1.0 INTRODUCTION

The aim of an effective drug delivery system is to achieve and sustain therapeutic blood level of the drug. The therapeutic effectiveness of a drug depends upon the ability of the dosage form to deliver the medicament to its site of action at a rate and amount sufficient to elicit the desired pharmacological response. This attribute of the dosage form is referred to as physiologic availability, biologic availability or simply bioavailability. For most drugs, the pharmacologic response can be related directly to the plasma levels.\(^1\)

Bioavailability of poorly water soluble drugs is a major problem in drug delivery.\(^2\) Oral administration is the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, cost-effectiveness, least sterility constraints and flexibility in the design of dosage form. In order for a drug to be absorbed into the systemic circulation following oral administration, it must be dissolved in gastrointestinal fluids. As a result, many of the generic drug companies are inclined more to produce bioequivalent oral drug products.\(^3\) The high costs and time involved in new drug development, expiry of patents for a significant number of drug molecules, ease of manufacturing and ready availability of technology for the production of oral drug products are also driving the generic pharmaceutical companies towards the development of bioequivalent oral dosage forms. However, the major challenge with the design of oral dosage forms lies with their poor bioavailability.\(^4\)

Currently, more than 60% of marketed drugs are oral products. Oral drug delivery is the most favorable route of drug administration, but not all drugs possess the desirable physic-chemical and pharmacokinetic properties. Therefore, low oral bioavailability is found in case of such drugs, which leads to high variability and poor control of plasma concentration and therapeutic effects. This has led to the choice of other route of administration, which may be more convenient and increase risk of non-compliance. Approximately, 30% of selling medicines and 40% of new chemical entities entering development programs had very low aqueous solubility or oral bioavailability to bring about satisfactory therapeutic efficacy. Thus, one of the major current challenges faced by the pharmaceutical industry involves the development of strategies to improve the aqueous solubility and dissolution rate of drugs.\(^5\)

Generally, due to poor bioavailability more than normally required oral dose is administered which leads to economic wastage, risk of toxicity and erratic, unpredictable responses. The main challenge over the years has been design techniques that will allow oral administration of most drugs irrespective of their properties to achieve a therapeutic systemic availability. There are several factors that influence oral bioavailability and these can be broadly divided in three categories namely drug properties, physiology of GIT and patient factors.

In order to improve bioavailability and achieve therapeutic goal, there is need to manipulate drug properties or interfere with these physiological barriers.
Manipulating drug properties is a more promising approach to pharmaceutical research in pharmacotherapy than the development of new drug entities as most new drug candidates face similar problems.\(^6\)

### 1.1 Bioavailability

Bioavailability is a measurement of the rate and extent of a therapeutically active drug that reaches the systemic circulation and is available at the site of action. The term bioavailability, one of the pharmacokinetic properties of drugs, is used to describe the fraction of an administered dose of unchanged drug that reaches the systemic circulation. By definition, when a medication is administered intravenously, its bioavailability is 100%. However, when a medication is administered via oral route, its bioavailability decreases due to incomplete absorption or first-pass metabolism. The measurement of the amount of the drug in the plasma at periodic time intervals indirectly indicates the rate and extent at which the active pharmaceutical ingredient is absorbed from the drug product and becomes available at the site of action. Bioavailability is one of the essential tools in pharmacokinetics, as it must be considered, when calculating dosages for non-IV routes of administration. It is expressed as either absolute or relative bioavailability.\(^7\)

Absolute bioavailability compares the bioavailability of the active drug in systemic circulation following non-intravenous administration (i.e., after oral, rectal, transdermal, subcutaneous, or sublingual administration), with the bioavailability of the same drug following intravenous administration. The comparison must be dose normalized (e.g. account for different doses or varying weights of the subjects); consequently, the amount absorbed is corrected by dividing the corresponding dose administered.

The absolute bioavailability is the dose corrected area under curve (AUC) non intravenous divided by AUC intravenous. The calculating bioavailability (F) for a drug administered by the oral route (po) is given in equation (1).

\[
F = \frac{[AUC]po \times \text{DoseIV}}{[AUC]IV \times \text{Dosepo}} \tag{1}
\]

A drug given by the IV route will have an absolute bioavailability of 1 (F =1), while drugs given by other routes usually have an absolute bioavailability of less than 1. If we compare the bioavailability of two drugs, it is called comparative bioavailability. Knowing the true extent of systemic absorption is also referred to as absolute bioavailability.\(^8\)

There is no regulatory requirement to define the intravenous pharmacokinetics or absolute bioavailability; however, regulatory authorities do sometimes ask for absolute bioavailability information of the extravascular route in cases in which the bioavailability is apparently low or variable, and there is a proven relationship between the pharmacodynamics and the pharmacokinetics at therapeutic doses.
Intravenous administration of a developmental drug can provide valuable information on the fundamental pharmacokinetic parameters of volume of distribution (Vd) and clearance (CL).\(^9\) Relative bioavailability is one of the measures used to assess bioequivalence between two drug products, as it is the Test/Reference ratio of AUC. The relative bioavailability was calculated by equation (2).

\[
\text{Relative Bioavailability} = \frac{[AUC]A \times \text{DoseB}}{[AUC]B \times \text{DoseA}} \quad \ldots (2)
\]

Bioavailability of a drug is largely determined by the properties of the dosage form, rather than by the drugs physico-chemical properties, which determine absorption potential. Differences in bioavailability among formulations of a given drug can have clinical significance, thus knowing whether drug formulations are equivalent is essential.\(^{10,11}\)

### 1.2 Factors Influencing Bioavailability

Various physiological factors reduce the availability of drugs prior to their entry into the systemic circulation. These factors are listed below,

a) Physical properties of the drug (hydrophobicity, pKa, solubility)

b) The drug formulation (immediate release, excipients used, manufacturing methods, modified release- delayed release, extended release, sustained release etc.)

c) If the drug is administered in a fed or fasted state

d) Gastric emptying rate

e) Circadian differences

f) Transporters: Substrate of an efflux transporter (e.g. P-glycoprotein)

g) Health of the GI tract

h) Enzyme induction/inhibition by other drugs/foods:

i) Enzyme induction (increase rate of metabolism) e.g. Phenytoin (antiepileptic) induces CYP1A2, CYP2C9, CYP2C19 and CYP3A4.

ii) Enzyme inhibition (decrease rate of metabolism) e.g. grapefruit juice inhibits CYP3A > higher nifedipine concentrations.

iii) Interactions with other drugs (e.g. antacids, alcohol, nicotine)

iv) Interactions with other foods (e.g. grapefruit juice, pomello juice)

i) Individual variation in metabolic differences

i) Age: In general, drugs metabolize more slowly in fetal, neonatal and geriatric populations.

ii) Phenotypic differences, enterohepatic circulation, diet, gender.

j) Disease state

i) e.g. hepatic insufficiency, poor renal function.

