1.1 INTRODUCTION

1.1.1 PARTICLE ENGINEERING

A particle has a small discrete quantity of matter that has an interface with the surrounding environment. Most often particles are associated with solid materials that have an interface with an enveloping gas or liquid. However, particles might just as easily be liquid droplets in air, bubbles in water or emulsions. There is no rule governing how large or small an object must be to be considered a particle. Some define particles as ranging from one nanometer to one millimeter. The study of particles at sizes below about 100 nanometers is part of the science of nanotechnology. In this size regime, many exciting properties begin to emerge along with many difficulties relating to the handling and dispersion of particles. As particles get smaller, attractive forces (Vander Waals) begin to dominate and particles become more likely to agglomerate into larger structures. One of the major consequences of reducing particle size is the increase in interparticle forces with respect to particle mass. This phenomenon manifests itself as greater cohesiveness in very fine powders and a tendency to aggregate in colloidal solutions. An example of this is the difference in the flow properties of wheat kernels versus wheat flour. The relatively large wheat kernels have a large mass in proportion to their surface area and the interparticle attraction. Thus, they are easily separated from one another as individual grains. The same mass of flour on the other hand, has a much higher surface area and individual particles have a much smaller mass. Thus, flour particles tend to stick together or "clump". Moisture or humidity exacerbates this tendency by adsorbing onto the surface and adding additional attractive forces. This has great significance in industrial and other technological applications of powders and colloids.\(^1\)\(^{-5}\)

Drug molecules with limited aqueous solubility are becoming increasingly prevalent in the research and development portfolios of discovery focused pharmaceutical companies. The increasing prevalence of poorly soluble drugs in development provides notable risk of new products demonstrating low and erratic bioavailability with consequences for safety and efficacy, particularly for drugs delivered by the oral route of administration. Molecules of this type can provide a number of challenges in pharmaceutical development and may potentially lead to slow dissolution in biological fluids, insufficient and inconsistent systemic exposure and consequent sub-optimal efficacy in patients. Although numerous strategies exist for enhancing the bioavailability of drugs with low aqueous solubility, the success of these approaches is not yet able to be guaranteed and is greatly dependent on the physical and chemical nature of the molecules being developed. Particle engineering offers a number of routes to improved solubility and dissolution rate, which can be adopted through an in-depth knowledge of crystallization processes and the molecular properties of active pharmaceutical ingredients.\(^6\)\(^{-8}\)

Advances in the pharmaceutical sciences have led to the establishment of a number of approaches for addressing the issues of low aqueous solubility. These strategies for improving and maximizing dissolution rate include micronisation to produce increased surface area for dissolution, the use of salt forms with enhanced dissolution profiles, solubilisation of drugs in co-solvents and micellar solutions, complexation with cyclodextrins and the use of lipidic systems for the delivery of lipophilic drugs. Although these techniques have been shown to be effective at enhancing oral bioavailability, the success of these approaches is dependent at times on the specific physicochemical nature of the molecules being studied. Solubilisation technologies such as micellar systems are reliant on the acceptable solubility and compatibility of
therapeutic molecules in a limited range of pharmaceutically acceptable excipients, whilst the increasing number of weakly ionisable and neutral molecules entering development constrains the opportunities for salt formation as a method of improving dissolution rate. Particle engineering approaches, which can potentially be applied to a wide range of crystalline materials, offer an alternative and potentially fruitful method for improving the solubility, dissolution rate and subsequent bioavailability of poorly soluble drugs. The ability to engineer materials with suitable dissolution characteristics, whilst maintaining suitable physical and chemical stability provides a strong driver for the utilisation of new and existing particle engineering approaches to drug delivery system design. The challenges of low aqueous solubility provide an ideal situation for the application of particle engineering techniques for improving bioavailability, whilst also developing stable and robust pharmaceutical products. Particle engineering has been described as the ‘exploitation of noncovalent interactions between molecular or ionic components for the rational design of solid-state structures that might exhibit interesting electrical, magnetic, and optical properties’. It is also recognised that it is becoming increasingly evident that the specificity, directionality, and predictability of intermolecular hydrogen bonds can be utilized to assemble supramolecular structures of, at the very least, controlled dimensionality. Molecules are built by connecting atoms with covalent bonds, solid-state supermolecules (crystals) are built by connecting molecules with intermolecular interactions.

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 particle formation and pretreatment processes in fine-tuning the required characteristics. Techniques such as micronisation, spray drying, spray freezing, supercritical fluid processing, spherical crystallization, solution atomization and crystallization by sonication (SAXS), sonocrystallization and melt sonocrystallization are introduced to provide particles with novel physicochemical properties. The alternative strategy of using particle engineering of pharmaceutical materials and drug delivery systems shows great promise in this area.

1.1.2 ORAL DRUG DELIVERY SYSTEM

Oral ingestion is the preferred route for administration of therapeutic agents, providing a convenient method of effectively achieving both local and systemic effects. Routes of drug administration that can be utilized in order to achieve systemic delivery of a drug include: parenteral, oral, buccal, transdermal, nasal and pulmonary. No single route matches all the physiological requirements of an “ideal” absorption site. But, considering surface area, low metabolic activity, contact time, blood supply, accessibility, lack of variability and permeability, relatively oral route is having more suitable characteristics for absorption of drugs. Among the pharmaceutical dosage forms, oral dosage forms are having maximum attribute of ideal dosage forms. Patients are usually accustomed to orally delivered drugs and find the method non-invasive. Today it is estimated that around 80% of all medications used utilize the oral route, in which tablets, capsules and granules continue to remain the dosage form of first choice. It is therefore important that oral drug delivery technology continues to advance and improve the safety and efficacy of treatment. Oral dosage forms represent the vast majority of the drug-delivery market because of the safety, efficacy, economic, and consumer compliance advantages they possess over alternative routes of delivery. Transdermal, injectable, and inhalation routes
possess significant regulatory, technical and compliance barriers to their economical application towards a wide a range of compounds.\textsuperscript{18}

In conventional oral drug delivery systems, there is very little control over release of drug. Effective concentration at target site can be achieved by intermittent administration of grossly excessive doses, which, in most situations, often results in constantly changing, unpredictable, and often sub- or supra therapeutic plasma concentrations leading to marked side effects.\textsuperscript{19}

Once-a-day formulations are a holy grail of sorts for scientists working with oral dosage forms. An ideal drug delivery system should steadily deliver a measurable and reproducible amount of drug to the target site over a prolonged period. Controlled release systems provide a uniform concentration of the drug at the absorption site and thus, after absorption allow maintenance of plasma concentrations within a therapeutic range, which minimizes side effects and also reduces the frequency of administration.\textsuperscript{20}

Controlled release (CR) dosage forms are defined as a technique or approach by which active pharmaceutical ingredients are made available to a specified target at a rate and duration designed to accomplish an intended effect. More specifically, an oral controlled release drug delivery system is, in principle, a device or dosage form that controls drug release into the absorption site in the gastrointestinal (GI) tract. It controls the drug absorption rate to achieve the desired plasma profiles defined by the steady-state pharmacology. A typical controlled release system is designed to provide constant or nearly constant drug levels in plasma with reduced fluctuation via slow release of drug over an extended period of time. An oral controlled release formulation should allow a reduction in dosing frequency as compared to that of a drug presented as a conventional dosage form.\textsuperscript{21}

With the growing need for optimization of therapy, controlled release technologies providing programmable delivery rates other than immediate input have increasingly become more important, especially for drugs for chronic use or with a narrow therapeutic index. Thus, understanding and utilizing the fundamentals of controlled release technologies are essential to the successful formulation research and development of a drug delivery product.

Controlled release products are formulations that release active drug compound into the body gradually and predictably over 12 to 24 hour period and that can be taken once or twice a day. It provides numerous benefits compared with immediate-release drugs, including greater effectiveness in the treatment of chronic conditions, reduced side effects, greater convenience, and higher levels of patient compliance due to a simplified dosing schedule. Therefore such systems form the major segment of drug delivery market.

As discovery research is very expensive and time consuming, some of the organizations are focusing on new drug delivery system to improve the efficacy and convenience of drug administration. After product patent protection, this activity picked up as they also get the benefit of patent protection.\textsuperscript{22} The driving forces behind this booming market can be divided into two main groups: patient related factors and market driven factors. The advantages of patient related factors include improved patient compliance due to a reduced dosing frequency, a decreased incidence and/or intensity of the side effects, a greater selectivity of pharmacological activity, and a more constant or prolonged therapeutic effect, as well as an increase of cost effectiveness. Market driven factors offer the benefits of product differentiation, market expansion, creating new markets, adding value to generics, protecting
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franchises and patent extension increased clinical value as well as extended product life.
Only a few years ago, CR was usually restricted to managing product life cycle, enhancing the proprietary position of marketed drugs and expanding and consolidating a product franchise. CR technologies were mostly licensed from small specialized drug-delivery companies. Although large pharmaceutical companies maintained internal CR formulation development (primarily as backup), the focus was on developing the next generation of new chemical entities (NCE’s). Nowadays, CR is considered earlier in the development of drug candidates, primarily because it has been recognized that CR formulations represent a cost-effective way to progress candidates, compared with eliminating the deficiencies of a compound using discovery approaches. The rapid progress of drug candidates using the most appropriate formulation approach [from first-in-human (FIH) studies to clinical proof-of-concept (POC)] is particularly important when investigating a novel pharmacology (i.e. first-in-class).

Today drug delivery companies are engaged in the development of multiple platform technologies for controlled release, delivery of large molecules, liposomes, taste-masking, oral fast dispersing dosage forms, technology for insoluble drugs and delivery of drugs through intranasal, pulmonary, transdermal, vaginal, colon and transmucosal routes. Drug delivery system represents a more sophisticated system, which may incorporate one, or a combination, of advanced technologies such as rate control, pulsatile release or bio-responsive release to achieve spatial and/or temporal delivery.

