



## NANOCARRIERS FOR THE TREATMENT OF HIV AND FUNGAL INFECTIONS

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### ABSTRACT

Infectious diseases like AIDS, tuberculosis had been threat to the mankind and the treatment for such diseases over the years had made the pathogens resistant to the drug therapy. Advent of nanotechnology based drug delivery systems however, has changed the picture. Drug delivery using nanocarriers has proved its potential by drastically improving the treatment for infectious diseases. This review provides an overview of different types of nanocarriers such as liposomes, polymeric micelles, dendrimers, solid lipid nanoparticles, polymeric nanoparticles and case studies involving the use of these nanocarriers in treating HIV and fungal infections.

**KEYWORDS:** Nanocarriers , HIV, Fungal infections, Liposomes, Polymeric micelles.

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## 1. INTRODUCTION

### 1.1 Infectious diseases

Infectious diseases are also called as transmissible or communicable diseases. Transmission of pathogen from one individual to another one can occur in various ways including physical contact, contaminated food, body fluids, objects, airborne inhalation, or through vector organisms. Infectious diseases are caused by pathogenic microorganisms which include bacteria, viruses, parasites or fungi. The diseases can be spread, directly or indirectly, from one person to another<sup>1</sup>.

### 1.2 Need of novel drug delivery systems

Diseases like AIDS, tuberculosis are major cause of death worldwide occurring due to pathogens. Because of the resistance developed by the causative organisms, harsh drug therapy is prescribed to eliminate the pathogens aggressively. But this kind of treatment strategy results into patient non compliance and severe side effects. To avoid above mentioned problems and for efficient drug delivery, novel drug delivery systems seem to be a plausible solution. Over past few years, drug delivery through nanocarriers has revolutionized the field of medicine because of their unique properties. The fact is well documented that nanocarriers may provide a promising drug delivery system to combat effectively with diseases like AIDS, TB, systemic mycoses etc.<sup>2</sup>

### 1.3 Fungal infections

The fungi are large diverse group of organisms, most of which are found as saprophytes in soil and on decaying organic matter. Fungi are eukaryotic and have rigid cell wall made up of polysaccharides, chitin and glycoproteins. They are heterotrophs i.e. depend on other cells for nourishment. Fungi exist in unicellular as well as multicellular forms. There are multiple varieties of fungi species found in nature but most of them are not harmful to humans, only some of them are responsible for causing infections of the body. Fungal infection of animals, including humans are also called 'mycoses'. Fungal infections are caused when

some species of fungi invades the tissue of the body. After entering into the body the microbes get favorable conditions for growth and start infecting tissues. Fungal infection can be superficial, localized skin infection or systemic infection. Two main forms of fungi are unicellular yeast and filamentous moulds. Fungi are opportunistic and can cause infection when immune system is weak and fungi are pathogenic also and may cause infection irrespective of the condition of immune system. Infections involving fungi may occur on the surface of the skin, in skin folds, and in other areas kept warm and moist by clothing and shoes. They may occur at the site of an injury, in mucous membranes, the sinuses, and the lungs. Fungal infections trigger the body's immune system, can cause inflammation and tissue damage, and in some people may trigger an allergic reaction<sup>3</sup>. Any person can get fungal infection but people who are at increased risk of getting the infection are the ones who already have AIDS, patients who got organ transplant, those who are on chemotherapy or have diabetes or lung disease. Such patients have weak immune system so they get easily affected by fungal species and the disease spreads rapidly as there is no strong defending system in the body. This has been the major cause of death of people who are already infected with HIV or cancer especially in developing countries<sup>3</sup>.

#### 1.3.1 Systemic mycoses

Many infections remain confined to a small area, such as between the toes, but others may spread over the skin and/or penetrate into deeper tissues. Such infections become very severe with time and if left untreated cause permanent damage or even death. Systemic mycoses occur due to pathogens originated primarily in the lungs and may spread to many organ systems. These infections may move into the blood and be carried throughout the body causing infection anywhere in the body<sup>3</sup>.

**Common deep or systemic infections are**

- Aspergillosis, caused by *Aspergillus fumigatus* or several other *Aspergillus* species. These fungi are commonly found in soil, plants, and house dust. They can cause fungal masses in the sinuses and lungs and, in some cases, can spread to the brain and bones<sup>3</sup>.
- Blastomycosis, caused by *Blastomyces dermatitidis* found in moist organic-rich soil<sup>3</sup>.
- Cryptococcosis, caused by *Cryptococcus neoformans* or rarely by another *Cryptococcus* species found in soil and are associated with bird droppings. Anyone may become infected, but the highest prevalence is in people who have HIV/AIDS<sup>3</sup>.
- Histoplasmosis, caused by *Histoplasma capsulatum* found primarily in the east and central U.S.; typically affects the lungs<sup>3</sup>.
- Candidiasis, caused by *Candida* species, which are part of the normal human flora, are found worldwide. Infections occur in the moist mucous membranes of the body<sup>3</sup>.
- Pneumocystis pneumonia, caused by *Pneumocystis jorveci* (formerly known as *Pneumocystis carinii*), found worldwide and most commonly affecting those with compromised immune systems, including those with HIV/AIDS<sup>3</sup>.

The drug of choice for treatment of such systemic fungal infections is Amphotericin B because of its large spectrum of activity and therapeutic effectiveness, but it can cause several adverse effects major being the nephrotoxicity. In last two decades new

important antifungal agents have been introduced like the echinocandins, the azoles. In addition, the development of novel drug delivery methods were also developed for established drug for more efficient treatment<sup>4</sup>.

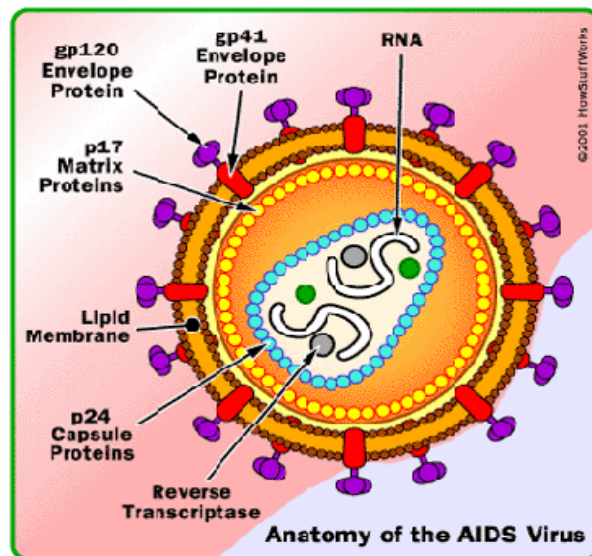
**1.4 HIV infection**

Human Immunodeficiency Virus (HIV) is commonly known as the causative agent of the disease Acquired Immunodeficiency Syndrome (AIDS). After its identification in 1983, much research has been conducted to understand its biology so as to develop effective strategies for preventive and therapeutic action against the disease. The understanding of transmission of virus, its pathogenesis are required to give important insights towards developing new and better treatment options such as finding novel drug delivery system for anti HIV drugs to combat with the pathogen effectively<sup>5</sup>.

**1.4.1 Human Immunodeficiency Virus (HIV)**

HIV is a retrovirus which has spherical shape and size about 100-150nm. It has membrane derived from host, a nucleocapsid, reverse transcriptase enzyme and genetic material in the form of RNA containing three structural genes. These genes are responsible for important group-specific antigens (gag gene), important viral enzymes such as reverse transcriptase, integrase and protease (pol gene), and the two glycoproteins present in the outer viral membrane, gp120 and gp41. Due to constant transcription errors, the virus has high polymorphism<sup>5</sup>.

