FORMULATION AND IN VITRO EVALUATION OF ACYCLOVIR LOADED POLYMERIC MICROPARTICLES: A SOLUBILITY ENHANCEMENT STUDY

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Abstract: Objective of present work was to formulate polymeric microparticles of acyclovir using β-cyclodextrin by solvent evaporation method and kneading technique. Four different ratios were fabricated in each case. Sodium lauryl sulfate (4%) was utilized as intestinal permeation enhancer in this study. Prepared microparticles were characterized for micrometric properties i.e., angle of repose, Hausner’s ratio, Carr’s index, bulk density and tapped density, entrapment efficiency, zeta size and zeta potential, Fourier transform infrared spectroscopy, differential scanning calorimetry, powder x-ray diffraction, scanning electron microscopy, transmission electron microscopy, optical microscopy and permeability studies across chicken intestine. Kinetic models: zero order, first order, Higuchi and Korsmeyer Peppas were applied on release data. Based upon the results of entrapment efficiency (81.25% and 74.50%), product yield (92.50% and 85.50%), permeability (85.18% and 82.05%), x-ray diffraction (amorphous nature), and solubility etc., (1 : 2) drug-polymer ratio was declared the best. Moreover, solid dispersions (1 : 2) had shown promising results. A new potential approach for solubility, bioavailability and permeability enhancement of acyclovir and other BCS class IV drugs was successfully established.

Keywords: acyclovir, solubility, permeability, β-cyclodextrin, solvent evaporation method, kneading technique.

To achieve therapeutic goals oral route still remains a preferable approach due to its simplicity, ease of administration, accurate and precise dosing. A majority of old chemical moieties i.e., 25–40% and 90% of newly discovered drugs exhibit inappropriate solubility and variable absorption across natural barriers that ultimately results in poor bioavailability of active pharmaceutical ingredient (API). Bioavailability of drugs with poor water solubility within the gastric mucosa can be enhanced by enhancing solubility of drug using different approaches (1). Among them, one of the approaches is altering nature of the drug i.e., from crystalline to amorphous form. Bioavailability of water insoluble drugs increases by increasing dissolved fraction consequently improving systemic availability of APIs (2).

Acyclovir [9-(2-hydroxyethoxy)-methyl]-guanine, is a drug of choice against herpes simplex virus (HSV), varicella zoster virus (VZV), Epstein bar virus (EBV), cytomegalovirus (CMV) and human herpes virus (HHV-6). It is activated by viral thymidine kinase enzyme into tri-phosphate form and inhibits viral DNA polymerase enzyme to stop viral replication. Acyclovir has oral bioavailability of only 10-30% due to its poor solubility (2.5 mg/mL, 37 ± 2°C) and low permeability. It is an ampholyte drug with two pKa values and is completely soluble at pH 1.2 and pH 7.4. Plasma half-life is about 2.5 h. So frequent dosing i.e., 200 mg five times a day is required to attain desired drug concentration within therapeutic levels. It has no fixed place in Biopharmaceutics Classification System (BCS) i.e., at 200 mg it is placed in BCS-III and at dose of 800 mg, it show properties of BCS-IV drugs (2, 3).

Drug polymeric complexes in the form of microparticles can be formulated by utilizing water soluble and water insoluble polymers of synthetic, semisynthetic and natural origin. Cyclodextrins (CD) are circular oligosaccharides containing 6-8 glucose units that are connected with each other by α-1,4 glucosidic linkages. CD’s are abundantly used for solubility, dissolution, stability and bioavailability enhancement. Among CD’s, β-cyclodextrin (β-CD) has major share in different fields because of low

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price, availability and biocompatibility. It presents itself as amphiphilic moiety i.e., hydrophilic outer surface due to presence of (−OH) groups and hydrophobic inner cone with less polarity. It has ability to grab therapeutic drug molecules with molecular weight 200 – 800 g/mol. β-CD is widely accepted as solubility, stability and bioavailability enhancing agent in the form of micro-sized particles i.e., microparticles (solid dispersions and inclusion complexes) (4).

A bunch of approaches has been presented in the literature to improve solubility and bioavailability of poorly soluble drugs. These include use of co-solvents, surfactants, cyclodextrins, salt formation, rapid dissolving tablets, pH maintenance, particle size reduction, hydrogel microparticles and lipids based delivery systems (5). Particles with micrometric size (microparticles) range can be used as potential candidates for solubility issues of hydrophobic drugs due to large surface area, wettability and wicking properties.