The technologies behind oral drug delivery have emerged from the mainstream of pharmaceutical industry and have become influential forces in their own right, as evidenced by the burgeoning “drug delivery companies” that are at the forefront of innovation and hold their own niche market.23

Drug delivery companies and their pharmaceutical industry partners are poised to reap the rewards of the multi-billion dollar drug delivery market, which is forecast to grow to about $543.8 billion, by 2010 an increase of 27.4% since 2005. The compound annual growth rate of the market in the period 2005-2010 is predicted to be 5%. Oral controlled release systems have around 50% share of total sale.24

Presently, the pharmaceutical industry is caught between the downward pressure on prices and the increasing cost of successful drug discovery and development. The average cost and time for the development of a new chemical entity are much higher (approximately $500 million and 10-12 years respectively) than those required to develop a novel drug delivery system (NDDS) ($20-50 million and 3-4 years respectively). The market for drug delivery systems has come a long way and will continue to grow at an impressive rate. Today’s drug delivery technologies enable the incorporation of drug molecules into new delivery systems, thus providing numerous therapeutic and commercial advantages.

Tomorrow’s drugs definitely will be more challenging in terms of the development of delivery systems, and pharmaceutical scientists will have to be ready for difficult task ahead. Significant changes in healthcare industry have led pharmaceutical companies to depend more heavily on advanced drug delivery technology to produce distinctive drugs and remain competitive. Pharmaceutical companies are employing low-risk strategies to develop existing medicines into improved therapies, with lower developmental costs and attrition rates.

Platform technology can work as a common base comprising of polymeric system with release modulator and able to accommodate the drugs with common
physicochemical / therapeutic properties with minimal changes. A platform drug delivery system allows a company to use one drug delivery system for several drugs. This builds an internal base of experience, which can shorten development, and scale-up times, improve quality control, and better utilize manufacturing capabilities. The utility of system is directly related to its complexity.

The developed platform technology should have following properties,

- Tailored to suit the drug: Drug delivery platforms are developed to address the wide range of active pharmaceutical ingredients (APIs) and their physicochemical characteristics such as solubility, compressibility and flowability as well as pharmacokinetic properties like half-lives, bioavailability, and other key characteristics.
- Release pattern: The degree to which a delivery system can achieve standard release profiles for a variety of chemically and physically diverse, pharmaceutically active molecules, is measure of delivery system’s efficacy and flexibility. The platform technology should allow the finished dosage form’s release profile to be manipulated by alterations in the formulation itself with minimum changes. Among the most challenging profiles, linear, zero-order, bimodal and pulsatile release of highly soluble actives over 12 to 24 hour period could be considered a reasonable performance standard against which delivery systems can be judged.
- Targeting: To develop the system suitable for drugs to be delivered in stomach (gastroretentive) and colon (colon targeted formulation).
- Dosing frequency: It should reduce the dosing frequency by creating once-a-day formulations for improved compliance and patient convenience.
- Improved plasma levels: It extends plasma concentration levels and provides a more linear release profile. It should maintain plasma drug profile within therapeutic window to reduce adverse effect and toxicity and increase efficacy.
- Low cost: The ingredients used in this system are commodity items produced in extremely large quantity and at very low cost. The platform technology comprises of excipients, coupled with standard tabletting, adds less direct cost of a tablet. It reduces the developmental time, which is very critical to launch the product in market by reducing the developmental time.
- Manufacturing ease: The ideal platform system should employ conventional equipment; involve minimum of excipients and processing steps; and be transferred easily between production facilities. The degree, to which this ideal requirement is achieved, is the direct reflection of the system’s design, the complexity of the system and its utility often being inversely related. No special tooling or engineering is required. This enables high quality, consistent, rapid scale-up and technology transfer to our development and marketing partners. The future of drug delivery lies in a technology that addresses these practical issues of formulation, scale-up and production, whilst provided even greater therapeutic effect. It can increase profitability by lowering the cost of goods as commonly used and made available for number of products, reduce inventory and improve the manufacturability.
- It should have high level of reproducibility, greater stability of the raw materials, and the finished dosage form, ease of scale up operation and well-established in-vitro- in-vivo correlations (IVIVC). These approaches use conventional technology to form swellable, erodible matrix tablets, caplets,
granules, patches, suspension or capsules that can yield first-order, bimodal and zero-order drug release profiles.

- Low risk inactive ingredients: Platform technology is composed of well understood polymers from the FDA's inactive ingredients list. This keeps the regulatory risks and hurdles of the formulation to an absolute minimum.
- Intellectual property: Platform technology can contribute proprietary intellectual property, know-how, including their flexible technologies, drug delivery and developmental expertise. This avoids delay due to patent clearance, as it is already passed through approval process
- Life-cycle management: Platform drug delivery technologies can be applied to meet many of the challenges of the next decade that pharmaceutical companies face in life-cycle management of both old and new drugs. It can maximize product potential and expand return on investment. Once platform is developed by research and tested, can be applied to number of product and maximize the profit.

1.1.3 LIPID LOWERING DRUGS

Lipids are important biomolecules. Cholesterol, for example, is an essential component of the human cell membrane and a precursor for steroid hormones and bile acids. Triglycerides also play an important role in transferring energy from food into body cells. However, any biomolecule in excess is not good for human health. Similarly, elevation of different forms of lipids in the bloodstream, a condition generally termed hyperlipidemia, causes a constant health problem. Because lipids are carried in the bloodstream, hyperlipidemia is always a threat to coronary arteries and the most important risk factor for coronary heart disease. However, to fight these problems, human wit has acquired several drugs, commonly known as lipid-lowering drugs. One group of drugs (statins) lowers cholesterol by interfering with the cholesterol biosynthetic pathway. On the other hand, fibrates decrease fatty acid and triglyceride levels by stimulating the peroxisomal β-oxidation pathway. Apart from these drugs, ezetimibe, which selectively inhibits intestinal cholesterol absorption, cholestyramine, colestipol, and colesevelam, which sequester bile acids, torcetrapib, which inhibits cholesterol ester transfer protein, avasimibe, which inhibits acyl-CoA: cholesterol acyltransferase, implitapide, which inhibits microsomal triglyceride transfer protein, and niacin, which modifies lipoproteins, are providing clinicians with several therapeutic options for lipid lowering. However, based on medical use, importance, and popularity, statins and fibrates are way ahead of the others. Recent experimental data have revealed that both statins and fibrates display a broad spectrum of activities in addition to their lipid-lowering properties. As a result, statins and fibrates are now being considered as possible medicines in a variety of human disorders.

1.1.3.1 Classifications

Most of the lipid-lowering drugs are classified mainly into two groups – statins and fibrates.

There are several classes of hypolipidemic drugs. They may differ in both their impact on the cholesterol profile and adverse effects. For example, some may lower the "bad cholesterol" low density lipoprotein (LDL) more so than others, while others may preferentially increase high density lipoprotein (HDL), "the good cholesterol". Clinically, the choice of an agent will depend on the patient's cholesterol profile, cardiovascular risk, and the liver and kidney functions of the patient, evaluated
Against the balancing of risks and benefits of the medications, in the United States, this is guided by the evidence-based guideline from the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII).

Established Classification

- Statins are particularly well-suited for lowering LDL, the cholesterol with the strongest links to vascular diseases. In studies using standard doses, statins have been found to lower LDL-C by 18% to 55%, depending on the specific statin being used. There is a risk of severe muscle damage (myopathy & rhabdomyolysis) with statins.
- Fibrates are indicated for hypertriglyceridemia. Fibrates typically lower triglycerides by 20% to 50%. Level of the good cholesterol HDL is also increased. Fibrates may decrease LDL, though generally to a lesser degree than statins. Similar to statins, there is a risk of severe muscle damage (myopathy & rhabdomyolysis) with fibrates.
- Niacin, like fibrates, is also well-suited for lowering triglycerides by 20-50%. It may also lower LDL by 5-25% and increase HDL by 15-35%. Niacin may cause hyperglycemia, and may also cause liver damage. The niacin derivative acipimox is also associated with a modest decrease in LDL.
- Bile acid sequestrants (resins) are particularly effective for lowering LDL-C by sequestering the cholesterol-containing bile acids released into the intestine and preventing their reabsorption from the intestine. It decreases LDL by 15-30% and raises HDL by 3-5%. It has little effect on triglycerides but can cause a slight increase. Bile acid sequestrants may cause gastrointestinal problems, and may also reduce the absorption of other drugs and vitamins from the gut.
- Ezetimibe (Zetia) is a selective inhibitor of dietary cholesterol absorption.
- Phytosterols may be found naturally in plants. Similar to ezetimibe, phytosterols reduce the absorption of cholesterol in the gut. Hence, they are most effective when consumed with meals. However, the precise mechanism of action of phytosterols differs from ezetimibe.
- Orlistat (Xenical): Its primary function is to prevent the absorption of about 30% of fats from the human diet; thereby reducing caloric intake (a drug designed to treat obesity) is by inhibiting Pancreatic lipase-an enzyme that breaks down triglycerides in the intestine.

Investigational Classification

- CETP Inhibitors (cholesteryl ester transfer protein inhibitors) are still under development. It is expected that these drugs will mainly increase HDL while lowering LDL.
- Squalene synthase inhibitor
- ApoA-1 Milano
- AGI-1067
- Mipomersen (completed 4 phase III trials - may file NDA in 2011)

1.1.3.2 STATINS

The statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and, thereby, suppress cholesterol biosynthesis (Fig. 1). In the 1970s, Dr. Endo and colleagues in Japan were studying how certain fungi protected themselves against others. As ergosterol, a derivative of cholesterol, is an essential component of fungi membrane, they were prompted to investigate if inhibition of cholesterol biosynthesis
was one such mechanism. In 1978, they reported the discovery of mevastatin, the first statin drug. Eventually, through the laboratory of Drs. Goldstein and Brown, these drugs emerged as the most effective means of reducing elevated levels of plasma cholesterol. There are currently seven statins available in pharmaceutical form – lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin, and pitavastatin. First-generation statins, such as lovastatin and mevastatin, were isolated from fungi. However, second- and third-generation statins have been developed by either modification of first-generation statins or chemical synthesis in the laboratory. In general, statins share similar chemical characteristics, with second- and third-generation statins having several aromatic rings and an aliphatic fatty acid side chain, and first generation statins having a decalin ring and an aliphatic side chain.

