Chapter 8

SUMMARY AND CONCLUSION
Developments in pharmaceutical technology and high i.v. throughput screening have led to a dramatic acceleration in drug discovery output. Clinical requirement for newly discovered molecules in order to have a therapeutic response in humans is that, they should have a good toxicity profile together with adequate biopharmaceutical/pharmacokinetic characteristics. The solubility and permeability related issues complicates the delivery of newly discovered molecules and too affects the delivery of many existing drugs. To counter these problems important advances are being made for improving the bioavailability of poorly soluble and permeable compounds, so that promising candidate drugs need no longer be abandoned or have their development stalled by sub-optimal formulation. However the main problem with available approaches is their unidirectional nature i.e. they can either increase dissolution of poorly soluble (hydrophobic) drug or increase permeation of (hydrophilic) drug.

Also none of the present approaches surmounts the problem of all class of BCS (biopharmaceutical classification system) drugs. Thus a combined novel approach simultaneously implementing/utilizing two different approaches in unique delivery system(s) was developed to serve a better solution to enhance bioavailability along with added benefits of nano and micro system.

The rationale behind the designing of these new nano and micro system was to simultaneously implement the cyclodextrin drug complexation power and the inherent properties of polymeric systems, in a unique delivery system of selected model drug molecules. The complexation with the cyclodextrin will permit the solubilization of poorly soluble drugs. Indeed, recent results from the literature highlight the enhancement of drug oral bioavailability by hydrophilic CDs through the inhibition of P-glycoprotein activity. Whereas the entrapment in the polymeric network will controls the release and is too expected to facilitate their absorption.

Two drugs simvastatin (SVS, hydrophobic) and rosuvastatin (RVS, hydrophilic) were selected as models for complexation with different cyclodextrins (β-CD, HP-β-CD, RM-β-CD) and further entrapment in the polymeric carriers (chitosan nanocarrier and eudragit RS100 microspheres). The resulting systems were thoroughly characterized for their physiochemical properties and also for their ability to associate and deliver the complexed drugs.
SUMMARY AND CONCLUSION

The selected work was divided into two phases. The first phase encompasses development, characterization and evaluation of pharmacodynamic potential of prepared drug (simvastatin and rosuvastatin) cyclodextrin (β-CD, HP-β-CD, RM-β-CD) complexes. The drug cyclodextrin complexes were prepared by physical mixture (PM0, kneading (KND), coevaporation (COEVP) and freeze drying (FZD) methods. Whereas in the second phase of the work, preparation and characterization of drug carriers (nanocarrier and microsphere) encapsulating prepared complex(s) in selected polymeric (chitosan and eudragit RS 100) network was carried out. Finally the carriers were evaluated for improvement in bioavailability and in-vivo viability of encapsulated drugs by animal studies on established model.

Preformulation studies were done to evaluate the purity of drug by melting point method while drug-polymer interactions studies were performed by fourier transform infrared spectroscopy (FTIR) studies to determine the compatibility of drugs with cyclodextrins and polymers used in the study. The observed melting point of drugs SVS (140°C) and RVS (129°C) were found to lie within given standard range. Also FTIR scans of both the drugs also showed similar characteristic peaks resembling to that of standard scans.

