CHAPTER 2:

LITERATURE REVIEW
2.1. Human Immunodeficiency Virus (HIV)

HIV is a lentivirus, and like all viruses of this type, it attacks the immune system. Lentiviruses are in turn part of a larger group of viruses known as retroviruses. Retroviruses are the exception because their genes are composed of Ribonucleic Acid (RNA). The name 'lentivirus' literally means 'slow virus' because they take such a long time to produce any adverse effects in the body. There are two types of HIV: HIV-1 and HIV-2. HIV-2 is less easily transmitted, and the period between initial infection and illness is longer in the case of HIV-2. Worldwide, the predominant virus is HIV-1, and generally when people refer to HIV without specifying the type of virus they will be referring to HIV-1. The relatively uncommon HIV-2 type is concentrated in West Africa and is rarely found elsewhere.¹

2.1.1. HIV life cycle

2.1.1.1. Entry

Outside of a human cell, HIV exists as roughly spherical particles (sometimes called virions). The surface of each particle is studded with lots of little spikes. HIV can only replicate inside human cells. The process typically begins when a virus particle bumps into a cell that carries on its surface a special protein called CD4. The spikes on the surface of the virus particle stick to the CD4 and allow the viral envelope to fuse with the cell membrane. The contents of the HIV particle are then released into the cell, leaving the envelope behind.

2.1.1.2. Reverse transcription and integration

Once inside the cell, the HIV enzyme reverse transcriptase converts the viral RNA into DNA, which is compatible with human genetic material. This DNA is transported to the cell's nucleus, where it is spliced into the human DNA by the HIV enzyme integrase. Once integrated, the HIV DNA is known as provirus
2.1.1.3. Transcription and Translation

HIV provirus may lie dormant within a cell for a long time. But when the cell becomes activated, it treats HIV genes in much the same way as human genes. First it converts them into messenger RNA (using human enzymes). Then the messenger RNA is transported outside the nucleus, and is used as a blueprint for producing new HIV proteins and enzymes.

2.1.1.4. Assembly, budding and maturation

Among the strands of messenger RNA produced by the cell are complete copies of HIV genetic material. These gather together with newly made HIV proteins and enzymes to form new viral particles. The HIV particles are then released or 'bud' from the cell. The enzyme protease plays a vital role at this stage of the HIV life cycle by chopping up long strands of protein into smaller pieces, which are used to construct mature viral cores. The newly matured HIV particles are ready to infect another cell and begin the replication process all over again. In this way the virus quickly spreads through the human body.

2.2. Acquired Immune Deficiency Syndrome (AIDS)

AIDS is a disease of the human immune system caused by the HIV. The illness interferes with the immune system, making people with AIDS much more likely to get infections, including opportunistic infections and tumors that do not affect people with working immune systems. This susceptibility gets worse as the disease continues. HIV is transmitted in many ways, such as: sexual intercourse; contaminated blood transfusions and hypodermic needles; and exchange between mother and baby during pregnancy, childbirth, and breastfeeding. It can be transmitted by any contact of a mucous membrane or the bloodstream with a bodily fluid that has the virus in it, such as the blood, semen, vaginal fluid, preseminal fluid, or breast milk from an infected person.

AIDS is the ultimate clinical consequence of infection with HIV. HIV primarily infects vital organs of the human immune system such as CD4+ T cells (a subset of T
cells), macrophages and dendritic cells. It directly and indirectly destroys CD4+ T cells. Once the number of CD4+ T cells per microliter of blood drops below 200, cellular immunity is lost. Acute HIV infection usually progresses over time to clinical latent HIV infection and then to early symptomatic HIV infection and later to AIDS, which is identified either on the basis of the amount of CD4+ T cells remaining in the blood, and/or the presence of infections.

The virus and disease are often referred to together as HIV/AIDS. The disease is a major health problem in many parts of the world, and is considered a pandemic, a disease outbreak that is not only present over a large area but is actively spreading. In 2009, the World Health Organization (WHO) estimated that there are 33.4 million people worldwide living with HIV/AIDS, with 2.7 million new HIV infections per year and 2.0 million annual deaths due to AIDS. In 2007, UNAIDS estimated: 33.2 million people worldwide were HIV positive; AIDS killed 2.1 million people in the course of that year, including 330,000 children, and 76% of those deaths occurred in sub-Saharan Africa. According to UNAIDS 2009 report, worldwide some 60 million people have been infected since the start of the pandemic, with some 25 million deaths, and 14 million orphaned children in southern Africa alone. However, with the adherence to HAART the efficacy rate of the available treatment has increased up to 85% against the AIDS as well as secondary diseases such as Kaposi’s sarcoma.

2.3. Antiretroviral (ARV) drug treatment

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV. The aim of antiretroviral treatment is to keep the amount of HIV in the body at a low level. This stops any weakening of the immune system and allows it to recover from any damage that HIV might have caused already. The treatment consists of drugs that have to be taken every day for the rest of a person’s life. If only one drug was taken, HIV would quickly become resistant to it and the drug would stop working. There are different classes of ARV drugs that act on different stages of the HIV life-cycle. (Table 2.1) HIV can easily develop resistance to individual ARV therapies, but it is harder for HIV to become drug-resistant when multiple ARV drugs with varied mechanisms of action are combined into a single HIV treatment. Taking
two or more ARV at the same time vastly reduces the rate at which resistance would develop, making treatment more effective in the long term.

Table 2.1. Classification of ARV drugs.

<table>
<thead>
<tr>
<th>Antiretroviral drug class</th>
<th>Mechanism of action</th>
<th>Generic name of drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion or Entry Inhibitors</td>
<td>Prevent HIV from binding to or entering human immune cells</td>
<td>Enfuvirtide, Maraviroc</td>
</tr>
<tr>
<td>Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)</td>
<td>NRTIs inhibit reverse transcription by being incorporated into the newly synthesized viral DNA strand as faulty nucleotides</td>
<td>Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abcavir, Emtricitabine, Tenofovir</td>
</tr>
<tr>
<td>Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)</td>
<td>NNRTIs inhibit reverse transcriptase by binding to an allosteric site of the enzyme</td>
<td>Efavirenz, Nevirapine, Loviride, Delavirdine, Etravirine, Rilpivirine, Lersivirine</td>
</tr>
<tr>
<td>Protease Inhibitors (PIs)</td>
<td>PIs target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for the final assembly of new virions.</td>
<td>Saquinavir, Ritonavir, Indinavir, Nelfinavir, Amprenavir, Tipranavir, Lopinavir, Darunavir, Atazanavir, Nelfinavir</td>
</tr>
<tr>
<td>Integrase Inhibitors</td>
<td>Inhibit the enzyme integrase, which is responsible for integration of viral DNA into the DNA of the infected cell.</td>
<td>Raltegravir</td>
</tr>
</tbody>
</table>

Taking a combination of three or more anti-HIV drugs is referred to as Highly Active Antiretroviral Therapy (HAART). The usual HAART regimen combines three or more different drugs such as two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor (PI), two NRTIs and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or other such combinations.11
2.4. Current limitations in ARV drug therapy

ARV drug therapy has contributed significantly to improved patient/disease management, its current use is associated with several disadvantages and inconveniences to the HIV/AIDS patient. Many ARV drugs undergo extensive first pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability. The half-life for several ARV drugs is short, which then requires frequent administration of doses leading to decreased patient compliance. A major limitation is that HIV is localised in certain inaccessible compartments of the body such as the CNS, the lymphatic system and within the macrophages. These sites cannot be accessed by the majority of drugs in the therapeutic concentrations required; and the drugs also cannot be maintained for the necessary duration at the site of HIV localisation. These sub-therapeutic drug concentrations and short residence time at the required sites of action contribute significantly to both the failure of eliminating HIV from these reservoirs, and the development of multidrug-resistance against the ARV drugs. The severe side effects associated with ARV therapy can therefore be attributed to the subsequent large doses essential for achieving a therapeutic effect, due to the inadequate drug concentrations at the site of action, and/or the poor bioavailability of several ARV drugs. These drugs also suffer from physico-chemical problems such as poor solubility that may lead to formulation difficulties. Strategies currently being investigated to overcome these limitations include the identification of new and chemical modification of existing chemical entities, the examination of various dosing regimens, as well as the design and development of novel drug delivery systems (NDDS) that can improve the efficacy of both existing and new ARV drugs. More specifically, in the past decade there has been an explosion of interest in the development of NDDS for the incorporation of ARV drugs as a way of circumventing the problems described above and optimising the treatment of HIV/AIDS patients. NDDS present an opportunity for formulation scientists to overcome the many challenges associated with ARV drug therapy. The nanometer size and high surface area to volume ratio which affect the pharmacokinetics and biodistribution of the associated drug molecule are main features of NDDS.
2.5. Nanotechnology in drug delivery system

Nanotechnology received a lot of attention with the never-seen-before enthusiasm because of its future potential that can literally revolutionize each field in which it is being exploited. In drug delivery, nanotechnology is just beginning to make an impact. Many of the current “nano” drug delivery systems, however, are remnants of conventional drug delivery systems that happen to be in the nanometer range, such as liposomes, polymeric micelles, nanoparticles, dendrimers, and nanocrystals. The importance of nanotechnology in drug delivery is in the concept and ability to manipulate molecules and supramolecular structures for producing devices with programmed functions.

