

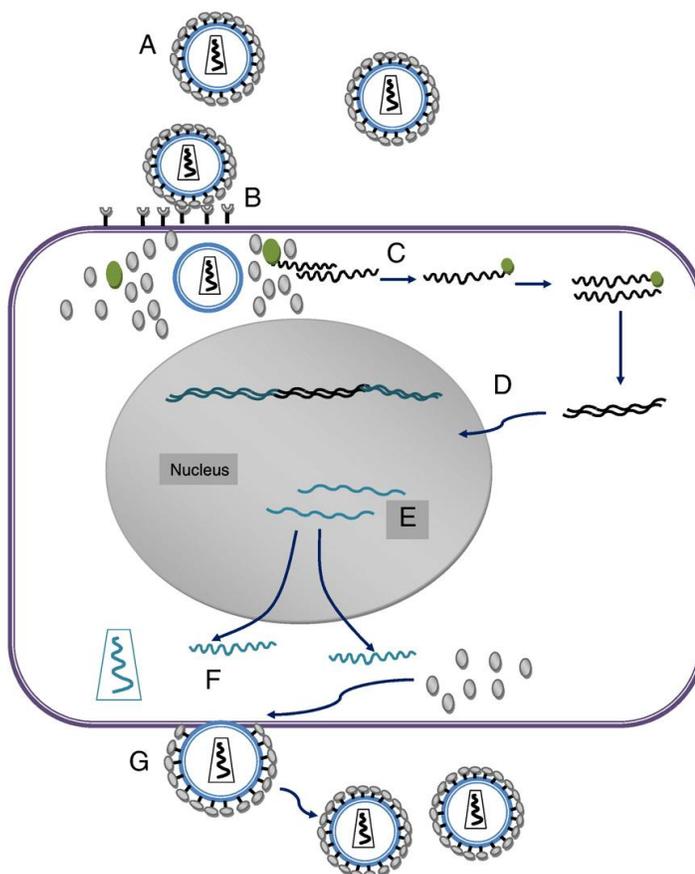
## 2.1 Human Immuno Deficiency Virus (HIV)

HIV is a lentivirus of the family Retroviridae, known for being the causative agent of Acquired Immuno Deficiency Syndrome (AIDS). HIV is of two types of HIV, HIV-1 and HIV-2, with HIV-1 being much more virulent, transmittable and prevalent. HIV-1 is the cause of the majority of HIV infections in the world. Extensive destruction of T-helper cells, macrophages, dendritic cells and other cellular components associated with cell-mediated immunity is caused due to HIV infected cells and results in compromised immune defense (1). As a result, HIV-infected patients are substantially more vulnerable to opportunistic infections. As per the data of WHO, in 2013, 35 million people worldwide lived with HIV/AIDS. HIV is considered as world's leading infectious killer that has taken lives of approximately 39 million people since its first discovery in 1981 till 2013 worldwide (2).

### 2.1.1 Pathogenesis of HIV

HIV is mostly transmitted by vaginal or anal sexual intercourse (3). Other means of transmission includes transfusion of contaminated blood products, sharing of contaminated needles among injected drug patients and transmission from mother-to-child during pregnancy, labor or breastfeeding (1). The infection due to HIV begins with the attachment of the virions (Figure 2.1) to the cell surface mediated by an interaction of the extracellular domain of HIV-1 gp120 and cellular receptors (4). After binding to the co-receptor, viral and cellular membranes fuse. As a result, the viral core is released into the cytoplasm of the cell. Viral uncoating involves cellular factors and viral proteins gag, nef and vif. Viral reverse transcriptase retro-transcribes the viral RNA genome into a full length double stranded DNA by the viral reverse transcriptase. The preintegration complex (5) docks the nuclear membrane directed by HIV-1 vpr, and enters the nucleus, through the nuclear pore (6, 7). Linear double stranded DNA in the pre-integration complex is inserted into the host chromosome by the viral integrase (8). In the early phase of replication cycle, only multiply-sliced mRNAs are produced and the regulatory proteins *tat*, *nef* and *rev* are expressed. The *env* gene is translated into the precursor protein gp160, which is glycosylated within the endoplasmic reticulum. The *gag-pol* gene

is primarily translated to produce the *gag* and *gag-pol* polyproteins. The *gag* polyprotein is proteolytically processed during the maturation of the virus into six structural proteins. These proteins rearrange and produce the mature virion. Later on, this complex buds through the plasma membrane and produces an immature virion (4).



**Figure 2.1 HIV replication steps.**

**A. HIV; B. Attachment of body cells; C. Reverse transcription; D. Integration; E. Protein synthesis and translation; F. Budding; G. Maturation and release.**

After initial infection and local amplification at the mucosal site, infected cells migrate to the regional lymph nodes, resulting in a mild initial viral amplification in naive T cells (9). The viral infection is then quickly disseminated by T cells to lymphoid organs, particularly the gut-associated lymphoid tissues (GALT), bone marrow and spleen. This is accompanied by a burst in the viral load (acute infection) (10). The gastrointestinal tract is particularly affected by the virus during the acute and early stages of infection, leading to a dramatic loss of CD4<sup>+</sup> and CD8<sup>+</sup> T cells which never quite

recover completely and remain despite antiretroviral treatment (11, 12). Individuals during this acute phase pose an increased risk for sexual transmission as result of high blood and genital viral load, with clear implications in the prevention of HIV transmission (13). CD4<sup>+</sup> cell levels recover soon after this phase. In the case of CD8<sup>+</sup> cells, there is a rise followed by a rapid recovery of normal levels. Intense immune response causes the levels of the virus to be down regulated, but never completely depleted, resulting in asymptomatic infection and clinical latent (14). During latency, the virus persists particularly in lymph node dendritic cells, resting CD4<sup>+</sup> memory cells and extra-vascular tissues. This state can evolve to a symptomatic clinical stage, designated by AIDS, which is characterized by decreased CD4<sup>+</sup> T cell counts and rising viral load. HIV genetic diversity increases noticeably with the progression of infection, due to intense error-prone reverse transcription and evolutionary pressure to evade the immune system (15). This new heterogeneous population significantly increases the generation of viruses which are resistant to cellular and humoral immune response, and represents a major challenge in the development of therapy and preventive strategies.

### **2.1.2 HIV Reservoir sites**

Even if current antiretroviral therapy is able to reduce the viral load to undetectable levels, HIV is able to persist in the human body in several reservoir sites. These may be defined as cellular or anatomical locations where a replication-form of the virus is persistently harbored with more stable kinetic properties than in the main pool of actively replicating virus (16). Reservoir sites are able to protect the virus from biological elimination pathways, immune response and/or antiretroviral drugs, making it impossible to eradicate the virus and achieve a cure with currently available therapy (16). The cellular reservoirs of HIV mainly include macrophages, resting CD4<sup>+</sup> T cells and follicular dendritic cells (16, 17). Among these, macrophages constitute one of the most important viral reservoirs outside the bloodstream and are able to transport HIV into the central nervous system, allowing this anatomical site to become infected (18). When CD4<sup>+</sup> T cells are largely depleted, these cells, that otherwise are irrelevant as source of HIV particles, constitute a key cell population in maintaining the HIV replication cycle (17). Cellular reservoirs are able to sustain HIV infection by allowing its residence in a

physical state capable of surviving for prolonged periods despite otherwise therapeutic levels of antiretroviral drugs (17). Anatomical reservoir sites for HIV include lymphoid organs (particularly the spleen, lymph nodes and GALT), Central nervous system, testicles and female genital tract (16, 17). The importance of lymphoid organs is directly related with their role in the circulation and production of lymphocytes and the abundant presence of HIV-susceptible immune cells, namely those able to constitute reservoirs as discussed above. In the case of anatomical reservoir sites, the problem is mainly to achieve and sustain adequate levels of antiretroviral agents within these spaces (17).

### 2.1.3 Treatment of HIV- HAART therapy

Upon identification of resistance of antiretroviral agents in individuals treated with single drug regimens, the concept of highly active anti-retroviral therapy (HAART) was introduced in 1990s. HAART therapy comprises of the use of combination of antiretroviral agents. The different categories of antiretroviral agents comprises the following classes of drugs. *Nucleoside reverse transcriptase inhibitors (NRTIs)*: Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir, Emtricitabine. *Nucleotide reverse transcriptase inhibitors (NtRTIs)*: Tenofovir. *Non-nucleoside reverse transcriptase inhibitors (NNRTIs)*: Nevirapine, Efavirenz, Etravirine. *Protease Inhibitors (PIs)*: Saquinavir, Indinavir, Ritonavir, Nelfinavir, Lopinavir, Atazanavir, Fosamprenavir, Tipranavir, Darunavir. *Entry Inhibitors/ Fusion Inhibitors*: Enfuvirtide, Maraviroc. *Integrase Inhibitors*: Raltegravir (1). The HAART therapy includes the combination of drug from the above categories in order to combat the resistance that develops in patient by the use of same drug/ same category of drug. FDA has approved various fixed dose combinations as mentioned in Table 2.1.

**Table 2.1 Various fixed dose combinations approved by FDA for treatment of HIV infection**

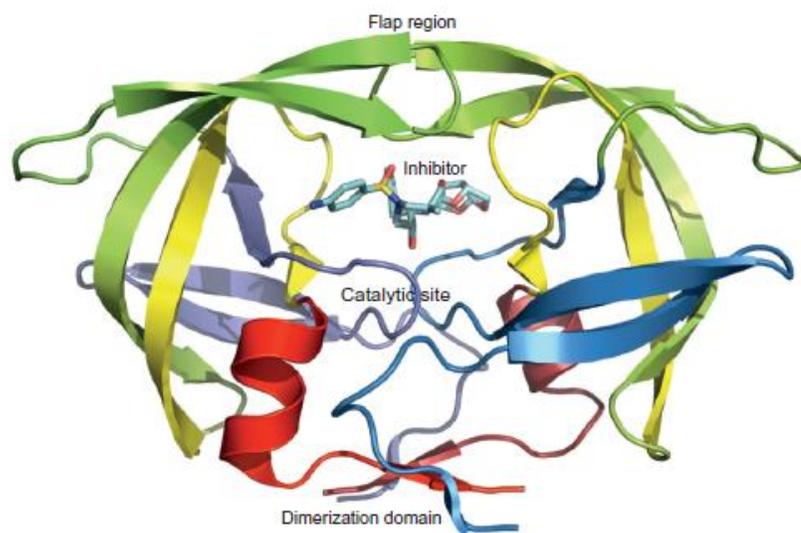
<b>Brand name</b>	<b>Drug names</b>	<b>Year of FDA approval</b>	<b>Company</b>
Combivir	Lamivudine + Zidovudine	1997	GlaxoSmithKline
Kaletra	Lopinavir + Ritonavir	2000	Abbott laboratories

Trizivir	Abacavir + Lamivudine + Zidovudine	2000	GlaxoSmithKline
Epzicom (in USA) Kivexa (in Europe and Russia)	Abacavir + Lamivudine	2004	GlaxoSmithKline
Truvada	Tenofovir + emtricitabine	2004	Gilead Sciences
Atripla	Emtricitabine + Tenofovir + Efavirenz	2006	Gilead sciences and Bristol-Myers Squibb
Complera (in USA) Eviplera (in Europe and Russia)	Emtricitabine + Rilpivirine + Tenofovir	2011	Gilead Sciences and Janssen Therapeutics
Stribild	Elvitegravir + Cobicistat + Emtricitabine + Tenofovir	2012	Gilead Sciences
Triumeq	Abacavir + Dolutegravir + Lamivudine	2014	ViiV Healthcare
Evotaz	Atazanavir + Cobicistat	2015	Bristol-Myers Squibb
Prezcobix	Darunavir + Cobicistat	2015	Janssen Therapeutics
Dutrebis	Lamivudine + Raltegravir	2015	Merck & Co.

