Summary
9.0 Summary
Cancer is a generic term for a large group of diseases that can affect any part of the body. Other terms used are malignant tumours and neoplasms. Curcumin, the most explored of the curcuminoids, has received increasing interest in recent years. Curcumin has tremendous potential for the treatment or prevention of cancer and various other diseases like Rheumatoid arthritis, Multiple sclerosis, Alzheimer, cataract formation, pulmonary toxicity and fibrosis. Unfortunately Curcumin suffers from poor biopharmaceutical properties such as, low solubility, poor permeability, metabolism in enterocytes and extensive first pass metabolism. This limits its oral bioavailability and hinders its successful application to the treatment and prevention of these pathologies, as therapeutic concentrations are difficult to achieve at the site of action. By a systematic application of modern formulation approaches these biopharmaceutical barriers can be successfully overcome and the true potential of Curcumin can be fully exploited. An industrially scalable product with enhanced bioavailability of curcumin has been developed, characterized and reported in this work.
Curcumin being a drug of herbal origin has been standardized for its identity purity and various contaminants that are required to be controlled as per the current regulatory guidelines. As part of characterization DSC, TGA, NMR, SEM and HPTLC fingerprinting has been carried out. The precise content of each of the three curcuminoids present in the extract was estimated by the HPLC method. It was found that the total curcuminoids content of the extract was 98.5% with 72.2% Curcumin, 21.7% Demethoxycurcumin and 4.7% Bisdemethoxycurcumin. A total of 6 heavy metals were analyzed in the extract by ICP-MS. Also no pesticides or Aflatoxins were detected in the drug.
Use of appropriate and validated analytical methods is essential for any formulation development exercise. Two methods; one on HPLC, for in vitro samples and the second on LCMSMS, for bioanalysis have been developed and validated as part of this work. The HPLC method was validated for linearity, range and filter validation. A correlation coefficient ($R^2 = 0.9993$) indicated functional linearity between concentration of curcumin and peak area. Filtered samples gave a recovery of 98.91 % (RSD of 1.75 %) for 100 ng/ml sample. Thus it was established that curcumin is not retained on the filter and Nylon membrane filters can be used for filtration of samples without loss of accuracy.
Due to the poor bioavailability of curcumin, very low concentrations are attained in plasma; thus a LC/MS/MS method has been developed and validated for the pharmacokinetic studies. A simple liquid-liquid extraction based method has been
Developed and fully validated as per the US FDA guidance for industry for validation of bioanalytical methods. Various parameters like Specificity, Linearity, Matrix Effect, Precision and Accuracy, Recovery of Standard, Stability, Dilution Integrity and Ruggedness have been studied and the method found to be meet the requirements laid down in the guidance and thus suitable for the analysis of curcumin in plasma samples.

As part of the pre-formulation studies forced degradation studies have been carried out for curcumin to establish its intrinsic stability, it was concluded that curcuminoids are stable against acid hydrolysis, extremely labile under base hydrolysis, labile under neutral hydrolysis, labile under oxidation and photolabile. These results provide valuable inputs in the design of the delivery system and also the packaging system to be used. For further development no alkaline excipients were studied and it was decided to have a final pack which minimizes contact with light and air to prevent oxidative or photo degradation.

As the first step in the development of the SNEDDS formulation solubility studies have been carried out in excipients such as oil phase, co-solvents, surfactants and co-surfactants. Based on the results of these solubility studies Transcutol, Capryol and Cremophor were selected for development of the SNEDDS. Though a higher solubility was seen in citronella oil it has not been studied further because of lack of safety data for use in oral formulations. Solubility in other commonly used oils like Olive oil, Arachis oil and Coconut oil was found to be too low for achieving significant drug loading in the formulation thus these were not studied further for the development of SNEDDS.

The effect of pH on the solubility of curcumin has also been studied. Curcumin has shown pH dependent solubility; higher solubility was seen in pH above seven; this can be attributed to the ionization of the phenolic groups and is in line with the solubility behavior of polyphenolic compounds. Though solubility is enhanced in alkaline medium it cannot be used as an approach for enhancing the oral bioavailability as curcumin has been shown to be extremely labile to alkali hydrolysis.

Compatibility of curcumin was studied with various excipients by isothermal stress testing. Curcumin was found to be compatible with most of the excipients studied. Maximum degradation of around 20 % was seen in lauroglycol and olive oil. Negligible degradation was observed in the excipients used in the final formulation like Transcutol, Cremophor, Caproyl 90 and PEG 200.