Successful delivery of drugs often requires processing in order to create more desirable physicochemical properties for effective drug delivery. Due to rapid advancements in nanotechnology, significant effort has been devoted to developing nanoparticle processes in order to address issues associated with the current pharmaceutical challenges. These challenges include delivering drugs with poor water solubility, target-specific drug therapy, cost-reduction and product lifecycle extension. In addition, these technologies offer a suitable means of delivering a wide range of drugs including small molecular weight drugs, as well as macromolecules such as proteins, peptides or genes by either localized or targeted delivery to the tissue of interest. Nanoparticle technologies have already had a significant impact on drug delivery systems in terms of improving the performance of existing drugs and enabling the use of new drug candidates.

2.6. Drug delivery systems selected for the study

2.6.1. Nanosuspension (NS)

NSs consist of an internal phase and an external phase in which the internal phase is dispersed uniformly throughout the external phase. The dispersed phase can be pharmaceutical solid or semi-solid colloidal particles with the particle size ranging from 10 to 1000 nm. NSs demonstrate special physical and chemical properties being different to the bulk materials. The main properties of drug NSs are increased
saturation solubility and increased surface area, both leading to an increase in the rate of dissolution. Nanocrystals are therefore useful in increasing the oral bioavailability where drug dissolution is the rate limiting step (Biopharmaceutics Classification System (BCS) class II drugs).

NS can be defined as colloidal dispersions of nano-sized drug particles produced by nanonization methods and stabilized by GRAS (Generally regarded as safe) listed stabilizers. The dispersion can be in water, aqueous solutions or non-aqueous solutions. The stabilizer character and concentration play an important role in creating a stable formulation. Too little stabilizer induces agglomeration or aggregation and too much stabilizer promotes Ostwald ripening.

In order to obtain a stable suspension, stabilizers are usually necessary to stabilize the nanoparticles against attractive forces between particles. These attractive forces, caused by dispersion or van der Waals forces, can increase significantly when the particles approach each other, eventually resulting in an irreversible agglomeration. This process can be slowed down by either enhancing the repulsive forces or reducing the attractive forces between the particles. There are two stabilization mechanisms imparting repulsive forces or energy barriers to a dispersion system—steric stabilization and electrostatic stabilization.

**Steric stabilization** is obtained by adsorbing polymers onto the particle surface which provides effective steric barriers to aggregation. Steric stabilizers commonly used in pharmaceutical systems include non-ionic polymers such as hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), povidone (PVP), polyvinyl alcohol (PVA), and poloxamer. Stabilizing polymers should have a strong surface affinity to the solid-liquid interface, and their polymer chains should be long enough to provide enough steric barriers at the interface, but not too large to impede dissolution. Non-ionic nonpolymeric surfactants such as polysorbate 80 (Tween 80) have also been used as stabilizers. In addition to stabilizing the system, these can also help as wetting and dispersant agents for very hydrophobic drugs.

However, smaller surfactants alone do not effectively provide enough barriers at the interface, and are more prone to a shift in size distribution to larger particles (Oswald ripening) and particle growth. Oswald ripening is a process that occurs due to differences in the dissolution rates of particles of different sizes. Since smaller
particles dissolve faster as described by the Prandtl equation, their contribution to the drug in solution phase is higher. Due to the dynamic nature of equilibrium of drug in solid and solution phase, larger particles begin to receive more drug from the solution phase and grow in size.

**Electrostatic stabilization** is obtained by adsorbing charged molecules onto the particle surface to provide effective electrostatic barriers to aggregation. Electrostatic stabilizers commonly used in pharmaceutical system include anionic surfactants or polymers such as sodium lauryl sulfate (SLS), and sodium di(2-ethylhexyl) sulfosuccinate (DOSS).²¹,²³ The negatively charged ions or molecules on the particle surface provide an electrical barrier to the particles. The combination of steric and electrostatic stabilization is often used to obtain long-term stabilization. For example, an increase in the stability of nanoformulations containing glycol copolymers was caused by the addition of SLS, an anionic surfactant. At high temperatures, the solubility of glycol copolymers in water is reduced, which leads to visible polymer aggregates (cloud point). Therefore, the ability to autoclave such formulations are limited. SLS in this case also acts as a cloud point modifier by raising the cloud point of the polymer, thus enhancing stability at higher temperature.²² Another example is in the case of viscosity increase in NSs due to flocculation. This problem could be minimized by the addition of anionic surfactants, such as SLS or DOSS, which would improve surface wetting and may also provide electrostatic stabilization.²¹ However, adding excessive surfactants should be avoided as this can cause increased solubility and Ostwald ripening, which is a phenomenon caused by the diffusion of smaller particles to form larger particles.

This technology can meet the drug development industry requirements, such as increasing solubility of poorly water soluble drugs; easily transferable to the production scale; cost effective and with little or no regulatory hurdles.²⁴

### 2.6.1.1. Properties of nanosuspension and formulation theory

NS can be formed by building drug particles up from the molecular dimensions as in precipitation, or by nanonizing the micron sized particles down as in milling.¹⁹ In either case, a new surface area ΔA, is created, and also is free energy (ΔG). ΔG = γs/l
ΔA, in which $\gamma_{s/l}$ is the interfacial tension. The system prefers to reduce this increase in surface area by either dissolving incipient crystalline nuclei, in the case of precipitation, or by agglomerating nanosized particles.

This tendency is resisted by addition of surface-active agents, which reduce the $\gamma_{s/l}$ and therefore the free energy of the system. Surface active agents are more effective when present at the time of creation of the new, fresh surface than if added afterwards. Nanocrystals are stabilized by two classes of surface-active agents; charged or ionic surfactants, which affect the electrostatic repulsion among the particles; and non-inonic polymers, which confer a steric repulsion i.e., they resist aggregation.$^{19}$

Nano-sizing of drugs results in increased dissolution velocity and saturation solubility. Dissolution velocity is an important parameter affecting the oral bioavailability of drugs. Poor water solubility correlates with slow dissolution rate ($dc/dt$), and inherently lower bioavailability. According to the Noyes Whitney equation: $dc/dt = D.A (Cs-C)/h$, where, $dc/dt$ is the dissolution velocity and it is proportional to the surface area ($A$) of the particle and saturation solubility ($Cs$). Saturation solubility is a compound specific constant and depends on the temperature and properties of dissolution medium. However, for a particle below 1-2 $\mu$m, the saturation solubility is also a function of particle size.$^{25}$ Nano-sizing increases the saturation solubility because the dissolution pressure (dissolving molecules) increases due to the strong curvature of the particles.$^{20}$ Both enlarging the surface area ($A$) and increasing the saturation solubility ($Cs$) can improve the drug dissolution rate. Oral NSs have been specifically used to increase the rate and extent of drug absorption.$^{26}$

In such an example, a comparison between a danazol NS (average particle size 169 nm) and a conventional danazol suspension (particle size 10 $\mu$m) was made. The NS showed higher Cmax and AUC values in a pharmacokinetic study conducted in dogs. The bioavailability of the NS was equivalent to that of a cyclodextrin solution formulation indicating that the dissolution rate limited bioavailability observed with the 10 $\mu$m suspension had been overcome.$^{27}$
2.6.1.2. Preparation of nanosuspension

There are several production techniques to manufacture NS. Typically, NSs can be produced by precipitation or disintegrating process and are stabilized by surfactant(s) or polymer(s). Owing to their small particle size, the NS can be given by different routes of administration. In addition NS can be converted into solid dosage forms intended for oral delivery (tablets, pellets or granules containing capsules) for increased patient convenience.

Precipitation and disintegration are so called bottom up and top down technologies in NS production. In a precipitation method the drug is dissolved in one solvent, which is subsequently poured into a non-solvent. There are two phases involved in precipitation process, the initial creation of nuclei and their subsequent growth into nanocrystals. Examples of this technique are hydrosols developed by Sucker (Novartis, previously Sandoz).

The basic advantage of the precipitation technique is that they use relatively simple, low cost equipment. Scale-up is relatively easy using static blenders or micromixers. Disadvantages are the use of organic solvents and difficulty in avoiding growth from nanocrystal to microcrystal dimensions during precipitation. In addition, drugs should be soluble in all organic solvents to produce nanocrystals. As a result of the disadvantages addressed, this method hasn’t been widely used by the pharmaceutical industry.

Commonly used methods for NS formation are disintegration processes, which are top down techniques. Drugs are disintegrated using two basic disintegration technologies; wet milling or high pressure homogenization principles.

2.6.1.2.1. Wet milling

Wet milling is a particle size reduction technology whereby drug crystals are comminuted using high-shear media mills in the presence of surface stabilizer(s) and grinding media. The grinding media consist of rigid media with an average size ranging from 0.4 to 3 mm. The grinding materials may be composed of glass, zirconium oxide, ceramics and plastics (e.g., cross-linked polystyrene resin). The
typical process temperature during is less than 40°C to prevent thermal degradation. In general, the technology involves pre-dispersing drug in aqueous solution containing hydrophilic stabilizers and then the slurry is wet milled with a grinding media over a specified time period. High energy shear forces and the forces generated during impaction mechanically break down drug crystals into nanometer-sized particles which are suspended in a polymer solution.