**Figure1**  
**Structure of HIV<sup>6</sup>**



Human retroviruses have four major types. They are human T cell leukemia viruses, HTLV-1, HTLV-2, and human immunodeficiency viruses, HIV-1 and HIV-2. HIV-1 and HIV-2 which belong to the subclass lentivirinae are responsible for causing acquired immunodeficiency syndrome (AIDS). HIV infection is spread due to the transfer of body fluids from blood transfusion, organ transplant, sexual contact, or perinatally from mother to offspring. Cellular entry of HIV virus involves three steps<sup>7</sup>

- 1) Attachment of the gp120 glycoprotein on HIV surface to the CD4 receptor present on the cell membrane. These receptors are expressed by the monocyte derived macrophages and T-lymphocytes
- 2) Interaction of the gp120 protein and CD4 complex with a coreceptor
- 3) Virus-cell membrane fusion mediated by transmembrane gp41 protein

Though the present antiretroviral therapy has capacity to reduce the viral load to quite low levels, HIV does persist in the human body in several reservoir sites. These are cellular or anatomical locations where a replication-form of the virus is constantly harbored. Because of the reservoir sites, the virus gets protected from biological elimination pathways, immune response and/or antiretroviral drugs. These

protections reduce the possibility to eradicate the virus and hence the infection due to which there is no chance to achieve a cure with currently available therapy. The CNS, lymphatic system, lungs, and genital organs are the anatomical sanctuary sites for HIV, because antiretroviral drug concentrations are not attained to required level within the respective anatomic spaces. This is the limiting factor which is due to the presence of barriers, such as the blood-brain barrier (BBB), blood-cerebrospinal fluid barrier, and blood-testes barrier. In addition many cell types have been recognised as important reservoirs for HIV but only some are clearly recognized as so. Few examples of these cells are macrophages, resting CD4+ T cells, and follicular dendritic cells (i.e., dendritic-like cells present in the lymph nodes)<sup>5</sup>. Initially single anti-HIV drug therapy (also known as Antiretroviral Therapy i.e. ART) was prescribed to patients infected with HIV but as the virus started developing fast resistance to the drug, soon there was need for better treatment option. Therefore three or more drugs were used simultaneously in the new treatment. This therapy is known as Highly Efficient Antiretroviral Therapy (HAART). The purpose of this therapy was to minimize the development of resistant strains by attacking the virus aggressively. Due to the high and frequent dosing, severe side effects and toxicity

was developed. But with the advent of novel drug delivery systems like nanocarriers, such problems have been solved and offer the most efficient therapy available today<sup>8</sup>.

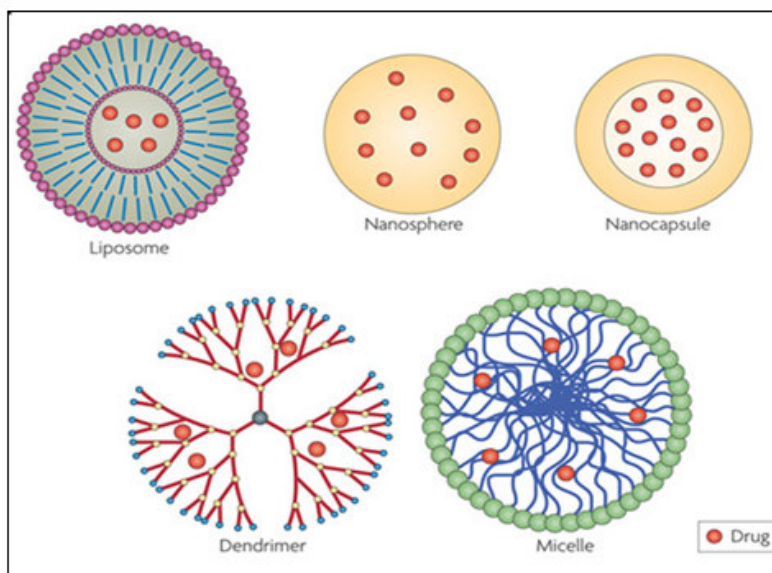
## 2. NANOCARRIERS

Nanomedicine or nanoformulation is a novel drug delivery system that consists of nanocarriers. The concept has been developed in the last decade and today finds applications in the treatment of some major diseases like infectious diseases and cancer. Nanocarriers are nanosize structures, size typically ranging from 1-100 nm, which are used as drug/ gene carrying agents. The drug can be encapsulated or adsorbed or chemically attached to the surface of nanocarriers. Because of very small

size, nanoparticles acquire specific chemical and physical properties<sup>9</sup>. The various parameters which play important role in exhibiting the required pharmacological effect are particle size, hydrophobicity, surface area, surface charge and crystallinity. Due to nano size, nanocarriers get access to most of the tissues in the body. Normal human cell size is of the order of 10 $\mu$ m so nanocarriers are very important agents for the treatment of intracellular diseases in which site of action for the drug is located inside the cell where drug targeting is difficult. Effective drug targeting is achieved with nanocarriers which results in enhanced pharmacological effect and reduced toxicity<sup>9</sup>.

### 2.1 Types of nanocarriers

Figure2  
Types of nanocarriers<sup>10</sup>



#### 2.1 Liposomes

Liposomes are spherical structures made of phospholipid bilayer and little bigger than conventional nanoparticles (90-150nm). Liposomes were the first nanocarrier used for drug delivery purpose. They are also referred as lipid vesicles. The phospholipid molecules are amphiphilic in nature i.e. they consist of hydrophilic head part and hydrophobic tail part. Within the liposome, phospholipid molecules are aligned opposite to each other such that

hydrophilic part is present on the extrimities. while hydrophobic chains point towards each other in the middle portion of bilayer. Due to this structure liposome gets divided into two parts- core hydrophilic region and hydrophobic region surrounding the core, hence both the types of drug, hydrophobic and hydrophic can be incorporated into the liposome. Surface modification can be done with polyethylene glycol (PEG units) depending upon the time of circulation required in blood. Cell membranes

are made up of phospholipids so liposomes are easily accepted by the cells. In spite of these features and advantages, their use is limited because of poor in vivo stability and leaky nature<sup>9</sup>.

### **2.1.1 Polymeric micelles**

Polymeric micelles are made from amphiphilic block copolymers or polymer-lipid conjugate or surface active molecules that self-associate to form structures having hydrophobic core, in aqueous solution. This property of micelles allows easy encapsulation of hydrophobic drug. The micelles have large drug carrying capacity, can respond to various stimuli like pH, temperature. Their small particle size (1-50 nm) and hydrophilic exterior makes them suitable candidate for various delivery routes. They are more stable when compared with liposomes<sup>9</sup>.

### **2.1.2 Dendrimers**

Dendrimers are globular polymeric macromolecules synthesized via series of controlled polymerization reaction having repeatedly branched structure. Size ranges from 10-100nm. As they possess multiple functional groups on their surface, they are ideal for targeted drug delivery. They can be engineered as required, by making the exterior groups hydrophilic they can be made water soluble, by making internal portion hydrophobic, any hydrophobic drug can be encapsulated in the interior. The main drawback of dendrimers is their polycationic surface which causes a toxic effect on the cell membranes<sup>9</sup>.

