6. SUMMARY AND CONCLUSION

The thesis entitled “Osmotic Controlled Release Oral Formulation of Nutraceuticals with Enhanced Solubility” describes the osmotic controlled release formulations of lycopene and curcumin through osmotically controlled asymmetric membrane capsule, with enhanced solubility using solid dispersion of drug. The thesis is divided into five chapters.

Chapter 1 deals with introduction about Conventional oral drug delivery systems and their disadvantages which are overcome by novel drug delivery system, historical aspects of various osmotic pumps like Rose Nelson Pump, Higuchi-Leeper Pump and Higuchi-Theeuwes Pump etc. This chapter also includes basic components of osmotic system, classification of osmotically controlled drug delivery system, formulation aspects, evaluation of osmotic pumps, concept of asymmetric membrane, its features, manufacturing method, basic introduction about nutraceuticals and its classification. The need of study along with its aim and objectives are also described in this particular chapter.

Chapter 2 comprises of extensive literature review based on osmotic drug delivery system, curcumin delivery and lycopene delivery. It also includes drugs profile and excipients profile.

Chapter 3 deals with material and method section of the thesis. It includes different methods used for preformulation studies, formulation, evaluation of developed systems, optimization study and bioavailability study. Chapter 4 and chapter 5 comprises of results and discussion part of the thesis.

In the preformulation study drug authentication was done by melting point, thin layer chromatography, UV and IR spectroscopy.

The calibration curve of lycopene and curcumin was prepared in different medium such as phosphate buffer (pH 7.4), distilled water and 0.1 N HCl (pH 1.2). All the calibration plots were found to be linear in concentration range of 5-30 µg/mL. A simple and sensitive HPLC method for the estimation of lycopene and curcumin were developed and validated. The calibration curve of curcumin was found to be linear in concentration range of 0.5 to 3µg/mL.

Solubility of lycopene and curcumin was determined by equilibrium solubility method and it was found that solubility of lycopene and curcumin was less in 0.1N HCl (pH 1.2) as compared to
phosphate buffer (pH 7.4). The solubility of lycopene and curcumin was modulated by forming inclusion complex with β-cyclodextrin.

The solid dispersions of lycopene with β-cyclodextrin (LSDs) in different weight ratios (1:1 to 1:5) and of curcumin with β-cyclodextrin in different weight ratios (1:1 and 1:2) were successfully prepared by solvent evaporation method. The solubility of lycopene and curcumin was significantly increased by forming solid dispersion with β-cyclodextrin, which may be due to conversion of crystalline form of lycopene and curcumin in amorphous form, which was indicated by X-ray diffraction study. X-ray diffractogram of inclusion complex of lycopene and curcumin revealed less intense peaks as compared with the X-ray diffractogram of lycopene and curcumin, which may be due to the inclusion of drug into β-cyclodextrin cavity. The cytotoxicity of lycopene, curcumin and their solid dispersion against MCF-7 cells were evaluated by MTT assay. The inhibitory rate of solid dispersion of lycopene and curcumin to MCF-7 cells was higher than free drug which is due to the enhanced solubilization of solid dispersion of lycopene and curcumin as compared to pure drug. Drug excipient compatibility studies were performed using TLC and IR spectra analysis. There was no extra spot of polymer in case of physical mixture revealed compatibility of drug and polymer. Further the Rf value was also found to be same as that of drug in physical mixture. IR spectra analysis of drug, carrier and combination of drug and carrier confirmed compatibility of drug and carrier as there was no shift found in vibrational or stretching bands of key functional groups in physical mixture. Measurement of the osmotic activity of the solid dispersion of lycopene was determined using a thistle tube. The final level of solution in tube was found to be the same as compared to the initial level of the solution, thus suggesting that the lycopene is osmotically inactive so there is need to incorporate osmogen into the formulation.

AMCs were prepared by dip coating method. AMCs were compared with conventional gelatin capsules (CGC) for appearance and geometric characterization. AMCs showed higher opacity as compared to CGCs and revealed high degree of similarity in dimensions with CGCs (p< 0.005). Capsule shell rupture force was determined and resulted in high rupture force (870.59 ± 2.15 g) for AMC suggested more rigidity of prepared AMC. The AMC had low moisture content and plasticizer content evident by high rupture force value indicated no influence of environmental conditions on AMC.
SEM photomicrographs of developed AMCs indicated outer dense region and inner porous region revealed the asymmetric nature of capsules which is desirable for osmosis. The pores in the membrane were created due to incorporation of plasticizer (glycerol), which acted as pore former.

*In-vitro* release studies were performed for developed AMCs in USP-II dissolution apparatus. The best formulation was selected based on the optimization studies using central composite design. The effect of independent variables such as ethyl cellulose ($X_1$), glycerol ($X_2$) sodium chloride ($X_3$) over the dependent variables like cumulative % drug release ($Y_1$) and correlation coefficient of drug release ($Y_2$) was assessed by using this design. On the basis of maximum value of % cumulative drug release ($Y_1$) and correlation coefficient of drug release ($Y_2$), optimized formulation was selected. $F_{18}$ was selected as optimized formulation having 85.63% of drug release and 0.9994 of correlation coefficient of drug. Further the $F_{18}$ was subjected to analyze the effect of osmotic pressure and agitational intensity on *in-vitro* drug release. Release studies of lycopene from AMC were performed to analyze mechanism of drug release indicated that the release was highly dependent on osmotic pressure of release medium. Lycopene release from AMC was independent of agitational intensity of dissolution media as there was non-significant difference in release of drug at different rpm ($p<0.01$, $f^2 = 98.17$). *In-vitro* release studies were performed to analyze the effect of any defect in the asymmetric membrane on the release kinetics of optimized formulation ($F_{18}$). *In-vitro* drug release profile of intentionally defected formulation was compared with optimized $F_{18}$ and there was no significant difference at $p<0.05$. *In-Vitro* release kinetics for the AMCs of solid dispersion of lycopene was determined by fitting the dissolution data to various kinetic models and best model was selected based on goodness of fit (regression coefficient). Among the various formulations, $F_{18}$ exhibited best pattern of zero order release with highest value of $r^2$ (0.9994).

