SUMMARY
AND
CONCLUSION
Viral diseases are one of the most acute health problems that the world is facing today. There were approximately 35.3 (32.2–38.8) million people living with AIDS in 2012 and has claimed more than 36 million lives so far. In India alone, there were about 2.2 million people living with HIV and about 1.5 lacs deaths in 2011. More than 530 million people have the virus that causes genital herpes (HSV-2). More than 290 million women have a human papillomavirus (HPV) infection.

Acyclovir, chemically known as 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one, is a purine nucleoside analogue, known for its potent and selective anti-viral activity against viruses of the herpes group. Acyclovir is a structural analog of deoxyguanosine. In addition to HSV, it has *in-vitro* activity against Varicella-Zoster Virus (VZV), Epstein - Barr virus (EBV), and Cytomegalovirus (CMV). This drug has been shown to be of clinical benefit when administered topically, orally, or parenterally for the prophylaxis and treatment of certain herpes virus infections. It is clinically indicated for the acute treatment herpes zoster, treatment of chicken pox, and genital herpes. Acyclovir is one of the treatments of choice for treatment of initial episodes and management of recurrent episodes of genital herpes.

Currently marketed oral formulations of acyclovir include immediate release (IR) capsules, tablets and suspension. In spite of its effective anti-viral activity, the use of acyclovir still has biopharmaceutics and pharmacokinetic limitations. Oral bioavailability of acyclovir is low (15 to 30%) and highly variable. It also has a short elimination half-life of 2.3 h. Absorption of acyclovir from the GIT is slow, variable and incomplete. Acyclovir is soluble in acidic pH and predominantly absorbed from the upper GIT. In commercially available IR dosage forms, the fraction of dose absorbed is very low due to short residence time of the dosage form at the absorption site. As a result, most of the administered drug is excreted in the feces (50–60%), in an unabsorbed form. These limitations necessitate frequent administration of acyclovir up to five times a day, leading to poor patient compliance, which in turn leads to reduced therapeutic efficacy and development of resistance. The recommended dosage regimen of acyclovir for the management of genital herpes is 200 mg every four hours, five times daily for 10 days.

Thus, there is a need for a dosage form, which will overcome the above said disadvantages of acyclovir therapy, increase success rate of the treatment and reduce
development of resistance. Development of a GR formulation with extended release (ER) of acyclovir and similar pharmacokinetic performance of IR dosage form will not only reduce the frequency of administration, but also possess similar or better anti-viral activity due to constant maintenance of plasma concentration of drug. Since absorption of acyclovir is restricted to upper parts of the GIT, developing a GR formulation will prolong the absorption and enhance its bioavailability.

In the present study an attempt was made to develop gastroretentive sustained formulations of acyclovir for improving its bioavailability and reducing the frequency of administration.

In first part of the study, pharmacokinetic simulations were performed to determine the dose and in-vitro drug release profile required for twice daily GR formulation of acyclovir (termed as SR formulation). Additionally, in-vitro drug release profile required for a once daily GR formulation of acyclovir (termed as OD formulation) was simulated considering the total daily dose of IR formulation (1000 mg) as the target dose.

In second part of the study, GR formulations of acyclovir were developed and optimized based on the dose and target release profile calculated using pharmacokinetic simulations. Two different approaches were used to develop GR formulations. In the first approach, formulations were based on a combination of swelling and mucoadhesion. In the second approach, gastroretention was attempted using floating mechanism. Both of these approaches were used SR as well as OD formulations. Attempt has also been made to evaluate the mechanism of gastroretention by studying swelling and matrix erosion kinetics, floating characteristics and quantitative evaluation of bioadhesion using texture analyzer. In-vivo radiographic and pharmacokinetic studies have been performed in rabbits to evaluate gastroretention and associated pharmacokinetic advantage over conventional dosage form.

