**CHAPTER-5**

**FORMULATION, *IN VITRO* AND *IN VIVO* EVALUATION OF OPTIMIZED IMMEDIATE RELEASE PRODUCTS OF CLOPIDOGREL BISULPHATE AND DOLUTEGRAVIR SODIUM**

**EXPERIMENTAL METHODOLOGY OF CLOPIDOGREL BISULPHATE AND DOLUTEGRAVIR SODIUM**

Preparation of optimized formulation of CBS & DTG immediate release tablets, there *in vitro* studies and *in vivo* pharmacokinetic studies of optimized and marketed formulations are discussed in the following sections:

5.1. Preparation of optimized immediate release formulation tablets

5.2. Comparison of optimized formulations with marketed formulations

5.3. *In vivo* pharmacokinetic studies of optimized and marketed formulations

**5.1. PREPARATION OF OPTIMIZED IMMEDIATE RELEASE FORMULATION TABLETS**

**5.1.1. PREPARATION OF OPTIMIZED FORMULATION OF CBS IMMEDIATE RELEASE TABLETS**

Solid inclusion complexes prepared by kneaded complex of CBS and HP-β-CD with soluplus (**1:1:1)** was selected as the optimized ratio for the formulation of tablets and the ANN predicted values for this formulation were in close agreement with the observed experimental values. Tablets weighing 300 mg equivalent to 75 mg of CBS were prepared by direct compression method. The ternary complex i.e., drug, cyclodextrin and soluplus polymer and diluents were passed through sieve no #22 (approximate particle size is 0.71 mm). All the above ingredients were properly mixed together in a polybag. Mannitol and magnesium stearate were passed through sieve #60, mixed and blended with initial mixture in a polybag. The powder blend was compressed into tablets by using a 16- station rotary tablet compression machine on 9 mm flat faced punches. The weight of the tablet was kept constant to 300mg. The composition of the tablet prepared from optimized formulation **C34** is shown in the **Table 5.1.1.1**.

**Table 5.1.1.1: Formulation of the optimized product (C34)**

|  |  |  |
| --- | --- | --- |
| **S. No** | **Composition** | **Quantity per tablet (mg)** |
| 1. | Complex of Clopidogrel Bisulphate- HP-β-CD- Soluplus  (75:75:75) | 225 |
| 2. | Mannitol | 45 |
| 3. | Micro crystalline cellulose | 24 |
| 4. | Cross povidone | 2.4 |
| 5. | Sodium lauryl sulphate | 1.2 |
| 6. | Magnesium stearate | 2.4 |
| 7. | **Total weight** | **300 mg** |

**5.1.2. PREPARATION OF OPTIMIZED FORMULATION OF DTG IMMEDIATE RELEASE TABLETS**

Solid inclusion complexes prepared by kneaded complex of DTG and HP-β-CD with soluplus **(1:2:1.5)** where the predicted values by the ANN model provided relatively accurate results was selected for the formulation of tablets. Tablets weighing 300 mg equivalent to 50mg of DTG sodium were prepared by direct compression method. The ternary complex i.e., drug, cyclodextrin and soluplus polymer and diluents were passed through sieve no #22 (approximate particle size is 0.71 mm). All the above ingredients were properly mixed together in a polybag. Talc and magnesium stearate were passed through sieve #60, mixed and blended with initial mixture in a polybag. The powder blend was compressed into tablets by using a 16- station rotary tablet compression machine on 9 mm flat faced punches. The weight of the tablet was kept constant to 300 mg. The composition of the tablet prepared from optimized formulation **F35** is shown in the **Table 5.1.2.1**.

**Table 5.1.2.1: Formulation of the optimized product (F35)**

|  |  |  |
| --- | --- | --- |
| **S. No** | **Composition** | **Quantity per tablet (mg)** |
| 1. | Complex of Dolutegravir sodium- HP-β-CD- Soluplus  (50:100:75) | 225 |
| 2. | Micro Crystalline Cellulose | 65.5 |
| 3. | Cross povidone | 2.5 |
| 4. | Sodium lauryl sulphate | 1.0 |
| 5. | Talc | 3.0 |
| 6. | Magnesium stearate | 3.0 |
| 7. | **Total weight** | **300 mg** |

**EVALUATION OF GRANULES**

Before tablet preparation, the mixture blend was assessed by pre-compression parameters like bulk density, tapped density, compressibility index, Hausner’s ratio and angle of repose. All the experiments were done in triplicate and the results are expressed as mean ± S.D as shown in **Table 5.1.1.2** for clopidogreland in **Table 5.1.2.2** for dolutegravir.

**Pre compression parameters**

**Angle of Repose**

Angle of repose was determined using fixed funnel method1. The powders were allowed to flow through the funnel fixed on a burette stand at definite height (h). The angle of repose (θ) was then calculated by measuring the height (h) and radius (r) of the heap of granules formed using the following formula:

Tan θ=h/r

Where, θ= angle of repose

h=height of the pile

r= radius of the pile

**Bulk density**

The bulk density of powder is dependent on particle packing and changes as powder consolidates1. Apparent bulk density was determined by pouring a weighed quantity of powder into a graduated cylinder and measuring the volume of packing.

Bulk density = Weight of the powder/ Bulk volume of the packing

**Tapped density**

Tapped density is defined as the mass of a powder divided by the tapped volume1. Tapped density was determined by tapping method. Weighed quantity of powder was placed in a graduated cylinder and tapped until no further change in volume of powder was seen, and then the volume of tapped packing was noted.

Tapped density = Weight of the powder/volume of the tapped packing

**Compressibility index**

The compressibility index of the powder was determined by the compressibility index equation1:

Carr’s index (%) = (Tapped density- Bulk density) × 100

Tapped density

**Hausner’s ratio**

Hausner’s ratio (H) is an index of ease of powder flow1. It is also called packing factor and 1calculated by the following formula:

Hausner's ratio = Tapped density /Bulk density

**EVALUATION OF TABLETS**

The prepared tablets were subjected to post compression parameters like hardness, friability, weight variation, drug content, wetting time, *in vitro* disintegrating time and *in vitro* dissolution rate and the results are expressed as mean ± S.D. in **Table 5.1.1.3** for clopidogreland **Table 5.1.2.3** for dolutegravir

**Hardness**

Hardness of the tablets was tested using a Monsanto hardness tester. Five tablets from each batch were tested for hardness2.

**Thickness**

Thickness of the tablets was determined using vernier callipers2. Five tablets from each batch were used, and an average value was calculated.

**Friability**

Friability of the tablets was determined in a Roche friabilator2, 3. 6.5g of sample should be taken, as our tablet weight is less than 650mg. Tablets were weighed initially (w1) and placed in the friabilator that revolves at a speed of 25 rpm, dropping those tablets at a distance of six inches height with each revolution and rotated in the friabilator for 100 revolutions. After the completion of rotations, tablets were dedusted and weighed (w2). The percent loss in weight or friability (f) is calculated by using the formula:

% F = (w1-w2)/w2×100

The experiment was done in duplicate in the values are expressed as mean ± S.D.

**Weight variation test**

Twenty tablets were randomly selected from each batch and individually weighed2. The average weight and standard deviation of twenty tablets were calculated and represented as mean ± S.D value.

**Drug content estimation**

Ten tablets were taken and the amount of drug present in each tablet was determined as per the below procedure.

Tablet was crushed in a mortar and was transferred to a 100 mL volumetric flask. The powder sample was dissolved in a solution of pH 3.0 and was mixed by using Remi mixer for 5 minutes. The sample was then filtered through a Whatman’s filter paper. The filtered solution, after appropriate dilution (1 to 10 mL) with 0.1 N HCl buffer was analyzed by the validated UV-Spectrophotometric method at λ max of 220nm for CBS and 258 nm for DTG. The experiment was done in triplicate and results are expressed as mean ± S.D.

