CHAPTER 1:

INTRODUCTION
Advances in combinatorial chemistry, biology and genetics in the recent years have led to a steady increase in the number of drug candidates under development. Due to the phospholipidic nature of cell membranes, a certain degree of lipophilicity is often a requirement for the drug compound, not only to be absorbed through the intestinal wall following oral administration but possibly also to exert its pharmacological action in the target tissue. While high lipophilicity is advantageous in terms of compound permeability, it intrinsically translates into poor aqueous solubility. Since the first step in the oral absorption process is dissolution of the drug compound in the gastrointestinal lumen contents, poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientists working on oral delivery of drug compounds.\textsuperscript{1,2}

Majority of new drug candidates have poor aqueous solubility. Poorly soluble drugs are a general problem in pharmaceutical drug formulation.\textsuperscript{3} Typical problems associated with poorly soluble drugs are a too low bioavailability and/or erratic absorption. In case of a too low bioavailability after oral administration, parenteral administration cannot solve this problem in many cases. Due to the poor solubility, intravenous injection as a solution is not possible. Parenteral administration as a micronized product (e.g. i.m. or i.p.) does not lead necessarily to sufficiently high drug levels because the solute volume at the injection site is too low.

Given the increasing number of compounds emerging from discovery programs having poor aqueous solubility and/or dissolution, pharmaceutical scientists are constantly seeking new formulation approaches in order to obtain an adequate oral bioavailability. Currently, novel possibilities are offered by the rapidly emerging field of nanoscience.

"Nanoscience and Nanotechnology: Opportunities and Uncertainties", reads "Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale."

British Standards Institution defines a nanoparticle/nanoparticulate as a "particle with one or more dimensions at the nanoscale", "nanoscale" being defined as "having one or more dimensions of the order of 100 nm or less"\textsuperscript{4}
Several strategies to improve the solubility and dissolution of poorly water soluble drugs have been developed and described in literature, which were at start primarily based on modifying the drug’s physicochemical properties. Many approaches have been used in an effort to increase the solubility, wetting and dissolution rate of poorly water soluble drugs including chemical modification (salt formation and prodrug), complexation with cyclodextrins, formation of solid dispersions and solubilization in surfactant systems, liposomes, nanosuspension (NS), lipid based delivery system, polymeric nanoparticles.\textsuperscript{5-11} Complexation is a known solubility enhancing strategy, though its use is restricted to a considerably small group of molecules. Liposomes often have poor shelf stability and insufficient (for lipophilic drugs) loading. Although these approaches have shown some success to improve bioavailability, since last decade, NS and self- microemulsifying drug delivery system (SMEDDS) have gained a great interest as a commercially feasible novel drug delivery system.

Methods to improve drug bioavailability may involve the alteration of various key factors that determine drug dissolution, as described by the Noyes-Whitney equation\textsuperscript{12}

\[ \frac{dM}{dt} = DA/h (C_s - C_t) \]

in which \( \frac{dM}{dt} \) represents the dissolution rate, \( A \) the specific surface area of the drug particle, \( D \) the diffusion coefficient, \( h \) the diffusion layer thickness, \( C_s \) the saturation solubility and \( C_t \) the drug concentration at time \( t \). That is, dissolution rate can be increased by increasing the surface area from where dissolution can take place, by decreasing the diffusional layer thickness and by altering the solubility of the drug. NS and SMEDDS can induce a considerable increase in dissolution rate as these strategies can simultaneously alter various of these factors.\textsuperscript{13-15}

1.1 Nanosuspension

Nanosizing refers to the reduction of the active pharmaceutical ingredient (API) particle size down to the sub-micron range. These particles have a size below 1 \( \mu \)m, typically a few hundred nanometers.\textsuperscript{16} The sub-micron particles are stabilized with surfactants or polymers in NSs, which can be further processed into standard dosage forms, such as capsules or tablets, suitable for oral administration.\textsuperscript{1} These nano-
formulations offer increased dissolution rates and enhance bioavailability of insoluble compounds (BCS Class II and IV).

