LITERATURE REVIEW
2. LITERATURE REVIEW

2.1 The Global HIV Epidemic

Human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS), universally referred to as HIV/AIDS, constitute one of the most infectious and chronic immunodeficiency disease that damages and ultimately destroys the immune system, have become a worldwide pandemic, with political and economic implications that surpass public health [1, 2]. This fatal disease is mostly rampant in developing countries and hence, its adverse impact on society and economy could not be ignored. [2-4]. According to an estimate, this disease incarcerates about 1500 people every day and has had a crippling effect in certain parts of the world making 33.2 million people living with HIV/AIDS globally [3].

Currently, more than 40 million people are infected, with more than 20 millions death till date as reported by World Health Organization. The prevalence of infection in southern and eastern Asia is at higher rate as the basic requirements to control or treat infection is limited in these regions [4, 5]. AIDS is one of the leading causes of death worldwide. HIV-1 is primarily transmission through sexual intercourse, vertical transmission from mother to child, or exposure to contaminated blood or blood products.

The risk of infection is generally higher in people with infected sexual partners, infants born to HIV-infected mothers, drug users who share HIV-contaminated needles [6-9]. AIDS was first detected in the United States in homosexual persons infected with Pneumocystis carinii pneumonia (PCP). Usually, PCP was most associated with persons having severe combined immune deficiency and in immune compromised cancer patients on chemotherapy. Unfortunately, numerous social stigmas associated with the syndrome played a role in dawdling the ability of public health agencies in the United States to aggressively screen at risk populations early on in the epidemic [7, 8].

The stated viral infection results in a chronic, progressive illness which assails the immune system and attacks CD4 cells, which are necessary to fight off illnesses. Eventually, the virus overwhelms the CD4 cells and depletes the count below normal levels (500-1500 cells/mm$^3$) to <200 cells/mm$^3$ of blood, resulting in opportunistic infection taking hold of weakened immune system; this justifies the rationale of using CD4 cell threshold to define AIDS [9]. After infection with HIV, there is usually a seroconversion illness followed by an asymptomatic stage which lasts months to years. This is succeeded by symptomatic phases which correlate with progressive
immunodeficiency which is dependent on the stage of the infection. The progression of HIV disease varies from person to person and depends on a number of factors including genetics and mode of transmission [5-7].

The quantity of virus in plasma, termed as viral load, is an important surrogate marker which predicts the rate of progression of disease. It is measured in RNA copies/ml. It is also used to assess response to drug therapy and may indicate the development of drug resistance. After seroconversion, patient develops a viral load set point [1-3]. The progression of HIV disease is slower till the viral load is lower and eventually clinical symptoms and opportunistic conditions are dawdled. Although, due to current efforts including AIDS counseling, educational tools and antiretroviral drug therapy the HIV infection has been transformed from a fatal to a manageable chronic infectious disease. Nevertheless, the mentioned statistics vividly corroborates that there is still a long way to go despite of the availability of a lot many preventive measures as the number of currently reported cases of HIV are still unmanageable. [4, 5].

Despite the increase in number of infected people, the most recent reports available from UNAIDS show a slight decrease in the pandemic. This can be ascribed to the decrease in number of new infections (2.7 million in 2007 vs. 3.0 million in 2001) since the beginning of the 21st century. These figures can be explained by the expanding access to antiretroviral drugs, especially in resource-limited settings, which has not only increased lifespan, but also the quality of life of HIV infected people. In the absence of an effective cure and prevention, access to antiretroviral therapy is the best preferences to affect the HIV pandemic. Nevertheless, the contemporary approaches for providing universal access to prevention and treatment are inadequate, necessitating the search for newer and improvised alternatives [2, 3, 5].

2.2 The HIV Basics

HIV-1, a lentivirus of the family retroviridae is mostly the causative agent of AIDS. During mid 20th century, simian immunodeficiency virus (SIV), the causative agent of HIV-1 might have transmitted from chimpanzees (predominant hosts) to human beings [11, 16]. SIV has multiple genetic variants and infects multiple simian species. This nanostructured virus (around 100–150 nm), comprises of a membrane derived from host, a nucleocapsid and genetic material in the form of RNA containing three structural genes. These genes code for important group-specific antigens (gag gene), essential viral
enzymes such as reverse transcriptase, integrase and protease (pol gene), and the two
glycoproteins present in the outer viral membrane, gp120 and gp41, which are responsible
for recognizing the CD4 receptor and the CCR5 or CXCR4 co-receptors of the host cell
membrane, and for virus/ cell fusion, respectively (env gene) [4-6, 62, 63]. These viral
structures present high polymorphism due to continuous constant transcription errors,
which leads to mutation, thus constituting a major source of antiretroviral-resistance
development.

Similarly, there are currently two different types of HIV, HIV-1 and HIV-2, which
are known to cause infection and disease in humans. Among other differences, HIV-2 is
almost similar to SIV-1, less common with slower progression to immunodeficiency, less
efficiently transmitted with poor pathogenicity than HIV-1 [64-68]. Depending on their
geographic distributions and origins HIV-1 has been classified into many groups and
subtypes. The subtypes A to J of group M are most prevalent worldwide, while in Africa
and Eastern Europe two new groups, N and O, have been identified recently. The group
M mostly comprises of epidemiologically and antigenically distinct subtypes namely B, C
and E [65, 66].

The subtype B has prevalence in North America, Europe, parts of South America
and India while subtype C is predominant in sub-Saharan Africa, and the subtype E is
typically established in southeastern Asia. These subtypes could have profound impact on
future vaccine endeavors [16-20]. AIDS in humans is the advanced stage of the disease
caused by HIV infection which results mainly from integration of the viral genome into
the host cell for the purpose of cell replication. The virus infects the host cell by binding
the viral gp120 protein to two trans-membrane receptors, i.e., CD4+ and either of the two
chemokine receptors, CCR5 and CXCR4. HIV infects macrophages and T-helper
lymphocytes (CD4+); but the characterizing facet of AIDS is the depletion of CD4+ cells
[6, 63]. T-tropic viruses prefer to replicate in T cells, while M-tropic viruses prefer the
macrophage. Of the HIV-1 viruses, M-tropic types predominate in the brain. The viral
genome contains three structural genes; gag, pol and env with six regulatory genes; tat,
rev, nef, vif, vpr and vpu. The virus utilizes some of these genes to maximize its
production using host cell resources [19, 21, 22].

DNA microarray studies have implicated HIV encoded Nef protein in this process.
This fact has been further endorsed by the findings that humans infected with the nef-
deleted form of HIV have remained disease free for several years. HIV has been quoted

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh
as a "master regulator" of cellular gene expression for augmenting the expression of its own genome [69-74]. In order to develop novel therapeutic strategies for the suppression or elimination of the virus a corroborative insight into these processes is critical. The immunopathogenesis of HIV/AIDS has been previously amply documented; from the time of infection to the end stage of the disease. The end stage of the disease may be characterized by a spectrum of diseases including opportunistic infections (such as *Pneumocystis carinii* and *Mycobacterium tuberculosis*), dementia and cancer. Along with the macrophages, lymph nodes, bone marrow, spleen and lungs, the CNS represents one of the most imperative anatomical sites of the virus after infection. This causes significant neuronal damage and loss that often leads to HIV associated dementia. Without treatment, HIV-1 infection is nearly uniformly fatal within 5–10 years [75].

### 2.2.1 The Anatomy of HIV

Each HIV has same morphology as that of cell membrane. The glycoproteins are the key factors for viral attachment and penetration to the host cell. [62, 63]. The inner core of the virus consists of nucleocapsid, surrounding the two identical RNAs with complete viral proteins and enzymes [76-79]. The mRNA comprises three segments: gag, pol and env polyproteins. The gag protein again splits into p24, p9 and p17, constituting the nucleocapsid. The pol protein generates protease, reverse transcriptase and integrase. The env protein forms gp160, further dividing to gp41 and gp120. These glycoproteins are basically responsible for viral attachment to the target cells [65], (Fig. 1).

![Figure 1: The Human immunodeficiency virus (HIV). [62]](image-url)
2.3 The Life cycle of HIV

The Human Immunodeficiency Virus is a single stranded RNA retrovirus, which can infect various cells, including CD4 bearing macrophages and T-helper lymphocytes inside the host [62-64]. There are several steps involved throughout the viral replication cycle which not only defines the various mechanisms involved in HIV life cycle but also provides an insight for the development of novel antiretroviral therapies (Fig. 2).

Step 1. Binding and Fusion

HIV infection starts with binding and fusion of virus to the host cell. The virus uses two types of receptors for attachment and viral entry at cellular level. The viral attachment completes through the binding of the protein gp120/gp 41 to the CD4 cells. Affinity of G-protein coupled chemokine receptors improves the interaction between CD4 and gp120, which plays a key role in cell migration. The coreceptors named CCR5 and CXCR4 are differentially expressed on subpopulations of CD4-expressing cells, including T lymphocytes, thymocytes, macrophages and dendritic cells. These CCR5/CXCR4 chemokine receptors induce a core change in the HIV gp 120/gp 41 complex by interaction with gp120 [82, 83, 84]. The conformational change exposes gp 41 to start fusion of the membranes.

Step 2. Reverse Transcription

Complexes of gp120-gp41 present on viral lipid envelope fuses with the lipid membrane of the target cell, which results in viral core entry into the cytoplasm of the host cell. The viral RNA then converts into double stranded DNA in the cytoplasm through reverse transcription by the help of retroviral enzyme reverse transcriptase (RT) [64].

Step 3. Integration

Once the process of formation of new HIV DNA is completed; a pre integration complex is transported to the host cell's nucleus where an HIV enzyme integrase plays a key role in linkage of viral DNA and host chromosomal DNA [63, 85]. The integrated HIV DNA is called provirus that may remain latent for several years.
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Step 4. Transcription
The provirus on activation uses a host enzyme called RNA polymerase to replicate the HIV genetic material, as well as shorter strands of messenger RNA (mRNA). This mRNA is used as a blueprint to make long chains of HIV proteins [86].