Moreover, permeability issues can be minimized by the addition of surfactants, chelating agents, fatty acids and cyclodextrins etc. Anionic surfactants i.e., sodium lauryl sulfate (SLS also called as sodium dodecyl sulfate) have an efficient wetting, solubility, dissolution, stability and permeability enhancement characteristics when these are available in nearby locality of dissolving products.

Solid dispersion (SD) is the dispersion of drug molecules into a non-reactive polymeric carrier by different approaches like melting, co-grinding, use of solvent and size reduction etc. Due to amorphous nature of SD’s these provide enhanced dissolution, increased surface area, improved wettability of active drug and inhibit recrystallization in physiological fluids of the body (6).

On the other hand, inclusion complexes host forms cone/cavity in which second molecule resides forming an inclusion complex. There stable interaction is strongly supported by Van der Waals forces, hydrogen bonding, electrostatic forces, hydrophobic interactions and charge transfer interaction (7).

In present work, efforts have been made to improve solubility, dissolution and bioavailability of acyclovir by developing β-CD and surfactant based polymeric microparticles i.e., SD’s and inclusion complexes (IC’s) with desired characteristics of solubility, dissolution and bioavailability.

EXPERIMENTAL

Materials

Chemicals utilized in this study included: acyclovir received as gift sample from Brooks Pharmaceuticals, Karachi, Pakistan, β-cyclodextrin 97% pure purchased from Sigma Aldrich, Germany, sodium lauryl sulfate obtained from Gray’s Pharmaceuticals, Islamabad, Pakistan. Methanol and hydrochloric acid of HPLC grade were purchased from Merck, USA. Double distilled water was freshly prepared by LC/MS Lab no. 25 of department. All the chemicals and solvents used were of analytical grade.

METHODS

Preparation of acyclovir – β-cyclodextrin solid dispersions

Acyclovir loaded β-cyclodextrin solid dispersions were obtained by modifying solvent evaporation method used by Frizon et al. (8). Five different ratios (drug and polymer) were selected i.e., 1 : 0.5, 1 : 1, 1 : 2, 1 : 3 and 1 : 4. Required quantities of β-CD, acyclovir (ACV) and sodium lauryl sulfate (1, 2, 4 and 6%) were weighed on electronic weighing balance (Shimadzu, AUW220D Japan). β-CD and sodium lauryl sulfate were poured into beaker containing water-methanol mixture (25 : 75, v/v) that was already placed on hot plate magnetic stirrer (30°C) at 100 rpm. Stirring was continued until clear solution was formed. Acyclovir 1% solution was prepared by pre-dissolving ACV in 0.1 mol/L HCl media and sonicated to completely solubilize it. Acyclovir solution was then poured into β-CD solution and stirring was continued at 100 rpm and 40°C until the whole solvent was evaporated. End product was spread on filter paper and placed in hot air oven (Memmert) at 50°C for complete drying overnight. Dried solid dispersions were sieved through sieve no. 80 to attain uniform particle size distribution. Formulations prepared by this method were tagged as SD1 (1 : 0.5), SD2 (1 : 1), SD3 (1 : 2), SD4 (1 : 3) and SD5 (1 : 4 ), respectively, and stored in air tight containers containing silica gel bags for further analysis (8).

Preparation of acyclovir – β-cyclodextrin inclusion complexes

Acyclovir loaded β-CD inclusion complexes were fabricated by kneading technique. Four different (drug-polymer) ratios were designed i.e., 1 : 0.5, 1 : 1, 1 : 2, 1 : 3 and 1 : 4. β-CD and sodium lauryl sulfate were accurately weighed and subjected to pestle and mortar by adding water-methanol (25 : 75) mixture dropwise to form paste. Acyclovir 1% solution was prepared in 0.1 mol/L HCl media as discussed above. SLS was used in 1, 2, 4 and 6% in
all four ratios, respectively. Dissolved ACV drug solution was added into β-CD, sodium lauryl sulfate paste and trituration was further continued for 3 h. Mortar was then placed into hot air oven (Memmert) at 50°C overnight for complete drying of the product. Fabricated product was sieved through sieve no. 80 to obtain particles of uniform size. Inclusion complexes thus formed were named as IC1 (1 : 0.5), IC2 (1 : 1), IC3 (1 : 2), IC4 (1 : 3) and IC5 (1 : 4). They were stored in air tight containers for further experimental work (9).

