Chapter 6
Summary & Conclusion

6.1. Summary and Conclusion

The oral route of drug administration is still preferred to the non-oral alternatives mainly for the reasons of lower production cost, better suitability for self-medication, a higher level of patient safety, and better patient compliance. One important prerequisite for successful oral therapy is the sufficient intestinal absorption of the orally administered drug from gastrointestinal tract. Intestinal drug absorption is controlled by, (i) dissolution rate and solubility, determining how fast a drug reaches a maximum concentration in the luminal intestinal fluid, and (ii) permeability coefficient, which relates to the rate at which dissolved drug will permeate the epithelium of the intestinal mucosa to reach the portal blood circulation. It has been found that, approximately 40% of newly synthesized drug candidates have poor water solubility and the oral delivery of such drugs can be frequently associated with implications of low bioavailability, high intra and inter subject variability, and lack of dose proportionality. To overcome these problems, various formulation strategies can be adopted including the use of surfactants, CDs, nanoparticles, solid dispersions, micronization, lipids, permeation enhancers etc. Nowadays, Microemulsifying drug delivery system and self-emulsifying drug delivery systems (SEDDS) have been explored widely as a delivery system by virtue of having considerable potential to enhance the oral bioavailability of a wide range of poorly water soluble drug molecules across the G.I. tract.

The objectives of this investigation were to prepare and characterize fast dissolved and rapidly absorbed oral microemulsions and CDs inclusion complexes of Cilostazol/Tadalafil and to assess their pharmacokinetic performance for oral drug delivery in rabbits. It was hypothesized that oral administration of Cilostazol/Tadalafil microemulsion/inclusion complex will result in to effective oral drug absorption, reduced dose dependent toxicity, reduce side effects, and rejuvenate their life in treatment of peripheral vascular disease (Cilostazol) and erectile dysfunctioning (Tadalafil).

Cilostazol 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl) butoxy]-3, 4-dihydro-2 (1*H*)-quinolinone, and several of its metabolites are cAMP PDE III inhibitors, inhibiting phosphodiesterase activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation. It is also used as an antithrombotic agent. For over a decade, Cilostazol has been considered the first choice of treatment for peripheral vascular disease. Cilostazol is commercially available only for oral administration which is the most preferred route from the viewpoint of patient compliance and convenience. However, following oral

administration, the absorption of the drug from the gastrointestinal tract is poor, slow and variable. The oral bioavailability of Cilostazol is very low because of its poor water solubility (3D/mL at room temperature.)[1]. Additionally it shows large deviation in its bioavailability between one subject and another subject when administered via orally.

Tadalafil, pyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-, (6R,12aR)-, an oral treatment for erectile dysfunction, is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5).The PDE 5 inhibitors are the first oral agents that are truly effective and safe. The long half life of Tadalafil (17.5 Hour) translates into a consideribaely longer period of clinical responsiveness than that of another PDE 5 inhibitors (4-5 Hour). This special pharmacokinetic profile of the Tadalafil is an effective aspect for patients, reliving them from any time constrains and consequently, making sexual activities more spontaneous and therefore more natural. The absorption of the drug after oral administration from the gastrointestinal tract is poor, slow and variable. The oral bioavailability of Tadalafil is low (16%)[2] . Also low oral bioavailability is associated with greater intersubject variability of plasma concentrations and, hence, poorer control of the effects of the drug.

Analytical Method Development for Cilostazol :-

- A UV spectrophotometric method for the estimation of Cilostazol was developed before formulating an oral microemulsion/inclusion complex. The absorbance of Cilostazol solutions was found to be linear in the concentration range of 5 – 50 µg/mL at 257 nm.
- The samples of Cilostazol for inclusion efficiency study, phase solubility study, *in vitro* dissolution study (Cilostazol in dissolution media), Cilostazol formulations and *in vitro* diffusion studies (Cilostazol in diffusion media) were analyzed using validated UV spectroscopic method. The samples for microemulsions and *in vitro* diffusion studies were analyzed by preparing dilution in methanol and measuring the absorbance at 257 nm. The ingredients used for microemulsion preparation or diffusion and dissolution media did not interfere with the proposed method.
- An already reported HPLC method[3] was modified and used for the estimation of Cilostazol. The mobile phase selected was acetonitrile:water (60:40) with flow rate of 0.4 mL/min on a C_{18} Phenomenax Luna column. The retention time of Cilostazol was found to be 12.2-12.4 min at detection wavelength 254 nm with no interfering peaks of the solvent. The method was found to be linear in the range of 1-50 µg/mL. The regressed calibration graph between mean area and concentration, exhibited correlation coefficient

(r^2) greater than 0.999. The method was validated for linearity (different days), accuracy (intra- and inter-day), precision (intra- and inter-day), robustness and recovery studies and the % RSD was found to meet the USP criteria (< 2%).