Each of these factors may vary from patient to patient (inter-individual variation) and indeed in the same patient over time (intra-individual variation).\(^{12}\) The rate and extent to which the active moiety (drug or metabolite) enters the general circulation, gaining access to the site of action. Although a drug may be absorbed completely, its rate of absorption may also be important. The most frequent
causes of low bioavailability are an insufficient time in the GI tract and the presence of competing reactions. Ingested drug is exposed to the entire GI tract for no more than 1 to 2 days and to the small intestine for only 2 to 4 h, unless gastric emptying is considerably delayed. If the drug does not dissolve readily or if the drug is incapable of penetrating the epithelial membrane (highly ionized and polar), there may be insufficient time at the absorption site. Not only is the bioavailability low in this case, but it tends to be highly variable. In addition, individual variations in age, sex, activity, genetic phenotype, stress, disease (achlorhydria, malabsorption syndromes), and previous gastrointestinal surgery can alter and further increase variability in drug bioavailability.

1.2.1 Poor aqueous solubility

Poor solubility of a drug is in most cases associated with poor bioavailability. The contents of GIT are aqueous and hence a drug having poor aqueous solubility has a low saturation solubility, which is typically correlated with a low dissolution velocity, resulting in poor oral bioavailability. Low bioavailability is the most common with oral dosage forms of poorly water-soluble, slowly absorbed drugs.

1.2.2 Inappropriate partition coefficient

Very hydrophilic drugs would not be able to permeate through the GIT mucosa and too lipophilic drug will not dissolve in the aqueous GIT contents. For optimum absorption, the drug should have sufficient aqueous solubility to dissolve in the GIT contents and also adequate lipid solubility to facilitate its partitioning into the lipoidal membrane and then into systemic circulation. Drugs having partition coefficient (log P) value in the range of 1 to 3 show good passive absorption across lipid membranes, and those having log P value greater than 3 or less than 1 have often poor transport characteristics.

1.2.3 First-pass metabolism

When a drug rapidly dissolves from a drug product and readily passes across membranes, absorption from most sites of administration tends to be complete. This is not always the case for drugs given orally. Before reaching the vena cava, a drug must move down the alimentary canal and pass through the gut wall and liver, which are common sites of drug metabolism, thus, the drug may be metabolized before it can be measured in the general circulation. This cause of a decrease in drug input is called the first-pass effect. A large number of drugs show low bioavailabilities owing to extensive first-pass metabolism. In many instances, the extraction is so complete that the bioavailability is virtually zero.13

1.2.4 Degradation in the gastrointestinal tract

Drug substances used as pharmaceuticals have diverse molecular structures and are therefore, prone to variable degradation pathways. Protein drugs in particular
are highly susceptible to inactivation due to the pH and the enzymes present in the GIT.\(^\text{14}\)

1.2.4.1 Low pH in stomach

Most drug substances are fairly stable at the neutral pH values found in the small intestine (disregarding enzymatic degradation) but can be unstable at low pH values found in the stomach.\(^\text{15}\) Knowledge of the stability of a drug in the pH range of 1-2 at 37°C is important in the formulation design of potentially acid labile drugs.\(^\text{16}\) At low pH, foscarnet decomposes via an acid-catalyzed decarboxylation; therefore, poor oral bioavailability (7-9%) might be due to decomposition of foscarnet in gastric acid.

1.2.4.2 Chemical reactions taking place in GIT

Various drugs cannot be administered orally because of their inactivation in GI fluids due to chemical reactions. Possible degradation pathways include hydrolysis, dehydration, isomerization, racemization, elimination, oxidation, photodegradation and complex interactions with excipients, food and other drugs, thiol/disulfide exchange reactions. A hydrolytic cleavage takes place particularly at low pH of the stomach. Various antibiotics, for instance, are hydrolyzed in the stomach. In case of thiol/disulfide bond bearing drugs, thiol/disulfide exchange reactions in particular with glutathione can inactivate them in the gastrointestinal tract.\(^\text{17}\)

1.2.4.3 Enzymatic Reaction

Various classes of drugs such as therapeutic peptides and nucleic acids are enzymatically degraded by proteases/peptidases and nucleases respectively. Proteases/peptidases are on the one hand based on luminally secreted proteases including pepsin, trypsin, chymotrypsin, elastase and carboxypeptidase A and B and on the other hand on membrane bound peptidases including various endo as well as amino and carboxypeptidases. In the colon numerous additional enzymes originating from the local microflora have to be taken into consideration. Teriparatide undergoes enzymatic degradation in the intestinal mucosa by enzymes like trypsin, chymotrypsin and pepsin.\(^\text{18}\)

1.2.4.4 Interaction with food

Drugs that undergo a significant first pass metabolism with a lower bioavailability ranging from 5% to 30% may be affected to a greater degree by grapefruit juice.\(^\text{19}\) Calcium, as well as food and dairy products containing high concentrations of calcium, may decrease the absorption of tetracyclines due to chelate formation in the gut.\(^\text{20}\)

1.2.5 Drug efflux pumps like p-glycoprotein
It is recently identified that drug efflux pumps like P-glycoprotein are playing a major role in altering the pharmacokinetics of various drugs. Due to selective distribution at the port of drug entry and exit, P-glycoprotein has been speculated to play a major physiological role in absorption, distribution and excretion of xenobiotics.

1.3 Techniques of Improving Bioavailability

One approach to improve the systemic availability of the drug is to deliver it by alternative routes of administration such as parenteral, nasal, vaginal, rectal or transdermal. However, improvement in the oral bioavailability of the drug is the most realistic approach, as it is the most preferred and convenient route of administration. There are various techniques available to improve the solubility and dissolution rate of poorly soluble drugs. Following is the classification of these techniques based on various approaches.