![Figure 1.1: Schematic diagram depicting the various functions of statins.](image)

1.1.3.2.1 Mode of action of statins

Inhibition of cholesterol biosynthetic pathway

Statins came into the limelight due to their inhibitory effect on cholesterol biosynthesis. In humans, cholesterol is synthesized from acetyl-CoA via multiple reactions. HMG-CoA reductase is the key rate-limiting enzyme of this biosynthetic pathway (Figure 1.1). Statins are structural analogues of HMG-CoA and thereby inhibit HMG-CoA reductase competitively with an affinity about 1000–10,000 times greater than that of the natural substrate. In addition to direct inhibition of cholesterol synthesis, statins have also been shown to lower plasma cholesterol levels indirectly due to up-regulation of the low-density lipoprotein (LDL) receptor.

Inhibition of small G protein activation

The activity of several proteins involved in intracellular signaling cascades is dependent on post-translational modification by isoprenylation. As described in Figure 1, isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate are intermediates in the cholesterol biosynthetic pathway. These...
intermediates serve as important lipid attachment molecules for the γ subunit of heterotrimeric G proteins and small G proteins, such as Ras, Rho, and Rac.\textsuperscript{34,35} Inactive GDP-bound Ras, Rho, and Rac are localized in the cytoplasm. After isoprenylation, these small G proteins are translocated to the membrane and converted to active GTP-bound forms. Subsequently, activated Ras, Rho, and Rac modulate functions of multiple downstream signaling molecules. Because mevalonate is a precursor of isoprenoids, statins inhibit the synthesis of isoprenoids and thereby suppress the activation of small G proteins.

**Suppression of proinflammatory molecules**

The idea of investigating the role of the mevalonate pathway in the regulation of inducible nitric oxide (NO) synthase (iNOS) and proinflammatory cytokines came from the fact that intermediates of this biochemical pathway are isoprenoids, which are known to play an important role in activating small G proteins like Ras and Rac as described above. Interestingly, Pahan et al.\textsuperscript{46} have shown that lovastatin inhibits the activation of NF-κB and the expression of iNOS and proinflammatory cytokines [tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6] in lipopolysaccharide (LPS)-stimulated rat primary astrocytes. In fact, this landmark finding has revolutionized statin research. Nowadays, statin drugs are being widely considered as potential therapeutic agents against various neuroinflammatory and neurodegenerative disorders. Because lovastatin inhibits HMG-CoA reductase, both mevalonate and farnesyl pyrophosphate (FPP) are capable of reversing the inhibitory effect of lovastatin on the expression of iNOS and the activation of NF-κB. However, addition of ubiquinone and cholesterol to astrocytes does not prevent the inhibitory effect of lovastatin. These results suggest that depletion of FPP, rather than end products of the mevalonate pathway, is responsible for the observed inhibitory effect of lovastatin on the expression of iNOS.

**Suppression of LPS-induced activation of NF-κB and expression of iNOS in glial cells by farnesyltransferase inhibitors** suggests an important role for the farnesylation reaction in the regulation of the iNOS gene. Consistent with a role of farnesylation in the activation of p21\textsuperscript{Ras}, a dominant-negative mutant of p21\textsuperscript{Ras}(S17N) also attenuated activation of NF-κB and expression of iNOS in rat and human primary astrocytes. Statins also block interferon (IFN)-γ-inducible and constitutive transcription of the major histocompatibility complex (MHC) class II transactivator (CIITA), which regulates nearly all MHC class II gene expression. Recently, Cordle and Landreth have also indicated that statins inhibit fibrillar Aβ-induced expression of iNOS in mouse BV-2 microglial cells by inhibiting isoprenylation of Rac. Taken together, these studies suggest that mevalonate metabolites regulate the expression of iNOS in glial cells via modulating isoprenylation of small G proteins.

**Stimulation of endothelial NOS**

In patients with atherosclerosis and hypercholesterolemia, endothelial function is known to be impaired due to decreased synthesis of endothelium-derived NO.\textsuperscript{47} In vascular walls, NO is synthesized from endothelial nitric oxide synthase (eNOS). Although statins inhibit the expression of iNOS, these drugs have been found to stimulate eNOS-derived NO production. This beneficial effect of statins is found to be independent of cholesterol lowering. Reversal of this effect by geranylgeranyl pyrophosphate but not FPP suggests that Rac/Rho but not Ras play a role in down-regulation of eNOS. In addition, Akt has been shown to phosphorylate eNOS and increase the production of NO. On the other hand, mevalonate, an intermediate of the cholesterol biosynthetic pathway, inhibits phosphatidylinositol-3 (PI-3) kinase and thereby attenuates the activation of protein kinase B (Akt). These studies suggest that
Statins may also favor the up-regulation of eNOS by inhibiting the synthesis of mevalonate and thereby activating the PI-3 kinase-Akt pathway. Furthermore, according to Feron et al., atorvastatin increases NO production by decreasing the expression of caveolin-1, a negative regulator of eNOS.

**Inhibition of migration and proliferation of smooth muscle cells**

Migration and proliferation of smooth muscle cells (SMCs) play an important role in the pathogenesis of atherosclerosis. Small G proteins, such as Ras and Rho, are known to promote SMC migration and proliferation. While Ras promotes cell cycle progression via activation of the MAP kinase pathway, Rho/Rho kinase induces cell proliferation via destabilization of the inhibitor of cyclin-dependent kinase, p27kip1. Because statins are capable of inhibiting the activation of Ras and Rho, these drugs also suppress SMC migration and proliferation.

**Inhibition of reactive oxygen species production**

Reactive oxygen species (ROS) play many important roles in intracellular signal transduction. Several inflammatory and degenerative stimuli induce the production of ROS via the activation of NADPH oxidase. NADPH oxidase is a five-subunit protein that generates superoxide from molecular oxygen and is composed of two membrane-bound subunits, gp91phox and p22phox, and at least two cytosolic subunits, p47phox and p67phox. Phosphorylation of p47phox results in translocation of the p47phox-p67phox complex to the membrane, where it interacts via multiple binding sites with gp91phox and p22phox. This complex remains incomplete without the participation of Rac, a small G protein, which is known to associate with p67phox and gp91phox. As mentioned above, statins inhibit geranylgeranylation of Rac and thereby attenuate NADPH oxidase-mediated generation of superoxide.

**Switching of T-helper cells**

CD4 T helper (Th) cells play an important role in controlling two different arms of immunity – cell-mediated immunity and antibody-mediated immunity. While Th1 cells play an important role in cell-mediated immunity, Th2 cells induce humoral or antibody-mediated immunity. The polarization of Th0 (naive) cells into functionally distinct subsets (Th1 and Th2) are characterized by the patterns of cytokines they produce, with Th1 cells producing IFN-γ, and Th2 cells producing IL-4 and IL-10. Sometimes, Th2 cells are able to negatively regulate Th1 cell-mediated responses, thus acting in an anti-inflammatory capacity. In healthy human beings, there is a proper balance between Th1 and Th2 cells. However, once the balance is lost, it leads to immune-related disorders. It has been suggested that altering the Th1/Th2 balance in vivo toward Th2 function could protect against Th1-type autoimmune disease. Interestingly, statins have been found to favor the polarization toward Th2. In experimental allergic encephalomyelitis (EAE), the animal model of multiple sclerosis (MS), statins induce the differentiation of neuroantigen-primed T cells from the Th1 to Th2 mode. While activated (tyrosine-phosphorylated) signal transducer and activator of transcription STAT4 has a key role in IL-12-dependent Th1 lineage commitment, activation of STAT6 is required for IL-4-dependent Th2 lineage commitment. Interestingly, atorvastatin treatment suppresses the formation of activated STAT4 but stimulates the activation of STAT6 in T cells from atorvastatin-treated or phosphate-buffered saline-treated mice.

**Destabilization of fibrillar amyloid-β peptides**

Fibrillar forms of amyloid-β (Aβ) peptide play an important role in the pathogenesis of Alzheimer's disease (AD). These are 39- to 43-residue peptides released due to proteolytic processing of the transmembrane precursor glycoprotein, amyloid precursor protein (APP). The amyloidogenic pathway requires that APP be
sequentially cleaved by β- and γ-secretases. β-Secretase cleaves APP close to the membrane to produce βAPPs (secreted), and a 12-kDa, C100 transmembrane stub, subsequently cleaved by γ-secretase to produce the Aβ peptide and a cytoplasmic fragment with a very short half life.\textsuperscript{54,55} On the other hand, α-secretase cleaves APP within the Aβ sequence thus preventing its formation. Statin treatment has recently been suggested to decrease amyloidogenic APP processing by reducing cellular cholesterol levels. Recent studies have suggested that treatment with statins or depletion of cholesterol appears to increase α-secretase cleavage of APP in cells, whereas β-secretase cleavage and secreted Aβ levels are decreased. In contrast, cholesterol enrichment leads to elevated amyloidogenic processing of APP. In agreement with this, Sidera et al. have demonstrated that high cellular cholesterol levels decrease the glycosylation of mature oligosaccharides in β-secretase leading to its inhibition. On the other hand, in the presence of lovastatin, the glycosylation process is stimulated, thereby attenuating the function of β-secretase. However, lovastatin does not inhibit β-secretase in vitro.

1.1.3.2.2 Therapeutic efficacy of statins

The current state of knowledge indicates that statins are not only lipid-lowering drugs. Due to multiple functions, these wonder drugs have emerged as possible medicines for many other chronic disorders including neurodegeneration, inflammation, demyelination, cancer, and diabetes. Below, I have tried to analyze a large body of information regarding possible treatment of several human disorders by statins.

Coronary artery disease

Data from several epidemiological studies have established statins as the most potent class of medicines for cardiovascular diseases. Being a cholesterol-lowering drug, statins are expected to ameliorate cardiovascular problems. However, in addition to lowering cholesterol, statins seem to ameliorate multiple problems in patients with atherosclerosis. For example, statins lower the levels of acute-phase proteins independent of their effects on cholesterol and thereby retard the deleterious effects of advanced atherosclerotic disease.\textsuperscript{70} There is increasing evidence that inflammation and the underlying cellular and molecular mechanisms contribute to the progression of atherosclerosis.\textsuperscript{71} The vascular inflammatory process seems to promote plaque rupture and atherothrombosis, resulting in clinical complications of atherosclerosis. Schillinger et al.\textsuperscript{72} have shown that the association between statin use and survival is markedly influenced by the inflammatory status of the patient, suggesting that a reduction of vascular inflammation or attenuation of the effects of inflammatory activity may be an important mechanism by which statins exhibit improved event-free survival. However, in addition to cholesterol-lowering and anti-inflammatory activities, improved endothelial function and plaque stabilization by statins in patients with atherosclerosis may also involve their anti-thrombotic, anti-proliferative, and anti-oxidative effects.