**Solubility studies**

Solubility studies were performed at pH 1.2 and pH 7.4. An excess amount of acyclovir – β-CD complex was poured into 1 mL of different solutions i.e., pH 1.2, pH 6.8 and water in separate screw capped glass vials. All solutions were subjected to mechanical shaking on thermo shaker incubator (MSC-100 China) operated at 400 rpm and 37 ± 5°C for 24 h to attain equilibrium. Each solution was then subjected to centrifugation at 6000 rpm for 10 min. Supernatant was collected by micropipette and filtered through 0.45 µm syringe filter (Sartorius). One mL of filtered solution was further diluted by distilled water and marked a dilution number accordingly. Each dilution was analyzed at 254 nm using UV-visible spectrophotometer (Shimadzu, Japan) against the same β-CD concentration in water for omission of any absorbance from polymer (10).

**Product yield**

Actual amount of ingredients incorporated into microparticles and amount of microparticles recovered at the end of each preparation method was noted (11). Product yield in percentage was calculated using following formulas: 

\[
\text{Mass loss} (\%) = \frac{M_i - M_f}{M_i} \times 100
\]

\[
\text{Product yield} = 100 - \text{mass loss}
\]

where \( M_i \) = initial weight of all ingredients, \( M_f \) = final weight of microparticles.

**Entrapment efficiency**

Microparticles containing ACV were weighed on electronic weighing balance (Shimadzu, AUW 220D, Japan), triturated, dissolved in phosphate buffer pH 7.4 and filtered. After filtration, aliquots were further diluted and absorbance was determined by using UV-visible spectrophotometer at 254 nm (11). The amount of acyclovir incorporated into microparticles was determined using the following equation:

\[
\text{Entrapment efficiency} = \frac{\text{Absorbance of sample containing 50 mg ACV}}{\text{Absorbance of 50 mg pure ACV}} \times 100
\]

**Micromeritic properties**

**Angle of repose**

Funnel method was used to calculate angle of repose. Prepared microparticle’s blend was poured through vertically placed funnel having aluminium sheet under it. Height (\( h \)) of cone was noted by scale and radius (\( r \)) was calculated by drawing the diameter of cone by pencil on aluminium sheet and then dividing it by 2. Angle of repose (\( \theta \)) was calculated using the following formula:

\[
\tan \theta = \frac{h}{r}
\]

Angle of repose less than 30° supports better flow properties of prepared microparticles.

**Bulk density**

Cylinder method was adopted for bulk density. Microparticles of known mass (\( M \)) were taken. These were poured into measuring cylinder and their bulk volume (\( V_b \)) was noted. Bulk density (\( \rho_b \)) was calculated using the following formula:

\[
\text{Bulk density (\( \rho_b \))} = \frac{M}{V_b}
\]

**Tapped density**

Cylinder method along with tapings i.e., 250 ± 15 taps from a height of 3 ± 0.2 mm was adopted for tapped density. Known mass was added into measuring cylinder and subjected for tapping. Tapped volume (\( V_t \)) was noted after tapping visually (12). Tapped density was calculated using the following formula:

\[
\text{Tapped density (\( \rho_t \))} = \frac{M}{V_t}
\]

**Carr’s compressibility index**

Carr’s index (\( I \)) was calculated by using results of tapped volume (\( V_t \)) and bulk volume (\( V_b \)) from their respective densities. It was calculated using the following formula:

\[
\text{Compressibility index (\( I \))} = \frac{V_t - V_b}{V_b} \times 100
\]

Carr’s index between 13 – 19% proves better flow of microparticles (12).

**Hausner ratio**

Ratio between tapped (\( \rho_t \)) and bulk (\( \rho_b \)) densities is known as Hausner ratio. The following formula was used for its calculation:

\[
\text{Hausner ratio} = \frac{\rho_t}{\rho_b}
\]

Value less than 1.25 provide evidence for good flow (12).
Instrumental analysis

Particle size and zeta potential determination

Particle size and zeta potential determination was made by single particle size analyzer (Zetasizer Nano-series ZEN3600, Malvern Instruments Ltd., U.K.) with software DTS-nano (8).

Microscopic analysis

Size analysis of prepared microparticles was performed using optical microscope (Nikon E200, Tokyo, Japan) equipped with (DCM-35 USB 2.0 and MINISEE IMAGE software, Scopetek Electric, Hangzhou, China). Samples were of white color so they were first soaked into black ink and dried on filter paper. Dried microparticles were spread and transparent tap was wrapped to fix particles on glass slide. Magnification power was set at 100X (13).

