Chapter-6

Summary and Conclusions

6.1 Summary

Complications of atherosclerosis are common cause of death worldwide. Atherosclerosis is not only prevalent in developed countries but also afflicting developing nation population. Financial burden to the world for the treatment of atherosclerosis is quite high. Hyperlipidemia is a dominant cause of progression of atherosclerosis. ATR is a blockbuster statin drug, preferably prescribed by physician for the treatment of atherosclerotic complications and hyperlipidemia due to its advantages over other statin drugs. Nevertheless; ATR do possess drawback like low bioavailability and side effects like mild myalgia to rhabdomyolysis. Polymeric nanoparticles are well established novel drug delivery system to improve bioavailability, efficacy and safety profile of incorporated drug.

Present thesis dealt with the preparation, optimization, *in vitro* and *in vivo* evaluation of ATR loaded Eudragit RSPO, PLGA and PCL nanoparticles to improve bioavailability, efficacy and safety profile of incorporated ATR.

Simple, robust, accurate, precise and inexpensive RP-HPLC analytical methods were developed and validated for analysis of ATR in experimental rat's plasma as well as in mobile phase. Solubility study of ATR with different surfactants in various buffer media were successfully carried out. ATR showed pH dependent solubility and solubility increased with pH elevation of media. Sodium lauryl sulphate exhibited good solubility enhancement profile than sodium taurocholate in anionic surfactants. All the solubility parameters of SLS were found to be better than STC. Cationic surfactant cetyl trimethyl ammonium bromide exhibited reduction in solubility of ATR due to insoluble adducts formation with the ATR. Nonionic surfactants demonstrated best solubility enhancement among all the surfactants. Moreover, D α-tocopherol polyethylene glycol 1000 succinate (TPGS) exhibited highest solubility enhancement in all five

surfactants. All the solubility parameters were found to be most prominent in TPGS. Tween 80 was proposed for the maintenance of sink condition in dissolution study of ATR due to its cost-effectiveness and comparable solubility enhancement property. On the other hand, based on stability, bioavailability enhancer and antioxidant properties of TPGS is used as one of the best stabilizer in fabricated nanoparticles colloidal systems.

ATR loaded eudragit RSPO nanoparticles (AERSNs) were prepared by emulsification solvent evaporation method using central composite design (CCD) as optimization tool. On the basis of preliminary study, four factors polymer content, stabilizer concentration, volume of chloroform and stirring speed were selected as independent variables with five levels (α=2). AERSNs were successfully optimized by using 4-factor, 5-level CCD for hydrodynamic mean diameter particle size (PS) and entrapment efficiency (EE). The optimized batch of AERSNs was prepared by using 75 mg of polymer, 1% (w/v) of PVA concentration, 6 ml of chloroform and 12000 rpm homogenization speed which resulted to form nanoparticles with 251±5 nm PS, 71.7±0.8% EE, 0.228±.009 PDI and +29.3±1.1 mV zeta potential. Optimized batch of AERSNs were lyophilized for solid state characterization. Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) evidenced for good compatibility of ATR within polymer matrix of formulation and also indicated amorphous nature of ATR inside matrix. Morphological study by transmission electron microscopy (TEM) and atomic force microscopy (AFM) demonstrated uniformly distributed spherical shape and comparable particle size of AERSNs. *In vitro* drug release profile showed 24 h sustained release profile in phosphate buffer solution (PBS; pH 7.4) with Fickian diffusion controlled drug release mechanism. Stability study indicated refrigerated conditions (4±1°C) as best storage condition for long term storage. Gas chromatography was performed for the estimation of residual organic solvent. Gas chromatograms of freshly prepared AERSNs indicated for absence of detectable chloroform level in colloidal system. Furthermore, the optimized

batch was evaluated by pharmacokinetic study in rats. Pharmacokinetic profile of ATR exhibited significant enhancement in C_{max}, AUC and MRT of AERSNs than drug suspension (Astin, marketed as tablet). Moreover, efficacy and safety profile indicating biochemical parameters were estimated and compared in different groups. AERSNs showed equal efficacy and significantly better safety profile at half dose of ATR as AERSNs than ATR suspension. Thus, the promising results provide a possibility of eventually using AERSNs formulation in the clinical setting with greater anti-atherosclerotic efficacy and better safety.