Wet milling often requires grinding for hours to days in order to achieve a desired size range of nanocrystalline particles. The level and type of stabilizer are important parameters to achieve nanoparticle size using this technology and should be investigated for each situation. It was found that higher molecular weight polymeric stabilizers were optimal for effective particle size reduction and shelf stability. Additionally, the size of the grinding media, flow rate and speed of the mill rotor can also be adjusted to achieve optimum results.

NanoSystems™ has commercialized its technology resulting in two products. The first product approved by the United States Food and Drug Administration (FDA) is the reformulation of Rapamune®, a lipophilic macrolide immunosuppressant (marketed by Wyeth Pharmaceuticals). Previously, Rapamune® (sirolimus) was only available as an oral solution which contains solubilizing agents such as tween 80, phosphatidylcholine, mono- or diglycerides and propylene glycol. The current oral formulation requires storage under refrigerated conditions and additional preparation steps prior to use. A new formulation is now available as a tablet dosage form in which the particle size of the drug substance is reduced to less than 200nm in order to improve drug’s water solubility. The new tablet formulation also enables more convenient administration and storage than the Rapamune oral solution. The second product is Emend® (aprepitant) developed as a new drug in a NanoCrystal® formulation. It is an antiemetic therapeutic agent used to prevent and control nausea and vomiting caused by chemotherapy treatment.

2.6.1.2.2. High pressure homogenization

The second most frequently used disintegration method is high pressure homogenization. The two existing homogenization methods applied are
microfluidisation and piston-gap homogenization. In microfluidisation, the suspension is sprayed with high velocity into specially designed homogenization chamber. Where, the flow of the suspension stream changes its direction a few times leading to particle collision and shear forces. A disadvantage of the technique is a relatively large fraction of microparticles in the final product thus losing the benefits of a homogenous suspension.

An alternative to wet milling and the microfluidisation is the piston-gap type high pressure homogenizers (e.g. Avestin, APV Gaulin, Stansted). DissoCubes® technology (trade name currently owned by Skye Pharma) was developed by Muller et al.\textsuperscript{31} using a piston-gap homogenizer.

The initial drug suspension contained in a cylinder of diameter about 3 mm, passes suddenly through a very narrow homogenization gap of 25 $\mu$m. During homogenization, the fracture of drug particles is brought about by cavitations, high shear forces and the collision of the particles against each other, breaking the microcrystals into nanocrystals.\textsuperscript{32} The implosion forces are sufficiently high to break down the drug microparticles into nanoparticles. Additionally, the collision of the particles at high speed helps to achieve nano-sizing of the drug.

Low temperature manufacturing is preferred. The addition of viscosity enhancers is advantageous in certain cases as increasing the viscosity increases the powder density within the dispersion zone (homogenization gap).\textsuperscript{32} The high pressure homogenizer can be operated at pressures varying from 100 to 2000 bars. A number of homogenization cycles usually 3, 5 or 10 cycles can be carried out to obtain the nanosized drug. However, the drug should be pre-milled to get the particle size below 25 $\mu$m in order to prevent blocking of the homogenization gap.

Characteristics of the particles (particle size distribution) are influenced by homogenizer type, applied pressure, number of homogenization cycles and hardness of the drug particles. Changes in drug crystallinity have been reported for high pressure homogenization technology.\textsuperscript{33} The use of high pressures can cause changes in the crystal structure and may also produce uncontrollable variations of amorphous structure.
2.6.1.3. Characterization tests

A prerequisite for the development of optimized NS is its characterization. A NS must have a minimal tendency for the agglomeration of particles. A suspension with slow sedimentation rate is preferable, provided the product is re-suspendable and homogeneous. In addition, NS products must be free from toxicity or irritation. A NS given by parenteral route must also be isotonic and non-pyrogenic.

2.6.1.3.1. Particle size distribution and charge

Particle size growth is mainly responsible for agglomeration. Precise sizing techniques can give useful information about the particle size distribution in NS. The frequently used techniques are laser diffraction and photon correlation spectroscopy (PCS), which are based on different operating principles to measure the particles size. Laser diffraction is fast and suitable for screening large numbers of samples, acquiring data in the useful range of 0.02-2,000 μm. However, input of the refractive index (RI) of the samples is required. PCS is also rapid, but only covers the range of 0.02-3 μm.24

The zeta potential is the potential at the surface of the hydrodynamic shear plane and can be obtained from electrophoretic mobility. Zeta potential is one of the methods to predict stability of suspensions since it measures the potential difference between the electrical double layer and the bulk liquid. Both the stabilizer and the drug govern the zeta potential of a NS.34 For electrostatically stabilized NS, minimum zeta potential should be ±30 mV, and for combined steric and electrostatic stabilization, it should be a minimum of ±20 mV.21,35

2.6.1.3.2. Particle morphology and crystalline state

The particle morphology assessment helps in understanding the morphological changes that a drug might undergo when subjected to nanosizing. In order to get an actual idea of particle morphology, scanning electron microscopy (SEM) can be applied.32
It is essential to investigate the extent of amorphous drug nanoparticles generated during the production of NS. The change in the physical state of the drug particle as well as the extent of the amorphous fraction can be determined by X-ray diffraction (XRD) analysis and can be supplemented by differential scanning calorimetry (DSC). DSC can detect the presence of crystallinity phase. Compared to the sharp melting peak of the drug crystal, the NS present a broader peak with a markedly lower maximum of temperature. Moreover, a decrease of the temperature maximum related to the NS melting peak is observed when the NS size decreases. The amorphous phase does not show any thermal event.

2.6.1.3.3. In Vitro dissolution to assess the in vivo performance

The bioavailability of a NS given by any route of administration depends on the dissolution of the drug. In vitro dissolution testing in a bio-relevant medium is very important to predict the drug in vivo performance (bioavailability and pharmacokinetics) of the drug (96, 97). Dissolution velocity of the nanocrystal can be affected by pH and the nature of the polymorph, which can in turn affect pharmacokinetics. Poor solubility is generally associated with poor dissolution rate and thus poor oral bioavailability. Nanosized drug can undergo faster dissolution than the un-milled drug because of enhanced surface area, thus increasing a drug’s bioavailability. Hecq et al. showed the enhanced dissolution rate through the nanosizing of nifedipine, 95% of the nanosized nifedipine was dissolved in 60 min compared to 5% for the un-milled nifedipine. The absolute bioavailability in fasted male beagle dogs of nanocrystalline danazol was found to be about 82.3%. However, the bioavailability of an aqueous suspension of conventional danazol particles was found to be just 5.1%. The increased dissolution and oral bioavailability resulting from nanosizing a drug can enhance the pharmacological activity of the drug. Kaysers et al. reported better efficacy with a NS compared to a liposomal formulation of amphotericin B. About 23% reduction in Leishmania donovani was achieved after oral administration of amphotericin B NS, but a 0% reduction was observed after oral administration of the
lipsomal dosage form. It has been demonstrated in rats that reducing drug particle size decreased the gastric irritation and increased the rate of absorption by about four-fold following oral administration of naproxen.\textsuperscript{39}

### 2.6.1.4. Benefits of nanosuspension

NS provide a convenient remedy for administering high doses of drug without the risks routinely associated with conventional formulations containing co-solvents.\textsuperscript{40} Table 2.2 shows benefits of NSs.\textsuperscript{19} Some products produced by NS technology in clinical development or available commercially are shown in Table 2.3.\textsuperscript{19,24}

**Table 2.2. Benefits of nanosuspensions**

<table>
<thead>
<tr>
<th>Physicochemical characteristic</th>
<th>Potential benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased drug amount in dosage form without harsh vehicles (extreme pH, co-solvents)</td>
<td>Intravenous: reduced toxicity, increased efficacy</td>
</tr>
<tr>
<td>Reduced particle size: increased drug dissolution rate</td>
<td>Oral: increased rate and extent of absorption, increased bioavailability of drug: area under plasma versus time curve, onset time, peak drug level, reduced variability, reduced fed/fasted effects. Pulmonary: increased delivery to deep lung</td>
</tr>
<tr>
<td>Solid state: increased drug loading</td>
<td>Reduced administration volumes; essential for intramuscular, subcutaneous, ophthalmic use</td>
</tr>
<tr>
<td>Solid state: increased stability</td>
<td>Increased resistance to hydrolysis and oxidation, increased physical stability to settling</td>
</tr>
<tr>
<td>Particulate dosage form</td>
<td>Intravenous: potential for intravenous sustained release via monocyte phagocytic system targeting, reduced toxicity, increased efficacy. Oral: potential for reduced first-pass hepatic metabolism</td>
</tr>
</tbody>
</table>
Table 2.3. Overview of nanosuspension based formulations (until 2009) of drugs in the market and in different clinical phases