Analytical methods (UV spectrophotometric method) were performed for the drugs in presence of cyclodextrins. The drugs solution in methanol showed absorption maxima at 240 nm for SVS and 244 nm for RVS in the presence of cyclodextrins. The study also confirmed that presence of cyclodextrins did not interfere with the assay. The Beer’s law was obeyed up to the concentration range of 10-20μg/ml. The regression equation gave a slope of 0.024 (SVS-β-CD), 0.040 (SVS- HP-β-CD), 0.041 (SVS-RM-β-CD), 0.0044 (RVS-β-CD), 0.0109 (RVS-HP-β-CD) and 0.0175 (RVS-RM-β-CD) with correlation coefficient 0.986, 0.990, 0.983 and 0.9967, 0.9991, 0.9982 for different SVS-CD (simvastatin-cyclodextrin) and RVS-CD (rosuvastatin-cyclodextrin) respectively. The phase solubility studies were done for determination of the exact molar ratios in which the drug(s) could make complex with CDs. The phase solubility diagrams for SVS and RVS both were found to be linear, corresponding to an AL-type profile with a slope less than 1 in all the cases, indicating that the inclusion complexes are of the first order with respect to the CDs (i.e. 1:1 stoichiometry). The apparent stability constants K_{1:1} calculated from phase
solubility diagram were found to be $542 \text{M}^{-1}$, $824 \text{M}^{-1}$, $838 \text{M}^{-1}$ for SVS-β-CD, SVS-HP-β-CD, SVS-RM-β-CD complexes and were $354 \text{M}^{-1}$, $884 \text{M}^{-1}$, $1429 \text{M}^{-1}$ for RVS-β-CD, RVS-HP-β-CD, RVS-RM-β-CD. The obtained values of apparent stability constants ($K_{1:1}$) indicate that the complexes formed between SVS, RVS and all the selected CDs are quite stable.

All the prepared complexes of SVS and RVS were characterized for drug content, enhancement of aqueous solubility and in-vitro dissolution studies. The solid state characterization to determine and confirm the formation of inclusion complexes in the solid state was done by fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and x-ray diffraction (XRD) studies. Based on result of all above parameters studied the optimum complex(s) of each of SVS and RVS were selected and finally evaluated for pharmacodynamic activity in rats to ensure the behavior of selected complexes in biological system.

In case of SVS-CDs complexes, SVS-β-CD complex prepared by physical method [SVS-β-CD (PM)] showed least (92±3.24%) while SVS-HP-β-CD and SVS-RM-β-CD complexes prepared by kneading method [SVS-HP-β-CD (KND), SVS-RM-β-CD (KND)] showed the highest drug content of 99±1.12%, 99±1.47% respectively. While amongst all the prepared complexes of RVS-CDs, complex of RVS with β-CD prepared by physical method [RVS-β-CD (PM)] showed least (15±2 %) while RVS-RM-β-CD complex prepared by freeze dying method [RVS-RM-β-CD (FZD)] showed highest (94±2%) percent drug content. The marginal increase in aqueous solubility of simvastatin and rosuvastatin amongst all the prepared SVS-CDs and RVS-CDs complexes was shown by simvastatin-β-CD and rosuvastatin-β-CD complexes prepared by physical mixture method (SVS-β-CD (PM) and (RVS-β-CD (PM) i.e. of 7.62 fold and 0.95 fold respectively in comparison to pure drug. While the highest drug solubility was shown by kneaded complex of SVS-HP-β-CD complex prepared by kneading method [SVS-HP-β-CD (KND)] of 11.96 fold and RVS-RM-β-CD complex prepared by freeze drying method [RVS-RM-β-CD (FZD)] of 9.34 folds.

The FTIR results of SVS-CDs suggests that the C=O group of lactone ring of SVS might be involved in the inclusion complexation with HP-β-CD and RM-β-CD. Additionally a decrease in intensity of characteristic peak of RM-β-CD at 1750 and
broadening of peak in the region of 1250-1500 also suggests interaction between SVS and RM-β-CD. Similarly like SVS FTIR spectra of RVS-CDs (HP-β-CD and RM-β-CD) demonstrate increase in broadband at the region of 3300cm$^{-1}$ to 3400cm$^{-1}$ due to hydrogen bonding as compared to plain HP-β-CD. FTIR spectra's of RM-β-CD also shows a shift of peak of –C-O-C group. These modifications clearly indicate the presence of host–guest interactions and suggest the formation of hydrogen bonds between SVS-CDs and RVS-CDs.