*In-vitro* release studies of asymmetric membrane capsules of solid dispersion of curcumin were performed in 2 groups as per the $2^3$ factorial design. The release profiles of Curcumin from all formulations in the dissolution medium were statistically compared with release rate profiles of the theoretical formulation (extra design checkpoint batch), which was obtained by using the polynomial equation. The statistical significance was tested at $P < 0.05$. The best formulation among the non-significant pairs of formulations was found to be AMC 6, which gave the value of $f_2$ (similarity factor) as 90.68.
In-Vitro release kinetics for the AMCs of solid dispersion of curcumin was determined by fitting the dissolution data to various kinetic models. While considering higher correlation coefficient value (R), the release data seems to fit Zero-order model better. According to correlation coefficient value (R) of release models, AMC 6 seems to be the best formulation. In-vitro release data, for AMC 6 formulation further analyzed for exponent of drug release and n = 0.4273 confirmed that release of curcumin from AMC 6 formulation was fickian type.

The effect of osmotic pressure of dissolution media was determined performing the in-vitro release studies of optimized formulation (AMC 6) in presence of dissolution medium of different osmotic pressure. In-vitro drug release was found to depend upon osmotic pressure of media. In-vitro release profile of intentionally defected formulation was compared with optimized formulation using one-way ANOVA. The calculated F-value (1.019) and t-value (0.06058) was found to be less than tabulated F-value (4.22) and t-value (2.054), respectively and P value was 0.9522, which was not significant. In-vitro release profile(s) at three different agitational speeds was compared using one-way ANOVA. The calculated F-value (0.002128) was found to be less than tabulated F-value (3.37), thus indicating that the change in agitational intensity do not have any significant effect on release profiles of asymmetric membrane capsules. This study describes the fact that the in vitro release from the asymmetric membrane capsules is independent of the hydrodynamic conditions of the body.

The pharmacokinetic parameters were determined by performing the in-vivo studies in rabbit model. Results of in-vivo pharmacokinetic studies exhibited discernible difference in the plasma drug concentration between both drugs and asymmetric membrane capsules of solid dispersion of both. The C\textsubscript{max} was found to be 16.5 ng/mL and 105.32 ng/mL with oral administration of lycopene and F\textsubscript{18} respectively after 12 h. The C\textsubscript{max} was found to be 22.09 ng/mL and 170.58 ng/mL with oral administration of curcumin and AMC6 respectively after 12 h. The results showed a significant difference between the maximum plasma drug concentrations between lycopene and F\textsubscript{18}, curcumin and AMC6. This may be due to the enhanced solubility and controlled release of drug from F\textsubscript{18} and AMC6 formulation. There was significant or noticeable difference between AUC (area under the curve) of lycopene (414.311 ngh/mL) and F\textsubscript{18} (1357.21 ngh/mL) and AUC (area under the curve) of curcumin (376.549 ngh/mL) and AMC6 (1547.88 ngh/mL) which is due to the more absorption of lycopene from F\textsubscript{18} formulation and curcumin.
from AMC6 formulation because of enhanced solubilization of drug from $F_{18}$ and AMC6. As evident from the results, the bioavailability of $F_{18}$ was 3.28 fold that of pure lycopene and the bioavailability of AMC6 was 4.11 fold that of pure curcumin. So therefore it is reasonable to conclude that asymmetric membrane capsules of solid dispersion of lycopene and curcumin enhances the absorption and retention time of lycopene and curcumin \textit{in-vivo} significantly.

Level A \textit{in vitro- in vivo} correlation was investigated by plotting the percent dissolved vs percent absorbed data. A good linear regression relationship (Level A) was observed between percent dissolved and percent observed with correlation coefficient ($r^2$) of 0.9775 in case of lycopene and 0.9637 in case of curcumin. Level A correlation represented a point to point correlation between \textit{in-vitro} dissolution rate and \textit{in-vivo} input rate, which meant that by using \textit{in-vitro} dissolution profile of lycopene and curcumin from asymmetric membrane capsules containing solid dispersion of lycopene and curcumin, one can also predict their \textit{in–vivo} performance.

The stability studies of optimized formulation (AMC 6 and $F_{18}$) were performed in accordance to ICH Q1 A guidelines for 6 months to investigate the influence of humidity and temperature on appearance and \textit{in-vitro} drug release of curcumin. Capsule texture and color remained unchanged and both formulations showed comparable release profile after 6 months also, which proved the stability of asymmetric membrane capsules. Thus, suggesting that there was no problem of stability for asymmetric membrane capsules of curcumin and lycopene.

Thus a novel oral formulation of lycopene and curcumin was successfully developed and evaluated with enhanced bioavailability due to increased solubility and controlled release.