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Drug Delivery Company</th>
<th>Route</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td>Anti-cancer</td>
<td>American BioScience</td>
<td>Intravenous</td>
<td>Phase III</td>
</tr>
<tr>
<td>Undisclosed multiple</td>
<td>Anti-infective</td>
<td>Baxter NANOEDGE</td>
<td>Oral, intravenous</td>
<td>Preclinical to Phase II</td>
</tr>
<tr>
<td>Undisclosed</td>
<td>Anticancer</td>
<td>Baxter NANOEDGE</td>
<td>Intravenous, oral</td>
<td>Preclinical to Phase II</td>
</tr>
<tr>
<td>Rapamune (sirolimus)</td>
<td>Immuno-suppressant</td>
<td>Elan Nanosystems</td>
<td>Oral</td>
<td>Marketed</td>
</tr>
<tr>
<td>Emend (aprepitant)</td>
<td>Anti-emetic</td>
<td>Elan Nanosystems</td>
<td>Oral</td>
<td>Marketed</td>
</tr>
<tr>
<td>Cytokine inhibitor</td>
<td>Crohn’s disease</td>
<td>Elan Nanosystems</td>
<td>Oral</td>
<td>Phase II</td>
</tr>
<tr>
<td>Diagnostic agent</td>
<td>Imaging agent</td>
<td>Elan Nanosystems</td>
<td>Intravenous</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>Thymectacin</td>
<td>Anticancer agent</td>
<td>Elan Nanosystems</td>
<td>Intravenous</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>Budesonide</td>
<td>Asthma</td>
<td>Elan Nanosystems</td>
<td>Pulmonary</td>
<td>Phase I</td>
</tr>
<tr>
<td>Tricor (fenofibrate)</td>
<td>Lipid lowering</td>
<td>Abbott Laboratories</td>
<td>Oral</td>
<td>Marketed</td>
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<tr>
<td>Fenofibrate</td>
<td>Lipid lowering</td>
<td>SkyePharma</td>
<td>Oral</td>
<td>Phase I</td>
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<td>Busulfan</td>
<td>Anticancer</td>
<td>SkyePharma</td>
<td>Intrathecal</td>
<td>Phase I</td>
</tr>
<tr>
<td>Megace ES (megestrol)</td>
<td>Weight gain</td>
<td>Elan Nanosystems</td>
<td>Oral</td>
<td>Marketed</td>
</tr>
<tr>
<td>Insulin</td>
<td>Diabetes</td>
<td>BioSante</td>
<td>Oral</td>
<td>Phase I</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>Mucosal vaccine adjuvant for herpes</td>
<td>BioSante</td>
<td>Oral</td>
<td>Phase I</td>
</tr>
</tbody>
</table>
2.6.2. Self-microemulsifying drug delivery system (SMEDDS)

For drug substances that exhibit poor water solubility but sufficient lipophilic properties, it will be beneficial to dose them in a predissolved state, e.g. in a lipid formulation,\textsuperscript{41-43} thereby reducing the energy associated with a solid-liquid phase transition and overcoming the slow dissolution process after oral intake. Lipid formulations include lipid solutions, emulsions, microemulsions, self-emulsifying drug delivery systems (SEDDS) or SMEDDS.\textsuperscript{44,45} A simple classification system of lipid formulations, based on the polarity of the blend, has been proposed by Pouton (Table 2.4).\textsuperscript{46,47} The most straightforward lipid-based formulation is a lipid solution, classified as a Type I formulation. The obvious advantage of this formulation is its relative simplicity. Nonetheless these formulations are highly dependent on the digestion process and suffer from low solvent capacity. Unless the drug is sufficiently lipophilic (log P>4), formulation as an oil solution is limited to highly potent compounds. Solvent capacity can be increased by adding surface active agents as is the case in type II and III formulations. In addition, the most polar formulations, comprising hydrophilic surfactants and represented by class III, often exhibit self-emulsifying properties.

The application of self-emulsifying systems has gained considerable interest after the commercial success of lipid-based formulations of cyclosporine A (‘Neoral Sandimmune\textsuperscript{TM}, previously known as ‘Sandimmune\textsuperscript{TM}’) and HIV protease inhibitors, saquinavir (‘Fortovase\textsuperscript{TM}’) and ritonavir (‘Norvir\textsuperscript{TM}’), which are commercially available as SMEDDS formulations.\textsuperscript{48-50}

SMEDDS are isotropic mixtures made up of oil, surfactant and sometimes cosurfactant or cosolvent. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result, the formation of which is improved by gentle agitation, \textit{in vivo} provided by gastrointestinal motility.\textsuperscript{51,52} The drug will be solubilised in the GI tract in oil droplets, the large amount and small size (submicron size) of which lead to a considerable increase of surface area from where drug dissolution can take place. Furthermore, these formulations are known to reduce inter- and intra-individual variations in bioavailability, which is believed to be caused by a decreased sensitivity of formulation performance to pre-absorptive solubilisation and dietary status.
SMEDDS can enhance drug absorption by a number of ancillary mechanisms, including reduction of gastric motility and alteration of the physical and biochemical barrier function of the gastro-intestinal mucosa. Considering the classification system as determined by Pouton, SMEDDS are generally categorized as Type III (B) formulations, although literature is not clear on this subject nor is the sub-classification well defined.53

2.6.2.1. Role of SEDDS/SMEDDS in improvement of oral absorption

SEDDS/SMEDDS partially avoids the additional drug dissolution step prior to absorption in the GI tract. They increase the amount of solubilized drug in the intestinal fluids resulting in good drug absorption. Apart from this, absorption of the drug may also be enhanced by using lipid based excipients in the formulation. There are several mechanisms through which increased absorption can be achieved such as: Retardation of gastric emptying time; Increase in effective drug solubility in lumen; Lymphatic transport of the drug; Enterocyte based drug transport; Increasing membrane permeability.54

Medium-chain glycerides induce structural and fluidity changes in the mucosal membrane thus resulting in significant permeability changes. Supporting to this, several in vitro studies have shown that medium-chain glycerides markedly affect the permeability of paracellular markers. Several physical and physiological factors, that may affect the drug absorption from this systems include: 1) whether drug is formulated in an oil or emulsified form and in the later form how it is distributed between the two phases, 2) the absorption pathway of the drug, 3) the nature and particle size of the in vivo emulsion, 4) the role of surfactants/enhancers 5) the metabolic pathway of oil and 6) the tendency of the formulation to slow gastric motility and to promote emptying of the gall bladder. The literature reports that the absorption of drugs from oral dosage forms containing oil(s)/lipid(s) is sometimes increased by the presence of a lipophilic solvent and sometimes remains unaffected or reduced if oil is non-digestible. So it can be predicted that effect of lipid(s) on drug absorption is dependent on the specific combination of drug and lipid involved. The nature of drug and that of lipid, as well as aqueous and lipid solubility of drug are
crucial factors that control drug release/absorption from lipid-based dosage formulation.

### Table 2.4. Typical properties of type I, II, III and IV lipid formulations

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Materials</th>
<th>Characteristics</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Oils without surfactants (e.g. tri-, di- and monoglycerides)</td>
<td>Non-dispersing, requires digestion</td>
<td>GRAS status; simple; excellent capsule compatibility</td>
<td>Formulation has poor solvent capacity unless drug is highly lipophilic</td>
</tr>
<tr>
<td>Type II</td>
<td>Oils and water insoluble surfactants</td>
<td>SEDDS formed without water-soluble components</td>
<td>Unlikely to lose solvent capacity on dispersion</td>
<td>Turbid o/w dispersion (particle size 0.25–2 μm)</td>
</tr>
<tr>
<td>Type IIIA</td>
<td>Oils, surfactants, cosolvents (both water insoluble and water soluble excipients)</td>
<td>SEDDS/SMEDDS formed with water soluble components</td>
<td>Clear or almost clear dispersion; drug absorption without digestion</td>
<td>Possible loss of solvent capacity on dispersion; less easily digested</td>
</tr>
<tr>
<td>Type IIIB</td>
<td>Oils, surfactants, cosolvents</td>
<td>SMEDDS with water-soluble components and low oil content</td>
<td>Clear dispersion, drug absorption without digestion</td>
<td>Likely loss of solvent capacity on dispersion</td>
</tr>
<tr>
<td>Type IV</td>
<td>Water-soluble surfactants and cosolvents (no oils)</td>
<td>Formulation disperses typically to form micellar solution</td>
<td>Formulation has good solvent capacity for many drugs</td>
<td>Likely loss of solvent capacity on dispersion; may not be digestible</td>
</tr>
</tbody>
</table>
For SMEDDS, it has been shown that the oil/water partition coefficient of the drug and droplet size can modulate drug release. The droplet size upon dilution with aqueous media is primarily controlled by the nature and concentration of the emulsifier, and phase diagrams of the oil/nonionic surfactant/ drug can be constructed to identify regions where maximum self-microemulsification occurs. The higher the concentration of emulsifier, the smaller the droplet sizes of the resulting emulsion and the faster is the drug release. The combination of small droplets along with a low oil/water partition coefficient will allow for an optimum drug release from SMEDDS. Similarly, drug release from microemulsion (o/w and w/o), depends on a number of process parameters, such as oil/aqueous phase ratio, the droplet size, the distribution of drug in the phases of microemulsion system and its diffusion rate in both phases. It is observed that the higher water/oil partition coefficient favors the higher bioavailability. It is therefore not surprising that not all water soluble or insoluble drugs can be formulated in water-in-oil microemulsion with a concomitant improvement of their intestinal absorption. Though direct determination of drug distribution between the aqueous and oil phases of microemulsion is difficult water/oil partitioning studies using the aqueous and oil phases of the corresponding microemulsion should be conducted and correlated to the observed oral bioavailability and/or in vitro permeability.52

2.6.2.2. Mechanism of self-emulsification

Conventional emulsions are formed by mixing two immiscible liquids namely water and oil stabilized by an emulsifying agent. When an emulsion is formed surface area expansion is created between the two phases. The emulsion is stabilized by the surfactant molecules that form a film around the internal phase droplet. In conventional emulsion formation, the excess surface free energy is dependent on the droplet size and the interfacial tension. If the emulsion is not stabilized using surfactants, the two phases will separate reducing the interfacial tension and the free energy.55 In case of SMEDDS, the free energy of formation is very low and positive or even negative which results in thermodynamic spontaneous emulsification. It has been suggested that self-emulsification occurs due to penetration of water into the Liquid Crystalline (LC) phase that is formed at the oil/surfactant-water interface into
which water can penetrate assisted by gentle agitation during self-emulsification. After water penetrates to a certain extent, there is disruption of the interface and a droplet formation. This LC phase is considered to be responsible for the high stability of the resulting nanoemulsion against coalescence.