Several production techniques like precipitation\textsuperscript{17}, jet milling, pearl milling\textsuperscript{18,19} and high-pressure homogenization (HPH)\textsuperscript{20,21} have been applied to produce NSs. Currently, media milling is preferred over HPH technique because it is easy to scale up to industrial pharmaceutical unit operations.\textsuperscript{22-26} Also, crystalline nature of the drug remains largely intact during the media milling processing, thus relieving any stability concerns. Furthermore, no organic solvent or harsh environment is needed. Recently, APIs have been successfully processed into NSs through media milling method by the pharmaceutical industry and some commercial products currently in the market include Emend\textsuperscript{®} (Aprepitant, Merck & Co.), Rapamune\textsuperscript{®} (Sirolimus, Wyeth), Tricor\textsuperscript{®} (Fenofibrate, Abbott) and Megace\textsuperscript{®} ES (Megestrol acetate, Par Pharmaceuticals).\textsuperscript{24,27,28}

NSs efficiently improve oral absorption of poorly soluble drugs and achieve a higher bioavailability compared to traditional formulation.\textsuperscript{15} Major advantages of NS technology are increased dissolution velocity, increased saturation solubility, versatility in surface modification and ease of post-production processing. NSs show adhesion to the gastrointestinal mucosa, prolonging contact time of the drug and thereby enhancing its uptake via the gastrointestinal tract (GIT).\textsuperscript{29-31}

Formulation of NS requires a careful selection of stabilizers. Stabilizers are needed to stabilize the nanoparticles against inter-particle forces and prevent them from aggregating. At the nanometer domain, attractive forces between particles, due to dispersion or van der Waals forces, come into play. This attractive force increases dramatically as the particles approach each other, ultimately resulting in an irreversible aggregation.

To overcome the attractive interaction, repulsive forces are needed. There are two modes of imparting repulsive forces or energetic barriers to a colloidal system-steric stabilization and electrostatic stabilization. Steric stabilization is achieved by adsorbing polymers onto the particle surface. As the particles approach each other, the osmotic stress created by the encroaching steric layers acts to keep the particles separate. Electrostatic stabilization is obtained by adsorbing charged molecules,
which can be ionic surfactants or charged polymers, onto the particle surface. Charge repulsion provides an electrostatic potential barrier to particle aggregation. Steric stabilization is often combined with electrostatic stabilization for additional repulsive contribution.

Common pharmaceutical excipients that are suitable for use as polymeric stabilizers include the cellulosics, such as hydroxypropylcellulose (HPC) and hydroxypropylmethylcellulose (HPMC), povidone (PVP K30), and pluronics (F68 and F127). The surfactant stabilizers can be non-ionic, such as polysorbate (Tween 80), or anionic, such as sodium lauryl sulfate (SLS) and docusate sodium (DOSS).

1.2. SMEDDS

SMEDDS is isotropic mixtures of an oil, surfactant, co-surfactant (or solubilizer), and drug. The basic principle of this system is its ability to form fine oil-in-water (o/w) microemulsions under gentle agitation following dilution by aqueous phases.\textsuperscript{32,33}

The spontaneous formation of an emulsion upon drug release in the GIT advantageously presents the drug in a dissolved form and the small droplet size provides a large interfacial surface area for drug absorption. Specific components of SMEDDS promote the intestinal lymphatic transport of drugs. Main mechanisms include increasing membrane fluidity to facilitate transcellular absorption, opening tight junction to allow paracellular transport, inhibiting P-gp and/or CYP450 to increase intracellular concentration and residence time by surfactants, and stimulating lipoprotein/chylomicron production by lipid.\textsuperscript{34-35}

Because of their unique solubilization properties, SMEDDS offer the following advantages:\textsuperscript{50}

1. Bioavailability enhancement of poorly aqueous soluble drugs: SMEDDS offer the opportunity to present lipophilic drugs to the gastrointestinal tract in a dissolved state, avoiding the dissolution step (which can limit absorption rate of BCS Class II and IV drugs).
2. Reduction in inter-subject and intra-subject variability.
3. Reduction of food effect.
4. Ease of manufacturing and scale up.
5. Ability to deliver peptides that are prone to enzymatic hydrolysis in GIT.
6. No influence of lipid digestion process.

