respective drug release profiles of different batches of gastroretentive tablets were used as test.

5.5.6 Release kinetics

Drug release data of selected batches were fitted into zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell equations (Costa and Lobo, 2001). To study the mechanism of drug release, the well-known exponential equation (Eq. 21, Korsmeyer equation) was used (Siepmann and Peppas, 2001), which is often used to describe the drug release behavior from polymeric systems.

\[
\log \left( \frac{M_t}{M_f} \right) = \log k + n \log t
\]

(21)

Where, \( M_t \) is the amount of drug released at time \( t \); \( M_f \) is the amount of drug released after infinite time; \( k \) is a release rate constant incorporating structural and geometric characteristics of the tablet; and \( n \) is the diffusional exponent indicative of the mechanism of drug release. \( n \) value is obtained from the regression line of a plot of log % drug released versus log time. A value of \( n = 0.45 \) indicates Fickian (case I) release; \( > 0.45 \) but \( < 0.89 \) for anomalous (non-fickian) diffusion, indicating a
combination of diffusion and erosion controlled drug release; and 0.89 or above indicates super case II type of release referring to erosion of polymeric chain.

### 5.5.7 Swelling index and matrix erosion

Swelling index and matrix erosion studies for formulations based on mucoadhesion mechanism were performed by a method similar to the one reported by Al-Taani and Tashtoush (2003), using USP type 2 dissolution test apparatus (VK7010 with VK8000 Auto sampler, Varian Inc., USA). The tablets were accurately weighed and dropped into the dissolution vessel containing 900 mL of 0.1 N HCl (pH 1.2) maintained at 37°C ± 0.5°C; speed of rotation was 50 rpm (n=3). At regular time intervals, the residual matrices were carefully removed from the dissolution vessel and weighed. Then the residual matrices were dried in a hot air oven at 60°C for 12 hours and reweighed. The percentage swelling index (% SI), i.e., the degree of swelling due to absorbed medium, was calculated using Eq. 22. Percentage erosion (% E) was calculated from Eq. 23.

\[
\% \text{SI} = \frac{\text{Weight of swollen tablet}}{\text{Weight of eroded tablet (after drying)}} \times 100 \quad (22)
\]

\[
\% \text{E} = \frac{\text{Initial weight of the tablet} - \text{Weight of eroded tablet (after drying)}}{\text{Initial weight of the tablet}} \times 100 \quad (23)
\]

### 5.6 Stability Studies

Stability studies of optimized formulations were conducted at accelerated stability test conditions as per ICH guidelines (ICH, 2003a). Tablets were packed in aluminum blisters (Minibliss, PAM-pak, India) and stored at 40°C/75% RH in stability chamber (NEC2355, Newtronic, India). After 1, 2, 3 and 6 months, samples were withdrawn and analyzed for description, assay, water content, content of guanine and *in-vitro* drug release.

### 5.7 Ex-Vivo Studies

#### 5.7.1 Measurement of mucoadhesive strength

Mucoadhesive strength of acyclovir gastroretentive tablets based on mucoadhesion mechanism was determined by using texture analyzer (TA.XT.Plus,
Stable Microsystems Ltd., UK). Immediately after slaughter, stomach of the goat was removed and cleaned using ice-cold Krebs ringer solution (KRB) (pH 6.8). The KRB contained 115 mM sodium chloride, 5.9 mM potassium chloride, 1.2 mM each of magnesium chloride, sodium dihydrogen phosphate and sodium sulfate, 2.5 mM calcium chloride, 25 mM sodium bicarbonate and 10 mM glucose per liter of solution.

A piece of gastric mucosa measuring about 2 cm x 2 cm was mounted securely in the tissue holder of texture analyzer, with mucosal side facing upwards. Tablet was fixed to the probe with the help of double-sided adhesive tape. The surface of the tablet was immersed in 0.1 N HCl for 30 seconds and then allowed to equilibrate for 90 seconds. The probe was lowered at a speed of 0.5 mm/s and allowed to be in contact with the mucosa with a force of 10 g for 300 seconds. Then the probe was withdrawn with a trigger force of 5 g. The force required to detach the formulation from the tissue surface was determined as the peak value in resultant force-time plot.