### **2.1.3 Solid lipid nanoparticles (SLNs)**

SLNs are formulated by solid lipids. While preparation, the melted lipids are either dispersed in water or aqueous surfactant solution. This is carried out with the help of various techniques such as high pressure homogenization or emulsification. SLNs are majorly used in parenteral dosage form and their particle size ranges between 50-500nm. SLNs have immediate drug release which is important when immediate suppression of pathogen is required. SLNs have high drug loading capacity and more stability than

liposomes which makes them better alternative<sup>9</sup>.

### **2.1.4 Polymeric nanoparticles**

Polymeric nanoparticle is a collective name used for nanospheres and nanoparticles. Polymeric nanoparticles are solid colloidal particles ranging from 10-500nm. Nanospheres possess matrix type structure in which drug can be absorbed, entrapped or dissolved. Nanocapsules have polymeric exterior and liquid core. They are synthesized by using different natural or synthetic polymers that are biocompatible and/or biodegradable. Polymeric nanoparticles have various unique properties: 1) structurally stable and synthesized with a precise size range, 2) other is particle properties like zeta potential, drug release profile can be modified with selecting polymer length, surfactant and solvent used, 3) the surface has functional groups that can be modified with drug moiety or targeting group. The advantage of Polymeric nanoparticles is drug release due to the progressive degradation of polymer. In addition, targeting becomes easy because polymers responding to particular microenvironment such as pH and redox have been found<sup>9</sup>.

## **3. NANOCARRIERS FOR TREATMENT OF FUNGAL INFECTIONS**

Fungal pathogens affect human body, causing superficial skin infections to deeply situated tissues. Systemic mycoses can broadly be classified into two types as: endemic diseases such as histoplasmosis, coccidioidomycosis, and blastomycosis and opportunistic diseases such as cryptococcosis, aspergillosis, and candidosis. The second type is found in patients with compromised immune system. The anti-fungal drugs that are normally used belong to a few chemical classes such as polyene antibiotics, azole derivatives, allylamines-thiocarbamates, morpholines and miscellaneous compounds such as 5-fluorocytosine and griseofulvin. Out of these majorly used are polyenes and azoles. Amphotericin B (AmB), nystatin and natamycin are the drugs belonging to polyene class. These are used in the treatment of human fungal

infections. But the only drug that has been used for ages and still finds significant commercial application is AmB. This is because AmB has large anti-fungal activity spectrum<sup>11</sup>.

### **3.1 Nanocarriers for treatment of systemic mycoses**

#### **3.1.1 Liposomes**

The effectiveness of liposomes as a carrier for anti-fungal drug has been fairly demonstrated in case of fungal diseases, such as candidiasis, aspergillosis and cryptococcosis because of reduction in the drug toxicity and increasing its therapeutic index. Liposome is effective in treating both intracellular (leishmaniasis and histoplasmosis) and extracellular (candidiasis and aspergillosis) systemic infections<sup>12</sup>. The causative agent of the disease is found only in reticuloendothelial macrophages and liposomes are easily taken up by reticuloendothelial system. The liposomal AmB shows less toxicity than AmB and hence can be given in higher doses<sup>11</sup>.

#### **A) AmBisome**

AmBisome is anti fungal product which contains AmB entrapped in small unilamellar liposomes. It is available in the form of lyophilized powder and has to be reconstituted with water prior to use. This creates liposomes of 60-70nm size. The liposomes after coming in contact with the fungal cells break open and AmB is released. AmB then binds to ergosterol in the fungal cell membrane leading to its disruption<sup>13</sup>. Cholesterol was incorporated into the formulation to increase stability and to retain AmB in the bilayer because cholesterol binds with AmB. Other ingredients of the formulation were antioxidants,  $\alpha$ -tocopherol and disodium succinate hydrate, and sucrose as isotonic agents. This formulation is approved for the therapy of febrile neutropenia, aspergillosis, candidiasis, and cryptococcosis. It is also indicated as a second-line therapeutic for visceral leishmaniasis. Liposomal delivery system for AmB is better as compared to AmB emulsions or colloidal formulations when parameters such as bioavailability and side-effects are considered. Because of such

advantages even though the cost of AmBisome is high, it has retained its importance<sup>14</sup>.

#### **B) Immunoliposome**

Immunoliposomes carrying AmB are liposomes having fungus-specific antibodies attached to their surface. This makes easy targeting towards fungal cells. Immunoliposomes were reported to decrease the mortality rate significantly in mice with invasive pulmonary aspergillosis as compared to conventional AmB liposomes (L-AmB). Immunoliposomes were more effective than AmB integrated with long-circulating liposomes. Treatment of murine candidiasis and cryptococcosis with AmB encapsulated immunoliposomes showed improved activity as compared to conventional AmB liposomes<sup>11</sup>.

#### **C) Long-circulating liposomes**

Long-circulating liposomes are the liposomes coated with hydrophilic polymer such as PEG. Such liposomes are also called '*stealth liposomes*'<sup>4</sup>. Coating with PEG increases the time period of liposome in systemic circulation. Since the duration in circulation is prolonged by the structural nature of stealth liposomes, more intact liposomes can get localized at the site of infection, hence improving the in vivo efficacy. In an experiment conducted with murine model of systemic candidiasis stealth liposomes with AmB were found to be effective than L-AmB<sup>11</sup>.

#### **3.1.2 Micelles**

The challenges in the delivery of antifungal agents are low water solubility and high toxicity. Anti-fungal agents such as AmB are hydrophobic in nature. Such drugs have low compatibility with hydrophobic cores of the micelles formed with conventional block copolymer. Dhembre et al reported that to improve solubility of AmB core forming blocks of micelle were derivatised with stearate side chains. AmB interacted strongly with the stearate side chains in the core of the micelles. As a result, AmB can be entrapped efficiently into the micelles core, resulting in subsequent sustained release in the external environment. Due to solubilised state of AmB in micelles, it was reported that the onset of hemolytic activity



of AmB toward bovine erythrocytes was delayed, compared to that of the free drug<sup>15</sup>. The copolymers used in the micelle formation can solubilize drugs, and lead to prolonged circulation, sustained drug release, and enhanced delivery to non-MPS sites<sup>4</sup>. Adams et al. prepared micelles using amphiphilic diblock copolymers based on methoxypoly (ethylene oxide)-*block* poly( L-aspartate), PEO-*b*-p(L-Asp). The polymer was derivatised to incorporate stearate side chains. The effects of stearate esterification were evaluated in terms of micelle stability and amphotericin B (AmB) encapsulation or release. The degree of stearate esterification relates to the relative self-aggregation state of encapsulated AmB as evidenced by absorption spectroscopy. When AmB is physically loaded into polymeric micelles, the onset of hemolytic activity toward bovine erythrocytes was delayed compared to the free drug. The level of stearate esterification (0, 46, or 91%) was seen to have prominent influence on the time-dependent hemolytic profile of AmB toward bovine erythrocytes. On the basis of the corresponding absorption spectra, it was speculated that encapsulated AmB may interact strongly with stearate side chains, resulting in sustained release. In a neutropenic murine model of disseminated candidiasis, kidney colony-forming unit determination revealed dose-dependent efficacy for the AmB encapsulated polymeric micelle formulation. The efficacy was not significantly different from that of Fungizone (marketed AmB preparation). Hence, AmB administered via a polymeric micelle formulation retained potent *in vivo* activity<sup>16</sup>. One very important advantage of PEO-*b*-poly (L-amino acid) micelles other than biocompatibility, biodegradability, and flexibility in L-amino acid composition is the ability to chemically modify the side chain structure of a poly (L-amino acid) block to adjust the properties of PEO-*b*-poly (L-amino acid) micelles. Simple acyl chains were attached onto PEO-*b*-PAsp by ester linkages to form amphiphilic block copolymer micelles that are compatible with polyene antibiotics like AmB. AmB has tendency to undergo self aggregation which affects the selectivity of attaching to cells. The monomers of the drug selectively bind to