Step 5. Translation and Envelope Processing
The translation of the viral mRNA results in the synthesis of three proteins: ENV gp 160, GAG p55 and GAG-POL p160. p55 and p160 are generated from the same mRNA strand by the process of ribosome frame shifting. The env (gp160) proteins pass through the Endoplasmic reticulum and Golgi apparatus to be processed into gp120 and gp41 HIV envelope proteins [64, 87].

Step 6. Assembly
The gag and gag-pol polyproteins associate with the inner surface of the plasma membrane and interact with gp41. Some viral RNA interacts with the nucleocapsid portion of p55. The p55 and p160 proteins interact and aggregate to form the virion. In crude terms an HIV enzyme called protease cuts the long chains of HIV proteins into smaller individual proteins [65, 66, 67]. As the smaller HIV proteins come together with copies of HIV's RNA, a new virion gets assembled. The assembly continues till the structure extrudes out of the cell.

Step 7. Extrusion/Budding
The newly assembled virus pushes out ("buds") from the host cell. In process of budding, the new virus captures part of outer envelope of the host cell. This envelope acts as a covering, is loaded with protein/sugar combinations named HIV glycoproteins carrying the gp 120 and gp 41 glycoproteins [67, 88]. These glycoproteins have the potential to bind CD4 and co-receptors. The immature virus extrudes out into the extracellular space.

Step 8. Maturation
To spread infection, newly budded virions undergo subsequent conformational changes for maturation, which involves systematic cleavage of Gag proteins and enzymes by the protease enzyme [65, 67, 68]. During viral maturation, protease helps in the
cleavage of Gag and Gag-Pol polyproteins in an ordered process. The protease contains two catalytic aspartic acid residues, which cleave the bond between two amino acids in a substrate. In brief, soon after the budding of the new HIV particle, the viral protease in p160 becomes active, resulting in the cleavage of p160 and p56 into the various subunits and generating the mature form of HIV. This processing of p160 and p56 by the viral protease is essential for the generation of infectious virus. The new matured and infectious copies of HIV move on to infect other cells [89-93].

Figure 2: The complete life cycle of HIV; including major classes of antiretroviral agents. [62]

2.4 The HIV-1 Infectious Process In-vivo

2.4.1 Acute infection in Adults

The most widespread way of infection is unsafe sexual activities, wherein virus affected the immune cells like CD4-expressing macrophages and T cells. The intravaginal inoculation of SIV has showed several probable outcomes that occur during the early phase of acute infection [1-3]. Virus usually attaches to host cells through binding of gp120 which then infects CD4 T cells. The infected cells transmitted through secretions
cause infection of sub mucosal macrophages and CD4 T cells. [3-5]. Co-infections resulting in mucosal ulcerations, like in herpes simplex virus infection, enhance the probability of infection through sexual transmission. Ruptures over the mucosal layer allow HIV-1 to cross squamous epithelium and local inflammation results in higher levels of T-cell activation, leading to enhanced viral integration and replication [94].

Dendritic cells, macrophages and CD4 T cells loaded with HIV migrate to the regional lymphoid tissues in the time span of 3 to 5 days. Direct contact between virus harboring cells and susceptible macrophages or CD4 T cells within the lymph nodes leads to rapid onset of viral replication within two weeks of exposure. The local inflammatory responses induced through the virus cause viral replication and viremia that leads to involvement of other lymphoid tissues and organs. [11-16]. Acute viremia exhibits significantly enhanced viral levels usually higher than 106 copies/mL plasma. Patients become highly infectious with abnormal blood level findings including a typical lymphocytosis, elevated liver enzymes, hyper gammaglobulinemia, elevated serum acute phase reactants, leucopenia, thrombocytopenia and inversion of the CD4 to CD8 T cell ratio [13].

2.4.2 Perinatal Infection

In children, the infection is generally occurred from mother to child. In the absence of ART targeted to interrupt transmission, approximately 20% to 30% percent of infants born to HIV infected women become infected [94, 95]. About 60-70% of infants get infected through transplacental circulation to the fetus form infected mothers [71-73]. Perinatally infected children have lower early levels of viral replication, slower attrition of CD4 Tcells and delayed clinical disease progression with an estimated rate of progression to AIDS of 8% per year. Ingestion of virus laded maternal breast milk attributes to further 15% to 20% of pediatric HIV-1 infection particularly in developing countries, where options for infant nutrition are limited. [96-99].

2.4.3 Clinical findings in HIV-1 Infection

Immunity mediated by T-cells gets altered on HIV infection; result in opportunistic infections and increased risk of malignancy related to altered immune functions. The infected persons have an increased risk of malignancies associated with viral co-infections, such as herpes virus, Kaposi sarcoma and B-cell lymphoma. Virus
directly can cause infections leading to progressive multi focal leukoencephalopathy, cardiomyopathy, nephropathy and chronic conditions within other organ systems [71-73]. Prediction of progression of disease could only be accessed through regular monitoring of blood levels for viral load and CD4 T-cells count.

A higher steady state levels of viral replication (>35,000 copies/mL) predicts higher risk (>60%) of development of AIDS within few years of infection. However only 8% of persons having HIV infection with a steady-state viral loads of less than 5000 copies/mL have developed AIDS within the same time frame [95]. The extent of suppression of immune system is based primarily on evaluation of CD4 T-cell counts which has a direct correlation with age (infants and children have higher total lymphocyte counts than adults). HIV infected child who exhibits normal CD4 T-cell counts has higher susceptibility for immune suppression and opportunistic infections. The demarcation of mild, moderate and severe immune suppression is based upon the relative percentages of CD4 T cell count throughout all the age groups. Mild immune suppression (>25% CD4 T cells), in general have no severe symptoms, however incidences of upper respiratory tract infections, allergic disease, muco-cutaneous candidiasis, lymphadenopathy and splenomegaly are frequently increased [75, 76]. Patients with moderate immune suppression (CD4 T cell count 15% to 24%) are at risk for panocytopenias, recurrent viral infections with *herpes simplex* and *varicella zoster* and systemic bacterial infections. Patients with CD4 T-cell count <15% have severe immune suppression and carry high risks of recurrent life threatening bacterial infection, extra pulmonary *cryptococcal* infection and other systemic fungal infections. [100-106].

### 2.5 Treatment and Prevention of HIV/AIDS

In current trends of AIDS therapy use of antiretrovirals (ARVs) is the best treatment for HIV-1 infection; which targets multiple steps in the viral life cycle. There is a list of various ARTs available which could either be used as alone or in combinations [107]. (Table 1, Table 2) These drug regimens can significantly delay the progression of AIDS and may prevent or reverse immune deficiency. Reverse transcriptase inhibitors could be primarily used as therapeutic regimen targeting the reverse transcription process of viral replication. RT inhibitors are classified into two groups, namely the nucleoside and nucleotide RT inhibitors (NRTIs) and the non-nucleoside RT inhibitors (NNRTIs). NRTIs, happened to be the first anti-HIV drugs used clinically [108, 109]. These drugs
undergo intracellular phosphorylation for activation and compete with endogenous nucleotides for incorporation into the growing viral DNA strand.

NRTIs lack a 3’ hydroxy terminal, so when an NRTI is incorporated into DNA the next phosphodiester bond will not be formed and the DNA strand is terminated. They can have a variety of agent-specific adverse effects as well as class specific toxicities including mitochondrial toxicity and lipodystrophy. Unlike NRTIs, NNRTIs bind directly to HIV-1 RT (require no intracellular phosphorylation) and have controlled impact on other cellular enzymes [107, 110]. Agents in this class have fewer long-term toxicities and longer half-lives resulting in more convenient dosing. The NNRTI class of drugs is the back-bone of many combination antiretroviral regimens and thus considered highly effective in controlling viral replication. Resistance to NNRTIs can occur quickly, however, with a single point mutation rendering the entire class nonfunctional; the feature that should be considered in patients who are anticipated to have difficulty with adherence [107, 108].

The HIV protease inhibitors another class of antiretroviral therapy binds selectively to HIV protease and prevents this enzyme from performing its normal function of cleaving viral polyprotein precursors into individual functional proteins. Inhibition of HIV protease leads to the formation of deformed HIV particles that do not replicate [111]. Historically, protease inhibitors carried a high pill burden and dosing frequency, significant gastrointestinal toxicity, and certain food restrictions. Newer drugs and newer formulations of older drugs have mitigated many of these problems. Protease inhibitors still are associated with long-term metabolic complications such as diabetes, insulin resistance, and fat redistribution, but in contrast to NNRTIs, have a high threshold to drug resistance, making a protease inhibitor anchored regimen more forgiving of lapses in adherence [112, 113].

The fusion inhibitor enfuvirtide represents the newest class of antiretroviral agents. It has been studied primarily in highly treatment-experienced patients and therefore is not recommended for first-line ART. It works by binding to the gp41 envelope protein of HIV to prevent it from mediating fusion of the viral and cell membranes [108, 109, 114]. Enfuvirtide is most effective in patients who have CD4 T-cell counts above 100 cells/mL; in these cases, enfuvirtide is combined with a standard regimen of drugs that includes one or two agents to which the patient’s virus is sensitive. Several clinical trials have shown the highly treatment experienced patients who received
an optimized background of boosted active protease inhibitor with enfuvirtide demonstrated higher rates of virologic suppression compared with those who received active protease inhibitors alone [111, 113, 115].

Patients who do not meet these criteria still may experience a virologic response and some immunologic benefit from enfuvirtide, however. These benefits must be weighed against the cost, difficulty of administration (twice-daily subcutaneous injections), and adverse effects (e.g., injection site reactions, hypersensitivity, and possible pneumonia). Integration of the viral DNA into a host cell genome is an essential step for HIV replication and maintenance of persistent infection. A number of compounds currently under investigation inhibit HIV-1 integrase, the enzyme necessary to accomplish this function [114-116].