2.6.2.3. SMEDDS components

SMEDDS are easily manufactured and physically stable isotropic mixture of oil, surfactant, cosurfactant and drug substances that are suitable for oral delivery in soft and hard gelatin capsules. Self-emulsifying formulations are easily dispersed in the GI tract, where the motility of stomach and small intestine provides the agitation necessary for emulsification. SMEDDS forms transparent microemulsion with a size of less than 100 nm. Small lipid droplet size and associated greater lipid surface are produced by SMEDDS formulation facilitating lipid digestion resulting in more rapid incorporation of the drug into the bile salt mixed micelles. The ultimate result is an increase in the degree and uniformity of drug absorption relative to that associated with simple lipid solution of drug. The improved drug absorption provided by SMEDDS depends upon maintenance of drug in solubilized state until it is absorbed from GIT. In intestine where lipid vehicle hydrolysis rate exceeds that of drug absorption, luminal drug precipitation can occur resulting in suboptimal and more variable drug absorption.

Self-emulsification has been shown to be specific to the nature of the oil/surfactant pair; the surfactant concentration and oil/surfactant ratio; and the temperature at which self-emulsification occurs. In support of these facts, it has also been demonstrated that only very specific pharmaceutical excipient combinations could lead to efficient self-emulsifying systems.

2.6.2.3.1. Oils

The oil represents one of the most important excipients in the SMEDDS formulation not only because it can solubilize marked amounts of the lipophilic drug or facilitate self-emulsification but also and mainly because it can increase the fraction of
lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract depending on the molecular nature of the triglyceride. Both long and medium chain triglyceride oils with different degrees of saturation have been used for the design of self-emulsifying formulations. Furthermore, edible oils which could represent the logical and preferred lipid excipient choice for the development of SMEDDS are not frequently selected due to their poor ability to dissolve large amounts of lipophilic drugs. Modified or hydrolyzed vegetable oils have been widely used since these excipients form good emulsification systems with a large number of surfactants approved for oral administration and exhibit better drug solubility properties. They offer formulative and physiological advantages and their degradation products resemble the natural end products of intestinal digestion. Novel semi synthetic medium chain derivatives, which can be defined as amphiphilic compounds with surfactant properties, are progressively and effectively replacing the regular medium chain triglyceride oils in the SEOFs.

2.6.2.3.2. Surfactants

Several compounds exhibiting surfactant properties may be employed for the design of self-emulsifying systems, the most widely recommended ones being the non-ionic surfactants with a relatively high hydrophilic–lipophilic balance (HLB). The commonly used emulsifiers are various solid or liquid ethoxylated polyglycolyzed glycerides and polyoxyethylene 20 oleate (Tween 80). Safety is a major determining factor in choosing a surfactant. Usually the surfactant concentration ranges between 30 and 60% w/w in order to form stable SMEDDS. It is very important to determine the surfactant concentration properly as large amounts of surfactants may cause GI irritation. The surfactant involved in the formulation of SMEDDS should have a relatively high HLB and hydrophilicity so that immediate formation of o/w droplets and/or rapid spreading of the formulation in the aqueous media (good self-emulsifying performance) can be achieved. Surfactants are amphiphilic in nature and they can dissolve or solubilize relatively high amounts of hydrophobic drug compounds. The lipid mixtures with higher surfactant and co-surfactant/oil ratios lead to the formation of SMEDDS. The formulation of w/o microemulsions for use as SEDDS or SMEDDS has been investigated using blends of low and high HLB
surfactants, which are commercially available and pharmaceutically acceptable, typically sorbitan esters and Tween 80. The oil phase comprised long or medium chain length glycerides.61

2.6.2.3. Co-solvents

The production of an optimum SMEDDS requires relatively high concentrations (generally more than 30% w/w) of surfactants. Organic solvents such as, ethanol, propylene glycol (PG), and polyethylene glycol (PEG) are suitable for oral delivery, and they enable the dissolution of large quantities of either the hydrophilic surfactant or the drug in the lipid base. These solvents can even act as co-surfactants in microemulsion systems. On the other hand, alcohols and other volatile co-solvents have the disadvantage of evaporating into the shells of the soft gelatin, or hard, sealed gelatin capsules in conventional SEDDS leading to drug precipitation. Thus, alcohol–free formulations have been designed52, but their lipophilic drug dissolution ability may be limited.

2.6.2.4. Formulation of SMEDDS

SMEDDS are composed of oil, hydrophilic surfactant, and a cosurfactant. The process of self-emulsification is only specific to certain combinations of pharmaceutical excipients. The primary step during formulation of a SMEDDS is the identification of specific combinations of excipients and constructing a phase diagram which shows various concentrations of excipients necessary for self-emulsification. Mutual miscibility of these excipients is also important for producing a stable liquid formulation. Selection of excipients for SMEDDS lies in identifying excipients combination which will solubilize the entire dose of drug in volume acceptable for unit oral administration. Excipients combinations yielding SMEDDS formulations are identified by construction of pseudo-ternary phase diagram. Pseudo-ternary phase diagram can be represented in a triangular format (triangle) which has three coordinates. Each coordinate represents one component of microemulsion system.
2.6.2.4.1. Drug incorporation

Poorly water soluble drugs are often a choice for SMEDDS based dosage form. It is essential that the therapeutic dose of the drug be soluble in an acceptable volume of self-emulsifying mixture. The uses of newer synthetic oils that are amphiphilic in nature can dissolve large quantities of the drug when compared to conventionally used pure vegetable oils or its derivatives. Surfactants also provide good solvency for the drug. Although, the cosolvent is capable of dissolving a large quantity of the drug, they may cause drug precipitation on aqueous dilution due to loss of solvent capacity. This necessitates performing equilibrium solubility measurements of the drug in the excipients under use. The drug may affect the self-emulsification efficiency by changing optimal oil/surfactant ratio. It may interact with the LC phase of some of the mixture components causing blockage of charge movement through the system or may penetrate the surfactant monolayer. The incorporated drug may increase or decrease the self-emulsifying efficiency or may not affect it at all. Hence SMEDDS should also be evaluated for its self-emulsification efficiency in the presence of the drug. SMEDDS are known to be more sensitive towards any changes in the ratio of excipients. Because of these reasons, pre-formulation solubility and phase diagrams should be thoroughly evaluated when choosing the optimized formulation.

2.6.2.4.2. Capsule compatibility

Liquid SMEDDS (L-SMEDDS) filled in hard and soft gelatin capsules are more acceptable as dosage forms. Presence of hygroscopic material in the liquid formulation may cause dehydration of capsule shell or polar molecules such as polyethylene glycol or alcohol may penetrate into the capsule shell. Thus it is necessary to investigate such effects at an early stage of development. Solid SMEDDS possess an advantage in this regard due to lack of contact of liquid material with the capsule shell.
2.6.2.5. Conversion of L-SMEDDS to Solid SMEDDS

L-SMEDDS can be filled in soft or hard gelatin capsule. Recently, there have been efforts by research groups working on SEDDS/SMEDDS to convert L-SEDDS/SMEDDS to S-SEDDS/SMEDDS. These Solid SEDDS/SMEDDS can be made into tablets or be encapsulated. The primary reason to formulate SEDDS/SMEDDS in a solid form is to consolidate the advantages of L-SMEDDS with convenience of solid oral dosage forms. Oral solid dosage forms have the following advantages: (a) low production cost (b) convenience of process control (c) high stability and reproducibility and (d) better patient compliance. Generally, the formulated SMEDDS are liquid in state, but sometimes it could be in a semisolid state depending on the physical state of excipients used. Researchers have adopted various techniques to obtain this conversion. S-SMEDDS also offers added versatility in terms of possible dosage forms. The following description elaborates various Liquid to Solid SMEDDS conversion techniques.

Spray drying: Spray drying is the most widely used technique to convert Liquid SEDDS/SMEDDS into solid state. In this method the Liquid SEDDS/SMEDDS is mixed with a solid carrier in a suitable solvent. The solvent is then atomized into a spray of fine droplets. These droplets are introduced into a drying chamber, where the solvent gets evaporated forming dry particles under a controlled temperature and airflow conditions. The process parameters required to be controlled are inlet and outlet temperature, feed rate of solvent, and aspiration and drying air flow rate. The dry particles can then be either filled into capsules or made into tablets after addition of suitable excipients. Various solid carriers that have been used for this purpose are: Aerosil 200 suspended in ethanol and aqueous solution of Dextran 40.