Based on the results of the solubility and compatibility studies Transcutol, Cremophor, and Caproyl 90 were shortlisted for the development of SNEDDS and were further taken for construction of ternary phase diagrams. Ternary phase diagrams were drawn.
for deciding Smix ratio and its ratio with oil phase in self emulsifying system. On the basis of solubility of curcumin in various excipients, Cremophor EL was selected as the surfactant, Transcutol HP as co-surfactant and Capryol 90 was selected as the oil phase. First three ternary phase diagrams were drawn to establish the best Smix ratio (ratio of surfactant to the cosurfactant i.e. the ratio of Cremophor and Transcutol). The Smix ratio of 2:1 (2 parts Cremophor EL and 1 part Transcutol) was found to produced the largest nanoemulsion zone. Thus the Smix ratio was fixed as 2:1 for further optimization. Due to regulatory binding Transcutol HP could not be added beyond its Inactive Ingredient Guideline limit so it was decided to replace a part of Transcutol HP with PEG 200 (selected on the basis of its solubility). Different ratios of Transcutol HP : PEG 200 were taken (1:3, 1:2, 1:1, 2:1, 3:1) and ternary phase diagrams were again constructed to determine what part of Transcutol can be substituted with PEG 200. Based on the pseudo ternary phase diagrams we decided to use Transcutol to PEG 200 (3:1) as with this ratio the largest nanoemulsion zone was observed. This enabled a 25% reduction in content of Transcutol in the formulation.

Due to the large conventional dose of curcumin and its poor solubility the amount of curcumin which can be loaded into the SNEDDS system was a critical parameter for selection of the final formulation. Solubility of curcumin in the five candidate formulations was studied and was found to range between 34 to 66 mg/ml. The five curcumin loaded formulations have been characterized for Droplet size, zeta potential, emulsification time, in vitro dissolution, morphology and thermodynamic stability. The candidate formulations evaluated were found to have average particle size ranging from 85 to 190 nm. Formulation A was found to have the lowest particle size at 85 nm and formulation D the largest at 190 nm. As particle size is a critical parameter in the bioavailability enhancement formulation C was selected for further studies and evaluation as it had the optimum balance of particle size and solubility curcumin.

The formulations were found to have negative Zeta potential. Zeta potential measurements though of great value to evaluate the stability of stabilized nano or microparticulate systems are of lesser relevance for self emulsifying systems as they are stored and supplied as a single phase, oily system which forms an emulsion spontaneously on coming in contact with water. As emulsion formation is a spontaneous process the emulsion is the more stable state. All the SNEDDS formulations were found to rapidly form clear emulsions under the test conditions. The emulsions formed were clear with a strong yellow color characteristic of curcumin.
Based on the results of solubility of curcumin in SNEDDS, particle size and its composition formulation C was selected as the final formulation for further characterization and evaluation. Formulation C gave the highest drug loading (61 mg/ml) with particle size of 90 nm, though formulation D gave higher drug loading (66 mg/ml) the particle size was found to be much larger at 200 nm.

The TEM image after dilution shows spherical particles having a diameter of around 90 nm. This shows that though the system does not contain a traditional oil phase but rather contains a water insoluble surfactant, Capryol 90, in its place a nanoemulsion has still been formed. This has been further reconfirmed by the AFM image of the system which shows similarly sized spherical particles having a smooth surface.

The effect of different diluents like distilled water, phosphate buffered saline (pH 6.8) and 0.1 N hydrochloride solution on the nano-emulsification process was studied. No significant change was seen in the particle size as a function of diluents used. This can be attributed to the nonionic nature of the surfactants used in the formulation as their behavior is not influenced by the pH of the dilution media.

Multi pH dissolution studies have been carried out on the optimized formulation. No effect of pH of the dissolution medium was observed on the release of curcumin from the formulation. Further $f_2$ values were calculated for the dissolution profiles, considering dissolution in 0.1 N HCl as the standard profile. The $f_2$ values were found to be greater than 50, thus statistically establishing that there is no difference in the dissolution behavior as a function of pH of the dissolution medium. The pH independent dissolution behavior can be attributed to the nonionic nature of the surfactants used in the SNEDDS formulation. Formulation C was subjected to thermodynamic stability tests like Centrifugation Study, Heating and cooling cycle, Freeze thaw cycle (Accelerated ageing). It was found that the Formulation C was thermodynamically stable and there were no signs of precipitation, creaming or cracking.

Accelerated stability studies at 40°C and 75% RH have been carried out for Formulation C. No significant changes were seen in the Assay and particle size of the formulation over the 6 months accelerated stability study with storage at 40°C and 75% RH. The formulation showed a slightly better performance in the glass bottle pack compared to the blister pack, which was anticipated as glass is a better barrier to oxygen and moisture compared to PVC blister. No visual changes like leakage or haziness of diluted formulation were seen in either of the two packs. It can be concluded that the formulation is stable and can be assigned a shelf life of at least 2 years.
The final optimized formulation was evaluated for its in vivo performance by conducting comparative bioavailability studies with curcumin suspension. The SNEDDS formulation was compared with an aqueous suspension of curcumin at equal dose level. A study with 10 animals in each group was conducted with a larger number of blood samples to get a complete plasma profile for pharmacokinetic modeling and evaluation. Blood samples were drawn at 0.15, 0.25, 0.5, 1, 2, 3, 4, 5, 8 and 10 hours. The additional time points helped to get sufficient data points for pharmacokinetic modeling as minimum 2 points are needed in the absorption phase. With this design we got 10 data points for the curcumin SNEDDS and 9 for the curcumin suspension. Also it was seen that higher Cmax values were attained in this study (120.825 ng/ml) compared to the curcumin suspension (14.63 ng/ml) due to in situ formation of the nanoemulsion.