The DSC studies revealed total disappearance of characteristic drug endothermal peak or maximum decrease in drug endotherm intensity was observed in scans of SVS-CDs complexes prepared by kneading SVS-β-CD (KND), SVS-HP-β-CD (KND) SVS-RM-β-CD (KND) and freeze drying methods SVS-β-CD (FZD), SVS-HP-β-CD (FZD) SVS-RM-β-CD (FZD). However the total disappearance of RVS endothermic peak was observed only in freeze dried complexes of RVS with HP-β-CD (RVS-HP-β-CD (FZD)) and RM-β-CD (RVS-RM-β-CD (FZD)).

After FTIR and DSC studies, as further supporting evidence for the formation of inclusion complex between SVS, RVS and CDs was obtained from x-ray diffraction studies. X-ray diffractogram showed that the complete amorphization of simvastatin was observed in complexes prepared by both kneading and freeze dried methods, with all selected CDs (β-CD, HP-β-CD and RM-β-CD). While x-ray diffractogram of complexes of RVS with HP-β-CD and RM-β-CD prepared by freeze drying method RVS-HP-β-CD (FZD), (RVS-RM-β-CD (FZD) respectively showed complete drug amorphization.

Results of in-vitro dissolution studies showed that the highest and rapid drug dissolution amongst all the prepared complexes of simvastatin is depicted by complex of SVS with HP-β-CD prepared by kneading method [SVS-HP-β-CD (KND)] which is 56.72% and 98.93 % after 15 and 120 minutes respectively in phosphate buffer pH 6.8. While the RVS-CDs complex prepared by freeze drying method [RVS-RM-β-CD(FZD)] showed highest and rapid drug dissolution which is 60% and 99.85% after 15 and 90 minutes respectively in phosphate buffer pH 6.8. It is obvious from overall dissolution results that the kneading method and freeze drying method were found to be best suited method while coevaporation and physical mixture showed less influence on formation of higher dissolution complexes for SVS and RVS.
Finally on evaluation of pharmacodynamic effect of selected complexes of SVS (SVS-HP-β-CD (KND) and SVS-RM-β-CD (KND)) results showed that after 7 days of treatment standard group showed approximately 11.5% decrease in elevated total cholesterol, and higher 91.65% increases in triglyceride (TG). In contrast, SVS-HP-β-CD (KND) and SVS-RM-β-CD (KND) complexes showed 2.93 and 2.70 fold decrease in total cholesterol, 3.27 fold and 3.15 fold increases in TG level of test group III and IV respectively. However after 15 days study, standard group showed approximately 2.54 fold decrease in total cholesterol and 2.36 fold increase in TG. On the other hand, test groups III and IV presented further 3.38 and 3.28 fold decrease in total cholesterol and less increase in TG in comparison with the standard group.

In case of RVS-CDs complexes, frieze dried complexes RVS-HP-β-CD (FZD) and RVS-RM-β-CD (FZD) showed approximately 13 % decrease in total cholesterol and 87 % increases in TG of standard group. In contrast, RVS-HP-β-CD (FZD) and RVS-RM-β-CD (FZD) showed 2.2 and 1.5 fold decrease in total cholesterol and 2.47 and 1.3 fold increases in TG level of test group III and IV after 7 days of treatment. While after 15 days of similar treatment standard group showed approximately 1.85 fold decrease in total cholesterol and 1.76 fold increase in TG. On the other hand, test groups III and IV further showed 2.4 and 1.8 fold decrease in total cholesterol and less increase in TG in comparison with the standard group. Thus both the selected complexes of each drug [SVS SVS-HP-β-CD (KND) and SVS-RM-β-CD (KND)] and RVS [RVS-HP-β-CD (FZD) and RVS-RM-β-CD (FZD)] showed enhanced solubility and significant increase in bioavailability as compared to pure drug. However based on overall results complexes SVS-HP-β-CD (KND) and RVS-HP-β-CD (FZD) prepared by kneading (KND) and freeze dried method (FZD) for SVS and RVS respectively were selected for development of drug delivery system(s).