Fourier transform infrared spectroscopy (FT-IR) analysis

FT-IR spectra of acyclovir, β-CD and prepared microparticles were recorded to check compatibility, complex formation or any sort of interaction by using FT-IR spectrophotometer (Tensor 27, Bruker, OPUS software). Samples were placed on crystal spot (zinc selenide) and arm was rotated downwards to generate fine disc of sample. Scanning range was kept within the range of 3500 to 1000 cm\(^{-1}\) at spectral resolution 4 cm\(^{-1}\) (8).

Thermal analysis (DSC and TGA)

In order to get the phase transition and weight loss preview, acyclovir, β-CD, sodium lauryl sulfate and fabricated microparticles i.e., inclusion complexes and solid dispersions were examined using simultaneous thermogravimetric analyzer and differential scanning calorimeter (TA Instruments, USA model Q600). Before loading sample, both reference pan and sample pan were tarred. Samples were prepared after trituration and sealing in aluminum pan. Heating rate was kept 10°C/min for 0 to 400°C. Thermal stability of microparticles was determined by thermal gravimetric analysis (TGA). In both cases nitrogen flow rate was kept 10 mL/min (8).

Powder X-ray diffraction analysis (PXRD)

PXRD was carried out to check the impact of both processes on crystallinity of the acyclovir. PXRD patterns using XRD xpert pro with software pan analytical*. Voltage applied was 38 kV with current of 26 mA and scanning was performed at 2θ range 15-65°. Microparticles were triturated before taking each scan (8).

Scanning electron microscopy (SEM)

Surface morphology of prepared microparticles was observed on electron microscope (Quanta 250) using maker fei® software. Accelerating current was kept at 20 kV. Powder of microparticles was fixed on support with carbon-glue and coated with gold using a SPI sputter module in a high-vacuum evaporator (8).

Transmission electron microscopy (TEM)

More deep insight on prepared microparticles was taken by using JEM 2100F field-emission transmission electron microscope (JEOL, Tokyo, Japan). Samples were observed after sprinkling on metallic grid of instrument and different magnifications were used to obtained fine view (14).

Dissolution profile

Drug release studies were carried out in 0.1 M HCl of pH 1.2 and phosphate buffer of pH 7.4. Weighed quantity of prepared microparticles was enclosed in empty hard gelatin capsules against Cap. Zalcovir. Proper sink conditions were maintained. USP type-II apparatus was used. It was operated at 50 rpm at 37 ± 5°C. Five mL of samples were taken from middle of basket by using 10 mL pipette (Pyrex*) at predetermined time intervals and replaced with fresh dissolution media each time. Samples were filtered through syringe filter (5 µm pore), diluted and analyzed on UV-VIS Spectrophotometer (Pharma Spec 1700, Shimadzu, Japan) at 254 nm wavelength. All measurements were made in triplicate (8).

Permeability assessment using chicken intestine

Composition of plasma membrane of intestinal epithelium is similar among many species. Chicken intestine was selected for permeability studies due to its ease of availability and very low cost. Developed method of Dias and co-authors (15) with slight modifications was used. Intestinal part (small intestine) of white female chicken (broiler) was obtained from nearby market and shifted to lab in sealed polythene bag. Sterile sharp edge surgical blade of carbon steel was used to cut a segment of 6 cm length. Eversion of intestinal segment was made by mounting and rolling back on glass rod. Isotonic solution (Macsol NS) was used for rinsing and removing mucous and debris from outer absorptive. One end was closed tightly with silk thread and 25-30 mL of phosphate buffer solution (pH 7.4) was poured as dissolution media for microparticles. Other end of intestine was also tied making it as intestinal pouch and hanged in centre of dissolution media with paddle of dissolution appara-
Formulation and in vitro evaluation of acyclovir loaded polymeric... 1315

Stability studies

Stability studies were carried out on optimized formulation SD3 and IC3 according to ICH (International Conference on Harmonization) guidelines for a period of 3 months at 40 ± 2°C/75% RH ± 5% conditions in stability chamber (Memmert Beschickung, 100–800, Japan).

RESULTS

Solubility studies

Acyclovir is an ampholyte having pKa values 2.27 and 9.25. It has solubility equal to 0.005 mM/L in water. It is more soluble at pH 1.2 as compared to pH 7.4. Higher solubility was seen at lower pH of 1.2 than higher pH values. From solubility studies of ACV and microparticles, i.e., IC’s and SD’s, there was significant increase solubility of drug in water (7.6 fold), pH 1.2 (9.5 fold) and pH 7.4 (6 fold) as shown in Figure 1.