5.8 In-vivo Studies

5.8.1 Gastroretention study using X-ray radiography

This study was based on X-ray radiography. The study was conducted on nine healthy rabbits weighing 1.8-2.4 kg. Three groups, each consisting of three animals each were made. Small sized (6.0 mm) conventional IR tablet formulation, small sized gastroretentive SR tablet (6.0 mm) based on mucoadhesion and floating were administered along with water to first, second and third groups, respectively. All the formulations contained 72.5 mg of acyclovir per tablet. To make the tablet X-ray opaque barium sulfate (10% w/w) was included. The small tablets were also characterized for shape, size, thickness, hardness, friability, drug content and in-vitro drug release. During the study, the subjects were not allowed to eat but water was available ad libitum. X-ray photography of abdominal region was taken at 0, 1, 1.5, 2, 4, 6, 8 and 12 h after administration of the tablets (Chary et al., 1999). All investigations were performed after approval of the Institutional Animal Ethics Committee of Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala and in accordance with the disciplinary principles and guidelines of CPCSEA.
5.8.2 Gastroretention study using fluorescence microscopy

Gastro retention property of developed formulation was also evaluated using fluorescence microscopy method. Similar protocol as used for X-ray radiography study was used. The study was conducted on nine healthy rabbits weighing 1.8-2.4 kg. Three groups, each consisting of three animals each were made. Small sized (6.0 mm) conventional IR tablet formulation, small sized gastroretentive SR tablet (6.0 mm) based on mucoadhesion and floating were administered along with water to first, second and third groups, respectively. 6-Carboxy fluorescein (6-CF) at 1% w/w concentration was included in all formulations as fluorescent marker.

During the study, the animals were not allowed to eat but water was available ad libitum. After 8 h of administration animals were sacrificed and the GI tract was isolated. Immediately after isolation, whole GI tract was divided into five sections: the stomach (section 1), duodenum (Section 2), jejunum (Section 3), ileum (Section 4) and remaining intestine (Section 5) (Morishita et al., 1993). The stomach was cut open, and each part of small intestine was turned inside out and homogenized with PBS 6.8 to dissolve 6-CF and centrifuged at 3000 rpm for 20 min (Remi equipment, Mumbai, India). The supernatant was analyzed fluorometrically at λ<sub>excitation</sub> 489 nm and λ<sub>emission</sub> 515nm to determine concentration of 6-CF. Also a 1 cm portion of each section was fixed as per the procedure reported by Jain et al. (2007) for fluorescence microscopy. Tissues were cut into small pieces and fixed in paraffin blocks by conventional methods. 5 μm thick sections were cut using microtome (Erma optical works, Tokyo, Japan) and examined under fluorescence microscope (Leica, DMRBE, Bensheim, Germany).

5.8.3 Pharmacokinetic study

Similar protocol as used for the GI retention study was used for pharmacokinetic study. At 0, 0.5, 0.75, 1, 1.5, 2, 4, 5, 6, 8, 10, 12, 14, 18 and 24 h time intervals blood was collected from ear vein in tubes coated with anti-coagulant and centrifuged at 4000 rpm for 10 min (Remi equipment, Mumbai, India). Acetonitrile was added to the supernatant to precipitate the proteins. The precipitated proteins were separated by centrifugation at 4000 rpm for 15 min. The supernatant was filtered through 0.45 mm filter and drug concentration was determined by HPLC method (USP, 2008a) using HPLC. Mixture of glacial acetic acid in water was used as mobile phase.
The injected fluid (20 μL) was eluted in C-18 column (250 X 4.6 mm, HyperSil) at room temperature and acyclovir content was analyzed at 254 nm. Standard solutions of concentrations ranging from 0.05 μg/ml to 20 μg/ml were prepared in triplicate and analyzed. A standard curve was prepared by plotting area counts against concentration and this standard curve was used to determine concentrations of samples analyzed for pharmacokinetic study. Pharmacokinetic parameters were calculated using non-compartmental analysis (WinNonlin 5.1.3, Certara, L.P., USA).

5.8.4 Statistical analysis

Data are expressed as the mean ± standard deviation (SD) and statistical analysis was carried out employing the student’s t-test (Sigma stat 3.5, STATCON and Witzenhausen, Germany). A value of p < 0.05 was considered statistically significant.