Cancer

The interest in studying the effects of statins on various forms of cancer stems from the facts that Ras is involved in at least 30% of all forms of cancer and that statins are capable of inhibiting the activation of Ras in various cell types.\textsuperscript{73} Statins also inhibit the growth of various cell lines either by induction of cell cycle arrest or apoptosis. In addition, lovastatin has been reported to reduce invasiveness of lymphoma cells, human glioma cells, melanoma cells, and NIH-3T3 cells in matrigel. Consistently, statins exhibit anti-tumor effects against melanoma, mammary carcinoma, pancreatic adenocarcinoma, fibrosarcoma, glioma, neuroblastoma, and lymphoma in various animal models, leading to either suppression of tumor progression, and/or inhibition
of the metastatic process. Consistently, in an epidemiological analysis, fewer cases of melanoma are observed in the lovastatin-treated group compared with the control group. In pre-clinical studies, statins also potentiate the anti-tumor effects of some cytokines and chemotherapeutics. However, clinical trial results do not display particularly encouraging prospect for statin therapy in cancer. In a phase II study by Kim et al., lovastatin (35 mg/kg body weight) was administered to patients with advanced gastric adenocarcinoma. Although this drug regimen leads to transient side effects, such as myalgia and elevated serum creatine phosphokinase, the anti-tumor effect was not very obvious. In another phase I-II trial of lovastatin by Larner et al. in patients with anaplastic astrocytoma and glioblastoma multiforme, high doses of lovastatin were well-tolerated with little anti-tumor activity. In the PROSPER trial, increased incidences for breast and colon cancer were also observed in the pravastatin-treated group. However, before writing off statins from cancer trials, it should be remembered that statins specifically target Ras and, therefore, these drugs may have a better success rate against Ras-dependent cancers.

**Diabetes**

Patients with type 2 diabetes have an atherogenic lipid profile, which greatly increases their risk of coronary heart disease (CHD) compared with people without diabetes. An estimated 92% of individuals with type 2 diabetes, without CHD, have a dyslipidemic profile. Consistently, the Heart Protection Study demonstrated an approximately 25% relative risk reduction of a first coronary event in patients with type 2 diabetes. In the Lescol Intervention Prevention Study (LIPS), routine use of fluvastatin in patients with type 2 diabetes led to a 47% reduction in the relative risk of cardiac death. An increased oxidative stress has been suggested to contribute to the accelerated atherosclerosis and other problems in diabetic patients. Accordingly, exposure of cultured aortic endothelial cells and SMCs to a high glucose level significantly increased the oxidative stress compared with a normal glucose level. This increase was completely blocked by treatment with pitavastatin. Subsequently, administration of pitavastatin in streptozotocin-induced diabetic rats attenuated the increased oxidative stress in diabetic rats to control levels. In addition to CHD, peripheral neuropathy is a frequent and major complication of diabetes. Interestingly, rosuvastatin restores nerve vascularity, including vessel size, in type II diabetic mice to the levels of nondiabetic mice by stimulating the expression of neuronal nitric oxide synthase (nNOS) in sciatic nerves. Although the mechanisms are poorly understood, these drugs also reduce the risk of leg ulcers and kidney disease that are common in diabetic patients.

**Osteoporosis**

Osteoporosis is the most common form of bone-degenerating malady in humans. Statins are also emerging as wonder drugs for bone disorders, such as osteoporosis. Bone morphogenetic proteins (BMPs) are cytokines that promote differentiation of mesenchymal stem cells into differentiated osteoblasts, and bone formation. Interestingly, statins have been found to stimulate the expression of BMP-2 and this phenomenon might be linked directly to the anabolic effect of statins on bone. In addition, IL-6 plays an important role in the pathogenesis of osteoporosis. Because isoprenoid-mediated activation of Ras is involved in the induction of IL-6, statins block IL-6 induction in various cell types by depleting isoprenoids. The role of statins in bone formation was shown in 1999 and, after that, observations of large groups of patients have pointed to a reduction in the risk of osteoporotic fractures with the use of statins compared to those using other lipid-lowering drugs or to the control group. Epidemiological analyses also indicate a reduction in the risk of
osteoporotic fractures with the use of statins, but whether using these drugs may have a beneficial effect on bone turnover is not yet known. We must therefore wait for larger prospective randomized clinical trials before prescribing these drugs in osteoporotic patients.

**Alzheimer's disease**

AD is a neurodegenerative disorder resulting in progressive neuronal death and memory loss. Neuropathologically, the disease is characterized by neurofibrillary tangles and neuritic plaques composed of aggregates of β-amyloid (Aβ) protein, a 40- to 43-amino acid proteolytic fragment derived from the amyloid precursor protein. In the early 1990s, the first hint about possible involvement of cholesterol in AD came from observations of enhanced prevalence of Aβ-containing senile plaques among subjects without dementia with coronary artery disease compared with individuals without dementia and heart disease. Although the underlying mechanism has not been identified, elevated levels of circulating cholesterol have been proposed to increase the risk of AD several fold. Subsequently, the cholesterol-AD nexus comes to the forefront with the direct evidence of increased levels of Aβ in cholesterol-fed New Zealand White rabbits, the small-animal model of human coronary artery disease. Interestingly, removing cholesterol from the diet of animals previously fed a cholesterol-enriched diet leads to significant reduction in brain Aβ levels, attesting an important role for cholesterol in stimulating the production of Aβ in vivo in the brain. Epidemiological studies also suggest that prior statin use in treating risk of coronary artery disease may reduce the risk of AD later in life. Recently, in a double-blind randomized trial with a 1-year exposure to atorvastatin (80 mg/day), Sparks et al. found that atorvastatin reduces circulating cholesterol levels and produces a positive signal on each of the clinical outcome measures (such as the Geriatric Depression Scale, the Alzheimer's Disease Assessment Scale, Clinical Global Impression of Change Scale, and Neuropsychiatric Inventory Scale) compared with placebo. However, results should be substantiated by a large multi-center clinical trial in order to establish statin therapy in AD.

**Multiple sclerosis**

MS is the most common human demyelinating disease of the central nervous system (CNS) of unknown etiology. A broad-spectrum inflammatory process in the CNS is believed to play an important role in the loss of myelin and myelin-producing cells. Evidence has emerged that statins have immunomodulatory effects in MS. Recent reports showed that statins prevent and reverse chronic and relapsing EAE, an animal model of MS. Several immunomodulatory properties of statins may account for their beneficial clinical effect. Statins decrease the migration of leukocytes into the CNS, inhibit MHC class II and co-stimulatory signals required for activation of proinflammatory T cells, induce a Th2 phenotype in T cells, and decrease the expression of inflammatory mediators in the CNS, including NO and TNF-α. Greenwood et al. have demonstrated that treatment of brain endothelial cells in vitro with lovastatin inhibits Rho-mediated transendothelial T cell migration. Consistently, they and others also demonstrate that in acute and relapsing-remitting mouse models of MS, lovastatin treatment inhibits leukocyte migration into the CNS and attenuates the development of both acute and relapsing clinical disease. Furthermore, in vitro experiments with human immune cells have shown an immunomodulatory profile of statins comparable to that of IFN-β. Consistent with this, an open-label clinical trial of simvastatin for MS reveals a significant decrease in the number and volume of new magnetic resonance imaging (MRI) lesions and a
favorable safety profile. As the evidence of the benefit of statins in MS is currently insufficient, large controlled clinical trials are needed.

Because statin treatment is being considered as a possible therapy for MS patients, it is worth mentioning that the rationale for statin treatment is MS patients should be justified. First, MS is a disease of the younger generation and, therefore, many MS patients do not experience any cholesterol-related problems before, during or after the time of MS attack. Second, the serum concentration of 24S-hydroxycholesterol reflecting brain cholesterol turnover may be a possible marker for neurodegeneration and demyelination in MS. Consistently, Teunissen et al. have demonstrated serum levels of 24S-hydroxycholesterol and lathosterol are lower in patients with primary progressive and in older relapsing remitting MS. Therefore, long-term use of statins in MS patients may eventually prove to be fatal.

Depression
A couple of studies demonstrate that long-term use of statin leads to reduced risk of depression in patients with coronary artery disease. They have demonstrated that risk of depression was 60% less in individuals using statins than in hyperlipidemic individuals not using lipid-lowering drugs. Interestingly, the use of non-statin lipid-lowering drugs yields a similar, but weaker effect. Although statins attenuate depression in susceptible patients, the molecular mechanisms associated with this beneficial effect of statin are not known. One could be the up-regulation of constitutive NOS (cNOS)-mediated NO production in brain cells by statins. As NO possesses the anti-depressant activity, statins may therefore suppress depression. Alternatively, another possible explanation could be the ‘feel-good’ effect of statins through improved quality of life due to decreased incidence of cardiovascular events.

1.1.3.3 FIBRATES
In contrast to statins, this group of drugs does not inhibit cholesterol biosynthesis. However, these drugs stimulate β-oxidation of fatty acids mainly in peroxisomes and partly in mitochondria. Therefore, this group of drugs is known to lower plasma levels of fatty acid and triacylglycerol. Clofibrate was the first such drug, developed in Japan in the 1960s. Eventually, the discovery of several other fibrate drugs such as ciprofibrate, bezafibrate, fenofibrate, and gemfibrozil has revolutionized lipid-lowering research. However, the enthusiasm has been short-lived, because prolonged use of some of these drugs like clofibrate and ciprofibrate causes peroxisome proliferation leading to hepatomegaly and tumor formation in the liver of rodents. Therefore, there are concerns about widespread use of these drugs in humans. Only gemfibrozil and fenofibrate, due to their milder effect on peroxisome proliferation, are being used as lipid-lowering drugs in humans.