Maximum plasma concentration \( (C_{\text{max}}) \) of 200 mg single dose of the IR formulation was 0.540 \( \mu \)g/ml. At steady state, maximum concentration \( (C_{\text{max-IR}}^{\text{SS}}) \), minimum concentration \( (C_{\text{min-IR}}^{\text{SS}}) \) and concentration at 12 h \( (C_{12\text{h-IR}}^{\text{SS}}) \) were 0.797, 0.097 and 0.318 \( \mu \)g/ml, respectively. \( C_{\text{max-IR}}^{\text{SS}} \) was considered as desired maximum steady state plasma concentration \( (C_{\text{max-des}}^{\text{SS}}) \) for SR formulation. Instead of \( C_{\text{min-IR}}^{\text{SS}} \), steady state
concentration of the IR formulation at 12 h \((C_{\text{SS}}^{12h-IR})\) was considered as desired minimum steady state plasma concentration \((C_{\text{min-des}}^{SS})\). The \(C_{\text{SS}}^{12h-IR}\) obtained was about 69\% of minimum steady state concentration reported for 200 mg dose, whereas \(C_{\text{min-IR}}^{SS}\) was only about 21\%. Moreover, the ratio of \(C_{\text{max-IR}}^{SS}\) to \(C_{\text{min-IR}}^{SS}\) was 251\%, which was much better than the ratio of \(C_{\text{max-IR}}^{SS}\) to \(C_{\text{min-IR}}^{SS}\) (822\%). Targeting lower ratio means targeting lower fluctuation. Therefore, \(C_{\text{12h-IR}}^{SS}\) was chosen over \(C_{\text{min-des}}^{SS}\), to target reduced fluctuation in plasma concentrations, a desirable property of any ER formulation.

Target dose for SR formulation simulated was 723 mg, which was considered as 725 mg for practical development. \(k^1\) for this formulation was determined to be 0.259 h\(^{-1}\). Steady state maximum and minimum plasma concentrations \((C_{\text{max}}^{SS} \text{ and } C_{\text{min}}^{SS})\) achieved for the above parameters upon multiple dose administration at 12 h intervals were 0.719 and 0.211 \(\mu g/ml\), respectively. These values were 90\% and 218\% of steady state maximum and minimum plasma concentrations of IR formulation, respectively. The fluctuation index of the SR formulation was 69\% of that of the IR formulation. Moreover, the overall plasma concentration profile was more consistent between individual doses in the case of SR formulation as compared to the IR formulation. The time for 50\% and 90\% of drug release \((t_{50\%} \text{ and } t_{90\%})\) for the target in-vitro drug release profile for SR formulation were determined to be 2.7 h and 8.9 h, respectively.

Determination of \(k^1\) followed by simulation of single dose and multiple dose pharmacokinetic parameters for 1000 mg OD formulation were performed for \(t_{\text{del}}\) of 8.88 h (same obtained for 723 mg SR formulation), 10, 12, 14 and 16 h. Simulation with 16 h \(t_{\text{del}}\) provided 81\% \(C_{\text{max}}^{SS}\) and highest \(C_{\text{min}}^{n}\) as compared to IR formulation among the simulations performed. The time for 50\% and 90\% of drug release \((t_{50\%} \text{ and } t_{90\%})\) for the target in-vitro drug release profile for OD formulation of 16 h \(t_{\text{del}}\) were 4.8 h and 16.0 h, respectively.

Formulation development studies were carried out with aforementioned targets of dose and in-vitro drug release profiles. Extensive preformulation studies were carried out before formulation development. Acyclovir was characterized for polymorphic form and solubility. Acyclovir was found to have crystalline nature as
determined by powder X-ray diffraction (pXRD). Acyclovir was found to be a hydrate form containing two molecules of water for every three molecules of acyclovir in the crystal lattice as determined through differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and water content determination by Karl-Fischer titration. In order to estimate acyclovir in experimental protocols using ultraviolet (UV) spectrophotometric method, calibration curves were prepared at \( \lambda_{\text{max}} \) (255 nm). The estimation procedure was found to be fairly reproducible and sensitive, and correlation coefficient of calibration curves was about 0.999 in all the cases. For measurement of acyclovir in plasma, more sensitive and accurate HPLC method reported in the United States Pharmacopeia (USP) was used.

Particle size distribution (PSD) of acyclovir was determined using laser diffraction method. The distribution was uniform within a narrow range of particle sizes. The mean particle size observed was 18.7 \( \mu \text{m} \). 90% and 10% of particles were below 58.5 \( \mu \text{m} \) and 4.2 \( \mu \text{m} \), respectively. Aqueous solubility of acyclovir was found to be dependent on the pH. Maximum solubility of 15.6 \( \pm 1.8 \) mg/ml was found at pH 1.2. Solubility at pH 2, 3, 4.5, 6.8 and 7.5 was found to be 3.7 \( \pm 0.2 \), 2.8 \( \pm 0.2 \), 2.4 \( \pm 0.1 \), 2.4 \( \pm 0.2 \) and 2.4 \( \pm 0.2 \) mg/ml, respectively.