Carrier is one of the most important entities essentially required for successful transportation of the loaded drug(s). Nano and micro carrier system, based on biodegradable and biocompatible polymeric systems have largely influenced the controlled and sustain drug delivery concepts. In present work we have investigated nanocarrier and microsphere systems for delivery of selected drug cyclodextrin complexes. We aimed to create a kind of new biodegradable nanocarriers of chitosan for the incorporation of HP-β-CD complexed simvastatin/rosuvastatin as model drugs.
The chitosan is selected as it is believed to facilitate dissolution/solubility of poorly soluble drugs in presence of cyclodextrin along with has potential of increasing transmucosal permeation, thereby enhancing bioavailability. This novel delivery system was suppose to increased dissolution of poorly water soluble model drug simvastatin/rosvastatin by HP-β-CD complexation and controlled delivery via encapsulation in chitosan nanocarrier.

Nanocarriers are sub-nanosized (below 1μm) colloidal structures composed of synthetic or semi synthetic polymers in which the active principle is dissolved, entrapped, encapsulated and/or to which the active principle is absorbed or attached. Among mucoadhesive polymers for nanocarriers chitosan based nanocarriers have been a focus of increasing attention in recent years, due to their desirable properties such as biocompatibility, bio and mucoadhesivity and hydrophilic character that facilitate the administration of poorly absorbable drugs. Chitosan has also shown to prolong the residence time of drug delivery systems at the site of drug absorption and is known to open transiently the tight junctions. A recognized feature of these nanosystems is their ability to overcome mucosal barriers. As a consequence, their application has been mainly centered in non invasive routes of administration via ocular, nasal, oral and oral mucosae.

Nanocarriers (NCs) of chitosan/tripolyphosphate (TPP) encapsulating drug cyclodextrin complex [SVS-HP-β-CD (KND)] and RVS-HP-β-CD (FZD)] were prepared by using the ionotropic gelation method. Initially preliminary studies were done to study polymer concentration (0.2–0.8%w/v) and concentration of cross-linking agent (0.5–2.5 mg/mL) suitable for nanocarrier formation. After initial preliminary studies optimum level of key variables such as ratio of polymer concentration to cross-linking agent (1:1–1:8 w/w) and stirring speed (300–900 rpm) were determined and optimized. On optimization of key variables effect of selected secondary variables viz. amount of selected drug-HP-β-CD complex (50–200 mg) and presence or absence of key ingredient cyclodextrin (HP-β-CD) was studied on various important formulation and physiochemical parameters of NCs.

On the basis of results of preliminary studies for nanocarriers of SVS and RVS chitosan concentration 0.3% w/v and TPP 2 mg/ml respectively resulted in nanocarriers with narrow particles size distribution, mean particle size less than 600 nm and smooth surface morphology.
The optimum TPP/chitosan ratios were found to be 1:4 and 1:5 respectively. For both SVS and RVS NCs it was observed that as the ratio of TPP/chitosan is increased from 1:1-1:5 nanocarriers with smaller sizes (612±4-464±3 nm and 600±5-451±2 nm) were produced. But when TPP/chitosan ratio increased beyond 1:5, it results in formation of large size (578±6 and 586±4 nm) NCs.

The results of varying stirring speed from 300-900 rpm on optimum formulations of SVS (SF-4, SF-5) and RVS (RF-4, RF-5) reveals decrease in particle size from 612±3-387±2 nm, 589±4-346±2 nm and 592±4-363±2 nm, 560±5-332±2 nm respectively. The polydispersity was also found to decrease for SNCs (simvastatin nanocarriers SF-4 and SF-5; 0.55-0.48 and 0.51-0.44 respectively) and RNCs (rosuvastatin nanocarriers RF-4 and RF-5; 0.52-0.44 and 0.48-0.41). The percent yield of SNCs and RNCs was too found to increase with increase in stirring speed from 300-900 rpm for both the formulations. The particle size, polydispersity and percent yield for optimized formulations of SNCs (SF-4, SF-5) obtained with stirring speed 700 rpm and for RNCs (RF-4, RF-5) obtained with stirring speed 900 rpm were found to be satisfactory and was chosen for further studies.