Adsorption to solid carriers: The L-SEDDS/SMEDDS can be made to adsorb onto free flowing powders that possess very large surface area and are capable of adsorbing high quantities of oil material. The adsorption can be done either by mixing L-SEDDS/SMEDDS and the adsorbent in a blender or by simple physical mixing. The resulting powders can be either filled into capsules or can be made into tablets after addition of appropriate excipients. The adsorbents are capable of adsorbing Liquid SEDDS up to 70 %w/w of its own weight. Solid carriers used for this purpose can be microporous inorganic substances, high surface area colloidal inorganic
substances or cross-linked polymers. Categories of solid adsorbents used are: silicates, magnesium trisilicate, talcum, crospovidone, cross-linked sodium carboxymethyl cellulose and cross-linked polymethyl methacrylate. Oral solid heparin and gentamicin SMEDDS were prepared using three kinds of adsorbents: microporous calcium silicate (Florite RE), magnesium aluminometa silicate (Neusilin US2) and silicon dioxide (Sylysia 320).

**Encapsulation of Liquid and Semisolid SEDDS:** It is one of the simplest techniques for conversion of Liquid SEDDS to solid oral dosage form. Liquid SEDDS can be simply filled in capsules, sealed using a microspray or a banding process. For a semisolid SEDDS, it is a four step process: (1) heating the semisolid excipients to at least 20°C above its melting point; (2) adding the drug in the molten mixture while stirring; (3) filling the drug loaded molten mixture into the capsule shell and (4) cooling the product to room temperature. The compatibility of the excipients used with the capsule shell should be well investigated. Lipid excipients compatible with the capsule shell are described by Cole et al. Capsule filling of SEDDS is suitable for low dose highly potent drugs and allows high drug incorporation.

**Extrusion Spheronization:** This is a solvent free technique that converts Liquid SEDDS into pellets using extrusion and spheronization processes. In this method the Liquid SEDDS is first mixed with a binder, followed by addition of water until the mass is suitable for extrusion. The extruded mass is then spheronized to form uniform sized pellets. The pellets are then dried and size separated. The relative quantity of water and Liquid SEDDS used in the process has an effect on size distribution, extrusion force, surface roughness of pellets, and disintegration time. High drug incorporation can be achieved by using this technique. Abdalla et al. used microcrystalline cellulose (MCC) as a binder in preparation of progesterone self-emulsifying pellets. A mixture of silicon dioxide, glyceryl behenate, pregelatinized starch, sodium croscarmellose, and MCC were used by Setthacheewakul et al. in the preparation of curcumin loaded SMEDDS pellets.

**Melt Granulation:** Melt Granulation is another solvent free technique for converting liquid SEDDS into solid form. In this method, Liquid SEDDS is mixed with a binder that melts or softens at relatively low temperature. This melted mixture can be granulated. This technique is advantageous since it does not require addition of a
liquid binder and subsequent drying unlike conventional wet granulation. The variables to be controlled in this process are impeller speed, mixing time, binder particle size, and the viscosity of the binder.\textsuperscript{64} A mixture of mono-, di- and triglycerides and esters of polyethylene glycol (PEG) called as Gelucire are used as binders to prepare immediate release pellets by melt granulation and as a self-emulsifying drug delivery system by capsule moulding or as powder obtained by cryogenic grinding.\textsuperscript{72}

### 2.6.2.6. Benefits of SMEDDS

Several benefits of SMEDDS over conventional formulations have been reported:

- Enhanced oral bioavailability enabling reduction in dose.
- More consistent temporal profiles of drug absorption.
- Selective targeting of drug(s) towards specific absorption window in GIT.
- Protection of drug(s) from the hostile environment in gut.
- Reduced variability including food effects.
- Protection of sensitive drug substances.
- Liquid or solid dosage forms.
- In SMEDDS, the lipid matrix interacts readily with water, forming a fine particulate oil-in-water (o/w) emulsion. The emulsion droplets will deliver the drug to the gastrointestinal mucosa in the dissolved state readily accessible for absorption. Therefore increase in AUC i.e. bioavailability and C max is observed with many drugs when presented in SMEDDS.
- Fine oil droplets empty rapidly from the stomach and promote wide distribution of drug throughout the intestinal tract and thereby minimizing irritation frequently encountered with extended contact of drugs and gut wall.
- Ease of manufacture and scale up is one of the most important advantage that make SMEDDS unique when compared to other drug delivery systems like solid dispersion, liposomes, nanoparticles etc.
- SMEDDS has potential to deliver peptides that are processed to enzymatic hydrolysis in GIT.
When polymer is incorporated in composition of SMEDDS it gives prolonged release of medicament.

SMEDDS formulation is composed of lipids, surfactants and co-solvents. The system has the ability to form an oil-on-water emulsion when dispersed by an aqueous phase under gentle agitation. SMEDDS present drugs in a small droplet size and well-proportioned distribution and increase the dissolution and permeability. Furthermore, because drugs can be loaded in the inner phase and delivered to the lymphatic system, can bypass first pass metabolism. Thus SMEDDS protect drugs against hydrolysis by enzymes in the GI tract and reduce the presystemic clearance in the GI mucosa and hepatic first-pass metabolism.73

2.6.3. Nanoparticles

The term ‘nanoparticle’ may be defined as a submicron drug carrier system which is generally (but not necessarily) composed of polymer. The polymer used may or may not be biodegradable even if the polymer biodegradability appears a main characteristic for drug delivery carriers. As a function of the morphological and structural organization of the polymer, we distinguish the ‘nanosphere’ which is a nanoparticle system with a matrix character and constituted by a solid core with a dense polymeric network, and the ‘nanocapsule’ which is formed by a thin polymeric envelope surrounding an oil or water filled cavity. Nanocapsules may, thus, be considered as a ‘reservoir’ system. Practically, the nanoparticles have a size around 200 nm and the drugs or other molecules may be dissolved into the nanoparticles, entrapped, encapsulated and/or adsorbed or attached. Figure 2.1 depicts various nanocarrier based drug delivery carrier systems used for effective drug delivery research.
**Figure 2.1.** Various nanomaterial-based drug delivery platforms: A. Polymeric nanoparticles/micelles B. Liposome C. Buckyball D. Carbon nanotube E. Colloidal gold nanoparticle F. Magnetic nanoparticle G. Quantum dots H. Multifunctional nanoparticle with metallic nanoparticle core (metallic nanoparticle) and semiconductor quantum dots surrounding the shell. Drug molecules can be attached to these carrier systems through encapsulation, mixing, covalent conjugation or electrostatic and affinity interactions.
2.6.3.1. PLGA Nanoparticles

Recently, nano-sized drug delivery systems especially biocompatible and biodegradable polymer nanoparticles have attracted considerable interest since they can offer a suitable means of delivering small molecular weight drugs, proteins or genes to a targeted tissue or organ.\(^{74,75}\) Nanoparticles are colloidal systems that have size typically in the range of 10-1000 nm in diameter, and drug can be entrapped in, adsorbed or chemically coupled onto the polymer nanoparticle matrix. On the other hand, a number of polymers have been investigated for formulating biodegradable nanoparticles, such as polylactide (PLA), poly(3-caprolactone) (PCL) and poly(lactide-co-glycolide) (PLGA) (Figure 2.2). They are biocompatible and biodegradable polymers approved by FDA and have been studied extensively.\(^{76,77}\)

![Diagram of molecular structures of lactide and glycolide based biodegradable polymers](image)

**Figure 2.2.** Molecular structure of lactide and glycolide based biodegradable polymers

PLGA is one of the most successfully used biodegradable nanosystem for the development of nanomedicines because it undergoes hydrolysis in the body to produce the biodegradable metabolite monomers, lactic acid and glycolic acid. Since
the body effectively deals with these two monomers, there is very minimal systemic toxicity associated by using PLGA for drug delivery or biomaterial applications. PLGA nanoparticles have been mostly prepared by emulsification–diffusion, solvent emulsion–evaporation, interfacial deposition and nanoprecipitation method (Figure 2.3). Briefly, in emulsification–diffusion method, the PLGA polymers are dissolved in organic solvent, poured and separated in aqueous phase having stabilizer and subsequently emulsified by homogenizer. In solvent evaporation method, the polymers are dissolved in volatile organic solvent and poured into continuously stirring aqueous phase with or without emulsifier/stabilizer and sonicated. Interfacial deposition methods have been used for the formation of both nanocapsule and nanospheres. The nanoparticles are synthesized in the interfacial layer of water and organic solvent (water miscible) and finally the nanoparticles are separated by centrifugations. Most commonly used method for the preparation of PLGA nanoparticles is nanoprecipitation. Polymer dissolved in acetone is added drop-wise into continuously stirring aqueous phase with or without emulsifier/stabilizer and consequently organic phase is evaporated under reduced pressure (Figure 2.3).

