STUDIES ON FORMULATION AND EVALUATION OF ORAL TARGETED DELIVERY OF ANTICANCER DRUG LOADED LIPID BASED SYSTEM

A THESIS

Submitted by

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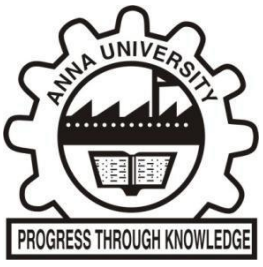

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CHAPTER 6

SUMMARY AND CONCLUSION

The thesis is well-organized, and a concise summary and conclusion of contents are presented below.

In **Chapter 1**, a broad introduction has been given with attention to the field of colorectal cancer, various solid dispersion techniques to enhance the solubility of poorly soluble drugs, the solid lipid nanoparticle, and response surface methodology and its significance was also discussed.

In **Chapter 2,** numerous works of literature related to CRC, SD techniques to enhance the solubility of the drug, SLN, and RSM and its application to targeted drug delivery for colon cancer has been presented.

In **Chapter 3,** the aim and objectives including the rationale for selection of drug and plan of work have been presented.

C**hapter 4** investigates the better optimized solid dispersion of curcumin with enhanced solubility, which can be employed to treat CRC better than pure CMN.

The phase solubility study results the stability constant (K_a) attained from the complex ranked in the order of 25 and 37 °C as PVP > P-407 > P-188 > PEG6000 > PEG4000 > GLR > BCD> MNT > SMP has been discussed. The phase solubility and *in silico* molecular modeling study in a snug fit with each other was revealed to be the better soluble complex formed for the enhancement of solubility, reported as poloxamer, PEG and GLR. Subsequently, the ideal 1:1 stochiometrically governed SD complex, further optimized by RSM-BBD, in the 1:5 ratio was revealed as better for solubility throughout.

Principally, the maximum solubility of SD complex of CMN-P-407 (1:5) enhanced solubility was around ~300-fold that of other carriers. The dissolution rate of CMN-SD with all carriers exhibited initial burst release followed by a low quantity of drug being released in the steady state, which is seen at all PM and SDs.

The FT-IR study revealed that SD with poloxamers show better complexation of hydrogen interaction with the drug. The results from PXRD and thermal analysis revealed the complex system of poloxamers to be better, and the complete disappearance of the endothermic peak of PEGs corresponding to CMN could be due to release of water molecules or conversion entirely into amorphous form or dissolution of the crystalline form into the molten carrier.

The RSM-BBD identifies the better complex P-407 (1:5) for further studies as showing better physicochemical properties over others, the hydrodynamic particle size of PM and SD revealed bi-modal size and uniformity of particle size distribution respectively. The ZP of PM and SD relate ideal complexes with CMN than PM. The topology of SEM image of SD surface appears to be porous and is observed as uniformly and homogeneously dispersed. The dyeing solution of SD complex shows the CMN solubility of the aqueous liquid.

Among all carriers, P-407 (1:5) complex of CMN showed better physicochemical properties so that the complex was studied further for cytotoxic contribution. The cytotoxic results of the MTT-reduction assay of pure CMN and CMN-SD-P-407 (1:5) on colorectal adenocarcinoma cells IC_{50} value was very much lower than pure CMN.

In **Chapter 5** preparation, coating and characterization of C-, C-SD-, CP-SD-SLN and its variables including *in vitro* and *in vivo* studies were

discussed. The drug release (72 h) from the prepared SLN in dialysis film membrane with initial burst release followed by a sustained release at pH 7.4 by diffusion was also discussed. In contrast, CP-SD-SLN in pH 1.2, and pH 4.5 showed the effect of pectin coating making the SLNs acid-resistant. The *in vitro* CMN release from SLN is diffusion controlled with super case II transport of sustained release.

The lyophilized SLN from FT-IR revealed the drug as being encapsulated into the lipid core. The thermal analysis study implies that the CP-SD-SLN successfully incorporated C-SD into the CP and pectin framework and the coating improves the physical properties of the lipid along with the stability of SLN.

Particle size in the order of C-SLN < C-SD-SLN < CP-SD-SLN exhibited greater size demonstrating that these particles were significantly more homogeneous, with similar zeta potential qualities. The C-SD-SLN regularly displayed a smooth spherical shape with a dense lipid matrix i.e. homogeneous. Moreover, CP-SD-SLN has a sufficient repulsive force to avoid aggregation/ accumulation in the course of long-term storage.

The prepared biopolymeric dual layer solid lipid nanoparticles (CP-SD-SLN) loaded with soluble curcumin enhanced the cytotoxicity of colorectal cancer for colon target delivery. The cytotoxic results of C-SD-SLN and CP-SD-SLN on SW480 and Caco-2 cell lines IC_{50} value were extremely lower than free CMN.

Apoptosis studies of SW480 and Caco-2 cells showed the cells undergoing cell death mainly by apoptosis with a small number of necrosis related death when treated with C-SD-SLN and CP-SD-SLN. The DNA fragmentation of adenocarcinoma cells treated with CP-SD-SLN show more explicit DNA ladders indicating the initiation of apoptosis. The result from flow

cytometry show the cells have undergone cell death by G_2/M phase arrest in both SW480 and Caco-2 cells. The *in vivo* toxicity of the zebrafish model showed that there is no malformation/abnormalities in CP-SD-SLN treated zebrafish egg embryos indicating that the formulation was safe throughout.

The CP-SD-SLNs initiated increases in the levels of the tumor-suppressing protein. The western blotting has proved that the CP-SD-SLNs contributed to prominent increment in PARP, caspase 3, and caspase 9 proteins in both SW480 and Caco-2 cells. The efficiency of CP-SD-SLN indicated its potential application for CRC treatment by enhancing the bioavailability of CMN via an oral route.

A brief narration of the soluble CMN loaded biopolymer coated SLN obtained described its effectiveness against human adenocarcinoma cells it as a promising carrier for the enhancement of both potential regression and target delivery when compared to free CMN.

The final formulation of CP-SD-SLN were tested in rats for its pharmacokinetic profile where it was varied from free curcumin. It was observed that free curcumin took around 1 h to achieve Cmax; in contrast, the colon-targeted formulation takes 24 h for the same, which reveals that the CP-SD-SLN is the promising formulation as it increases the systemic bioavailability. The colon targeting potential of the CP-SD-SLN was further studied by the organ distribution estimation that showed the highest amount of drug reached the colon in 24, 48, and 72 h compared to the stomach and small intestine. Therefore, CP-SD-SLN targets the colon and can be effective in preventing the development of colon cancer.

To summarize the current study, the present work deals with the selection of the best carrier, solid dispersion preparation technique and its governing variables, solid lipid nanoparticle polymers and its preparation

technique and governing variables, in preparing the correct dosage form of selected drugs by experimental design. Numerous characterization investigations, instrumental analysis, and cytotoxicity analysis including *in vivo* were carried out to judge the effectiveness of new formulation. It is proposed that these new formulations are more significant and more effective than existing formulationsof the pure drug.

In the future, investigators may pay attention to experimental design and consider other functional variables to achieve better models. The scale-up technique to pilot scale and large scale is an interesting work on any product, which needs a high budget and higher skill. In future, the researcher in the field may take up this work to develop an experimental design to scale up the process variables in the formulation of a multiparticulate drug delivery system.