At present, there are four drug products, Sandimmune® and Sandimmun Neoral® (cyclosporin A), Norvir® (ritonavir), and Fortovase® (saquinavir) on the pharmaceutical market, the active compounds of which have been formulated into specific self-emulsifying formulations, triggers much more attention on SMEDDS. SMEDDS formulations are normally prepared as liquids and dispensed in form of soft or hard gelatin capsule filled which give rise to some drawbacks such as interaction of the fill with the capsule shell, risks of leakage from into hard gelatin filled capsules, limited shelf-life. In recent years, there is a growing trend to formulate solid SMEDDS (S-SMEDDS) by adsorbing liquid SMEDDS (L-SMEDDS) onto suitable solid carriers. Such S-SMEDDS can be easily filled in capsules and overcome the disadvantages of liquid formulations. On oral administration, they readily form microemulsion in vivo; presenting the drug in nano-sized and ‘ready to absorb’ form. There are a limited number of publications reporting the oral bioavailability of solid SEDDS or SMEDDS. Formulation of L-SMEDDS requires a careful selection of oil, surfactant and cosurfactant. Selection of excipients should be optimized considering solubility, phase diagram and self-emulsification property.

1.3. Nanoparticles

Another focus of research is the development of nanoparticle technologies to improve and enable drug targeting. Most drugs currently on the market are delivered in a non-specific manner throughout the whole body, rather than directly to the site of action where they are needed. This may result in unintentional side effects or toxicity in other tissues. Site specific targeting (both passive and active targeting) can reduce systemic toxicity by enabling drugs to accumulate selectively in the target tissue. As a result, the local concentration of the drug at the site of action will be high, while its concentration in non-target tissue will be below a certain minimum level to prevent side effects. In addition, targeting ability may allow for lower dosing requirements which also potentially decrease side effects of the drug while maintaining the same
therapeutic results. The need to achieve selective delivery of drugs to specific areas of
the body has been recognized for many years.

Polymeric nanoparticles can be identified as submicron size (<1 μm) colloidal
 carriers. Compared with other colloidal carriers, polymeric nanoparticles hold
significant promise for the advancement of treating diseases and disorders. They have
attractive physicochemical properties such as size, surface potential, and hydrophilic-
hydrophobic balance, and for this reason they have been recognized as potential drug
 carriers for bioactive ingredients such as anticancer drugs, vaccines, oligonucleotides,
and peptides. Their widespread use for oral delivery also aims at improving the
bioavailability of drugs with poor absorption characteristics, reducing GI irritation
caued by drugs, and assuring stability of drugs in the GIT. Thus, these characteristics
of nanoparticles qualify them as a promising candidate in drug delivery technology.\textsuperscript{46}

Out of a large array of particulate carriers, polymeric nanoparticles are well-
established for drug delivery, specifically poly(lactic-co-glycolic acid (PLGA)-based
nanoparticles due to their well-known inherent advantages. PLGA is a food and drug
administration (FDA) approved biodegradable and biocompatible polymer that had
been widely used in the manufacturing of surgical sutures and in several controlled
release drug products for human use.\textsuperscript{47,48} PLGA nanoparticles represent an interesting
carrier system for the transport of antiviral drugs to monocytes/macrophage in an
attempt to reduce the required dose, minimize toxicity and side effects, and improve
the delivery of substances, which have insufficient intracellular uptake.\textsuperscript{48}

1.4 Rationale of project

Worldwide, over 60 million people are reported to be infected with the Human
Immunodeficiency Virus (HIV).\textsuperscript{49} The High Activity Antiretroviral Therapy
(HAART) introduced in 1996 combines at least three antiretroviral (ARV) drugs\textsuperscript{50-52}
and, for over a decade, has been used to extend the lifespan of the HIV-infected
patients. In this context, the formerly fatal HIV-associated disease, acquired
immunodeficiency syndrome (AIDS), has become a manageable chronic infection in
most developed countries.\textsuperscript{53} Chronic intake of HAART is mandatory to control HIV
infection\textsuperscript{54}; without it, viral replication resumes several weeks after withdrawal.
Epidemiology reveals that optimal therapeutic results are attained when treatment adherence levels are greater than 95% (no more than two doses missed monthly in a twice-a-day regime); adherence levels below 95% could diminish therapeutic effectiveness by 50%.\textsuperscript{51,52} The frequent administration of several drugs in relatively high doses is a main cause of patient compliance and a hurdle toward the fulfillment of the pharmacotherapy.\textsuperscript{55}