***In vitro* disintegration time**

The process of breakdown of tablet into smaller pieces is called disintegration. *In vitro* disintegration time was performed by apparatus specified in USP4. Distilled water was used as the medium for disintegration medium. The temperature was maintained at 37± 2ºC and the time in seconds taken for the complete disintegration of the tablet, with no palpable mass remaining in the apparatus, was measured and represented.

***In vitro* dissolution studies**

*In vitro* dissolution study was performed by using USP type II dissolution test apparatus (paddle type) [Lab, India (DS-8000), Mumbai] at 75 rpm5. A volume of 900mL containing 0.1 N HCl with 0.5% SLS was used as dissolution medium which was maintained at 37±0.5ºC. Aliquots of samples (5mL) were withdrawn at specific time intervals up to 1 hour and were filtered. Fresh sample (5mL) of dissolution medium was replaced into the dissolution vessel in order to maintain the sink condition. The amount of drug dissolved was determined by UV spectrophotometer by measuring the absorbance of the sample at 220 nm for CBS and at 258 nm for DTG. Studies were performed in triplet and the average percentage drug release with standard deviation was calculated and reported.

**RESULTS**

Pre compression parameters of granules and post compression parameters of CBS tablets are given in **Table 5.1.1.2 and Table 5.1.1.3.**

**Table 5.1.1.2: Evaluation of pre-compression parameters of CBS granules**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Formulation** | **Angle of repose (θ)\*** | **Bulk density\***  **(gm/cm3)** | **Tapped density\* (gm/cm3)** | **Compressibility Index\* (%)** | **Hausner’s ratio\*** |
| **OPTIMIZED (C 34)** | 28.23± 0.03 | 0.51 ± 0.30 | 0.62 ± 0.01 | 15.67 ± 0.28 | 1.19 ± 0.48 |

\* mean ±S.D, n=3

**Table 5.1.1.3: Evaluation of post-compression parameters of CBS tablets**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Formulation** | **Thickness\***  **(mm)** | **Hardness\* (Kg/cm2)** | **Friability\*\* (%)** | **Weight variation\*\* (%)** | **Drug content # (%)** | **Disintegration time## (sec)** |
| **OPTIMIZED (C 34)** | 3.05 ± 0.16 | 3.8 ± 0.31 | 0.37± 0.32 | 2.1± 0.34 | 96.04± 0.64 | 142± 3 |

\* mean ±S.D, n=5

\*\* mean ±S.D, n=20

# mean ±S.D, n=3

## mean ±S.D, n=6

Pre compression parameters of granules and post compression parameters of DTG tablets are given in **Table 5.1.2.2 and Table 5.1.2.3**

**Table 5.1.2.2: Evaluation of pre-compression parameters of DTG granules**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Formulation** | **Angle of repose (θ)\*** | **Bulk density\***  **(gm/cm3)** | **Tapped density\* (gm/cm3)** | **Compressibility Index\* (%)** | **Hausner’s ratio\*** |
| **OPTIMIZED (F 35)** | 27.31± 0.30 | 0.36 ± 0.15 | 0.59 ± 0.01 | 12.23 ± 0.82 | 1.22 ± 0.01 |

\* mean ±S.D, n=3

**Table 5.1.2.3: Evaluation of post-compression parameters of DTG tablets**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Formulation** | **Thickness\***  **(mm)** | **Hardness\* (Kg/cm2)** | **Friability\*\* (%)** | **Weight variation\*\* (%)** | **Drug content # (%)** | **Disintegration time## (sec)** |
| **OPTIMIZED (F 35)** | 2.85± 0.04 | 3.20± 0.06 | 0.34± 0.32 | 1.19± 0.24 | 97.56± 0.45 | 135±1 |

\* mean ±S.D, n=5

\*\* mean ±S.D, n=20

# mean ±S.D, n=3

## mean ±S.D, n=6

**5.2. COMPARISON OF OPTIMIZED AND MARKETED FORMULATIONS**

*In vitro* dissolution profiles of optimized and marketed formulations were compared along with predicted responses obtained through ANN and the results are shown in the **Table 5.2.1** to **5.2.2** and **Figure 5.2.1** to **5.2.2** for CBS tablets and the results of DTG tablets are shown in the **Table 5.2.3 to 5.2.4** and **Figure 5.2.3 to 5.2.4**.

**Fit factor test (f1 and f2)**

Under appropriate test conditions, a dissolution profile can characterize the product more precisely than a single point dissolution test. Moore and Flanner proposed a model independent mathematical approach to compare the dissolution profile using two factors, f1 and f26,7.

https://1.bp.blogspot.com/-PPervl5Th_E/Wo2Cm4xHnqI/AAAAAAAAAJs/lsHd8X9RKu0mVcWmjjE_mR4Jajgf_y4dQCLcBGAs/s1600/F1%2Bfactor.PNG

https://4.bp.blogspot.com/-4YxFglErExs/Wo2C6NROf-I/AAAAAAAAAJw/ivkp2kzHqOEan1Suv0YUQZPzyMjCVkXoQCLcBGAs/s1600/f2%2Bfactor.PNG

Where Rt = cumulative percentage dissolved at time point t for reference product

Tt = cumulative percentage dissolved at time point t for test product

n = number of pool points.

The factor f1 is proportional to the average differences between the two profiles, whereas factor f2 is inversely proportional to the average squared difference between the two profiles, with emphasis on the larger difference between the time points. The factor f2 measures the closeness between the two profiles. Because of the nature of measurement f1 was described as difference factor, and f2 as similarity factor. For curves to be considered similar, f1 values should be close to 0, and f2 values should be close to 100. Generally, f1 values up to 15 (0-15) and f2 values greater than 50 (50-100) ensure sameness or equivalence of the two curves and thus of the performance of the test and reference products.

The independent method is most suitable for dissolution profile comparison when three to four or more dissolution time points are available. As further suggestions for the general approach, the following recommendations should also be considered:

* The dissolution measurements of the test and reference batches should be made under exactly the same conditions.
* The dissolution time points for both the profiles should be same (e.g., 10, 20, 30, 40, 50 and 60 min).

Application of fit factor tests (f1 and f2), under appropriate test conditions is more suitable than a single point dissolution test for the accurate characterization of a dissolution profile. The *in vitro* release profile of optimized and innovator product of clopidogrel bisulphate tablets were compared for dissimilarity factor (f1) and similarity factor (f2).

**COMPARISON OF OPTIMIZED AND MARKETED FORMULATION OF CBS TABLETS**

The results for comparison of optimized and marketed formulation of CBS tablets are given below

**Table 5.2.1: Comparison of *in vitro* dissolution profiles of optimized and marketed formulation of clopidogrel bisulphate**

|  |  |  |  |
| --- | --- | --- | --- |
| **Time**  **(min)** | **% Drug release for clopidogrel bisulphate** | | |
| **Optimized** | **Predicted** | **Marketed** |
| 10 | 54.60 | 62.82 | 16.48 |
| 20 | 71.82 | 78.12 | 36.74 |
| 30 | 85.29 | 91.03 | 52.12 |
| 40 | 92.56 | 94.44 | 85.98 |
| 50 | 94.61 | 97.02 | 92.09 |
| 60 | 100.43 | 101.56 | 97.46 |

C:\Users\user\Desktop\prasanti Plots\27 Jan 2020\Excel Plots\Fig9.emf

**Figure 5.2.1: Comparison of dissolution profiles of optimized formulated CBS, predicted values obtained through ANN and marketed formulation of CBS tablets.**

**C:\Users\user\Desktop\prasanti Plots\27 Jan 2020\Excel Plots\Fig10.emf**

**Figure 5.2.2: Comparison of first order dissolution plots of optimized formulated CBS, predicted valves obtained through ANN and marketed formulation of CBS tablets**