#### 2.1.4 Protease Inhibitors (PIs)

Since the discovery of HIV up to 2015, 26 anti-HIV compounds have been approved by the US Food and Drug Administration (FDA). Among these compounds, 10 are HIV protease inhibitors. The AIDS-related mortality has dropped sharply and AIDS has gradually become a controllable, chronic disease. HIV protease inhibitor is one of the most important components in the combination therapy. In the preferred antiretroviral combination regimens, protease inhibitor-based therapy has resulted in a lower level of resistance compared with non-nucleoside reverse-transcriptase inhibitor (NNRTI)-based therapy. However, the need for lifelong treatment and the frequently associated side effects of HIV protease inhibitors severely reduces patient compliance, which is one of the obstacles in the treatment of HIV/AIDS patients. Although the toxic effects of HIV protease inhibitors could result from drug-drug interactions and overdose, the off-target adverse drug effects of therapeutic doses is a major concern in drug design (19).

In the HIV life cycle, protease is an essential element for viral maturation. The HIV protease is a homodimeric aspartyl protease, and each monomer is composed of 99 amino acid residues with a catalytic *Asp* at position 25 (Figure 2.2). HIV-1 protease cleaves *Gag* and *Gag-Pol* polyprotein precursor encoded by the HIV-1 virus genome at nine processing sites to produce mature active proteins. The *Pol* polyproteins is first cleaved off from the *Gag-Pol* polyproteins and then further digested into protease, reverse transcriptase (p51), RNase H (p15), and integrase. The active site is not fully exposed, being covered by two flexible  $\beta$ -hairpin flaps. The flaps need to open to allow the substrates to access the active site. The HIV-1 protease enzyme activity can be inhibited by blocking the active site of the protease (19).



**Figure 2.2 The HIV-1 protease complex with an inhibitor**

Although the success of PIs has been remarkable, there are only ten of these compounds currently approved by the FDA as antiviral agents due to several problems (Table 2.2). First, there is a problem of antiviral drug resistance. The high mutation rate caused by the lack of proofreading activity of the viral reverse transcriptase, the dynamic viral replication in HIV-positive individuals, together with potential dual infection and insufficient effect of drugs lead to rapid transformation of viral species resistant to the currently used inhibitors (20). Secondly, the clinical use of PIs is affected by their high price and by problems of tolerability, toxicity, and adherence. The PIs were found to

interact with other molecules, particularly in the lipid metabolism and trafficking pathways (20). Consequently, the side effects of PIs are very frequent and often so serious that the drug toxicity may sometimes represent even a greater risk for patients than the HIV infection itself. The presence of side effects together with the pill burden negatively influences the patient's adherence and hence contributes to the development of resistance. All PIs in current clinical practice have been approved only for oral administration, but their absorption in the gastrointestinal tract and diffusion through anatomical barriers is usually poor to moderate. This effect is partly due to their extensive binding to plasma proteins (90-99%), partly due to the binding to P-glycoprotein and consequent efflux of the drug (20). These factors make their effective concentration low which might lead to ongoing viral replication and enable the emergence of drug-resistant variants of the virus. It is therefore very important to combat the pharmacokinetic limitations associated with PIs. All PIs are administered at very high doses which again add to the dose related toxicities and drug adverse effects related to high dose. In order to enhance the pharmacokinetics of protease inhibitors, Ritonavir is always administered with all protease inhibitors except Nelfinavir. Recently, another pharmacokinetic enhancer, Cobicistat, has been approved for use in combination with few protease inhibitors.

**Table 2.2 Overview of the inhibitors of HIV protease approved for clinical use**

<b>Generic name</b>	<b>Recommended daily dose</b>	<b>Most common side-effects</b>	<b>Position in the present therapeutic arsenal</b>
Ritonavir (RTV)	100-200 mg BID as pharmacokinetic booster of various PIs	Nausea, diarrhea, abdominalgia, hyperlipidemia, lipodystrophy syndrome	Practically only pharmacoenhancing of various PIs
Saquinavir	1000 mg + RTV 100 mg BID	diarrhea, hyperlipidemia, lipodystrophy syndrome	second-line HAART therapy
Indinavir	800 mg + RTV 100 mg BID	nephrolithiasis, lipodystrophy syndrome, hyperlipidemia, hepatotoxicity	second/third-line HAART therapy in case of resistance or intolerance
Nelfinavir	1250 mg BID	diarrhea, hyperlipidemia,	second/third-line

		lipodystrophy syndrome	HAART therapy in case of resistance or intolerance, approved for therapy of children
Lopinavir	400 mg + RTV 100 mg BID	diarrhea, hyperlipideamia, lipodystrophy syndrome	first line option for PI based HAART regimen
Amprenavir	600 mg + RTV 100 mg BID	diarrhea, toxoallergic rash, hyperlipidemia, lipodystrophy syndrome	replaced by its prodrug fosamprenavir
Fos-amprenavir	700 mg + RTV 100 mg BID	diarrhea, toxoallergic rash, hyperlipidemia, lipodystrophy syndrome	first-line option for PI based HAART regimen
Atazanavir	300 mg + 100 mg RTV once a day or 400 mg once a day	hyperbilirubinemia, ECG abnormalities (1° atrioventricular block)	first-line option for PI based HAART regimen
Tipranavir	500 mg + RTV 200 mg BID	toxoallergic rash, hepatotoxicity, intracranial hemorrhage, lipodystrophy syndrome, diarrhea	second-line HAART therapy in case of resistance
Darunavir	600 mg + RTV 100 mg BID or 800 mg + RTV 100 mg once a day	nausea, diarrhea, hyperlipidemia, headache, toxoallergic rash	first-line option for PI based HAART regimen

## 2.2 Oral drug delivery

Oral drug delivery is the most preferred route of drug administration as it is the simplest and the easiest way for any patient to take a medication. It is cost effective requiring non-sterile conditions for manufacture and varieties of dosage forms are available like tablets, hard gelatin and soft gelatin capsules and powder. It is a non-invasive, painless route of administration. For drugs with high doses and requiring long term administration like that in HAART therapy, oral route is the only cost effective and preferred route of administration (21).

Despite these potential advantages, oral drug delivery poses several difficulties like instability in the gastric environment, poor absorption and poor permeability due to

mucus membrane. It is due to the various undesirable physicochemical properties of the drug such as enzymatic degradation, poor stability in gastric low pH environment, large molecular size, poor aqueous solubility, poor permeability in crossing intestinal barrier, short plasma half life, immunogenicity (22). In order to overcome these barriers, several approaches have been used like nanoparticulate carriers (22), lipid based delivery (23), sustained and controlled drug release (24), microparticle (25) and microemulsions (26) etc.

Nanoparticles as drug carriers have become the focus of attention in delivery of various categories of drugs. The nanoparticles possess certain advantages such as greater stability during storage, stability *in-vivo* after administration and ease of scale up without an aseptic process for oral administration. Nanoparticles are particulate dispersions with a size in range of 10-1000 nm in which the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix (27). The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release kinetics of pharmacologically active ingredients in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen (22).

The advantages of nanoparticulate drug delivery systems are listed below (22):

1. Both passive as well as active drug targeting is possible by employing suitable particle size and surface characteristics of nanoparticles.
2. Nanoparticles control and sustain the release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve an increase in drug therapeutic efficacy and a reduction in side effects.
3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
4. Site-specific targeting can be achieved by attaching targeting ligands to the surfaces of particles or by using magnetic guidance.

5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular, etc.

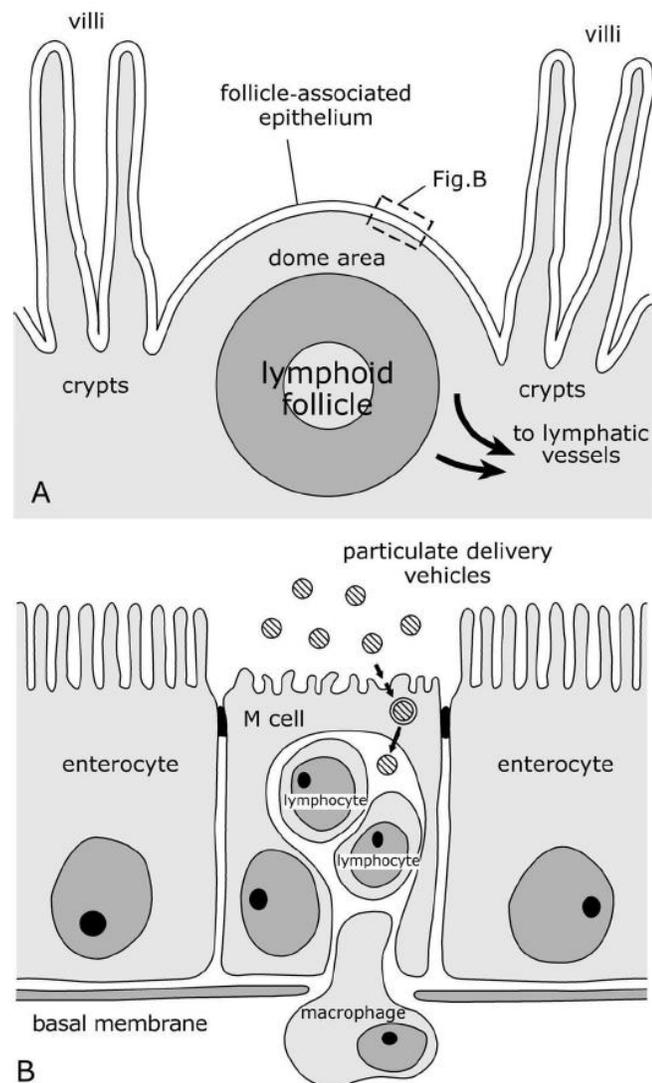
Nanoparticles are usually dispersed in liquid. Such a system can be administered to humans for example by injection, by the oral route, or used in ointments and ocular products. Alternatively, nanoparticles can be dried to a powder and delivered orally in form of dried-powder, tablet, capsule or pellets (28).