Regardless of the remarkable progress made in ARV pharmacotherapy, HIV is able to conserve its replication machinery in anatomical and intracellular sites where the ARV drugs have restricted access. HAART does not eliminate these reservoirs, nor prevent their generation and hence, a rebound in viral plasma levels occurs upon HAART withdrawal.\textsuperscript{56} CD4+ T lymphocytes are the best investigated reservoir. Others reservoirs are the cells of the mononuclear phagocyte system (e.g., monocytes/macrophages, dendritic cells and Langerhans cells), the brain, hepatocytes and the gastrointestinal tract. In this framework, the use of nanoparticles has arguably become the most attractive research avenue for targeting monocytes/macrophages.\textsuperscript{57} Macrophages possess various receptors such as fucos receptors, manniosyl, 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. Therefore, targeting of ARV drugs to HIV infected macrophages could be an attractive approach for improving the therapeutic efficacy and reducing the toxicity of ARV bioactives.\textsuperscript{46}

1.4.1. Selection of drug

Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) drugs were first introduced in 1998. The mechanism of action involves the non-competitive binding of the drug to the reverse transcriptase enzyme. According to the guidelines, HAART usually includes at least one NNRTI as a first-choice drug. The British HIV Association, the US Department of Health and Human Services (DHSS) and the International AIDS Society (IAS) guidelines indicate Efavirenz (EFV) as the preferred NNRTI. EFV is also the NNRTI of election recommended by the WHO for the initial treatment of
children above the age of 3. Molecular structure of EFV [(S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2(H)-3,1-benoxazin-2-one].

According to the biopharmaceutical classification system guidance by FDA, EFV comes under a class II category drug, i.e., it has low solubility and high intestinal permeability.\textsuperscript{58,59} It is a crystalline lipophilic solid with an aqueous solubility of 3-9 \( \mu \text{g/ml} \) and with a low intrinsic dissolution rate of 0.037 \( \text{mg/cm}^2/\text{min} \). Hence, it has very low bioavailability.\textsuperscript{60,61} To achieve effective therapy against viral diseases for orally administered drugs, it is essential that the drug should be adequately and consistently absorbed. Therefore, the recommended dose of EFV in adults is 600 mg q.d. The frequent administration of several drugs in relatively high doses is a main cause of patient incompliance.\textsuperscript{62} The reason for this is very low solubility of EFV hinders its administration, absorption and biodistribution. Thus, there is need to have some innovative formulation approach to enhance the bioavailability.

### 1.5. Hypothesis

It was hypothesized that NS and SMEDDS formulations of EFV might lead to improved oral bioavailability due to enhanced solubility, dissolution and, thus absorption.

Another hypothesis was that PLGA nanoparticles of EFV coupled with mannose can be utilized to target mannosyl receptor on macrophages for site-specific delivery.

These formulations would help to improve clinical utility, decrease the dose and frequency of dosing, reduce side effects and improve therapeutic efficacy of EFV.

### 1.6. Aims and objectives

The first aim of study was to develop stable formulations of EFV for improvement of oral bioavailability by improving its solubility, dissolution and absorption properties. The detail objectives of this study were as below:
To develop formulations of NS and SMEDDS loaded with EFV.
- Optimization of the various formulation and process parameters by factorial design study.
- To characterize the prepared formulations for particle size, zeta potential and its morphological properties by Scanning Electron Microscopy and Transmission Electron Microscopy.
- To study the Differential Scanning Calorimeter thermograms and X-ray diffraction patterns of excipients and optimized formulations.
- To carry out stability studies at various temperature conditions.
- To carry out in vitro dissolution study of optimized formulations and compared with standard EFV and marketed formulation.
- To carry out in situ intestinal perfusion study in rats for absorption of EFV from optimized formulations and compare with marketed formulation.
- To carry out parallel artificial membrane permeability assay (PAMPA) for permeability of EFV from optimized formulations and compared with standard EFV and marketed formulation.
- To carry out in vivo pharmacokinetic (bioavailability) study from optimized formulations to compare with standard EFV and marketed formulation.

The second aim of study was to develop EFV loaded mannose (MN) incorporated PLGA nanoparticles (MN-PLGA NPs) for site-specific delivery to macrophages. The detailed objectives were as follows:

- To carry out MN incorporation in PLGA polymer.
- To formulate MN-PLGA NPs loaded with ARV drug EFV.
- Optimization of the various formulation and process parameters by factorial design.
- To characterize the prepared formulations for entrapment efficiency (%), particle size, zeta potential and its morphological properties by transmission electron microscopy.
- To carry out in vitro diffusion study from optimized formulation.
- To carry out stability studies at various temperature conditions.
- To carry out drug uptake study in peritoneal macrophages.
- In vivo biodistribution study of optimized formulation in rats.
REFERENCES