**Table 5.2.2: Drug release kinetics of optimized and marketed formulations of CBS**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **PRODUCT** | **ZERO ORDER** | | **FIRST**  **ORDER** | | | **DE30%** |
| K0 | r | K1 (min1) | r | T50 (min) |
| OPTIMIZED | 0.870 | 0.974 | 0.057 | 0.995 | 11.58 | 39.38 |
| PREDICTED | 0.801 | 0.948 | 0.059 | 0.994 | 12.05 | 38.98 |
| MARKETED | 0.978 | 0.951 | 0.006 | 0.972 | 14.90 | 29.99 |

**COMPARISON OF OPTIMIZED AND MARKETED FORMULATION OF DTG TABLETS**

The results for comparison of optimized and marketed formulation of DTG tablets are given below

**Table 5.2.3: Comparison of *in vitro* dissolution profiles of optimized and marketed formulation of dolutegravir sodium**

|  |  |  |  |
| --- | --- | --- | --- |
| **Time**  **(min)** | **% Drug release for dolutegravir sodium** | | |
| **Optimized** | **Predicted** | **Marketed** |
| 10 | 53.57 | 58.86 | 21.23 |
| 20 | 74.14 | 77.12 | 35.55 |
| 30 | 86.64 | 90.11 | 52.18 |
| 40 | 95.02 | 91.50 | 70.25 |
| 50 | 97.52 | 96.08 | 85.49 |
| 60 | 100.23 | 101.42 | 90.23 |

C:\Users\user\Desktop\prasanti Plots\27 Jan 2020\Excel Plots\fig11.emf

**Figure 5.2.3: Comparison of dissolution profiles of optimized formulated DTG, predicted values obtained through ANN and marketed formulation of DTG tablets.**

**C:\Users\user\Desktop\prasanti Plots\27 Jan 2020\Excel Plots\Fig12.emf**

**Figure 5.2.4: Comparison of first order dissolution plots of optimized formulated DTG, predicted valves obtained through ANN and marketed formulation of DTG tablets**

**Table 5.2.4: Drug release kinetics of optimized and marketed formulations of DTG**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **PRODUCT** | **ZERO ORDER** | | **FIRST**  **ORDER** | | | **DE30%** |
| K0 | r | K1 (min1) | r | T50 (min) |
| OPTIMIZED | 0.890 | 0.960 | 0.066 | 0.994 | 10.37 | 39.58 |
| PREDICTED | 0.874 | 0.934 | 0.062 | 0.985 | 11.14 | 40.24 |
| MARKETED | 1.165 | 0.990 | 0.043 | 0.982 | 15.83 | 30.28 |

For the drug clopidogrel bisulphate designed formulation, blend of drug (CBS: HP-β-CD: soluplus) ternary complex and excipients was prepared and evaluated for pre compression properties as shown in **Table 5.1.1**.**2** Bulk density was found to be **0.51 ± 0.30 gm/cm3** and tapped density was found to be **0.62 ± 0.01gm/cm3**. From the density data, % compressibility was calculated and was found to be **15.67% ± 0.28.**  Hausner’s ratio was found to be **1.19 ± 0.48** and angle of repose was found to be **28.23 ± 0.03** **θ0**. All the parameters are within the prescribed limits and indicating good flow property. Hence, tablets were prepared by using direct compression technique.

The data obtained for post compression parameters such as hardness, friability, weight variation, uniformity of content, thickness, and disintegration time are shown in **Table 5.1.1.3.** The hardness was found to be **4.2 ± 0.31 Kg/cm3** indicating good mechanical strength to withstand physical and mechanical stress conditions while handling. The thickness of the tablet was found to be **3.05 ± 0.16 mm**. The friability value was found to be less than 1%. The % drug content was found to be **96.04% ± 0.64** and percentage weight variation was found to be 2.1% with a low standard deviation of 0.34.

The results of *in vitro* dissolution studies of optimized and for innovators are shown in **Table 5.2.1**, the dissolution of CBS followed first order kinetics. Plots of log % drug remaining Vs time were found to be linear. From the slopes of linear plots, the dissolution rates were calculated. The dissolution kinetics data are given in **Table 5.2.2**, the dissolution rate constant k1 and DE30% for **C 34** was found to be **0.995 and 39.38**.

The *in vitro* release profiles of optimized and optimized formulations were compared for f1 and f2. The values **f1=12.32** and **f2=59.58**, show that there is similarity between both the profiles and it can be observed from **Figure 5.2.1** that optimized formulation is showing better dissolution profile compared to marketed product.

The *in vitro* drug release kinetic data is shown in **Table 5.2.2**.The correlation coefficient of first order kinetics was greater than the correlation coefficient of the zero order kinetics indicating that the drug release followed first order kinetics. The release rate constant of optimized was found to be higher than the marketed product. Dissolution efficiency values of optimized and marketed DE30% were calculated from the dissolution data. The % DE30 value **(39.38)** for the optimized formulation was found to be higher than marketed product **(29.99)**.

For the drug dolutegravir sodium designed formulation, blend of drug (DTG: HP-β-CD: soluplus) ternary complex and excipients was prepared and evaluated for pre compression properties as shown in **Table 5.1.2**.**2** Bulk density was found to be **0.36 ± 0.15 gm/cm3** and tapped density was found to be **0.59 ± 0.01gm/cm3**. From the density data, % compressibility was calculated and was found to be **12.23% ± 0.61.**  Hausner’s ratio was found to be **1.22 ± 0.01** and angle of repose was found to be **27.23 ± 0.3** **θ0**. All the parameters are within the prescribed limits and indicating good flow property. Hence, tablets were prepared by using direct compression technique.

The data obtained for post compression parameters such as hardness, friability, weight variation, uniformity of content, thickness, and disintegration time are shown in **Table 5.1.2.3.** The hardness was found to be **3.2 ± 0.06 Kg/cm3** indicating good mechanical strength to withstand physical and mechanical stress conditions while handling. The thickness of the tablet was found to be **2.85 ± 0.04 mm**. The friability value was found to be less than 1%. The % drug content was found to be **97.56% ± 0.43** and percentage weight variation was found be 1.19% with a low standard deviation of 0.24.

The results of *in vitro* dissolution studies of optimized and for marketed are shown in **Table 5.2.3**, the dissolution of DTG followed first order kinetics. Plots of log % drug remaining Vs time were found to be linear. From the slopes of linear plots, the dissolution rates were calculated. The dissolution kinetics data are given in **Table 5.2.4**, the dissolution rate constant k1 and DE30% for **F35** was found to be **0.994 and 39.59**.

The *in vitro* release profiles of optimized and marketed formulations were compared for f1 and f2. The values **f1=10.38** and **f2=55.48**, show that there is similarity between both the profiles and it can be observed from **Figure 5.2.3** that optimized formulation is showing better dissolution profile compared to marketed product.

The *in vitro* drug release kinetic data is shown in **Table 5.2.4**.The correlation coefficient of first order kinetics was greater than the correlation coefficient of the zero order kinetics indicating that the drug release followed first order kinetics. The release rate constant of optimized was found to be higher than the marketed product. Dissolution efficiency values of optimized and innovator’s DE30% were calculated from the dissolution data. The % DE30 value **(39.58)** for the optimized formulation was found to be higher than the marketed product **(30.28)**.

