Chapter – 6

DISCUSSION OF RESULTS
In the present work, studies for the design, development and characterization of oral controlled release formulations of antiretroviral drugs were carried out. According to the proceedings of the study so far, the methodology had been discussed and thus the results of the methods were detailed and discussed here in this chapter.

6.1. ANALYTICAL METHODS

6.1.1. UV Spectroscopic method:

For the estimation of Lamivudine, Emtricitabine & Tenofovir DF in the assay and dissolution samples of the formulations, a suitable and convenient analytical method should be required. For this purpose, an accurate and reproducible spectrophotometric method was developed in distilled water, 0.1N HCl and Phosphate Buffer pH 6.8. The proposed UV methods had been validated for two parameters, i.e., Reproducibility and Interference Study. The reproducibility was determined by % RSD values (≤ 2). For the interference study, the drug and polymer were mixed in fixed proportions and assayed for any interference.

a) Lamivudine:

The \( \lambda_{\text{max}} \) for LAM was found to be 270 nm in distilled water, phosphate buffer pH 6.8 and 280 nm in 0.1N HCl (shown in figures 5.1-5.3). All these values were correlated with the literature values. The calibration curves had been plotted based on their Beer’s limits obeyed in the concentration range of 4-20 \( \mu \)g/mL in distilled water, 2-14 \( \mu \)g/mL in 0.1 N HCl and phosphate buffer pH 6.8. All the results showed good linearity \( (R^2=0.997-0.999) \) and reproducibility \( (% \text{RSD} \leq 2) \) and were given in the tables 5.1, 5.3 & 5.5 and shown in the figures 5.10-5.12. The calculated drug content of the interference study was detailed in tables 5.2, 5.4 & 5.6, indicated that none of the polymers and other materials interfere with the method of estimation.

b) Emtricitabine:

The \( \lambda_{\text{max}} \) for EMT was found to be 280 nm in distilled water, phosphate buffer pH 6.8 and 290 nm in 0.1N HCl (shown in figures 5.4-5.6). All these values were correlated with the literature values. The calibration curves had been plotted based on their Beer’s limits obeyed in the concentration range of 5-25 \( \mu \)g/mL in distilled water, phosphate buffer pH 6.8 and 2-16 \( \mu \)g/mL in 0.1 N HCl. All the results showed good linearity \( (R^2=0.999) \) and reproducibility \( (% \text{RSD} \leq 2) \) and were given in the tables 5.7, 5.9 & 5.11 and shown in the figures 5.13-5.15. The calculated drug content of the interference study was detailed in tables 5.8, 5.10 & 5.12, indicated that none of the polymers and other materials interfere with the method of estimation.
c) **Tenofovir DF:**

The $\lambda_{\text{max}}$ for TDF was found to be 260 nm in distilled water, 0.1N HCl and phosphate buffer pH 6.8 (shown in figures 5.7-5.9). All these values were correlated with the literature values. The calibration curves had been plotted based on their Beer’s limits obeyed in the concentration range of 5-40 $\mu$g/mL in distilled water, 5-35 $\mu$g/mL in 0.1 N HCl and 5-30 $\mu$g/mL in phosphate buffer pH 6.8. All the results showed good linearity ($R^2=0.998-0.999$) and reproducibility (%RSD $\leq 2$) and were given in the tables 5.13, 5.15 & 5.17 and shown in the figures 5.16-5.18. The calculated drug content of the interference study was detailed in tables 5.14, 5.16 & 5.18, indicated that none of the polymers and other materials interfere with the method of estimation.

Thus, these methods were found to be suitable for the estimation of Lamivudine, Emtricitabine & Tenofovir DF contents in the assay and dissolution samples of the formulations.

### 6.1.2. HPLC method

The HPLC method had been developed for the analysis of combination dosage forms of Lamivudine with Tenofovir and Emtricitabine with Tenofovir for their simultaneous estimation and also for the estimation of Lamivudine and Emtricitabine individually in biological samples (rabbit plasma).

#### 6.1.2.1. Simultaneous estimation of Lamivudine with Tenofovir DF

The developed HPLC method described the estimation of Lamivudine and Tenofovir DF. The method was validated and was found to be accurate and reproducible. The chromatographic peaks obtained were shown in figures 5.19-5.23 were better defined, resolved and almost free from tailing and fronting. The retention times obtained for LAM and TDF were 2.12 and 5.45 min respectively (Table 5.19).

The calibration curves were plotted by basing on their linearity in the concentration range of 50-350 $\mu$g/mL for both the drugs. The results showed good linearity ($R^2=0.999$) and reproducibility (%RSD $\leq 2$) and were given in the tables 5.20 & 5.21 and shown in the figures 5.24-5.25. This indicates that the method developed under the selected conditions was efficient enough to estimate either of the drugs in their combinations.

#### 6.1.2.2. Simultaneous estimation of Emtricitabine with Tenofovir DF

The developed HPLC method described the estimation of Emtricitabine and Tenofovir DF. The method was validated and was found to be accurate and
reproducible. The chromatographic peaks obtained were shown in figure 5.26-5.30 were better defined, resolved and almost free from tailing and fronting. The retention times obtained for EMT and TDF were 2.98 and 5.88 min respectively (Table 5.22).

The calibration curves were plotted by basing on their linearity in the concentration range of 30-210 µg/mL for Emtricitabine and 450-315 µg/mL for Tenofovir DF. The results showed good linearity (R²=0.998-0.999) and reproducibility (%RSD ≤2) and were given in the tables 5.23 & 5.24 and shown in the figures 5.31-5.32. This indicates that the method developed under the selected conditions was efficient enough to estimate either of the drugs in their combinations.

