Conclusion

9. CONCLUSION
Candesartan cilexetil is an orally administered ACE inhibitor for the treatment of hypertension and cardiac failure, but its solubility, stability and oral bioavailability are poor. The objective of our investigation was to formulate a self microemulsifying drug delivery system (SMEDDS) of candesartan cilexetil using minimum surfactant concentration that could improve its solubility, stability and oral bioavailability. The composition of optimized formulation [C7IIB] consist of Capryol 90 as oil, Labrasol as surfactant and Captex 500 as cosurfactant, containing 32 mg of candesartan cilexetil showing drug release for liquid SMEDDS formulation (99.91%), droplet size (9.15 nm), Zeta potential (-23.2), viscosity (0.8824 cP) and infinite dilution capability. In-vitro drug release of the C7IIB was highly significant (p <0.05) as compared to marketed conventional tablet (M). The C7IIB was further used for the preparation of various Solid SMEDDS(S-SMEDDS) formulations (Tablet). These tablets were prepared via adsorption to solid carrier technique, using optimized liquid SMEDDS formulation [C7IIB] whereas Aeropearl 300 pharma as optimized adsorbents. The resulting S-SMEDDS tablet exhibited particle size (78.3 nm) whereas the liquid SMEDDS showed (9.15 nm). The in vitro release was almost similar for the S-SMEDDS as well liquid i.e. 78.32% and 84.6% respectively within 5 min. Also, one of the main objective to enhance the oral bioavailability of drug (15%) which was enhanced to 1.78 folds. In conclusion, our studies illustrated that adsorption to solid carrier technique could be a useful method to prepare the solid SMEDDS tablets from liquid SMEDDS, which can improve oral absorption of candesartan cilexetil, nearly equivalent to the liquid SMEDDS, but better in the formulation stability, drugs leakage and precipitation, etc.
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➢ The solubility of candesartan cilexetil was found to be highest in Capryol 90 (80.12±4.04mg/mL) as compared to other oils while in water it was (0.09±0.01mg/mL). Thus, Capryol 90 was selected as the oil phase for the development of the formulation.

➢ From the prepared liquid SMEDDS formulations, C7 and C4 are clear whereas remaining became cloudy on dilution.

➢ Addition of higher concentration of co-surfactant (C) as compared to concentration of surfactant (A, B and D) showed poor microemulsion.

➢ From the results of pseudoternary phase diagram it was revealed that formulation C7IIB covers the maximum microemulsion region as compared to other formulations whereas other formulations makes microemulsion which are unstable on dilution and have poor microemulsion region.

➢ Higher concentration of oil in SMEDDS may provide greater opportunity for the solubilization and incorporation of higher concentration of candesartan cilexetil.

➢ It was observed that the viscosity of all the formulations is less than 1 cp which shows that all SMEDDS forms o/w microemulsion.

➢ All the formulations showed similar pH values in the range of 5.1 to 6.0; thus pH is not affecting stability. Therefore it can be assumed that drug is not diffusing in the external phase and remains in the oil phase. Since, water is the external phase entire system showed pH of water. Candesartan cilexetil is unstable in alkaline pH. Here the formulations show acidic to neutral pH which is suitable for stability of Candesartan cilexetil.

➢ It was observed that formulation C1, C3, C5 and C6 did not pass the thermodynamic stress tests and thus were dropped for further study.

➢ Formulation C7IIB was found out to have minimum average particle size 9.15 nm in water.
Conclusion

- The optimal batch C7IIB had the least zeta potential i.e. -23.2 mV with highest zeta potential towards negative side. The zeta potential governs the stability of microemulsion, it is important to measure its value for stability samples. The high value of zeta potential indicates electrostatic repulsion between two droplets. DLVO theory states that electric double layer repulsion will stabilize microemulsion where electrolyte concentration in the continuous phase is less than a certain value.

- Formulation C7 has % transmittance value greater than 99% which indicates the high clarity of microemulsion.

- The results show that formulations C3ID and C3IB does not pass the test as they have PDI more than 0.3 whereas remaining all formulations pass the test as they have PDI less than 0.3.

- The formulation C7IIB showed highest release rate among all the liquid SMEDDS formulations i.e. 92.01% in 10 min which is highest among all batches. The in-vitro study concludes that release of candesartan cilexetil was greatly enhanced by SMEDDS formulation. The batch C7IIB was thus taken for further studies and comparison.

- The formulation C7IIB has the maximum release rate at all the time as compared to the Atacand Tablet (M) and pure drug (S).

- From Two way ANOVA it can also be said that the change in the time and compositions of various formulations i.e. combination of oil and S/CoS have significant effect on the release rate of the formulation.

- From the stability studies it was revealed that formulation C7IIB is more stable as compared to marketed tablet Atacand.

- The values clearly prove that after the stability study, formulation C7IIB doesn’t show significant difference. After 3 months stability study the particle size of C7IIB was found to be 10.87 nm in water and the initial particle size was 9.15 nm, so no significant difference was found. The PDI was found to be 0.221 initially and 0.186 after stability.
Conclusion

study. The zeta potential was initially found to be -23.2 mV and after stability study it was found to be -22.2 mV.

- This result indicates that all the excipients used are compatible and hence form stable microemulsion with almost same particle size. From, all th results batch C7IIB was selected as optimized formulation and further used for solidification and convertin it into a solid dosage form (Tablet).