Besides nanoparticles, nanoemulsion also has important application in drug delivery. Nanoemulsions are novel drug delivery systems consisting of emulsified oil and water systems with mean droplet diameters ranging from 50 to 1000 nm. Usually, the average droplet size is between 100 and 500 nm and can exist as oil-in-water (o/w) or water-in-oil (w/o) form, where the core of the emulsion is either oil or water, respectively. Nanoemulsions are made from pharmaceutical surfactants that are generally regarded as safe (GRAS) (29). The capacity of nanoemulsions to dissolve large quantities of low soluble drugs along with their mutual compatibility and ability to protect the drugs from hydrolysis and enzymatic degradation make them ideal drug delivery vectors (30). The major advantages of nanoemulsions as drug delivery carriers include increased drug loading, enhanced drug solubility and bioavailability, reduced patient variability, controlled drug release, and protection from enzymatic degradation (31).

### **2.2.1 Physiological considerations in oral absorption across intestinal epithelium**

The human intestinal epithelium is a highly absorptive covered with villi on the apical side that increases the absorptive surface area of the Gastro intestinal tract to around 300-400 m<sup>2</sup> (32). It consists of a single layer of epithelial cells that majorly consists of enterocytes interspersed by a mucus secreting goblet cells. Enterocytes control the passage of macromolecules and pathogens, while it also allows the digestive absorption of dietary nutrients. Goblet cells secrete the mucus gel layer, a viscous fluid composed primarily of highly glycosylated proteins (mucins). In addition, the intestinal epithelium also contains specialized cells known as peyer's patch. Peyer's patch are Organised-Mucosa associated lymphoid tissues (O-MALT) and are located throughout the gastrointestinal tract. It consists of lymphoid follicles arranged either singly or as

cluster to form distinct structures than epithelium. The epithelium underlying the lymphoid follicles is termed the follicle-associated epithelium (FAE) and is chiefly distinguished from the intestinal epithelium at other sites chiefly by the presence of the specialized antigen sampling M cells (Figure 2.3). Together, the FAE, lymphoid follicles and associated structures form the antigen sampling and inductive sites of the mucosal immune system (33).



**Figure 2.3 Schematic transverse sections of peyer's patch lymphoid follicle and overlying follicle associated epithelium (FAE)**

The general structure of intestinal organized mucosa-associated lymphoid tissues (O-MALT) is represented by the schematic transverse section of a Peyer's patch lymphoid follicle and associated structures in (Figure 2.3A). The FAE is characterized by the presence of specialized antigen sampling M cells (Figure 2.3B)

M-cells are typically characterized by two features. Firstly, they have sparse irregular microvilli on their apical surface. This facilitates the adsorption of both microorganisms and inert particles to their surfaces. Secondly, they possess a basolateral cytoplasmic invagination which creates a pocket containing one or more lymphocytes and occasional macrophages. M cells are specialized for antigen sampling, but they are also exploited as a route of host invasion by many pathogens (33). Furthermore, M cells represent a potential portal for oral delivery of peptides and proteins and for mucosal vaccination, since they possess a high transcytotic capacity and are able to transport a broad range of materials, including nanoparticles. Uptake of particles, microorganisms and macromolecules by M-cells, have been described to occur through adsorptive endocytosis by way of clathrin coated pits and vesicles, fluid phase endocytosis and phagocytosis. In addition, M cells, compared with normal epithelial cells have reduced levels of membrane hydrolase activity, which can influence the uptake of nanoparticles. Although less numerous than enterocytes, M cells present enhanced transcytosis abilities which has made them attractive candidates for oral drug delivery applications (34).

The absorption of drug across the intestine can occur by 4 distinct mechanisms. In general, the drug molecule can be absorbed from the intestinal epithelium or the particulate uptake can also occur from the intestine. 4 different pathways for absorption are mentioned below. Multiple pathways could be involved in the drug absorption while absorption through each pathway is dependent on different physical characteristics such as molecular weight, hydrophobicity, ionization constants and pH stability of absorbing molecules as well as biological barriers that restrict protein absorption from the GI tract (22).

a) Paracellular transport

Paracellular transport is the pathway of substances across an epithelium by passing through the intercellular spaces in between epithelial cells. Paracellular

transport is passive and results from diffusion. This transport is under the control of tight junctions which constitutes the major rate limiting barrier towards the paracellular transport for permeation of ions and larger substances (35). The dimension of the paracellular space is on the order of 10 Å. The average size of aqueous pores created by epithelial tight junctions is approximately 7-9 Å for the jejunum, 3-4 Å for the ileum, and 8-9 Å for the colon in the human intestine (36). This data suggests that solutes with a molecular radius exceeding 15 Å (approximately 3.5 kDa) cannot be transported via this route (37). Furthermore, tight junctions comprise only about 0.01% of the total absorption surface area of the intestine (38).

b) Transcellular transport

Transcellular transport occurs through the intestinal epithelial cells by transcytosis, a process by which particles are taken up by cells. A typical example is the movement of glucose from the intestinal lumen to extracellular fluid by epithelial cells. This starts with an endocytic process that takes place at the cell apical membrane. Then, particles are transported through the cells and released at the basolateral pole (39). The basolateral membrane is thinner and more permeable than the apical membrane because the protein-to-lipid ratio is very low in the basolateral membrane. Transport of particles by the transcellular transport depends on several factors: (i) physicochemical properties of particles, such as size, lipophilicity, hydrogen bond potential, charge, surface hydrophobicity or the presence of a ligand at the particle surface and (ii) the physiology of the GI tract. The transport through enterocytes and M-cell of peyer's patch occur by transcellular route (40, 41).

c) Carrier-mediated transport (Active transport)

Drugs are transferred across the cell membrane or entire cell and then released from the basal surface of the enterocyte into circulation (42). The process is utilized by small hydrophilic molecules (43). Active absorption requires energy-dependent uptake of specific molecules by carriers. The carriers recognize target molecules through membrane receptors and transport them across the membranes into the GI epithelium, even against the concentration gradient and in trace quantities. For

example, small di/tripeptides (including  $\beta$ -lactam antibiotics and angiotensin-converting enzyme (ACE) inhibitors), monosaccharides, and amino acids are transported transcellularly by a carrier-mediated transport process (44).

d) Receptor-mediated transport

In receptor-mediated endocytosis, a specific receptor on the cell surface binds tightly to the extracellular macromolecule (the ligand) that it recognizes; the plasma-membrane region containing the receptor-ligand complex then undergoes endocytosis, becoming a transport vesicle. Receptor ligand complexes are selectively incorporated into the intracellular transport vesicles; most other plasma-membrane proteins are excluded. Endocytosis generally occurs through clathrin-coated pits while less common is caveolae-coated pits. This transportation, in general, is known as endocytosis and comprises phagocytosis, pinocytosis, receptor-mediated endocytosis (clathrin-mediated), and potocytosis (nonclathrin-mediated) (45).

### **2.2.2 Physicochemical considerations in the transport across intestinal epithelium**

Majority of orally administered drugs gain access to the systemic circulation by absorption into the portal blood. Solubility and lipophilicity are main parameters while determining the oral formulation strategy. For absorption, the drug needs to be in the solubilized form in the GI fluids. Moreover, it should have good permeability in order to permeate through the epithelium. However, with the advent of novel drug delivery strategies, various physicochemical parameters such as particle size, surface charge etc should also be considered since particulate uptake takes place through the epithelium. Hence, the physicochemical properties of drug as well as carrier are important for absorption.

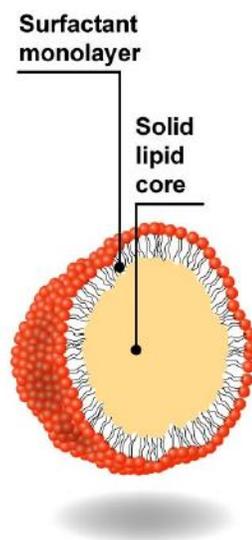
Particle size of carriers is an important parameter determining the uptake of particles through the intestinal region mainly the peyer's patch. Various studies have been conducted in order to enhance the uptake of particles through peyer's patch. A work done by **Desai et al.** (46) revealed that for biodegradable particles made polylactic acid polyglycolic acid copolymer, the particle size of 100 nm shows enhanced uptake by GALT (Gut associated lymphoid tissue) in comparison to 500 nm, 1  $\mu$ m and 10  $\mu$ m

particles. In another work done by **Awaad et al.** (47), thiol-organosilica nanoparticles of size 95, 130 and 200 nm were greatly taken up by peyer's patch in comparison to larger particles of size 340, 695 and 1050 nm. Moreover, the study revealed two pathways of particle uptake- the transcellular pathway and paracellular pathway. A study done by **Jani et al.** (48) revealed that polystyrene microsphere of size 50 and 100 nm showed enhanced gastrointestinal uptake in comparison to larger particle of size 300 nm and 3 $\mu$ m.

The surface charge also plays a significant role upon uptake of particulate delivery systems. Work done by **Tabata and Ikada et al.** (49) have shown that the maximum uptake of particles occurs with a zeta potential of -70mV. This zeta potential also shows an excellent stability and avoids coagulation of the particles due to the high surface charge (50). Based on fluorescence microscopy, anionic liposomes formulated using phosphatidyl serine (PS) were also shown to have improved uptake by peyer's patches compared to liposomes formulated without PS (51). Many other studies have shown that negatively charged particle had higher GI uptake in comparison to positively charged particles (52-54).

Another important parameter is the type of excipient used in the formulation. The increase in bioavailability of many drugs given with high-fat meals is well known (55). This enhanced absorption is due to a large extent to the endogenous lipid digestion process and the solubilisation of lipid digestion products, and presumably the co-administered drug, by bile salts and mixed micelles. More recent data has shown that high-fat meals inhibit transporters, and consequently, may substantially increase the extent of absorption of drugs which are known to be substrates for these transporters (56). A challenge exists therefore to simulate the 'food' effect through strategic design of lipid-based formulations. Recently it has been suggested that the fed state may be induced by the oral administration of exogenous lipids in quantities within the capacity of a unit dose delivery system (57, 58). Thus, selection of correct excipient especially in lipid carrier systems could prove an additional advantage of enhanced absorption of drug.