Table 1: Single antiretroviral medications.

<table>
<thead>
<tr>
<th>Class of ARV</th>
<th>Generic Name</th>
<th>Trade Name</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleoside RTIs</td>
<td>Abacavir (ABC)</td>
<td>Ziagen</td>
<td>300 mg bid</td>
</tr>
<tr>
<td></td>
<td>Didanosine (ddI)</td>
<td>Videx</td>
<td>400 mg qd (250 mg qd if &lt; 60 kg)</td>
</tr>
<tr>
<td></td>
<td>Emtricitabine (FTC)</td>
<td>Emtriva</td>
<td>200 mg qd</td>
</tr>
<tr>
<td></td>
<td>Lamivudine (3TC)</td>
<td>Epivir</td>
<td>150 mg bid</td>
</tr>
<tr>
<td></td>
<td>Stavudine (d4T)</td>
<td>Zerit</td>
<td>40 mg bid (30 mg bid if &lt; 60 kg)</td>
</tr>
<tr>
<td></td>
<td>Zalcitabine (ddC)</td>
<td>Hivid</td>
<td>0.75 mg tid</td>
</tr>
<tr>
<td></td>
<td>Zidovudine (AZT)</td>
<td>Retrovir</td>
<td>300 mg bid</td>
</tr>
<tr>
<td>Nucleotide RTIs</td>
<td>Tenofovir</td>
<td>Viread</td>
<td>300 mg qd</td>
</tr>
<tr>
<td>Non Nucleoside RTIs</td>
<td>Delavirdine</td>
<td>Rescriptor</td>
<td>400 mg tid</td>
</tr>
<tr>
<td></td>
<td>Efavirenz</td>
<td>Sustiva</td>
<td>600 mg qd</td>
</tr>
<tr>
<td></td>
<td>Nevirapine</td>
<td>Viramune</td>
<td>200 mg qd x 14 days, then 200 mg bid</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Atazanavir (ATV)</td>
<td>Reyataz</td>
<td>400 mg qd or (ATV 300 mg + RTV 100 mg qd)</td>
</tr>
<tr>
<td></td>
<td>Darunavir (DRV)</td>
<td>Prezista</td>
<td>DRV 600 mg + RTV 100 mg bid</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Class of ARV</th>
<th>Generic Name</th>
<th>Trade Name</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleoside RTIs</td>
<td>Zidovudine + Lamivudine</td>
<td>Combivir</td>
<td>One tablet bid</td>
</tr>
<tr>
<td></td>
<td>Zidovudine + Lamivudine + Abacavir</td>
<td>Trizivir</td>
<td>One tablet bid</td>
</tr>
<tr>
<td>Nucleotide RTIs</td>
<td>Emtricitabine + Tenofovir</td>
<td>Truvada</td>
<td>200/300 mg qd</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Lopinavir + Ritonavir (LPV/R)</td>
<td>Kaletra LPV</td>
<td>400 mg bid + RTV 100 mg bid</td>
</tr>
</tbody>
</table>

Table 2: Fixed dose combinations of antiretroviral medications.
2.5.1 HIV/AIDS Drug therapy and Limitations of ART

Although the development of drugs for HIV infection has undergone substantial progress, numerous uncertainties persist about the best way to manage this disease. In current research scenario, the different ARVs could be well classified under categories such as nucleoside reverse transcriptase inhibitors (NRTI), nucleotide reverse transcriptase inhibitors (NtRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) [113, 115, 116]. First antiretroviral drugs were introduced for therapy in the late 1980s and early 1990s. Furthermore, the concept of highly active antiretroviral therapy (HAART) comprising the intense use of combination drug regimens was introduced in the late 1990s to combat with the after fast development of antiretroviral resistance in individuals treated with single drug regimens. Extensive use of HAART has dramatically increased expectancy and quality of life and has also reallocated the status of AIDS from a rapid-progressing to a chronic disease [117]. Undoubtedly, antiretroviral treatment is currently the best option for prolonged and maximal viral suppression, and preservation of the immune system after HIV infection onset. About 30 individual drugs and fixed-dose combinations are available in market to treat HIV infection [114, 115-117].

The choice between this variety of drugs and drug regimens is not easy and depends of multiple variables related to drug pharmacological and toxicological properties, therapy costs, disease staging and progression, drug resistance status and patient characteristics. Even though HAART regimens confer considerable anti-HIV activity, often its success may be compromised by several impeding factors [116, 117, 118]. Despite the significant contribution of ARV drug therapy towards improved patient/disease management, nevertheless its contemporary use is associated with several shortcomings and hassles to the HIV/AIDS patient. Many ARV drugs undergo extensive first pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability. Several ARV drugs have short half-life which leads to frequent administration of doses and thus decreased patient compliance. Moreover dose of the ARV towards the higher end, further potentiates the inconvenience of the patient. Beside these facts, current antiretroviral therapy suffers from a myriad of other hiccups including adverse effects associated with prolonged treatment periods, poor drug-regimen compliance, drug resistance, drug-drug interactions, poor drug pharmacokinetics, viral levels rebound after therapy cessation and costs [117-119].
Early anticipations that combination ART would exterminate HIV have been unaccomplished. The major limitation in achieving the stated goal is that despite effective therapy, the HIV can persist in latent and inaccessible cellular and anatomical reservoir sites (CNS, lymphatic system and macrophages) for many years [106, 108]. The majority of drugs in the required therapeutic concentrations cannot access these sites and also cannot be maintained for the necessary duration at the site of HIV localization. Thus failure of ART to completely eliminate HIV from these reservoirs is due sub-therapeutic drug concentrations and short residence time at the required sites of action. Drug resistance is the most common cause of antiretroviral treatment failure and has been described for virtually every antiretroviral drug either alone or in combinations which are currently been used in therapy [111, 113, 114]. The inability to attain effective and/or sustained drug levels with currently used formulations and drug-schedules is an imperative aspect for the emergence of resistance to HIV/AIDS therapy, thus contributing to ineffective viral suppression (even if at undetectable levels by contemporary assays), particularly in reservoir sites.

Such issues are important hurdles that need to be resolved in order to optimize the activity of antiretroviral drugs and avoid drug resistance. In addition, myriad of factors such as inadequate drug concentrations at the site of action consequently requiring large doses for achieving a therapeutic effect, and/or the poor bioavailability of several ARV drugs often leads to severe side effects associated with ARV therapy [120]. These drugs also suffer from physicochemical problems such as poor solubility that may lead to formulation difficulties. One of the most problematic issues associated with the use of multi-drug regimens is that each of drugs often possesses considerable toxicity and this may delay therapy initiation or determine its interruption. The type, severity and frequency of clinical adverse events are variable and dependent on individual drugs, drug regimens and patients. The problem of drug toxicity is even more astounding if a nearly perfect compliance of drug regimens for long periods is essential for effective viral suppression chronically, with interruption of drug treatment frequently resulting in increased morbidity and mortality. Also, the fact that interactions between antiretroviral drugs or with other drugs are frequent and highly complex is of utmost relevance for the management of treatment course [119, 121].

Despite the arduous efforts made in last decade to provide complete cure to the disease, the universal access to antiretroviral therapy is still a distant realization. Of late
various strategies are being investigated to overcome these limitations. These include the identification of new and chemical modification of existing chemical entities, the examination of various dosing regimens, as well as the design and development of novel drug delivery systems (NDDS) that can improve the efficacy of both existing and new ARV drugs [122]. In the past decade, there has been renewed interest in the development of NDDS for the incorporation of ARV drugs for circumventing the problems described above and optimizing the treatment of HIV/AIDS patients.

2.5.2 Novel Drug Delivery Strategies for ARV drugs

Nevertheless, the contribution of antiretroviral therapy to treat the untreatable is irrefutable. It has been restricted by several factors, such as its inherent toxicity, insufficient efficacy, and drug resistance. Some of the aforementioned issues have been addressed and minimized by the development and recent approval of innovative or improved drugs however the remarkable ability of HIV to resist the new therapeutic options has limited success [123-125]. The meager physicochemical properties of most of these antiretroviral drugs (e.g. poor solubility, permeability, and stability) results in poor absorption, low biodistribution, and short antiretroviral effect, thus contributing to poor clinical outcome. In order to solve these problems, several new and improved delivery systems and dosage form have to be investigated [126, 127].

In accordance with the stated requirements of treatment; the urge for the development of controlled release drug delivery systems could not be ignored, in order to achieve the therapeutic drug concentration till the extended frame of time. That will not only prevent the plasma drug fluctuations which is considered to be the major root cause for development of drug resistance, but also provide freedom from large amount of doses as well as multiple number of administration of the therapeutic regimen; ultimately leading to efficacious drug therapy, enhanced bioavailability and patient compliance [128-130]. In recent years considerable attention has been focused on the development of controlled release drug delivery systems (CDDS), as the population of patients with chronic complications has increased; which needs the prolonged treatment regimens for better management of disease [130-133].
2.5.2.1 Controlled Drug Delivery Systems

The basic rationale for controlled drug delivery is to alter the pharmacokinetics and pharmacodynamics of pharmacologically active moieties by using novel drug delivery systems or by modifying the molecular structure and physiological parameter inherent in selected route of administration. It is desirable that the duration of drug action become more a designed property of a rate controlled dosage form and less or not at all, a property of the drug molecules inherent kinetic properties [134]. Thus optimal design of controlled release systems requires a thorough understanding of pharmacokinetics and pharmacodynamics of drug [134, 135].

