9. Summary and Conclusion
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The objective of the present research study was to formulate, develop and optimize microemulsion systems for oral delivery of poorly water soluble active pharmaceutical ingredients. Microemulsions are expected to be absorbed through the lymphatic route and hence the first pass hepatic metabolism can be decreased. Moreover, because the drug would be in solubilized form and in a fine state of sub-division, the absorption and bioavailability can be increased significantly. The most popular alternative and commercially viable form of microemulsion is self-microemulsifying drug delivery system (SMEDDS). This system is able to rapidly self-microemulsify in GI fluids and forms fine O/W microemulsions under the gentle agitation by GI tract movements. Solid SMEDDS would show higher kinetic and thermodynamic stability profile, better patient compliant, transport and storage as compared to liquid SMEDDS formulation.

We have explored design and development of oral microemulsions, SMEDDS and Solid SMEDDS for poorly water soluble drugs.

The drugs selected for present investigation were Felodipine and Valsartan. Felodipine was selected for formulation and development of microemulsion and Valsartan was selected for formulation and development of SMEDDS and Solid SMEDDS. We have employed Capmul MCM/Tween 20/PEG 400 system to formulate microemulsion system containing poorly water soluble drug Felodipine. We have employed Capmul MCM/Tween 80/PEG 400 system to formulate Self Microemulsifying drug delivery system containing poorly water soluble drug Valsartan. The optimized liquid SMEDDS formulation of Valsartan was converted into free flowing powder by adsorption of liquid onto solid carriers. The solid carriers used include Colloidal silicon dioxide i.e. Aerosil 200 alongwith either Lactose monohydrate or Microcrystalline cellulose (MCC) i.e. Avicel PH101.

The drugs in our work were identified and characterized by FTIR and UV spectroscopy and were found to be pure.
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The excipients with higher solubilizing efficiency for drug are selected for formulation development. Solubility of drugs was determined in different oils, surfactants and co-surfactants. The highest solubility of Felodipine and Valsartan was found in capmul MCM. Hence it was selected as oil phase for both drugs. The solubility of Felodipine was also very high in Tween 20 (surfactant) and PEG 400 (co-surfactant). Hence these components were selected as surfactant and co-surfactant for microemulsion system preparation of Felodipine. The solubility of Valsartan was almost similar in PEG 400 and Transcutol P. All three surfactants i.e. Tween 80, Peceol and Labrasol show comparable solubility of Valsartan. Thus all of them were selected for formulation development. Thus Tween 80, peceol and Labrasol were selected as surfactants and PEG 400 and Transcutol P as co-surfactants for Valsartan SMEDDS development.

The drug and surfactant compatibility study was designed to evaluate the effect of surfactant and co-surfactant on the physical and chemical stability of drugs. Felodipine did not show any signs of incompatibility with surfactant and co-surfactant mixture. There were no significant losses of potency (less than 10%) in any of the samples indicating chemical stability. Valsartan with Peceol : PEG 400 and Labrasol : PEG 400 combination showed incompatibility with surfactants during 1 month study. Thus they were eliminated from further studies. The remaining S:CoS combinations passed the drug-Surfactant compatibility test and were taken for further studies.

The optimization of surfactant: co-surfactant (S/CoS) ratio was performed using pseudoternary phase diagrams by water titration method. Among all S/CoS ratio studied for Felodipine microemulsion, 2:1 ratio formed better microemulsion region and more water incorporation to form visually clear microemulsion compared to 1:1 ratio and almost similar to 3:1 and 4:1 ratio and hence selected for further development and in all the cases concentration of oil was kept less than 20%v/v.

Valsartan SMEDDS was prepared using three different systems considering the solubility study of the drug in various surfactants and cosurfactants (V1, V2 and V3). In system V1, composition B prepared with 2:1 ratio of S/CoS forms better SMEDDS compared to other two formulations. The systems V2 show comparatively smaller microemulsion
region and composition A with 3:1 ratio of S/Cos forms better SMEDDS compared to other two formulations. In System V3, composition A prepared with 3:1 ratio of S/CoS forms better SMEDDS compared to other two compositions. The Microemulsion region of V1 was higher than V3 in spite of having Tween 80 as surfactant in both systems which may be due to the different co-surfactants used in the compositions.

Phase diagrams studies indicated that the incorporation of Felodipine does not affect the microemulsion region in the phase diagram. It was found that incorporation of Valsartan in the system led to a slight reduction in the area of microemulsion in the phase diagram. The possible reason for this is orientation and participation of Valsartan at the interface with surfactants due to low aqueous solubility and high surfactant mixture solubility of Valsartan. The reduction in the area of microemulsion formation could be due to valsartan influenced interaction of surfactant and co-surfactant with oil.

