10.1 SUMMARY

10.1.1 Selection of particle engineering method
Advances in drug delivery systems require specially engineered drug particles to meet biopharmaceutical and processing needs. Accordingly, development of engineered drug particles has become major research due to limitations of conventional drug particles. In present study, engineered drug particles were prepared by spherical crystallization technique. Spherical crystallization is the novel technique that can directly transfer the fine crystals produced in the crystallization into a spherical shape. It is a simple process and inexpensive enough for scaling up to a commercial level. Using this technology, physicochemical properties of pharmaceutical crystals were dramatically improved for pharmaceutical processing because of their excellent flowability and compressibility. Moreover, critical granulation steps (wet granulation or dry granulation) involved in manufacturing of tablets can be avoided. Spherical crystals produced by spherical crystallization method have tremendous advantages for advanced oral drug delivery systems.

10.1.2 Selection of candidate drugs
As an objective of study was to develop a unique method for preparation of engineered drug particles alone/or in combination with and without additives by using spherical crystallization technique, so hypolipidaemic drugs (Atorvastatin calcium, fenofibrate, ezetimibe and atorvastatin calcium – fenofibrate combination) were selected for study. All three selected hypolipidaemic drugs have different mechanism of actions and are also available in market as a fixed dose combination to provide synergistic effect. Preformulation study showed that all three drugs has poor aqueous solubility and low flowability which is challenge for development of solid oral dosage forms and therefore candidate drugs were selected for development of spherical crystals to resolve the problems.

10.1.3 Preformulation studies
Characterisation of the hypolipidaemic drugs were carried out by using scanning electron microscopy, Fourier transformed infrared spectroscopy, Differential scanning calorimetry and X-ray powder diffractometry. Scanning electron microscopy images of the hypolipidaemic drugs (Atorvastatin calcium, Fenofibrate, Ezetimibe and Physical mixture of Atorvastatin calcium – Fenofibrate) showed irregular shaped stone like crystal shapes. Fourier transformed infrared (FTIR) spectra showed all principal peaks of the hypolipidaemic drugs (Atorvastatin calcium, Fenofibrate, Ezetimibe and Physical mixture of Atorvastatin calcium – Fenofibrate) which indicate the identification of individual drugs. Differential scanning calorimetry (DSC) results showed sharp endothermic peak of Atorvastatin calcium, Fenofibrate, Ezetimibe at 162.47°C, 80.54°C, 163.17°C respectively which indicate the melting point of drugs which were further again confirmed by melting point determination study. DSC results of physical mixture of Atorvastatin calcium – Fenofibrate showed shape endothermic peaks at 81.55°C and 162.47°C which indicates no chemical changes or interactions occurs between physical mixture of Atorvastatin calcium – Fenofibrate. X-ray powder diffractometry (XRPD) results of the hypolipidaemic drugs (Atorvastatin calcium, Fenofibrate, Ezetimibe and Physical mixture of Atorvastatin calcium – Fenofibrate) showed intense and large number of peaks indicates that all drugs are crystalline in nature. Assay value of the hypolipidaemic drug (Atorvastatin calcium, Fenofibrate, Ezetimibe) were found
between 99% - 101% indicates that all drugs were in pure form and the aqueous solubility of all drugs were found between (0.02 – 0.11 mg/mL) indicates all hypolipidaemic drug (Atorvastatin calcium, Fenofibrate, Ezetimibe) were poorly water soluble drugs and also having low wettability. Data of bulk density, tapped density, carr’s index, hausner ratio and angle of repose showed all the hypolipidaemic drugs (Atorvastatin calcium, Fenofibrate, Ezetimibe) were having very low flowability as well as low packability, compressibility. Drug-excipient compatibility study results showed the hypolipidaemic drug (Atorvastatin calcium, Fenofibrate, Ezetimibe) and excipient combinations with different proportions were stable after 2 week and 4 week on 60°C. No significant difference in description and assay observed within samples therefore all excipients used in study were compatible with the hypolipidaemic drugs.