Controlled drug delivery systems (CDDS) serve two functions; it involves targeted delivery of the drug to specific tissues or organs as well as delivers the drug in a constant and therapeutic rate for a prolonged period of time. Thus the CDDS are used to enhance the therapeutic response by expressing the more consistent drug levels in blood plasma than conventional dosage forms [136]. They release the drug at predetermined and controlled rate for a definite period of time with enhanced potential of therapeutic efficacy and reduction in dosing frequency so as the adverse reactions. They result in drug levels within the therapeutic window avoiding higher systemic toxic levels; providing patient compliance and overall effective therapeutic regimens [134, 136].

(A) Drug release Mechanism of Controlled release Systems

An ideal controlled release mechanism for a drug delivery device is the one that exhibits zero order release kinetics, i.e. the release of drug is independent of concentration. However, with decreasing of drug level in the device, its release usually slows down. Thus, in most controlled release systems, drug release exhibits two phases: an initial phase and a second phase that relates to the rapid depletion of the drug from the device [135, 137]. There are various mechanisms involved in the controlled release systems by which a drug can be released from a delivery system. They may be diffusion, erosion and degradation. Diffusion occurs when the drug travels through the polymer matrix into the external environment (Fig. 3). The diffusion can happen on a macroscopic scale through pores in the polymer matrix, or on a molecular level, by involving polymer chains. In a polymeric matrix drug and the polymers have been mixed to form a homogeneous polymeric mesh or network to control the release of drug there from at a predetermined rate till specific period of time [137, 138].

*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh.*
As the drug diffusion continues, its rate normally decreases, mainly because the drug has a longer distance to travel and thus requires a prolonged duration to diffuse out of the polymeric matrix. Diffusion-controlled systems usually showed good stability in the biological environment [137, 139]. In the diffusion-controlled systems, the polymer matrices must allow the drug to diffuse through the pores or macromolecular structure of the polymer into the biological environment without introducing any change in the polymer itself. It is also possible to design a drug delivery system such that it is incapable of releasing its agent until it is placed in an appropriate biological environment [138, 140].

Swelling-controlled systems are initially dry, after being placed in the body; they absorb water or other body fluids through the capillary action and then swell. Swelling increases the aqueous solvent content within the formulation and the polymer mesh size, enabling the drug to diffuse through the swollen network into the external biological environment [139-141] (Fig. 4). Most of the materials used in swelling-controlled systems are based on hydrogels. They can absorb ample amount of fluid and, at equilibrium, typically comprise 60–90% fluid with only 10–40% polymer. One of the most notable and constructive feature is that a change in the environment surrounding the delivery system can trigger polymer swelling [142].
Dependent on the polymers used, the environmental change can involve pH, temperature, or ionic strength. The system can either shrink or swell upon a change in any of these environmental factors. For most cases, the structural changes of polymers are reversible and repeatable when additional changes in the external environment take place [140, 141]. The controlled release systems described above are based on polymers, the chemical structures of which do not change during the course of drug release.

In erosion controlled prolonged release systems, the rate of drug release is controlled by the erosion of a matrix in which the drug is dispersed. The erosion can be simply described as a continuous liberation of both drug and excipient comprising the matrix from the surface of the dosage form by surface erosion [141, 143]. The consequence will be a continuous reduction in matrix weight during the course of the release process (Fig. 5).
(B) Routes of Controlled drug delivery

Because of the significant advantages that controlled release systems provide, the list of controlled release products continues to grow, particularly in cost-neutral or cost-advantageous situations. There are some cases where controlled drug delivery is actually an enabling technology for a drug i.e. a drug may be unstable, ineffective or highly toxic without the controlled delivery devices but after designing a modified release drug delivery systems, there performance is unexceptionally increased and accepted for better therapeutic efficacy, patient compliance as well as minimized adverse effects [135, 136, 139]. Controlled release systems have already been found in a wide variety of applications in human medication, which have been delivered through various routes. Brief descriptions of some of these routes are as follows.

(i) Oral Delivery

Oral administration is the most widely used route because of its simplicity, convenience and patient compliance. Problems, however, include the hostile environment of the gastrointestinal route as well as poor solubility of the polymeric materials in the various cellular barriers. Adding to these constraints is the commonly substantial intra and inter subject variability associated with some of these factors [134, 143]. Generally, these factors cannot be controlled and hence severely limit the design of oral drug delivery systems. Various approaches include using polymers that degrade preferentially in the colon, nanoparticles that can be taken up by intestinal Peyer’s patches and bioadhesives that interact strongly with intestinal mucosa [137, 138]. Some advanced and important classes of oral controlled-release dosage forms may include multiple unit particulate systems (matrix as well as reservoir), hydrogels and osmotic pumps with their specific mechanism of action and therapeutic index [136, 137, 139].

(ii) Transdermal Delivery

In the past, topically applied dermatological drugs were used for localized treatment of skin diseases only. Recently, due to a better understanding of the anatomy and physiology of the skin as well as a more thorough understanding of percutaneous absorption, the limited permeability of human skin has also been utilized for systemic drug administration [134]. Transdermal patches, together with oral forms, are the most extensively developed because both transdermal and oral delivery are non-invasive.
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Almost all prescription medicines fall into these two categories. An advantage that transdermal delivery provides is that the drug enters the systemic circulation directly, avoiding first pass metabolism and gastrointestinal incompatibilities. Drugs such as scopolamine, hyosine, nitroglycerin, and clonidine have been successfully administered via this route [139, 140].

(iii) Buccal/Sublingual Delivery

Buccal drug delivery seems to combine the advantages of transdermal and oral delivery. Drug can be absorbed from the oral cavity through the oral mucosa either sublingually (under the tongue) or buccally (between the cheek and gingival). In general, rapid absorption from these routes is observed because of the thin mucous membrane and perfused blood supply [134]. For highly hydrophilic drugs, which also suffer from extensive pre-systemic elimination and require a rapid onset of action, sublingual or buccal administration may offer advantages over oral administration. After absorption, drug is transported through the deep lingual vein or facial vein. Thus, the buccal and sublingual routes can be used to bypass hepatic “first-pass” elimination [139, 141].

Conventional buccal and sublingual dosage forms are generally short acting. As the sublingual administration often interferes with eating, drinking, and talking, this route is generally considered unsuitable for prolonged administration. Compounds administered by either the buccal or sublingual route include steroids, barbiturates, papain, trypsin, and streptokinase-streptodornase and nitroglycerin [136, 139].

(iv) Parenteral Delivery

Administration of drugs at specific targets is possible by many more routes with variable dosage forms. However maintenance of in vivo therapeutic drug concentration for extended period of time is a challenging task. Fluctuations in blood plasma levels of drug are frequent whether the drug is given orally or even by intravenous route [144-148]. Drug toxicity and sub therapeutic concentrations may always happen to the patients whenever the administered drug concentration is above or below the minimum effective concentration (MEC). For troubleshooting of the stated problem a number of controlled drug delivery systems (CDDS) like transdermal, nasal, ocular, oral were investigated. But due to the problem of poor absorption and low bioavailability by all these non-injectable
routes have made the parenteral route the most viable route for drug delivery of drugs for extended period of time [149, 150].

(a) Specifications for Parenteral Delivery

Modified release parenteral drug delivery systems have some significant specifications with reference to design, development as well as administration as they are complex products and are designed for targeted as well as extended release. There are certain specific parameters which have to be taken into consideration during design and development of parenteral extended release formulations [147, 151]. Optimization of duration of action is one of the specific parameter which must be considered in accordance with the potency and physicochemical properties of drug as well as controlled release technology employed. The quantity of drug required, which is dependent on its potency and efficacy of the delivery system; in collaboration with the type and amount of rate controlling excipient will determine the extension of release from the dosage form [145, 146]. Rate controlling excipients /polymeric materials, which are to be used in formulation of controlled release dosage form, must fulfill certain specific requirements such as compatibility with drug, tissue compatibility, biodegradation kinetics, mechanical properties, ease of applicability and approval from regulatory bodies [145-151].

These excipients/polymeric materials must biologically degrade into non-obnoxious and non-toxic metabolites within specific duration of time preferably close to the duration of action of the designed dosage form. The design and developmental of controlled release parenterals could be done by selecting any of the methods available depending upon the physicochemical properties of the drug and the polymer [148, 150]. However the method must fulfill the standards of pharmaceutical processing and should be reproducible to produce the predictable release pattern and content uniformity with purity and integrity of the entrapped drug. Depending upon the method used for preparation of controlled release parenterals, different organic solvents might be used during the manufacturing process, which should not be present in the final dosage form but if still there is a possibility of the presence of the residual solvent in the final dosage form, that should be quantified by means of gas chromatography and should remain within the limits assigned by the regulatory agencies [151, 152].

Above all these specifications, consequent manufacturing of the long-acting parenteral controlled release dosage form is a tedious task which requires a strategy
involving diversity of disciplines ranging from medicine and chemistry to regulatory affairs and marketing. Besides all these complexity the most necessary thing after the manufacturing of the dosage form is the cost-effectiveness, which should ensure the accessibility of the formulation to the society [147-151].

2.5.3 Biodegradable Parenteral Microspheres

Out of the various means of formulating prolonged release parenteral preparations, the use of biodegradable microspheres is the most suitable one. Biodegradable polymeric parenteral microspheres are an emerging trend of formulation development which facilitates prolonged residence of medication in the body with significant therapeutic adherence and convenience benefits to patients [147]. In the era of early 1970s, biodegradable polymers were first used for sustained release parenteral drug delivery. The US Food and Drug Administration (FDA) have granted approval for the use of these polymers in humans as implantable devices, surgical sutures and drug delivery systems [147, 148].

The delivery of small molecules, proteins, and macromolecules by formulating parenteral microspheres using biodegradable polymers such as polycaprolactone (PCL), polylactide (PLA) and poly (lactide-co-glycolide) (PLGA) have been studied comprehensively and successfully used for the treatment of a score of diseases [150]. Microspheres as drug delivery devices confer several advantages such as enhanced stability of protein therapeutics, continuous and controlled drug release, reduced dosage, diminished systemic side effects, reduced possibility of dose dumping, less frequency of administration; ultimately leading to increased patient compliance as well as enhanced therapeutic benefits [147, 148]. These biodegradable materials can degrade within the body through natural biological processes with time, eliminating the need to be removed after the drug release has been completed.