A series of formulations were developed with varying ratios of oil, surfactant and co-surfactant as shown in chapter 5. All formulations were prepared by varying ratio of Oil in three levels i.e 5%, 7.5% and 10% v/v. For Felodipine microemulsion, Formulations F1 – F9 were prepared using Capmul MCM as oil, Tween 20 as surfactant and PEG 400 as co-surfactant to optimize the concentration of oil and $S_{mix}$. In all the formulations, the level of Felodipine was kept constant (i.e. 20 mg/ml of Felodipine). For Valsartan SMEDDS, Formulations V1 (A, B and C) were prepared using Capmul MCM as oil, Tween 80 as surfactant and PEG 400 as co-surfactant. Similarly formulations V2 (A, B and C) were prepared with Capmul MCM as oil, Labrasol as surfactant and Transcutol P as co-surfactant. Third system containing formulations V3 (A, B and C) were prepared using combination of Capmul MCM, Tween 80 and Transcutol P as an oil, surfactant and co-surfactant respectively. The concentration of Valsartan in all SMEDDS formulation was 40 mg/ml.

These 9 batches each of microemulsion and SMEDDS were successfully characterized and evaluated as described in chapter 6. These formulations were characterized for appearance, clarity, thermodynamic stability, dispersibility, droplet size distribution and zeta potential, PDI, conductivity, assay, pH, and viscosity.
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Based on the results of % Transmittance, thermodynamic stability and dispersibility studies, formulations batches F4, F6, F7, F8 and F9 and system V2 were rejected whereas remaining formulations were further subjected to droplet size determination.

The droplet size analysis of the Felodipine Microemulsion showed that the mean droplet size of F1, F2, F3 and F5 were below 100 nm. However, F2 showed significantly smaller droplet size than others. It was found that Valsartan SMEDDS formulations V1A, V1B, V1C and V3A, V3B formed SMEDDS having particle size less than 100 nm. The least droplet size was observed in the formulation batch V1A with mean particle size 11.86nm in water. The analysis also revealed that the droplet size was significantly increased by increasing the concentration of Capmul MCM. Based on Zeta potential values, it was found that formulation F2 and V1A were within the ideal range of -10 to -30 mV.

The data of PDI for Felodipine microemulsion showed that the formulations F1, F2 and F3 have PDI less than 0.3 whereas only V1A was found to have PDI value less than 0.3.

The dye solubility test was performed on Felodipine microemulsion confirmed the type of microemulsion to be o/w. The conductivity test on SMEDDS formulations revealed that the SMEDDS Formulation V1A has the highest conductivity 98.59 as compared to all other SMEDDS systems which confirm the lowest droplet size of the formulation.

The amount of the drug content in all formulations of microemulsion and SMEDDDS was in the range of 98-99% of the added amount. The results of assay revealed suitability of the system for high entrapment of drug in the internal phase.

All the formulations showed similar pH values in the range of 6.0 to 6.6 in case of Felodipine microemulsion and in the range 5.8 to 6.3 in case of Valsartan SMEDDDS. Therefore it can be assumed that drug is not diffusing in the external phase and remains in the oil phase

The viscosity of Felodipine microemulsions were in the range of 0.882 – 0.898 whereas the viscosity of SMEDDDS formulations was found to be in the range of 0.894 – 1.018 which is similar to that of water i.e. 1.0. This reveals that all the formulations are very clear, transparent and low viscous liquids.
Upon investigating the effect of Felodipine on droplet size of microemulsion, it was found that the globule size decreased with the decrease in the Felodipine loading.

The Felodipine microemulsions showed fairly similar mean globule size within range of 15–22 nm when diluted with various dissolution media differing in pH (pH 7, 1.2 and 6.8). The time required for formation of microemulsions after dilution with various dissolution media was less than 2 minutes.

Dynamic surface tension of Felodipine microemulsion F1, F2, F3 and F5 was measured using the maximum bubble pressure technique. The DST of microemulsion F2 was found to be more stable compared to F3 and F5. The experiment exhibited a higher dynamic surface tension for formulation F2. These findings indicated that formulation F2 with 45% v/v surfactant concentration is more stable than other formulations under the study.

Considering the droplet size, zeta potential, PDI, DST and other characteristics, Felodipine microemulsion formulation F2 was selected as optimized formulation and subjected to TEM study. The study revealed nearly uniform desired globule size with round and slight elliptical shape. There was absence of coalescence after 100 times dilution which suggests the physical and thermodynamic stability of the formulation. Microemulsion F2 has shown percolation phenomena at higher temperature and in dilution of excess to 1000 times with the dispersion medium which suggest instability and unsuitability of the system in these drastic conditions.

