CHAPTER-9

Summary and Conclusions

9.1 SUMMARY

Colorectal cancer (CRC) is most common form of cancer with high morbidity and mortality worldwide. Its symptom is worsening constipation or bloody stool. When such symptoms arise, complete treatment is late and 5 years survival is <60% as well as a periodic colonoscopy is desired. Major treatment for CRC is surgery, radiation, or chemotherapy which is applied as adjuvant therapy in many cases. Chemotherapeutic agents such as oxaliplatin, 5-flourouracil, leucovorin, and irinotecan are known, however, they are associated with severe side effects. Therefore, to avoid these side effects, there is an urgent need to discover more potent anticancer agents with no side effects on normal organs/tissues.

Recent studies have shown that curcumin (CUR) and naringenin (NAR), one of the most promising natural chemotherapeutic agents, are used extensively to treat a variety of solid tumors, including stomach, breast, intestine and especially colorectal carcinoma. CUR and NAR are potentially involved in the reduction of carcinogenesis by protecting DNA damage (induction), inhibiting tumor growth (progression) and regressing tumor invasion (proliferation). CUR is a yellow color polyphenolic natural compound extracted from plant Curcuma longa whereas NAR is one of the naturally occurring flavonoids present in citrus fruits, cherries, grapefruits, tomato and cocoa, respectively with no discernible toxicity. In spite of vast practical applications and therapeutic effectiveness of CUR and NAR in a variety of experimental models, its clinical applications have been hampered due to its extreme water insolubility and instability in physiological medium. Low aqueous solubility of CUR and NAR results in poor bioavailability, poor permeability and extensive first pass metabolism before

reaching into the systemic circulation. To overcome these problems, nano-drug delivery system is expected to yield more promising clinical applications for the delivery of CUR as well as NAR.

Present thesis is an attempt to design and optimize lipopolysaccharide based oral nanocarriers and also the polymeric nanoparticles for the enhancement of bioavailability and anticancer efficacy of two drugs i.e. CUR and NAR.

Simple, rapid, accurate, precise and inexpensive UV-visible spectrophotometric as well as HPLC analytical methods were developed and validated for analysis of CUR and NAR *in-vitro* as well as *in-vivo*.

The physical mixture in an equimolar ratio (1:1:1) was prepared manually by mixing CUR, soluthin MD® (SMD) and poloxamer 188 (PLX188) thoroughly for 10 min in a mortar and pestle until a homogeneous mixture was obtained. The sample was passed through 40# mesh and stored in a desiccator till further studies. The CUR encapsulated lipopolysaccharide based nanocarriers (C-LPNCs) was prepared by high speed homogenization technique as previously reported by Noack *et. al.* (2001) with little modification. A L_9 (3^4) Taguchi orthogonal experimental design (TOED) and statistical analysis was used to define the optimal conditions regarding the selected factors to develop C-LPNCs with minimal mean particle size (PS) and high percent entrapment efficiency (%EE). The experimental design involved four relevant independent factors each in three levels. The factors were amount of CUR, SMD concentrations, PLX188 concentrations and homogenization speed. The four parameters each at three levels would give only nine experimental runs. Each of the nine experiments was performed in triplicate, corresponding to a total of 27 experimental runs which were randomized to prevent any bias. The Prepared optimized C-LPNCs were evaluated for solid state characterizations (by FTIR, DSC and *p*XRD), surface morphology (by TEM and AFM), PS (108±3.4 nm), zeta potential (-20.4±0.91 mV), polydispersity index (0.167±0.02), %EE (65.29±1.00%), drug loading (0.149±0.07%), drug distribution within the nanocarriers, photophysical

properties, stability at room temperature for 3 months at 25±2°C/60%±5% RH. and *in-vitro* drug release study resulted in >80% drug release at the end of 24 hr in PBS, pH 7.4. Further, estimated pharmacokinetic parameters revealed ~130-fold increase in oral bioavailability and cytotoxicity studies demonstrated ~23-fold reduction in 50% cell growth inhibition when treated with optimized C-LPNCs as compared to PM and pure CUR. *In-vivo* anticancer study performed with optimized C-LPNCs showed significant increase in efficacy as compared to PM and pure CUR.

Preparation of physical mixture and formulation of CUR encapsulated Eudragit E 100 nanoparticles (CENPs): The PM in an equimolar ratio (1:1:1) was prepared manually by mixing CUR, Eudragit E100 (EE100) and PLX188 thoroughly for 10 min in a mortar using pestle until a homogeneous mixture was obtained. The sample was passed through 40# mesh and stored in a desiccator till further use. The CENPs were prepared by emulsification-diffusion-evaporation method by Fessi *et al.* (1989) using Drug-polymer ratio, amount of ethyl acetate, homogenization speed and PLX 188 concentration as variables. A L_9 (3^4) TOED and statistical analysis was used to define the optimal conditions regarding the selected factors to develop CENPs with minimal PS and high %EE. The experimental design involved four relevant independent factors each in three levels. The four parameters each at three levels would give only nine experimental runs. Each of the nine experiments was performed in triplicate, corresponding to a total of 27 experimental runs which were randomized to prevent any bias. The Prepared optimized CENPs were evaluated for solid state characterizations (by FTIR, DSC and pXRD), surface morphology (by TEM and AFM), PS (248.40±3.89nm), zeta potential (28.9±0.47mV), polydispersity index (0.212±0.013), %EE (65.77±3.17%), drug loading (0.559±0.026%), drug distribution within the nanocarriers, photophysical properties, stability at room temperature for 3 months at 25±2°C/60%±5% RH and *in-vitro* drug release, CENPs exhibited >70% drug release at the end of 24 hr in PBS, pH 7.4. Further, observed pharmacokinetic data revealed ~95-fold increase in oral bioavailability and cytotoxicity studies data demonstrated ~19-fold reduction in 50% cell growth

inhibition when treated with optimized CENPs as compared to PM and pure CUR. The *in-vivo* anticancer study performed with CENPs showed significant increase in efficacy as compared to PM and pure CUR.