1.1.3.3.1 Mode of action of fibrates
Activation of nuclear hormone receptors
One of the hallmarks of functions of fibrate drugs is the activation of peroxisome proliferator-activated receptor (PPAR). PPARs are a group of three nuclear hormone receptor isoforms, PPAR-γ, PPAR-α, and PPAR-δ, encoded by different genes. However, fibrate drugs like clofibrate and fenofibrate have been shown to activate PPAR-α with tenfold selectivity over PPAR-γ. Bezafibrate acts as a pan-agonist that shows similar potency on all three PPAR isoforms. WY-14643, the 2-aryl-thioacetic acid analogue of clofibrate, is a potent murine PPAR-α agonist as well as a weak PPAR-γ agonist. Although these drugs activate PPARs, direct binding of these drugs with PPARs has not been demonstrated. However, in response to fibrate drugs,
PPAR-α heterodimerizes with retinoid X receptor-α (RXR-α), and the resulting heterodimer modulates the transcription of genes containing peroxisome proliferator-responsive elements (PPREs) in their promoter sequence. In addition to fibrates, a number of natural ligands, such as polyunsaturated fatty acids (PUFAs), leukotriene B4 (LTB4), 8-S-hydroxy eicosatetraenoic acid (8-S-HETE), and prostaglandin J2 (PGJ2), are also known to activate PPARs. In the absence of ligands, all three isoforms of PPAR bind to various transcription co-repressors, such as nuclear receptor co-repressor (NCoR) and silencing mediator for retinoid and thyroid hormone receptor (SMRT), and histone deacetylases (HDACs) in a DNA-independent manner. On the other hand, ligand-mediated activation of PPARs leads to dissociation of co-repressors and concomitant association with various co-activators, such as steroid receptor co-activator 1 (SRC1) and histone acetylases (CBP/p300). Recent studies have also identified a PPAR-α-interacting cofactor (PRIC) complex containing many co-activators, such as PPAR-binding protein (PBP), PPAR-interacting protein (PRIP), PRIP-interacting protein with methyltransferase domain (PIMT), and others.

Figure 1.2: Lipid-lowering and anti-inflammatory functions of fibrate drugs

Stimulation of fatty acid oxidation
Fatty acids are β-oxidized mainly in mitochondria. Only very long chain and long-chain fatty acids are β-oxidized in peroxisomes. After chain shortening in peroxisomes, fatty acids are believed to be transported into mitochondria for complete β-oxidation. However, fibrate drugs are known to stimulate mainly peroxisomal β-oxidation. Accordingly, after clofibrate treatment, peroxisomal fatty acid β-oxidation increases up to 20-fold in the liver of rodents. Hepatocytes isolated from clofibrate-fed rats also oxidize more and esterify less of incoming fatty acids than do normal hepatocytes. This increase in fatty acid oxidation is particularly
striking for very long chain fatty acids (>C22:0), as these are particularly β-oxidized in peroxisomes. This stimulatory effect is mediated by PPAR-α, and a PPRE, consisting of an almost perfect direct repeat of the sequence TGACCT spaced by a single base pair, has also been identified in the upstream regulatory sequences of each of the genes involved in peroxisomal β-oxidation. In addition to stimulating β-oxidation, fibrate drugs are also known to stimulate fatty acid ω-oxidation in the liver, and they prevent or reduce the effects of some inhibitors of fatty acid oxidation, such as 4-pentenoyl-carnitine. Fibrates also increase the activity of acyl-CoA synthetase and the CoA content of liver while the level of malonyl-CoA, the precursor of de novo fatty acid synthesis, goes down. Apart from stimulating fatty acid oxidation-associated molecules, fibrates also increase lipolysis via PPAR-α-dependent up-regulation of lipoprotein lipase.64

Peroxisome proliferation and hepatocarcinogenesis
Fibrates are also termed peroxisome proliferators, because prolonged administration of fibrates to rodents typically leads to proliferation of peroxisomes and hepatomegaly. Continuous administration of fibrate drugs to rodents for 40–50 weeks also leads to the formation of hepatic tumor.42,63 However, the mode of action underlying fibrate-induced hepatocarcinogenesis has not yet been fully delineated. In response to fibrate drugs, PPAR-α is believed to mediate alterations in gene expression that eventually lead to increased cell proliferation, decreased apoptosis and increased signaling for replicative DNA synthesis in the liver. These alterations ultimately enable mutant cell populations to proliferate and become neoplastic. It is also known that a number of proteins required for transition into the S phase of the cell cycle are increased by fibrates, probably via the involvement of PPAR-α. However, functional PPREs have not been characterized in gene promoters of these regulatory molecules. Fibrate drugs have been suggested to induce oxidative stress, which ultimately contributes to an increase in hepatocyte proliferation and oxidative DNA damage. This hypothesis gains momentum as fibrates induce marked up-regulation of peroxisomal acyl-CoA oxidase, the fatty acid β-oxidizing enzyme that produces H2O2, without concomitant increase in the peroxisomal marker catalase, the H2O2-degrading enzyme.

Suppression of proinflammatory molecules
Similar to statins, fibrate drugs also inhibit the production of different proinflammatory molecules. Fibrates repress cytokine-induced IL-6 production in SMCs, iNOS activity in murine macrophages, and VCAM-1 expression in endothelial cells. The physiological relevance of these observations is further corroborated by the demonstration that fibrates lower plasma levels of inflammatory cytokines such as IL-6, TNF-α, and IFN-γ in patients with atherosclerosis. Interestingly, not only fibrate, but also PPAR-γ ligands65 have been reported to inhibit production of inflammatory cytokines by monocytes/macrophages in vitro.

Fibrate drugs also exhibit an anti-inflammatory effect in brain cells. For example, according to Xu et al.,66 all the fibrate drugs tested (ciprofibrate, fenofibrate, gemfibrozil, and WY-14643) inhibit cytokine-induced production of NO in microglia in a dose-dependent manner. Xu et al. also demonstrated that fibrates inhibit the secretion of the proinflammatory cytokines IL-1β, TNF-α, IL-6, and IL-12 p40 and the chemokine MCP-1 by LPS-stimulated microglia. Although mechanisms behind the anti-inflammatory effect of fibrates are currently unknown, these drugs may limit inflammation in part by inducing the expression of IkBα, which blocks the activation of NF-κB, a transcription factor critical in the activation of a variety of proinflammatory molecules.67
We have also demonstrated that gemfibrozil and clofibrate inhibit the expression of iNOS and the production of NO in human astrocytes. Although gemfibrozil induces PPRE-dependent reporter activity in human astrocytes, this drug inhibits the expression of iNOS independent of PPAR-α. Gemfibrozil has been found to markedly inhibit the activation of different proinflammatory transcription factors, such as NF-κB, AP-1, and C/EBPβ, which are required for the transactivation of the human iNOS promoter.

Switching of T helper cells

Being important immunomodulators, fibrates also modify functions of T cells. Fibrates are ligands of PPAR-α and resting T cells express PPAR-α. Marx et al.68 have demonstrated that fibrates alone are sufficient to inhibit IL-2, TNF-α, and IFN-γ production by activated CD4+ T cells. Fibrates also induce splenocyte production of IL-4, a cytokine important in the differentiation of Th2 cells that are generally believed to protect against the development of EAE. In addition, WY-14643, the synthetic agonist of PPAR-α, has been shown to induce apoptosis of lymphocytes, which may protect against autoimmune diseases by ablating autoreactive lymphocytes. Lovett-Racke et al. have demonstrated that fibrates suppress the differentiation of Th1 cells while promoting the differentiation of neuroantigen-primed T cells toward the Th2 mode. Although underlying mechanisms are poorly understood, a recent study suggests that PPAR-α also plays a physiologic role in regulating T-bet, an inducible transcription factor important in the initiation of cytokine gene transcription, particularly Th1 cytokines. This study demonstrates that PPAR-α present in the cytoplasm of T cells is able to negatively regulate the transcription of T-bet that favors the production of IFN-γ by T cells. This regulation occurred independently of DNA binding, suggesting that there may be several mechanisms by which PPAR-α can influence T cell activation and cytokine production.

1.1.3.3.2 Therapeutic efficacy of fibrates

Discovery of multiple functions of fibrates has allowed clinicians to consider fibrates as potential therapeutic agents for various pathological states including atherosclerosis, obesity, diabetes, inflammation, and demyelination. Here, I present the current state of knowledge regarding the treatment of several chronic diseases by fibrates.

Coronary heart disease

Fibrates were introduced for treatment of hyperlipidemia. Trials with fibrates have shown a reduction in CHD risk through modification of atherogenic dyslipidemia. The benefit is believed to be due to an increased clearance of very low density lipoprotein-cholesterol, a decrease in triglycerides, an increase in plasma high-density lipoprotein (HDL)-cholesterol via decreased exchange of triglyceride and HDL-cholesterol by the cholesterol ester transfer protein (CETP), and a reduction of hepatic cholesterol biosynthesis. Consistently, in several clinical trials, fibrate drugs alone have been found to cause a significant decrease in triglycerides (20–50%) and an increase in plasma HDL-cholesterol (14–20%). Although the reduction in low-density lipoprotein (LDL)-cholesterol by fibrates always remains marginal (5–15%),80-82 in a study by Winkler et al.83 fenofibrate lowers atherogenic small dense LDL more effectively than atorvastatin. However, in general, fibrates seem to be particularly effective in patients for whom a disturbance of the triglyceride-HDL axis is the primary lipid disorder.

In addition to lipid-lowering activity, fibrates are also anti-inflammatory. IL-6 has been shown to play an important role in the pathogenesis of atherosclerosis. Biswaset
al. reported that IL-6 induces monocyte chemotactic protein-1 expression in peripheral blood mononuclear cells and U937 macrophages. Thus, suppressing the secretion of IL-6, fibrates may indirectly inhibit the production of potent chemokines involved in monocyte recruitment into the subendothelial space, resulting in less foam cell formation.

In some instances, for better overall outcome, fibrates are also administered in combination with statin. According to Chapman, a large percentage of CHD patients on statins alone still succumb to the disease. In a randomized, double-blind, placebo-controlled crossover trial with atorvastatin and fenofibrate in patients with combined hyperlipidemia, the combination therapy was found to be safe and had beneficial additive effects on endothelial function. However, combination therapy may sometimes lead to an impairment in drug clearance, as the clearance of statin drugs from the body requires cytochrome P450-mediated chemical modification. In addition, gemfibrozil is known to inhibit cytochrome p450 and thereby may cause faulty clearance of statins. Therefore, caution must be exercised when prescribing combination therapy for CHD patients.