fungus cells lead to their disruption thus killing them but the soluble AmB aggregates bind to fungus as well as healthy cells in non-selective way exhibiting toxicity in both types of cells. When PEO-*b*-poly(*b*-benzyl L-aspartate) was modified to PEO-*b*-PAsp by stearate side chains, it resulted in high capacity for AmB in the micelles and solubilization of the drug in a deaggregated state, as per the absorption spectroscopy. Therefore there was a reduction in toxicity in terms of hemolysis due to membrane damage while still maintaining potent antifungal activity in a neutropenic murine model of systemic candidiasis. Nyctatin also has tendency to aggregate but it has higher critical aggregation concentration. Hence it is easier to deaggregate nystatin than AmB. Moderately hydrophobic Pluronics deaggregate and solubilize nystatin but not AmB, indicating potential of nystatin for the treatment of systemic fungal diseases. It has also been proven that PEO-phospholipid micelles readily deaggregate and solubilize AmB. PEO-phospholipids are generally regarded as safe and could move quickly into humans, assuming that the aggregation state hypothesis holds true. Hence it was expected PEO-phospholipid micelles to release AmB more rapidly than ones based on PEO-*b*-PAsp<sup>17</sup>. Naik et al. developed a stable, controlled release AmB lyophilized mixed micelle (MM) formulation using hydrogenated soya phosphatidylcholine (HSPC) and bile salts in monomeric form. The preparation was assessed for therapeutic performance and side effects. The three MM formulations of AmB were prepared using sodium deoxycholate (NDC)/sodium taurocholate (NTC)/sodium cholate (NC), and HSPC. MM formulations were lyophilized using sucrose as a cryoprotectant and analyzed for per cent micelle yield, per cent drug loading and per cent entrapment efficiency. Comparative *in vitro* diffusion studies, hemolytic activity, and minimum inhibitory concentration (MIC) were conducted using cellophane membrane, human red blood cells and *Candida albicans* respectively between developed MM formulations and marketed formulation (Fungizone). *In vivo* studies of MM formulations were also carried out on *Candida albicans*



infected white albino rats and compared with Fungizone. The optimized molar ratio of bile salt: HSPC was found to be 8:11. Among all MM formulations prepared, NDC/ HSPC formulation found to have maximum per cent drug loading (4.96+/-0.8%), per cent entrapment efficiency (93.2+/-1.5%) and per cent micelle yield (96.4+/-1.4%). The in vitro drug release studies of developed MM formulations showed close to zero-order diffusion kinetics. All MM formulations showed improved therapeutic index and reduced side effects compared to reference formulation Fungizone. The NDC/HSPC MM formulation was found to have least hemolytic activity, MIC and mortality rate at all dosage levels. It was predicted that enhanced antifungal activity and reduced toxicity of AmB was due to higher cellular uptake of the drug by fungal cells of infected tissues from MM formulations. Therefore, it was suggested that AmB MM formulation could be a safe and effective viable alternative for the treatment of systemic fungal infections<sup>18</sup>. Han et al prepared nanoparticles i.e. mixed micellar nanoparticle consisting of amphotericin B (AmB) and poly styrene-block-poly ethylene oxide (PS-block-PEO) by using a high pressure homogenizer method. The nanoparticles were investigated for nephrotoxicity, antifungal activity and self-aggregation status of the drug in the nanoparticle. When the mixed micellar preparation having AmB along with PS-block-PEO was intravenously administered to rats, nephrotoxicity was significantly reduced in terms of transmission electron microscopy of tubular cells and creatinine clearance. Antifungal activity of AmB did not change, when the drug was in the form of mixed micellar nanoparticle compared to both conventional formulation and AmB micelle produced by same method without PS-block-PEO. AmB molecules were present in the monomeric form in the mixed micellar nanoparticle with PS-block-PEO up to the therapeutic level of the drug (1-3 mM). It was predicted that the reduced nephrotoxicity of AmB in mixed micellar nanoparticle was due to the existence of the drug as monomeric form in the nanoparticle. The formulation was thought as a promising alternative for the treatment of fungal diseases in patients who are at risk of

renal dysfunction<sup>19</sup>. AmB is a membrane-active drug mainly used for the treatment of systemic mycoses. But the limitations for the use of AmB are poor water solubility and potential for serious systemic toxicities. It has been demonstrated that the aggregation state of AmB is responsible for toxicity. Yu BG and colleagues prepared micelles in which AmB was solubilized in micelles based on poly (ethylene oxide)-block-poly (beta-benzyl-L-aspartate) (PEO-block-PBLA), using a dialysis method of drug loading. The aggregation state of AmB was tested by electronic absorption spectroscopy. AmB loaded in PEO-block-PBLA micelles were found to be non-hemolytic for concentrations up to 15 microgram/ml. AmB in case of Fungizone initiates hemolysis at 1 microgram/ml. The onset of hemolysis depends on the respective critical aggregation concentrations (CACs) of AmB. The antifungal activity of the AmB-loaded PEO-block-PBLA micelles were four to eight times higher than Fungizone in terms of minimal inhibitory concentrations (MICs). PEO-block-PBLA was found to have no antifungal activity for concentrations up to 200 microgram/ml. The basis for the increase in antifungal activity of AmB-loaded PEO-block-PBLA micelles were unclear. But it was predicted that the activity was due to a stabilizing effect of the polymeric micelles against auto-oxidation of the AmB heptaene moiety or alternatively, an enhancement in membrane disruption of fungal cells<sup>20</sup>.

### **3.1.3 Polymeric nanoparticles**

#### **A) Chitosan-dextran sulfate nanoparticles**

Tiyaboonchai et al. prepared polymeric nanoparticles using positively charged polymer chitosan(CH) and negatively charged polymer dextran sulfate(DS) and zinc sulfate as cross linking and hardening agent. The process used for developing particles was polyelectrolyte complexation technique. The AmB loaded particles showed small polydispersity index 0.2 indicating small particle size distribution. The size of particles varied between range 600-800nm. The zeta potential value was -32mV indicating strong negative charge on particle surface. Scanning electron microscopy made it

clear that the particles were spherical in shape and had smooth surface. The in vitro dissolution studies revealed rapid drug release suggesting most AmB was adsorbed onto the particle surface and very little amount actually got entrapped into the particles. This also suggests that AmB has moderate interaction with cross linked polymers of nanoparticles. In vivo studies were conducted to compare renal toxicity between Fungizone (marketed AmB formulation) and the manufactured nanoparticles. The mice were injected with both formulations having high dose. The results showed decreased renal toxicity with AmB polymeric nanoparticles than Fungizone. The manufacturing method has advantages like ease of manufacturing and mild preparation condition, use of biodegradable polymers and production in aqueous media avoiding organic solvents<sup>21</sup>.