10.1.4 Development & optimization of particle engineered spherical crystals
Spherical crystals were prepared by quasi-emulsion solvent diffusion system (QESDS). The choice of the best solvent was done based on the available literature on solubility of drugs and miscibility of the solvent. Methanol, dichloromethane and water were selected as good solvent, bridging liquid and bad solvent, respectively. Selection of the bridging liquid should be such that it should be immiscible with the poor solvent i.e. water and the drug should have slight solubility in it. The impact of agitation speed on formulation of spherical crystals was such that on increase in agitation speed, spherical crystals with smaller diameter and rough surface were produced, which could be due to high shear force of stirrer. When the agitation speed was reduced, large irregular crystals were produced, where the shear energy may not be sufficient for the formation of good crystals. Various spherical crystals were prepared to select optimum speed of rotation. The impact of agitation speed on formulation of spherical crystals was such that on increase in agitation speed to 1000 ± 50 rpm, spherical crystals with smaller diameter and rough surface were produced. Fine powder was present along with irregular shaped crystals. The crystals with good spherical shape and flowability were produced at agitation speed of 750 ± 50 rpm. When the agitation speed was reduced to 500 ± 50 rpm, large irregular shaped crystals were produced.

The practical yield of spherical crystals prepared of atorvastatin calcium (ATR), fenofibrate (FNO), ezetimibe (EZM) and combination of atorvastatin calcium & fenofibrate (AFC) alone and with methacrylic acid copolymers (Eudragit L100, Eudragit S100, Eudragit EPO, Eudragit RSPO, Eudragit RLPO and combination of Eudragit RSPO & RLPO), hydrophilic polymers (polyvinyl alcohol, povidone, poloxamer, polyethylene glycol, hydroxypropyl cellulose, hydroxypropyl methylcellulose) and superdisintegrants (sodium starch glycolate, crospovidone and croscarmellose cellulose) were satisfactory and all were above 85 %. Drug content were observed in all spherical crystals above 95% and all spherical crystals showed improved aqueous solubility as compared with of plain drug. Plain drugs showed a significantly higher angle of repose in comparison with all the spherical crystals. The Carr’s index revealed that the flowability of the plain drugs was significantly poor than that of the all spherical crystals. The Hausnar ratio of the all spherical crystals was found to be less than 1.25. Powder bed hydrophilicity study revealed that the spherical crystals showed a significantly shorter rising time of water to its surface as compared with plain. The crushing strength of spherical crystals is significantly higher than that of plain drugs.
In-vitro dissolution studies of spherical crystals were showed faster dissolution rate as compared to plain drugs. Improvement in dissolution rate is due to improvement of aqueous solubility by preparation of spherical crystals by incorporation of various polymers. But the dissolution rates of spherical crystals were depended on the characteristics of polymers. Spherical crystals prepared with different polymethacrylate polymers showed pH dependent drug release profiles such as faster drug release profile in pH 6.8 phosphate buffer of spherical crystal prepared with Eudragit L100. The reason for this faster drug release in Eudragit L100 was linked to the increase in surface area due to spherical crystallization, better wettability and better solubility due to incorporation of Eudragit L100 in spherical crystallization process. Eudragit L100 is anionic copolymerization products of methacrylic acid and methyl methacrylate. The ratio of free carboxyl groups to the ester is approximately 1:1. It is readily soluble in neutral to weakly alkaline conditions (pH 6-7) and form salts with alkalis, thus affording film coats which are resistant to gastric media but soluble in intestinal fluid. The retards drug release profiles were observed in spherical crystals prepared by incorporation of Eudragit RLPO, Eudragit RSPO & Eudragit EPO due to solubility characteristics of Eudragit RLPO, Eudragit RSPO & Eudragit EPO respectively in pH 6.8. Further dissolution study carried out in 0.1N HCl showed faster drug release profile in spherical crystals prepared with Eudragit EPO. Eudragit EPO is cationic polymer based on dimethylaminoethyl methacrylate and other neutral methacrylic acid esters. It is soluble in gastric fluid as well as in weakly acidic buffer solutions. Whereas the retards drug release profiles were observed in spherical crystals prepared with Eudragit RLPO, Eudragit RSPO & Eudragit L100 due to solubility characteristics of Eudragit RLPO, Eudragit RSPO & Eudragit L100 respectively in 0.1N HCl. Eudragit RL and Eudragit RS are copolymers synthesized from acrylic acid and methacrylic acid esters with Eudragit RL having 10% of functional quaternary ammonium groups and Eudragit RS having 5% of functional quaternary ammonium groups. The ammonium groups are present as salts and give rise to pH-independent permeability of the polymers. Both polymers are water-insoluble and films prepared from Eudragit RL are freely permeable to water, whereas films prepared from Eudragit RS are only slightly permeable to water. In In-vitro dissolution study of spherical crystals prepared by incorporation of hydrophilic polymers such as poloxamer, polyvinyl alcohol, povidone, polyethylene glycol, hydroxypropyl methyl cellulose and hydroxypropyl cellulose were showed improved aqueous solubility and faster dissolution rate. The reason for this faster drug dissolution was linked to the increase in surface area due to spherical crystallization, better wettability of the spherically agglomerated crystals due to incorporation of the hydrophilic polymers and also because of spherical agglomerated crystals has a more porous internal structure exhibit a faster drug release rate than those of the less-porous agglomerates. In-vitro dissolution study of spherical crystals prepared by incorporation of superdisintegrants such as sodium starch glycolate, crospovidone and croscarmellose sodium were showed slightly improved aqueous solubility and dissolution rate but flowability, compressibility, particle size etc. were improved significantly as compared with plain drugs. Microscopy study of plain drugs (atorvastatin calcium, fenofibrate, ezetimibe and combination of atorvastatin calcium & fenofibrate) and its spherical crystals showed plain drugs were irregular and stone like shape as compared with the spherical crystals. The IR spectra of all the tested samples showed the prominent characteristic peaks of plain drugs respectively. The XRPD scan of plain drugs
showed intense peaks of crystallinity, whereas the XRPD pattern of the spherical crystals exhibited halo pattern with less intense and more denser peaks compared with plain drugs. In the DSC studies, plain drugs showed a sharp endotherm at corresponding to its melting point but a decrease, although little, in the enthalpy changes of spherical crystals when compared with that of plain drugs.