- Cilostazol estimation in plasma was performed using the above-mentioned method after liquid-liquid extraction. There were no interfering peaks of plasma at the retention time of Cilostazol. The method was used for pharmacokinetic and stability studies and the SD was found to be not more than 4 (specified limit is < 5 in biological samples).
- Cilostazol microemulsions (CME 1 and CME 2) were successfully prepared using the titration technique followed by construction of pseudo-ternary phase diagrams. The formulations were prepared by screening out a suitable drug vehicle as well as the solubility profile, accordingly different oils, surfactants and co-surfactants were selected.

Development of Formulation for Cilostazol:-

- Microemulsions were formulated at different S:CoS ratios such as 1:1, 1:2 and 1:3 and its pseudo-ternary phase diagrams were plotted. According to the data obtained, selected excipients and its composition for ME preparation are mentioned in Table 1.

Table 1: Excipients and its composition for the preparation of CME 1 and CME 2.

System	Oil (%)	Surfactant (%)	Co-surfactant (%)	Aqueous phase (%)	Amount of Cilostazol (mg/mL)
CME 1 (S: CoS ratio 3:1)	Capmul MCM C8 (15%)	Labrafil M1944 (26.25%)	Transcutol P (8.75%)	Water (50%)	10
CME 2 (S: CoS ratio 1:1)	Capmul MCM C8 (13.8%)	Tween 20 (16.24%)	Transcutol P (16.24%)	Water (53.72%)	10

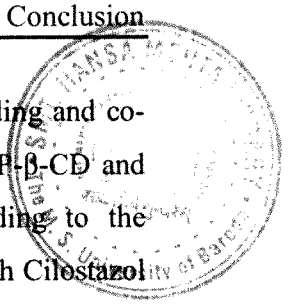
- Prepared CME 1 and CME 2 were characterized by globule size, zeta potential, % Transmittance, pH, viscosity and conductivity measurement. The results obtained from the globule size and zeta potential measurement were fairly reproducible within the range of ± 5 nm / ± 2 mV respectively. The % Transmittance of the both ME systems were found to be >99 % at 630nm which proved the clarity and transparency of the systems. The pH of the prepared MEs systems were similar to the pH of water which proved the ME systems were stable at stomach condition. Viscosity of CME 1 and CME 2 were

found to be 26.6 and 29.39 cps. From the supportive data of Viscosity and electroconductivity, it can be concluded that the ME systems were purely o/w type.

- The Stability of CME 1 and CME 2 as per stomach condition was studied by diluting the MEs with 10 parts, 100 parts and 1000 parts of distilled water and determining their globule size, zeta potential and % Transmittance. After each dilution, the globule size was increased because of decreased interface between oil and water phase by adding excess of water. Zeta potential was reduced up to -7.5 mV because with the increasing anionic phase the negative charge was increased into the ME. Diluted MEs showed decreased % Transmittance (between 98.7 to 99.70%) because of increase in the globule size.
- CME 1 and CME 2 were subjected to accelerated centrifugation for assessing the stability of the formed microemulsions. No appreciable change was seen before and after centrifugation for 15 min at accelerated conditions. The globule size of the CMEs in top, middle and bottom layer for CME 1 and CME 2 were within ± 5 nm from the initial values. The data clearly suggested that CMEs were physically stable under the testing conditions. All the batches of ME were having globule size less than 85 nm and zeta potential close to -2 mV or less. It was also observed that ME having zeta potential with a negative value gives reasonably good physical stability with regards to phase separation. It was concluded the CMEs which are bicontinuous, w/o or o/w were found to be stable.
- Drug retention study was performed on physically stable ME by subjecting CMEs at 30°C / 65% RH and 40°C / 75% RH. The MEs were assessed for globule size, size distribution, zeta potential, percent transmittance, and drug content. When globule size was evaluated up to six months, it was found that globule size for all CME formulations were within the range of ± 5 nm from the initial values and no abnormal changes in the globule size were noticed at both the accelerated testing conditions. The zeta potential values were also found to be consistent and within the range ± 5 mV from the initial values. The data clearly indicated that the formulations were found to be thermodynamically stable at accelerated conditions. Percent transmittance at 630 nm for all the selected experimental batches were found to be greater than 99% which indicated the clarity of the tested ME and indirectly gave an indication that no inversion, phase separation or cracking of the prepared CMEs were observed. Drug content for different CME formulations were found to be more than 95 %. The data clearly demonstrated that there was no appreciable degradation at 30°C / 65% RH and 40° C/ 75% RH. The results conclusively demonstrate the selected CMEs are physically and chemically stable at

accelerated stability conditions. The formulations were found to meet the general monograph of Pharmacopoeia and criteria stipulated therein for the liquid preparations.