1.3.1 Physical modifications

a) Particle size reduction micronization and nanonization
b) Modification of the crystal habit polymorphs and pseudopolymorphs
c) Drug dispersion in carriers eutectic mixtures, solid dispersions and solid solutions
d) Inclusion complexation

1.3.2 Chemical modifications

a) Change in pH of system
b) Salt formation

d) Addition of solubilizer

1.3.3 Formulation Based Approaches

a) Co-crystallization
b) Co-solvency

c) Hydrotrophy

d) Ultra rapid freezing
f) Porous microparticles technology

e) Use of novel drug delivery systems like microemulsion, SMEDDS, solid lipid nanoparticles.

1.3.4 Modification of partition coefficient

a) Ester formation
b) Novel formulation approaches like liposomes, niosomes and microemulsion

c) Use of novel drug delivery systems like microemulsion, SMEDDS, solid lipid nanoparticles.

1.3.5 Avoidance of hepatic first pass metabolism

a) Co-administration with another drug
b) Prodrugs to reduce presystemic metabolism

1.3.6 Avoidance of degradation in gastrointestinal tract

a) Avoidance of degradation in stomach - Enteric coating of tablet.
b) Avoidance of degradation in intestine - Enhancement of residence time in stomach
   i) Floating drug delivery systems
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ii) Use of mucoadhesive polymers

iii) Avoidance of degradation in stomach and intestine- colon targeted drug delivery system

1.3.7 Inhibition of P-glycoprotein efflux

a) By using P-glycoprotein inhibitors
b) Use of surfactant
c) Use of dendrimers

1.4 Bioavailability Enhancement Using Nanoparticles

The last century has witnessed a pronounced increase in life span. This revolution was mainly led, in early stages, by the discovery of drugs for the treatment of incurable pathologies. However, due to their high hydrophobicity, more than 50% of the drugs approved for use display low aqueous solubility, constituting nowadays one of the main hurdles to attain convenient absorption and bioavailability. Based on drug permeability characteristics, the Biopharmaceutic Classification System (BCS) categorizes hydrophobic drugs for oral administration in to Class II and Class IV. Class II drugs are poorly water-soluble entities with high permeability. In this context, enhancement of the solubility of these drugs usually correlates well with improved bioavailability. Class IV comprises poorly water-soluble molecules with low permeability. In this case, a further modification is required to attain appropriate transfer through body membranes. Thus, limited water solubility represents a serious drawback in the design of formulations not only for oral but also for parenteral and topical route of administration. Another aspect of concern stems from the limited chemical or biological stability of the drugs in the physiological environment.

Thus, one of the major current challenges facing the pharmaceutical industry involves the development of strategies to improve the aqueous solubility and dissolution rate of drugs. Drug solubility can be enhanced using traditional approaches such as designing prodrug, reducing particle size by micronization, cosolubilization by micellization, complexation, solid dispersions and use of solubilizing excipients. Thus, different technological strategies have been investigated in order to prolong the exposure to the drug. Nanotechnology has opened the possibility of controlling and manipulating structures at the molecular level and has led to the creation of novel surface architectures and materials. Nanotechnology, utilising science at the nanoscale is not new. In the 4th Century A.D., the Romans applied gold and silver nanoparticles to colour the glass cups. In the last 35 years, the growth of nanotechnology has opened several new vistas in medical sciences, especially in the field of drug delivery as new moieties are coming handy for treating diseases.

Recently, major research efforts have been focused on the development of nanotechnology based drug delivery systems including biodegradable polymeric nanoparticles, smart polymeric micelles, nanocrystals or nanosuspension, and nanoemulsion to enhance the dissolution rate of poorly soluble drugs and improve their oral bioavailability.
The input of today’s nanotechnology is that it allows real progress to achieve temporal and spatial site-specific delivery. The market of nanotechnology and drug delivery systems based on this technology will be widely felt by the pharmaceutical industry. In recent years, the number of patents and products in this field is increasing significantly like Caelyx, Doxil, Transdrug, Abraxane, etc. In January 2005, US FDA approves ABRAXANE® for breast cancer treatment, the first nanoparticle system for drug delivery.  

A basic requirement for the use of nanoparticles and other synthetic systems as drug delivery systems for human therapy is their biodegradability and biocompatibility. The challenge of modern drug therapy is the optimization of the pharmacological action of drugs, coupled with the reduction of their toxic side effects in vivo. A controlled size distribution (monodisperse distribution of size), for accurate drug administration, is a central need for the use of nanoparticles in drug delivery systems. Moreover, the absence of toxic residues in the final nanosystem is required and therefore stronger restrictions to the type of methods used for nanoparticles formation exist.

The main goals are to improve their stability in the biological environment, to mediate the biodistribution of active compounds, improve drug loading, targeting, transport, release and interactions with biological barriers. The nanodrug delivery is the targeting of diseased cells and the release of drugs into specific portions of the body. By making use of the special properties of dendrimers, nanoshells and nanotubes, we can destroy diseased cells without severe side effects and causing harm to healthy cells within the body. The designing of nanodevices, nanomachines, nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.

Several nano-oriented approaches are being intended (e.g. nanoparticle engineering) in order to optimize the technological aspects of drugs. The use of these processes has dramatically enhanced dissolution rates in vitro and in vivo bioavailabilities of many drugs. Another important strategy is the design of nanocarriers. Among the technological alternatives, the most broadly implemented are polymeric nanoparticles, dendrimers, polymeric micelles and polymersomes.

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the methods of preparation of nanoparticles, nanospheres or nanocapsules can be obtained. The devices and systems produced by chemical and/or physical processes having specific properties. There are wide advantages of nanoparticles like, better drug utilization, specific site of drug release, easy handling of nanoparticles prepared in the powder form, good control over size and size distribution, good protection on
the encapsulated drug, longer clearance times increased therapeutic efficacy of drugs, limiting side effects, retention of drug at the active site, reduces size of drug nanoparticles, allowing for greater dissolution of the drug in water and improved bioavailability, significantly increases drug solubility in the supercritical solvent, improving productivity, particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration, control and sustain release of the drug during the transportation and at the site of localization, controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents and used by various routes of administration including oral, nasal, parenteral, intra-ocular etc. But some of the disadvantages like due to small size and large surface area can lead to particle aggregation and discontinuation of therapy is not possible, when administration of drugs by intravenously.