6.1.2.3. Estimation of Lamivudine in Rabbit plasma

The developed HPLC method described the estimation of Lamivudine in rabbit plasma. The method was validated and was found to be accurate and reproducible. The chromatographic peaks obtained were shown in figure 5.33 & 5.34 were better defined, resolved and almost free from tailing and fronting. The retention times obtained for Lamivudine and Stavudine (IS) were 6.05 and 8.12 min respectively (Table 5.25).

The calibration curve was plotted by basing on the linearity in the concentration range of 0.05–1.5 µg/mL for Lamivudine and the results showed good linearity (R²=0.999) and reproducibility (%RSD ≤2) and were given in the table 5.26 and shown in the figure 5.35. This indicates that the method developed under the selected conditions was efficient enough to estimate the Lamivudine in rabbit plasma.

6.1.2.4. Estimation of Emtricitabine in Rabbit plasma

The developed HPLC method described the estimation of Emtricitabine in rabbit plasma. The method was validated and was found to be accurate and reproducible. The chromatographic peaks obtained were shown in figure 5.36 & 5.37 were better defined, resolved and almost free from tailing and fronting. The retention times obtained for Emtricitabine and Lamivudine (IS) were 5.16 and 7.36 min respectively (Table 5.27).

The calibration curve was plotted by basing on the linearity in the concentration range of 0.025–0.8 µg/mL for Emtricitabine and the results showed good linearity (R²=0.997) and reproducibility (%RSD ≤2) and were given in the table 5.28 and shown in the figure 5.38. This indicates that the method developed under the selected conditions was efficient enough to estimate the Emtricitabine in rabbit plasma.
6.2. PREFORMULATION STUDIES

6.2.1. Organoleptic properties:

The Lamivudine was a white crystalline powder, whereas Emtricitabine and Tenofovir were amorphous powder in the state. All the drugs were white to off-white in color, odorless and bitter to taste. All these results were given in table 5.29.

6.2.2. Solubility:

The results of solubility studies were shown in table 5.30 and figures 5.39-5.41. These results indicated that the Lamivudine and Emtricitabine were freely soluble in water and 0.1N HCl whereas Tenofovir was sparingly soluble in water and soluble in 0.1N HCl according to the I.P. description of solubility. The good solubility of Lamivudine and Emtricitabine indicated that they might have good bioavailability and do not show any solubility related bioavailability problems. The pH – solubility profiles of the drugs indicated that the solubility decreased upon pH increased, this might be attributed to the alkaline nature of the drugs.

6.2.3. Partition coefficient:

The results of partition coefficient were shown in the table 5.31. The very less partition coefficient value of Lamivudine and Emtricitabine indicated that its permeability might be poor so that the bioavailability might be affected.

6.2.4. Melting point:

The melting point was determined by capillary tube method and also by DSC studies was found to be 178 °C for Lamivudine, 154 °C for Emtricitabine and 116 °C for Tenofovir DF and these results were given in table 5.31. All these results were complying with the specifications mentioned in the pharmacopoeia and literature which indicated that the obtained drug was in its purest form.

6.2.5. Loss on drying:

The loss on drying for Lamivudine, Emtricitabine, and Tenofovir DF were found to be 0.05%, 0.12% and 0.14% respectively (given in table 5.31). The results obtained from the loss on drying of drugs, it was found to be complying with the pharmacopoeia specifications i.e., LOD not more than 0.50 %.

6.2.6. Flow properties:

The three drugs were evaluated for various derived properties such as bulk density, tapped density and flow properties such as angle of repose, Hausner’s ratio and Carr’s index, all the results together were shown in table 5.32. The flow properties altogether indicated that Lamivudine has good flowability which might be
attributed to the large particle size and crystalline nature whereas Emtricitabine and Tenofovir DF showed very poor flowability which might be attributed to the small particle size and amorphous nature.

6.2.7. Drug – Excipient compatibility studies:

6.2.7.1. Physical observation:

After one month storage of physical mixtures of the drugs with proposed excipients, no characteristic physical change was observed in all the drugs and drug-excipient admixtures and the results were given in table 5.33. Further studies on drug-excipient interactions were done using FTIR and DSC.

6.2.7.2. FTIR

Compatibility studies of drug & polymer were conducted by employing FTIR studies.

a) Lamivudine

The FTIR spectra of pure Lamivudine and the physical mixtures of drug and polymers, i.e., *Anacardium* gum, *Delonix* regia gum, *Moringa* gum, EC N50, EC N100, Eudragit RSPO, Eudragit RLPO, lactose and PVP K30 were carried out and the results were shown in figures 5.43-5.53.

The following characteristic peaks of the pure Lamivudine were observed with the spectra of pure Lamivudine as well as the physical mixtures with excipients.

- **Ketone, C=O stretching**: $1632.82\text{ cm}^{-1}$
- **Alcoholic, O-H stretching**: $3319.62\text{ cm}^{-1}$
- **Amine, N-H stretching**: $3252.42\text{ cm}^{-1}$ & $3182.88\text{ cm}^{-1}$
- **Symmetrical C-O-C**: $1122.06\text{ cm}^{-1}$
- **Aromatic C=C**: $1488.09\text{ cm}^{-1}$

As the identical principle peaks were observed in all the cases, it was confirmed that no interaction was existed in between the drug and polymers.

b) Emtricitabine

The FTIR spectra of pure Emtricitabine and the physical mixtures of drug and polymers i.e., EC N22, HEC, EC N100, Eudragit RSPO, Eudragit RLPO, lactose and PVP K30 were carried out and the results were shown in figures 5.54-5.62.