- Adsorption to solid carrier technique was used and adsorbent Aeroperl 300 pharma (A2) was selected as optimized adsorbent as it require only 60 mg to convert liquid SMEDDS (0.2 ml containing 32 mg drug) into free flow powder. Also, A2 showed better results for other tests like powder flow properties as well as in-vitro dissolution.

- Thus, solid state characterization was performed for Aeroperl 300 pharma based free flow powder by SEM. Surface of Aeroperl 300 pharma was rough before and after adsorption of liquid SMEDDS a smooth surface was observed which indicate that liquid smedds was adsorbed on the surface of Aeroperl 300 pharma.

- Further solid SMEDDS formulation i.e. tablets was prepared using the mixture of optimized liquid SMEDDS (C7IIB) and adsorbent Aeroperl 300 pharma (A2).

- Various tablet evaluation parameters of batch T1 to T9 were found to be satisfactory and within the specification for candesartan cilexetil tablet.

- All batches shows approximately 70% drug released within 5 minutes but among this batch T4 shows 78.32 % drug released in 5 minutes which was faster as compare to other batches.

- The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as drug absorption. Particle size of the batch T4 in water was found to be 78.3 nm which was higher as compare to optimized liquid SMEDDS formulation C7IIB which has particle size 9.15 nm.
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- Zeta potential of the formulation was found to be -17.4 mV for the batch T4 which was higher as compared to optimized liquid SMEDDS formulation C7IIB which has zeta potential value -23.2 mV.
- The DSC thermogram was taken for pure drug Candesartan cilexetil and optimized Solid SMEDDS formulation (T4). Pure drug substance shows a sharp endothermic peak at 169°C which shows the highly crystalline behavior of drug. Whereas, no peak was observed in S-SMEDDS formulation which shows the change in melting behavior of drug and inhibition of crystallization following granulation using lipid surfactants.
- A full factorial design was applied for the tablet formulations. The effect of various diluents (Lactose monohydrate, Mannitol and MCC) and the concentration of pregelatinized starch were kept as independent variables X1 and X2 respectively. The dependent variables were DT, T70 and T90. The data shows that values are strongly dependent on the selected independent variables.
- In-vitro drug release of batch T4 was compared with the marketed tablet formulation i.e. Atacand 32 mg tablet (M). Marketed formulation shows just 32.47% drug release in 5 min whereas tablets of batch T4 shows 78.32% drug release in same time. This data clearly indicate that by formulating S-SMEDDS formulation of candesartan cilexetil, solubility and thus dissolution profile of candesartan cilexetil was increased.
- In-vitro drug release of batch T4 was also compared with the optimized liquid SMEDDS formulation C7IIB and marketed formulation M. Batch T4 shows almost same dissolution profile as that of batch C7IIB. Initial drug release was found to be slightly slower in T4 as compared to C7IIB.
- Accelerated stability study (40°C ± 2°C /75% ± 5% RH) and real time stability study (25°C ± 2°C /60% ± 5% RH) was performed on batch T4 for a period of three months. No significant changes were observed in appearance, average weight, hardness, thickness and friability of the tablets for both the condition.
- Assay was decreased to 84.76% in the sample stored at accelerated condition after three months which indicate that formulation were not stable at higher temperature. So other evaluations were not performed on the sample stored at accelerated condition. After three
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months storage of batch T4 at real time condition assay value was fond to be 98.83% which indicate formulation were stable at this condition.

- Initial value for particle size of the batch T4 was 78.3 nm and after stability it was found to be 79.2 nm. Zeta potential value after stability was found to be -17.1 mv and the initial value was -17.4 mv so data indicate that formulation was stable.

- *In-vivo* studies were performed using optimized S-SMEDDS (T4) and marketed sample Atacand (M). The decrease in systolic blood pressure was observed in DC3 rats [receiving high dose S-SMEDDS suspension] which showed 26.75±0.336% and DC4 rats [receiving high dose plain drug suspension] showed 18.0±0.358% decrease in systolic blood pressure. Thus, significant enhancement in antihypertensive activity was clearly observed attributed to microsizing of candesartan cilexetil.

- Thus, it was confirmed that Candesartan cilexetil decreases blood pressure in a dose-dependent manner and hence decrease in pressor effect can be directly correlated with the amount of drug that reaches systemic circulation i.e. bioavailability of drug. Based on this pharmacodynamic study, it could be concluded that bioavailability of drug was higher from S-SMEDDS suspension in comparison to plain drug suspension.

- *In vivo* pharmacokinetic behaviors of candesartan cilexetil with SMEDDS (T4) and marketed formulation (Atacand) were studied in rat. Plasma concentration $C_{\text{max}}$ and $\text{AUC}_{0\rightarrow t}$ are significantly increased for S-SMEDDS than those for the Atacand suspension. $T_{\text{max}}$ is decreased for S-SMEDDS(T4) and it was 1 h for S-SMEDDS(T4) and 1.36 h for Atacand formulation. Relative bioavailability is increased 1.78-fold.

- Increased bioavailability of S-SMEDDS (T4) may due to its lymphatic transport through transcellular pathway. It is also reported that the long-chain oils promote lipoprotein synthesis and subsequent lymphatic absorption. On the basis of *in vitro* and *in vivo* correlation, it can be assumed that increase in release profile of candesartan cilexetil from S-SMEDDS (T4) can lead to increase of bioavailability of candesartan cilexetil.

From all above results it can be concluded that the proposed objective of the present research work of enhancing bioavailability of candesartan cilexetil, a low solubility antihypertensive drug, by improving solubility of drug was achieved successfully.