For evaluating the comparative bioavailability of the SNEDDS formulation against the curcumin suspension the Tmax (time to peak plasma concentration), Cmax (peak plasma concentration) and AUClast have been compared. It is essential to compare all three parameters to evaluate both the rate and extent of bioavailability enhancement. As two delivery systems may have the same extent of absorption (AUC) while having varying rate of drug absorption. Though conventionally AUC infinity (extrapolated AUC) should be used for comparison of bioavailability AUClast (AUC till the last time point) has been used because in the case of the curcumin suspension the contribution of the predicted AUC (AUC_%Extrap_obs) is 73% of AUC infinity. Ideally the contribution of the predicted data should be less than 20% of total AUC. It has been seen that the Cmax has increased 8 fold from 14.63 ng/ml for the suspension to 120.825 ng/ml for the SNEDDS and the AUC has increased 4 fold from 59.55 hr*ng/ml for the suspension to 240.78 hr*ng/ml for the SNEDDS. Also the rate of absorption has been enhanced as can be seen from the Tmax being reduced to 15 minutes for the SNEDDS compared to 60 minutes for the suspension. So it can be concluded that a fourfold increase in bioavailability has been achieved with the SNEDDS formulation. MTT assay on human lung cancer lines further ascertained the efficacy of curcumin against lung cancer and it was found that IC 50 value of curcumin SNEDDS was nearly half of what we obtained from curcumin. This indicated that SNEDDS helped in enhancing the efficacy of curcumin against lung cancer. The IC 50 value can be of help in ascertaining the plasma concentration of curcumin desired for treatment of lung cancer thereby calculating the dose of curcumin. The present study confirmed that the SNEDDS formulation was superior to other formulation approaches and could be used as a possible solution to deal with poor bioavailability of curcumin.
Conclusion
10.0 Conclusion

As per the literature Curcumin has tremendous potential for the treatment or prevention of cancer and several other diseases. Curcumin posses a favorable safety profile, supported by hundreds if not thousands of years of use by humans as a spice and in traditional medicines, despite of the diverse activities. Unfortunately Curcumin suffers from poor biopharmaceutical properties such as, low solubility, poor permeability, metabolism in enterocytes and extensive first pass metabolism. This limits its oral bioavailability and hinders its successful application to the treatment and prevention of these pathologies, as therapeutic concentrations are difficult to achieve at the site of action. By a systematic application of modern formulation approaches these biopharmaceutical barriers can be successfully overcome and the true potential of Curcumin can be fully exploited to alleviate and prevent the suffering due to these and possibly other diseases. Such an industrially scalable product with enhanced bioavailability of curcumin has been developed, characterized and reported in this work.

The key conclusions drawn from this work are summarized below:

1. A HPLC method for the analysis of curcuminoids has been partially validated and has been used for the analysis of curcumin in various in vitro samples like solubility studies, dissolution studies and stability studies.

2. A simple, specific and sensitive; LC/MS/MS based bioanalytical method has been developed and validated for the analysis of curcumin in plasma samples.

3. The intrinsic stability of curcumin has been studied and it has been shown to be stable against acid hydrolysis, extremely labile under base hydrolysis, labile under neutral hydrolysis, labile under oxidation and photolabile.

4. Solubility of curcumin has been studied in various oil and excipient and found to have low solubility in most with the exception of few.

5. Curcumin has been shown to have a pH dependent solubility profile; being more soluble in alkaline pH than in acidic pH.

6. Excipient compatibility studies have been carried out for curcumin with various excipients and it was found to be compatible with excipients used in the final formulation – Transcutol HP, Capryol 90, PEG 200 and Cremophor EL.

7. A stable and industrially scalable SNEDDS formulation has been developed for curcumin using established excipients within reported levels.
8. The SNEDDS formulation has been shown to give rapid, reproducible, complete and pH independent release of curcumin producing a nanoemulsion with particle size in the range of 90 nm.

9. The SNEDDS formulation has also been subsequently adsorbed on Colloidal Silicon Dioxide and the same has been filled into capsules.

10. The SNEDDS formulation has been shown to have a significantly higher bioavailability in rats in comparison to curcumin suspension showing a 4 times increase in AUC and 8 times increase in Cmax.

11. MTT assay on human lung cancer lines has further ascertained the efficacy of curcumin against lung cancer which indicated that SNEDDS helped in enhancing the efficacy of curcumin against lung cancer.