### 2.3 Solid lipid nanoparticles



**Figure 2.4 Schematic representation of a solid lipid nanoparticle**

Solid lipid nanoparticles (SLN) are a class of particulate drug carriers made from lipids that remain in the solid state at room and body temperatures. SLNs have mean particle size between 50-1000 nm (59). They are biocompatible and biodegradable in nature and are used in various administration routes like oral, topical and parenteral. SLN can be obtained by substituting the liquid lipid (oil) of the *o/w* nanoemulsions by a solid lipid. In general, a solid core offers many advantages in comparison to a liquid core. Emulsions and liposomes usually show lack of protection of encapsulated drugs, and drug releases as a burst (emulsions) or non-controlled (from liposomes) (59). SLN possess a solid lipid matrix identical to polymeric nanoparticles. In addition, SLN are of low cost, the excipients and production lines are relatively cheap, and the production costs are not much higher than those established for the production of parenteral emulsions (60).

A limiting factor for *in vivo* performance of poorly water-soluble drugs for oral administration is their resistance of being wetted and dissolved into the fluid in the GIT (apart from potential drug degradation in the gut). Thus, the increase in the dissolution rate of poorly water-soluble drugs is relevant for optimizing bioavailability. Over the last 10 years, poorly water-soluble compounds are formulated in lipid nanoparticles for drug administration (61). The features of lipid nanoparticles for oral and peroral delivery are

related with their adhesive properties. Once adhered to the GIT wall, these particles are able to release the drug exactly where it should be absorbed. In addition, the lipids are known to have absorption-promoting properties not only for lipophilic drugs, such as Vitamin E, Repagline (62), Puerarin (63). Hydrophilic drugs can also be incorporated in SLN; nevertheless, the affinity between the drug and the lipid needs to be analyzed. Therefore, loading hydrophilic drug in SLN is a challenge, due to the tendency of partitioning the encapsulated molecules in the water during the production of nanoparticles (64). Successful examples of loading of hydrophilic drug into nanoparticles are Zidovudine (64), Insulin (63), Tretinoin (65), and Diminazene (66).

### **2.3.1 Preparation techniques for SLNs (67, 68)**

#### **1. Hot homogenization technique**

In this technique, the homogenization is performed at temperatures above the melting point of the solid lipid. High shear mixing device like Ultraturrax is used for preparation of pre-emulsion of drug loaded lipid melt and the aqueous emulsifier phase (same temperature). The characteristics of the final product is affected by those of pre-emulsion to a large extent and it is desirable to obtain droplets in the size range of a few micrometers. In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase. However, high temperatures also accelerate the degradation rate of the drug and the carrier. Increasing the homogenization time or the number of cycles often results in an increase of the particle size due to particle coalescence which occurs as a result high kinetic energy of the particles. The primary product is a nanoemulsion due to the liquid state of the lipid which on cooling at room temperature leads to solid particles. Due to the small particle size and the presence of emulsifiers, lipid crystallization may be highly retarded and the sample may remain as a super cooled melt for several months.

#### **2. Cold homogenization**

The first step is same as in hot homogenization process that includes the solubilization or dispersing of the drug in the melt of the lipid. The drug containing melt is rapidly cooled which favours the homogeneous distribution of drug in the solid matrix. Low temperatures increase the fragility of the lipid and, therefore,

particle comminution. The solid lipid microparticles are dispersed in a chilled emulsifier solution. The pre-suspension is subjected to high pressure homogenization at or below room temperature resulting in formation of solid lipid nanoparticles.

### 3. Ultra sonication or high speed homogenization

SLNs are also prepared by ultrasonication or high speed homogenization techniques. The technique is used for deagglomerating and dispersing the particles. Probe sonication is suitable for producing dispersion, which can remain in suspension form for a number of months. The primary emulsion is prepared which is sonicated using the ultra sonicator. This results in decrease in the emulsion globules sizes. The internal phase is usually an organic solvent containing the drug and the lipid, which get dispersed in the outer phase containing stabilizer. The evaporation of organic solvent results in precipitation of drug and lipid into particle form. Final particle size depends on the size of globules during emulsification. For smaller particle size combination of both ultrasonication and high speed homogenization is required. It reduces shear stress but has some disadvantages like potential metal contamination, physical instability like particle growth upon storage. In this method, probe sonicator or bath sonicator is used.

### 4. Micro emulsion technique

Gasco and co-workers developed SLN preparation techniques which are based on the dilution of microemulsions. By stirring at 65-70°C, an optically transparent mixture is obtained which is typically composed of a low melting fatty acid (stearic acid), an emulsifier (polysorbate 20, polysorbate 60, soy phosphatidylcholine and sodium taurodeoxycholate), co-emulsifiers (sodium mono octylphosphate) and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring. Typical volume ratios of the hot microemulsion to cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the microemulsion.

### 5. Solvent emulsification- evaporation technique

The lipophilic material is dissolved in a water-immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticles dispersion is formed by precipitation of the lipid in the aqueous medium

giving the nanoparticles. The solution is emulsified in an aqueous phase by high pressure homogenization. The organic solvent is removed from the emulsion by evaporation under reduced pressure.

#### 6. Solvent emulsification- diffusion technique

Here, lipids are dissolved in a water miscible solvent or water miscible solvent mixture and rapidly injected through an injection needle into an aqueous phase containing surfactants. A violent spreading is observed because of the miscibility of both aqueous phase and water miscible organic phase. As the solvent get diffused in aqueous phase, lipid will be precipitated as nanoparticles. Normally used solvents in this method are acetone, isopropyl alcohol, and methanol.

#### 7. Double emulsion technique

Warm w/o/w double microemulsion can be prepared in two steps. Firstly, w/o microemulsion is prepared by adding an aqueous solution containing drug to a mixture of melted lipid, surfactant and co-surfactant at a temperature slightly above the melting point of lipid to obtain a clear system. In the second step, preformed w/o microemulsion is added to mixture of water, surfactant and cosurfactant to obtain a clear w/o/w system. SLNs can be obtained by dispersing the warm micro double emulsions in cold then washed with dispersion medium by ultra filtration system. Multiple emulsions have inherent instabilities due to coalescence of the internal aqueous droplets within the oil phase, coalescence of the oil droplets, and rupture of the layer on the surface of the internal droplets. In case of SLNs production, they have to be stable for few minutes, the time between the preparations of the clear double microemulsion and its quenching in cold aqueous medium, which is possible to achieve.

#### 8. Membrane contactor technique

The present study investigates a new process for the preparation of SLN using a membrane contactor, to allow large scale production. The lipid phase is pressed, at a temperature above the melting point of the lipid, through the membrane pores allowing the formation of small droplets. The aqueous phase circulates inside the membrane module, and sweeps away the droplets forming at the pore outlets. SLN are formed by the following cooling of the preparation to room temperature. The

influence of process parameters (aqueous phase and lipid phase temperatures, aqueous phase cross-flow velocity and lipid phase pressure, membrane pore size) on the SLN size and on the lipid phase flux is investigated. Also, vitamin E loaded SLN are prepared, and their stability is demonstrated.

#### 9. Solvent injection technique

It is a novel approach to prepare SLN, which has following advantages over other production methods like use of pharmacologically acceptable organic solvent, easy handling and fast production process without technically sophisticated equipment. It is based on lipid precipitation from the dissolved lipid in solution. In this technique the solid lipid was dissolved in water-miscible solvent (eg. ethanol, acetone, isopropanol) or a water miscible solvent mixture. Then this lipid solvent mixture was injected through an injection needle into stirred aqueous phase with or without surfactant. The resultant dispersion was then filtered with a filter paper in order to remove any excess lipid. The presence of emulsifier within the aqueous phase helps to produce lipid droplets at the site of injection and stabilize SLN until solvent diffusion was complete by reducing the surface tension between water and solvent.

#### 10. Supercritical fluid technology

The supercritical fluid technology is a new technique and has advantage of solventless processing. It is a new production technique for nanoparticles of water-insoluble drugs in combination with lipids, characterization and development of lipid nanosuspension formulations, and examination of the possibility of delivering the prepared nanosuspension as aerosols for inhalation using Aradigm's AERx Single Dose Platform with micron-sized nozzles and the all mechanical AERx Essence with submicron- sized nozzles. The continuous supercritical fluid extraction of emulsions method was used for particle precipitation of solid lipid nanoparticles. Ketoprofen and Indomethacin were used as model compounds in formulation with lipids such as tripalmitin, tristearin and Gelucire 50/13 (74).

**Table 2.3 Different techniques employed for preparation of SLNs**

Method	Drug	Lipid	Surfactant	Reference
Hot homogenization	Tilmicosin	Hydrogenated	Poly vinyl	(69)

		castor oil	alcohol	
Cold homogenization	Vinorelbine bitartrate	Glyceryl monostearate	Lecithin, Oleic acid	(70)
Ultra sonication or high speed homogenization	Curcuminoid	Migyol <sup>®</sup> 812, Trimyristin, Tristearin	Poloxamer 188	(71)
Micro emulsion technique	Atazanavir	Stearic acid	Pluronic <sup>®</sup> F68	(72)
Solvent emulsification- evaporation technique	Doclofenac sodium	Glyceryl monostearate	Polyvinyl alcohol	(73)
Solvent emulsification- diffusion technique	Cyclosporine	Gelucire 44/14	Polyvinyl alcohol	(74)
Double emulsion technique	Zidovudine	Stearic acid	Poly vinyl alcohol	(64)
Membrane contactor technique	-	Gelucire 44/14	Tween 20	(75)
Solvent injection technique	Doxorubicin HCl	Tristearin	Soya lecithin	(76)
Supercritical fluid technology	Indomethacin	Tripalmitin	Soya lecithin	(77)

### 2.3.2 Composition of solid lipid nanoparticles

Solid lipid nanoparticles typically consist of solid lipid and stabilizer. The *in-vivo* fate of SLNs depends on the properties of the solid lipids selected. Hence, the solid lipid selection is an important criterion. A surfactant is added in order to stabilize the lipid particles in the aqueous media. A combination of surfactant is also preferred sometimes.

#### Solid lipids:

Lipid can be defined as fatty or waxy organic compounds. Generally they are soluble in nonpolar and insoluble in polar solvents. These lipids used for preparation of SLNs are usually physiological lipids (biocompatible and biodegradable) with low acute and chronic toxicity (78). Their typical constituents are free fatty acids, free fatty alcohols, glycerol esters of fatty acids and waxes. Phospholipids, glycolipids and

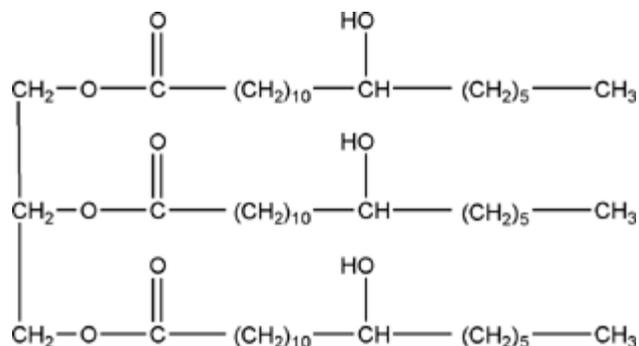
sphingophospholipids are also included in this group. The lipid matrix itself determines the particles pharmaceutical properties as it is the structure that stores, transports and releases the drug. Examples of different group of lipids used in preparation of solid lipid nanoparticles are shown in Table 2.4.