- *In vitro* diffusion studies of CME 1 and CME 2 were carried out to evaluate relative diffusion behaviour. The diffusion studies were carried out by dialysis bag and intestinal permeability study technique. The data revealed that **CME 2 have substantially higher diffusion among all formulations across the dialysis bag (60.00%) and intestinal mucosa (57.32%).** Cilostazol microemulsions (CME 2) showed 2.85-fold higher diffusion compared to marketed formulation (Pletoz – 50) and 6.58-fold higher diffusion compared to Cilostazol solution by dialysis bag study and 2.88-fold higher diffusion compared to marketed formulation (Pletoz – 50) and 7.49-fold higher diffusion compared to Cilostazol solution by intestinal permeability study. This may be attributed to the fact that microemulsion enhances transport of drug across mucosa.
- The results obtained from characterization, physical and chemical stability and *in-vitro* diffusion study suggest that **the CME 2 formulation was promising and further selected for *in-vivo* pharmacokinetic study.**
- The pharmacokinetic (*in vivo* absorption) study was carried out in New Zealand rabbits (fasted over night). Suspension of Pletoz-50, pure Cilostazol, and Cilostazol microemulsion (CME 2) were administered orally to the rabbits and the blood samples were collected from the marginal ear vein at hourly basis after the administration. The heparinised blood samples were immediately centrifuged at 40000 rpm for 15 minutes and separated plasma was stored at -20 $^{\circ}$C. Plasma samples collected from the rabbits and were analyzed using pre- developed reverse phase HPLC method. The average drug plasma concentration values were determined from the calibration curve. As per the obtained results, **C_{max} of CME 2 was 2.65 fold higher than pure Cilostazol and 1.43 fold higher than marketed formulation (Pletoz – 50).**
- The value of **$T_{1/2}$ was also decreased for CME 2 (10.10 Hr.)** compared with pure Cilostazol (12.68) and Pletoz-50(11.83) which indicated that *in vivo* absorption of CME 2 was rapid and higher compared to pure Cilostazol and Pletoz-50.



- Cilostazol-CDs Inclusion complexes were successfully prepared using kneading and co-precipitation methods. The complexing agent selected were β-CD, γ-CD, HP-β-CD and DM-β-CD according to their suitability for oral administration. According to the solubility studies data, suitable ratios like (1:1, 2:1 and 3:1) of CDs along with Cilostazol were selected to form inclusion complexes.
- The phase solubility study for the complex formation between Cilostazol and CDs in aqueous solution (HCl buffer pH 1.2, water and Phosphate buffer pH 6.8) was carried out at 37° C. The extremely low solubility of Cilostazol (0.101± 0.004 µg/mL in water at 37° C) was linearly increased with the increase in CDs concentration, giving rise to A_L - type solubility diagrams with strictly linear ascent having a regression values(r^2) >0.99. This linear Cilostazol-CDs correlation, suggest the formation of a 1:1 (mol/mol) Cilostazol-CDs inclusion complexes at the different pH values and all these results indicate that Cilostazol-CD complexes (1:1 molar ratio) were sufficiently stable in phosphate buffer pH 6.8 and the values of stability constant were more than 100 M^{-1}.Values of stability constant were less than 100 M^{-1}in water and HCl buffer pH 1.2 indicating that the inclusion complexes of Cilostazol-CDs were not stable in these solutions. The phosphate buffer pH 6.8 showed highest solubility (393.52 ± 4 µg/mL) of Cilostazol and stability constant (390.071 ± 35M^{-1}) of Cilostazol-DM-β-CD inclusion complex (1:1 molar ratio).
- The % inclusion efficiency of 1:3 Cilostazol-CDs inclusion complexes were more than 98.5 ± 1.50% compared with the another inclusion complexes and all physical mixtures having a values in the range of 49.2 ± 1.53 to 89.4 ± 1.33 which indicated that drug was uniformly distributed in all 1:3 inclusion complex whereas, the another inclusion complexes and physical mixtures did not show satisfactory drug incorporation.
- Prepared inclusion complexes were characterized by FTIR, DSC and XRD analysis which suggested that 1:3 ratio of Cilostazol-CDs inclusion complexes prepared by co-precipitation method having a less intense and reduced peak compared with pure Cilostazol.
- *In vitro* dissolution studies were performed to evaluate relative solubility behavior of different formulations of Cilostazol. From the phase solubility study, phosphate buffer pH 6.4 was found to be suitable dissolution medium as it showed maximum drug release. Dissolution study was carried for all Cilostazol-CDs physical mixtures, Cilostazol-CDs inclusion complexes prepared by co-precipitation and kneading methods, pletoz-50 and