The following characteristic peaks of the pure Emtricitabine were observed with the spectra of pure Emtricitabine as well as the physical mixtures with excipients.

- **Ketone, C=O stretching**: $1690.43\text{ cm}^{-1}$
- **Alcoholic, O-H stretching**: $3415.95\text{ cm}^{-1}$
Amine, N-H stretching : 3207.24 cm\(^{-1}\) & 3080.36 cm\(^{-1}\)
Symmetrical C-O-C : 1167.26 cm\(^{-1}\)
Aromatic C=C : 1441.62 cm\(^{-1}\)
C-F stretching : 1218.60 cm\(^{-1}\)

As the identical principle peaks were observed in all the cases, it was confirmed that no interaction was existed in between the drug and polymers.

c) Tenofovir DF

The FTIR spectra of pure Tenofovir DF and the physical mixtures of drug and polymers i.e., HPMC E15, HPMC E5, starch, SSG, PVP K30 and lactose carried out and the results were shown in figures 5.63-5.69.

The following characteristic peaks of the pure Tenofovir DF were observed with the spectra of pure Tenofovir DF as well as the physical mixtures with excipients.

Ketone, C=O stretching : 1681.45 cm\(^{-1}\)
Aromatic C=N stretching : 1755.69 cm\(^{-1}\)
Amine, N-H stretching : 3227.43 cm\(^{-1}\) & 3108.40 cm\(^{-1}\)
Symmetrical C-O-C : 1159.60 cm\(^{-1}\)
Aromatic C=C : 1422.26 cm\(^{-1}\)

As the identical principle peaks were observed in all the cases, it was confirmed that no interaction was existed in between the drug and polymers.

6.2.7.3. DSC

DSC studies were also performed to study the interaction between drug & excipients.

a) Lamivudine

DSC studies of pure Lamivudine and the physical mixtures of drug and polymers were carried out and the results were shown in figures 5.70 – 5.77.

The following endothermic peaks were observed in the thermograms of pure Lamivudine as well as the physical mixtures with excipients.

Lamivudine : 180.5\(^{0}\)C
Lamivudine + Tenofovir DF : 182.3\(^{0}\)C
LAM + Anacardium gum : 181.4\(^{0}\)C
LAM + Delonix regia gum : 182.2\(^{0}\)C
LAM + Moringa gum : 182.8\(^{0}\)C
LAM + EC 100cps : 180.3\(^{0}\)C
LAM + Eudragit RSPO : 177.8\(^{0}\)C
**LAM + Eudragit RLPO** : 178.1\(^{0}\)C

No significant change in the melting point of Lamivudine from the thermogram of pure Lamivudine with that of the thermograms of its physical mixtures was observed. So, it was confirmed that no interaction was existed in between the drug and polymers.

**b) Emtricitabine**

DSC studies of pure Emtricitabine and the physical mixtures of drug and polymers were carried out and the results were shown in figures 5.78-5.82.

The following endothermic peaks were observed in the thermograms of pure Emtricitabine as well as the physical mixtures with excipients.

- Emtricitabine : 154.9\(^{0}\)C
- Emtricitabine + Tenofovir DF: 146.62\(^{0}\)C
- EMT + EC : 154.4\(^{0}\)C
- EMT + Eudragit RSPO : 153.6\(^{0}\)C
- EMT + Eudragit RLPO : 155.8\(^{0}\)C

No significant change in the melting point of Emtricitabine from the thermogram of pure Emtricitabine with that of the thermograms of its physical mixtures was observed. So, it was confirmed that no interaction was existed in between the drug and polymers.

**c) Tenofovir DF**

DSC studies of pure Tenofovir DF and the physical mixtures of drug and polymers were carried out and the results were shown in figures 5.83-5.86.

The following endothermic peaks were observed in the thermograms of pure Tenofovir DF as well as the physical mixtures with excipients.

- Tenofovir DF : 116.5\(^{0}\)C
- TDF + Starch : 117.5\(^{0}\)C
- TDF + SSG : 120.9C
- TDF + HPMC E5 : 118.8\(^{0}\)C

No significant change in the melting point of Tenofovir DF from the thermogram of pure Tenofovir DF with that of the thermograms of its physical mixtures was observed. So, it was confirmed that no interaction was existed in between the drug and polymers.
6.3. PURIFICATION, PHYSICOCHEMICAL & PHYTOCHEMICAL CHARACTERIZATION OF NATURAL GUMS

The selected natural gums were collected and purified according to the method prescribed and the gums were kept for physicochemical analysis.

All the three natural gums showed nearly the neutral pH (6.21-7.12). The viscosity of 2% w/v solution at room temperature, 31 °C was found to be high for Delonix regia gum when compared to Anacardium gum and Moringa gum whereas the swelling index value for Delonix gum was found to be less when compared to Anacardium gum and Moringa gum. All these results were given in the table 5.34.

The obtained purified gums were subjected to phytochemical characterization to evaluate the chemical nature of the gums. All the three natural gums passed the molish test, fehling’s test and ruthenium red test confirming that the three gums contain carbohydrates, reducing sugars and gum properties respectively and the results were given in the table 5.35.

6.4. DESIGN, DEVELOPMENT & EVALUATION OF CR FORMULATIONS
6.4.1. DESIGN, DEVELOPMENT AND EVALUATION OF LAMIVUDINE CONTROLLED RELEASE MATRIX TABLETS.

The Lamivudine matrix tablets for controlled release by using an embedment technique by employing natural gums as release retarding agents at various concentrations were successfully formulated (LET1-LLET12). After that, three formulations (LET10A-LET12A), which contain a fixed quantity of natural polymers, i.e., at which the maximum controlling of the drug release was observed, were made into tablets by using the wet granulation method to drive out the efficiency of embedment technique over wet granulation method.