**Table 2.4 Various types of solid lipids used for SLNs preparation (67, 79)**

Triacylglycerols	Acyl glycerols	Fatty acids	Waxes	Hard fat types	Others
Tricaprin	Glyceryl monostearate	Stearic acid	Cetyl palmitate	Witepsol W35	Castor oil
Trilaurin	Glyceryl behenate	Palmitic acid	Bees wax	Witepsol H 35	Hydrogenated castor oil
Trimyristin	Glyceryl palmitostearate	Decanoic acid	Carnuba wax	Witepsol h45	Cacao butter
Tripalmitin	Glyceryl monooleate	Behenic acid			Goat fat
Tristearin	Glyceryl distearate				Anhydrous milk fat

#### Hydrogenated castor oil (HCO) Profile (80)

- *Synonyms:* Castor wax, Cutina HR, Castorwax MP 70, Castorwax MP 80
- *Chemical name and CAS Registry number:* Glyceryl-tri-(12-hydroxystearate) [8001-78-3]
- *Empirical formula and molecular weight:* C<sub>57</sub>O<sub>9</sub>H<sub>110</sub>, 939.50
- *Structural formula*



**Figure 2. 5 Structure of Hydrogenated castor oil**

- *Description:* HCO occurs as a fine, almost white or pale yellow powder or flakes. The PhEur 6.0 describes HCO as the oil produced by the hydrogenation of virgin castor oil, in presence of a nickel catalyst. It mainly consists of the triglyceride of 12-hydroxystearic acid.
- *Typical properties:*
  - Acid value  $\leq 5$
  - Density 0.98-1.10 /cm<sup>3</sup>
  - Flash point 316°C
  - Moisture content  $\leq 0.1\%$
  - Particle size distribution 97.7 %  $\geq 1000 \mu\text{m}$  in size for flakes
  - Solubility: Practically insoluble in water, soluble in acetone, chloroform and methylene chloride.
- *Stability:* HCO is stable at temperature up to 150°C. Clear, stable, chloroform solutions containing up to 15% w/v of hydrogenated castor oil may be produced. Hydrogenated castor oil may also be dissolved at temperatures greater than 90°C in polar solvents and mixtures of aromatic and polar solvents, although the hydrogenated castor oil precipitates out on cooling below 90°C. Hydrogenated castor oil should be stored in a well-closed container in a cool, dry place.
- *Safety:* Hydrogenated castor oil is used in oral and topical pharmaceutical formulations and is generally regarded as an essentially nontoxic and nonirritant material. Acute oral toxicity studies in animals have shown that hydrogenated castor oil is a relatively nontoxic material. Irritation tests with rabbits show that hydrogenated castor oil causes mild, transient irritation to the eye. LD<sub>50</sub> (rat, oral): >10 g/kg.

**Stabilizers:**

When one of the two immiscible phases is dispersed into another, an interfacial boundary is formed. The surface energy at this boundary is expressed as Gibbs free energy (G). At constant temperature and pressure, G depends on the surface area (A) and the interfacial tension ( $\gamma$ ).

$$\Delta P = P_{\text{inside}} - P_{\text{outside}} = \frac{2\gamma}{r}$$

Where  $P_{\text{inside}}$  is the pressure inside the droplet,  $P_{\text{outside}}$  is the pressure outside the droplet,  $\gamma$  is the surface tension and  $r$  is the radius of the spherical droplet (81). Hence, as the droplets become smaller their radius decreases which results in proportional increase in the pressure. Therefore, more energy will be required to produce smaller and smaller particles. Surface active compounds, due to their amphiphilic structure, exhibit a tendency to accumulate at phase boundary and form monomolecular layer around the droplets/particles. This normally results in system stabilization by lowering the surface tension, decrease in the Gibbsfree energy and the Laplace pressure. However, surfactants properties are predetermined by their chemical structure and they lower the surface tension only to a certain limit. Further decrease can be achieved with the incorporation of co-surfactants (82). The commonly used surface active agents employed for SLN preparation is given in Table 2.5.

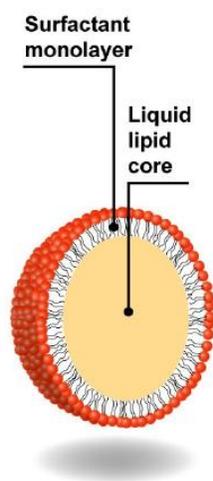
**Table 2.5 Commonly used stabilizers for SLN preparation (67, 79)**

<b>Phospholipids</b>	<b>Ethylene oxide/propylene oxide</b>	<b>Sorbitan ethylene/propylene oxide</b>	<b>Alkylaryl polyether alcohol polymers</b>	<b>Bile salts</b>	<b>Alcohols</b>
Soya lecithin	Poloxamer 188	Polysorbate 20	Tyloxapol	Sodium cholate	Ethanol
Egg lecithin	Poloxamer 182	Polysorbate 60		Sodium glycocholate	Butanol
Phosphatidyl choline	Poloxamer 407	Polysorbate 80		Sodium taurocholate	
	Poloxamer 908			Sodium taurodeoxycholate	

## 2.4 Nanoemulsion

Nanoemulsions are dispersion of oil and water where the dispersed phase droplets are in the nanosized range with mean droplet diameter ranging from 50-1000 nm and stabilized with a surface active film. It is also termed as sub-micron emulsion or mini-

emulsion. The nanosizes of the droplets prevent creaming or sedimentation occurring on storage and droplet coalescence. Nanoemulsions provide much larger oil-in-water contact area due to the nanosized droplet compared to classical emulsions, which facilitates drug release from the dispersed droplets. They are the most advanced nanoparticle systems for the systemic delivery of biologically active agents for controlled drug delivery and targeting. Nanoemulsions were initially used to provide energy to patients. Nanoemulsions are also promising vehicles for drug administration because of their features of sustained release, drug targeting and reduced toxicity (83). Nanoemulsions have already been developed for various drug including Diclofenac (84), Flurbiprofen (85), Primaquine (86), Paclitaxel (87), Docetaxel (88), Indinavir (89), Saquinavir (90), Etoposide (91) etc.



**Figure 2.6 Schematic representation of a nanoemulsion droplet**

The advantages of formulating lipophilic drugs in o/w nanoemulsion are obvious. The oil phase of the emulsion systems can act as a solubilizer for the lipophilic compound. Therefore, solubility of lipophilic drugs can be significantly enhanced in a nanoemulsion system, leading to smaller administration volumes compared to an aqueous solution (92).

#### **2.4.1 Methods of preparation of Nanoemulsion**

1. High pressure homogenization

This technique makes use of high-pressure homogenizer/ piston homogenizer to produce nanoemulsions of extremely low particle size (up to 1  $\mu\text{m}$ ). During this process, several forces, such as hydraulic shear, intense turbulence and cavitation, act together to yield nanoemulsions with extremely small droplet size. The resultant product can be re-subjected to high pressure homogenization until nanoemulsion with desired droplet size and polydispersity index is obtained. The production of small droplets (submicron) requires application of high energy. The emulsion is preferably prepared at high volume fraction of the disperse phase and diluted afterwards. However, very high phase volume ratios may result in coalescence during emulsification, but more surfactant could be added to create a smaller reduction in effective surface tension and possibly diminishing recoalescence.

## 2. Microfluidization

Microfluidization is a patented mixing technology, which makes use of a device called microfluidizer. This device uses a high pressure positive displacement pump (500 - 20,000 psi), which forces the product through the interaction chamber, consisting of small channels called “micro channels”. The product flows through the micro channels on to an impingement area resulting in very fine particles of submicron range. Homogenizer is used to form coarse emulsion which is then introduced into a microfluidizer to form stable nanoemulsion. The bulk emulsion is then filtered through a filter under nitrogen to remove large droplets resulting in a uniform nanoemulsion.

## 3. Phase inversion Temperature (PIT) technique

It has been observed that polyethoxylated surfactants tend to become lipophilic on heating owing to dehydration of polyoxyethylene groups. This phenomenon forms a basis of nanoemulsion fabrication using the PIT method. In the PIT method, oil, water and nonionic surfactants are mixed together at room temperature. This mixture typically comprises o/w microemulsions coexisting with excess oil, and the surfactant monolayer exhibits positive curvature. When this macroemulsion is heated gradually, the polyethoxylated surfactant becomes lipophilic and at higher temperatures, the surfactant gets completely solubilized in the oily phase and the initial o/w emulsion undergoes phase inversion to w/o emulsion. The surfactant

monolayer has negative curvature at this stage. This method involves heating of the components and it may be difficult to incorporate thermolabile drugs.

#### 4. Solvent displacement method

The water miscible organic phase such as acetone is poured into an aqueous phase containing surfactant to yield spontaneous nanoemulsion by rapid diffusion of organic solvent. The organic solvent is removed from the nanoemulsion by a suitable means, such as vacuum evaporation. Solvent displacement methods can yield nanoemulsions at room temperature and require simple stirring for the fabrication. Hence, researchers in pharmaceutical sciences are employing this technique for fabricating nanoemulsions mainly for parenteral use. However, the major drawback of this method is the use of organic solvents, such as acetone, which require additional step of their removal from nanoemulsion. Furthermore, a high ratio of solvent to oil is required to obtain a nanoemulsion with a desirable droplet size. This may be a limiting factor in certain cases.

#### 5. Self- Nanoemulsification method

This method generates nanoemulsions at room temperature without use of any organic solvent and heat. Kinetically stable nanoemulsions with small droplet size (~50 nm) can be generated by the stepwise addition of water into solution of surfactant in oil, with gentle stirring and at constant temperature. The spontaneous nanoemulsification has been related to the phase transitions during the emulsification process and involves lamellar liquid crystalline phases or D-type bicontinuous microemulsion during the process. Nanoemulsions obtained from the spontaneous nano-emulsification process are not thermodynamically stable, although they might have high kinetic energy and long-term colloidal stability.

### **2.4.2 Composition of lipid nanoemulsion**

Nanoemulsions are prepared using oils, surfactants, co-surfactants and aqueous phase.