**Figure 2.3.** Different method for preparation of PLGA nanoparticles: PLGA nanoparticles were synthesized by emulsion diffusion, solvent evaporation and nanoprecipitation methods.
PLGA nanoparticles have been used to develop the proteins and peptides nanomedicine, nano-vaccines, nanoparticles based gene delivery system, nano-antigen and growth factor, etc.\textsuperscript{82,83} Surface modification of PLGA, drug encapsulation methods and particle size, additives added during formulation, molecular weight of drug, ratio of lactide to glycolide moieties has strong influence on the release and effective response of formulated nanomedicines.\textsuperscript{84} The acidic nature of PLGA monomers is not suitable for some sensitive drugs or bioactive molecules.\textsuperscript{85} However, the approaches to overcome these problems have been developed. PLGA nanomedicine formulations are blended with alginate, chitosan, pectin\textsuperscript{86}, poly(propylenefumarate)\textsuperscript{87}, polyvinylacohol\textsuperscript{88}, poly(orthoester)\textsuperscript{89} etc. The approval of PLGA has been granted by US Food and Drug Administration (USFDA) for human use and nanomedicines.\textsuperscript{90}

PLGA is approved by FDA for therapeutic use in humans. Various preparation methods have been optimized for PLGA nanoparticles synthesis and numerous cancer related drugs have been incorporated in PLGA. These loaded nanoparticles protect poorly soluble and unstable payloads from the biological milieu and are small enough for capillary penetrations, cellular internalization and endosomal escape.\textsuperscript{82} Furthermore, their surface is modified for targeted delivery of molecules to tumor or other tissues.\textsuperscript{91} The larger size of PLGA nanoparticles is advantageous as multifunctional imaging and probes which incorporate encapsulated cancer drug, release, imaging, and targeting in a single nanoparticles platform.\textsuperscript{92}

The performance of these nanoparticles is not completely satisfactory and great effort is needed to improve its physiochemical properties and synthesis process. The properties of nanoparticles as precursor of good nanomedicine are particle size, size distribution, surface morphology, surface chemistry, surface charge, surface adhesion, surface erosion, interior porosity, drug diffusivity, drug encapsulation efficiency, drug stability, drug release kinetics and hemodynamic. The surface charge of the nanoparticles is important for the cellular internalization of the NPs, clustering in blood flow, adherence, and interaction with oppositely charged cells membrane.\textsuperscript{93} PLGA nanoparticles are frequently used for the encapsulation of various drugs and their successful delivery in vivo. PLGA nanoparticles loaded with hydrophobic poorly soluble drugs are most commonly formulated by nanoprecipitation. Drug release and effective response of PLGA nanoparticles are influenced by (i) the surface
modification, (ii) the method of preparation, (iii) the particle size, (iv) the molecular weight of the encapsulated drug and (v) the ratio of lactide to glycolide moieties.\textsuperscript{94} The cancer related drug paclitaxel, doxorubicin, 5-fluorouracil, 9-nitrocamptothecin, cisplatin, triptorelin, dexamethasone, xanthone, etc., have been successfully encapsulated on PLGA nanoparticles.\textsuperscript{94}

The CD4\textsuperscript{+} T lymphocyte is the major target for infection by HIV-1. Cells of the mononuclear phagocyte system also serve as a reservoir for HIV. Macrophages are mature, non-proliferating and immunologically active cells that can be productively infected with HIV-1 and HIV-2.\textsuperscript{95-97} Altered cellular functions in the macrophage population may contribute to the development and clinical progression of AIDS. Evidence has accumulated that cells of the macrophage lineage are vectors for the transmission of HIV-1. The placental macrophage is likely to be the primary cell type responsible for vertical transmission of HIV-1.\textsuperscript{98} An important property of HIV-1 for mucosal transmission is the ability to infect macrophages.\textsuperscript{99} Because of the important role of cells of the monocytes/macrophage lineage in the pathogenesis of HIV-1, fully effective ARV must react with monocytes/macrophage in addition to other targets.

Macrophages possess various receptors such as fucose receptors, mannosyl, galactosyl, and many others. Mannose receptors are present at the surface of monocyte macrophages, alveolar macrophages, astrocytes in brain, hepatocytes in liver and so on.\textsuperscript{100-102} Therefore, targeting of ARV drugs to HIV infected macrophages could be an attractive approach in improving the therapeutic efficacy and reducing the toxicity of ARV bioactives.\textsuperscript{103}

Polymeric nanoparticles have been used to target ARVs to (i) macrophages/monocytes\textsuperscript{104-106} and (ii) CNS\textsuperscript{107} which act as viral reservoir sites during HIV infection.\textsuperscript{108} Macrophages have been reported to be a major cause of dissemination of the infection in the body in the later stages of the disease during which there is a continuous depletion of CD4\textsuperscript{+} T lymphocytes.\textsuperscript{109} During this period, virus production from these mature non-proliferating macrophages/monocytes is dramatically enhanced without being affected by the lethal effect of the replicating virus. Nanoparticulate mediated targeting of macrophages is well known and has been reported by several authors.\textsuperscript{105,110,111} Following i.v. administration, nanoparticles are removed from the blood circulation by macrophages.\textsuperscript{74} The recognition of particles by
macrophages is mediated by a process called opsonization.\textsuperscript{112} When the distance between the particles and the opsonins is sufficiently small, they can bind to the surface of particles by any of the interactions such as van der Waals, electrostatic, ionic etc. After binding to the surface, particles become recognizable by macrophages and phagocytosis takes place.\textsuperscript{112}

PLGA-nanoparticles are internalized in cells partly through fluid phase pinocytosis and also through clathrin-mediated endocytosis. PLGA-nanoparticles rapidly escape the endo-lysosomes and enter the cytoplasm within 10 min of incubation. This facilitates interactions of nanoparticles with the vesicular membranes leading to transient and localized destabilization of the membrane resulting in the escape of nanoparticles into the cytosol.\textsuperscript{113} The body recognizes hydrophobic particles as foreign. The reticulo-endothelial system (RES) eliminates these from the blood stream and takes them up in the liver or the spleen. This process is one of the most important biological barriers to nanoparticles-based controlled drug delivery.\textsuperscript{94} The binding of opsonin proteins present in the blood serum to injected nanoparticles leads to attachment of opsonized particles to macrophages and subsequently to their internalization by phagocytosis.\textsuperscript{112}

PLGA nanoparticles can act as potential drug carriers to improve the delivery of ARV agents to the mononuclear phagocyte system in vivo, overcoming pharmacokinetic problems and enhancing the activity of drugs for treatment of HIV infection and AIDS. PLGA nanoparticles are worth investigating in this area of research.

\subsection*{2.6.3.2. Characterization of nanoparticles}

Characterization of the nanoparticle carrier systems to thoroughly understand the properties is essential before putting them to pharmaceutical application. After preparation, nanoparticles are characterized at two levels. The physicochemical characterization consists of the evaluation of the particle size, size distribution, and surface properties (composition, charge, hydrophobicity) of the nanoparticles. The biopharmaceutical characterization includes measurements of drug encapsulation, in vitro drug release rates, and in vivo studies revealing biodistribution, bioavailability, and efficacy of the drug.
There are many sensitive techniques for characterizing nanoparticles, depending upon the parameter being looked at; laser light scattering (LLS) or photon correlation spectroscopy (PCS) for particle size and size distribution; scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) for morphological properties; X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) for surface chemistry; and differential scanning calorimetry (DSC) for thermal properties. Parameters such as density, molecular weight, and crystallinity affect release and degradation properties, whereas surface charge, hydrophilicity, and hydrophobicity significantly influence interaction with the biological environment.

2.6.3.2.1. Particle size and morphology

Nanoparticle size is critical not only in determining its release and degradation behaviour but also in determining the efficacy of the therapeutic agent by affecting tissue penetration or even intracellular uptake. Particle size can be determined by PCS, SEM, TEM, AFM.

PCS is a technique employed to determine the mean particle size (PCS diameter) and size distribution (polydispersity index, PI) in Malvern Zetasizer Nanoseries-ZS. It is a light scattering experiment in which the statistical intensity fluctuations in light scattered from the particles are measured. These fluctuations are due to the random brownian motion of the particles. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surround them. The parameter calculated is defined as the translational diffusion coefficient (usually given as D). The particle size is then calculated from the translational diffusion coefficient by the Stokes-Einstein equation.

PCS diameter gives information about the average particle size. The measured PCS diameter is based on the intensity of scattered light and therefore is not identical to the numeric diameter except in case of monodisperse particle suspensions. For polydisperse samples, PCS diameter is larger because it is based on the scattering intensity of the particles.
2.6.3.2. Crystallinity

The physical state of both the drug and the polymer are determined because this will have an influence on the in vitro and in vivo release characteristics of the drug. The crystalline behaviour of polymeric nanoparticles is studied using XRD and thermo-analytical methods such as DSC and differential thermal analysis (DTA).\textsuperscript{115,116} DSC and XRD techniques are often combined to get useful information on the structural characteristics of both drugs and polymers.

2.6.3.2.3. Surface charge

Zeta potential is measure of the surface charge of the nanoparticles. The zeta potential value can influence particle stability and mucoadhesion as well as intracellular trafficking of nanoparticles as a function of pH. High zeta potential values, either positive or negative, should be achieved to ensure stability and to avoid aggregation of the particles. The extent of surface hydrophilicity can then be predicted from the values of zeta potential.\textsuperscript{82} Surface charge is generally determined by well-known electrophoresis method with the help of Zetasizer.\textsuperscript{117}

2.6.3.3. Commercial product based on Poly(lactic acid) (PLA), Poly(glycolic acid) (PGA), and PLGA

The first FDA-cleared PLGA product was the Lupron Depot drug-delivery system (TAP Pharmaceutical Products, Lake Forest, Illinois), a controlled release device for the treatment of advanced prostate cancer that used biodegradable microspheres of 75:25 lactide/glycolide to administer leuprolide acetate over a period of 4 months (replacing daily injections).\textsuperscript{118} A list of commercial products is presented in Table 2.5.
### Table 2.5. FDA-approved and under development (until 2009) drug delivery products using PLGA.