6.4.1.1. Studies on Precompression Blends

Granules of formulations were prepared by mainly embedding technique and also by wet granulation. These granules were evaluated for various derived properties such as bulk density, tapped density and flow properties such as angle of repose, Hausner’s ratio and Carr’s index. All these results together were shown in table 5.36.

The bulk density of the granules was found to be in between 0.909–0.943g/mL and the tapped density was found to be in between 1.020–1.046g/mL. The Carr’s index and Hausner’s ratio of the granules were found to be in between 9.16–12.51% and 1.106–1.129 respectively. The angle of repose of the granules was found in between 19°.08°–24°.36°.
The difference in the bulk and tapped densities indicated that the granules were having good flowability and packageability and it was confirmed by the results of Carr’s index and Hausner’ ratio. The results of the angle of repose indicated that the granules of all the formulations were having good to excellent flowability. These studies combined indicated that the granules of all formulations and of both techniques were suitable for compression.

6.4.1.2. Physical evaluation studies:

The LAM matrix tablets were subjected to different physical evaluation tests such as thickness, hardness, tensile strength, friability and the results obtained were shown in table 5.37. The thickness of the tablets was found to be in between 4.39–4.52mm and these results would be a sign of ease in swallowing. The hardness was found to be in between 5.02 – 5.78 kg/cm²; the tensile strength of the tablets was found to be in between 5.81*10⁵ – 6.76*10⁵ N/m² and the tablets satisfied friability requirement, as the % friability values were less than 1% (0.32-0.57). These results showed that the tablets had enough strength to overcome the external stresses caused by packaging and transportation. The drug content estimations showed values in the range of 97.89 to 102.34%, which reflects good uniformity of the mixing of the drug with excipients during the tabletting process. The tablets passed weight variation test as the % weight variation was within the Pharmacopoeia limits of ± 5% of the weight.

6.4.1.3. Wetting time:

The results of wetting time (shown in table 5.37) indicated that the tablets were sufficiently compressed for the penetration of dissolution medium which might control the drug dissolution rate. From the results, it was indicated that upon increasing in the concentration of natural gum, the wetting time was also increased which might be attributed to the increased control of the dissolution rate.

6.4.1.4. Dissolution studies:

a) Dissolution studies of formulations prepared by embedment technique

The formulations were prepared with increasing amounts of natural gums as release retarding agent. The results of dissolution studies of formulations LET1-LET12 were given in tables 5.38 & 5.39 and these results indicated that upon increasing the amount of natural gum, the dissolution rate constant was decreased and attained more controlled release. The formulations containing Delonix regia gum (LET1, LET4, LET7 & LET10) showed better-controlled release when compared to Anacardium occidentale gum and Moringa oleifera gum and the drug release was
controlled up to 18 hrs by the *Delonix regia* gum (LET10). This might be due to the low swelling behavior of *Delonix regia* gum when compared to *Anacardium occidentale* gum and *Moringa oleifera* gum, as the low swelling index make polymer chains open only partially so that the drug bound with polymer chains releases slowly. The order of efficiency of controlling the drug release was found to be

*Moringa oleifera* gum < *Anacardium occidentale* gum < *Delonix regia* gum

The results of the drug release kinetics of all formulations were given in table 5.41 indicated that all the formulations were found to be linear with zero order kinetics. The $R^2$ values of Higuchi plots were found to be far away from 1, thus indicated that the drug release from the matrix tablet formulations was not pure Higuchi's diffusion. This was further confirmed by the Peppas n values which were in the range of 0.761- 1.710, thus, the drug release from the matrix tablet formulations might be through anomalous diffusion i.e., diffusion is combined with either dissolution or erosion.

**b) Dissolution studies of formulations prepared by wet granulation method**

The formulations which show the more controlled release from each gum were selected and the same formulae were used to prepare the same by using wet granulation method. Formulations LET10, LET11 and LET12 were showing drug release up to 18hrs, 16 hrs and 12 hrs respectively. So, these formulations were also prepared by wet granulation method and the dissolution profiles were compared to study the efficiency of wet granulation.

The formulations contain *Delonix regia* gum (LET10A) showed drug release up to 16 hrs whereas *Anacardium occidentale* gum (LET11A) and *Moringa oleifera* gum (LET12A) showed release up to 14 and 10 hrs respectively.

The results of the drug release kinetics of these formulations were given in table 5.41 indicated that all the formulations were found to be linear with first order kinetics. The $R^2$ values of Higuchi plots indicated that the diffusion associated with the drug release from the matrix tablet formulations was anomalous diffusion i.e., diffusion is combined with either dissolution or erosion which was further confirmed by the Peppas n values. This might be due to high aqueous solubility of Lamivudine, which always make the drug to release from controlled release dosage forms by dissolution mechanism, and limits the drug to release by diffusion mechanism.
From the above results, it was concluded that formulation LET10 (containing 400mg of *Delonix regia* seed endosperm gum) was optimized formulation as it controlled the drug release for the desired period i.e. upto 18 hrs.

From these results, the embedment technique was found to be more effective for the efficient incorporation of drug in the polymer matrix than wet granulation technique for the preparation of controlled release formulations. These results also indicated that the gums obtained from seeds were more efficient in controlling the drug release than the gums obtained from barks.