On varying the amount of drug [SVS-HP-β-CD (FZD) and RVS-HP-β-CD (FZD) complexes] from 50-200 mg in optimized formulations SF-4, SF-5 and RF-4, RF-5 results demonstrated an increase in the average size of nanocarriers from 390±2 to 425±1 nm, 348±1 to 377±2 nm for formulations of simvastatin SNCs-1 to SNCs-4, SNCs-A to SNCs-D and from 363±2 to 390±2 nm, 332±2 to 358±2 nm for formulations of rosuvastatin RNCs-1 to RNCs-4, RNCs-A to RNCs-D respectively. The percent yield (54.2±2-76±3%, 57.1±3-79±2% and 58.1±2-84±2%, 64.1±1-86.0±1%) and loading capacity (8.8±0.09-16.9±0.02%, 9.6±0.05-18.8±0.02% and 12.1±0.06-20.9±0.05%, 13.5±0.08-23.7±0.03%) was also found to increase with increase in amount of drug for SNCs-1- SNCs-4, SNCs-A- SNCs-D and RNCs-1- RNCs, RNCs-A- RNCs-D. However with increase in amount of drug incorporated there was no significant change in zeta potential was observed for different batches of NCs (SNCs and RNCs). A comparison of blank nanocarriers with drug loaded NCs (SNCs and RNCs) showed that the presence and absence of CD/CD complexed drug has minor effect on size of NCs and CDs do not interfere with the NCs formation process.
**SUMMARY AND CONCLUSION**

*In-vitro* release behavior of selected nanocarrier formulations SNCs (SNCs-3, SNCs-4, SNCs-C, SNCs-D) and RNCs (RNCs-3, RNCs-4, RNCs-C, RNCs-D) showed initial burst followed by nearly sustained release for 24 hrs. Results of release profile for all formulations indicate that the release involves biphasic release pattern characterized by an initial fast release phase (burst release) followed by a delayed release and the plateau level. The burst release lasted for 30 and 20 minutes for SNCs and RNCs releasing 29.76±1.08%, 28.69±0.78%, 27.73±1.08%, 26.25±3.04% and 33.87, 33.04, 31.94, 30.65% of the drug from formulations SNCs-3, SNCs-4, SNCs-C, SNCs-D and RNCs-3, RNCs-4, RNCs-C, RNCs-D respectively. While the plateau level was obtained approximately after 1 and 2 hrs for SNCs and RNCs respectively and lasted up to 6-7 hrs. Further after 6 hrs the formulations showed sustained released pattern which was maintained up to 24 hrs.

Based on high drug loading capacity (LC) and percent yield with optimum particle size in nano range as well on basis of dissolution profiles of NCs (SNCs and RNCs) formulation SNCs-4, SNCs-D of simvastatin and RNCs-4, RNCs-D of rosuvastatin were further investigated for physical state of encapsulated drug(s)-CD complex and uniform molecular dispersion of drug cyclodextrin complex in prepared NCs by differential scanning calorimetry (DSC) and X-ray diffractometry (XRD). The disappearance of characteristic and/or reduced endothermic peak and signals of DSC and XRD studies confirm the presence of rosuvastatin and simvastatin in complex form, inside the SNCs formulations (SNCs-4, SNCs-D) and RNCs formulations (RNCs-4 and RNCs-D), suggesting the possible interaction such as Vander wall interactions, hydrogen bonding between drugs and HP-β-CD formed during complexation still persists. Results of DSC and XRD studies taken together suggests formation of molecular dispersion or amorphous nano dispersion of encapsulated complexes within the chitosan matrix of the nanocarriers producing a marked decrease in crystallinity of SVS and RVS and/or confers a nearly amorphous state of drug.