pure Cilostazol. The %Cumulative drug release of all physical mixtures, inclusion complexes, pletoz-50 and pure Cilostazol were mentioned in Table 2.

Table 2: Maximum % cumulative drug release of all physical mixtures, inclusion complexes, Pletoz-50 and pure Cilostazol.

Method	Cilostazol-CDs Ratios	Maximum % cumulative drug release ± SD			
		Cilostazol-β-CD	Cilostazol-γ-CD	Cilostazol-HP-β-CD	Cilostazol-DM-β-CD
Physical Mixture	1:1	45.35 ± 1.59	51.98 ± 2.62	61.55 ±1.45	53.22 ±0.13
	1:2	56.67 ±1.93	56.09 ± 1.55	66.56 ±1.59	69.76 ±0.25
	1:3	69.32 ± 1.75	71.13 ± 1.24	72.34 ±2.14	78.62 ±1.78
Co-precipitation Method	1:1	69.32 ± 1.75	67.87 ± 1.82	78.32 ±0.830	78.21 ±2.56
	1:2	68.88 ± 1.56	78.95 ± 1.64	83.72 ± 1.24	88.55 ±2.14
	1:3	76.49 ± 1.09	95.20 ± 2.31	83.72 ± 1.24	**98.16 ± 2.24**
Kneading Method	1:1	47.83 ± 1.47	63.99 ± 1.04	55.76 ±1.85	61.96 ±2.85
	1:2	63.76 ± 1.65	66.4 ± 2.33	70.5 ±2.67	73.61 ±1.33
	1:3	73.28 ± 1.93	87.08 ± 2.02	79.89 ±1.24	84.86 ±1.23
PLETOZ – 50		46.56 ± 2.1			
Pure Cilostazol		32.98 ± 0.91			

The data shows that, **inclusion complex of Cilostazol-DM-β-CD in the ratio of 1:3, prepared by co-precipitation method showed maximum drug release (98.16 ± 2.24%).**

- Cilostazol-DM-β-CD inclusion complex (1:3 ratio) showed 2.10-fold higher drug released compared to marketed formulation (Pletoz–50) and 2.97-fold higher drug release compared to Cilostazol solution.
- The values of DE_{120min} and T_{50} study for all Cilostazol-CDs physical mixtures, inclusion complexes, Cilostazol and marketed formulation (Pletoz–50) indicated that inclusion complex of **Cilostazol-DM-β-CD in the ratio of 1:3, prepared by co-precipitation method having highest dissolution efficiency (89.59%) among the all inclusion complexes.** Reduction in the time taken for 50 % dissolution (**T_{50} value-11.81 min.**) indicated that the inclusion complex having a rapid and higher dissolution rate and it was further selected for stability study.

- Stability study of Cilostazol-DM-β-CD in the ratio of 1:3 was carried out as per ICH Q1A (R2) and SUPAC IR guidelines. The sample was stored at 30° C ± 2° C / 65% ± 5% relative humidity (RH) and 40° C ± 2° C / 75% ± 5% RH for 6 months time duration. The samples for *in vitro* dissolution study were collected at 1, 2, 4 and 6 months respectively. As per the result obtained, change of cumulative percentage of drug release after 6 months was from 4.0 % to 12.0%. The dissolution curve did not show any significant changes in dissolution patterns indication stability of Cilostazol in the inclusion comeplx.
- The results of student's t-test and similarity factor (f_2) show insignificant difference between the dissolution profiles and the calculated f_2 values for Cilostazol-DM-β-CD was higher than 50 indication stability of formulated product on storage.
- The pharmacokinetic (*in vivo* absorption) study was carried out in New Zealand rabbits (fasted over night) using the method as described for CME 2. As per the obtained results, **C_{max} of Cilostazol-DM-β-CD inclusion complex was 4.11 fold higher than pure Cilostazol and 2.23 fold higher than marketed formulation (Pletoz – 50).**
- The value of **$T_{1/2}$ was also decreased in Cilostazol-DM-β-CD inclusion complex (7.46 Hr.) compared with pure Cilostazol (12.68) and Pletoz-50(11.83).**Decreased values of $T_{1/2}$ for Cilostazol-DM-β-CD inclusion complex indicated that, *in vivo* absorption were rapid and higher compared to pure Cilostazol and Platoz-50.