**6.4.1.5. Stability studies**

The formulation which showed good *in vitro* performance i.e., LET10 subjected to accelerated stability studies. These studies were carried out to investigate the effect of temperature on the physical properties of the tablets and on drug release from the matrix tablet. The results of these studies were given in tables 5.42 & 5.43. The results indicated that there were no physical changes observed in the matrix tablets after storage. It was also observed that there was no significant change in the drug release from the matrix tablets. The controlled drug release characteristics of the matrix tablets remain unaltered. Thus, the controlled release matrix tablets designed were found to be quiet stable.

**6.4.1.6. Statistical Analysis**

The drug release rate constants of all the formulations prepared with different gums at different concentrations were subjected to ANOVA to investigate whether the type and concentration of gum significantly influence the drug release rate from the matrix tablets or not. The ANOVA results indicated that both the type and concentration of gums significantly influenced the drug release rate, but the influence of the concentration of gums was more than that of the type of gum. The influence of concentration of gums was found to be significant at P < 0.01 and the influence of type of gum was found to be significant at P < 0.1.

**6.4.2. DESIGN, DEVELOPMENT AND EVALUATION OF EMTRICITABINE CONTROLLED RELEASE COMPRESSION COATED TABLETS.**

The Emtricitabine controlled release tablets were formulated by the compression coating technology. The compression coated tablets were prepared with varying amount of coating granules. Then the obtained tablets with various thickness of coating layer were studied for the effect of thickness of coating layer on drug release rate.
6.4.2.1. Studies on Precompression Blends

The granules of core tablet and coating polymers of EC, HEC and mixtures of EC with lactose at different concentrations were evaluated for various derived properties such as bulk density, tapped density and flow properties such as angle of repose, Hausner’s ratio and Carr’s index. All these results together were shown in table 5.45.

The bulk density of the granules was found to be in between 0.918–0.932g/mL and the tapped density was found to be in between 1.025–1.042g/mL. The Carr’s index and Hausner’s ratio of the granules were found to be in between 9.85–11.91% and 1.108–1.132 respectively. The angle of repose of the granules was found in between 20.0.341–240.231.

The difference in the bulk and tapped densities indicated that the granules were having good flowability and packageability and it was confirmed by the results of Carr’s index and Hausner’ ratio. The results of the angle of repose indicated that the granules of all the formulations were having good to excellent flowability. These studies combined indicated that all the granules were suitable for compression.

6.4.2.2. Physical evaluation studies:

The granules of HEC fail to produce intact tablets upon the final stage of compression and the tablets were found to be a lack of strength and crumbled upon pressing between fingers. So, these tablets (ECT4–ECT6) were not considered for further studies.

The Emtricitabine compression coated tablets were subjected to different physical evaluation tests such as thickness, hardness, tensile strength, friability and the results obtained were shown in table 5.46. The thickness of the tablets was found to be in between 2.92–3.26mm and these results would be a sign of ease in swallowing. The hardness was found to be in between 3.31–4.92 kg/cm²; the tensile strength of the tablets was found to be in between 7.04*10⁵–9.44*10⁵ N/m² and the tablets satisfied friability requirement, as the % friability values were less than 1% (0.24-0.42). These results showed that the tablets had enough strength to overcome the external stresses caused by packaging and transportation. The drug content estimations showed values in the range of 97.56%–102.23%, which reflects good uniformity of the mixing of the drug with excipients during the tabletting process. The tablets passed weight variation test as the % weight variation was within the Pharmacopoeia limits of ± 5% of the weight.
6.4.2.3. Dissolution studies:

The results of dissolution studies of formulations ECT1-ECT15 were given in tables 5.47 & 5.48 and these results indicated that upon increasing the thickness of the coating, the dissolution rate constant was decreased and attained more controlled release.

The formulations containing ethyl cellulose as coating polymer (ECT1-ECT3) with varied thickness, showed high controlled release i.e., only 16-30% of drug release was found in 18 hrs this indicated that the coat composed of only ethyl cellulose controlled the drug release to greater extent than desired. Then lactose was incorporated in the coat in various proportions along with ethyl cellulose. The formulations containing EC: lactose in 1:1 ratio (ECT7-ECT9) showed the 100% drug release within 4-6 hrs only. The formulations containing EC: lactose in 2:1 ratio (ECT10-ECT12) controlled the release up to 6-12 hrs. The formulations containing EC: lactose in 3:1 ratio (ECT13-ECT15) controlled the release up to 8-20 hrs. These results showed that by altering the amounts of ethyl cellulose and lactose in the coat formulation, the drug release was changing and hence EC: lactose in 3:1 ratio (ECT13-ECT15) was found to be a suitable composition for the desired release rate. Again the influence of the amount of ethyl cellulose and lactose blend in the coat on the release rate was studied by taking 50, 75 and 100 mg. Upon increasing the amount of coating blend, the thickness of the coat was increased and hence the drug release was found to be delayed. At an amount of 75 mg of coating blend of EC: Lactose at 3:1 ratio (ECT14), the drug release was found to be up to 18 hrs which was considered to be optimum. The order of efficiency of controlling the drug release was found to be

\[ \text{EC: lactose (1:1) < EC: lactose (2:1) < EC: lactose (3:1) < Ethyl cellulose} \]

Though ECT 13 (100 mg polymer in coat; 91.22% after 18 hrs) showed more control in release than that of ECT14 (100 mg polymer in coat; 99.87% after 18 hrs), the later was considered to be optimum since it contained less amount of polymer in the coat which resulted in tablets with less weight which is always advantageous.