2.5.3.1 Controlled release by Polymeric Microspheres

The mechanisms to control the release rate by a controlled delivery vehicle include dissolution, diffusion, erosion or chemical reactions, swelling, osmosis and external forces such as ultrasound and magnetic force. In polymeric microspheres, major mechanisms normally include polymer degradation and drug diffusion [147]. In addition to controlling the drug release rate, releasing the drug at a desired site or drug targeting
can also be very important, particularly for the ailments requiring targeted delivery like AIDS as well as cancer chemotherapy [148, 151].

(A) Control of Release rate

Almost all commercial biodegradable polymers for drug delivery have hydrolysable backbones and mainly undergo hydrolytic degradation. The possibility of enzyme degradation has also been considered. Two types of degradation are possible, namely bulk degradation and surface erosion. In bulk degradation, the rate of water penetration into the polymer matrix is faster than that of polymer degradation, so that degradation takes place throughout the whole matrix [152, 153]. In contrast, in surface erosion, water cannot enter the polymer matrix readily and the matrix has to erode gradually from the surface to the core. Surface erosion is normally desirable for drug release because of its ability to offer zero-ordered release kinetics, i.e. constant drug release. However, up to now all the USA Food and Drug Administration (FDA) approved biodegradable polymers undergo the bulk degradation mechanism [151, 154].

Bulk degradation normally experiences three stages. In the beginning, the backbone of the polymer is hydrolyzed and its molecular weight decreases gradually. At this stage (stage I) the strength and integrity of the delivery device is intact. After some time, the device is hydrolyzed so greatly that the strength is lost (stage II) and the mass is subsequently dissolved (stage III). The dissolved mass can be further metabolized and eliminated from the human body eventually [152]. The degradation rate of polymeric microspheres is dictated by their hydrophilicity since the degradation mechanism is mostly hydrolysis. Therefore, faster degradation can be caused by more hydrophilic molecular structure and more amorphous state of the polymer, and smaller particle size and higher porosity of the polymeric microspheres [153-155].

By assuming that the driving force of diffusion is the concentration gradient, one may describe the drug diffusion in polymer matrix by Fick’s first law in which the diffusive flux is proportional to the drug concentration gradient. The coefficient is termed as the intrinsic diffusivity of the drug in the polymer matrix. It is a constant of proportionality or may be a function of concentration [147, 148]. Considerations based on the thermodynamics of irreversible processes indicate that a more fundamental driving force is the gradient of chemical potential of the drug. The resulted Fick’s first law has
the similar form as the concentration based on the drug diffusive flux is proportional to the chemical potential gradient [149, 153].

The coefficient, i.e. the chemical potential based diffusivity, is less dependent on the concentration of the drug. A distinction between two types of situations has been made with respect to drug diffusion in polymeric matrix. In one type, the pores in the polymer matrix are smaller than or of the same order magnitude as the mean free path of the drug molecules [149-153]. The molecular network constituting the polymer matrix takes part at the molecular level in frictional interactions with the drug molecules. In the other type, the pores in the polymer matrix are much larger than the mean free path of the drug molecules. The system containing these pores may be regarded as comprising two phases: the polymer phase represents little more than a solid container for the diffusing fluid phase within which the transport process occurs. The polymer phase only serves to define the geometry of the diffusing channels [149, 154, 155].

The drug diffusion rate in the polymer matrix depends on the temperature, the molecular structure of the drug and the polymer, the presence of other components and so on. Likewise, an elevated temperature increases the mobility of the polymer chain and the free volume in the polymer matrix, thereby leading to higher drug diffusivity [148, 152]. Drug molecules of low molecular weight readily pass through polymer molecules of high molecular weight, giving large drug diffusivity. In addition, the presence of a plasticizer increases the drug diffusivity, because it reduces the polymer inter-chain interactions and can serve as a diluent. A generic kinetics of drug release from polymeric microspheres has three stages.

In the first stage, the drug attached to, or loosely entrapped near, the microsphere surface is desorpted and gives rise to a fast release, which is known as initial burst. This is followed by the second stage in which drug mainly diffuses out through the free volume in the polymer matrix [147]. In the subsequent and final third stage, the polymer degradation mechanism becomes predominant, implying that the drug most likely escapes from the polymer matrix together with the degraded polymer [153-155]. These three phases are sometimes not so distinctly divided. For instance, certain parameters may be adjusted to make the diffusion faster and desorption slower, thereby leading to more sustained release, which is desirable in most clinical cases which require extended therapeutic regimen from days to weeks to months; as in case of AIDS we need constant
therapeutic drug plasma level not only to maintain the viral load to undetectable levels but also to prevent disease progression [147, 153].

(B) Control of Release site

It is estimated that only less than 1% of the intravenously administered dose of a free drug reaches the desired site, while the remaining majority causes toxicity in various tissues and organs. This fact indicates a large room for research on targeted drug delivery devices which are able to release the drug at selected organs, tissues, cells or even intracellular compartments [148]. Polymeric microspheres can be used as a targeted device. The particle size of polymeric microspheres is critical to dictate their delivery route and biological fate. Microspheres of particle size less than 10 μm are desirable for intravenous injection. Smaller particle size is required for oral, transdermal, ocular and nasal delivery [151-153].

Microspheres in size range of 10-100 μm can be used for subcutaneous or intramuscular administration while particle size over 100 μm has been employed for implantation. Once entering systemic circulation, a large portion of the polymeric microspheres is taken by RES [147, 148]. In general, smaller particle size helps to reduce the RES uptake. RES targeting is desirable in some cases to reduce systemic side effects. Besides, microspheres with size range of 20-50 μm are able to provide targeting to tumor via the so-called chemo-immobilization process [60, 156, 158]. For instance, to treat liver cancer, polymeric microspheres are injected to the liver artery, and are trapped in the microvasculature of the liver. In comparison with the microvasculature of normal tissue, that of tumor is much leakier and will thus trap most of the microspheres.

Surface modification is another means to control the biological fate of the polymeric microspheres. Bioadhesive polymers such as polyanhydride copolymers of fumeric and sebacic acid can increase the absorption of the polymeric microspheres at certain sites. On the other hand, coating the polymeric microspheres with some hydrophilic polymers may avoid the uptake by RES [60]. Theoretically; a number of ligands can be coupled onto the surface of the microspheres to allow targeting to tumor cells through recognition of proteins involved in receptor-mediated cell entry [158].
2.5.4 Efficacy of Parenteral Microspheres in treating HIV/AIDS

Detailed discussion has been already carried out over the root cause of incomplete eradication of HIV so as total cure of AIDS. It is a well known fact that the current medication regimen has many drawbacks, including the higher dose with frequent dosing, missing to which leads to fluctuation in drug plasma levels with enhanced viral load in blood and ultimately developing the drug resistance. Moreover the HIV hidden into the host reservoirs could not be targeted with the current ARV therapy [147]. Needless to state that complete eradication of HIV and total cure of the disease require not only the controlled and prolonged level of ARVs in the blood but also require targeting of ARVs to the hidden HIV into the inaccessible compartments of the body.

Administration to deliver the drug in the form of parenteral depot formulation will potentially avoid the plasma level fluctuations of the drug thus inhibiting the possibilities of drug resistance. Parenteral biodegradable microspheres will provide an impetus in the treatment of HIV disease in case of neonatal infections from HIV positive mother, unconscious patients unable to take oral medications and moreover the patients with damaged hepatic or gastric systems due to complications associated with HIV infections which are the major indications associated with the disease [147].

Primarily, owing to low degradation rates of the biodegradable polymers, these novel drug carriers encapsulating ARVs will deliver a sustained dose in vivo milieu for varying periods of time, usually ranging from few days to weeks to months. Besides this, these can also be used as targeted drug delivery systems to the host reservoirs of hidden HIV for complete eradication of the same leading towards maximum therapeutic benefits [147, 148]. ARV loaded polymeric microparticles for targeting to the inaccessible compartments/reservoirs have consequently emerged in the current era which seems to be the only tool to overcome the problem of inadequate drug concentration, lower residence time at the targeted site as well as the drug resistance [145, 146].

Passive targeting of drugs through biodegradable polymeric microspheres exhibiting narrow range of particle size distribution might be one of the precise approaches for achieving therapeutic drug concentration for extended period of time with minimized chances of drug resistance and cellular toxicity. The major advantage of controlled release biodegradable polymeric systems as targeted delivery is the ability to lower the necessary dosage, facilitating the uptake of antiviral drugs in optimum concentration [60, 158]. It significantly exhibits a reduction in side effects with desired
therapeutic benefits through complete eradication of HIV from host reservoirs enabling a considerably improved AIDS therapy. Some controlled release long acting parenteral dosage forms based on the biodegradable microspheres, which are approved by FDA as well are available in the market like Lupron Depot® (Leuprolide acetate), Nutropin Depot® (Recombinant human growth hormone), Decapeptyl® (Triptorelin), Sandostatin LAR Depot® (Octreotide acetate) and Zoladex® etc. [148]

2.6 Biodegradable Polymers

A myriad of polymers are employed as excipients, drug delivery systems, bandage, suture, or packing materials in pharmaceutical arena. Synthetic nondegradable polymers were primarily employed as drug carriers, diffusion barriers, or protective coatings for parenteral depot systems [159]. But, with the advent new polymeric materials, which degrade in a biological environment displayed immense potential as drug carriers. For an ideal drug delivery, a biodegradable polymer and its degradation products ought to be biocompatible and toxicologically safe [159, 160]. Invariably, the biodegradable polymers are beneficial, especially in instances, wherein the removal of the spent device is either inconvenient or even impossible.