The *in-vivo* study was performed on rats to evaluate the pharmacodynamic potential of developed formulation against plain drug(s) (SVS and RVS) using poloxamer-407 induced hyperlipidemia model. A higher decrease in TC (total cholesterol) and TG (triglyceride) and marked elevated protective HDL (high density lipids) levels by treated groups (SNCs-4, SNCs-D and RNCs-4, RNCs-D) in 3 phases after 24, 48
and 96 hrs were observed as compared to standard group (plain SVS and RVS). The observed results confirms complete dissolution of SVS and RVS, along with increased permeation of chitosan NCs which could have increased absorption and thereby led to a higher plasma drug concentration (higher bioavailability). Results clearly indicate that the formulations SNCs-4 and RNCs-4 showed comparatively less decrease in TC and TG level as well as less increase in HDL level too, as comparison to respective formulation SNCs-D and RNCs-D. Results of storage stability studies of selected nanocarriers formulations carried out according to ICH guidelines showed that all SNCs and RNCs formulations were found to be stable under both normal and accelerated conditions.

Microspheres are solid spherical particles ranging in size from 1 to 1000 μm containing dispersed drug in light solution or microcrystalline form. Microspheres are novel systems preferable over existing systems as they can be ingested or injected; can be tailored for desired release profiles and in some cases can even provide organ-targeted release. Eudragit RS100, a copolymer synthesized from acrylic and methacrylic acid esters with quaternary ammonium groups was selected as another polymer. Since Eudragit RS 100 film is slightly permeable, drug release through the film is retarded. Now it is also clear that incorporation of drug/CD mixtures or complexes into polymeric matrices can improve hydration of the matrix and modify drug solubility and diffusivity, leading to an enhanced or retarded drug release from the polymeric system. We aimed to develop eudragit microspheres encapsulating drug cyclodextrin complex in highly soluble form will led the sustained release, decreasing dosing frequency and will to enhance bioavailability (via. increased permeability) thereby decreasing potential hazards of statins reported recently. Finally, it could be interesting to study potential of eudragit microspheres as possible candidates for having sustained released and bioavailability enhancing property should also be investigated to prove their dual nature.

Eudragit microspheres (MPS) encapsulating selected simvastatin-CD complex prepared by kneading (KND) method [SVS-HP-β-CD (KND)] and rosuvastatin-CD complex prepared by freeze drying (FZD) method [RVS-HP-β-CD (FZD)] were prepared by slight modification of emulsification and a solvent evaporation technique in an acetone/liquid paraffin solvent system. First of all preliminary studies were done to determine polymer concentration (10–30%) and drug polymer ratio (1:1–1:5) for
the formation of MPs using acetone/liquid paraffin solvent system. The optimization parameters studied were effect of solvent (liquid paraffin Vs acetonitrile), stirring speed (5000-12000 rpm), volume of continuous Phase (30-50ml), amount of drug-CD complex (50-200 mg) and absence/presence of cyclodextrin. The prepared MPs were characterized for particle size, morphology, percent yield and association efficiency (AE). Based on results of characterization parameters optimum MPs formulations of SVS and RVS were selected for in-vitro drug release studies. Finally two formulations each of SVS (SMPs) and RVS (RMPs) with highest and lowest initial burst and drug release were evaluated for in-vivo antihyperlipidemic effects on poloxamer-407 induced hyperlipidemia model of rats.

Preliminary study based on random trials showed that polymer concentrations 10%, 20% and 30% with drug polymer ratio 1:4-1:5; 1:3-1:5 and 1:2-1:5 showed formation of simvastatin MPs (SMPs-4, SMPs-5, SMPs-8, SMPs-9, SMPs-10, SMPs-12, SMPs-13, SMPs-14, SMPs-15) respectively. Also the mean particle size of MPs was found to increase with increase in polymer concentration as well as with increase in drug polymer ratio. However on replacement of continuous phase solvent acetone with acetonitrile remarkable decrease in size of SVS MPs along with increase in association efficiency (AE) and percent yield of MPs was observed. Thus RVS MPs were also prepared using acetonitrile as continuous phase solvent in order to get smaller particle size along with increase in association efficiency (AE) and percent yield of RVS MPs.