**5.3. *IN VIVO* PHARMACOKINETIC STUDIES OF OPTIMIZED AND MARKETED FORMULATIONS**

The ability to rapidly generate (absorption, distribution, metabolism, elimination) ADME data is vital at all stages of the drug discovery process. Preliminary stage metabolism and pharmacokinetic data can provide guidance to discovery stage medicinal chemistry program, leading to selection of development candidate with optimized ADME properties. In the progression from drug discovery to development, success rate increasingly relies on the ability to rapidly identify quality molecules that possess the desired attributes of bioavailability, chemical tractability, selectivity and potency. The technological advances like combinatorial chemistry and functional genomics have now given drug discovery scientists the ability to deliver a large number of lead compounds for final optimization/selection. Turning a chemical lead into a marketable drug requires a balance of potency, safety and pharmacokinetics; which are traditionally low throughput processes. Safety and pharmacokinetic studies can be expedited by higher throughput approaches (cassette dosing, sample pooling) to sample preparation and analysis, and also by decreased time to assay development and validation. The objective of the pharmacokinetic studies is to describe the time course of drug concentration in blood in mathematical terms. It helps

1. To evaluate the performance of pharmaceutical dosage forms in terms of the rate and amount of drug they deliver to the blood
2. To adjust the dosage regimen to produce and maintain therapeutically effective blood concentrations with minimal or no toxicity.

*In vivo* pharmacokinetic studies for the optimized formulation and marketed formulations were carried out as follows:

**Animals**

Young, healthy male Wistarrats weighing 250±10 gm were obtained from the institutional animal house and were housed in appropriate stainless steel cages in standard laboratory conditions with regular 12hr day-night cycle in well-ventilated room with an average temperature of 25-28°C and relative humidity of 40-60%. Standard pelleted laboratory chow (Goldmohar Laboratory Animal Feeds, Chandigarh, India), food and water allowed *ad libitum.* Ethical guidelines for maintenance and experimental studies with animals were followed. All experiments were conducted in accordance with the guidelines laid by local ethical committee of the institute for animal experiments. The study protocols were also approved by the Institutional Animal Ethics Committee (IAEC) bearing the registration number: ECR/993/AP/Re/S/06.

**5.3.1. Clopidogrel bisulphate Formulations**

**Reference formulation**

The marketed product (Plavix) contained 97.875 mg of clopidogrel bisulphate per tablet which is equivalent to 75 mg of clopidogrel. It also contains hydrogenated castor oil, hydroxypropyl cellulose, mannitol, microcrystalline cellulose and polyethylene glycol 6000, ferric oxide, hypromellose 2910, lactose monohydrate, titanium dioxide and triacetin.

**Test formulation**

Formulation **C-34**, containing 75 mg of clopidogrel per tablet. This optimized formulation contains the drug, HP-β-CD and soluplus in the ratio **1:1:1** and also contains mannitol, micro crystalline cellulose, cross povidone, sodium lauryl sulfate and magnesium stearate.

**Experimental design**

A cross over, non-blinded, open labelled experimental design was followed. Six rats received one treatment (sample). Blood samples were taken from each rat over a period of three hours. Both the samples were tested in all the six rats during the study.

***In vivo* study protocol**

* Young, healthy male Wistarrats weighing 250±10 gm, were fasted overnight with free access to water for at least 12 hr before dosing.
* The optimized formulation was disintegrating in an average time of 142 seconds.
* Hence, it was reasoned that, triturating the tablet into a powder and taking the weight of powder (6.72mg) equivalent to the calculated dose of the drug was an acceptable way of administration of the formulation to the animal.

CBS tablet powder was suspended in an aqueous solution. It was administered orally by catheter to the rats in a dose of approximately 6.72 mg calculated as per the body weight of each rat. The blood samples (approximately 500 µL) were collected in heparinised tubes at 0 hr, 0.5, 1.0, 1.5, 2.5, 4, 6, 8, 10, 12, 14, 16 and 18 hrs respectively. After collection, the samples were immediately placed in an ice flask till further use. All samples were centrifuged at 2500 RPM for 10 min. The plasma was separated into clean tubes and was frozen at -20ºC till further use. CBS content was determined by the HPLC method.

CBS concentrations in plasma following the administration of marketed and optimization products are given in **Table 5.3.1.1** and **5.3.1.2** and are shown in **Figure 5.3.1.1**. The results are tabulated in **Table 5.3.1.3.**

**5.3.2. Dolutegravir sodium Formulations**

**Reference formulation**

The marketed product (Tivicay) contained 52.6 mg of dolutegravir sodium per tablet which is equivalent to 50 mg of dolutegravir. It also contained mannitol, microcrystalline cellulose, povidone, sodium starch glycolate Type A, sodium stearylfumarate, polyvinyl alcohol – part hydrolyzed, titanium dioxide, macrogol 3350, talc, and iron oxide yellow.

**Test formulation**

Formulation **F-35**, containing 50 mg of dolutegravir per tablet. This optimized formulation contains the drug, HP-β-CD and soluplus in the ratio **1:2:1.5** and also contains micro crystalline cellulose, crosspovidone, talc and magnesium stearate.

**Experimental design**

A cross over, non-blinded, open labelled experimental design was followed. Six rats received one treatment (sample). Blood samples were taken from each rat over a period of three hours. Both the samples were tested in all the six rats during the study.

***In vivo* study protocol**

* Young, healthy male Wistarrats weighing 250±10 gm, were fasted overnight with free access to water for at least 12 hr before dosing.
* The optimized formulation was disintegrating in an average time of 134 seconds.
* Hence, it was reasoned that, triturating the tablet into a powder and taking the weight of powder (6.75mg) equivalent to the calculated dose of the drug was an acceptable way of administration of the formulation to the animal.

DTG tablet powder was suspended in an aqueous solution. It was administered orally by catheter to the rats in a dose of approximately 6.75 mg calculated as per the body weight of each rat. The blood samples (approximately 500 µL) were collected in heparinised tubes at 0 hr, 1, 2, 4, 8, 12, 16, 20 and 24 hrs respectively. After collection, the samples were immediately placed in an ice flask till further use. All samples were centrifuged at 2500 RPM for 10 min. The plasma was separated into clean tubes and was frozen at -20ºC till further use. DTG content was determined by the HPLC method.

DTG concentrations in plasma following the administration of marketed and optimization products are given in **Table 5.3.2.1** and **5.3.2.2** and are shown in **Figure 5.3.2.1**. The results are tabulated in **Table 5.3.2.3**.

**Determination of C max and t max**

The values of peak plasma concentration (C max) and timerequired to achieve the peak plasma concentration (t max) were recorded from the plasma concentration versus time curves.

**Determination of elimination rate constant (Kel) and biological half life (t1/2):**

The elimination rate constant is the rate at which drug is cleared from the body assuming first-order elimination. To perform this calculation, the concentration-time data was plotted with a linear x-axis and a logarithmic y-axis (Semi logarithmic graph).Time versus plasma concentration data was plotted on a semi logarithmic graph paper. The elimination rate constant (Kel) was calculated from the slope of the linear line in the elimination phase (the ‘best fit’ linear regression line for the 151 points in the elimination phase was drawn by the method of least squares). The corresponding biological half-life was calculated using the equation, t1/2 = 0.693/Kel

**Determination of Percentages Absorbed to Various Times and Absorption Rate Constant (Ka):**

Percentages absorbed to various times and absorption rate constant (Ka) were calculated from plasma concentration data by the method described by Wagner and Nelson 1,2. The equation developed for the determination of absorption rate from blood data is

Where,

= absorption rate

= Apperent volume of distribution

= rate of change of blood concentration (*C b*) with respect to time t

= Elimination rate constant

The equation may be integrated between the limits of t= 0 and t = T and divided by Vd to give

Where

AT = amount of drug absorbed to time T,

CT = blood concentration at time T.

The quantity under the integral sign is the area under the blood concentration versus time curve between the indicated limits.

When the successive values of AT / Vd are calculated, a maximum or asymptotic value [AT/Vd]∞ is obtained. The maximum asymptotic value is divided into successive values of AT / Vd to yield percentage absorbed data i.e,

% absorbed as a function of time

A graph of log percent unabsorbed Vs time is a linear plot, the slope of which is equal to – Ka / 2.303 from which the absorption rate constant ( Ka) was calculated. The results are given in **Table 5.1.3.3**.