**Obesity**

Obesity itself is a disease and is a serious risk factor for many other chronic complications, such as diabetes, hypertension, dyslipidemia, and cardiovascular diseases. People become obese when the body takes in more calories than it burns off and those extra calories are stored as fat. Due to its direct stimulatory effect on the catabolism of fat, fibrates have been used as primary or adjunct therapy for several years to control obesity. In obese prone (OP) rats, fenofibrate treatment significantly (p < 0.05) reduces food intake, weight gain, feed efficiency, and adiposity to the levels seen in control obesity-resistant rats. Fenofibrate treatment also increases whole-body fatty acid oxidation, and stimulates the expression of carnitine palmitoyl transferase I, the enzyme involved in the entry of fatty acyl-CoA into mitochondria, in the liver of OP rats.

Obesity is often associated with leptin resistance, as evidenced by hyperleptinemia. Leptin is a 16-kDa protein secreted by fat cells that regulates feeding and energy expenditures by acting at sites primarily within the CNS. Obesity in humans and rodents is almost always associated with a resistance to, rather than a deficiency of, leptin. In fact, leptin itself is elevated in obesity. Leptin resistance arises from impaired leptin transport across the blood-brain barrier (BBB) and defects in leptin receptor signaling. Interestingly, gemfibrozil restores leptin transport across the BBB and in diet-induced obese rats, gemfibrozil significantly reduces the leptin level.

**Diabetes**

As mentioned above, patients with type 2 diabetes are at particularly high risk of atherosclerotic events. The Diabetes Atherosclerosis Intervention Study and the St. Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention study clearly show that fibrates improve cardiovascular outcomes in patients with type 2 diabetes. In addition to lowering cardiovascular risk, fibrates may also improve insulin sensitivity in diabetic patients. Fat metabolism and sugar homeostasis are inherently related. Insulin is recognized for its role in promoting glucose uptake. However, insulin is also capable of regulating the catabolism of triglycerides through its inhibition of hormone-sensitive lipase. On the other hand, lipid abnormalities also have profound effects on glucose homeostasis. For example, according to Schulman, abnormal accumulation of triglycerides and fatty acyl-CoA in muscle and liver may result in insulin resistance. In a number of animal models, fibrates have been shown to lower plasma triglycerides, reduce adiposity and improve hepatic and muscle
steatosis, thereby improving insulin sensitivity. Although fibrate drugs are widely used to treat hypertriglyceridemia in patients, surprisingly, their effects on insulin sensitivity in humans have not been thoroughly examined. Another putative beneficial effect of fibrates in diabetes that has not been much appreciated is reduction in inflammation. Subclinical inflammation always plays an important part in the pathogenesis of type 2 diabetes, primarily as a mediator of obesity-induced insulin resistance. In this connection it is worth mentioning that fibrates are also capable of reducing inflammation.

Multiple sclerosis
A recent study also suggests that fibrate drugs, such as gemfibrozil and fenofibrate, may be considered as possible therapeutics for MS. The EAE animal model is particularly useful in testing new therapeutic intervention in MS. Lovett-Racke and colleagues have demonstrated that these drugs are able to prevent and treat the disease process of EAE in mice. Although underlying mechanisms are poorly understood, anti-inflammatory property, suppression of Th1 activity, and promotion of the Th2 response might be involved in fibrate-mediated attenuation of the EAE disease process.

Fibrate drugs like ciprofibrate, clofibrate, fenofibrate, and gemfibrozil induce the proliferation of peroxisomes in rats and mice. Continuous administration of these drugs to the rodents for 40–50 weeks also leads to the formation of hepatic tumor. However, induction of hepatic tumor promotion by fibrate drugs has not been demonstrated in humans, other primates or guinea pig, species which have lost their ability to synthesize ascorbate due to inherent loss of the gulonolactone oxidase gene. Braun et al. have reported that the evolutionary loss of the gulonolactone oxidase gene may contribute to the missing carcinogenic effect of peroxisome proliferators in humans since ascorbate synthesis is accompanied by H2O2 production, and consequently its induction can be potentially harmful. Furthermore, recent studies have also revealed that humans have considerably lower levels of PPAR-α in liver than rodents, and this difference may, in part, explain the species differences in the carcinogenic response to peroxisome proliferators. Therefore, hepatic tumor formation may not be a concern in humans. However, combination therapy of cerivastatin and gemfibrozil may cause myopathy and rhabdomyolysis, suggesting that such a combination therapy should be prescribed cautiously.

1.1.4 SPHERICAL CRYSTALLIZATION
In the past, the pharmaceutical industry did not feel that they need to improve the manufacturing efficiency. The elementary reason was that the most important mission for the pharmaceutical industry had been to rapidly bring new products to the market. Nowadays, rising of the energy prices and the inefficient manufacturing have made pharmaceutical companies face cost pressures. Therefore, the pharmaceutical industry needs to improve the performance of their manufacturing operations. Particle design for solid pharmaceutical dosage forms involves improving the efficiency of the manufacturing processes and giving a high degree of functionality to the drug or excipient particles. Tablet is very specific dosage form, accounting for 50 % of all oral drug delivery system and 70 % of all pharmaceutical preparations produced. Direct tabletting of pharmaceutical materials is a modern method in tablet manufacturing. Such manufacturing of tablets involve simple mixing and compression of powders, which results in a number of overall benefits including time, cost and energy savings. Direct tabletting as a technique has been successfully applied to numerous drugs on the industrial scale. The success of any procedure resulting in
mechanical properties for tabletting is strongly affected by the micromeritic properties of the crystals used. Compressing a drug directly requires good micromeritic properties, such as flowability, and a good reproducible compressibility. Especially, the flowability of needle-shaped or plated-shaped crystals is very poor and these crystals are difficult to handle. Kawashima suggested obtaining the size enlargement of particles during the crystallization step by controlling crystal agglomeration with controlled properties. He introduced this technique into pharmaceutical manufacturing and showed that spherically dense agglomerates could be produced and were suitable for direct tabletting and defined it as spherical crystallization. The traditional drug manufacturing procedures (granulation) involves following steps: crystallization → filtration → drying → formulated powders blending → granulation → drying → tabletting. This is a slow and time consuming process, where as in spherical crystallization the process could be reduced to: crystallization → filtration → drying → dry blending → tabletting. It means less equipment and space, lower labor costs, less processing time, and lower energy consumption in the direct tabletting process. This technique is also reputed to improve the detectability, bioavailability, and dissolution rate of some poorly soluble drugs like celecoxib and fenbufen. So spherical crystallization can be defined as “a novel particle engineering technique by which crystallization and agglomeration can be carried out simultaneously in one step to transform crystals directly into compacted spherical form.” Besides being producing spherical crystals it also enables co-precipitation of drugs and encapsulating polymers in the form of spherical particles. The spherical crystallization technique also involves the use of a bridging liquid that imparts compressibility by acting as granulating fluid. Thus spherical crystallization is a method that helps to achieve good flowability and compressibility. Some drugs have also been recrystallized by the spherical agglomeration technique using polymeric materials to modify their release profile.

1.1.4.1 Benefits of the spherical crystallization process
- A spherical shape of the final product formed drastically improves the micromeritic property of the drug crystals.
- Improvement in wettability and dissolution rate of some drugs were found by utilization of this process.
- This technique could enable subsequent process such as separation, filtration, drying etc to be carried out more efficiently.
- Furthermore the resultant agglomerated crystals could be easily compounded with other pharmaceutical powders due to spherical shapes.

1.1.4.2 Techniques of spherical crystallization
Spherical crystals can be obtained by two different techniques, either by typical spherical crystallization technique or non typical spherical crystallization technique. Non typical spherical crystallization technique can also be considered as the traditional crystallization process (salting-out, cooling, precipitation, etc.). This process is carried out by controlling the physical and chemical factors. Typical spherical crystallization employs three solvents: one is the drug dissolution medium i.e. good solvent; another is a medium which partially dissolves the drug and have wetting property i.e. bridging liquid; and the last one is immiscible with the drug substance i.e. bad solvent. The spherical crystallization has been applied to several drugs, and it has been found that the product properties are quite sensitive to the amount of the bridging liquid. With decreasing amount of bridging liquid in the three-solvent system, the median diameter of agglomerated crystals increased, having a wider size distribution. Less than the optimum amount of bridging liquid
produces plenty of fines and more than optimum produces very coarse particles. So the amount of bridging is the critical process parameters in crystallization process.\textsuperscript{108} The median diameter of agglomerates decreased with increasing content of good solvent.\textsuperscript{107} Also the choice of bridging liquid, the stirring speed and the concentration of solids (or of the solute) are of importance. So various parameters optimized for this are type, amount and mode of addition of bridging liquid, temperature, and agitation speed to get maximum amount of spherical crystals. The two most commonly used techniques of spherical crystallization are wet spherical agglomeration method (WSA), quasi-emulsion solvent diffusion method (QESD, Transient emulsion). But there are two extensions of these techniques, ammonia diffusion system (ADS) and crystal-co-agglomeration technique (CCA). Another technique of this process is Neutralization, where first fine crystals form by neutralization then it will agglomerate by the help of a bridging liquid.\textsuperscript{109}

1.1.4.2.1 Wet spherical agglomeration method (WSA)

Here the good and the poor solvents are freely miscible and interaction (binding force) between the solvents is stronger than drug interaction with the good solvent, which leads to precipitation of crystals immediately. Bridging liquid collects the crystals suspended in the system by forming liquid bridges between the crystals due to capillary negative pressure and interfacial tension between the interface of solid and liquid. WSA method proceeds in three steps as shown in Figure 1.3.\textsuperscript{103} The first one is the selection of the crystallization method to precipitate crystals from solution, i.e., thermal method (temperature decrease or evaporation), physicochemical methods (addition of another solvent, salting out) and chemical reaction. The second step is the choice of the wetting agent that will be immiscible with the solvent of crystallization. Finally, the third step is the hardening of the agglomerates.

**Figure 1.3**: Steps involved in Spherical agglomeration (SA).

Chow \textit{et al} postulated some general guide lines for the spherical agglomeration of drugs.\textsuperscript{110}

- For compounds that are water soluble, a water-immiscible organic solvent is used as the external medium and salt solutions of high concentration without common ions can be used as the bridging liquid.
- For compounds that are soluble in one or more organic solvents water is employed as the external phase and a water-immiscible organic solvent as the bridging liquid.
- For compounds that are only soluble in water-miscible organic solvents a saturated aqueous solution of the compound can serve as the external phase and an organic solvent mixture as the bridging solvent.
For compounds that are insoluble in water or any organic solvents a water-immiscible organic solvent can act as the external phase and a 20% calcium chloride solution as the bridging liquid. In addition, a binding agent such as PVP or PEG is required for agglomeration since the powders are not sufficiently soluble in the bridging liquids to allow binding through recrystallization and fusion.