<table>
<thead>
<tr>
<th>Product</th>
<th>Polymer</th>
<th>Active ingredient</th>
<th>Indication</th>
<th>Route of administration</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutropin Depot®</td>
<td>PLGA</td>
<td>Human growth hormone</td>
<td>Growth deficiencies</td>
<td>SC/IM</td>
<td>Marketed</td>
</tr>
<tr>
<td>Sandostatin LAR®</td>
<td>PLGA-glucose</td>
<td>Octreotide acetate</td>
<td>Acromegaly</td>
<td>SC/IM</td>
<td>Marketed</td>
</tr>
<tr>
<td>Trelstar™ Depot</td>
<td>PLGA</td>
<td>Triptorelin pamoate</td>
<td>Prostate cancer</td>
<td>IM</td>
<td>Marketed</td>
</tr>
<tr>
<td>Decapeptyl®</td>
<td>PLGA</td>
<td>Triptorelin pamoate</td>
<td>Prostate cancer</td>
<td>IM</td>
<td>Marketed</td>
</tr>
<tr>
<td>Pamorelin</td>
<td>PLGA</td>
<td>Leuprolide acetate</td>
<td>Prostate cancer</td>
<td>SC</td>
<td>Marketed</td>
</tr>
<tr>
<td>Oncogel®</td>
<td>PEG-PLGA-PEG</td>
<td>Paclitaxel</td>
<td>Solid tumors</td>
<td>Intratumoral injection</td>
<td>Clinical trial; Phase II</td>
</tr>
<tr>
<td>Atridox®</td>
<td>PLGA</td>
<td>Doxycycline hyclate 10%</td>
<td>Chronic adult periodontitis</td>
<td>Topical</td>
<td>Marketed</td>
</tr>
<tr>
<td>Sanvar® SR</td>
<td>PLGA</td>
<td>Vapreotide</td>
<td>Esophageal bleeding varices (EVB)</td>
<td>SC/IM</td>
<td>Clinical trial; Phase III</td>
</tr>
<tr>
<td>Lupron depot</td>
<td>PLA</td>
<td>Leuprolide acetate</td>
<td>Prostate cancer, endometriosis</td>
<td>SC/IM</td>
<td>Marketed</td>
</tr>
<tr>
<td>Zoladex</td>
<td>PLA</td>
<td>Goserelin acetate</td>
<td>Prostate cancer, endometriosis</td>
<td>SC</td>
<td>Marketed</td>
</tr>
</tbody>
</table>

### 2.7. Model Drug – Efavirenz

Efavirenz (EFV) is an HIV-1 specific, NNRTI. EFV is chemically described as (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one. Its empirical formula is $\text{C}_{14}\text{H}_9\text{ClF}_3\text{NO}_2$ and its structural formula is shown in Figure 2.4. EFV is a white to slightly pink crystalline powder with a molecular mass of 315.68. It is practically insoluble in water ($< 10 \mu\text{g/ml}$).\textsuperscript{119}
2.7.1. Physicochemical properties

State: solid\textsuperscript{120}
Melting Point: 139-141°C
Predicted water solubility: 8.55e-03 g/l
Log P: 4.6
pK\textsubscript{a}: 10.2

2.7.2. Mechanism of action

EFV inhibits the activity of viral RNA-directed DNA polymerase (i.e., reverse transcriptase).\textsuperscript{119} Antiviral activity of EFV is dependent on intracellular conversion to the active triphosphorylated form. The rate of EFV phosphorylation varies, depending on cell type. It is believed that inhibition of reverse transcriptase interferes with the generation of DNA copies of viral RNA, which, in turn, are necessary for synthesis of new virions. Intracellular enzymes subsequently eliminate the HIV particle that previously had been uncoated, and left unprotected, during entry into the host cell. Thus, reverse transcriptase inhibitors are virustatic and do not eliminate HIV from the body. Even though human DNA polymerase is less susceptible to the pharmacologic effects of triphosphorylated EFV, this action may nevertheless account for some of the drug's toxicity.
2.7.3. Pharmacokinetics

2.7.3.1. Absorption and distribution

Peak EFV plasma concentrations of 1.6-9.1 µM were attained by 5 hours following single oral doses of 100 mg to 1600 mg administered to uninfected volunteers.\textsuperscript{119} Dose-related increases in $C_{\text{max}}$ and AUC were seen for doses up to 1600 mg; the increases were less than proportional suggesting diminished absorption at higher doses. In HIV-1-infected patients at steady state, mean $C_{\text{max}}$, mean $C_{\text{min}}$, and mean AUC were dose proportional following 200-mg, 400-mg, and 600-mg daily doses. Time-to-peak plasma concentrations were approximately 3-5 hours and steady-state plasma concentrations were reached in 6-10 days. EFV is highly bound (approximately 99.5-99.75%) to human plasma proteins, predominantly albumin. In HIV-1 infected patients who received 200 to 600 mg once daily for at least one month, cerebrospinal fluid concentrations ranged from 0.26 to 1.19% (mean 0.69%) of the corresponding plasma concentration. This proportion is approximately 3-fold higher than the non-protein-bound (free) fraction of EFV in plasma.

2.7.3.2. Metabolism

Studies in humans and in vitro studies using human liver microsomes have demonstrated that EFV is principally metabolized by the cytochrome P450 system to hydroxylated metabolites with subsequent glucuronidation of these hydroxylated metabolites. These metabolites are essentially inactive against HIV-1. The in vitro studies suggest that CYP3A and CYP2B6 are the major isozymes responsible for EFV metabolism. EFV has been shown to induce CYP enzymes, resulting in the induction of its own metabolism.

2.7.3.3. Elimination

The elimination pathway for EFV was mainly through the faeces. Approximately 14-34% of the radiolabel was recovered in the urine and 16-61% was recovered in the feces. Nearly all of the urinary excretion of the drug was in the form of metabolites.
The majority of the compound was recovered in faeces as the 8-hydroxy glucuronide. Less than 1% was excreted in urine as unchanged EFV.

2.7.4. Adverse effect

Psychiatric symptoms, including insomnia, nightmares, confusion, memory loss, and depression, are common, and more serious symptoms such as psychosis may occur in patients with compromised liver or kidney function. The most common adverse effects are rash, dizziness, nausea, headache, fatigue, insomnia, and vomiting. A general guideline about EFV and pregnancy states that EFV can cause birth defects and should not be used in women who might become pregnant. A later study, however, found no increased risk of overall birth defects among women exposed to EFV during the first trimester of pregnancy compared with exposure to other antiretroviral drugs.

2.7.5. Literature review

Some of the research articles on EFV are as follow:

- King J. et al has carried out a randomized crossover study to determine relative bioequivalence of tenofovir, emtricitabine, and efavirenz (Atripla) fixed-dose combination tablet compared with a compounded oral liquid formulation derived from the tablet.

- Chiappetta D. A. et al investigated the synergistic performance of mixed polymeric micelles made of linear and branched poly(ethylene oxide)-poly(propylene oxide) for the more effective encapsulation of the anti-HIV drug efavirenz. Chiappetta D. A. et al also investigated oral pharmacokinetics of the anti-HIV efavirenz encapsulated within polymeric micelles.

- Rajesh Y.V. et al. investigated the impact of superdisintegrants on efavirenz release from tablet formulations. Efavirenz tablets of different doses were prepared by a wet granulation process using different superdisintegrants such as croscarmellose sodium (CCS), sodium starch glycollate (SSG) and
crosspovidone (CP) to evaluate the role of different disintegrants on the in vitro release of EFV.\textsuperscript{128}

- Chiappetta D. A. et al investigated a highly concentrated and taste-improved aqueous formulation of efavirenz for a more appropriate pediatric management of the anti-HIV therapy.\textsuperscript{129}

- Balkundi S. et al studied nano-formulations of crystalline indinavir, ritonavir, atazanavir, and efavirenz by wet milling, homogenization or sonication with a variety of excipients.\textsuperscript{130}

- Sathigari S. et al investigated the physicochemical characterization of efavirenz-cyclodextrin inclusion complexes. This study was to characterize the inclusion complexes of EFV with beta-cyclodextrin (beta-CD), hydroxypropyl beta-CD, and randomly methylated beta-CD to improve the solubility and dissolution of EFV.\textsuperscript{131}

- Dutta T. et al has studied poly (propyleneimine) dendrimer based nanocontainers for targeting of efavirenz to human monocytes/macrophages in vitro.\textsuperscript{132} Dutta T. et al also investigated the targeting of efavirenz loaded tuftsin conjugated poly(propyleneimine) dendrimers to HIV infected macrophages in vitro.\textsuperscript{133}

- Destache C. J. et al has developed PLGA nanoparticle of three ARV drugs. Poly-(lactic-co-glycolic acid) nanoparticles containing ritonavir, lopinavir, and efavirenz were fabricated using multiple emulsion-solvent evaporation procedure.\textsuperscript{134}

The present study was carried out to develop NS, SMEDDS to improve oral bioavailability of EFV. Mannose incorporated PLGA nanoparticles of EFV were developed for site-specific drug delivery.
REFERENCES


Chapter 2 LITERATURE REVIEW


120 [http://www.drugbank.ca/drugs/DB00625](http://www.drugbank.ca/drugs/DB00625).