**Determination of Area under curve (AUC):**

The area under the plasma concentration vs. time curve from 0 to t hrs AUC 0-t  was measured by applying the trapezoidal rule. The area under the curve from 0 to ∞ can be calculated by dividing the plasma concentration at the last time point with .

AUC 0- ∞ was calculated using the equation as given below,

=

= +

Where Ct  is plasma concentration of drug at t hrs.

**Determination of mean residence time (MRT):**

The tendency of the drugs and its metabolite to remain in the body can be assessed by measuring the mean residence time (MRT).The mean residence time (MRT) can be defined as the average amount of time spent by the drug molecules in the body before being eliminated (under constant clearance conditions), provided that the drug in the organs of elimination is always in equilibrium with drug in plasma. The MRT is considered as the statistical moment anology to the half life (t 1/2).

MRT is calculated from plasma drug concentrations using statistical moment analysis by the following equation,

Where AUMC is the area under the ‘First moment curve’ and is obtained from a plot of the product of the drug concentration in plasma and time Vs time from zero to infinity.

AUC is the area under the zero moment curves and is obtained by plotting the drug concentration in plasma Vs time from zero to infinity

**RESULTS OF *IN VIVO* PHARMACOKINETIC STUDIES OF CBS OPTIMIZED AND MARKETED FORMULATIONS**

The results of *in vivo* pharmacokinetic studies of CBS optimized and marketed formulations are discussed below.Plasma concentrations of clopidogrel following oral administration of marketed formulation and optimized formulation to six male Wistar rats are given in **Table 5.3.1.1 and 5.3.1.2.** Time Vs Plasma concentration curves of clopidogrel following oral administration of marketed table and optimized formulation in male Wistar rats are shown in **Figure 5.3.1.1.**

**Table 5.3.1.1: Plasma concentrations of clopidogrel following oral administration of marketed tablet to six male Wistar rats**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Plasma concentration (ng/ml ) in rats for marketed tablet** | | | | | | | | | |
| **Time (hr)** | **Male Wistar Rats** | | | | | | **Mean** | **S.D** | **% CV** |
| **1** | **2** | **3** | **4** | **5** | **6** |
| 0.5 | 321.6 | 446.9 | 308.53 | 378.8 | 319.49 | 366.22 | 356.92 | 35.64 | 10.63 |
| 1 | 907.24 | 856.41 | 786.42 | 801.263 | 754.15 | 926.65 | 838.69 | 72.07 | 8.26 |
| 1.5 | 1326.4 | 1463.46 | 1269.84 | 1286.35 | 1259.87 | 1349.82 | 1325.96 | 83.23 | 5.70 |
| 2.5 | 1813.71 | 1923.52 | 1571.31 | 1725.97 | 1584.09 | 1822.46 | 1740.17 | 91.23 | 8.08 |
| 4 | 1456.79 | 1554.39 | 1328.40 | 1463.79 | 1358.67 | 1477.98 | 1440.00 | 86.11 | 5.76 |
| 6 | 1045.62 | 1192.41 | 963.65 | 982.34 | 814.77 | 1026.13 | 1004.15 | 81.09 | 11.24 |
| 8 | 918.29 | 1021.17 | 746.33 | 734.28 | 776.41 | 916.23 | 852.11 | 59.91 | 12.67 |
| 10 | 558.92 | 663.34 | 362.59 | 463.55 | 389.81 | 544.72 | 497.16 | 49.22 | 8.47 |
| 12 | 154.25 | 218.54 | 162.37 | 196.86 | 167.24 | 192.24 | 181.92 | 66.22 | 9.11 |
| 14 | 63.5 | 84.06 | 52.57 | 42.21 | 49.60 | 58.86 | 58.47 | 61.40 | 4.88 |
| 16 | 35.36 | 40.24 | 33.36 | 36.09 | 30.58 | 39.12 | 35.79 | 13.27 | 2.51 |
| 18 | 17.53 | 20.25 | 19.37 | 17.45 | 15.30 | 18.61 | 18.08 | 5.85 | 1.79 |

**Table 5.3.1.2: Plasma concentrations of clopidogrel following oral administration of optimized formulation to six male Wistar rats**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Plasma concentration (ng/ml ) in rats for optimized tablet** | | | | | | | | | |
| **Time (hr)** | **Male Wistar Rats** | | | | | | **Mean** | **S.D** | **% C.V** |
| **1** | **2** | **3** | **4** | **5** | **6** |
| 0.5 | 495.31 | 622.27 | 484.56 | 575.65 | 503.35 | 528.13 | 534.87 | 38.30 | 10.04 |
| 1 | 1061.64 | 1032.42 | 966.26 | 996.26 | 940.24 | 1019.98 | 1002.86 | 50.04 | 9.81 |
| 1.5 | 1484.52 | 1633.75 | 1448.58 | 1460.58 | 1448.80 | 1552.18 | 1504.73 | 64.32 | 8.08 |
| 2.5 | 1983.34 | 2109.64 | 1759.7 | 1923.14 | 1768.89 | 2031.15 | 1929.31 | 76.49 | 9.37 |
| 4 | 1515.53 | 1624.31 | 1412.66 | 1567.52 | 1449.96 | 1596.17 | 1527.69 | 51.82 | 5.41 |
| 6 | 1196.61 | 1287.42 | 1027.48 | 1036.46 | 1062.63 | 990.53 | 1100.18 | 43.15 | 11.50 |
| 8 | 910.65 | 1011.45 | 841.54 | 834.02 | 880.35 | 709.88 | 864.64 | 96.17 | 9.87 |
| 10 | 626.89 | 845.34 | 544.19 | 658.51 | 576.24 | 635.58 | 647.79 | 29.26 | 7.56 |
| 12 | 217.56 | 393.61 | 242.23 | 253.01 | 251.56 | 220.66 | 263.10 | 39.81 | 13.64 |
| 14 | 93.69 | 88.75 | 78.57 | 69.48 | 64.18 | 71.74 | 77.73 | 62.70 | 9.43 |
| 16 | 40.34 | 50.85 | 38.21 | 47.64 | 51.24 | 41.36 | 44.94 | 7.93 | 3.41 |
| 18 | 21.27 | 32.02 | 20.53 | 22.13 | 23.59 | 19.28 | 23.13 | 4.18 | 3.03 |

**Figure 5.3.1.1: Time Vs Plasma concentration curves of clopidogrel following oral administration of marketed table and optimized formulation in male Wistar rats**

**Table 5.3.1.3: Pharmacokinetic parameters (Mean ±S.D) of clopidogrel following oral administration of marketed tablet and optimized formulation in male Wistar rats**

|  |  |  |
| --- | --- | --- |
| **Pharmacokinetic parameters** | **Marketed product** | **Optimized formulation** |
| C max (ng/ml) | 1740.17 | 1929.31 |
| T max (hr) | 2.5 | 2.5 |
| Kel (/hr) | 0.115 | 0.124 |
| Ka (/hr) | 5.42 | 6.63 |
| AUC 0-t (ng hr/ml) | 13197.38 | 15334.07 |
| AUC 0-∞ (ng hr/ml) | 13287.38 | 15467.97 |
| MRT 0-12 (hr) | 6.48 | 6.50 |
| MRT 0-∞ (hr) | 6.54 | 6.58 |
| AUMC 0-t (ng hr/ml) | 85665.78 | 99747.05 |
| AUMC0-∞ (ng hr/ml) | 87074.18 | 101856.37 |
| Biological half life (t1/2  hr) | 6.02 | 5.57 |