1.1.4.2.2 Quasi-emulsion solvent diffusion method (QESD)
This technique is usually applied for the preparation of microspheres. Here interaction between the drug and the good solvent is stronger than that of the good and poor solvents; hence the good solvent drug solution is dispersed in the poor solvent, producing quasi emulsion droplets, even if the solvents are normally miscible. This is because of an increase in the interfacial tension between good and poor solvent. Then good solvent diffuses gradually out of the emulsion droplet into the outer poor solvent phase. The counter diffusion of the poor solvent into the droplet induces the crystallization of the drug within the droplet due to the decreasing solubility of the drug in the droplet containing the poor solvent. The steps involved in QESD are shown in Figure 1.4.

Figure 1.4: Steps involved in Quasi emulsion Solvent Diffusion (QESD)

1.1.4.2.3 Crystal-co-agglomeration technique (CCA)
Applications of spherical crystallization to obtain directly compressible agglomerates without diluents are restricted to water insoluble large-dose drugs only. Most of the excipients, such as diluents and disintegrating agents, are hydrophilic in nature; hence, incorporation of these excipients in the agglomerates formed using organic bridging liquid is difficult. Because of this limitation, spherical crystallization could not be applied to obtain agglomerates of low-dose or poorly compressible materials. To overcome these limitations of spherical crystallization Kadam et al. developed the crystallo-co-agglomeration (CCA) technique. It is a modification of the spherical crystallization technique in which a drug is crystallized and agglomerated with an excipient or with another drug, which may or may not be crystallized in the system. The agglomeration is performed using bridging liquid. The process enables design of agglomerates containing two drugs or a low-dose or poorly compressible drug in combination with diluents. The difference in the physicochemical properties of the drug molecules and the excipient becomes the major challenge in the selection of a solvent system for the crystallo-co-agglomeration.

1.1.4.2.4 Ammonia diffusion system (ADS)
In this technique ammonia-water system is used as the good solvent and bad solvent is selected depending upon the drugs solubility in that solvent. The ammonia-water also acts as a bridging liquid. This technique usually meant for amphoteric drugs which cannot be agglomerated by conventional procedures. The whole process is
completed in three stages. First, the drug dissolved in ammonia water is precipitated while the droplets collect the crystals (Figure 1.5 I). Simultaneously, ammonia in the agglomerate diffuses to the outer organic solvent (Figure 1.5 II). Its ability to act as a bridging liquid weakens and subsequently spherical agglomerates are formed (Figure 1.5 III).

Figure 1.5: Steps involved in Ammonia Diffusion System (ADS).

1.1.4.2.5 Neutralization technique (NT)
This technique involves the formation of fine crystals by neutralization and consequently their agglomeration by a bridging liquid. Spherical crystallization of tolbutamide and phenytoin were reported by this technique. The drug was dissolved in alkaline solution and then poured into an acidic solution containing polymers and bridging liquid under constant agitation. The drug crystals are precipitated out by neutralization of the base with acid. Then the precipitated crystals were simultaneously agglomerated with the incorporated polymer through the wetting action of the bridging liquid.

1.1.4.3 The principle steps in the process of spherical crystallization
1.1.4.3.1 Flocculation Zone
In this zone, the bridging liquid displaces the liquid from the surface of the crystals and these crystals are brought in close proximity by agitation; the adsorbed bridging liquid links the particles by forming a lens bridge between them. In these zone, loose open flocs of particles are formed by pendular bridges and this stage of agglomeration process where the ratio of liquid to the void volume is low and air is the continuous phase, is known as the pendular state. Mutual attraction of particles is brought about by surface tension of the liquid and the liquid bridges. The capillary stage is reached when all the void space within the agglomerate is completely filled with the liquid. An intermediate state known as the funicular state exists between the pendular and capillary stage. The cohesive strength of agglomerate is attributed to the bonding forces exerted by the pendular bridges and capillary suction pressure.

1.1.4.3.2 Zero Growth Zone
Loose floccules get transferred into tightly packed pellets, during which the entrapped fluid is squeezed out followed by squeezing of the bridging liquid onto the surface of small flocs causing poor space in the pellet of completely filled with the bridging liquid. The driving force for the transformation is provided by the agitation of the slurry causing liquid turbulence, pellet-pellet and pellet-stirrer collision.

1.1.4.3.3 Fast Growth Zone
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The fast growth zone of the agglomerates takes place when sufficient bridging liquid has squeezed out of the surface on the small agglomerates. This formation of large particles following random collision of well-formed nucleus is known as coalescence. Successful collision occurs only if the nucleus has slight excess surface moisture. This imparts plasticity on the nucleus and enhances particle deformations and subsequent coalescence. Another reason for the growth of agglomerates size is attributed to growth mechanisms that describe the successive addition of material on already formed nuclei.

1.1.4.3.4 Constant Size Zone

In this zone agglomerates cease to grow or even show slight decrease in size. Here the frequency of coalescence is balanced by the breakage frequency of agglomeration. The size reduction may be due to attrition, breakage and shatter. The rate determining step in agglomeration growth occurs in zero growth zones when bridging liquid is squeezed out of the pores as the initial flocules are transformed into small agglomerates. The rate determining step is the collision of particle with the bridging liquid droplets prior to the formation of liquid bridges. The rate is governed by the rate of agitation. The strength of the agglomerates is determined by interfacial tension between the bridging liquid and the continuous liquid phase, contact angle and the ratio of the volumes of the bridging liquid and solid particles.

1.1.4.4 Need for spherical crystallization

Developing novel methods to increase the bioavailability of drugs that inherently have poor aqueous solubility is a great challenge to formulate solid dosage form. Mechanical micronization of crystalline drugs and incorporation of surfactants during the crystallization process are the techniques commonly used to improve the bioavailability of poorly soluble drugs. The micronization process alters the flow and compressibility of crystalline powders and cause formulation problems. Addition of surfactant generally led to less significant increase in aqueous solubility. To overcome this problem Kawashima developed a spherical crystallization technique that led to improving the flow and direct compressibility of number of microcrystalline drugs.

1.1.4.5 Advantages of spherical crystallization

- Spherical crystallization technique has been successfully utilized for improving of flowability and compressibility of drug powder.
- This technique could enable subsequent processes such as separation, filtration, drying etc to be carried out more efficiently.
- By using this technique, physicochemical properties of pharmaceutical crystal forms are dramatically improved for pharmaceutical process i.e. milling, mixing and tableting because of their excellent flowability and packability.
- This technique may enable crystalline forms of a drug to be converted into different polymorphic form having better bioavailability.
- For masking of the bitter taste of drug.
- Preparation of microsponge, microspheres and nanospheres, microbaloons, nanoparticles and micro pellets as novel particulate drug delivery system.
1.2 LITERATURE SURVEY

1.2.1 Maheshwari Manish et al studied development of melt sonocrystallization technique for ibuprofen agglomerates and characterization of their physicochemical, micromeritic and compressional properties. Melt sonocrystallization process was developed for ibuprofen in which ibuprofen melt was poured in deionized water maintained at 25°C and simultaneously subjected to ultrasonic energy. The agglomerates obtained were evaluated using Scanning electron microscopy (SEM), Differential scanning calorimetry (DSC), X ray powder diffractometry (XRPD), Fourier transformed infrared spectroscopy (FTIR), intrinsic dissolution rate, BET analysis, solubility, image analysis, Heckel plot analysis and friability studies. The irregular agglomerates with porous surface were obtained. The agglomerates comprised of crystals having different crystal habits such as needles, plates, and some hollow tubes. Solubility, specific surface area and intrinsic dissolution rate increased with the treatment of ultrasonic energy. SEM and XRPD confirmed crystal habit changes. Improvement in compressional properties and reduction in sticking was observed due to the change in crystal habit. Crystal habit changes and lattice defects during processing have caused favorable changes in the physicochemical and compressional properties of the drug.120

1.2.2 Martyn D. Ticehurst et al were observed micronised particles of revatropate hydrobromide agglomerate when stored in uncontrolled conditions. Dynamic vapour sorption (DVS), isothermal microcalorimetry, microscopy and particle size measurement by laser diffraction have been used to study micronised revatropate hydrobromide. The rate and extent of agglomeration were dependent on the energy of the micronisation process, the sampling point for bulk within the mill and the humidity during storage. The agglomeration was attributed to the recrystallisation of disordered regions on the particles of revatropate hydrobromide generated during micronisation. This recrystallisation was assessed qualitatively and quantitatively, compared against spray-dried amorphous material, using DVS and isothermal microcalorimetry, respectively. A correlation was established between the energy of micronisation and the level of disorder within the micronised powder. A comparison of the DVS profiles of freshly prepared and aged micronised revatropate hydrobromide suggests an increased physical stability for the aged material.121

1.2.3 Herbert Chiou et al were produced salbutamol sulfate (SS) as a model anti-asthmatic drug using high-gravity controlled precipitation (HGCP) through antisolvent crystallisation. An aqueous solution of SS was passed through a HGCP reactor with isopropanol as antisolvent to induce precipitation. Spray drying was employed to obtain dry powders. Scanning electron microscopy, X-ray powder diffraction (XRD), density measurement, thermal gravimetric analysis, and dynamic vapour sorption were carried out to characterise the powder physical properties. The aerosol performance of the powders was measured using an Aeroliser connected to a multiple stage liquid impinger operating at 60 L/min. The HGCP SS particles were elongated with 0.1μm in width but varying length of several μm, which formed spherical agglomerates when spray dried. The particles showed the same XRD pattern and true
density (1.3 g/cm$^3$) as the raw material, indicating that they belonged to the same crystalline form. However, the spray dried agglomerates had a much lower tapped density (0.1 g/cm$^3$) than the raw material (0.6 g/cm$^3$). Compared with the powder obtained by spray drying directly from an aqueous solution, the SS powders obtained from HGCP were much less hygroscopic (0.6% versus 10% water uptake at 90% RH). The in vitro aerosol performance showed a fine particle fraction FP_Loaded and FP_Emitted up to 54.5±4.9% and 71.3±10.0%, respectively. In conclusion, SS powder with suitable physical and aerosol properties can be obtained through antisolvent HGCP followed by spray drying.$^{122}$