Entrapment efficiency and product yield

Results of entrapment efficiency and product yield are shown in Table 1. Results indicated that

![Figure 1. Column graph of solubility of ACV](image)

<table>
<thead>
<tr>
<th>Codes</th>
<th>Entrapment efficiency (%)</th>
<th>Product yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD1 (1 : 0.5)</td>
<td>53.25 ± 0.25</td>
<td>67.00 ± 0.40</td>
</tr>
<tr>
<td>SD2 (1 : 1)</td>
<td>59.14 ± 0.15</td>
<td>80.00 ± 0.30</td>
</tr>
<tr>
<td>SD3 (1 : 2)</td>
<td>81.25 ± 0.50</td>
<td>92.50 ± 0.70</td>
</tr>
<tr>
<td>SD4 (1 : 3)</td>
<td>75.50 ± 0.25</td>
<td>91.50 ± 0.10</td>
</tr>
<tr>
<td>SD5 (1 : 4)</td>
<td>73.40 ± 0.85</td>
<td>91.75 ± 0.25</td>
</tr>
<tr>
<td>IC1 (1 : 0.5)</td>
<td>49.50 ± 0.10</td>
<td>64.00 ± 0.50</td>
</tr>
<tr>
<td>IC2 (1 : 1)</td>
<td>57.20 ± 0.30</td>
<td>78.50 ± 0.25</td>
</tr>
<tr>
<td>IC3 (1 : 2)</td>
<td>78.50 ± 0.25</td>
<td>85.50 ± 0.15</td>
</tr>
<tr>
<td>IC4 (1 : 3)</td>
<td>72.20 ± 0.50</td>
<td>86.20 ± 1.00</td>
</tr>
<tr>
<td>IC5 (1 : 4)</td>
<td>70.00 ± 0.25</td>
<td>86.80 ± 0.50</td>
</tr>
</tbody>
</table>

Table 1. Results of entrapment efficiency and product yield.
both of these parameters were ranged between 53.25 to 81.25% and 49.50 to 78.50% for SD’s and IC’s, respectively (Table 1). Optimum entrapment efficiency and product yield were observed for SD3 and IC3 formulations, i.e., 81.25 and 78.50% and 92.50 and 86.80%, respectively.

**Micromeritic properties**

In case of solid dispersions, angle of repose was found between 23.21° and 28.21° while in case of inclusion complexes it was ranged from 25.21° to 28.21° indicating overall better flow of powder blend. Bulk density of all microparticles formulations were ranged between 0.621-0.658 g/mL. Tapped density was calculated and found between 0.738-0.771 g/mL. Hausner ratio was found within 1.16 to 1.19 for all developed formulations. Similarly, Carr’s index values were noted in the range of 16.03 to 19%. Individual results are presented in Table 2.

**Particle size and zeta potential determination**

There was no net charge on fabricated microparticles thereby confirming their stability. All
the particulate complexes were having neutral value of charge on them. Microparticles were of micro-metric scale having particle size in the range of 1–1000 µm as shown in Figure 2. Optical microscopic studies also proved micrometric range. Results are shown in Figure 3.

**Fourier transform infrared spectroscopy**

FT-IR spectra of each constituent as well as finished products i.e., solid dispersions and inclusion complexes were recorded. Results are shown in Figure 4. Acyclovir FTIR spectrum was very similar to be presented in the literature exhibiting characteristic peaks of primary and secondary amines at 3438 and 3179 cm⁻¹, one C=O band was seen at 1706 cm⁻¹, two bands at 1609 and 1629 cm⁻¹ were of amino groups. O-H bands were recognized at 1541 and 1574 cm⁻¹ and the presence of band at 1344.95 cm⁻¹ confirmed ñCH group of acyclovir.

IR spectrum of β-CD showed an evident wide absorption band due to vibrations of O-H bonds in primary C-OH groups at 3292 cm⁻¹, vibrations of C-H bonds hosted in -CH and -CH₂ groups presented absorption peak at 2927 cm⁻¹ and due to vibrations of C-O bonds in ether and hydroxyl groups, peaks appeared at 1021 to 1077 cm⁻¹(16).