1.5 Polymers Used to Design Nanoparticles

A wide spectrum of synthetic and natural polymers are available for nanoparticle formation, but their biocompatibility and biodegradability are the major limiting factors for their use in the drug delivery area. Biodegradable polymers are advantageous in many ways over other materials for use in drug delivery systems such as nanoparticles. They can be fabricated into various shapes and sizes, with tailored pore morphologies, mechanical properties and degradation kinetics to suit a variety of applications. By selecting the appropriate polymer type, molecular weight, and copolymer blend ratio, the degradation/erosion rate of the nanoparticles can be controlled to achieve the desired type and rate of release of the encapsulated drug.\textsuperscript{54}

In spite of development of various synthetic, semi synthetic and natural polymers still enjoy their popularity in drug delivery. The main advantage of these degradable polymers is that they are broken down into biologically acceptable molecules that are metabolized and removed from the body via normal metabolic pathways; some of the polymers are listed in Table 1.1.

1.5.1 Natural polymers

Natural polymers offer the advantage of established history of safety, low cost, aqueous solubility and biocompatibility with both, the human body as well as drugs and other formulation components. Mostly they are water-soluble, but can be transformed into nanoparticles by means of denaturation, leading to cross-linking. In case of charged groups being present in the material, the use of oppositely charged counter-ions also leads to formation of particles by electrostatic neutralization.

Chitosan is a deacetylated chitin that is of great interest as a functional material of high potential in various areas including the biomedical field. It is reported that chitosan can increase the paracellular permeability of intestinal epithelia, which
attributed to chitosan polymers the property of transmucosal absorption enhancement. Because of low production costs, biocompatibility, and very low toxicity, chitosan is a very interesting excipient for vaccine delivery research. For these reasons, chitosan has been selected as the polymer of choice in the present research.

1.5.2 Synthetic polymers

Synthetic polymers offer better reproducibility of the chemical characteristics of the synthesized nanoparticles as compared to the natural polymers. Synthetic polymers from the ester family, such as poly(lactic acid), poly(hydroxybutyrate), poly(caprolactone), poly(dioxanone), or other families such as poly(cyanoacrylates), poly(acrylic acid), poly(anhydrides), poly(amides), poly(ortho esters), poly(ethylene glycol) and poly(vinyl alcohol) are suitable for drug delivery due to their biodegradability, special release profiles and biocompatibility.

The PLGA copolymer is degraded in body by hydrolytic cleavage of ester linkage into lactic acid and glycolic acid at a very slow rate. The acids easily metabolize in the body via Krebs’ cycle and are eliminated as carbon dioxide and water. It is a well characterized polymer, its degraded sub products are non toxic, it provides controlled drug release profiles by changing the PLGA copolymer ratio, which affects the crystallinity (low crystallinity, more amorphous polymer means more fast degradation) of PLGA. For these reasons, PLGA has been selected as the polymer of choice in the present research.

PLGA of different molecular weights (from 10 kDa to over 100 kDa) and different copolymer molar ratios (50:50, 75:25 and 85:15) is available in the market. Molecular weight and copolymer molar ratio influence the degradation process and release profile of the drug entrapped. In general, low molecular weight PLGA with higher amounts of glycolic acid offer faster degradations rates.

Table 1.1: Polymers used in nanoparticles

<table>
<thead>
<tr>
<th>Natural Hydrophilic Polymers</th>
<th>Synthetic Hydrophilic Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Polysaccharides</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Alginate</td>
</tr>
<tr>
<td>Albumin</td>
<td>Dextran</td>
</tr>
<tr>
<td>Lecithin</td>
<td>Chitosan</td>
</tr>
<tr>
<td>Legumin</td>
<td>Agarose</td>
</tr>
<tr>
<td>Vicilin</td>
<td></td>
</tr>
</tbody>
</table>
1.6 Preparation Methods of Nanoparticles

Numerous methods were existing for the manufacture of nanoparticles allowing extensive modulation of their structure, composition and physicochemical properties. Two main procedures can be followed to form polymeric nanoparticles, namely top-down and bottom-up techniques. The top-down methods use size reduction to obtain controlled-size nanoparticles. This size reduction is based on the application of strong shear stress by wave sound emission (sonication), high pressure (microfluidization) and high speed agitation (homogenization).

The choice of preparation methods essentially depends on the raw materials intended to be used and on the solubility characteristic of active compound to be associated with the particles. Regarding raw materials, criteria are biocompatibility, degradation behavior, choice of administrative route, desired release profile of drugs. The bottom-up methods start from individual molecules to form nanoparticles by polymerization. The main drawbacks of the bottom-up methods are the presence of residual sub-products in the final nanoparticles that can impart toxicity to the nanoparticles. To overcome these limitations, top-down methods were developed using naturals and synthetic polymers. The emulsion evaporation, salting out, nanoprecipitation, ionotropic gelation and emulsion diffusion are the main top-down methods used to form polymeric nanoparticles. Processes used for the preparation of polymeric nanoparticles summaries in Table 1.2.

<table>
<thead>
<tr>
<th>Process</th>
<th>Particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single emulsion</td>
<td>(Particle size depends on the size of dispersion used)</td>
</tr>
<tr>
<td>Double emulsion</td>
<td>100-1000 nm</td>
</tr>
<tr>
<td>Spray drying</td>
<td>&gt;200 nm</td>
</tr>
<tr>
<td>Gas- antisolvent precipitation</td>
<td>400-600 nm</td>
</tr>
<tr>
<td>Nanoprecipitation</td>
<td>&gt;100 nm</td>
</tr>
<tr>
<td>High pressure homogenisation</td>
<td>&gt;300 nm</td>
</tr>
</tbody>
</table>
1.6.1 Dispersion-based processes

1.6.1.1 Wet milling

Wet milling is an attrition-based process, in which the drug is dispersed first in an aqueous-based surfactant solution. The resulting suspension is subjected to wet milling using a pearl mill in the presence of milling media.

1.6.1.2 High-pressure Homogenization

High pressure homogenization is based on the principle of cavitation (i.e., the formation, growth and implosive collapse of vapor bubbles in a liquid. In this process, a drug presuspension (containing drug in the micrometer range) is prepared by subjecting the drug to air jet milling in the presence of an aqueous surfactant solution. The main advantage of high-pressure homogenization is that, it is suitable for both large and laboratory-scale production because high-pressure homogenizers are available in various sizes. In addition, homogenization creates negligible nanoparticle contamination, which is one of the most important objectives of a nanoparticle production process. A limitation of this process is that the pressure used is so high that in some cases, the crystal structure changed.