Analytical Method Development for Tadalafil:-

- Analytical method based on UV spectrphotometry was developed for the estimation of Tadalafil before formulating an oral microemulsion/inclusion complex. The absorbance of Tadalafil solutions was found to be linear in the concentration range of 2 – 24 µg/mL at 284.5 nm.
- The samples of Tadalafil for inclusion efficiency study, phase solubility study, *in vitro* dissolution study (Tadalafil in dissolution media), Tadalafil formulations and *in vitro* diffusion studies (Tadalafil in diffusion media) were analyzed using validated UV spectroscopic method at 284.5 nm. The ingredients used for microemulsion preparation or diffusion and dissolution media did not interfere with the proposed method.
- An already reported HPLC method[4] was modified and used for the estimation of Tadalafil. The mobile phase selected was water containing 0.1 mM of glacial acetic acid (pH 2.5 – 2.7):acetonitrile (60:40) with flow rate of 1.0 mL/min on a C_{18} Phenomenax Luna column. The retention time of Tadalafil was found to be 11.7-11.9 min at detection wavelength 280 nm with no interfering peaks of the solvent. The method was found to be linear in the range of 0.5 -50 µg/mL. The regressed calibration graph between mean area and concentration, exhibited correlation coefficient (r^2) greater than 0.999. The method was validated for linearity (different days), accuracy (intra- and inter-day), precision (intra- and inter-day), robustness and recovery studies and the % RSD was found to meet the USP criteria (< 2%).
- Tadalafil was analyzed in presence of plasma using the above-mentioned method after liquid-liquid extraction. There were no interfering peaks of plasma at the retention time of Tadalafil. The method was used for pharmacokinetic and stability studies and the SD was found to be not more than 4 (specified limit is < 5 in biological samples).
- A spectrofluorophotometric method for the estimation of Tadalafil was developed. Fluorescence spectrum of Tadalafil in 0.1 M methanolic sulphuric acid is showed excitation wavelength at 315 nm and emission wavelength at 332 nm. The calibration curves for Tadalafil showed linearity in the concentration range of 10-50 ng/mL with correlation coefficient of 0.9998. The method was validated and found to be accurate, precise and sensitive with the % RSD < 2% as per USP craiteria.
- Estimation of Tadalafil in plasma was performed by spectorflurophotometric method after precipitation of proteins and no interfering peaks of plasma were found in the spectrum. The SD was found to be less than 4.3 (specified limit is < 5 in biological samples).

Development of Formulation for Tadalafil:-

- Tadalafil microemulsions (TME 1, TME 2 and TME 3) were successfully prepared using the titration technique followed by construction of pseudo-ternary phase diagrams. Solubility study was carried and they were selected on the merit of highest solubility of Tadalafil.
- Microemulsions were formulated at different S:CoS ratios such as 1:1, 1:2 and 1:3 and its pseudo-ternary phase diagrams were plotted. According to the data obtained, selected excipients and its composition for ME preparation are mentioned in Table 3.

Table 3: Excipients and its composition for the preparation of TME 1,TME 2 and TME 3.

System	Oil (%)	Surfactant (%)	Co-surfactant (%)	Aqueous phase (%)	Amount of Tadalafil (mg/mL)
TME 1 (S: CoS ratio 3:1)	Capmul MCM C8 (12 %)	Tween 20 (25.00 %)	Transcutol P (8.33 %)	Water (55.55 %)	15
TME 2 (S: CoS ratio 3:1)	Capmul MCM C10 (16.66 %)	Tween 80 (25.00 %)	Transcutol P (8.33 %)	Water (50.00%)	15
TME 3 (S: CoS ratio 3:1)	Capmul MCM C10 (15.09 %)	Tween 20 (28.30 %)	Transcutol P (9.43 %)	Water (47.16 %)	15