<table>
<thead>
<tr>
<th>Code</th>
<th>Angle of repose (°)</th>
<th>Bulk density (g/mL)</th>
<th>Tapped density (g/mL)</th>
<th>Hausner's ratio</th>
<th>Carr's index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD1</td>
<td>23.21 ± 0.769</td>
<td>0.621 ± 0.005</td>
<td>0.739 ± 0.012</td>
<td>1.19 ± 0.012</td>
<td>19.00 ± 0.772</td>
</tr>
<tr>
<td>SD2</td>
<td>26.21 ± 0.991</td>
<td>0.632 ± 0.002</td>
<td>0.738 ± 0.011</td>
<td>1.16 ± 0.005</td>
<td>16.77 ± 0.844</td>
</tr>
<tr>
<td>SD3</td>
<td>24.21 ± 0.811</td>
<td>0.637 ± 0.001</td>
<td>0.757 ± 0.002</td>
<td>1.18 ± 0.001</td>
<td>18.83 ± 0.922</td>
</tr>
<tr>
<td>SD4</td>
<td>28.21 ± 0.521</td>
<td>0.661 ± 0.001</td>
<td>0.767 ± 0.004</td>
<td>1.16 ± 0.002</td>
<td>16.03 ± 0.976</td>
</tr>
<tr>
<td>SD5</td>
<td>25.80 ± 0.432</td>
<td>0.665 ± 0.003</td>
<td>0.782 ± 0.015</td>
<td>1.17 ± 0.003</td>
<td>14.96 ± 0.784</td>
</tr>
<tr>
<td>IC1</td>
<td>27.21 ± 0.724</td>
<td>0.639 ± 0.002</td>
<td>0.758 ± 0.004</td>
<td>1.18 ± 0.003</td>
<td>18.62 ± 0.916</td>
</tr>
<tr>
<td>IC2</td>
<td>28.21 ± 0.715</td>
<td>0.645 ± 0.003</td>
<td>0.749 ± 0.001</td>
<td>1.16 ± 0.001</td>
<td>16.12 ± 0.736</td>
</tr>
<tr>
<td>IC3</td>
<td>25.21 ± 0.803</td>
<td>0.642 ± 0.004</td>
<td>0.760 ± 0.003</td>
<td>1.18 ± 0.002</td>
<td>18.38 ± 0.806</td>
</tr>
<tr>
<td>IC4</td>
<td>28.21 ± 0.759</td>
<td>0.658 ± 0.003</td>
<td>0.771 ± 0.001</td>
<td>1.17 ± 0.001</td>
<td>17.17 ± 0.772</td>
</tr>
<tr>
<td>IC5</td>
<td>23.65 ± 0.673</td>
<td>0.672 ± 0.001</td>
<td>0.789 ± 0.003</td>
<td>1.17 ± 0.002</td>
<td>14.82 ± 0.638</td>
</tr>
</tbody>
</table>

S.D. (Standard deviation) ± Mean, n = 3.
Thermal analysis (DSC and TGA)

DSC thermogram of β-CD has shown two endothermic peaks at 81.25 and 324.95°C corresponding to loss of moisture of crystallization and phase transition (solid to liquid), respectively. One exothermic peak was originated at 382.31°C, while in TGA curves of β-CD, three step mass losses were seen i.e., at 109.44°C and 3.95 min there was only 13.03% weight loss occurred, above melting point 326.86°C and 14.86 min 18.35% mass loss occurred and at 359.21°C and 16.44 min mass loss observed was 84.28%. DSC of ACV has depicted less prominent peak at 90.88°C that relates to loss of moisture and broad endothermic peak at 256.80°C (with enthalpy variation of 0.05210 J/g) presenting its melting point. In TGA, prominent decomposition was observed at 268.69°C (Δm = 93.89%) (17).

Results for fabricated IC’s and SD’s have been shown in Figure 5.

Powder X-ray diffraction analysis (PXRD)

ACV exists in the literature with six different crystal morphologies i.e., I, II, II, IV, V and VI. Nature of pure ACV powder, crystalline or amorphous was confirmed by PXRD studies. ACV has proved its crystalline nature due to prominent peaks at 2θ = 10.34°, 23.65°, 25.98°, 26.39° and 29.15°. Crystalline nature of CD was proved by the presence of sharp peaks at 2θ = 10.34°, 17.45°, 24.85°, 28.75°, 36.15° and 42.27° (17). All the results are shown in Figure 6.

Scanning electron microscopy (SEM)

Photomicrographs of IC’s and SD’s were scanned at various powers were taken to analyze

![Figure 4. FTIR spectra of A - β-cyclodextrin, B - acyclovir, C - sodium lauryl sulfate, D - physical mixture, E - solid dispersions and F - inclusion complexes](image-url)
surface morphology. Irregular shape and size variant microparticles were seen but these were within pharmacopoeial limits of micrometric scale. All the results are presented in Figure 7.