In the beginning, the biodegradable polymers were used as biomaterials for the manufacture of absorbable sutures and orthopedic fixture materials. Afterward, they fascinated an immense consideration for drug delivery and tissue engineering [161]. Primarily, biodegradable polymers can be classified based upon the mechanism of erosions. Undoubtedly, ‘degradation’ refers to the chemical process of bond cleavage, while ‘erosion’ implies a physical phenomenon dependent on dissolution and diffusion processes.

Two mechanisms of polymer erosion can be distinguished as surface and bulk erosion. However, the relative extent of surface or bulk erosion varies depending on the chemical structure and composition of the polymer backbone. The erosion mechanism generally depends on the rate of water permeation and consequently on the diffusion of degradation products [159-161]. This is often considered to be a desirable mechanism in drug delivery because the kinetics of erosion, and hence the rate of drug release, is highly reproducible as well as predictable and moreover in this method the drug substance is not exposed to an acidic environment generated by hydrolytic cleavage products, e.g., from polyesters. In an ideal surface erosion process, the erosion rate is directly proportional to
external surface area. Surface erosion can lead to zero-order drug release as long as diffusional release is limited and the overall shape remains constant [162, 163].

Surface-eroding devices usually degrade by enzymatic or hydrolytic cleavage starting from the surface. In an ideal case, the interior of the device contains unchanged polymer in the dry state. Bulk erosion occurs when water molecules are able to permeate into the bulk of the polymer matrix at a faster rate than erosion [161, 163]. As a consequence, polymer molecules in the bulk may be hydrolyzed and the kinetics of polymer degradation/erosion is more complex than surface eroding polymers. Bulk-eroding devices degrade from the ‘inside’ and hence water uptake precedes degradation of the polymer mostly by hydrolytic cleavage reactions. Consequently, the molecular weight of the polymer decreases until the cleavage products become water soluble. The interior of the devices retains drug and cleavage products in an aqueous milieu [164].

The majority of biodegradable polymers used in controlled drug delivery undergo bulk erosion, including the very important materials, poly (esters). Controlled-release systems improve efficacy of drug by enhancing the therapeutic activity while diminishing the intensity of side effects and frequency of drug administration required during treatment [161, 164]. Besides biodegradability, as the material for a drug delivery device, a polymer should have satisfactory biocompatibility and bioabsorption, easily controllable drug release, satisfactory mechanical properties, easy processing, acceptable shelf life and ease of sterilization. A variety of biodegradable polymers have been synthesized for the controlled release of different drugs. The selection and design of a suitable biodegradable polymer is the foremost demanding step for the development of a parenteral drug delivery system [163, 165].

2.6.1 Natural and Modified natural Polymers

Biologically degradable polymers include natural, modified natural and synthetic polymers. The natural polymers were used from ancient ages of time to deliver drugs either for a tailored release or for targeted delivery to the specific location. Natural polymers remain attractive primarily because they are natural products of living organisms, readily available, relatively inexpensive capable of multitude of chemical modifications [165, 166]. A majority of research investigations with reference to natural polymers as matrices in drug delivery systems have focused on two potential components; proteins (e.g. collagen, gelatin and albumin) and polysaccharides (e.g. starch, dextran,
inulin, cellulose and hyaluronic acid). Collagen, by virtue of its unique structural properties has been fabricated into wide variety of forms including crosslinked films, meshes, fibers and sponges [164, 167]. It’s phenomenal properties as a biomaterial offers several advantages: biocompatibility and non toxicity in most tissues; it has well documented structural, physical, chemical and immunological properties; it could be processed into a variety of forms and last but significantly it has the property to be readily isolated and purified in large quantities as well [165, 166]. Although certain properties of collagen including variability in drug release kinetics, tissue irritation due to residual cross linking agents, chances of occurrence of antigenic response activity, poor dimensional stability due to swelling \textit{in vivo} and low elasticity have adversely influenced its use as a drug delivery carrier [163, 166, 167].

However non-collagenous proteins including gelatin, albumin and casein were profusely used and exploited as drug delivery devices to develop various nano and microparticulate systems. Microspheres developed from albumin have been extensively used in diagnostic nuclear medicine for the evaluation of organ function and circulatory studies with various routes of administration [165, 168]. The significant features of albumin include its readily availability, lack of toxicity and non-antigenicity and moreover its biodegradation into natural products. Similarly gelatin also provides several advantages in drug delivery systems: weaker binding to drugs, less potential for drug degradation and lower antigenicity [169].

Natural polymers, particularly in the form of microspheres, have an important role in the controlled release of drugs and their targeting to selective sites. But before the widespread use of natural polymers several issues have to be sorted out [170], including greater understanding of drug-polymer interactions and their effect on shelf-life stability, better understanding of the kinetics of drug release, additional animal studies to determine local tissue response, more effective ways to control burst phenomenon, biodegradation rates and metabolic fate and most importantly well designed clinical studies to assess the therapeutic efficacy in accordance to current therapeutic regimens [167-170].

Discussing the modified natural polymers, it was revealed that they are natural polymers that are altered in order to suit a particular application. The reason for modification is that these polymers often take longer time to degrade within the body [168, 170]. By adding polar functionalities to the polymers, the problem could be overcome since the polar groups are more flexible and can therefore promote the
degradation of the polymers. The addition of functional groups may change the physical and chemical properties of the polymers. In the process of modifications of natural polymers, the nature and extent of modification should be considered [170, 171]. If a polymer is modified in excess, the natural polymer may not degrade easily. In addition, the added functional groups may be converted to toxic degradation products [169, 172].

2.6.2 Synthetic Polymers

As per the previous discussion a lot many issues have been raised including the difficulties in purification, scale-up or commercial manufacturing which have limited the use of natural biodegradable polymers. While concurrently the synthetic biodegradable polymers have proved its potential with wide range, applicability, availability and cost effectiveness [170-173]. Over the past few decades, synthetic polymers have been actively studied for use in drug delivery systems. Several classes of synthetic polymers have been proposed, which include poly-(ester)s, poly-(anhydride)s, poly-(carbonate)s, poly-(amino-acid)s, poly-(amide)s, poly-(urethane)s, poly-(ortho-ester)s, poly-(imino-carbonate)s, and poly-(phosphazene)s [171, 172, 174].

2.6.2.1 Poly (ester)s

Aliphatic poly (ester)s are the most extensively investigated class of polymers pertaining to toxicological and clinical data comprise consisting of lactic and glycolic acid. [175]. The high molecular weight polymers of glycolic and lactic acid are produced by ring-opening polymerization of their cyclic dimers in the presence of catalysts such as stannous octoate. Lactic acid comprises of an asymmetric α-carbon which is typically described as D or L form and thus three different plausible polymers include poly (L-lactic acid), poly (D-lactic acid), and poly (DL-lactic acid). Thus far, poly (L-lactide) and poly (DL-lactide) have received the most attention among these poly (lactide)s (PLAs) [172-175].

The diverse properties of the polymers including mechanical, thermal, and biological are markedly influenced by their stereochemistry. Poly (lactide-co-glycolide) (PLGA) embodies the “gold standard” of biodegradable polymers. The properties of these copolymers can be tailored by varying the ratio of PLA and poly (glycolide) [173-176]. The mechanism of degradation in poly (ester)s is classified as bulk degradation with random hydrolytic cleavage of the ester bond linkages in the polymer backbone. The
accumulation of acidic degradation products in the polymeric matrix as a result of reduced diffusion especially in larger devices eventually leads to an autocatalytic effect on degradation of device [172, 175, 176]. Chemical structures of various homopolymers and copolymers are as follows:

\[
\begin{align*}
\text{Poly (L-lactide)} & \quad \text{Poly (d, l-lactide)} \\
\text{Polyglycolide} & \quad \text{Poly (lactide-co-glycolide)}
\end{align*}
\]

Figure 6: Various biodegradable polyesters.

(A) PGA, PLA and PLGA

Currently most frequently used biodegradable polymers for drug delivery include poly (glycolic acid) (PGA), poly (lactic acid) (PLA) and poly (lactide-co-glycolide) (PLGA), which is typically attributable to their long history of use as medical sutures [175, 177]. PGA is the simplest linear aliphatic polyester which is usually synthesized from the dimer of glycolic acid i.e. glycolide. The first totally synthetic absorbable suture was developed by PGA. It is highly crystalline (45-55%) with a high melting point (220-225°C) and a glass transition temperature of 35-40 °C [171, 173, 176]. Owing to its high degree of crystallization, PGA is insoluble in most organic solvents. Fibers made from PGA exhibit high strength and modulus and these being extremely stiff, thus can’t be used as sutures. Glycolide has been copolymerized with lactide, the dimmer of lactic acid, to reduce the stiffness of the resulting fibers [178].

PLA is normally synthesized from lactide monomers. Lactide exists as two optical isomers, d and l. L-lactide is the naturally occurring isomer, and dl-lactide is the synthetic blend of d-lactide and l-lactide. Poly (l-lactide) is about 37% crystalline with a melting point of 175-178 °C and a glass transition temperature of 60-65 °C. The degradation of
poly (l-lactide) is very slow, requiring more than 2 years to be completely absorbed [175, 176, 178]. It exhibits high tensile strength and low elongation and consequently has a high modulus that makes it more suitable for load-bearing applications such as sutures.

Poly (dl-lactide) is an amorphous polymer exhibiting a random distribution of both isomeric forms of lactic acid, and accordingly is unable to arrange into an organized crystalline structure. The versatile properties of this material such as lower tensile strength, higher elongation, and a much more rapid degradation time, make it more suitable as a drug delivery system [177-179].

Using the properties of PGA and PLA as a starting point, it is possible to copolymerize the two monomers to extend the range of the homopolymer properties. PLGA has been developed for both sutures and drug delivery applications. It is imperative that the copolymer composition and the mechanical and degradation properties of the materials do not bear a linear relationship between them. For instance, a copolymer of 50% glycolide and 50% dl-lactide degrades faster than either homopolymer [174-179].