Compatibility of acyclovir with different excipients was determined by storing mixture of the drug with individual excipients packed in glass vials and low density polyethylene (LDPE) bags to 40\(^\circ\)C/75\% RH and 60\(^\circ\)C for 15 and 30 days. Slight increase in impurity (guanine) was observed in drug-carbomer mixture packed in LDPE bags at 40\(^\circ\)C/75\% RH, but similar increase was not observed in samples packed in glass vials. Since the sample packed in glass vials are completely protected from moisture, but not that packed in LDPE bag, it can be concluded that the increase in impurity in LDPE bag is due to absorption of moisture. Impurity levels increased in drug-citric acid mixture also, but well below the limit of 2\% specified in USP. No increase in the impurity levels were found with other excipients.

Angle of repose and compressibility index of acyclovir were 41.6\(^\circ\) \( \pm 1.3\)\(^\circ\) and 39.8\% \( \pm 1.8\)\%, respectively, indicating its poor flow property. Hence wet granulation process was selected to improve flow properties. As the dose of acyclovir was high (725 mg or 1000 mg), quantity of excipients has to be less so as not to increase the size of the tablet, which otherwise will be very difficult to swallow. Low concentration of
polymers may also distribute in non-uniform manner within the matrix and lose control over the drug release. Wet granulation will also improve the uniformity of the polymer distribution and result in intimate contact between drug and polymer. All the polymers used in the preparation were hydrophilic and swell in presence of water. This property will make drying process of the wet mass after granulation very difficult, if water is used as granulating solvent. Hence wet granulation was performed using isopropyl alcohol as solvent. Isopropyl alcohol is a widely used granulating solvent in pharmaceutical industry. It is a very safe solvent to use in oral dosage forms and has been classified as a solvent with low toxic potential by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). GR tablets were subjected to in-process characterization such as average weight, uniformity of weight, thickness, hardness and friability. In-vitro drug release was determined in 900 ml of 0.1N HCl using USP dissolution test apparatus 2 (paddle) at 50 rpm. In-vitro drug release profiles were compared with target profiles using similarity factor (f2).

SR formulations that are based on swelling and mucoadhesion approach contained polyethylene oxide (Polyox WSR 303), carbomer (Carbopol 974P) and sodium alginate (Keltone HVCR). Carbomer, polyethylene oxide and sodium alginate were used as mucoadhesive–release controlling polymers either individually and/or in combination. These polymers have good gel forming ability and high degree of swelling, which will assist retention of tablets in stomach without allowing them to pass through pyloric sphincter. Moreover their chemical structures allow them to form numerous hydrogen bonds, which is very crucial for strong mucoadhesion. They are widely used in drug products and cosmetics as release controlling polymers and viscosity modifiers.

The SR tablets of all the formulated batches were compact, smooth and shiny in appearance. Weight variation and friability were found to comply with the official limits in all batches. Tablets were of high mechanical strength as inferred from the values of friability testing (0.05-0.11%). There was no capping or lamination observed in any of the formulations. In-vitro drug release and their physical behavior during in-vitro drug release were found to be dependent on the type and concentration of polymer(s) used. Drug release decreased when the concentration of carbomer was increased from 7.5% to 10%. Further increase in carbomer concentration to 15%
resulted in much faster drug release than 10% carbomer and closer to 7.5% carbomer. Initial swelling of the batch containing 15% carbomer was high, but the tablets split into two portions in longitudinal axis due to internal stresses created by high degree of swelling. This resulted in increased surface area due to newly exposed surfaces and subsequently in a faster drug release profile. Batches containing polyethylene oxide alone did not swell much and followed an erosion pattern throughout the duration of release. Drug release was inversely proportional to the concentration of polyethylene oxide. Polyethylene oxide had a better release controlling effect than carbomer up to 6 h at same polymer concentration of 10%. In batches containing combination of carbomer and polyethylene oxide, drug release decreased with increasing proportion of polyethylene oxide. This is in line with the observation that polyethylene oxide controls the drug release better than carbomer. Batches containing combination of carbomer and sodium alginate at equal concentrations split during drug release, though their drug release profiles were closer to the target. Drug release profile of batch containing 10% carbomer and 3.5% sodium alginate was much slower than the target. Six out of eleven batches prepared had acceptable $f_2$ values ($\geq 50$). Out of the six batches with acceptable $f_2$, three batches split during drug release studies due to high swelling. These three batches were not considered suitable for gastroretention due to the smaller size of split portions as compared to whole tablet. Drug release profile of the batch containing 7.5% carbomer and 2.5% polyethylene oxide was closest to the target and the $f_2$ value of this batch was 85. Rapid swelling and controlled erosion of GR formulation are essential for better gastroretention. Swelling is an important characteristic of polymer that controls the drug release and increases the GI retention of GR tablets. Swelling is also an important parameter for mucoadhesion property of formulation. A number of studies reported showed the direct relationship between swelling and mucoadhesion. This batch possessed highest swelling index (255.7% in 0.75 h) with sustained erosion profile and hence, this formulation was selected as optimized formulation for in-vivo study. To avoid the gastric emptying, size of the dosage form should be greater than about 13 mm. The optimized SR formulation maintained the size greater than 13 mm up to 8 h. Drug release of optimized SR formulation AGR-6 followed first order kinetic model and its drug release mechanism was anomalous transport (non-fickian). As the quantity of polyethylene oxide is increased, $n$ value increased towards case II transport,
that is, towards erosion controlled zero order release. Visual observation of drug release profiles of these batches also concurred with this observation.