**Transmission electron microscopy (TEM)**

Microparticles shape and more close morphology was seen in transmission electron microscopy images. Solid dispersions were dense carrying ACV with irregular shape but with definite boundaries, on the other hand, inclusion complexes were more uniform than solid dispersions. All results have been included in Figure 8.

**Dissolution studies**

Dissolution studies were conducted to assess release behavior at different pH values (pH 1.2 and 7.4). Slightly lower release profiles were seen at pH 7.4 as compared to pH 1.2 depending upon degree of ionization. SD’s and IC’s exhibited 90.75% and 93.12% at pH 1.2, respectively, while in case of ACV capsule drug release was only 59% during 3 h of study. At pH 7.4, 88.75% and 92.50% release was seen in case of inclusion complexes and solid dispersions, respectively. Results of best formulations i.e., IC3 and SD3 along with marketed product (Zaclovir) are shown in Figure 9. Drug release kinetic models i.e., zero order, first order, Higuchi and Korsmeyer Peppas were applied on data obtained up to maximum release period i.e., 60 min by using DD Solver® Excel based Add in program. Results are shown in Table 3.

**Permeability studies**

ACV is weakly permeable across intestinal mucosa. To overcome such issue, fixed quantity...
(4%) of sodium lauryl sulfate was incorporated into each ratio of finished microparticles. Optimum percentage of diffused drug was noted in IC3 and SD3 i.e., 82.05% and 85.18%, while Zalcovir marketed capsule has only shown 36.89% diffusion. Results are presented in Figure 10.

Stability studies
No change in dissolution, permeability profile and physical morphology was observed after stability studies.

DISCUSSION

Solubility studies
At pH 1.2, reason for high solubility was that at this pH ACV exists in ionized form. Moreover, SLS incorporated for permeation enhancement has excellent wetting, solubility, dissolution and stability properties. It might have contributed for enhanced solubility of ACV with rising ratios of β-CD. Solubility studies revealed 1 : 1 complex formation in aqueous solution because of stability constant obtained from phase solubility diagram. Kumari et al. have made efforts for solubility enhancement of nelfinavir mesylate using different ratios of β-CD in the form of solid dispersions and inclusion complexes. At pH 1.2 there was 5.32-fold rise in solubility while at higher pH 3.64-fold rise was seen that supported solubility results of present study (18).

Entrapment efficiency and product yield
Entrapment efficiency of SD’s and IC’s was increased from 53.25% to 81.25% and 49.50% to 78.50%, respectively (1 : 0.5 to 1 : 2), after that a decline was noted in case of 1 : 3 and 1 : 4. Main reason behind this was that increasing polymeric content results in more compact or packed polymer coat that limit further entrapment of ACV. Product yield was 92.50%, 86.80% for SD3 and IC3. Study conducted by Tummala et al. exhibited higher entrapment of 5-flurouracil (69.18%) within polymeric network up to 1 : 3 drug-polymer ratio, then there
was a decline in results of entrapment efficiency (32.18%) (11).

**Micrometric properties**

Angle of repose is associated with density, surface area and friction coefficient of tested powders. All prepared microparticles were having angle of repose 23.21 to 28.21° that was less than official limit of 30° exhibiting excellent flowability. Bulk and tapped densities proved better flow due to more dense particles, on the other hand, if particles are fine (< 100 µm) cohesiveness occurs. Hausner ratio was less than 1.25 confirming good flow of microparticles. Similarly, Carr’s compressibility
index values i.e., 16.03% to 19% also favored flow criteria. Results of micromeritic properties of Sarfraz et al. i.e., angle of repose (22.40 to 26.80°), bulk and tapped densities (less than 1 g/mL), Hausner ratio (less than 1.21) and Carr’s index (14 to 17.51%) were according to official limits as in this study (12).

Particle size and zeta potential determination

Particle size was variable with polymer (β-CD) concentration, degree of agitation and stirring speed. Microparticles were of irregular shape but ranged within the micrometric scale (1-1000 µm). Microscopic evaluation also proved micro-sized particles. Due to smaller size and larger surface area, microparticles major part of atoms become closer to core and also become more reactive that ultimately pushes solubility profile to higher values. Zeta potential also effect particle size as it indicates repulsion between particles. Neutral values assure stability of products while lower values results in aggregation. It was neutral among 0 to 40000 counts thereby proving stability of microparticles. Stable microparticles can easily be dispersed that enhances the solubility of incorporated therapeutic moiety.