#### **B) Polyethylamine-dextran sulfate nanoparticles**

Tiyaboonchai et al. developed polymeric nanoparticles using two oppositely charged polymers polyethylenimine (PEI) and dextran sulfate (DS) and zinc sulfate as a stabilizing agent. Complex coacervation was used as manufacturing technique and the encapsulated drug was AmB. The nanoparticles possessed a zeta potential of approximately +30 mV and had a narrow size distribution in the range 100±600 nm with a polydispersity index of 0.2. Electron microscopy showed spherical nanocapsules with a smooth surface. Processing parameters, such as the pH of the PEI solutions, ratio of the two polymers, the concentrations of DS and zinc sulfate, all played a significant role in controlling particle size. In vitro dissolution studies demonstrated a rapid drug release that is dependent on the model drug solubility. The nanoparticles showed greater than 100 times reduction in toxicity as compared with pure AmB but were still able to kill *Candida albicans*. The method of preparation offers advantages like ease of manufacturing and mild preparation conditions, use of absolute aqueous processing conditions, use of biocompatible polymers, ability to control particle size, and a high level of drug entrapment. The experiment suggests that

PEI – DS nanoparticulate drug delivery system may provide a viable method for delivery of AmB that helps getting rid of the liposome manufacturing which is costly and complex technique<sup>22</sup>.

#### **3.1.4 Lipid nanoparticles**

Jung et al. prepared AmB containing lipid nanoparticles ((LNPs). Three types namely plain, anionic and peglyted nanoparticles were prepared by using spontaneous emulsification and solvent evaporation method. Lipid nanoparticles were chosen as drug carriers because a hydrophobic drug like AmB which is poorly soluble in water could be entrapped with high efficiency. The mean particle size of the LNPs obtained, ranged from 72.9 to 159.1 nm according to a variation of their lipid composition. The PEG-LNPs had zeta potential value of the surface equal to  $-50.4 \pm 5$  mV. Entrapment efficiency of the drug in the PEG-LNPs was up to  $76.5 \pm 5\%$ . Cytotoxicity of all the AmB-entrapping LNPs was tested against human kidney cells and was found to be lower than the commercialized AmB-formulations such as Fungizone and AmBisome. Hematotoxicity of all the nanoparticles was tested with red blood cells and was found to be much lower than that of Fungizone but comparable to AmBisome. *In vitro* antifungal activity of all the LNPs was tested against *Candida albicans* and *Aspergillus fumigates* and was found to be better than the commercialized AmB formulations. All the AmB-entrapping LNPs increased circulation half life of AmB in blood circulation which was comparable to AmBisome. The *in vivo* antifungal activity of PEG-LNPs against *Aspergillus fumigates* – infected mice was superior to that of AmBisome. The experiment showed that drug-entrapping LNPs, especially PEG-LNPs, can be used for entrapping poorly water-soluble drugs and enhancement of therapeutic efficacy by modulating pharmacokinetic behaviors and/or drug-related toxicities<sup>23</sup>. Sheikh S et al. prepared the formulation of lipid nanoparticles containing DS AmB without using any detergent or toxic organic solvents. Electron microscopy and particle size determination revealed that the AmB containing nanoparticles had a

homogeneous population of nanosized particles below 100 nm. Hemolysis testing indicated that the prepared formulation causes significantly less toxicity to red blood cells than Amphotericin B deoxycholate and was comparable to Ambisome. A maximum daily dose of the prepared formulation of 5 mg/kg in rabbits and 10 mg/kg in mice for 28 days showed no symptoms of toxicity, mortality or significant body weight reduction. The dose of nanosomal AmB and Ambisome were injected intravenously at 2 mg/kg consecutively for 5 days into *Aspergillus fumigates* infected mice. The treatment showed 90% survival with nanosomal Amphotericin B and only 30% survival with Ambisome after 10 days of fungal infection. However, all of the 10 control mice which were not treated with Amphotericin B died within 5 days of fungal infection. Hence lipid nanoparticles as carriers of AmB were found to be safe, cost effective and also provide an alternative option for treatment of fungal disease<sup>24</sup>.

#### **4. HIV INFECTION AND TREATMENT**

Even after 30 years of efforts, researchers haven't able to come up with complete cure for HIV. Zidovudine was the first drug, which was approved by the US FDA in 1987, leading to the approval of a total of 25 drugs till 2010<sup>8</sup>.

##### **4.1 Traditional approach towards HIV treatment**

Initially Antiretroviral Therapy (ART) was implemented to combat with HIV in which the medicine had only one active principle. But this was found to be ineffective due to easy development of drug resistance by the virus<sup>5</sup>.

##### **4.2 Current anti-HIV treatment**

To overcome the drug resistance problem, the new medicine was incorporated with at least three drugs so that the virus will be aggressively killed and there will be least chance for resistance to occur. This technique was named as HAART (Highly Active Anti Retroviral Therapy). The technique surely was better than traditional one and improved life expectancy

and quality of life but the high dose and frequency gave rise to severe side effects. The treatment also failed for those people who develop resistance to even combination of drugs. The treatment is expensive which prevents its widespread use in poor or developing countries of the world<sup>25</sup>.

##### **4.3 Need of nanocarriers for the treatment**

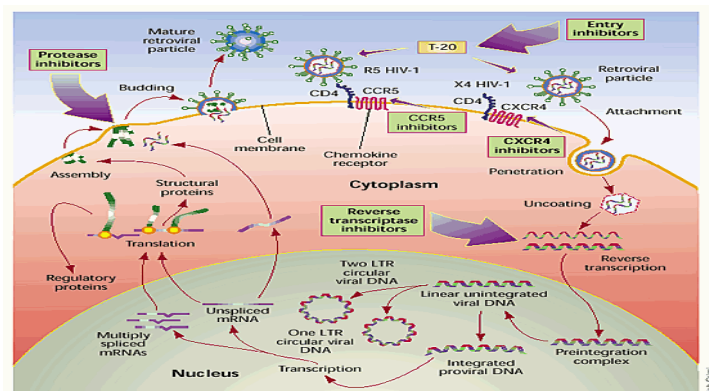
Due to above mentioned problems; efforts were continued in the direction to find the most efficient medicine or rather the cure. When the established drug delivery systems couldn't give satisfactory results, there was need to develop novel drug delivery system which will effectively treat the disease by eliminating the problems with previous techniques. When nanotechnology was reported to have important application in drug delivery, various formulation strategies were adopted for anti-HIV nanocarrier development.

##### **4.4 Anti-HIV (Anti retroviral) drugs and their mechanism of action**

HIV is a retrovirus. Its replication cycle and the different stages involved in it are shown in the figure below. The drugs act on virus so that the replication cycle is stopped from that stage leading to attenuation of growth of virus in the cell. The drugs have been categorized depending upon the stage at which they act:

- 1) Entry inhibitors- They prevent the entry of virus into the cell.
- 2) Reverse transcriptase inhibitors- Reverse transcriptase is an important enzyme in virus that helps in making DNA from RNA. These drugs act on the enzyme to prevent their action and thus stop virus multiplication. They are of 2 types- Nucleotide reverse transcriptase inhibitors (nRTIs) and Non-nucleoside reverse transcriptase inhibitors (NNRTIs).
- 3) Protease inhibitors- These drugs bind to the active site of the HIV protease and inhibit it, which results in the production of immature and non-viable proteins, instead of infectious particles<sup>25</sup>.