Mucoadhesive strength was measured using texture analyzer. Mucoadhesive strength of the optimized formulation was much higher than the marketed IR tablet formulation, Zovirax (19.3 ± 4.7 g vs. 9.3 ± 0.8 g). Mucoadhesive strength decreased when the concentration of polyethylene oxide increased. The optimized formulation was stable under accelerated stability test conditions (40°C/75% RH) up to 6 months. There was no increase in the impurity (guanine) and no significant change in the assay and water content. Drug release profiles were similar to the initial profiles as observed through acceptable \( f_2 \) values.

For the development of OD formulations based on mucoadhesion and swelling mechanism, polyethylene oxide (Polyox WSR 303) alone or in combination with carbomer (Carbopol 974P) was used based on the knowledge gained from the development of SR formulation. All the developed formulations had good physical properties and complied with official limits of weight variation and friability. Gradual reduction in the in-vitro drug release was observed when quantity of polyethylene oxide was increased. This was similar to the observation with SR formulation made using the same approach. Batch containing 8.7% carbomer and 6.7% polyethylene oxide had highest \( f_2 \) of 72 as compared with the target profile. This batch also maintained constant swelling and erosion over 8 h indicating its ability to resist passage through pyloric sphincter. This batch was considered as most optimal OD formulation based on mucoadhesion and swelling approach.

SR formulation based on floating approach was optimized based on floating lag time and the duration of floating in addition to the similarity of in-vitro drug release to the target profile. Sodium bicarbonate based gas generation mechanism was used to achieve floatation. Hypromellose (different viscosity grades) and polyethylene oxide (Polyox WSR 303) alone and/or in combination were used as release controlling polymers in this approach. Formulation with basic polymeric composition containing combination of hypromellose and polyethylene oxide did not float in the absence of sodium bicarbonate. Various batches were prepared to study the effect of quantity of sodium bicarbonate, quantity of polymer, viscosity of polymer and intra or extragranular addition of sodium bicarbonate. Batch containing 2.4% hypromellose (Methocel K100 MCR), 3.3% polyethylene oxide, 6.6% intragranular sodium
bicarbonate and 8.5% extragranular sodium bicarbonate was found to have optimal floating lag time (162 ± 17 sec), duration of floating (up to 12 h) and highest $f_2$ for *in-vitro* drug release among all batches (74). This batch was selected as optimal SR formulation based on floating approach. Drug release mechanism of this optimized batch fitted best with Korsmeyer-Peppas model with the release kinetics following anomalous transport (non-fickian).

For the development of OD formulation based on floating approach, combination of hypromellose (Methocel K100 MCR) and polyethylene oxide (Polyox WSR 303) were used, based on the knowledge gained from the development of floating based SR formulation. Optimization of formulation was carried out by intra / extragranular addition of sodium bicarbonate, inclusion of sodium starch glycolate as wicking agent, inclusion of mannitol as hydrophilic diluent, alteration of quantity of hypromellose and inclusion of citric acid to enhance formation of carbon dioxide. Batch containing 1.9% hypromellose, 6.6% polyethylene oxide, 6.3% mannitol, 9.5% extragranular sodium bicarbonate and 3.8% citric acid was found to have optimal floating lag time (55 sec), duration of floating (more than 24 h) and highest $f_2$ for *in-vitro* drug release among all batches (57). This batch was selected as optimal once daily GR formulation based on floating approach. Its drug release fitted best with Higuchi model. Drug release mechanism of all batches were by anomalous transport (non-fickian) indicating combination of diffusion and erosion based drug release.