Preparation of physical mixture and formulation of NAR encapsulated lipopolysaccharide based nanocarriers (N-LPNCs): The PM in an equimolar ratio (1:1:1) was prepared manually by mixing NAR, SMD and PLX188 thoroughly for 10 min in a mortar using pestle until a homogeneous mixture was obtained. The sample was passed through 40# mesh and stored in a desiccator till further use. The N-LPNCs was prepared by high speed homogenization technique as previously reported by Noack *et al.* (2001) with little modification. A L_9 (3^4) TOED and statistical analysis was applied to define the optimal conditions regarding the selected factors to develop N-LPNCs with minimal PS and high %EE. The experimental design involved four relevant independent factors each in three levels. The factors were amount of NAR, SMD concentrations, PLX188 concentrations and homogenization speed. The four parameters each at three levels would give only nine experimental runs. Each of the nine experiments was performed in triplicate, corresponding to a total of 27 experimental runs which were randomized to prevent any bias. The Prepared optimized N-LPNCs were evaluated for solid state characterizations (by FTIR, DSC and pXRD), surface morphology (by TEM and AFM), PS (176±2.35nm), zeta potential (-13.14±1.22mV), polydispersity index (0.136±0.03), %EE (70.83±4.55%), drug loading (0.347±0.02%), stability at room temperature for 3 months at 25±2°C/60%±5% RH and *in-vitro* drug release. The prepared optimized formulation exhibited >85% drug release at the end of 24 hr in PBS, pH 7.4. Further, observed pharmacokinetic parameters indicated ~116-fold increase in oral bioavailability and *in-vitro* cytotoxicity studies in terms of 50% cell growth inhibition, demonstrated 21-fold reduction in cell growth inhibition when treated with N-LPNCs as compared to PM and pure NAR. The *in-vivo* anticancer study performed with optimized N-LPNCs showed significant increase in efficacy as compared to PM and pure NAR.

Preparation of physical mixture and formulation of NAR encapsulated Eudragit E 100 nanoparticles (NENPs): The PM in an equimolar ratio (1:1:1) was prepared manually by mixing NAR, EE100 and PLX188 thoroughly for 10 min in a mortar using pestle until a homogeneous mixture was obtained. The sample was passed through 40# mesh and stored in a desiccator till further use. The NENPs were prepared by emulsification-diffusion-evaporation method by Fessi *et al.* (1989) using Drug-polymer ratio, amount of ethyl acetate, homogenization speed and PLX 188 concentration as formulation variables. A L_9 (3^4) TOED and statistical analysis was used to define the optimal conditions regarding the selected factors to develop NENPs with minimal PS and high %EE. The experimental design involved four relevant independent factors each in three levels. The factors were drug-polymer ratio, amount of ethyl acetate, homogenization speed and PLX188 concentrations. The four parameters each at three levels would give only nine experimental runs. Each of the nine experiments was performed in triplicate, corresponding to a total of 27 experimental runs which were randomized to prevent any bias. The Prepared optimized NENPs were evaluated for solid state characterizations (by FTIR, DSC and pXRD), surface morphology (by TEM and AFM), PS (430.42±5.78nm), zeta potential (32.1±2.56mV), polydispersity index (0.283±0.089), %EE (68.83±3.45%), drug loading (0.612±0.035%), stability at room temperature for 3 months at 25±2°C/60%±5% RH and *in-vitro* drug release. The optimized formulation exhibited >75% drug release at the end of 24 hr in PBS, pH 7.4. Further, observed pharmacokinetic data revealed~96-fold increase in oral bioavailability and cytotoxicity studies data demonstrated~16-fold reduction in 50% cell growth inhibition when treated with optimized NENPs as compared to PM and pure NAR. The *in-vivo* anticancer study performed with NENPs showed significant increase in efficacy as compared to PM and pure NAR.

9.2 CONCLUSIONS

In conclusion, SMD and EE 100 are shown to be an excellent drug delivery carrier. Both *in-vitro* and *in-vivo* experiments demonstrated a marked increase in anticancer efficacy by C-LPNCs, CENPs, N-LPNCs and NENPs when compared

with PM of all formulations as well as pure drugs (CUR and NAR). Furthermore, it has been proved that all the four types of formulations shown better *in-vitro* cytotoxicity as demonstrated by the GI_{50} values. The pharmacokinetic studies revealed that all the four formulations provided high oral bioavailability as compared to pure CUR as well as NAR aqueous suspension. Furthermore, enhanced accumulation of all four types of formulations in tumor tissue occurred which is attributed to combined effect of enhanced oral bioavailability and enhanced EPR effect of nanoparticles. Increased formulations accumulation in tumor cells led to enhanced anticancer efficacy as well as survival time of mice. Therefore, the present research opens new era for successful utilization of maltodextrin based LPNCs and polymeric nanoparticles as a potential carrier to improve oral bioavailability as well as anticancer efficacy of hydrophobic chemotherapeutic drug(s) in the treatment of cancer, especially CRC.

When all the four types of developed formulations (C-LPNCs and NLPCs vs CENPs and NENPs) were compared, results suggest that lipopolysaccharide based nanocarriers have more promising potential as carrier for enhancing the bioavailability as well as anticancer efficacy of poorly water soluble chemotherapeutic drugs than the polymeric nanoparticles.