**RESULTS OF *IN VIVO* PHARMACOKINETIC STUDIES OF DTG OPTIMIZED AND MARKETED FORMULATIONS**

The results of *in vivo* pharmacokinetic studies of optimized and marketed formulations are discussed below.Plasma concentrations of dolutegravir following oral administration of marketed formulation and optimized formulation to six male Wistar rats are given in **Table 5.3.2.1 and 5.3.2.2.** Time Vs Plasma concentration curves of dolutegravir following oral administration of marketed table and optimized formulation in male Wistar rats are shown in **Figure 5.3.2.1.**

**Table 5.3.2.1: Plasma concentrations of dolutegravir following oral administration of marketed tablet to six male Wistar rats**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Plasma concentration (ng/ml) in rats for marketed tablet of dolutegravir sodium** | | | | | | | | | |
| **Time (hr)** | **Male Wistar Rats** | | | | | | **Mean** | **S.D** | **% C.V** |
| **1** | **2** | **3** | **4** | **5** | **6** |
| 1 | 554.09 | 429.18 | 531.19 | 527.01 | 557.27 | 514.30 | 518.84 | 42.81 | 10.96 |
| 2 | 802.61 | 721.73 | 823.12 | 750.73 | 730.91 | 760.67 | 764.96 | 36.62 | 5.74 |
| 4 | 1564.22 | 1458.22 | 1596.56 | 1543.12 | 1482.78 | 1471.53 | 1519.40 | 70.04 | 5.78 |
| 8 | 1259.34 | 1240.17 | 1263.07 | 1274.57 | 1249.41 | 1255.33 | 1256.98 | 29.20 | 10.38 |
| 12 | 1094.55 | 1125.10 | 1047.76 | 1025.24 | 1039.82 | 1089.12 | 1070.26 | 45.89 | 6.62 |
| 16 | 776.87 | 768.69 | 762.68 | 749.88 | 780.59 | 773.92 | 768.77 | 96.16 | 8.87 |
| 20 | 128.11 | 120.81 | 126.85 | 119.76 | 117.34 | 109.27 | 120.35 | 41.54 | 7.41 |
| 24 | 21.98 | 18.15 | 11.57 | 16.07 | 19.06 | 10.09 | 16.15 | 80.55 | 8.03 |

**Table 5.3.2.2: Plasma concentrations of dolutegravir following oral administration of optimized formulation to six male Wistar rats**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Plasma concentration (ng/ml) in rats for Optimized formulation of dolutegravir sodium** | | | | | | | | | |
| **Time (hr)** | **Male Wistar Rats** | | | | | | **Mean** | **S.D** | **% C.V** |
| **1** | **2** | **3** | **4** | **5** | **6** |
| 1 | 735.57 | 719.54 | 737.55 | 742.55 | 738.56 | 719.53 | 732.21 | 29.20 | 6.29 |
| 2 | 983.82 | 1010.84 | 970.79 | 967.77 | 956.78 | 960.78 | 975.13 | 28.10 | 5.18 |
| 4 | 1738.15 | 1696.15 | 1781.16 | 1753.65 | 1697.15 | 1711.27 | 1729.58 | 31.13 | 4.04 |
| 8 | 1436.21 | 1429.12 | 1461.12 | 1482.29 | 1434.26 | 1460.82 | 1450.63 | 48.95 | 7.72 |
| 12 | 1318.11 | 1319.11 | 1307.11 | 1298.08 | 1279.09 | 1294.41 | 1302.65 | 53.98 | 8.05 |
| 16 | 955.79 | 961.79 | 964.78 | 966.76 | 968.78 | 996.78 | 969.11 | 73.04 | 10.78 |
| 20 | 307.14 | 322.15 | 331.51 | 329.13 | 318.48 | 335.31 | 323.95 | 39.38 | 8.54 |
| 24 | 69.27 | 51.23 | 58.19 | 65.72 | 50.79 | 50.5 | 57.61 | 26.96 | 9.21 |

**Figure 5.3.2.1: Time Vs Plasma concentration curves of dolutegravir following oral administration of marketed table and optimized formulation in male Wistar rats**

**Table 5.3.2.3: Pharmacokinetic parameters (Mean ±S.D) of dolutegravir following oral administration of marketed tablet and optimized formulation in male Wistar rats**

|  |  |  |
| --- | --- | --- |
| **Pharmacokinetic parameters** | **Marketed product** | **Optimized formulation** |
| C max (ng/ml) | 1519.40 | 1729.58 |
| Tmax (hr) | 4 | 4 |
| Kel (/hr) | 0.0506 | 0.0508 |
| Ka (/hr) | 4.66 | 5.56 |
| AUC 0-t (ng hr/ml) | 18233.50 | 22821.91 |
| AUC 0-∞ (ng hr/ml) | 18465.62 | 22919.07 |
| MRT 0-12 (hr) | 12.91 | 12.59 |
| MRT 0-∞ (hr) | 12.93 | 12.67 |
| AUMC 0-t (ng hr/ml) | 235525.67 | 287352.26 |
| AUMC0-∞ (ng hr/ml) | 236282.91 | 291153.11 |
| Biological half life (t1/2  hr) | 13.67 | 12.63 |

**DISCUSSIONS**

For the drug **clopidogrel bisulphate**, the *in vivo* experiments were carried out as per a crossover design, (n=6), in healthy Wistar male rats, at a dose of 300mg. The plasma concentration was determined by the HPLC method as described in chapter 2.

Pharmacokinetic parameters were determined, after the oral administration of clopidogrel marketed tablets. The absorption rate constant Ka was found to be 5.42 hr-1, the elimination rate constant Kel was found to be 0.115 hr-1 and the corresponding biological half life (t1/2) was found to be 6.02 hours. The MRT was found to be 6.48 hours. A peak plasma concentration 1740 ng/ml was observed at 2.5 hours after administration of clopidogrel marketed tablet.

When the optimized formulation was administered orally, plasma concentration of clopidogrel was found to be significant when compared to the marketed formulation. A peak concentration of 1929.31 ng/ml was observed at 2.5 hours. The elimination rate constant for Kel for clopidogrel was found to be 0.124hr-1, and the corresponding biological half life (t1/2) was found to be 5.57 hours. The MRT was found to be 6.50 hours. The absorption rate constant (Ka) was found to be 6.63 hr-1.

All the pharmacokinetic parameters of absorption (**Table 5.3.1.3**) viz. Ka, C max, AUC and AUMC indicated that rapid absorption and higher bioavailability of optimized formulation than the marketed formulation. Higher C max is observed with the similar Tmax values when compared to marketed formulation.

AUC 0-t and AUMC 0-∞ were found to be 13197.38 ng.hr ml-1 and 85665.78 ng.hr ml-1 respectively for marketed tablet and 15334.07 ng.hr ml-1  and 99747.05 ng.hr ml-1 respectively for optimized formulation. AUC 0-t and AUMC 0-∞ were found comparable of optimized formulation with marketed formulation.

The pharmacokinetic data were subjected to statistical analysis (P < 0.05). The results of statistical analysis indicated significant difference in C max, Kel, Ka, AUC and AUMC values among marketed and optimized formulation. The optimized formulation showed significantly higher plasma concentration, faster onset of action, more extent and rate of absorption, higher rate of elimination, equivalent residence time in the body, low t ½ and more bioavailability when compared with the marketed formulation. So it may be concluded that optimized formulation tablets of clopidogrel improves absorption and bioavailability. This result may be attributed to the modified composition of the optimized formulation viz. clopidogrel, HP-β-CD and soluplus in a ratio of (**1:1:1**)

For the drug **dolutegravir sodium**, the *in vivo* experiments were carried out as per a crossover design, (n=6), in healthy Wistar male rats, at a dose of 300mg. The plasma concentration was determined by the HPLC method as described in chapter 2.