In-vitro release of Felodipine Microemulsion formulation F2 was studied by using dialysis bag method in phosphate buffer (pH 6.8). This in vitro study concludes that release of Felodipine was very fast by microemulsion as approximately 85% of the drug release was achieved in 1 hour. The in-vitro dissolution behavior of the Valsartan SMEDDS was studied in phosphate buffer (pH 6.8). T85% of SMEDDS formulations was found to be varied between 15 to 20 min and the lower T85% was found in formulation V1A i.e. 15 minutes which satisfy the US-FDA guidance for immediate release products. The dissolutions of SMEDDS were very fast as approximately 100% drug release was achieved in less than 45 min.
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On the basis of all the characterization results for Valsartan SMEDDS, formulation V1A was found suitable as optimized SMEDDS. The three main criteria, particle size, PDI and zeta potential of the system V1A fulfills the requirement of SMEDDS. The other characteristics like dilutability, viscosity, pH and conductivity of V1A also support the formation of o/w microemulsion upon dilution. T\textsubscript{85\%} of the formulation V1A was found out to be lower compared to other formulation. Hence V1A was selected for further study.

The \textit{in-vitro} intestinal permeability study of optimized formulations viz Felodipine microemulsion F2 and Valsartan SMEDDS V1A revealed that the drug diffused at a faster rate from the microemulsion/SMEDDS than from the plain drug suspension. \textit{In-vivo} absorption study of Felodipine microemulsion revealed that the oral bioavailability of Felodipine microemulsion F2 was increased 9.85 fold than that of plain drug suspension of Felodipine. Based on \textit{in-vitro} release and \textit{in-vivo} absorption studies, it can be said that microemulsion and SMEDDS offers a promising approach to increase solubility and bioavailability of poorly water soluble drugs.

The optimized liquid SMEDDS formulation (V1A) was converted into free flowing powder by adsorption of liquid onto solid carriers. The solid carriers used include Colloidal silicon dioxide i.e. Aerosil 200 alongwith either Lactose monohydrate or Microcrystalline cellulose (MCC) i.e. Avicel PH101. Both water soluble (lactose monohydrate) and insoluble (microcrystalline cellulose as Avicel PH 101) adsorbents and combination of them (1:1 mixture) were used. The prepared formulation batches of solid SMEDDS (S-SMEDDS) SS1, SS2 and SS3 were characterized for flow property and \textit{in-vitro} drug release. The optimised formulation was assessed for reconstitution properties morphology and solid state characterization.

The flowability study revealed that only formulation SS1 showed satisfactory flowability as its angle of repose value was less than 35. The in-vitro release study revealed that the fastest release was obtained with formulation SS1 i.e. 99.81\%. Nearly, 60\% of drug was released in first 10 min. SS1 showed good emulsification time within a min. It was concluded that the formulation SS1 containing Lactose monohydrate showed the faster
release due to its hydrophilic nature. The drug dissolution study also indicated that the self microemulsifying property of the formulation remains unaffected by the conversion of the formulation to solid.

Comparisons indicated that $T_{85\%}$ of formulation SS1 consisting of lactose monohydrate is found to be significantly different statistically ($P = <0.001$) from SS2 consisting of Avicel PH 101. Similarly formulation SS3 has shown significantly different dissolution profile difference ($P = <0.05$) in terms of $T_{85\%}$ with SS1 and SS2. The data revealed that the type of adsorbents carrier in the formulations has the significant effect on the release rate of the formulation.

The comparison of the of in-vitro drug release of optimized formulation of S-SMEDDS i.e. SS1 with the marketed conventional tablet formulation (Valzar) and optimized liquid SMEDDS formulation i.e. V1A revealed that $T_{85\%}$ of marketed formulation Valzar is significantly different from both solid and liquid SMEDDS formulations i.e. SS1 and V1A respectively ($P<0.001$). Not surprisingly, the comparison between V1A and SS1 does not show significant difference with each other ($P = 0.077 > 0.05$). The results clearly indicated that the dissolution profile of solid and liquid SMEDDS was similar with each other but significantly different and better than marketed formulation (Valzar).

Reconstitution properties of S-SMEDDS have shown that adsorbing the liquid SMEDDS on solid carrier seem to have a significant effect on droplet size and PDI. Still the solid SMEDDS preserved the self-microemulsification performance of the liquid SMEDDS.

The comparative morphological analysis of solid SMEDDS consisted of well separated particles. Moreover, the particle showed a nearly spherical shape with shallow dents which is considered advantageous to enhance the dissolution rate of Valsartan.

The solid state characterization of S-SMEDDS showed that Valsartan in the solid SMEDDS was in the amorphous state or disordered crystalline phase of a molecular dispersion state in the matrix.

Optimized formulations F2 (Felodipine Microemulsion), V1A (Valsartan SMEDDS) and SS1 (Valsartan Solid SMEDDS) were subjected to stability studies. It was found that all
formulations were robust to dilution and pH changes. The stability studies revealed that formulation F2 and V1A were able to retain original droplet size of microemulsion system. Also it was clarified that solidification has preserved self microemulsification property of the SS1 formulation during stability studies. All the characteristics like droplet size, zeta potential, %Transmission, pH and assay support the conclusion that all three formulations F2, V1A and SS2 were found stable even after 6 months period of chemical stability. The result indicates that all the excipients used are compatible and hence form stable microemulsion/SMEDDS with almost same particle size.

These all results suggest that the microemulsions are physically and chemically stable on storage.