1.6.1.3 Emulsification Technology

Emulsification also can be used to prepare nanoparticle suspensions. In this method, the drug solution in an organic solvent is dispersed in the aqueous phase containing surfactant. This step is followed by the evaporation of organic solvent under reduced pressure, which results in the precipitation of drug particles to form a nanoparticle suspension, which is stabilized by the added surfactant.

1.6.2 Emulsion-Solvent Evaporation

Polymer and drug is dissolved in a suitable volatile solvent, which is miscible with water. This solution is emulsified in an aqueous solution containing stabilizer (mostly surfactants) by conventional emulsification techniques. Droplet size can be further reduced by using a high energy source. Continuous emulsification under mixing prevents coalescence of organic droplets and allows the spontaneous evaporation of the solvent at room temperature and the formation of the colloidal particles. Following evaporation of organic phase under reduced pressure or vacuum produces a fine aqueous dispersion of nanoparticles. These can be collected by centrifugation, washed to remove residual stabilizer and can be freeze dried for storage.
As this approach is limited to certain mainly water insoluble drugs, a variation of the first method has been developed for the encapsulation of more hydrophilic drugs. The so-called double-emulsion-technique is thus very interesting for the entrapment of peptides, proteins and nucleic acid sequences. Here, water-in-oil-in-water (w/o/w) emulsions are used, incorporating hydrophilic drugs in an inner aqueous phase. The polymer is dissolved in the organic phase and a first mixing step forms a water-in-oil emulsion which is thereafter emulsified in a second, outer aqueous phase. Upon evaporation of the organic phase the polymer precipitates on the surface of the inner aqueous droplets, thereby entrapping the drug dissolved therein.\cite{55}

### 1.6.3 Nanoprecipitation (Solvent displacement or solvent diffusion)

A solution of polymer, drug and lipophilic stabilizer (surfactant) in a semi-polar solvent miscible with water is injected into an aqueous solution (being a non-solvent or anti-solvent for drug and polymer) containing another stabilizer under moderate stirring. Nanoparticles are formed instantaneously by rapid solvent diffusion and the organic solvent is removed under reduced pressure. The velocity of solvent removal and thus nuclei formation is the key to obtain particles in the nanometer range instead of larger lumps or agglomerates.

Fessi et al. proposed a simple and mild method yielding nanoscale and monodisperse polymeric particles without the use of any preliminary emulsification. Both, solvent and nonsolvent must have low viscosity and high mixing capacity in all proportions, e.g. acetone and water. Another delicate parameter is the composition of the solvent/polymer/water mixture limiting the feasibility of nanoparticle formation. The only complementary operation following the mixing of the two phases is to remove the volatile solvent by evaporation under reduced pressure. One of the most interesting and practical aspect of this methods is its capacity to be scaled up from laboratory to industrial amounts, since they can be run with conventional equipment.

The nanoprecipitation method introduced by Fessi and co-workers,\cite{56} the particle formation is based on precipitation and subsequent solidification of the polymer at the interface of a solvent and non-solvent. Thus, the process is often called solvent displacement or interfacial deposition. The polymer is dissolved in a water miscible organic solvent (or solvent mixture) and added to an aqueous solution, in which the organic solvent diffuses shown in figure 1.1. Particle formation is spontaneous, because the polymer precipitates in the aqueous environment.

Subsequently, as the solvent diffuses out from the droplets, the polymer precipitates. Finally, the organic solvent is typically evaporated with the help of a vacuum or rotatory evaporator. No emulsification step (which is usually part of a nanoparticle preparation process), laborious processing conditions. The size of the nanoparticles prepared by nanoprecipitation varies typically from 100 to 500 nm.\cite{57}
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Figure 1.1: Schematic illustration of the nanoprecipitation process

Usually, surfactants or stabilizers are included in the process to modify the size and the surface properties, or to ensure the stability of the nanoparticle dispersion (especially during the early stages of the precipitation). However, presence of surfactants/stabilizers is not indispensable for the formation of the particles. The drug substance to be encapsulated is, depending on its solubility, dispersed as an aqueous solution or dissolved in the organic solvent evaporation. The nanoprecipitation technique suffers from poor encapsulation efficacy of hydrophilic drugs, because the drug can diffuse to the aqueous outer phase during polymer precipitation. By modifying the solubility of the drug by changes in pH, the encapsulation has been increased. An accelerated precipitation rate of the polymer, modified solvent composition and increase in the molecular weight of the polymer are among the other means to improve the encapsulation efficiency. So, nanoprecipitation method has been selected in the present research work.

1.6.4 Salting out

The salting out techniques was introduced and patented by Blindschaedler and Ibrahim et al. In this method toxic solvents are avoided. Here acetone is used, which can be easily removed by cross flow filtration in the final stage. The preparation methods consist of adding under mechanical stirring, an electrolyte saturated solution containing a hydrocolloid, generally a PVA, as stabilizing and viscosity increasing agent to an acetone solution of polymer. This PVA is compatible with several electrolytes. The saturated aqueous solution, prevents acetone from diffusing in to water by a salting out process after the preparation of O/W emulsion. Sufficient water or aqueous solution of PEG is added to allow the complete diffusion of acetone in to the aqueous phase, thus inducing the formation of nanospheres.
1.6.5 Emulsification diffusion

This method is a modification of salting out procedure. It was first described and patented by Leroux et al., wherein large amount of salts in aqueous phase are avoided to eliminate the problem of compatibility. Here, partially water soluble solvent is used, which is previously saturated in water to ensure the thermodynamic equilibrium. Polymer is dissolved in the water saturated solvent containing stabilizer and the organic phase is emulsified under agitation. The subsequent addition of water leads to diffusion in to the external phase, which in turn forms nanoparticles.