10.1.5 Development & evaluation of advanced drug delivery system

Developed spherical crystals were used for formulation of Atorvastatin calcium orally disintegrating tablets 40 mg, Fenofibrate gastroretentive floating tablets 160 mg, Ezetimibe rapid release tablets 10 mg and Fixed dose combination of atorvastatin calcium 20 mg & fenofibrate 160 mg tablets by direct compression method.

Developed free flowing, direct compressible particle engineered atorvastatin calcium spherical crystals prepared with Eudragit L100 (ATRL100) were used for development of atorvastatin calcium orally disintegrating tablets because spherical crystals of ATRL100 were showed pH dependent solubility and rapid dissolution in pH 6.8 phosphate buffer and very retard dissolution in 0.1N HCl due to Eudragit L100 which is having solubility above pH 6. Orally disintegrating tablets were rapidly disintegrates in mouth where atorvastatin calcium has high solubility (low acidic pH of saliva), also disintegrated undissolved drug particles were rapid pass through stomach (rapid gastric emptying) which will provide faster absorption of atorvastatin calcium in upper intestinal tract and reduces first pass metabolism of atorvastatin calcium. Tablet formulation (B. No. AT04) was found excellent among all due to rapid drug release profile and desirable stability. Hence developed atorvastatin calcium orally disintegrating tablets will enhance oral bioavailability of drug and improve patient compliance.

Developed particle engineered fenofibrate spherical crystals prepared with poloxamer (FNOPLM) were used for development of fenofibrate gastroretentive floating tablets. Spherical crystals of fenofibrate (FNOPLM) were showed significantly improved aqueous solubility as compared to plain fenofibrate also spherical crystals were showed improvement in flow property and compressibility of fenofibrate which is important for direct compression method. Tablets (B. No. FT14) were found excellent among all due to desirable drug release profile and stability. Kinetic modeling studies showed that the dissolution profile of B. No. FT14 was fitted to Bekker and Lonsdale, First-order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models. The results of Fit test, coefficient of determination, level of significance were used to select the most appropriate model. The release profile of the best batch, FT14 fitted best to the Korsmeyer-Peppas model ($F=1251.84$) with a coefficient of determination ($R^2 = 0.9937$), Significance level ($P < 0.0001$) and release exponent ($n = 0.5234$) indicating Fickian diffusion. Although Higuchi and First order were also showed good coefficient of determination but failed in Fit test. Fickian diffusion indicates that driving force was chemical gradient and release mechanism, was diffusion. In it, the water transport was controlled by a concentration gradient. It confirmed that the developed polymeric system was of swellable type and drug was mainly released by diffusion. Therefore developed fenofibrate gastroretentive floating tablets provide better aqueous solubility, maximum GI residence time and direct compression manufacturing process which will tremendously reduce manufacturing cost as well as reduce absorption variability and enhance oral bioavailability of fenofibrate.
In present study, particle engineered ezetimibe spherical crystals prepared with polyethylene glycol (EZMPEG) were used for development of ezetimibe rapid release tablets because spherical crystals prepared with polyethylene glycol were showed significantly improved aqueous solubility of ezetimibe as well as improved physico-chemical properties like particle size, particle shape and flow properties which is suitable for direct compressible tablet formulation. Tablet formulation (B. No. ET04) was found excellent among all due to rapid drug release profile and desirable stability. Therefore developed ezetimibe rapid release tablet provide faster dissolution rate which will improve oral bioavailability of ezetimibe and direct compression manufacturing process which will significantly reduce manufacturing cost.