The results of the drug release kinetics of all formulations were given in table 5.49 indicated that all the formulations were found to be linear with zero order kinetics. The \( R^2 \) values from Higuchi’s plots were found to be far away from 1, thus indicated that the drug release from the matrix tablet formulations was not pure Higuchi’s diffusion. This was further confirmed by the peppas n values which were in
the range of 0.747-1.430, thus the drug release from the compression coated tablet formulations might be through anomalous diffusion i.e., diffusion is combined with either dissolution or erosion for the formulations ECT7-ECT15, this might be due to high aqueous solubility of Emtricitabine, which always make the drug to release from controlled release dosage forms by dissolution mechanism, and limits the drug to release by diffusion mechanism; whereas the for the formulations ECT1-ECT3, the peppas n values were in the range of 0.048-0.149, thus indicated that the drug release mechanism in these formulations was found to be fickian diffusion, this might be because of the highly hydrophobic nature of ethyl cellulose, that bound the drug so firmly that caused the drug to release in controlled manner by diffusion mechanism though it is freely soluble.

6.4.2.4. Stability studies

The formulation which showed good in vitro performance i.e., ECT11 subjected to accelerated stability studies. These studies were carried out by investigating the effect of temperature on the physical properties of the tablets and on drug release from the compression coated tablet. The results of these studies were given in table 5.50 & 5.51. The results indicated that there was no visible and physical changes observed in the compression coated tablets after storage. It was also observed that there was no significant change in the drug release from the compression coated tablets. The controlled drug release characteristics of the compression coated tablets remain unaltered. Thus, the controlled release compression coated tablets designed were found to be quiet stable.

6.4.2.5. Statistical Analysis

The drug release rate constants of all the formulations prepared with different coating thickness at different coating compositions were subjected to ANOVA to investigate whether the coating thickness and coating compositions significantly influence the drug release rate from the compression coated tablets or not. The ANOVA results indicated that both the coating thickness and coating compositions significantly influenced the drug release rate, but the influence of coating thickness was more than that of coating compositions. The influence of coating thickness was found to be significant at P < 0.001 and the influence of coating compositions was found to be significant at P < 0.05.
6.4.3. DESIGN, DEVELOPMENT AND EVALUATION TENOFOVIR DF IMMEDIATE RELEASE TABLETS.

6.4.3.1. Studies on Precompression Blends

Granules of TIR formulations were prepared by wet granulation method. These granules were evaluated for various derived properties such as bulk density, tapped density and flow properties such as angle of repose, Hausner’s ratio and Carr’s index. All these results together were shown in table 5.53.

The bulk density of the granules was found to be in between 0.927–1.007g/mL and the tapped density was found to be in between 1.042–1.111g/mL. The Carr’s index and Hausner’s ratio of the granules were found to be in between 9.17–12.56% and 1.101–1.149 respectively. The angle of repose of the granules was found in between 19°.28–24°.56.

The difference in the bulk and tapped densities indicated that the granules were having good flowability and packageability and it was confirmed by the results of Carr’s index and Hausner’s ratio. The results of the angle of repose indicated that the granules of all the formulations were having good to excellent flowability. These studies combined indicated that the granules of all formulations and of both techniques were suitable for compression.

6.4.3.2. Studies on Physical evaluation tests

The Tenofovir DF immediate release tablets were subjected to different physical evaluation tests such as thickness, hardness, tensile strength, friability and the results obtained were shown in table 5.54. The thickness of the tablets was found to be in between 2.67–3.78mm and these results would be a sign of ease in swallowing. The hardness was found to be in between 2.81–3.89 kg/cm²; the tensile strength of the tablets was found to be in between 5.43*10⁵–7.12*10⁵ N/m² and the tablets satisfied friability requirement, as the % friability values were less than 1% (0.55-0.88). These results showed that the tablets had enough strength to overcome the external stresses caused by packaging and transportation. The drug content estimations showed values in the range of 97.63%–102.89%, which reflects good uniformity of the mixing of the drug with excipients during the tableting process. The tablets passed weight variation test as the % weight variation was within the Pharmacopoeia limits of ± 5% of the weight.
6.4.3.3. Studies on Disintegration test

The Tenofovir DF immediate release tablets were subjected disintegration test and the results obtained were shown in table 5.54. The formulations TIR1-TIR8 fail to disintegrate within the pharmacopoeial limit i.e., below 15 min. This indicated that the polymer HPMC E15 showed more binding properties even in the concentration range of 1-5%, and HPMC E5 also showed more binding properties in the concentration range of 3-5%, but in the formulations contained HPMC E5 at 1-2% pass the disintegration test. All the other formulations passed the disintegration test and the formulations TIR19 & TIR20 were disintegrated with in >5min.

6.4.3.4. Dissolution studies

The formulations which were failed in disintegration test (TIR1-TIR8) were not considered for dissolution test. The results of dissolution studies of formulations TIR9-TIR20 were given in tables 5.55 & 5.56 and these results indicated that the formulations contains sodium starch glycolate were rapidly disintegrated and releases the drug immediately when compared to formulations containing starch as disintegrant. The formulations TIR19 and TIR20 were passed the dissolution test as per pharmacopoeial limits i.e. NLT 85% (the D value is 80% and the limit to pass the test in Stage 1 is NLT D+5%) in 45 min. Though both formulations satisfied the dissolution limits, the formulation TIR19 was considered to be optimum as it contained less amount of SSG (4%) than in the TIR20 (5%).

6.4.3.5. Stability studies

The formulation which showed good in vitro performance i.e., TIR19 subjected to accelerated stability studies. These studies were carried out by investigating the effect of temperature on the physical properties of the tablets and on drug release from the tablet. The results of these studies were given in table 5.58 & 5.59. The results indicated that there were no physical changes observed in the tablets after storage. Thus, the tablets designed were found to be quiet stable.