1.6.6 Coacervation or ionic gelation method

Much research has been focused on the preparation of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, and the other is a polyanion sodium tripolyphosphate. The chitosan solutions are then added drop wise under constant stirring to the solutions containing other counterions i.e sodium tripolyphosphate solution in water shown in figure 1.2. The low molecular weight counterions (e.g. CaCl$_2$, BaCl$_2$, MgCl$_2$, CuCl$_2$, ZnCl$_2$, CoCl$_2$, pyrophosphate, tripolyphosphate, tetrapolyphosphate, octapolypophosphate, hexametaphosphate were available, we selected sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases. Due to the complexation between oppositely charged species, polysaccharides undergo ionic gelation and precipitate to form spherical particles. The nanoparticles were collected by centrifugation, filtration, washed with distilled water and freeze dried. Ionic gelation method has been selected in the present research work because method is very simple and mild. In addition, reversible physical cross linking by electrostatic interaction instead of chemical cross linking avoids the possible toxicity of reagents and other undesirable effects.
1.6.7 Desolation method

This solution of the polymer and drug to be entrapped are poured in to water, resulting in the spontaneous formation of nanoparticles of size between 90-200 nm. Polyacrylic nanoparticles can be prepared by dissolving relatively hydrophilic copolymer such as Eudragit RS or Eudragit RL in water miscible solvents such as acetone and ethanol.\textsuperscript{74}

1.6.8 Supercritical fluid technology

Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents, which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro and nanoparticles because supercritical fluids are environmentally safe.\textsuperscript{75,76}

1.7 Factors Influencing Preparation of Nanoparticles

1.7.1 Solvent selection

The top-down method requires the dissolution of the polymer in the aqueous or organic phase. The solvent selection is restricted to the method used; for example, nanoprecipitation and emulsion diffusion use water-soluble solvents (i.e. acetone, benzyl alcohol). The method selected to form the nanoparticles was nanoprecipitation method, in which the polymer (PLGA) was dissolved in the organic solvent. The solvents have been extensively used with this method to dissolve the PLGA (i.e. acetone, methylene chloride, dichloromethane, chloroform). A solvent that could be used as an acetone. The low toxicity, low
boiling point (77°C) are the main advantages of using acetone to dissolve the polymer and hydrophobic drug.  

1.7.2 Surfactant selection

The stability of the organic droplet (acetone and PLGA) in water, during the precipitation, is insured by the addition of surfactants. A wide spectrum of surfactants are available for the stabilization of droplets, ionic surfactants (cationic, anionic, zwitterionic) and nonionic surfactants. The nonionic surfactants are macromolecules formed by copolymers or tri polymers (amphiphilic), which can form stable micelles due to the hydrophobic-hydrophilic interactions with the two phases. The major nonionic surfactants used in the nanoprecipitation method are poly(vinyl alcohol), poloxamer and poloxamines family, (F68, F127 and others) and tween 80. Pluronic F 68 an nonionic surfactant with the melting point of about 55°C, has been selected, because it’s having properties like mucoadhesive force, low toxicity, less skin irritation, good drug release characteristics and compatibility with other chemical.

1.7.3 Processing Parameters

The method and material selection, as well as the synthesis parameters play an important role in forming nanoparticles of controlled physical and chemical properties. Process parameters like sonication time, amount of surfactant, PLGA concentration, evaporation conditions, and purification play a key role in determining the final nanoparticle size. Synthesis parameters were selected as follows; the PLGA concentration used was 0.25% w/v (PLGA /acetone) and stirring speed based on previous published studies and preliminary studies.

In ionic gelation method, sonication time (droplet size reduction), concentration of chitosan, concentration of sodium tripolyphosphate, controlled pH, stirring speed is important in final nanoparticle size. The processing parameters were selected based on previous published studies, acidic phase (pH 4-6) chitosan solution, chitosan / STPP weight ratio should be within the range 1:1-2:1 in order to obtain a high yield of nanoparticles, the sonication time was 30 minutes, which were proven experimentally to form small size nanoparticles. Stirring speed fixed on the basis of preliminary studies.

1.8 Characterization of Nanoparticles

Particle size and Particle size distribution are the most important characteristics of nanoparticle systems. They determine the in vivo distribution, biological fate, toxicity and the targeting ability of nanoparticle systems. In addition, they can also influence the drug loading, drug release and stability of nanoparticles given in Table 1.3.
1.8.1 Surface properties of nanoparticles

Surface charge of nanoparticles was determined by zeta potential measurement on a Malvern Zetasizer 2000 HS (Malvern, UK). Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in a dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e., the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate. So, colloids with ± 30 high zeta potential (negative or positive) are electrically stabilized, while colloids with low zeta potentials tend to coagulate or flocculate.

1.8.1.1 Scanning Electron Microscopy

Scanning Electron Microscopy is a limited tool to characterize nanoparticles. Nevertheless, SEM can yield valuable information regarding the purity of a nanoparticle sample as well as an insight on their degree of aggregation. Moreover, when nanoparticles are part of secondary and tertiary nanostructures, SEM becomes a valuable tool to assess their location.

1.8.1.2 Transmission Electron Microscopy

Transmission Electron Microscopy has enabled the direct imaging of atomic structures in solids and surfaces. Nanometer sized particles are commonly present in many different types of materials and the use of TEM allows for gathering information about particle size, shape and any surface layers or absorbates. More recently, changes in nanoparticle structure as a result of interactions with gas, liquid, or solid-phase substrates, can now be monitored by this technique. For instance, the study of nanoparticles can be greatly improved with the use of aberration-corrected lenses, enabling image resolutions at levels, sometimes lower than 1 Å. This level of image resolution yields a new level of understanding of the behavior of matter at the nanoscale.

1.8.1.3 Atomic force microscopy

Atomic force microscopes are employs a sharp, cantilever-mounted probe to raster scan surfaces. Image resolution can be very high. Scientists have observed subatomic scale features, but depends on various factors including tip sharpness, acoustic isolation of the instrument, sampling medium, AFM controller precision etc. The tip and sample positions are manipulated relative to each other with piezoelectric or other (e.g. electromagnetic coils) actuators. The AFM precisely controls the tip location on the sample by managing the voltage applied to the scanners. These are arranged either with three independent, orthogonal piezoelectric blocks or in a tube configuration. Piezoelectric scanner performance can be limited because of nonlinearities in the scanner material creep, noise, and drift in the high voltage supply, or thermal drift of the AFM apparatus itself.
1.8.2 Particle size determination

Particle size determination was performed with two different methods, dynamic light scattering (Malvern Zetasizer 2000 HS) and scanning electron microscopy; a new sizing method was evaluated. The applied new analytical tool was an asymmetric flow field-flow fractionation unit with a multi angle light scattering detector. Dynamic light scattering is also often referred as photon correlation spectroscopy or quasi elastic light scattering. In DLS experiments, the Brownian motion of the analytes within the dispersion medium is detected. More precisely, this is done by measuring the angular distribution of time-dependent scattered light intensity due to density and/or concentration fluctuations. From these fluctuations an auto correlation function is derived, which is inverted to determine the diffusion coefficient of the analyzed sample. The diffusion coefficient in turn represents the velocity of the analytes Brownian motion. The size of the analyte is now calculated based on the measured velocity with respect to two further factors having significant impact on this calculation; medium viscosity and temperature.