**Figure3**  
**Viral replication cycle<sup>26</sup>**



## 5. NANOCARRIERS FOR ANTI-HIV/AIDS TREATMENT

### 5.1 Liposomes

Liposomes are lipid vesicles made of phospholipid bilayer. They can be categorised as multilamellar vesicles (MLVs), small unilamellar vesicles (ULVs) and large unilamellar vesicles (LUVs). Cholesterol is generally used in the formulation so as to adjust membrane rigidity and stability. E.g. stearylamine and dicetyl phosphate liposomes<sup>27</sup>.

#### 5.1.1 Liposome surface modification and targeting

Liposomes are easily taken up by reticuloendothelial system (RES) and disappear quickly from blood circulation. This property was used for antiretroviral drug delivery to macrophages<sup>28</sup>. Liposome surface modification can be done in order to improve its properties such as long term circulation in blood, active targeting. It has been found that when liposome surface is coated with hydrophilic polymer such as PEG (sterically stabilized liposome) circulation time increases because PEG incorporation prevents interactions with plasma proteins, thus retards recognition and removal by the RES<sup>29</sup>. Also by attaching targeting ligands such as antibody, antibody segments, aptamer, peptides and small molecule ligands to the surface of the liposomes, antiretroviral delivery can be improved further. They can selectively bind to microorganisms or infected cells and then release the drug entrapped within to kill or inhibit the growth of the virus<sup>27</sup>. Still more specific targeting of HIV-infected cells, such as CD4+

cells, could also be achieved by coupling antibodies on the surface of liposomes (immunoliposomes) which have affinity for specific receptors. Thus the combination of conventional liposomes, sterically stabilized liposomes and immunoliposomes in the formulation represents very important approach in effective delivery to most HIV affected cells<sup>30</sup>. Clayton R et al. had prepared sterically stabilized pegylated liposomes coated with targeting ligands derived from the Fab' fragment of HIV-gp120-directed monoclonal antibody F105, and also evaluated them as vehicles for targeted delivery of a novel HIV-1 protease inhibitor. It was found that these immunoliposomes were selectively taken up by HIV-1 infected cells. The experiments showed that immunoliposomes giving higher antiviral activity than free drug or simple liposomes. The conclusion that derived was by combining a targeting moiety with drug-loaded liposomes, efficient and specific uptake by non-phagocytic HIV-infected cells was facilitated resulting in less toxic treatment for HIV<sup>31</sup>. Bronshtein T et al. studied Cell derived liposomes expressing CCR5. The liposomes were prepared from cytoplasmic membranes of cells expressing CCR5 which is human receptor for gp120, that is found on the surface of HIV virions and HIV-infected cells. The specific targeting and cytotoxicity of these liposomes was studied. CCR5 that was expressed on the surface of the cell-derived liposomes were biologically active and correctly oriented. To demonstrate the system efficacy, EDTA was selected as liposomal

encapsulate. The cell-derived liposomes containing EDTA led showed 60% reduction in the viability of gp120-expressing cells while showing no effect on control cells that do not express gp120 hence giving specific targeting and cytotoxic effect towards gp120-expressing HIV model cells<sup>32</sup>.

### 5.1.2 Liposomal charge

Cationic liposomes are associated with efficient cellular delivery of drug load. Electrostatic interactions between positively charged liposomes and the negatively charged cell membranes and cell surface proteoglycans improve cell uptake. But cationic liposomes can cause cytotoxicity which limits their safety for clinical use. As a result interest for drug delivery has been given to neutral and anionic liposomes. Negatively charged lipids such as phosphatidylserine (PS) and phosphatidylglycerol (PG) are mainly recognized by macrophages. Negative charge can also be obtained by the incorporation of dicetylphosphate (DCP). Studies comparing phosphatidylcholine (neutral) and PS-composed liposomes have shown that negative liposome formulations have improved macrophage intake<sup>33</sup>.

### 5.1.3 Drug loaded liposomal systems

Lakshmi et al. developed liposomal drug delivery system for nevirapine, which is a hydrophobic non-nucleoside reverse transcriptase inhibitor. Liposomes were manufactured from egg phospholipids using thin film hydration. By optimizing the parameters of the process, they got spherical liposomes below 200 nm with a narrow polydispersity. It was observed that the size of the liposomes and the encapsulation efficiency of the drug increased simultaneously with the increasing ratio of drug and lipid and maximum stability was observed at the physiological pH. It was found that within the first 20 minutes 40, 60 and 100% of the drug was released when placed in phosphate buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) or when ultrasound was applied, respectively. In conclusion they proposed that nevirapine-loaded liposomal formulations had improved targeted delivery of

the anti-retroviral drugs selectively to compartments and cells and decreases systemic toxic side effects<sup>34</sup>. Ramana and colleagues studied stability of saquinavir loaded liposomes. The liposomes prepared had surface modified by incorporating PEG. Encapsulation efficiency of the anti-retroviral drug saquinavir was found to be approximately 33%. Liposomes exhibited sustained release of the drug. The PEGylated liposomes loaded with saquinavir were found to be less cytotoxic as compared to the non-PEGylated liposomes or free drug proving the potential of the liposomes as a sustained drug-release system<sup>35</sup>. Use of liposomes as nanocarrier for anti retroviral drug has important advantages like specific targeting, natural acceptance by RES, capability of entrapping hydrophilic as well as hydrophobic drug but there are certain drawbacks of this nanocarrier that limit its use. The hydrophilic drug loading capacity of drug is small due to small core volume (approximately 15 $\mu$ L). Long term physical and biological stability is one of the challenging issue<sup>28</sup>. As same as many antiviral agents, liposomes has a limited ability to cross the blood-brain barrier<sup>30</sup>. Hence there are some advantages and some disadvantages, the nanocarrier use of liposomes is restricted to certain formulations.

### 5.2 Solid lipid nanoparticles (SLNs)

SLNs are generally made from lipids that are in solid or semisolid phase at the room temperature and surfactants for emulsification. Solid lipids used in SLN formulations have fatty acids like palmitic acid, decanoic acid, and behenic acid; triglycerides like trilaurin, trimyristin, and tripalmitin; steroids (e.g. cholesterol), partial glycerides (e.g. glyceryl monostearate and glyceryl behenate) and waxes (e.g. cetyl palmitate)<sup>27</sup>.

#### 5.2.1 Uses

Sustained drug release and site specificity for drug delivery can be achieved by changing the properties of SLNs, such as their lipid composition, size, and surface charge. SLNs can be formulated to release drug in response to an external condition, such as pH and temperature. SLNs have several advantages such as relative

ease of production, sterilization, and scale-up, avoidance of the use of organic solvents, low-cost excipients, and biocompatibility. Compared with nanoemulsions which are liquid lipid encapsulations of the drug, SLNs containing the lipid in the solid state have greater drug stability and better control over drug-release kinetics<sup>28</sup>. CNS is one of the important sanctuary sites for the HIV as mentioned earlier and hence targeting of anti-HIV/AIDS drugs to the brain or CNS is an important issue for drug delivery. The blood brain barrier (BBB) prevents access of anti-HIV/AIDS drugs to the brain because of the tight endothelial cell junctions of the brain capillaries and the presence of efflux transporters on the cell surface. Nanocarriers can gain access to brain by endocytosis/phagocytosis. They also get moved away from the vicinity of efflux pumps due to their size<sup>36</sup>.