Results of preliminary studies done by considering selected range of formulation and process parameters for RVS MPs showed nearly same results with little difference as were obtained in case of SVS MPs. However the average particle size of RVS MPs was greater than those of SVS MPs with similar polymer concentration and drug polymer ratio. The mean particle size of RVS (RVS-HP-β-CD complex) MPs (RF-1, RF-2, RF-3, RF-4, RF-5, RF-6, RF-7, RF-8, RF-9) ranged from 152±1.8–69±1.2 μm while mean particle size of 135± 2.6-57± 1.0 μm was obtained for SVS (SVS-HP-β-CD complex) MPs. Thus based on the above obtained results with initial experimentation under selected range of parameters MPs formulations with association efficiency (AE) 65% and above 75% along with mean particle size below 100μm of SVS and RVS respectively were selected for further optimization of remaining variables.
The study of effect of varying stirring speed from 5000-12000 rpm on SVS MPs (SMPs-8, SMPs-9, SMPs-12, SMPs-13, SMPs-14) and RVS MPs (RMPs-1A-5A) showed that the average particle size decreases from 87±1.1-30±0.9 nm, 68±1.3-32±1.30 nm, 76±1.2-26±0.7 nm, 61±1.3-24±0.5 nm, 57±1.0-23±0.7 nm and 98±1.5-65±2.2 nm, 86±2.1-56±2.0 nm, 90±1.1-59±1.5 nm, 78±0.8-48±0.9 nm, 69±1.2-36±1.1 nm respectively with increasing stirring speed. However the majority of SVS MPs obtained with stirring speed 12000 rpm were broken and of rough outer morphology. Also with increase in stirring speed percent yield and association efficiency were found to increase for SVS MPs and RVS MPs both. Thus considering overall results the SVS MPs obtained with 8000 rpm stirring speed (SMPs-8A, SMPs-9A, SMPs-12A, SMPs-13A, SMPs-14A) and RVS MPs (RMPs-1C-5C) obtained with 12000 rpm were considered optimum in terms of size, morphology and were intact.

On the other hand increase in association efficiency was more with low drug polymer ratio and lower polymer concentration. This can be credited to higher availability drug per unit of polymer at low drug polymer ratio.

On varying volume of continuous phase (from 30-50 ml) there was only slight difference in morphological and physiochemical parameters of different prepared batches of SVS and RVS MPs. Thus the SVS MPs formulations (SMPs-8A2, SMPs-9A2, SMPs-12A2, SMPs-13A2, SMPs-14A2) and RVS MPs formulations (RMPs-1CE-5CE) with lowest particle size and high percent yield were considered optimum formulations for studies ahead.

Increase in the amount of drug (SVS-HP-β-CD complex and RVS-HP-β-CD complex from 100-200 mg) in selected optimized formulations demonstrate increase in size, association efficiency, and percent yield of optimum SVS MPs formulations. As there was a marginal increase in above parameters with increase in amount of drug from 150-200 mg for SVS MPs, formulations obtained with amount of drug 150 mg (SMPs-8A22, SMPs-9A22, SMPs-12A22, SMPs-13A22, SMPs-14A22) were considered optimum for SVS MPs. While for RVS MPs increase in amount of drug to 200 mg results in high amount of broken MPs along with rough outer morphology surface so formulations with amount of drug (RVS-HP-β-CD complex) 100 mg (RMPs-1CE to 5CE) were considered optimum. The effect of absence/presence of cyclodextrin on physiochemical parameters of prepared MPs reveals a noteworthy positive effect on size, association efficiency and percent yield of SVS as well as RVS...
MPs in presence of cyclodextrin (CD). Also the MPs prepared in presence of CD were of smoother outer morphology in comparison to MPs obtained in absence of cyclodextrin.

The *in-vitro* released data obtained shows that all the formulation of SVS and RVS MPs showed more or less initial burst. Formulations of SVS and RVS with low polymer concentration (20%, SMPs-8A22 and RMPs-1CE) and low drug polymer ratio (1:2 and 1:3, SMPs-12A22, SMPs-8A22 and RMPs-3CE and RMPs-4CE) showed more burst release while formulations with high polymer concentration and high drug polymer ratio (30%, 1:4, SMPs-14A22 and RMPs-5CE) showed low initial burst release. However all the selected formulations showed decreased or sustained release up to 24 hrs approximately.