ATR loaded poly (dl lactide-co-glycolic acid) (PLGA) nanoparticles (APLNs) were attempted to sustain the drug release, to improve its oral bioavailability, efficacy and safety profile. PNs were prepared by nano-precipitation method using D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) as a stabilizer and optimized by linear CCD. The effect of independent variables like polymer content, stabilizer concentration, volume of organic phase and stirring speed were studied on PS and EE of APLNs. The formulation composition of optimized batch was found to be 125 mg PLGA, 0.31% of stabilizer (TPGS), 1250 rpm stirring speed and 8.0 ml of organic phase (acetone). PS and EE were found to be 207 nm and 81.3% respectively. The drug polymer compatibility study has been carried out by FT-IR, DSC and PXRD to observe the compatibility between drug and formulation component at normal room temperature condition. DSC and PXRD also showed the amorphous nature of drug in formulation. The TEM revealed spherical shape of the PNs. AFM confirmed the uniform spherical nanoparticles distribution in APLNs. The *in vitro* drug release study of the optimized batch has shown sustained drug release up to 72 h in PBS (pH 7.4). The release kinetic was best fitted in zero order release kinetic model indicating erosion controlled release mechanism of ATR from APLNs. Gas chromatography of APLNs exhibited absence of detectable acetone in nanoparticles. Pharmacokinetic study exhibited a significant enhancement in drug bioavailability following the oral intake of PNs formulation as compared to the drug suspension. Furthermore, APLNs were evaluated for efficacy and safety

study at preclinical set up in rats. Efficacy was found to be equal and safety markedly improved at even 50% reduced dose of ATR in APLNs than the drug suspension in rats. Henceforth, APLNs need to be evaluated under clinical conditions to scale up it into commercial product for the benefit to patient.

ATR loaded poly (ε-caprolactone) nanoparticles (ALPNs) were prepared to enhance oral bioavailability, efficacy and safety profile of drug. ALPNs were prepared by nanoprecipitation technique while formulation and process parameters were optimized using central composite factorial design. The optimized ALPNs were investigated through *in vitro* (solid state characterization, morphological, drug release and stability studies) and *in vivo* (pharmacokinetic, efficacy and safety study) performance in rats. The optimized ALPNs having 197±5 nm particle size, 0.213±0.012 polydispersity index and 75.6±3.2% entrapment efficiency, did not exhibit any physicochemical interaction of drug with carrier through FT-IR studies. The PXRD, DSC and electron diffraction pattern had substantiated the amorphous character of ATR encapsulated in nanoparticles. Smooth and homogeneous spherical shape of nanoparticles was evidenced in morphological analysis using TEM and AFM. *In vitro* drug release profile of ALPNs showed 96 h sustained release and pharmacokinetic profile in rats exhibited significant enhancement in bioavailability, C_{max} and mean resident time of drug. Stability study demonstrated that the refrigerated conditions were suitable for long term storage of formulations. ALPNs exhibited similar efficacy (plasma lipid profile and glucose level) and markedly improved biochemical safety profiles (creatinine, blood urea nitrogen, creatinine kinase, lactate dehydrogenase and aspartate amino transferase) in rats of ALPNs dose compared to orally administered ATR.

6.2 Conclusions

Solubility study of ATR with different surfactants in various buffer media demonstrated Tween 80 as a better surfactant to maintain sink condition in dissolution study of drug and TPGS as better stabilizer in colloidal nanoparticle system. Polymeric nanoparticles have proven their potential as an excellent novel drug delivery carrier. *In vivo* study demonstrated equal efficacy of AERSNs, APLNs and ALPNs at 50% reduced dose of ATR as compared with ATR drug suspension. All excipients of formulations belong to generally regarded as safe material. All the three formulations were found significantly safer than its conventional dosage form as evidenced by estimation of safety indicating biochemical parameters. The potential of AERSNs, APLNs and ALPNs was also confirmed by absence of any hepatotoxicity in liver tissue histology study. The promising results therefore provide a possibility of eventually using these formulations in the clinical setting with better efficacy and safety profile.

On the basis of cost effectiveness, biodegradability; TPGS advantages over PVA; and comparative *in vitro* release, pharmacokinetic, efficacy and safety results of all the developed formulations (AERSNs, APLNs and ALPNs). It is further inferred that ALPNs possessed promising potentials and merits over AERSNs and APLNs.