*In-vivo* radiographic study was conducted on healthy albino rabbits to determine gastric retention time of the SR formulations in comparison with IR tablets. For this study, tablets of 6.0 mm diameter and 110 mg weight were prepared for optimized batches of SR formulations based on swelling and mucoadhesion, and floating. These batches were selected as optimized batches due to *in-vitro* characteristics, acceptable drug release characteristics and their swelling and matrix erosion profiles. To make the tablets X-ray opaque, barium sulfate in 10% w/w concentration was included. Images were captured at different time points to find the location of the tablet. Gastric residence time of GR formulations was found to be 480 min in comparison with 90 min for conventional IR tablet. From this study, it is clear that the conventional IR tablet was quickly emptied from stomach, but the retention of GR formulation was prolonged up to 8 h. The increased gastric retention time of GR
formulation was due to the synergistic mechanism of swelling and mucoadhesion or floating. These results were in accordance with the results of in-vitro studies.

In addition to the X-ray radiographic study, fluorescence marker based simple technique was used to determine the gastric retention properties of optimized SR formulations. Small tablets of optimized SR and IR formulations were labeled with fluorescent marker 6-carboxy fluorescein (6-CF) and administered to rabbits. At the end of 8 h, rabbits were sacrificed and fluorescence intensity at different parts of the GIT was determined using fluorescence assay. 6-CF was not detected from the stomach, duodenum and jejunum regions of the rabbits administered with IR formulation. About 32% of 6-CF was recovered from intestine below ileum. In contrast, fraction of 6-CF recovered from stomach, duodenum and jejunum regions of rabbits administered with optimized mucoadhesive and floating SR formulations were 65% and 58%, respectively. In stomach alone rabbits administered with optimized mucoadhesive and floating SR formulations contained about 17% 6-CF. In contrast, SR formulations are retained in stomach for more prolonged period of time than IR formulation and also ensure slow release of the 6-CR resulting in presence of much higher amount of marker even at 8 h.

Pharmacokinetic study of optimized SR formulations was performed in rabbits using tablets of 6.0 mm diameter. $t_{\text{max}}$ of SR formulations of both approaches was 4 h, which is significantly longer than that of IR formulation (1.5 h). $C_{\text{max}}$ of mucoadhesive, floating and IR formulations were $292.6 \pm 7.5$, $278.9 \pm 10.2$ and $295.4 \pm 5.9$ ng/ml, respectively. Bioavailability of mucoadhesive and floating GR formulations was significantly higher (2.6-fold and 2.3-fold, respectively) than IR formulation. Plasma concentration profile of GR formulations indicated more sustained absorption as compared to IR formulation. The results were very well in agreement with results of gastroretention studies. Results of this single dose study were also simulated to steady state using superposition method. Both the SR formulations were able to achieve more than 80% of $C_{\text{max}}^{\text{ss}}$ and more than 170% of $C_{\text{min}}^{\text{ss}}$ of the IR formulation. The initial pharmacokinetic simulations (Chapter 6.1) revealed that the daily dose required for SR formulation is 1450 mg as against 1000 mg of IR formulation, but optimized formulations exhibited better performance at much lower doses than IR formulation. This is due to more than 2-fold bioavailability of SR formulations as compared to IR formulation. Prolonged gastric retention of SR formulations resulted in continuous
supply of the drug to the upper small intestine, prolonging the absorption of the drug and enhancing its bioavailability.

Due to poor oral bioavailability (15 to 30%) of acyclovir from conventional oral formulations and high frequency of administration, there is a pressing need to develop a GR formulation to improve patient compliance and therapeutic efficacy. Poor oral bioavailability of acyclovir from conventional formulations is due to its narrow absorption window in the upper parts of the GIT. Considering its favorable solubility in acidic pH and absorption window in upper parts of the GIT, acyclovir is a suitable candidate for GR drug delivery system. As the gastric residence time of GR formulation is prolonged, small amounts of the drug is continuously released in the absorbable regions of the GIT, thereby increasing its bioavailability. Since the C_{\text{max}} of optimized GR formulations are similar to the IR formulation, similar anti-viral activity can be expected. At the same time, prolonged absorption and higher bioavailability of these formulations will help in reducing the dosing frequency of currently marketed IR formulations. Developed GR formulation might represent a better alternative for sustained and more efficacious delivery of acyclovir, improve patient compliance and reduce development of resistance.