Figure 7: Chemical structure and synthesis of Poly (lactic acid), Poly (glycolic acid), and Poly (lactic-co-glycolic acid). [175]
(B) PLA and PEG Copolymers

Although the PLA and PLGA are now commonly used, but still research is going on for designing and synthesizing new polymers for the application of drug delivery. One very promising strategy is to copolymerize PLA and poly (ethylene glycol) (PEG). PEG has been known as an excellent biomaterial due to its biocompatibility, hydrophilicity and flexibility [177-180]. It is also referred as poly (ethylene oxide) (PEO) at high molecular weight. Copolymerization of hydrophobic PLA and hydrophilic PEG can provide a balance between the two opposite parts. Furthermore, different supramolecular structures can be achieved by different monomer combinations and preparation processes to meet various medical requirements [173, 176, 179]. The PEG chains minimize non-specific fouling of the device surface with bio-components such as proteins. The uptake of nanoparticles by the reticuloendothelial system (RES) can be reduced. Di-block PLA-PEG copolymer can also form micelles in aqueous environment with PEG on the surface.

In contrast to surfactant micelles, these polymeric micelles are more stable, have a lowered critical micellar concentration, and have a slower rate of dissociation, thus permitting the retention of loaded drugs for a longer period of time and, eventually, achieving higher accumulation of a drug at the target site. Furthermore, they have a size range of several tens of nanometers with a considerably narrow distribution, which is crucial in determining their body disposition [178, 181]. A family of star-block copolymers from multi-arm PEO and l-lactide or l-lactide/glycolide has been recently reported. In vitro degradation test results on these polymers show that the biodegradation consists of an initial slow-rate period in the first 2-3 weeks, which makes them an excellent drug carrier, and an exhaustive degradation period, which provides the way for renal excretion [182].

Thermo-sensitive hydrogels have been prepared from either PLA-PEO di-block or PEO-PLA-PEO tri-block polymers. The hydrogel can be loaded with bioactive molecules in an aqueous phase at an elevated temperature (around 45 °C), where a sol is formed [173-178]. The polymer is injectable as well when used in this form. Upon subcutaneous injection and ensuing rapid cooling to body temperature at 37 °C, the polymer form a gel that can act as a sustained-release matrix for drugs. The gel-sol transition temperature can be well controlled by the molecular weight of PLA segment. Both high molecular weight proteins and low molecular weight hydrophobic drugs can be loaded and released [177-183].
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The release rate is controllable by the initial drug loading and the polymer concentration. In one investigations Biotin has been conjugated to the PLA-PEG copolymer to form a new polymer PLA-PEG-biotin. In this new polymer, the PLA component provides structural integrity to the fabricated devices [181-184]. The PEG block acts as a hydrophilic coating to avoid the uptake of RES. The third part of the polymer, i.e. biotin moiety, allows facile surface engineering using aqueous solution of avidin. Avidin posses a tetrameric structure with four binding sites for biotin [175, 179, 183]. One of these sites of avidin is utilized for the binding of biotin, while biotinylated ligand motifs get bound to other available free binding sites, which in turn be used for targeting to tumor cells.

2.6.2.2 Poly (ethylene glycol) block Copolymers

Poly (ethylene glycol) entails excellent and outstanding biocompatibility which can be ascribed to its hydrophilic nature. This attribute leads to formation of hydrogen bonds between water and the polymer chains, and also inhibits protein adsorption [185-187]. Consequently, the presence of poly (ethylene glycol) chains at the surface of a parenteral device prolongs the biological events such as endocytosis or phagocytosis which in turn increases blood circulation times. One of the emerging uses for inclusion of PEG in a controlled release system arises from its interaction with protein [188-190]. It has been established that the conjugation of proteins with PEG provide prolonged protein circulation life, reduced immunogenicity and antigenicity as well.

PEG chains at the surface provide the incorporated substance longer circulation time in the body by prolonging biological events such as endocytosis, phagocytosis, liver uptake and clearance, and other adsorptive processes. PEG can be tailored with a range of terminal functionalities, which lead to its easy incorporation into copolymer systems [184, 188, 190]. Further, the synthetic modification of PEG is facilitated by the presence of chain-end hydroxyl groups. Besides, the block copolymers of poly (ethylene glycol) and PLA or PLGA have been synthesized for the encapsulation of various APIs. Di-block PLA-PEG and tri-block PLA-PEG-PLA systems have been synthesized and characterized with various PLA contents [183, 184, 191]. A thermo-sensitive PLA-PEO hydrogel has been evolved that exhibit temperature dependent gel-sol transition for use as injectable drug delivery systems [187-191].
2.6.2.3 Poly (anhydride)s

Although, Poly (anhydride)s contains water-sensitive bonds, yet it’s relatively more hydrophobic than the poly (ester)s which eventually leads to reduced water permeation into the polymer bulk. Poly (anhydride)s predominately undergoes surface erosion by cleavage of the anhydride bonds at the surface of the device [177-189]. The widely studied poly (anhydride)s is based on sebacic acid, p-(carboxyphenoxy) propane, and p-(carboxyphenoxy) hexane. In recent trends and implication of the polymeric delivery, interstitial administration of an antitumor agent using a loaded polymeric disc composed of poly (carboxyphenoxypropane-sebacic acid) has been successfully performed [188, 191].

2.6.3 Properties of Biopolymers

In current research trends the biodegradable polymers have its own place and added advantage due to its very specific phenomenal properties. The lactide and glycolide homo and copolymers have potentially exhibited wide versatility and applicability in controlled drug delivery [159-167]. A broad spectrum of these polymers in accordance to performance characteristics can be obtained by precise and careful combination of certain variables which are as follows-

- Molecular weight of polymer
- Linearity of polymer chain
- Ratios of co-polymer
- Stereochemistry of the monomer

2.6.3.1 Molecular weight of Polymer

Various classes of biopolymers including polyesters are commonly available in a wide range of molecular weights, commercially. The stated property affects biodegradation and resultant release profile and ultimately affects the performance characteristics of the delivery system [163-167]. Higher molecular weight leads to increment in polymeric viscosity of the solutions and hence affects not only the microsphere size and sphericity but also the entrapment efficiency. Biological properties of these aliphatic polyesters have been studied by various teams of researchers who conclude about the biocompatibility and histocompatibility of these polymers [165-169].
A group of researchers have disclosed that PLA was found to be suitable for surgical sutures and vascular grafts because it elicited no immunological response due to the absence of peptide chain and the biodegradable nature of the polymer. PLA and PLGA are degraded to lactic acid and glycolic acid which are ultimately excreted as CO$_2$ and H$_2$O from the body [163, 173-178]; exhibiting no toxicity. Histopathological studies have shown mild inflammatory reaction after the administration of lypressin-loaded polymeric microcapsules. However the exact cause of the reaction is not known as it may be due to irritation, or actual chemical reaction of polymeric components with body [173-177].

2.6.3.2 Linearity of Polymer chain

Polymer chain linearity plays a key role in determination of hydrophilicity/hydrophobicity of the polymeric component. The outcome of the chain linearity affects the hydrophilicity of the polymer, which ultimately affects the degradation rate leading to tailor the release profile of the API [161-167]; entrapped within the finished dosage form developed from the same polymer. The major associated phenomenon of chain linearity i.e. the extent of block or random structure present in the copolymer also affects the rate of hydration. The rate of hydration will exhibit a governing role over degradation pattern of the finished polymer block which will significantly alter the time frame of controlling the release rate of entangled candidate drug substance [162, 164, 166].

2.6.3.3 Ratios of Co-polymer

Factors affecting the biodegradation pattern as well as profile of various polymers depend over different co-polymer ratios, leading to different crystallinities, glass transition temperature (Tg) and hydrophilicity. The crystallinity of the co-polymers of lactide and glycolide depend on the molar ratio of two monomer components. PLGA containing < 70% glycolide content are amorphous [171-179]. PLA, because of an additional methyl group is more hydrophobic than other glycolide polymer, which leads to lower water uptake and hence results in slower degradation patterns. Generally, the copolymers having the 50:50 ratios degrade quicker than not only the homopolymers but also from other ratios proving its least stable tendency over any other ratio or the homopolymer [188-192]. Different polymer forms exhibits varying range of
crystallinities, different hydrophilic behavior and solubility profiles [174]. These parameters ultimately affect the biodegradation and release profiles. Crystallinity and water uptake are considered as the key factors in determining the rates of in vivo degradation. The water uptake increases as glycolide ratio in copolymer increases [169-173].

2.6.3.4 Stereochemistry of the Monomer

The crystallinity of the co-polymers not only depends on the molar ratio of two monomer components but also relies on stereochemistry of the monomer. The racemic poly (DL-lactide) is less crystalline and thus has lower melting point than the stereo-regular forms of the same, i.e., D-PLA and L-PLA [184-192]. Researchers have revealed that crystalline domains and stereo-irregularity inhibit the degradation phenomenon of the polymer. Stereoregular or racemic lactides and co-polymers with < 50% glycolides are soluble in organic solvents as halogenated hydrocarbons, tetrahydrofuran (THF), dioxane etc. whereas glycolide rich polymers are soluble in exotic solvents like hexafluoroisopropanol [183-187].

2.6.4 Degradation of Biopolymers

Biodegradable polymers may degrade through various means including enzymatic degradation, hydrolytic degradation, and microbial degradation. It is generally now admitted that in the case of aliphatic polyesters such as PLA, PGA and their copolymers, enzyme involvement is unlikely at the early stages of degradation in vivo or under outdoor conditions [159-166]. However, enzymes contribute at the later stages, especially when soluble by-products are released. In contrast, for rubbery polymers like cross-linked PCL, enzymes seem to be active from the very beginning via surface erosion phenomena. Hydrolytic degradation of aliphatic polyesters involves four main phenomena, namely water absorption, ester cleavage, diffusion of soluble oligomers and solubilization of fragments [163-174]. Some microorganisms use lactic acids, PLA oligomers and polymers, and PLGA copolymers as sole carbon and energy sources under controlled or natural conditions.