Based on above results of *in-vitro* release studies two optimum formulation each of SVS MPS (SMPs-8A22, SMPs-14A22) and RVS MPs (RMPs-1CE, RMPs-5CE) one with highest initial burst, highest drug release and another with lowest initial burst, lowest drug release were evaluated for presence of drug in complexed form with cyclodextrin and uniform molecular dispersion of drug cyclodextrin complex within polymer matrix of prepared MPs by differential scanning calorimetry (DSC) and x-ray diffractometry (XRD). The absence of the crystalline peaks of SVS and RVS in the DSC scans and x-ray spectra of the selected SVS and RVS MPs formulations indicates the presence of drug in same amorphous form. These results taken together suggest that the drug (SVS-HP-β-CD and RVS-HP-β-CD complex) encapsulation process did not affect the nature of drug-CD complex.

Finally results of *in-vivo* antihyperlipidemic effect of selected formulations of SVS MPs (SMPs-8A22 and SMPs-14A22) showed significant change in TC and TG of 8.89 and 5.85 fold in control group whereas the standard and treated groups showed around 6.69, 5.36 fold (standard) and 5.8, 4.24 fold (group III), 6.0, 4.38 fold (group IV) increase in TG and TC levels respectively for SVS MPs. While in case of RVS MPs (RMPs-1CE and RMPs-5CE) the standard and treated groups showed around 5.98, 4.61 (standard group), 5.2, 3.5-fold (group III), 4.5, 3.1-fold (group IV) increase in TG and TC levels respectively after 24 hrs of poloxamer-407 injection. However after 48 hrs the control and both standard groups (of SVS and RVS; group I and II) did not showed any significant difference in TC levels. While there was a difference of 1.16, 1.09 fold in TC and 1.31, 1.22 folds in TG levels of the treated groups (group
III and IV) with SVS MPs in comparison to control group. Whereas the RVS MPs demonstrated difference of 1.23, 1.14 folds in TC and 1.29, 1.20 folds in TG levels of the treated groups (group III and IV) in comparison to the difference of control group with standard group. At the end of 4 days of treatment, the TC and TG levels of all the groups reduced to the baseline except SVS and RVS standard groups.

Also the HDL levels of treated groups of SVS and RVS were found higher than control and standard group after 24 and 48 hours. It is obvious from results that there is a less difference (increase) in the HDL (2.38 folds more) levels after 48 hr of poloxamer-407 injection in standard group (4.54 fold increase in HDL) as compared to the control group (2.16 fold increase in HDL) while both treated groups (group III and IV) demonstrated significant increase in the HDL levels (4.86 and 5.11 folds). HDL levels in control group and standard group decreased to baseline after 4 days of injection. Overall results clearly indicate that formulation SMPs-14A22 and RMPs-ICE showed more decrease in TC and TG level in comparison to SMPs-8A22 and RMPs-5CE respectively.

Results storage stability studies of selected microspheres carried out according to ICH guidelines showed that the prepared system was found to be stable under both normal and accelerated conditions.

In conclusion, the cyclodextrin complexed novel delivery systems for model drugs simvastatin and rosuvastatin were developed and evaluated in-vitro and in-vivo. The work successfully demonstrates the possibility to entrap cyclodextrin complexed model drugs within chitosan nanocarriers and eudragit RS 100 microspheres using simple techniques. These new approach permits to enhance the entrapment of hydrophobic and hydrophilic drugs by forming molecular inclusion complexes with cyclodextrins in aqueous media. The developed technique could be of interest for increasing the absorption of poorly soluble and poorly permeable drugs through the complexation followed by entrapment in the polymer matrices. Also the prepared systems could be administered effectively to achieve desired therapeutic potential alleviating side effects and poor bioavailability with improved patient compliance. The same could be established by extensive pharmacokinetic and pharmacodynamic studies on human volunteers and patients.