Table 1.3: Methods for characterization of nanoparticles

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>METHODS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td></td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td></td>
<td>SEM combined with energy dispersive</td>
</tr>
<tr>
<td></td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td></td>
<td>Freeze fracture electron microscopy</td>
</tr>
<tr>
<td></td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>Density</td>
<td>Helium compression pycnometry</td>
</tr>
<tr>
<td>Crystallinity</td>
<td>X-ray diffractometry</td>
</tr>
<tr>
<td></td>
<td>Differential Scanning colorimetry</td>
</tr>
<tr>
<td>Surface charge</td>
<td>Electrophoresis</td>
</tr>
<tr>
<td></td>
<td>Laser Doppler anemometry</td>
</tr>
<tr>
<td></td>
<td>Zeta potential measurement</td>
</tr>
<tr>
<td>Hydrophobicity</td>
<td>Hydrophobic interaction chromatography</td>
</tr>
<tr>
<td></td>
<td>Contact angle measurement</td>
</tr>
<tr>
<td>Surface properties</td>
<td>Static secondary ion mass spectrometry</td>
</tr>
<tr>
<td>Surface Element analysis</td>
<td>X-ray Photoelectron spectroscopy</td>
</tr>
<tr>
<td></td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td></td>
<td>Fourier transform infra red spectroscopy</td>
</tr>
</tbody>
</table>
1.8.3 Drug loading

Ideally, a successful nanoparticulate system should have a high drug loading capacity thereby reducing the quantity of matrix materials for administration. Drug loading can be done by two methods:

a) Incorporation method: Incorporating at the time of nanoparticles production.

b) Adsorption / Absorption technique: Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution.

Drug loading and entrapment efficiency very much depend on the solid state drug solubility in matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of end functional groups (ester or carboxyl). The macromolecule or protein shows greatest loading efficiency, when it is loaded at or near its isoelectric point, when it has minimum solubility and maximum adsorption. For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading.

1.8.4 Drug release

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on:

a) Solubility of drug

b) Desorption of the surface bound/ adsorbed drug

c) Drug diffusion through the nanoparticle matrix

d) Nanoparticle matrix Erosion/degradation

e) Combination of erosion/diffusion process

1.9 Nanoparticles for Lipid Lowering Drugs

Cardiovascular diseases remain the biggest cause of deaths worldwide, though over the last two decades. Cardiovascular mortality rates have declined in many high income countries but have increased at an astonishingly fast rate in low and middle income countries. Cholesterol is one of the body fats (lipids). Lipids are transported through the plasma compartment in lipoprotein, complex water soluble molecule consisting of core of cholestryl ester and triglyceride covered by a surface monolayer of phospholipids, free cholesterol and apolipoprotein.

Cholesterol and triglyceride are important building blocks in the structure of cells and are also used in making hormones and producing energy. To some extent, the cholesterol level in blood depends on what you eat, but it is mainly dependent on how the body makes cholesterol in the liver. Having too much cholesterol in the blood is not a disease in itself, but can lead to the hardening and narrowing of the arteries (atherosclerosis) in the major vascular systems. For the sake of simplicity,
there are two sorts of cholesterol: a 'good' sort called high-density lipoprotein (HDL) and a 'bad' sort called low-density lipoprotein (LDL). HDL has a useful effect in reducing cholesterol and taking it back to the liver. HDL actually protects against atherosclerosis. LDL can contribute to diseases of the heart and circulation (cardiovascular disease). It is the proportion of LDL cholesterol to HDL cholesterol that influences the degree to which atherosclerosis is likely to cause cardiovascular risk.

1.9.1 Clinical significance

Elevation of the total cholesterol values in plasma is considered to be a prime risk factor for coronary heart disease. Increased triglyceride and VLDL values are taken as primary risk factors. A low serum triglyceride level is suggestive of intravascular lipolysis and enhanced formation of HDL. Hypertriglycerideremia, on the other hand indicates less effective intravascular lipolysis and a reduced formation of HDL, which is associated with higher atherogenic risk. Elevated LDL is suggestive of atherogenic risk. Low level of HDL-cholesterol indicate high risk of coronary heart disease. Total cholesterol / HDL-cholesterol ratio should be below 5.0, and then it is usually regarded as a risk factor in the development of ischemic heart disease.90

Table 1.4: Normal ranges of CH, LDL-C, HDL-C, and triglycercide

<table>
<thead>
<tr>
<th>Atherosclerotic risk</th>
<th>CH</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>Triglyceride</th>
<th>LDL-C/HDL-C ratio</th>
<th>CH/HDL-C Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desirable / low risk</td>
<td>&lt; 200</td>
<td>&lt; 130</td>
<td>&gt; 60</td>
<td>&lt; 150</td>
<td>0.5-3.0</td>
<td>3.3-7.1</td>
</tr>
<tr>
<td>Borderline/ moderate risk</td>
<td>200 - 240</td>
<td>130 - 160</td>
<td>35-60</td>
<td>150-200</td>
<td>3.0-6.0</td>
<td>7.1-11</td>
</tr>
<tr>
<td>Elevated level / high risk</td>
<td>&gt; 240</td>
<td>&gt; 160</td>
<td>&lt; 35</td>
<td>&gt; 200</td>
<td>&gt; 6.0</td>
<td>&gt;11.0</td>
</tr>
</tbody>
</table>

1.9.2 Major risk factors

Both hereditary and environmental factors affect the cholesterol level. Cholesterol levels can run in families. If the inherited cholesterol levels are very high, this is called familial hypercholesterolaemia (FH). Familial combined hyperlipidaemia (FCH) is where the triglyceride levels are very high as well. Diets that are high in saturated fat (cakes, pastry, meat, dairy products) raise cholesterol. Middle-aged women and men who smoke, have a much higher risk of suffering a heart attack. As we get older, cholesterol levels rise. Before menopause, women tend to have lower total cholesterol levels than men of the
same age. After menopause, however, women's LDL levels tend to rise, reduced metabolism due to thyroid problems, liver disease, kidney diseases, diabetes and alcohol abuse. Occasionally a medical condition may cause an elevation of cholesterol levels in the blood.