Spherical crystals of atorvastatin calcium & fenofibrate combination prepared with poloxamer (ATRPLM) were used for development of tablets because spherical crystals prepared with poloxamer were showed significantly improved aqueous solubility of atorvastatin calcium & fenofibrate as well as improved physico-chemical properties like particle size, particle shape and flow properties which is suitable for direct compressible tablet formulation. Tablet formulation (B. No. CT04) was found excellent among all due to faster drug release profile and desirable stability. Therefore developed fixed dose combination atorvastatin calcium (20 mg) & fenofibrate (160 mg) tablet provide faster dissolution rate and combination therapy will improve oral bioavailability of atorvastatin calcium & fenofibrate and direct compression manufacturing process which will significantly reduce manufacturing cost.

10.1.6 *In-vivo evaluation study*

Randomized, two way, parallel study were carried out using male adult rat (n = 6) to finding out pharmacokinetic profile of developed spherical crystals of fixed dose combination of atorvastatin calcium & fenofibrate prepared by incorporation of poloxamer [Test samples] as compared to plain physical mixture of atorvastatin calcium & fenofibrate [Reference samples]. In-vivo study in rat shows $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ of both the drugs (Atorvastatin calcium & fenofibrate) of test samples were improved as compared to reference samples. Inter-subject variability also significantly reduced of test samples than that of reference samples. $T_{\text{max}}$ of both the drugs (Atorvastatin calcium & fenofibrate) of test samples were shorter as compared to reference samples. The enhancement in rate of absorption (shorter $T_{\text{max}}$, higher $C_{\text{max}}$ & AUC) of developed spherical crystals prepared by incorporation of hydrophilic polymer (poloxamer) was due to improvement in wettabillity, solubility and dissolution profile compared to plain drug because the rate of absorption and bioavailability of poorly water soluble drugs is often controlled by the rate of dissolution of the drug in the gastrointestinal tract. Therefore in-vivo study was demonstrated that the improvement in oral bioavailability of combination of atorvastatin calcium and fenofibrate can obtain by spherical crystallization method.
10.2 CONCLUSION OF RESEARCH WORK

In this study, we can concluded that the successful development of a unique particle engineering method for preparation of spherical crystals of single drug such as atorvastatin calcium (ATR), fenofibrate (FNO), ezetimibe (EZM) and novel method for development of spherical crystals of combination of atorvastatin calcium & fenofibrate (AFC) prepared by incorporation of different additives. Prepared spherical crystals were showed excellent physico-chemical properties as compared with plain drugs therefore suitable for direct compression method. Spherical crystals showed an improvement in the solubility, dissolution rate, packability, compactibility, wettability, flowability and crushing strength as compared with pure drug. There is no chemical modification of drugs takes place by spherical crystallization method.

Present study also proved that successful development of a stable formulation of advanced drug delivery systems like Atorvastatin calcium orally disintegrating tablets 40 mg, Fenofibrate gastroretentive floating tablets 160 mg, Ezetimibe rapid release tablets 10 mg and Fixed dose combination of atorvastatin calcium 20 mg & fenofibrate 160 mg tablets prepared by using developed spherical crystals of drugs. In vivo study confirmed that the developed spherical crystals of fixed dose combination of atorvastatin calcium & fenofibrate were improved the drug oral absorption rate and bioavailability as compared to plain unprocessed drugs.