### 5.2.2 Drug loaded SLNs

Singh et al tried to formulate SLN with Zidovudine (an anti-human immunodeficiency viral agent) which is hydrophobic drug and improve the entrapment efficiency of the drug. The SLN emulsion was prepared with stearic acid by w/o/w double-emulsion solvent-evaporation method. Different triglycerides alone and in different combinations, with/without stearic acid were used to prepare SLN using a similar procedure. Two operating parameters, polyvinyl alcohol concentration and amount of lipid were found to have impact on the particle size and entrapment efficiency (EE) of the SLN. 27% was the maximum EE was found with particle size of 621 nm which was significantly higher than that reported earlier. This work showed that SLN can be used for entrapment of hydrophilic drug<sup>37</sup>. Dodiya and colleagues prepared Saquinavir (SQ) loaded SLNs and studied their characteristics. The aim of study was to prepare SLN and nanosuspension (NS) of saquinavir which is poorly water soluble drug to increase its bioavailability. The study showed SLNs were having particle size  $215 \pm 9$  nm and entrapment efficiency  $79.24 \pm 1.53\%$ . Pharmacokinetics and biodistribution studies of orally administered SQSLN in mice exhibited higher plasma level concentration compared to saquinavir microsuspension. The relative bioavailabilities

observed for saquinavir NS and saquinavir SLN were 37.39% and 66.53%, respectively, compared to 18.87% bioavailability obtained after administration of saquinavir microsuspension, proving suitability of nanoparticulate formulations for improving bioavailability<sup>38</sup>. Aji Alex et al. prepared Lopinavir loaded solid lipid nanoparticles (SLN) for intestinal lymphatic targeting. SLN with mean particle size of 230 nm (polydispersity index,  $PDI < 0.27$ ) and surface electrical charge of approx.  $-27$  mV, were produced by a hot homogenization process followed by ultrasonication. From the intestinal lymphatic transport study it was observed that SLN increased the cumulative percentage dose of lopinavir secreted into the lymph, which was 4.91-fold higher as compared to conventional drug solution in methyl cellulose 0.5% (w/v) as suspending agent. The accelerated stability testing of the prepared formulation was carried out and showed the shelf life is 21.46 months<sup>39</sup>. Shegokar R and Singh KK prepared stavudin loaded SLNs. The purpose of study was to assess the potential of lipid nanoparticles for active delivery of an antiretroviral drug to lymphatic tissues. Characterization of different physicochemical parameters of SLNs such as appearance, particle size, polydispersity index and zeta potential was conducted. The targeting efficiency of the prepared nanoparticles was tested by carrying out ex vivo cellular uptake studies in macrophages which showed several times enhanced uptake than the pure drug solution. The developed nanoparticles showed prolonged residence in splenic tissues. The conclusion derived was stavudine entrapped lipid carriers can be exploited for effective and targeted delivery to HIV infected sites which could ultimately increase the therapeutic safety and reduce side effects<sup>40</sup>. Chattopadhyay N et al. studied delivery of atazanavir by human brain endothelial line. Protease inhibitors (PIs) show low brain permeability. So if HIV viral replication is kept untreated, it can lead to HIV-encephalitis and antiretroviral drug resistance. The purpose of this study was to develop and evaluate a lipid nanoparticle system for enhanced brain delivery of the potent and frequently

used protease inhibitor, atazanavir, using a well characterized human brain endothelial cell line representing the blood-brain barrier. Solid lipid nanoparticles (SLNs) were prepared with a thin film hydration technique and analyzed for the drug encapsulation efficiency, particle size, morphology, zeta potential and drug release. Spherical shaped SLNs with an average particle size of approximately 167 nm were formed. Delivery of atazanavir by SLNs led to a significantly higher accumulation by the endothelial cell monolayer as compared to the drug aqueous solution. The study concluded that SLNs could be a promising drug delivery system to brain for drugs like atazanavir and potentially other PIs<sup>41</sup>. It was also reported in few studies that the modification of surface charge of SLNs for enhancing drug delivery to the CNS must be applied with care because positively charged SLNs and high amounts of negatively charged SLNs were observed to increase the cortical cerebrovascular volume of rats significantly *in situ* in brain perfusion experiments, indicating a compromise in the integrity of the BBB<sup>28</sup>.

### 5.3 Dendrimers

Dendrimers are highly ordered and regularly branched globular macromolecules produced by stepwise iterative approaches. The structure of dendrimers has three distinct regions: a focal moiety or a core part, layers of continuously branched units emerging from the core, and functional end groups present on the exterior of repeat units<sup>27</sup>.

#### 5.3.1 Uses and special features of dendrimers

Small size (typically less than 100 nm), narrow molecular weight distribution, and easy incorporation of targeting ligands make them useful nanocarriers for drug delivery. They have minimal polydispersity and high functionality. They have a highly branched three-dimensional architecture. Dendrimers not only act as carriers for drug delivery of antiretroviral agents, but they themselves also act as antiretrovirals. Dendrimers possessing anti retroviral property can be synthesized by having certain functional groups on their surface. Such dendrimers prevent binding of the virus to cell. The dendrimers that are water-soluble can be used as effective carriers of antiretrovirals which can

be entrapped in the dendrimer structure. The antiretroviral agents or their prodrugs can also be attached covalently to the surface of the dendrimers, either alone or by combining with other molecules, such as targeting moieties and fluorescent tags. Multivalent dendrimeric systems have been of much interest in the field of antiviral therapy because such dendrimers showed reduced cytotoxicity when compared with nonconjugated poly (propyleneimine) dendrimers *in vitro*<sup>28</sup>.

#### 5.3.2 Dendrimers in HIV

Witvrouw et al. prepared Polyanionic dendrimers and evaluated them for their antiviral effects. Phenylidicarboxylic acid and naphthylidissulfonic acid dendrimers were found to inhibit the replication of human immunodeficiency virus type 1. HIV strains containing mutations in the envelope glycoprotein gp120 (engendering resistance to known adsorption inhibitors) displayed reduced sensitivity to the dendrimers. The conclusion derived from the experiment was that Polyanionic (i.e., Polysulfonate) dendrimers are able to inhibit the replication of HIV by interfering with both virus adsorption and later steps (Reverse Transcriptase/Integrase) in the virus replicative cycle<sup>42</sup>. Weber N and colleagues developed amino-terminated carbosilane dendrimers (CBS) as a means to protect and deliver siRNA. Initially, stability studies showed that CBS bind siRNA via electrostatic interactions. Dendrimer-bound siRNA was found to be resistant to degradation by RNase. CBS/siRNA dendriplexes were shown to silence GAPDH expression and reduce HIV replication in SupT1 and PBMC. These results showed that dendrimers such as CBS to deliver and transfect siRNA into lymphocytes will hence allow the use of RNA interference as an important alternative therapy for HIV infection<sup>43</sup>.