1.9.3 Symptoms of high cholesterol in the blood stream

Atherosclerosis is the build up of cholesterol and fat (fatty deposits or plaques) in the artery walls. The arteries become narrow and hardened, their elasticity disappears and it becomes difficult for blood to flow through. These fatty plaques can rupture, causing blood to clot around the rupture. If blood can not flow to a part of the body, the tissue dies. The following are all symptoms of cardiovascular disease. They depend on the degree of narrowing, the likelihood that the plaque is going to rupture (vulnerability), and the organ supplied by the affected arteries.91

In the brain, a blood clot (thrombus) may block an artery or a smaller blood vessel may rupture, causing local haemorrhage (bleeding), either will result in a stroke. In the heart, narrowed coronary arteries cause angina and ruptured plaques cause blood clots that can lead to a heart attack. This may lead to reduced heart function if a significant amount of heart muscle is damaged. If the carotid arteries in the neck become narrow, clots may form and float to the brain. This can result in a stroke or repeated 'mini-strokes' (transient ischaemic attacks or TIAs). It's common for those most affected by atherosclerosis to have the disease in several arteries, including, the aorta, the main artery in the chest and abdomen, renal arteries, mesenteric (intestinal) vessels.102

1.9.4 Prevention

Measures to prevent cardiovascular disease may include, low fat high fiber diet including, whole grains and plenty of fresh fruit and vegetables, Tobacco cessation and avoidance of second hand smoke, limit alcohol consumption to the recommended daily limits, lower blood pressures, if elevated through the use of antihypertensive medications, decrease body fat (BMI) if overweight or obese and increase daily activity to 30 minutes of vigorous exercise per day at least five times per week.

1.10 Statin as Antihyperlipidemic Drug

Statins block the production of cholesterol in the liver itself. These drugs are the first line of treatment for most people with high cholesterol. Side effects can include intestinal problems, liver damage, and in a few people, muscle tenderness or weakness. Mevastatin was the first HMG-CoA reductase inhibitor isolated from Penicillium citrinum. Other statins simvastatin, lovastatin and pravastatin are also fungal derivatives, while atorvastatin, cerivastatin, fluvastatin, pitavastatin and rosvastatin are fully synthetic compounds.92
The use of statins (simvastatin, pravastatin, lovastatin, fluvastatin, rosvastatin and atorvastatin) has become the preferred method for treating elevated LDL-C levels in children and adolescents. In fact, their use is generally safe and well tolerated. However, it must be remembered that cholesterol is an essential structural component of cells, a precursor for steroid hormones, vitamin D metabolites, bile acids and brain growth. Statins or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (HMGR) inhibitors, have been described as the most effective class of drugs to reduce serum cholesterol levels, and have been shown to significantly reduce cardiovascular events and mortality in patients with or without coronary artery disease.

1.11.1 Pharmacological action of statin on the Mevalonate (Cholesterol) Pathway

The action of the statins as cholesterol lowering agents is mainly due to its inhibitory effect on the mevalonate or the cholesterol pathway. These agents prevent the cholesterol formation cascade by its action on the HMGR enzyme thereby leading to reduced serum cholesterol levels. To understand its action more thoroughly, we must first discuss the mevalonate pathway which leads to cholesterol formation.

1.11.2 Biosynthesis of Cholesterol

Cholesterol synthesis occurs in the cytoplasm and microsomes from the two-carbon acetate group of acetyl-CoA. The process has five major steps (as shown in figure 1.3);

a) Acetyl-CoAs are converted to HMG-CoA
b) HMG-CoA is converted to mevalonate
c) Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO₂. IPP is converted to Geranylpyrophosphate. Further, Geranylpyrophosphate (GPP) is converted to Farnesylpyrophosphate (FPP).
d) FPP to Squalene
e) Squalene is converted to Lanosterol, which further yields Cholesterol.

The acetyl-CoA utilized for cholesterol biosynthesis is derived from an oxidation reaction (eg, fatty acids or pyruvate) in the mitochondria and is transported to the cytoplasm. Acetyl-CoA can also be derived from cytoplasmic oxidation of ethanol by acetyl-CoA synthetase. All the reduction reactions of cholesterol biosynthesis use nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Acetyl-CoA units are converted to mevalonate by a series of reactions that begins with the formation of HMG-CoA. Unlike the HMG-CoA formed during ketone body synthesis in the mitochondria, this form is synthesized in the cytoplasm. However, the pathway and the necessary enzymes are the same as those in the mitochondria.
1.11.3 Mechanism of action of statin

3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) act by blocking the HMG-CoA reductase enzyme, which catalyzes the rate-limiting step in de novo cholesterol synthesis. All statins are competitive inhibitors of HMG-CoA reductase with respect to the binding of the substrate, HMG-CoA, but not for that of the co-enzyme NADPH, suggesting that their HMG-CoA-like moieties bind to the HMG-CoA-binding portion of the enzyme active site.

1.11.4 Effects on Thrombosis

Statins are considered to play a pivotal role in modulating the levels of important elements in the process of thrombosis. Statins have varying effects on different prothrombotic factors, such as tissue factor pathway inhibitor, tissue factor, platelet aggregation, blood and plasma viscosity, fibrinogen and plasminogen activator inhibitor 1 (PAI-1). Long-term statin treatment leads to a significant reduction of fibrinogen levels, normalization in thrombin generation, and a reduction of platelet aggregation induced by ADP in hypercholesterolemic patients.

1.11.5 Pharmacokinetics

Lovastatin and simvastatin are administered as lactone prodrugs, and are enzymatically hydrolysed in vivo to their active, hydroxy-acid form. All statins are...
absorbed rapidly following administration; reaching peak plasma concentration (Tmax) within 4 h. Food intake has a variable effect on statin absorption.  

**1.11.6 Pharmacodynamics**

Lovastatin is the most effective at lowering LDL-C, with reductions of up to 63% reported with a daily dose of 40 mg. In general, statins are well tolerated and serious adverse events are rare. The most serious adverse effect associated with statin therapy is myopathy, which may progress to fatal or nonfatal rhabdomyolysis.