6.4.4. DESIGN, DEVELOPMENT AND EVALUATION MICROCAPSULES OF LAMIVUDINE AND EMTRICITABINE

Lamivudine and Emtricitabine microcapsules were prepared by emulsion solvent evaporation method. The microcapsules were prepared from four different polymers i.e., Eudragit RLPO, Eudragit RSPO, Ethyl Cellulose 50 cps and Ethyl Cellulose 100 cps at three different concentrations of polymers i.e., 1:0.5, 1:1 and 1:2.
The microcapsules prepared with ethyl cellulose polymers needed the application of temperature for the complete evaporation of solvent evaporation and the formed microcapsules were smaller in size with a smooth texture. But those prepared with Eudragit polymers were dried without the need of temperature and surface was not uniform with small pits. This might be attributed to the possible formation of small droplets at higher viscosity and the controlled evaporation of the solvent from the microcapsules of the first case under moderate temperature.

6.4.4.1. Studies on flow properties:

Microcapsules of Lamivudine and Emtricitabine were evaluated for various derived properties such as bulk density, tapped density and flow properties such as angle of repose, Hausner’s ratio and Carr’s index.

a) Lamivudine microcapsules:

The bulk density of the Lamivudine microcapsules (LMC) was found to be in between 0.912–0.959 g/mL and the tapped density was found to be in between 1.137–1.184 g/mL. The Carr’s index and Hausner’s ratio of the microcapsules were found to be in between 18.12–21.85 and 1.231–1.273 respectively. The angle of repose of the microcapsules was found in between 33°.56¹–37°.54¹. All these results were given in the table 5.60.

b) Emtricitabine microcapsules:

The bulk density of the Emtricitabine microcapsules (LMC) was found to be in between 0.912–0.959 g/mL and the tapped density was found to be in between 1.131–1.174 g/mL. The Carr’s index and Hausner’s ratio of the microcapsules were found to be in between 18.06–21.62% and 1.233–1.279 respectively. The angle of repose of the microcapsules was found in between 33⁰.56¹–37⁰.54¹. All these results were given in the table 5.67.

The difference in the bulk and tapped densities indicated that the microcapsules were having fair flowability and good packageability and it was confirmed by the results of Carr’s index and Hausner’ ratio. The results of the angle of repose indicated that the granules of all the formulations were having fair to good flowability. These studies combined indicated that all the microcapsules were suitable for compression.

6.4.4.2. Studies on other physical properties:

The results of the swelling index were shown in tables 5.61(for LMC) and 5.68 (for EMC). These results indicated that upon an increase in the concentration of
the polymer, the swelling capacity was found to be reduced. This might be because of the more strengthened polymer network in the microcapsules of higher concentration which made the water difficult to absorb into the microcapsules. This behavior was observed in all the four polymers. There is no significant difference was observed in the swelling index among these polymers, which might be because all these polymers are water insoluble.

The percentage yield results were shown in tables 5.61 (for LMC) and 5.68 (for EMC). All the microcapsules of different formulations were prepared by solvent evaporation technique and from the results, more than 80% yield, in any case, indicated that the solvent evaporation technique was highly effective for the preparation of microcapsules.

The results of entrapment efficiency were shown in tables 5.61 (for LMC) and 5.68 (for EMC). These results indicated that upon an increase in the concentration of the polymer, the entrapment efficiency was found to be enhanced as the higher amount of polymer allows more amount of drug to be entrapped in its matrix. Again the entrapment efficiency of the microcapsules of different polymers indicated that it was improved upon an increase in the molecular weight of the polymer. The increasing order of efficiency of the polymers was observed as

\[ \text{Eudragit RLPO} < \text{Eudragit RSPO} < \text{Ethyl Cellulose N50} < \text{Ethyl Cellulose N100} \]

6.4.4.3. Studies on Scanning Electron Microscopy (SEM) Analysis:

The results of the SEM analysis were shown in fig 5.135 & 5.136 (for LMC) and 5.155 & 5.156 (for EMC). The SEM images of microcapsules of formulations LMC5 & EMC5 (Eudragit RSPO at 1:1 ratio, 15 mL chloroform) showed that the texture was rough and the microcapsules were observed to be porous and tortuous. The SEM images of microcapsules of formulations LMC11 & EMC11 (Ethyl cellulose N100 at 1:2 ratio, 15 mL chloroform) showed that the texture was continuous with no observed porosity. This difference in the texture and intactness between the capsules prepared with both the polymers might be because of the nature of molecular strands in the polymer structure.

6.4.4.4. Studies on Dissolution test of microcapsules:

The results of the dissolution studies of microcapsule formulations LMC1-LMC12 were shown in tables 5.62 & 5.63 and figures 5.137 & 5.138. The results of the dissolution studies of microcapsule formulations EMC1-EMC12 were shown in tables 5.69 & 5.70 and figures 5.157 & 5.158.
The results indicated that upon increasing the concentration of polymer, the more controlled drug release was observed. This might be because of the increased thickness of a coat of polymer on the microcapsules upon an increase in its concentration, which might hinder the drug to release to a greater extent. The microcapsules prepared with ethyl cellulose polymers of either viscosity showed lesser drug release rate than that of microcapsules prepared with either of the eudragit polymers. This might be due to lesser viscosity and high permeability of eudragit polymers than those of ethyl cellulose polymers. Further the drug release from microcapsules prepared with eudragit RSPO was more controlled than that of microcapsules prepared with eudragit RLPO, which might be due to less permeability of the former than that of the later polymer. The drug release from microcapsules prepared with ethyl cellulose 100cps was more controlled than that of microcapsules prepared with ethyl cellulose 50cps, which might be due to the higher viscosity of the former than that of the later polymer.