#### 5.4 Micelles

Micelles have hydrophilic exterior and hydrophobic core, water insoluble or slightly soluble drugs can be encapsulated into them. Chiappetta DA and colleagues did a study in order to investigate the synergistic performance of mixed polymeric micelles made of linear and branched poly(ethylene oxide)-



poly(propylene oxide) for the more effective encapsulation of the anti-HIV drug efavirenz. The synergistic solubilization capacity of the micelles was checked. The study reported a sharp solubility increase from 4 µg/ml up to more than 33 mg/ml, representing a 8430-fold increase. Moreover, the drug-loaded mixed micelles displayed increased physical stability over time in comparison with pure poloxamine ones. Overall observations confirmed the enormous versatility of the poloxamer/poloxamine systems as Trojan nanocarriers for drug encapsulation and release by the oral route<sup>44</sup>. Uchman M and colleagues prepared micelles differing in morphology from double-hydrophilic block copolymer poly(ethylene oxide)-block-poly(methacrylic acid), PEO-PMA, and two types of fluorescein-[3-cobalt(III) bis(1,2-dicarbollide)] conjugates, GB176 and GB179, in alkaline buffer. GB176 molecule consists of fluorescein attached to the metallacarborane anion. In GB179 molecule, the fluorescein moiety connects two metallacarborane anions. The micelles were studied by static and dynamic light scattering, fluorometry and atomic force microscopy. As the metallacarborane conjugates act as potent inhibitors, of HIV protease the presented system is important from the point of view of drug delivery<sup>45</sup>. Chiappetta DA and colleagues prepared Efavirenz loaded micelles using poly(ethylene oxide)-poly(propylene oxide) block copolymers. The study was aimed towards improving the aqueous solubility and the oral bioavailability of the drug. The results showed that, Efavirenz which is poorly water soluble, when entrapped in polymeric micelles of different poly(ethylene oxide)-poly(propylene oxide) block copolymers significantly increases the oral bioavailability of the drug and decreases the interindividual variability<sup>46</sup>. Chiappetta DA et al. prepared a concentrated formulation of the first-line antiretroviral drug efavirenz by encapsulating into micelles for improving anti-HIV treatment in paediatrics. The aqueous solubility of the drug was found to be increased by 8400 times (up to 34mg/mL) and preliminary preclinical data indicated the significantly greater oral bioavailability with respect to an extemporaneous suspension and an oleous

solution (similar to the only "commercially available" pediatric formulation). The study showed that the micellar size and the bioavailability attained were inversely related<sup>47</sup>.

### **5.5 Polymeric nanoparticles**

Different materials have been used in the synthesis of polymeric nanoparticles, and material choice in some cases depends upon application. Synthetic or semisynthetic polymers, such as poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(alkyl)cynoacrylates, poly(ethylene glycol-co-(lactic-glycolic acid)), poly(caprolactone), and poly(methyl methacrylate), are frequently used for the manufacture of nanoparticles intended for drug delivery. Out of these, PLA and PLGA have been approved for human use by the Food and Drug Administration (FDA). A wide range of drugs having different levels of hydrophilicity or hydrophobicity can be incorporated into these polymers, and their release characteristics can be manipulated to meet dosing requirements. The performance of the polymer can be changed according to application by controlling the molecular weight or copolymer composition. This has impact on properties such as degradation rate, thermal sensitivity, and pH sensitivity. These polymers have the capacity to sustain drug release for several weeks. Drug loading is mainly done by encapsulation, entrapment, or dissolution/dispersion. Because of biodegradable and biocompatible nature of polymeric nanoparticles, they have become very attractive candidates for drug delivery<sup>28</sup>. E.g. poly lactic-co-glycolic acid (PLGA) nanoparticles, poly(ethylene oxide) (PEO)-modified poly(epsilon-caprolactone) (PCL) nanoparticles<sup>27</sup>.

#### **5.5.1 Drug encapsulated polymeric nanocarriers**

Shah and Amiji prepared biodegradable polymeric nanoparticles for intracellular delivery of saquinavir. The aim of study was to develop poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticles loaded with saquinavir which is an anti-HIV protease inhibitor for intracellular delivery. The nanoparticles were prepared by a solvent displacement process. The formed nanoparticles

were characterized for size, surface charge, and surface presence of PEO chains. The results showed spherically shaped nanoparticles with smooth surface with a mean particle diameter of approximately 200 nm. When administered with the prepared nanoparticle formulation showed significantly higher intracellular saquinavir concentrations than from aqueous solution. This study showed that PEO-PCL nanoparticles provide a versatile platform for encapsulation of saquinavir and subsequent intracellular delivery<sup>48</sup>. Belletti D et al. prepared Tenofovir (PMPA) loaded nanoparticles by using poly-(D,L-lactide-co-glycolide) (PLGA) and/or chitosan (CH). Tenofovir (PMPA) is an acyclic nucleoside phosphonate analog which is one of the most important drugs used for HIV treatment. For effective drug loading, CH was added in the first inner emulsion or in the external phase during the second emulsion of water/oil/water (W/O/W) nanoparticles. In vitro studies showed a limited control on drug release in phosphate buffer (pH 7.4). In acidic conditions (pH 4.6), an initial burst effect was observed followed by a slow drug release. The study concluded that the PLGA/CH nanoparticles should be an effective and attractive anti-HIV drug carrier for studying the cellular uptake and drug delivery to target cells such as macrophages<sup>49</sup>. Destache CJ and colleagues conducted an experiment in which the drug release from free antiretroviral drugs such as ritonavir, lopinavir and efavirenz injected intraperitoneally in mice was compared with the same drug loaded nanoparticles (NPs). The observations showed the free drug concentrations peaked 4 h post-injection (ritonavir  $3.9 \pm 3.05$ , lopinavir  $3.4 \pm 2.5$  and efavirenz  $1.8 \pm 0.63$   $\mu\text{g/mL}$ ) and were eliminated by 3 days. Poly(DL-lactide-co-glycolide) NP administered mice had detectable ritonavir, lopinavir and efavirenz concentrations in all tissues for 28 days. Treatment of monocyte-derived macrophages with the nanoparticles showed sustained inhibition of HIV-1 replication. It was concluded that drug encapsulated nanoparticles could give sustained drug release and inhibited HIV-1 replication than free drug treatment<sup>50</sup>.

## 6. SUMMARY AND FUTURE PERSPECTIVES

Systemic fungal infections result in a severe kind of infection in human body. They can even prove fatal if not treated in time. They are a great cause of fear and mortality in immunocompromised patients. Amphotericin B is largely used in the treatment of systemic mycoses. The main problem associated with the drug is renal toxicity which can be minimized if targeting of the drug is effectively achieved. Also, AmB is hydrophobic so it should be delivered preferably in lipidic environment where it can solubilize. Nanocarriers provide solutions to many such problems associated with the drug and various other nanocarrier based formulations are in development for the other antifungal drugs. With respect to AIDS, the cure is still a distant dream for the researchers. One of the important problems associated with anti-HIV therapy is the development of resistance to the drug. Hence HAART uses the combination of drugs to minimize this problem. In case of treatment with the nanocarriers, generally single drug is integrated into nanocarrier. But recently the efforts towards manufacturing nanocarriers possessing a combination of antiretroviral drugs have been begun and demonstrated efficacy with minimal toxicity<sup>51</sup>. These developments will pave the way for further advantages in addition to the targeting using nanocarriers. Looking at the advantages and efficiency associated with nano drug delivery systems, more research should be directed towards more efficacious HIC treatment with minimum toxicity. Though, nanocarriers also possess some disadvantages like susceptibility to aggregation and unsuitability for less potent drugs. Additionally, because of nano size, they may gain access to unintended environments with harmful consequences, e.g. it can cross the nuclear envelope of a cell and cause unintended genetic damage and mutations<sup>52</sup>. But the advantages of nanocarriers outweigh their disadvantages; their use in medicinal field is inevitable. Current research trend suggests that the next decade may see extensive research for finding treatment strategy for HIV and fungal infection using nanocarriers.

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