- The prepared TME 1, TME 2 and TME 3 were characterized by globule size, zeta potential, % Transmittance, pH, viscosity and conductivity measurement. The results obtained from the globule size and zeta potential measurement were fairly reproducible within the range of ± 5 nm / ± 2 mV respectively. The % Transmittance of the both ME systems were found to be >99 % at 630nm which proved the clarity and transparency of the systems. The pH of the prepared MEs systems were similar to the pH of water which proved the ME systems were stable at stomach condition. Viscosity of TME 1, TME 2 and TME 3 were found to be 29.2, 31.33 and 28.5 cps respectively. From the supportive data of viscosity and electroconductivity, it can be concluded that the ME systems were purely o/w type.

- The Stability of TME 1,TME 2 and TME 3 as per stomach condition were was studied by diluting the MEs with 10 parts, 100 parts and 1000 parts of distilled water and determining their globule size, zeta potential and % Transmittance. After each dilution, the globule size was increased because of decreased interface between oil and water phase by adding excess of water. Zeta potential was reduced up to -8.1 mV because with the increasing anionic phase the negative charge was increased into the ME. Diluted MEs showed decreased % Transmittance (between 98.66 to 99.08%) because of increase in the globule size.
- TME 1, TME 2 and TME 3 were subjected to accelerated centrifugation for assessing the stability of the formed microemulsions. No appreciable change was seen before and after centrifugation for 15 min at accelerated conditions. The globule size of the TMEs in top, middle and bottom layer for TME 1, TME 2 and TME 3 were within ± 5 nm from the initial values. The data clearly suggested that TMEs were physically stable under the testing conditions. All the batches of MEs were having globule size less than 50 nm and zeta potential close to -8 mV or less. It was also observed that ME having zeta potential with a negative value gives reasonably good physical stability with regards to phase separation. It was concluded the TMEs which are bicontinuous, w/o or o/w were found to be stable.
- Drug retention study was performed on physically stable ME by subjecting TMEs at 30°C / 65% RH and 40°C / 75% RH. The MEs were assessed for globule size, size distribution, zeta potential, percent transmittance, and drug content. When globule size was evaluated up to six months, it was found that globule size for all TME formulations were within the range of ± 5 nm from the initial values and no abnormal changes in the globule size were noticed at both the accelerated testing conditions. The zeta potential values were also found to be consistent and within the range ± 5 mV from the initial values. The data clearly indicated that the formulations were found to be thermodynamically stable at accelerated conditions. Percent transmittance at 630 nm for all the selected experimental batches were found to be greater than 99% which indicated the clarity of the tested ME and indirectly gives an indication that no inversion, phase separation or cracking of the prepared TMEs were observed. Drug content for different TME formulations were found to be more than 95 %. The data clearly demonstrated that there was no appreciable degradation at 30°C / 65% RH and 40° C/ 75% RH. The results conclusively demonstrate the selected TMEs are physically and chemically stable at accelerated stability conditions.

The formulations were found to meet the general monograph of Pharmacopoeia and criteria stipulated therein for the liquid preparations.

- *In vitro* diffusion studies of TME 1, TME 2 and TME 3 were carried out to evaluate relative diffusion behaviour. The diffusion studies were carried out by dialysis bag and intestinal permeability study technique. The data revealed that, **TME 3have substantially higher diffusion among all formulations across the dialysis bag (65.95%) and intestinal mucosa (63.37%).** Tadalafil microemulsion (TME 3) showed 2.77-fold higher diffusion compared to marketed formulation (Tadora – 20) and 4.81-fold higher diffusion compared to Tadalafil solution by dialysis bag study and 3.00-fold higher diffusion compared to marketed formulation (Tadora – 20) and 5.19-fold higher diffusion compared to Tadalafil solution by intestinal permeability study. This may be attributed to the fact that microemulsion enhances transport of drug across mucosa.
- The results obtained from characterization, physical and chemical stability and *in-vitro* diffusion study suggest that **the TME 3 formulation was promising and further selected for *in-vivo* pharmacokinetic study.**
- The pharmacokinetic (*in vivo* absorption) study was carried out in New Zealand rabbits (fasted over night). Suspension of Tadora-20, pure Tadalafil, and Tadalafil microemulsion (TME 3) were administered orally to the rabbits and the blood samples were collected from the marginal ear vein at hourly basis after the administration. The heparinised blood samples were immediately centrifuged at 40000 rpm for 15 minutes and separated plasma was stored at -20 °C. Plasma samples collected from the rabbits and were analyzed using pre- developed reverse phase HPLC method. The average drug plasma concentration values were determined from the calibration curve. As per the obtained results, **C_{max} of TME 3 was 3.00 fold higher than pure Tadalafil and 2.33 fold higher than marketed formulation (Tadora – 20).**
- The value of **$T_{1/2}$ was also decreased for TME 3 (9.42 Hr.)** compared with pure Tadalafil (16.99) and Pletoz-50(16.96) which indicated that *in vivo* absorption of TME 3 was rapid and higher compared to pure Tadalafil and Tadora-20.