#### **Oils:**

The oils used in nanoemulsions preparation includes Miglyol 812, soyabean oil, triacetin, capmul MCM, Captex 355, Captex 8000, Witepsol, Myritol 318, Isopropyl

myristate, Capryol 90, sefsol 218, olive oil, castor oil etc (29). Solubility of the drug in the oil phase is an important criterion for the selection of oils. This is particularly important in the case of oral formulation development, as the ability of nanoemulsion to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in the oil phase. Drug loading in the formulation is a very critical design factor in the development of nanoemulsions for poorly soluble drugs, which is dependent on the drug solubility in various formulation components. An understanding of factors influencing drug loading capacity while maintaining the capability of the system to undergo monophasic dilution with water and minimizing the tendency for drug precipitation or crystallization in diluted systems is essential to the design of stable and appropriately low-volume nanoemulsion systems for drug delivery applications (93, 94). Novel semi-synthetic medium chain derivatives (as amphiphilic compounds) having surfactant properties are progressively and effectively have replaced the regular medium chain triglyceride oils (95, 96).

#### **Soyabean oil profile (80)**

- *Synonyms:* Refined soya oil, Lipex 200, Lipex 107
- *Chemical name and CAS Registry number:* Soyabean oil [8001-22-7]

*Empirical formula and molecular weight:* A typical analysis of refined soybean oil indicates the composition of the acids, present as glycerides, to be: linoleic acid 50–57%; linolenic acid 5–10%; oleic acid 17–26%; palmitic acid 9–13%; and stearic acid 3–6%. Other acids are present in trace quantities.

- *Functional category:* Oleaginous vehicle, solvent
- *Description:* Soybean oil is a clear, pale-yellow colored, odorless or almost odorless liquid, with a bland taste that solidifies between -10 and -168 °C.
- *Typical properties*
  - Autoignition temperature: 445 °C
  - Density: 0.916-0.922 g/cm<sup>3</sup> at 25 °C.
  - Flash point: 282°C
  - Freezing point: -10°C to -16 °C

Hydroxyl value: 4-8

Solubility: Practically insoluble in ethanol (95%) and water; miscible with carbon disulfide, chloroform, ether and light petroleum

- *Stability*: Soyabean oil is stable material if protected from atmospheric oxygen. It should be stored in a well-filled, airtight, light resistant container at a temperature not exceeding 25 °C.
- *Safety*: Soyabean oil is widely used orally and intramuscularly as drug vehicle; parenterally in emulsion formulation; and also consumed as edible oil. Generally, soyabean oil is regarded as an essentially non toxic and nonirritant material.

### **Surfactants:**

Surfactants used for stabilizing nanoemulsions may be non ionic, zwitterionic, cationic and anionic. The surfactants may include Capryol 90, Gelucire 44/14, 50/13, Cremophor RH 40, Imwitor 191, 742, 780 k, 928, 988, Labrafil CS, M, 2125 CS, Lauroglycol 90, PEG MW > 4000, Plurol Oleique CC 497, Poloxamer 124 and 188, Softigen 701, 767, Labrasol, Cremophor EL, Tween 20, Tween 60, and Tween 80, etc (29). Components of nanoemulsion-based systems are associated with toxicity concerns. Large amounts of surfactants may cause gastrointestinal and skin irritation when administered orally and topically, respectively. Therefore, the proper selection of surfactants is essential. Rational use of the minimum concentration of the surfactant in the formulation is advocated. Nonionic surfactants are relatively less toxic than their ionic counterparts and typically have lower critical micelle concentration (CMCs). Also, o/w nanoemulsion dosage forms for oral or parenteral use based on nonionic surfactants are likely to offer *in vivo* stability (97). Therefore, proper selection of surfactants is a crucial factor. Another important criterion is the selection of surfactant with proper hydrophile-lipophile-balance (HLB) value. Hydrophilic surfactants and co-surfactants are considered to prefer the interface and to lower the necessary energy to form the nanoemulsions, thereby improving the stability. For instance, the required HLB value to form o/w nanoemulsion is greater than 10 (98). The right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion upon dilution with water. The type and nature of the surfactant is also an important factor for consideration; nonionic

surfactants are usually selected since they are known to be less affected by pH and changes in ionic strength, are generally regarded as safe, and are biocompatible; ionic surfactants are less commonly used due to toxicological concerns. Solubilization of oil with the surfactant is also an important factor. It is not necessary that the same surfactant that has good solubilizing power for drugs would have equally good affinity for the oil phase. Surfactant–oil miscibility can thus give an initial indication on the possibility of nanoemulsion formation with this system.

**Co-surfactants:**

Cosurfactants are added to obtain nanoemulsion systems at low surfactant concentration (99). Short-to medium-chain-length alcohols (C3-C8) are commonly added as cosurfactants, which further reduce the interfacial tension and increase the fluidity of the interface (100). They also increase the mobility of the hydrocarbon tail and allow greater penetration of the oil into this region. Alcohols may also increase the miscibility of the aqueous and oily phases due to its partitioning between these phases. Co-surfactants used in nanoemulsions include Transcutol P, glycerin, ethyleneglycol, ethanol, propanol, ethanol, isopropyl alcohol, n-butanol, PEG 400, Carbitol, and propylene glycol. Nanoemulsion area is often used as the assessment criterion for the evaluation of cosurfactants. The larger the size of the nanoemulsion field, the greater the nanoemulsification efficiency of the system (29).

**2.5 Characterization of solid lipid nanoparticles and lipid nanoemulsion**

Proper characterization of formulations is necessary to control the product quality, stability and release kinetics. Hence, accurate and sensitive characterization methods should be used. There are several important characterization techniques as follows.

**2.5.1 Particle/ globule size (78, 101)**

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful and widely used techniques for the particle size measurement of lipid nanoparticles. PCS is also known as dynamic light scattering. The fluctuation of the intensity of the scattered light, caused by the particles movement, is measured by this

technique. PCS is relatively accurate and sensitive method. However, only size range from few nanometers to about 3  $\mu$  can be measured by PCS. This size range is enough to characterize lipid nanoparticles as well as nanoemulsion. On the other hand, LD can measure bigger particle sizes ( $>3 \mu$ ). LD covers a broad range from nanometer to lower millimeter range. This method is based on dependence of the diffraction angle on the particle radius. Smaller particles lead to more intense scattering at high angles than the larger particles.

### **2.5.2 Polydispersibility index (78, 101)**

As SLNs are usually polydisperse in nature, measurement of polydispersibility index (PDI) is important to know the size distribution of the nanoparticles and nanoemulsion. The lower the PI value, the more monodispersed the nanoparticle dispersion is. PI can be measured by PCS.

### **2.5.3 Zeta potential (78, 101)**

The zeta potential (ZP) indicates the overall charge a particle acquires in a specific medium. Stability of nanoparticles and nanoemulsion during storage can be predicted from the ZP value. The ZP indicates the degree of repulsion between close and similarly charged particles in the dispersion. High ZP indicates highly charged particles. Generally, high ZP (negative or positive) prevents aggregation of the particles due to electric repulsion and electrically stabilizes the nanoparticle dispersion. The ZP of the nanodispersions can be determined by PCS.

### **2.5.4 Shape and morphology (78, 101)**

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) are very useful techniques to determine the shape and morphology of lipid nanoparticles as well as nanoemulsion. These techniques can also determine the particle/ globule size and size distribution. SEM utilizes electron transmission from the sample surface, whereas TEM utilizes electron transmission through the sample. In contrast to PCS and LD, SEM and TEM provide direct

information on the particle shape and size. Although normal SEM is not very sensitive to the nanometer size range, field emission SEM (FESEM) can detect nanometer size range. However, sample preparation (e.g., solvent removal) may influence the particle shape. Cryogenic FESEM might be helpful in this case, where liquid dispersion is frozen by liquid nitrogen and micrographs are taken at the frozen condition. AFM technique is also gaining popularity for nanoparticle characterization. AFM directly provides structural, mechanical, functional, and topographical information about surfaces with nanometer- to angstrom-scale resolution. In this technique, the force acting between a surface and a probing tip results in a spatial resolution of up to 0.01 nm for imaging.

### **2.5.5 Crystallinity and polymorphism (78)**

Determination of the crystallinity of the components of SLN formulations is crucial as the lipid matrix as well as the incorporated drug may undergo a polymorphic transition leading to a possible undesirable drug expulsion during storage. Lipid crystallinity is also strongly correlated with drug incorporation and release rates. Differential scanning calorimetry (DSC) and X-Ray diffractometry (XRD) are two widely used techniques to determine the crystallinity and polymorphic behavior of the components of the SLNs. DSC provides information on the melting and crystallization behavior of all solid and liquid constituents of the particles, whereas XRD can identify specific crystalline compounds based on their crystal structure. Another two techniques, infrared and Raman spectroscopy are also useful to investigate structural properties of lipids. However, they have not been extensively used to characterize SLNs.

### **2.5.6 Determination of incorporated drug (68)**

The amount of drug encapsulated per unit weight of nanoparticles is determined after separation of the free drug and solid lipids from the aqueous medium. This separation can be carried out using centrifugation, filtration or gel permeation chromatography. In centrifugation filtration, the filters such as U'trafree-MC (Milipore) or Utrasart-10 (Sartorius) are used along with classical centrifugation techniques. The degree of encapsulation can be assessed indirectly by determining in the supernatant after centrifugation filtration / ultracentrifugation of SLN suspension or alternatively by

dissolution of the sediment in an appropriate solvent and subsequent analysis. In gel permeation chromatography Sephadex<sup>®</sup> and Sepharose<sup>®</sup> gels are used for removal of free drug from SLN preparations. First, preliminary calibration of column is carried out using SLNs and free drug. SLN preparations are applied to the column and washed with suitable buffer. Fractions containing SLNs can be collected and analyzed for the actual drug content after dissolution/ extraction with appropriate solvent. Drug content can also be determined directly in SLNs by extracting the drug with suitable solvent under optimum conditions and subsequent analysis of aqueous extract.

### **2.5.7 *In-vitro* assessment of drug release from nanoparticles and nanoemulsion (68, 101)**

*In-vitro* drug release could be studied using dialysis tubing. The formulation is placed in pre-treated dialysis tubing which can be hermetically sealed. The dialysis sac is then dialyzed against a suitable diffusion medium at room temperature. The samples are withdrawn from the diffusion medium at suitable intervals, centrifuged and analyzed for the drug content using a suitable analytical method. In another method called reverse dialysis, a number of small dialysis sacs containing 1 ml of diffusion medium are placed in SLN dispersion.