Pharmacokinetic parameters were determined, after the oral administration of clopidogrel marketed tablets. The absorption rate constant Ka was found to be 4.66 hr-1, the elimination rate constant Kel was found to be 0.0506 hr-1 and the corresponding biological half life (t1/2) was found to be 13.67 hours. The MRT was found to be 12.91 hours. A peak plasma concentration 1519.40 ng/ml was observed at 4 hours after administration of dolutegravir marketed tablet.

When the optimized formulation was administered orally, plasma concentration of dolutegravir was found to be significant when compared to the marketed formulation. A peak concentration of 1729.58 ng/ml was observed at 4 hours. The elimination rate constant for Kel for dolutegravir was found to be 0.0548 hr-1, and the corresponding biological half life (t1/2) was found to be 12.63 hours. The MRT was found to be 12.59 hours. The absorption rate constant (Ka) was found to be 5.57 hr-1.

All the pharmacokinetic parameters of absorption (**Table 5.3.2.3**) viz. Ka, C max, T max, AUC and AUMC indicated that rapid absorption and higher bioavailability of optimized formulation than the marketed formulation. Higher C max is observed with the similar Tmax values when compared to marketed formulation.

AUC 0-t and AUMC 0-∞ were found to be 18233.50 ng.hr ml-1 and 235525.67 ng.hr ml-1 respectively for marketed tablet and 22821.91 ng.hr ml-1  and 287352.26 ng.hr ml-1 respectively for optimized formulation. AUC 0-t and AUMC 0-∞ were found comparable of optimized formulation with marketed formulation.

The pharmacokinetic data were subjected to statistical analysis (P < 0.05). The results of statistical analysis indicated significant difference in C max, Kel, Ka, AUC and AUMC values among marketed and optimized formulation. The optimized formulation showed significantly higher plasma concentration, faster onset of action, more extent and rate of absorption, higher rate of elimination, equivalent residence time in the body, low t ½ and more bioavailability when compared with the marketed formulation. So it may be concluded that optimized formulation tablets of dolutegravir improves absorption and bioavailability. This result may be attributed to the modified composition of the optimized formulation viz.dolutegravir, HP-β-CD and soluplus in a ratio of (**1:2:1.5**)

**CONCLUSIONS**

The optimised formulation in this research project was tablets prepared with a solid inclusion complex containing a hydrophilic polymer. The objective of the work was to enhance the solubility of the drugs namely clopidogrel bisulphate and dolutegravir sodium. It was shown in Chapter 3 that C 34, a complex including clopidogrel, HP-β-CD and soluplus prepared by kneading method, showed an 14.95 fold increase and F 35, a complex including dolutegravir, HP-β-CD and soluplus, prepared by kneading method, showed an 14.75 fold increase in dissolution over that of the pure drug. The optimized tablets contained this complex and showed a faster dissolution profile than the marketed tablet, where tested *in vitro*, over a period of 60 minutes. It released more than 100% of its drug content in 60 minutes.

The *in vivo* experiments were carried out as per a cross over design in six male Wistar rats. All the pharmacokinetic parameters of absorption viz., Ka, C max, t max, AUC and AUMC indicated similar rate of absorption and higher bioavailability when compared to commercial tablet. Higher C max at similar t max values, faster onset of action, more extent and rate of absorption, higher rate of elimination, equivalent residence time in the body, low t ½ and more bioavailability was observed with the optimized formulations of clopidogrel and dolutegravir when compared to their respective marketed formulations. This result may be attributed to the modified composition of the optimized formulation C-34 contains the clopidogrel, HP-β-CD and soloplus in a ratio of (1:1:1) for CBS and optimized formulation F-35 contains the dolutegravir, HP-β-CD and soloplus in a ratio of (1:2:1.5) for DTG.

**5.3. COMPARISON WITH PAST WORK**

In the present work, complexation of the BCS Class II drugs clopidogrel bisulphate (CBS) and dolutegravir sodium (DTG) each with two cyclodextrins namely β-CD and HP-β-CD were studied individually and the effect of hydrophilic polymers namely PVP K30, PEG 6000 and SOLUPLUS on the dissolution enhancement of the respective drugs was also investigated. Application of artificial neural networks to study the influence of cyclodextrins on the dissolution enhancement of the drugs CBS and DTG in the presence of hydrophilic polymers was done. The predicted responses obtained through ANN were almost similar to the experimental results with minimum difference.

1. **Smita G8** applied artificial neural network to predict the percentage extraction of diclofenac at different levels of various parameters. The emulsion liquid membrane (ELM) was prepared using n-heptane as an organic solvent, Di-2-ethylhexyl phosphoric acid (D2EHPA) as a carrier, and span 80 as a surfactant. An artificial neural network (ANN) model is proposed in the present study to examine the effect of stripping phase concentration, surfactant concentration, carrier concentration, homogenizer speed and stirring speed. Multilayer perceptron (MLP) model is used to predict the percentage extraction at different parameters conditions. The neural network proved as a very promising method for the purpose of process simulation. At optimum conditions of all parameters the maximum 96 % extraction is possible within 30 minutes.

# Mohammed T A *et al*9 developed effects of polymers on complexation efficiency of aceclofenac-beta cyclodextrin inclusion complex, aceclofenac is a poorly water soluble analgesic drug. An effort has been made to enhance the solubility through forming inclusion complex with an aim to improve the complexation efficiency of β-CD. The inclusion complex was formed by kneading method. Hydrophilic polymers such as PVP, SCMC, HPMC and hydrophobic polymer such as Ethyl Cellulose (EC) were used. Phase solubility studies were carried out to evaluate the solubilizing power of β-CD along with the optimized concentration of polymers. Complexation efficiency and stability constant was calculated from the phase solubility studies. Higher values of solubility constant for ternary complexes clearly prove the beneficial effects of added polymers. Complexation efficiency was enhanced maximum by EC but the dissolution rate followed the following sequence PVP>HPMC>SCMC>EC.

# Narendra Kumar P *et al*10 had developed a Duloxetine hydrochloride (DXH) suffers from poor solubility and thereby poor absorption, which ultimately leads to poor bioavailability. In present study, an attempt has been made to formulate and characterize DXH complex, using β-CD and different hydrophilic polymers in order to enhance its solubility and dissolution rate. Phase solubility study was used to investigate the interaction of the drug in binary systems (DXH-β-CD) as well as ternary systems (DXH-β-CD-hydrophilic polymer). It was observed that solubilization of DXH by β-CD was further enhanced by using HPMC K4M at 0.1% w/v concentration. Several methods were used to prepare ternary complex of DXH-β-CD-HPMC K4M. Ternary complex prepared by co-evaporation method containing DXH-β-CD-HPMC K4M in the ratio of 1:1.10:0.01 has shown the fastest dissolution rate (53.65 ± 2.83% in 5 min) as compared to pure DXH (3.03 ± 1.88% in 5 min) as well as other methods used to prepare these complexes.

# [Vieira A C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Vieira%20AC%5BAuthor%5D&cauthor=true&cauthor_uid=26076609) *et al*11 developed the multicomponent system with cyclodextrins and hydrophilic polymers for delivery of effavirenz. Among several drug delivery systems, the multicomponent systems with cyclodextrins and hydrophilic polymers are a promising alternative for increasing the aqueous solubility of the drug. The present study aimed to develop and characterize in a ternary system of EFZ, M-β-CD and PVP K30. The results showed that the solid ternary system provided a large increase in the dissolution rate which was greater than 80% and was characterized by DSC, TG, XRD, FT-IR and SEM. The use of the ternary system (EFZ, M-β-CD and PVP K30 1%) proved to be a viable, effective and safe delivery of the drug. The addition of the hydrophilic polymer appeared to be suitable for the development of a solid oral pharmaceutical product, with possible industrial scale-up and with low concentration of CDs.