Fourier transform infrared spectroscopy

FTIR-spectrum of β-CD and ACV physical mixture, peaks position and shapes were carried out. In case of IR-spectrum of solid dispersions, C-OH absorptive peak was observed at 3287 cm⁻¹, C-H (2923 cm⁻¹), amine groups (1597 and 1697 cm⁻¹). IR spectrum of inclusion complexes have shown C-OH absorptive peak at 3306 cm⁻¹ and amino groups have represented their identity at 1629 and 1717 cm⁻¹. These shifting of peaks demonstrated complex formation.
between ACV and β-CD. A study by Marinescu et al. revealed the presence of β-CD characteristic transmittance peaks at C-OH (3368 cm⁻¹) and C-H (2927 cm⁻¹). In complexed form peak shifting and variability in intensities was seen accordingly (16).

**Thermal analysis**

DSC of IC’s revealed that exothermic peak at 81.25°C was absent and peak at 382.31°C was shifted to 380.04°C. In TGA there was only 0.44% mass loss in first 3.56 min, 4.22% up to 12.24 min and 15.46% in 13.88 min as compared to TGA of pure β-CD. In case of SD’s, exothermic peak at 382.31°C was absent and peaks at 81.25°C, 324.95°C were shifted to 92.88°C and 242.22°C in DSC studies. In TGA of SD’s, there was only 0.16, 7.20 and 14.14% mass loss occurred at 3.48, 10.77 and 14.19 min. ACV peak at 256.60°C was absent in both cases ensuring that it was encapsulated into β-CD cavity and stability was promoted.

**Powder X-ray diffraction analysis (PXRD)**

PXRD of formulated microparticles was performed to check status of crystallinity. Relatively, semi crystalline nature was seen due to peaks lower intensities in case of inclusion complexes, while in the case of solid dispersions, different patterns with least or uniform peak intensities were seen thereby proving amorphous state.

**Scanning electron microscopy (SEM)**

SEM images revealed that IC’s and SD’s were of irregular shape having enlarged surface area. Micrometric size range and high surface area occurred due to size reduction, which has prominent effect on solubility enhancement and rapid release of active ingredient.

**Transmission electron microscopy (TEM)**

Transmission electron microscopy proved successful loading of ACV in microparticles, size range within micrometric scale and shape of developed ACV microparticles.

**Dissolution studies**

Dissolution studies of all developed ratios were performed at pH 1.2 and pH 7.4 to check dependence upon solubility, degree of ionization, pKa values and also to evaluate release pattern in gastric fluid and in intestinal region. Higher release or % ACV in dissolved form was noticed in case of SD’s i.e., 93.12 and 90.75% at pH 1.2 in the first hour. Slightly lower release results occurred in case of IC’s i.e., 92.5 and 88.75% at pH 7.4, respectively, within first 60 min. At higher pH values, dissolution of ACV from SD’s and IC’s was increased as compared to free/pure ACV due to impact of β-CD and manufacturing techniques. So physical binding of ACV in the form of solid dispersions and inclusion complexes imparted a positive effect. In-vitro drug release kinetic declared first order model as best fit model depending upon R² value i.e., SD3 (0.9937), IC3 (0.9939) and Cap. Zaclovir (0.9928). According to Korsmeyer Peppas model, values of (n) were 0.254 (SD3) and 0.300 (IC3), while in the case of ACV tablet 0.764 so proving Fickian diffusion while tablets induces anomalous transport. Pedotti et al. concluded that dissolution profile of complexed ACV was greater at high pH when compared with pure ACV. Similarly, complexed ACV has shown even higher dissolution at pH 1.2 (19).

**Permeability studies**

SLS was incorporated in a fixed amount (4% of total weight of each formulation). β-CD enhances bioavailability of ACV at absorptive surface, while SLS causes opening of tight junctions, extract intracellular lipids or prevent active efflux ultimately promoting permeability of ACV across intestinal membrane. Moreover, at higher pH values ACV exists in unionized form that has more capability to cross intestinal mucosa. Higher permeability results were seen in case of SD’s 85.18% while Zaclovir exhibited only 36.89% diffusion. Dias et al. have shown that by increasing surfactant concentration, permeability of ACV increases markedly due to an increase of partition coefficient that results in higher ACV content penetration (15).

**CONCLUSION**

Co-incorporation of β-CD and SLS into developed microparticles (SD’s & IC’s) significantly promoted solubility, bioavailability and permeability of acyclovir. Simple techniques i.e., kneading method and solvent evaporation technique were utilized that ultimately resulted in reduced cost of therapy, improvement of patient compliance and decrease of dosing frequency. This approach can also be utilized for other BCS Class IV drugs of particular interest.

**REFERENCES**


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