- Tadalafil-CDs Inclusion complexes were successfully prepared using kneading and co-precipitation methods. The complexing agent selected were β-CD, γ-CD, HP-β-CD and DM-β-CD according to their suitability for oral administration. According to the solubility studies data, suitable ratios like (1:1, 2:1 and 3:1) of CDs along with Tadalafil were selected to form inclusion complexes.
- The phase solubility study for the complex formation between Tadalafil and CDs in aqueous solution (HCl buffer pH 1.2, water and Phosphate buffer pH 6.8) was carried out at 37° C. The extremely low solubility of Tadalafil (0.98± 0.004 µg/mL in water at 37° C) was increased in a concentration depending manner by adding CDs. The data obtained by this study, showed enhanced solubility of Tadalafil in β-CD containing aqueous solution (HCl buffer pH 1.2) shown the A_P type of isotherm having a regression values (r^2) < 0.99, which was having a positive deviation from linearity which indicated a continuous increase in the stoichiometry of the complex that was, the original 1:1 complex tends to associated with further guest molecule, forming 2:3 compositions etc,. The solubility of the Tadalafil increased linearly with the increase of γ-CD, HP-β-CD and DM-β-CD concentration, giving rise to A_L - type solubility diagrams with strictly linear ascent with a regression values (r^2) > 0.99. The results indicated that Tadalafil-CDs complex (1:1 molar ratio) were sufficiently stable in HCl buffer pH 1.2 and the vales of stability constant were more than 100 M^{-1}. Highest solubility (39.49 ± 2.47 µg/mL) of Tadalafil and stability constant (351.88 ± 21 M^{-1}) of Tadalafil-β-CD inclusion complex (1:1 molar ratio) were found in HCl buffer pH 1.2.
- The % inclusion efficiency of 1:3 Tadalafil-CDs inclusion complexes were more than 96.2 ± 0.82% compared with the another inclusion complexes and all physical mixtures having a values in the range of 60.2 ± 0.11 to 91.48 ± 0.77 which indicated that drug was uniformly distributed in all 1:3 inclusion complex whereas, the another inclusion complexes and physical mixtures did not show satisfactory drug incorporation.
- Prepared inclusion complexes were characterized by FTIR, DSC and XRD analysis which suggested that 1:3 ratio of Tadalafil-CDs inclusion complexes prepared by kneading method having a less intense and reduced peak compared with pure Tadalafil.
- *In vitro* dissolution studies were performed to evaluate relative solubility behavior of different formulations of Tadalafil. From the phase solubility study, HCl buffer pH 1.2 was found to be suitable dissolution medium as it showed maximum drug release. Dissolution study was carried for all Tadalafil-CDs physical mixtures, Tadalafil-CDs

inclusion complexes prepared by co-precipitation and kneading methods, Tador - 20 and pure Tadalafil. The %Cumulative drug release of all physical mixtures, inclusion complexes, Tadora - 20 and pure Tadalafil were mentioned in Table 4.

Table 4: Maximum % cumulative drug release of all physical mixtures, inclusion complexes, Tadora - 20 and pure Tadalafil.

Method	Tadalafil-CDs Ratios	Maximum % cumulative drug release ± SD			
		Tadalafil-β-CD	Tadalafil-γ-CD	Tadalafil-HP-β-CD	Tadalafil-DM-β-CD
Physical Mixture	1:1	59.58 ± 2.61	19.84 ± 2.42	35.11 ± 2.11	20.82 ± 3.34
	1:2	68.92 ± 2.55	20.82 ± 1.86	50.31 ± 1.92	23.17 ± 2.51
	1:3	75.36 ± 3.11	24.14 ± 1.88	62.44 ± 1.58	28.72 ± 1.01
Co-precipitation Method	1:1	67.65 ± 2.01	24.56 ± 2.82	60.00 ± 2.54	29.13 ± 1.58
	1:2	74.98 ± 2.53	28.44 ± 2.73	69.09 ± 3.22	30.10 ± 2.99
	1:3	86.72 ± 1.99	29.83 ± 1.93	75.45 ± 3.25	34.15 ± 0.89
Kneading Method	1:1	78.32 ± 2.76	25.39 ± 3.56	67.35 ± 2.11	26.50 ± 3.04
	1:2	83.72 ± 3.33	28.16 ± 2.83	78.12 ± 1.21	39.25 ± 2.85
	1:3	**90.38 ± 1.25**	46.14 ± 1.35	86.95 ± 1.88	63.46 ± 1.34
TADORA – 20		44.33 ± 0.62			
Pure Tadalafil		17.06 ± 1.11			