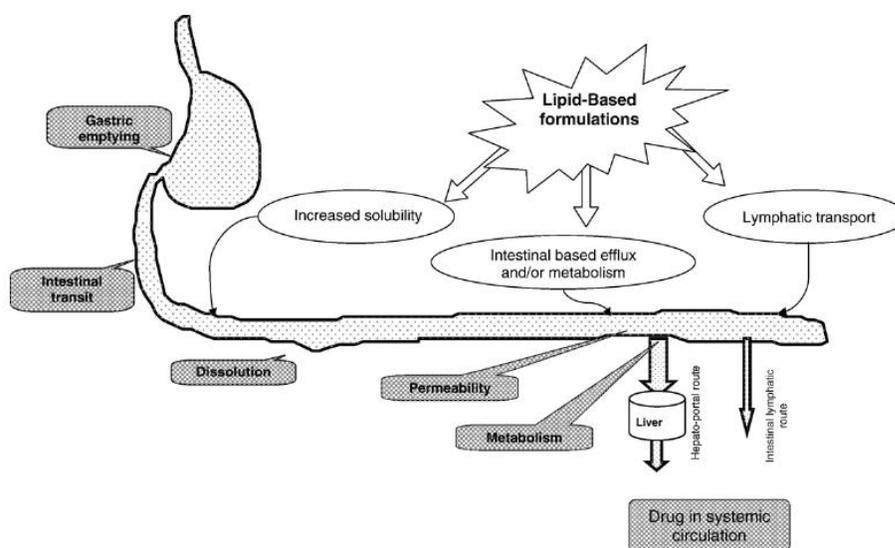
### **2.5.8 Short term stability of nanoemulsions (102)**

Stability studies are performed on nanoemulsions by storing them at refrigerator and room temperatures over a few months. During this period of storage, various parameters including creaming, coalescence, phase separation and/or precipitation, globule size, polydispersibility index, zeta potential and drug entrapment are measured. Insignificant changes in these parameters indicate formulation stability. This study is generally done for screening of stable nanoemulsion formulation. Accelerated stability studies can also be performed. In this case, nanoemulsion formulation is kept at accelerated temperatures and samples are withdrawn at regular intervals and analyzed for drug content by HPLC. The amount of drug degraded and remaining in nanoemulsion formulation is determined at each time interval.

## **2.6 Bioavailability enhancement by SLNs and lipid nanoemulsion**

Lipid based delivery systems are now widely employed for enhancing bioavailability of poorly soluble drugs. The primary role of lipids in oral delivery is the solubilization and emulsification of the drug in gastro-intestinal tract leading to higher absorption. A number of drug like Raloxifene hydrochloride (103), Simvastatin (104), Saquinavir (105), Lovastatin (106), and Praziquantel (107) have been incorporated into SLNs formulation in order to increase their oral bioavailability. However, few studies have reported mechanism of higher absorption of poorly soluble drugs apart from its increased portal circulation through use of SLN formulation. One such study was done by Aji Alex et al. (108) who formulated SLNs of Lopinavir using Compritol 888 ATO. They found an increase in relative bioavailability by 213.45 % in comparison to Lopinavir suspension. There was an increase in the lymphatic transport of Lopinavir by 5 fold in comparison to suspension form which indicated that the SLNs have capacity for increasing the oral bioavailability of drug like Lopinavir through increased lymphatic uptake. Similarly, bioavailability and intestinal lymphatic uptake of methotrexate was found to be enhanced by incorporating it into solid lipid nanoparticles made from compritol 888 ATO (109). The bioavailability of poorly soluble drug Candesartan cilexetil (CC) has also been improved by incorporating it into SLN formulation (110). They found that the absorption of CC- SLNs was only 2.8 % of that in intestine indicating the stability of prepared SLNs in the gastric media. The Caco-2 uptake study indicated that clathrin and caveole mediated endocytosis pathways were involved in the uptake of CC-SLNs. Moreover, after blockade of lymphatic flow, there was decrease in plasma concentration of CC which indicated that CC was also transported via lymphatic system. Thus, the uptake of CC-SLNs was through the internalization into enterocytes and transport into systemic circulation via portal circulation and intestinal lymphatic pathway. Similarly, oral bioavailability of Praziquantel was improved by loading into SLN formulation and lymphatic uptake study indicated that the SLNs were transported by lymphatic route in addition to portal circulation (111). Thus, lipophilic drug are the best candidates for incorporating into SLNs in order to improve their oral bioavailability.

Nanoemulsions are also an attractive carrier for lipophilic drug in order to increase their oral bioavailability (90), sustained release (112) and targeting (113, 114). Oral bioavailability of many poorly soluble drug have been increased by incorporating them into nanoemulsion formulation. Vyas et al. incorporated Saquinavir into nanoemulsion formulation using edible oil- flax-seed oil and safflower oil. It not only resulted into increased bioavailability by approximately 2 fold, but also increased brain uptake by 1.5-3 folds (90). Another potential advantage associated with the LNEs is the use of correct excipient for particular drug. The good example is the use of Tween-80 into lipid nanoemulsion as emulsifier and as P-gp inhibitor and has been used for enhancing permeation of P-gp substrate drug Indinavir (102). Another drug, Primaquine has shown increased oral bioavailability and anti-malarial activity in comparison to plain drug by incorporation it into lipid nanoemulsion formulation prepared using medium chain triglyceride containing Miglyol 812 (86). Thus, lipid based formulations can positively influence the drug absorption in a number of ways including: increasing solubilization capacity, preventing precipitation on intestinal dilution, enhancing membrane permeability, inhibiting efflux transporters, reducing CYP enzymes, enhancing chylomicron production and lymphatic transport (115). The critical steps in oral drug absorption are illustrated (115).



**Figure 2.7 Schematic representation of the critical steps in oral drug absorption and the possible influences of lipid based formulations.**

## 2.7 CD4 receptor targeting

A major tissue pool of HIV during HAART therapy is in the lymph nodes and lymphoid tissues where the majority of HIV target cells reside. Virus isolated from lymphoid tissues, blood CD4<sup>+</sup> T cells and plasma are all equally sensitive to anti-HIV drugs (116, 117), particularly protease inhibitors (118). These data suggests that at effective plasma drug concentrations, insufficient drug exposure to lymphoid tissue may be one of the key reasons in the inability to completely eliminate residual virus. As 98% of circulating lymphocytes reside in the lymphatic system (119), lower sub-therapeutic levels in the lymphatic system may allow low and persistent levels of viral replication and increase the probability of developing and harboring drug resistant virus (120).

Within a lymph node, less than 30% of the total cells are HIV target cells expressing the major HIV receptor CD4 (121); however, all cells are likely exposed to drug with the non-targeted formulations. Thus, various authors have attempted to prepare targeted formulation containing the receptor that selectively bonds to CD4 cell. This increases the binding affinity with the HIV target cells, prolongs residence time in CD4<sup>+</sup> T cells and thus enhances the exposure of drug to HIV infected cells (120). Nanocarriers have been successful in achieving this goal. Targeting using surface-modified nanocarriers occurs conceptually at two levels, at the organ/ tissue and cell levels. At the organ/tissue level, nanocarrier size and surface properties determine which organ/tissue will be targeted by the nanocarriers (i.e., preferentially retained) and how long they will be retained. The dependence of nanocarrier size-flow-organ/ tissue filtration is a hallmark of passive targeting. The most successful targeted drug therapies will ultimately prove to be a combination of passive and active targeting. Once retained in a tissue or organ, a nanocarrier displaying a targeting moiety on its surface would have a much higher specificity than the non cell-targeted nanocarrier because it has been enriched twice, once at the organ/tissue level (passive targeting) and once at the cellular level (active targeting) (119).

**Duzqunes et al.** (122) developed targeted nanocarriers to target HIV-1 infected cells via covalently coupled soluble CD4. **Pollock S et al.** (123) also demonstrated increased uptake of liposomal carrier encapsulating anti-HIV drug through the attachment

of soluble CD4 on carrier surface. Another work done by **Slepshkin et al.** (124) demonstrated that a synthetic peptide from the complementarily determining region 2 (CDR-2)-like domain of CD4 could bind specifically to HIV-infected cells and mediate the binding of peptide-coupled liposomes to these cells. This demonstrated the feasibility of using synthetic peptides to target nanocarrier containing antiviral drugs to HIV-infected cells. Apart from soluble CD4 and synthetic peptides, another ligand, gp-120 directed monoclonal antibody F105 has also been shown by **Clayton R et al.** (125) to target liposomal nanocarrier to HIV-infected cells. Another ligand mouse anti-HLA-Dr antibody Fab fragments has been shown by **Gange et al.** (126) and **Desormeaux et al.** (127) to enhance accumulation of carrier on HIV target cells.

The most popular among these ligands to target HIV infected cells are the synthetic peptides. Synthetic peptides can be synthesized rapidly either automatically or manually in a reproducible manner (128). Various peptides having affinity of binding to CD4 receptor containing cells have been synthesized. **Florence et al.** (129) synthesized a 27 residue peptide which displayed an affinity for the antigen (CD4) of 0.9 nM compared to 2 nM for the parent antibody ST40. **Carlo Zanatto et al.** (130) derived another synthetic peptide having 23 amino acid sequence- YNKRKRIHIGPGRAFYTTKNIIG and showed 3 times more binding to CD4 expressing Molt-4 cells in comparison to control. **Aron Endsley and Rodney J.Y.Ho** (120) formulated Indinavir loaded lipid nanoparticles, covalently attached 4 peptides derived synthetically on the surface of prepared nanoparticles and compared their affinity for binding to CD4 expressing cells. They found that peptides having sequence CKGIRIGPGRAYAAE and CARRPKFYRAPYVKNHPNVWGPWVAYGP showed an enhanced binding of nanoparticles to CD4 expressing cells in comparison cells not expressing CD4 receptors. The results are demonstrated the ability of CD4 binding peptides to be efficiently incorporated onto drug-associated nanoparticles.

## **2.8 Drug profiles**

### **2.8.1 Darunavir (131-135)**

**Category:** Anti-HIV agent, HIV protease inhibitor

**CAS NO:** 206361-99-1

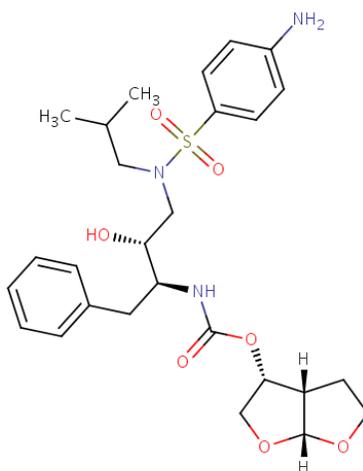
**Proprietary name:** TMC 114, Darunavirum

**Molecular formula:** C<sub>27</sub>H<sub>37</sub>N<sub>3</sub>O<sub>7</sub>S

**Molecular weight:** 547.664

**IUPAC name:** (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)4-aminobenzenesulfonamido]-1-phenylbutan-2-yl]carbamate

**Structural formula:**



**Figure 2. 8 Structural formula of Darunavir**

**Physicochemical properties:** Darunavir is a white solid powder

**Solubility:** As ethanolate salt, Darunavir is very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol and freely soluble in acetone and dichloromethane.