# Jayshree T B *et* al12 had designed to study solubility properties of inclusion complexes of atorvastatin, with β-CD and HP-β-CD and to analyze the effect of hydrophilic polymer on complexation, aqueous solubility and dissolution of drug. The phase solubility curves were classified as an AL type for both binary and ternary systems showed that atorvastatin solubility increased linearly as a function of β-CD concentration indicated formation of the inclusion complex. The highest improvement in solubility, drug content were observed in inclusion complex prepared with HP-β-CD and polymer by common solvent evaporation method. The findings confirmes the addition of small amounts of hydrophilic polymers improves solubilizing and complexing ability of cyclodextrin which further related to increased release of drug in dissolution medium. This study signifies the use of hydrophilic polymers in combination with HP-β-CD for the formation of inclusion complex of atorvastatin.

1. **Chowdary K P R *et al*13** studied the complexation of etoricoxib with two CDs, β-CD and HP-β-CD for enhancing its solubility. The effect of three hydrophilic polymers namely PVP, HPMC and PEG on the complexation and solubilizing efficiencies of cyclodextrins was also investigated. The aqueous solubility of etoricoxib was linearly increased as a function of the concentration of β-CD and HP-β-CD alone and in the presence of hydrophilic polymers, PVP, HPMC and PEG. The increase in solubility is due to the formation of a 1: 1 M complex in solution in each case. The complexes formed between etoricoxib–CD were quite stable. Addition of hydrophilic polymers has markedly enhanced the complexation efficiency and solubilizing efficiency of both; βCD and HPβCD. The order of hydrophilic polymers in enhancing the complexation efficiency and solubilising efficiency was PVP > HPMC > PEG with both; βCD and HPβCD. Addition of hydrophilic polymers has markedly enhanced the solubilizing efficiency of both; β-CD and HP-β-CD. HP-β-CD exhibited higher solubilizing efficiency, when compared to β-CD, both; alone and in the presence of hydrophilic polymers. Hence, a combination of CDs and hydrophilic polymers is recommended for enhancing the complexation and solubilizing efficiencies of CDs and to enhance the solubility of etoricoxib, a BCS class II drug.
2. **Hirlear R S *et al*14** developed the effect of complexation of irbesartan (IRB), a practically water-insoluble drug, with cyclodextrins in presence of different concentrations of water-soluble polymers (PEG 4000 and PVP K-90) on the dissolution rate of the drug has been investigated. Phase solubility studies were carried out to evaluate the solubilizing power of βCD in association with water-soluble polymers towards IRB and to determine the apparent stability constant (KS) of the complexes. Improvement in KS value for ternary complexes (IRB–β-CD–polymers) clearly proved the benefit on the addition of water-soluble polymer to increase complexation efficiency. The dissolution rate of the drug from ternary systems containing PEG 4000 and PVP K-90 was higher as compared to the binary system.

**REFERENCES**

1.Srinath K R, Pooja C, Palaniswamy P, Vamsy Krishna A, Aparna S and Ali SS. Formulation and evaluation of effervescent tablets of paracetamol, International Journal of Pharmaceutical Research and Development, 2011; 3(3): 76-104.

2. Shoukri R A, Ahmed I S and Shamma R N. *In vitro* and *in vivo* evaluation of nimesulide lyophilized orally disintegrating tablets, European Journal of Pharmaceutics and Biopharmaceutics, 2009; 73(1): 162–171.

3. Venkateswara Reddy B. Formulation and evaluation of immediate release tablets of irbesartan. Journal of Pharmaceutical and Biological Research, 2015; 3(1): 211-216.

4. Rajalakshmi G, Vamsi CH, Balachandar R and Damodharan N. Formulation and evaluation of diclofenac potassium effervescent tablets. International Journal of Pharmaceutical and Biomedical Research, 2011; 2(4): 237- 243.

5. Nandini B and Sushma S. Formulation, evaluation and optimization of effervescent granules to be reconstituted into suspension of levetiracetam for sustained release, International Journal of Pharmaceutical Sciences Review and Research, 2013; 20(2): 181-186.

6. Moore J W and Flanner H H. Mathematical comparison of curves with an emphasis on *in vitro* dissolution profiles, Pharmaceutical Technology, 1996; 20(6): 64-74.

7. Vinod P S, Yi Tsong, Jen Pei L and Pradeep S. *In vitro* dissolution profile comparison - statistics and analysis of the similarity factor, f2, Pharmaceutical Research, 1998; 15(6): 889-896.

1. Smita G. Application of artificial neural network for the extraction of a non-steroidal anti-inflammatory drug through emulsion liquid membrane, International Journal of Engineering Technology Science and Research, 2018; 5(1): 1245-1251.
2. Mohammed Tahir A, Poonam R and Sadath A. Effects of polymers on complexation efficiency of aceclofenac-beta cyclodextrin inclusion complex, International Journal of Pharma and Biosciences, 2017; 8 (4): 21-29.

# Pinakin P, Narendra Kumar P, Sachin Kumar S and Manish Kumar. Formulation and characterization of ternary complex of poorly soluble duloxetine hydrochloride, Journal of Applied Pharmaceutical Science, 2015; 5(6):88-96

1. [Vieira A C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Vieira%20AC%5BAuthor%5D&cauthor=true&cauthor_uid=26076609), [Ferreira Fontes D A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Ferreira%20Fontes%20DA%5BAuthor%5D&cauthor=true&cauthor_uid=26076609), [Chaves L L](https://www.ncbi.nlm.nih.gov/pubmed/?term=Chaves%20LL%5BAuthor%5D&cauthor=true&cauthor_uid=26076609), [Alves L D](https://www.ncbi.nlm.nih.gov/pubmed/?term=Alves%20LD%5BAuthor%5D&cauthor=true&cauthor_uid=26076609), [Freitas Neto J L](https://www.ncbi.nlm.nih.gov/pubmed/?term=de%20Freitas%20Neto%20JL%5BAuthor%5D&cauthor=true&cauthor_uid=26076609), [De La Roca Soares M F](https://www.ncbi.nlm.nih.gov/pubmed/?term=de%20La%20Roca%20Soares%20MF%5BAuthor%5D&cauthor=true&cauthor_uid=26076609), [Soares Sobrinho J L](https://www.ncbi.nlm.nih.gov/pubmed/?term=Soares-Sobrinho%20JL%5BAuthor%5D&cauthor=true&cauthor_uid=26076609), [Rolim L A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Rolim%20LA%5BAuthor%5D&cauthor=true&cauthor_uid=26076609) and [Rolim Neto P J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Rolim-Neto%20PJ%5BAuthor%5D&cauthor=true&cauthor_uid=26076609). Multicomponent systems with cyclodextrins and hydrophilic polymers for the delivery of efavirenz, [Carbohydrate Polymers](https://www.ncbi.nlm.nih.gov/pubmed/26076609), 2015; 40: 130-133.
2. Jayshree T B, Swati L N, Rashmi T V, Jayashri M G and Umekar M J. Effect of hydrophilic polymer on solubility and dissolution of atorvastatin inclusion complex, International Journal of Pharmaceutical and Chemical Sciences, 2012; 1 (1): 374-385.
3. Chowdary K P R and Seetha Devi A. Effect of hydrophilic polymers on the complexation and solubilizing efficiencies of cyclodextrins, International Journal of Chemical Sciences, 2011; 9(2): 510-516.
4. [Rajashree S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Hirlekar%20RS%5BAuthor%5D&cauthor=true&cauthor_uid=19562489) H,  [Suneeta N](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sonawane%20SN%5BAuthor%5D&cauthor=true&cauthor_uid=19562489) S and [Vilasrao J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Kadam%20VJ%5BAuthor%5D&cauthor=true&cauthor_uid=19562489) K. Studies on the effect of water-soluble polymers on drug–cyclodextrin complex solubility, [American Association of Pharmaceutical Sceintists Pharm Sci Tech](https://www.ncbi.nlm.nih.gov/pubmed/19562489), 2009; 10(3): 858-863.