The data shows that, **inclusion complex of Tadalafil-β-CD in the ratio of 1:3, prepared by kneading method showed maximum drug release (90.38 ± 1.25%).**

- Tadalafil-β-CD inclusion complex (1:3 ratio) showed 2.03-fold higher drug release compared to marketed formulation (Tadora–20) and 5.29-fold higher drug release compared to Tadalafil solution.
- The values of DE_{120min} and T_{50} study for all Tadalafil-CDs physical mixtures, inclusion complexes, Tadalafil and marketed formulation (Tadora-20) indicated that inclusion complex of **Tadalafil-β-CD in the ratio of 1:3, prepared by kneading method having highest dissolution efficiency (64.72%)** among the all inclusion complexes. Reduction in the time taken for 50 % dissolution **(T_{50} value-30.20 min.)** indicated that the inclusion complex having a rapid and higher dissolution rate and it was further selected for stability study.

- Stability study of Tadalafil-DM-β-CD in the ratio of 1:3 was carried out as per ICH Q1A (R2) and SUPAC IR guidelines. The sample was stored at 30° C ± 2° C / 65% ± 5% relative humidity (RH) and 40° C ± 2° C / 75% ± 5% RH for 6 months time duration. The samples for *in vitro* dissolution study were collected at 1, 2, 4 and 6 months respectively. As per the result obtained, change of cumulative percentage of drug release after a 6 months was from 4.0 % to 9.0%. The dissolution curve did not show any significant changes in dissolution patterns indication stability of Tadalafil in the inclusion comeplx.
- The results of student's t-test and similarity factor (f_2) show insignificant difference between the dissolution profiles and the calculated f_2 values for Tadalafil-β-CD was higher than 50 indication stability of formulated product on storage.
- The pharmacokinetic (*in vivo* absorption) study was carried out in New Zealand rabbits (fasted over night) using the method as described for TME 3. As per the obtained results, **C_{max} of Tadalafil-β-CD inclusion complex was 1.81 fold higher than pure Tadalafil and 1.41 fold higher than marketed formulation (Tadora – 20).**
- The value of **$T_{1/2}$ was also decreased in Tadalafil-DM-β-CD inclusion complex (11.23 Hr.)** compared with pure Tadalafil (16.99) and Tadora - 20(16.96).Decreased values of $T_{1/2}$ for Tadalafil-β-CD inclusion complex indicated that, *in vivo* absorption were rapid and higher compared to pure Tadalafil and Tadora-20.
- Finally the enhancement of oral absorption rate may be explained in terms of (1) the huge specific surface area of the microemulsion droplets(droplet size less that 90 nm) and CD inclusion complex (2) improved permeation because of the presence of surfactant, which reduced the interfacial tension, (3) the stability of the microemulsion in the gastrointestinal tract,(4) improved dissolution because of the hydrophilic exterior surface of CD and(5) the stability of inclusion complex in the gastrointestinal tract.

To conclude, oral microemulsions and CD inclusion complexes of Cilostazol and Tadalafil were successfuly prepared and demonstrated the rapid and higher absorption rate through the G.I.T. in rabbits. **Amongst the various formulations (MEs and Inclusion complexes) of Cilostazol and Tadalafil, Cilastazol-DM-β-CD (1:3) prepared by Co-precipitation method and TME 3 microemulsion having S:CoS ratio (3:1) were found to be most promising for future development and commercial utilization.** Enhanced solubility and absorption rate may reduce/eliminate dose dependent side effects of both the drug for the treatment of peripheral vascular (Cilostazol) and ED (Tadalafil) diseases. However, clinical studies with special focus on toxicity evaluation on chronic use of the

developed formulations is necessary for establishing suitability in clinical practice in the treatment of peripheral vascular disease and ED.

6.2. References

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