**Log P:** 2.82

**pKa:** 2.39

**Mechanism of action:** Darunavir is a HIV protease inhibitor which prevents HIV replication by binding to the enzyme's active site, thereby preventing the dimerization

and the catalytic activity of the HIV-1 protease. Darunavir selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in virus-infected cells, which prevents the formation of mature infectious virus particles. Structural analyses suggests that the close contact that Darunavir has with the main chains of the protease active site amino acids (Asp-29 and Asp-30) is an important contributing factor to its potency and wide spectrum of activity against multi-protease inhibitor resistant HIV-1 variants. Darunavir can also adapt to the changing shape of a protease enzyme because of its molecular flexibility. Darunavir is known to bind to two distinct sites on the enzyme: the active site cavity and the surface of one of the flexible flaps in the protease dimer.

**Pharmacokinetics:** It is absorbed orally with bioavailability of only 37 %. Darunavir undergoes hepatic metabolism and is extensively metabolized by CYP enzymes, primarily by CYP3A. So, it is always administered with Ritonavir to reduce its metabolism and increase the bioavailability. Presence of food increases the bioavailability of Darunavir. It is a substrate of ABC transporters like P-gp and thus have low permeability resulting in increased enzymatic extent and lower bioavailability. When combined with Ritonavir, its terminal elimination half life was 15 h. Darunavir is approximately 95% bound to plasma proteins. Darunavir binds to plasma alpha 1-acid glycoprotein (AAG).

**Therapeutic uses and administration:** Darunavir is indicated in combination therapy for HIV/AIDS (HAART). It is always administered with low dose Ritonavir.

**Adverse effects:** Hepatitis, fever, severe skin reactions, rashes, hyperglycemia, exacerbation of existing diabetes mellitus, lipodystrophy.

**Contraindications:** It is contraindicated with drugs that are primarily metabolized by CYP3A. Concomitant use of Darunavir with drugs like Alfuzosin, Ranolazine, Disopyramide, Mexiletine, Rifapentine, Dasatinib is contraindicated.

**Formulations available:** It is available as 70, 150, 300, 400 and 600 mg tablets. It is also available in suspension formulation and strength of 100 mg/ml.

**Research work done on Darunavir:**

**Thommes et al.** (136) formulated pellet formulation of Darunavir using wet extrusion/spheronisation with K-carrageenan or microcrystalline cellulose as pelletisation aid. The relative bioavailability of Darunavir versus the reference tablet was found to be 155% with k-carrageenan pellets.

**Spandana et al.** (137) formulated solid self-microemulsifying drug delivery system (s-snedds) of Darunavir for improving dissolution and enhancing bioavailability. S-snedds were prepared using Capmul MCM, tween 80 and transcitol P. Dissolution was increased by 3 fold in comparison to plain drug. Oral bioavailability in wistar rats significantly improved in comparison to plain drug.

**Meshram et al** (138) formulated biodegradable polymeric nanoparticles of combination of drugs- Darunavir and Atazanavir sulfate in order to achieve reduced toxicity, longer intracellular drug residence and decreased dosing variability. Nanoparticles were prepared by nanoprecipitation using polycaprolactone as polymer. The *in-vivo* study showed a controlled release for 24 hr with about 2 fold increase in area under curve for both drugs in comparison to pure drug suspension.

**Thommes et al** (139) prepared 800 mg of Darunavir in a single unit dosage. The tablets were prepared by hot melt extrusion technique. Poloxamer 188 and 407 were used to modify dissolution properties of Darunavir and a higher solubilization for poloxamer 188 was obtained. A zero order drug release from pure Darunavir extrudates was found.

### 2.8.2 Atazanavir Sulfate (130, 140-142)

**Category:** Anti-HIV agents, HIV protease inhibitors

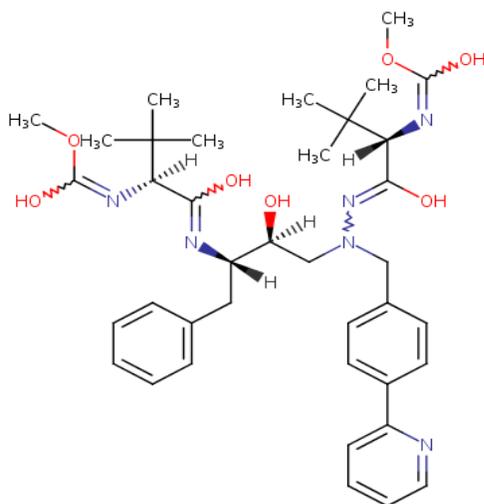
**CAS NO:** 206361-99-1

**Proprietary name:** BMS-232632, Atazanavirum

**Molecular formula:** C<sub>38</sub>H<sub>52</sub>N<sub>6</sub>O<sub>7</sub>

**Molecular weight:** 704.855, Monoisotropic: 704.387

**IUPAC name:** methyl N-[(1S)-1-([(2S,3S)-3-hydroxy-4-[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethylbutanehydrazido] -1-phenylbutan-2-yl] carbonyl) -2,2 dimethyl propyl] carbamate

**Structural formula:****Figure 2. 9 Structural formula of Atazanavir sulfate**

**Physicochemical properties:** Atazanavir sulfate is a white solid powder

**Solubility:** Free base slightly water soluble, freely soluble in methanol, sparingly soluble in dichloromethane. The solubility of Atazanavir sulfate is pH dependent and is highest at pH 1.9. As pH increases, the solubility decreases and is practically insoluble at pH 6.8.

**Log P:** 4.54

**pKa:** 11.92

**Mechanism of action:** Atazanavir sulfate selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells by binding to the active site of HIV-1 protease. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Atazanavir is not active against HIV-2.

**Pharmacokinetics:** Atazanavir is rapidly absorbed with a  $T_{max}$  of approximately 2.5 hours. Administration of Atazanavir with food enhances bioavailability and reduces pharmacokinetic variability. Oral bioavailability is 60-68%. It is 86% bound to human serum proteins (alpha-1-acid glycoprotein and albumin). Protein binding is independent of concentration. Atazanavir is extensively metabolized in humans, primarily by the liver.

The major biotransformation pathways of Atazanavir in humans consisted of monooxygenation and dioxygenation. Other minor biotransformation pathways for Atazanavir or its metabolites consisted of glucuronidation, N-dealkylation, hydrolysis, and oxygenation with dehydrogenation. *In-vitro* studies using human liver microsomes suggested that Atazanavir is metabolized by CYP3A. Elimination half-life in adults (healthy and HIV infected) is approximately 7 hours (following a 400 mg daily dose with a light meal). Elimination half-life in hepatically impaired patients following a single 400 mg dose is 12.1 hours.

**Therapeutic uses and administration:** Atazanavir is used in combination with other antiretroviral agents for the treatment of HIV-1 infection, as well as postexposure prophylaxis of HIV infection in individuals who have had occupational or nonoccupational exposure to potentially infectious body fluids of a person known to be infected with HIV when that exposure represents a substantial risk for HIV transmission.

**Adverse effects:** Headache, cardiac conduction abnormalities, hyperbilirubinemia, nephrolithiasis, cholelithiasis, nausea, jaundice, sclera icterus, vomiting, diarrhea, stomach pain, rashes, fatigue, fever, or trouble sleeping.

**Contraindications:** Atazanavir may interact with digoxin, antibiotics, antifungals, antidepressants, blood thinners, calcium channel blockers, cholesterol-lowering medicines, drugs that weaken the immune system, heart rhythm medications, insulin or oral diabetes medication, medicines to treat erectile dysfunction, other HIV /AIDS medicines, or stomach acid reducers. Many drugs like Rifampicin, Irinotecan, Efavirenz, Triazolam, Ergotamine, Cisapride, Pimozide, Indinavir and Nevirapine are contraindicated with Atazanavir therapy.

**Formulations available:** It is available in capsule dosage form at strengths of 100, 150, 200 and 300 mg and 50 mg powder form.

#### **Research work done of Atazanavir:**

**Gurinder et al.** (143) formulated Atazanavir loaded Eudragit RL 100 nanoparticles to improve oral bioavailability. Eudragit RL 100 was used for maximizing

the oral bioavailability of Atazanavir and prolonging the residence time in order to obtain sustain drug release. The nanoparticles were prepared by nanoprecipitation technique. The optimized formulation gave mean particle size of 465 nm. Transmission electron microscopy demonstrated uniform shape and size of particles. *In-vivo* pharmacokinetics studies indicated increase in AUC<sub>0-24</sub> (2.91 fold) compared to pure drug. In-situ study ascribed the significant enhancement in absorption and permeability.

**Niladri et al.** (72) prepared solid lipid nanoparticles of Atazanavir sulfate for enhancing brain delivery by increasing its permeability. Solid lipid nanoparticles were preparing by microemulsion technique using stearic acid as solid lipid and Pluronic® F68 as emulsifier. Spherical nanoparticles with mean average particle size of 167 nm were formulated. The morphology of prepared nanoparticles was confirmed by Transmission electron microscopy and scanning electron microscopy. The release of Atazanavir from SLNs in phosphate buffer saline pH 7.4 indicated an initial burst release of 17 % in 1 hr followed by gradual release up to 46 % after 24 hr. Delivery of [<sup>3</sup>H] Atazanavir by SLNs led to a significantly higher accumulation by the endothelial cell monolayer as compared to the drug aqueous solution.

**Suddhasattya et al.** (144) developed and validated RP-HPLC method for determination of Atazanvir sulfate in tablet dosage form using C<sub>18</sub> column Phenominex (250 mm x 4.6 mm, 5 µm particle size) with a mobile phase consisting of a mixture of 900 ml of methanol with 100 ml distilled water, pH adjusted to 3.55 with acetic acid. The detection was carried out at 249 nm and retention time of 8.3 min was obtained. Linearity was observed from 10-90 µg/ml, r<sup>2</sup> of 0.999 with equation  $y = 23.427 + 37.73x$ .

**Pavan et al.** (145) examined Atazanavir nanoparticles in targeting of folic acid (FA) receptor and permit cell based drug depots using pharmacokinetic and pharmacodynamic studies. FA receptor targeted poloxamer 407 nanocrystals containing Ritonavir-boosted Atazanavir significantly increased drug bioavailability and pharmacodynamic by 5 and 100 times respectively. The drug levels were high in spleen and lymph nodes in comparison to plain drug and untargeted nanoparticles. The data also mirrored potent reductions in viral loads, tissue viral RNA and numbers of HIV-1p24+

cells in infected and treated animals. The authors concluded that FA-P407 coating of Atazanavir nanoparticles readily facilitated drug carriage and antiretroviral responses.

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