1.2.4 Ravindra S. Dhumal et al were produced fine elongated crystals of salbutamol sulphate (SS) by sonocrystallization for pulmonary delivery and compare with micronized and spray dried SS (SDSS) for in-vitro aerosolization behavior. Application of ultrasound during anti-solvent crystallization resulted in fine elongated crystals (Sonocrystallized SS; SCSS) compared to aggregates of large irregular crystals obtained without sonication. Higher sonication amplitude, time, concentration and lower processing temperatures favored formation of smaller crystals with narrow particle size distribution (PSD). SCSS was separated from dispersion by spray drying in the form of loose aggregates (SDSCSS). The fine particle fraction (FPF) of formulations with coarse lactose carrier in cascade impactor increased from 16.66 % for micronized SS to 31.12 % for SDSS (obtained by spray drying aqueous SS solution) and 44.21 % for SD-SCSS, due to reduced cohesive/adhesive forces and aerodynamic size by virtue of elongated shape of crystals. SD-SCSS was stable without any change in crystallinity and aerodynamic behavior for 3 months at 40°C/75% RH, but amorphous SDSS showed recrystalization with poor aerosolization performance on storage. Sonocrystallization, a rapid and simple technique is reported for production of SS crystals suitable for inhalation delivery.$^{123}$

1.2.5 Helena Schiavone et al were studied performance of SCF-engineered budesonide and albuterol sulfate powder blends in passive dry powder inhalers (DPI) relative to micronized drug blends. A number of lactose grades for inhalation were screened and the appropriate carrier and drug-to-lactose blending ratio were selected based on drug content and emitted dose uniformity. Aerosol performance was characterized by Andersen cascade impaction. Blend formulations of SEDS (solution enhanced dispersion by supercritical fluids) budesonide and albuterol exhibited a significant drug content uniformity (7–9% RSD) improvement over micronized drug blends (16–20% RSD). Further, the SEDS formulations demonstrated higher emitted dose and reduced emitted dose variability (10–12% RSD) compared to micronized powders (21–25% RSD) in the Turbospin, albeit without significant enhancement of the fine particle fraction. In contrast, SEDS powders exhibited increased fine particle fractions over micronized blends in the Clickhaler; improvements were more pronounced with albuterol sulfate. The performance enhancements observed with the SEDS powders are attributed to their increased surface smoothness and reduced surface energy that are presumed to minimize irreversible drug–carrier particle interactions, thus resulting in more efficient drug detachment from the carrier particle.
surface during aerosolization. As demonstrated for budesonide and albuterol, SEDS may enhance performance of lactose blends and thus provided an attractive particle engineering option for the development of blend formulations for inhalation delivery.\textsuperscript{124}

1.2.6 Anant R. Paradkar et al were prepared amorphous discreet nanoparticles by sonoprecipitation method for enhancing oral bioavailability of cefuroxime axetil (CA), a poorly water-soluble drug. CA nanoparticles (SONO-CA) were prepared by sonoprecipitation and compared with particles obtained by precipitation without sonication (PPT-CA) and amorphous CA obtained by spray drying. Spray drying present broad particle size distribution (PSD) with mean particle size of 10 \textmu m and low percent yield, whereas, precipitation without sonication resulted in large amorphous aggregates with broad PSD. During sonoprecipitation, particle size and yield improve with an increase in the amplitude of sonication and lowering the operation temperature due to instantaneous supersaturation and nucleation. The overall symmetry and purity of CA molecule was maintained as confirmed by FTIR and HPLC, respectively. All the three methods resulted in the formation of amorphous CA with only sonoprecipitation resulting in uniform sized nanoparticles. Sonoprecipitated CA nanoparticles showed enhanced dissolution rate and oral bioavailability in Wistar rat due to an increased solubility attributed to combination of effects like amorphization and nanonization with increased surface area and reduced diffusion pathway.\textsuperscript{125}

1.2.7 Robert O. Williams et al were developed a novel cryogenic spray-freezing into liquid (SFL) process to produce microparticulate powders consisting of an active pharmaceutical ingredient (API) molecularly embedded within a pharmaceutical excipient matrix. In the SFL process, a feed solution containing the API was atomized beneath the surface of a cryogenic liquid such that the liquid-liquid impingement between the feed and cryogenic liquids resulted in intense atomization into microdroplets, which were frozen instantaneously into microparticles. The SFL micronized powder was obtained following lyophilization of the frozen microparticles. The objective of this study was to develop a particle engineering technology to produce micronized powders of the hydrophobic drug, danazol, complexed with hydroxypropyl-b-cyclodextrin (HPbCD) and to compare these SFL micronized powders to inclusion complex powders produced from other techniques, such as co-grinding of dry powder mixtures and lyophilization of bulk solutions. Danazol and HPbCD were dissolved in a water/tetrahydrofuran cosolvent mixture prior to SFL processing or slow freezing. Identical quantities of the API and HPbCD used in the solutions were co-ground in a mortar and pestle and blended to produce a co-ground physical mixture for comparison. The powder samples were characterized by differential scanning calorimetry (DSC), powder X-ray diffraction (XRD), Fourier transform infrared spectrometry (FTIR), scanning electron microscopy, surface area analysis, and dissolution testing. The results provided by DSC, XRD, and FTIR suggested the formation of inclusion complexes by both slow-freezing and SFL. However, the specific surface area was significantly higher for the latter. Dissolution results suggested that equilibration of the danazol/HPbCD solution prior to SFL processing was required to produce the most soluble conformation of the
resulting inclusion complex following SFL. SFL micronized powders exhibited better dissolution profiles than the slowly frozen aggregate powder. Results indicated that micronized SFL inclusion complex powders dissolved faster in aqueous dissolution media than inclusion complexes formed by conventional techniques due to higher surface areas and stabilized inclusion complexes obtained by ultrarapid freezing.

1.2.8 Kirk A. Overhoff et al were developed an ultra-rapid freezing (URF) technology to produce high surface area powders composed of solid solutions of an active pharmaceutical ingredient (API) and a polymer stabilizer. A solution of API and polymer excipient(s) is spread on a cold solid surface to form a thin film that freezes in 50 ms to 1 s. This study provides an understanding of how the solvent’s physical properties and the thin film geometry influence the freezing rate and consequently the final physicochemical properties of URF-processed powders. Theoretical calculations of heat transfer rates are shown to be in agreement with infrared images with 10 ms resolution. Danazol (DAN)/polyvinylpyrrolidone (PVP) powders, produced from both acetonitrile (ACN) and tert-butanol (T-BUT) as the solvent, were amorphous with high surface areas (28–30 m$^2$/g) and enhanced dissolution rates. However, differences in surface morphology were observed and attributed to the cooling rate (film thickness) as predicted by the model. Relative to spray-freezing processes that use liquid nitrogen, URF also offers fast heat transfer rates as a result of the intimate contact between the solution and cold solid surface, but without the complexity of cryogen evaporation (Leidenfrost effect). The ability to produce amorphous high surface area powders with submicron primary particles with a simple ultra-rapid freezing process is of practical interest in particle engineering to increase dissolution rates, and ultimately bioavailability.

1.2.9 Wolfgang Schlocker et al were studied potential of air jet milling for the preparation of protein-loaded microparticles in industrial quantities. The model protein horseradish peroxidase was incorporated via co-precipitation in carbomer (1:100) and a poly(methacrylate) (Eudragit L100-55) (1:100) used as carrier matrix. Co-precipitation of the model protein and each polymer in aqueous solution was achieved either by a pH-shift or by the addition of various non-solvents. Dried protein/polymer complexes (desiccator under vacuumization at 4°C with silica blue gel) were ground with an air jet mill and resulting microparticles were investigated regarding protein load, remaining protein activity, size distribution and shape. Results of this study showed that the polymer used and the method of co-precipitation has a great impact on protein load. Using carbomer a maximum protein load of 60±1% was achieved, whereas in case of Eudragitw L100-55 the maximum was 78±5%. Using petroleum ether, isopropanol or tetrahydrofuran as non-solvents led to significantly higher protein loads than a pH shift from 7 to 5, 4 and 3.5, respectively. Determination of the remaining protein activity after milling showed that the grinding air pressure (GAP) has a major impact on protein stability. In case of Eudragit L100-55 at a GAP of 4.5 bar peroxidase activity was almost completely lost, whereas 42±1% loss in activity was determined at a GAP of 2.5 bar. The mean particle size of protein/carbomer and protein/poly(methacrylate) particles was determined to be 3.6–5.2 and 4.5–8.7
µm at a GAP of 2.5 bar and 2.7–3.1 and 2.4–3.1 mm at a GAP of 4.5 bar, respectively. Generally, 90% of all particles were in the range of 3–16 µm. All particles were of spherical shape exhibiting a non-porous surface. According to these results, air jet milling seems to represent a novel method for the large-scale production of protein drug loaded microparticles.\textsuperscript{128}

1.2.10 Gerrit S. Zijlstra et al were formulated cetorelix acetate, as an adhesive mixture for use in dry powder inhalation. To achieve the highest possible deposition efficiency we investigated both the influence of different micronization techniques and different inhalers. The Novolizer with an air classifier as the powder de-agglomeration principle and the ISF inhaler were used for in vitro deposition experiments (cascade impaction). Micronization by milling as the classical approach and micronization by spray drying and spray freeze drying as advanced particle engineering techniques were investigated to determine whether advanced techniques are necessary to obtain high fine particle fractions (FPF) for this specific drug. It was found that the effects obtained with a certain micronization technique depended on the complex interaction of the physical characteristics of the drug substance with the type of formulation chosen, as well as with the de-agglomeration principle used. The combination of particle engineering by spray drying and the use of the air classifier technology resulted in a fine particle fraction of 66%, while spray freeze drying yielded extremely fragile particles resulting in a FPF of only 25%. The behaviour of the milled material showed similar trends as the spray dried material but FPF values were lower. It was concluded that when a drug is to be formulated as a powder for inhalation with high fine particle fractions, it is profitable to use advanced particle engineering techniques, however the applied technique should be tuned with the characteristics of the formulation type and process as well as with device development.\textsuperscript{129}
1.3 REFERENCES


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