Though LMC9/EMC9 (1:2 drug polymer ratio) showed similar control in release than that of LMC11/EMC11 (1:1 drug polymer ratio), the later was considered to be optimum since it contained less amount of polymer in the coat which resulted in tablets with less weight which is always advantageous.

The results of the drug release kinetics of LMC formulations were given in table 5.64 indicated that all the formulations were found to be linear with zero order kinetics. The $R^2$ values from Higuchi’s plots were found to be far away from 1, thus indicated that the drug release from the microcapsules was not pure Higuchi's diffusion. This was further confirmed by the peppas n values which were in the range of 0.554-1.078, thus, the drug release from the microcapsules might be through anomalous diffusion i.e., diffusion is combined with either dissolution or erosion.

The results of the drug release kinetics of EMC formulations were given in table -5.71 indicated that all the formulations were found to be linear with zero order kinetics. The $R^2$ values from the Higuchi’s plots were found to be far away from 1, thus indicated that the drug release from the microcapsules was not pure Higuchi's diffusion. This was further confirmed by the peppas n values which were in the range of 0.795–1.195, thus, the drug release from the microcapsules might be through anomalous diffusion i.e., diffusion is combined with either dissolution or erosion.
This anomalous diffusion might be due to high aqueous solubility of Lamivudine/Emtricitabine, which always make the drug to release from controlled release dosage forms by dissolution mechanism, and limits the drug to release by diffusion mechanism

6.4.4.5. Stability studies

The formulations which showed good \textit{in vitro} performance i.e., LMC11 & EMC11 were subjected to accelerated stability study. These studies were carried out by investigating the effect of temperature on the drug release from the microcapsules. The results of these studies were given in table 5.65 (for LMC11) and 5.72 (for EMC11). The results indicated there was no significant change in the drug release from the microcapsules. The controlled drug release characteristics of the microcapsules remain unaltered. Thus, the designed controlled release microcapsules were found to be quiet stable.

6.4.4.6. Statistical Analysis

The drug release rate constants of all the formulations prepared with different polymers at different drug-polymer ratios were subjected to ANOVA to investigate whether the drug polymer ratio and type of polymer significantly influence the drug release rate from the microcapsules or not. The ANOVA results indicated that both the drug polymer ratio and the type of polymer significantly influenced the drug release rate at \( P < 0.001 \).

6.4.5. DESIGN, DEVELOPMENT AND EVALUATION BILAYERED TABLETS OF LAMIVUDINE with TENOFOVIR DF (LT) AND EMTRICITABINE with TENOFOVIR DF (ET)

The bilayered tablets of LT & ET were formulated by considering the optimized formulation of microcapsules of LAM/EMT i.e. LMC11/EMC11 and the immediate release tablet of TDF i.e. TIR19 compressed together into tablets.

The LT & ET bilayered tablets were subjected to different physical evaluation tests such as thickness, hardness, tensile strength, friability and the results obtained were shown in table 5.74. The tablets satisfied the hardness, tensile strength and friability requirement. These results showed that the tablets had enough strength to overcome the external stresses caused by packaging and transportation. The drug content estimations values reflect good uniformity of the mixing of the drug with excipients. The tablets passed weight variation test as the \% weight variation was within the Pharmacopoeia limits of \( \pm 5\% \) of the weight.
The results of dissolution studies of formulations LT and ET were given in tables 5.75 & 5.76 and shown in figures 5.178 & 5.180. These results indicated that there was no significant change was observed for the drug release profile of microcapsules, before and after upon tableting microcapsules.

The results of stability studies were given in table 5.77, 5.78 and 5.79. The results indicated there was no significant change in the drug release from the bilayered tablets. The controlled drug release characteristics of the microcapsules remain unaltered even before and after tableting. Thus, the designed controlled release bilayered tablets were found to be quiet stable.

6.5. IN VIVO PHARMACOKINETIC EVALUATION OF SELECTED FORMULATIONS OF LAMIVUDINE AND EMTRICITABINE

The Lamivudine CR matrix tablet (LET10) and Emtricitabine CR microcapsules (EMC11) were selected for the in vivo clinical study. The plasma kinetic data was assessed with “pk solutions 2.0” software.

Figures 5.183 and 5.185 showed the comparative mean plasma concentration-time profiles of the test (CR formulation) and reference (pure drug) of Lamivudine and Emtricitabine respectively. Tables 5.81 and 5.83 showed the kinetic data of Reference and test formulations of Lamivudine and Emtricitabine respectively.

The $C_{max}$ values of the test formulations (T) were less when compared with those of Reference (R). The $T_{max}$ values of the test formulations (T) were high when compared with those of Reference (R), this clearly indicated the rate of the drug absorption from the test formulations was controlled which might be because of the controlled release of the drug from the test formulations. After achieving $T_{max}$, the reference drug underwent rapid elimination but the test formulation showed gradual decrease in the plasma drug concentration which further indicated the absorption continued for a prolonged period.

The increase in $AUC_{0-t}$ was observed in the test formulation (T) when compared to reference drug (R). This clearly indicated, the availability of drug is more in case of test formulation.

The decrease in elimination rate constant ($K_{el}$) from reference drug (R) to test formulations (T) indicated presence of drug in the body in therapeutically effective concentrations for a long period.
There was a difference in $T_{\text{max}}$ and $C_{\text{max}}$ was observed when compared among individual subjects which might be due to intersubject variability. This was observed in both test and reference formulations.

The overall $C_{\text{max}}$, $T_{\text{max}}$, $AUC_{0-t}$ and $K_{el}$ were completely different between test and reference formulations indicated the successful development of controlled release formulations of both Lamivudine and Emtricitabine.