Aliphatic poly esters comprising of lactide/glycolide polymer chains undergo biodegradation through bulk erosion which are cleaved by hydrolysis to the monomeric acids and ultimately get eliminated from body through Krebs cycle, chiefly as carbon


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dioxide and in urine [167]. Since the hydrolysis rate is dependent only on significant changes in temperature and pH or presence of catalysts so eventually petty divergence is discernible in the rate of degradation at different body sites [169]. The role of enzyme in the biodegradation of the polymers has been still ambiguous. Hitherto reports nullified the involvement of enzymes during the bioerosion of lactide/glycolide polymers occurring by means of hydrolysis, yet recent investigation has suggested that the enzymes do play a considerable role in the breakdown of these polymers in body. However much of these speculation are based on the difference observed between in vitro and in vivo disintegration rates rather than direct study. [161-166].

**Table 3: Biodegradation time of lactide/glycolide polymers.**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Degradation time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly (l-lactide)</td>
<td>18 - 24</td>
</tr>
<tr>
<td>Poly (dl-lactide)</td>
<td>12 - 16</td>
</tr>
<tr>
<td>Poly (glycolide)</td>
<td>2 - 4</td>
</tr>
<tr>
<td>Poly (dl-lactide-co-glycolide) (50:50)</td>
<td>2</td>
</tr>
<tr>
<td>Poly (dl-lactide-co-glycolide) (65:35)</td>
<td>3 - 4</td>
</tr>
<tr>
<td>Poly (dl-lactide-co-glycolide) (85:15)</td>
<td>5</td>
</tr>
<tr>
<td>Poly (dl-lactide-co-glycolide) (90:10)</td>
<td>2</td>
</tr>
</tbody>
</table>

Lactide glycolide polymers show wide range of hydrophilicity which makes them versatile in designing controlled release system. It has been demonstrated by various researchers that the efficiency of water up take increase as the glycolide ratio in the co-polymer increases [188-193]. Table 3 clearly indicated the effect in degradation tenure varying with the copolymer ratio of lactic and glycolic acid. In current trends and implications, synthesis of lactide/glycolide polymers branched with different polyols polyvinyl alcohol and dextran acetate with significant change in the degradation profile of these polymers from that of linear polylactides were also reported [179, 181-194].

**2.6.5 Selection criteria for Biodegradable Polymers**

Formerly, the researchers usually used hydrophobic polylactic acid (PLA) for drug microencapsulation with biodegradable polymers. However, for the last two
decades, poly (lactic-co-glycolic) acid (PLGA) has been frequently employed in the area of research and commercialization. [189-193]. The advantage of PLA resides in its tendency to degrade slowly and release of drug in a controlled manner over days to months (e.g., Trenantone®), Comparatively the rate of biodegradation of PLGA is faster than PLA and provides the tailored release of the drug till a month or two (Enantone®) which was frequently required for ancient long acting parenteral formulations of routinely administered drugs [188-193].

The criteria of selecting a biodegradable polymer for effective therapy could be made easier by optimizing following steps: best route of administration and precise amount of microspheres equivalent to the dose of the drug [191-194]; expected release rate for therapeutic efficacy; feasibility of administration of total required dose to maintain therapeutic drug blood level for desired frame of time. Nevertheless, the release of the drug must be faster that rate of degradation of the used polymer. These specific parameters are valuable for selecting a suitable biodegradable polymer for developing controlled release parenteral systems [189-195].

2.6.5.1 Polymer degradation Behavior

PLGA is the most studied biodegradable polymer for development of microspheres. The water holding capacity, rate of biodegradation, ratio of manufacturing components and crystallinity of the polymer acts as major responsible factor for optimum behavior of the developed formulation [161-169]. Particle size distribution, pH and temperature of the medium significantly affect the degradation of the polymer. Moreover the most frequent and prominent use of PLGA is always supported by its USFDA approval for human benefits [185, 188, 196]. PLGA (50:50) generally provides prolonged release up to 4-6 weeks which has the flexibility to shorten the release duration as per the requirement of the therapy by altering the ratios of the components of the polymer [174-177]. Water holding capacity and degradability of the polymer depends upon the type of end group i.e. free end groups have much more swelling tendency than the capped one [147, 148].

The *in-vivo* degradation of the polymer was always found to be faster in comparison to *in-vitro* release behavior. This could be attributed to the effect of biological substances of the body which are supposed to trigger the degradation process *in-vivo* [186-193]. Acidic byproducts of the degraded polymer may also catalyze the degradation
process. It has been inferred by various means including spectroscopy, confocal microscopy, and chromatographic analysis that acidic degradation products of the polymer remain inside the polymer [191-197]. The acidic climate present inside the microspheres provides the stability of some hydrophobic drugs below pH 4.0, whereas it is considered to be a drawback for the stability of drugs sensitive to hydrolysis [194-197].

2.6.5.2 Polymer mixtures and Alternative PLGA co-polymers

In some instances it was observed that PLGA (50:50) and copolymers holds the hydrophobic drug tightly prolonging the release. A faster release for the hydrophobic anticancer drug was discerned due to higher porosity eventually resulted through the use of a block polymer instead of PLGA (50:50) [189-193]. PLGA in combination with glucose as initiator leads the market as Sandostatin LAR\textsuperscript{®} Depot. Continuous release of peptides/hydrophobic drugs was attained through the mixture of high and low molecular weight polymers in various ratios [193-197]. The incessant drug release through the mentioned processes decreased the lag period of polymer loss and release. The situation in general arises after significant burst release of the surface bound drug as well as in case of limited diffusion controlled release from dense polymeric matrix [198]. The importance of mixed or blended polymers could be justified by the fact that PLGA with less molecular weight has the tendency of faster biodegradation leading to generation of more number of pores and channels to allow quicker drug release [194-198]. The simultaneous availability of hydrophilic carboxyl group generated through biodegradation process provides an increment in water uptake capacity of the polymer [198, 199, 200].

2.6.5.3 Polymer properties Influencing Drug release

Drug release from a polymeric matrix is an important phenomenon in selecting the polymer for the development of polymeric microparticles as it will significantly affect the criteria of selection of polymer, as per the need of the delivery system. Drug release could be potentially affected by various factors including drug nature, polymer properties and morphology of the matrix [177-183]. Two important polymer properties that influence drug release are a) Molecular weight and molecular weight distribution. It reflects the size and size distribution of a polymer. The drug diffusion rate decreases with increasing molecular weight, the critical factor that governs drug diffusion is determined by the overall microstructure of the polymer in the presence of diffusing and other foreign
species. Glass transition temperature can be modified by changing the strength of the secondary forces within the polymer [182-187]. This can be accomplished by introducing an additive, such as a plasticizer, to the polymer. However it could also be modified through formation of copolymer.

Drug transportation is modeled by migration of the penetrant through holes or free volume within the polymer. Therefore, the rate of transport is based on the probability of creating a hole of sufficient size to accommodate the penetrant, and the probability for this penetrant to have sufficient energy to enter this hole. At temperatures significantly higher than the Tg, both probabilities must be taken into account to estimate diffusivity [196, 199]. However, near the Tg, the amount of free volume is small, so the probability of encountering holes of sufficient size dominates mass transfer. In most cases, when the temperature is lower than the glass transition temperature, the amount of free volume is small and the redistribution of holes within the polymer is negligible because segmental motion is virtually nonexistent.

2.6.5.4 Effect of Drug properties and Preparation process on Polymer characteristics

The method of preparation and the interaction between drug and the biodegradable polymer in some instances acts as key factor affecting the polymer properties and the developed microspheres [188]. Sometimes resulting in faster drug release due to accelerated polymer degradation e.g., thioridazine, an amine-catalyzed hydrolysis of the polymeric matrix during the manufacturing process with faster release was inferred. The phenomenon could be minimized through o/w emulsification at minimum temperatures or by preparation of a salt leading to minimum nucleophilic effect of the drug [181-183].

Contrary to above, the microparticles comprising of a water-miscible, N-acetyl cysteine showed an enhanced catalytic degradation of PLGA (50:50) which could be dependent on plasticization of the polymer [193-197]. Moreover it was also reported that the emulsification supported with ultrasonication also led to faster biodegradation due to decrease in molecular weight of the polymer [189-195].

Sterilization through radiation of estradiol-loaded microparticles resulted in decreased molecular weight of the polymer leading to quicker release of drug and presence of drug degradation products. However a slower release could be observed in case of hydrophobic drugs developing ionic bonds with carboxyl group of polymer or
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interact through the hydrophobic bonds with the polymeric matrix [196-199]. In some cases enhanced solubility of the drug could be observed with end-group capped polymers, higher lactide content, and lower molecular weight of the end capped polymer [181-185].

In another case of ketoprofen, hydrogen bonding was observed on dissolving in 20% PLGA which acts as plasticizer leading to augmentation of ketoprofen via minimized chain-chain interaction of the polymer. For instance, the release of progesterone from PLA vis-a-vis 85/15 PLGA microparticles was found to be faster rather than slower which can be attributed to a rough surface with highly porous PLA particles. The unique appearance of the microparticle could be attributed to faster precipitation of PLA leading to inability of microspheres to shrink and make the common smooth surface [197-200].

Selection of polymer for the development of controlled release biodegradable microparticles depends on the factors such as biocompatibility and toxicity, degradation rate and time, degradation products, biocompatibility as well as toxicity of the degradation products, chemical, physical, and mechanical properties, processing parameters and requirements, compatibility of the drug with the polymer, required sterilization methods, glass transition temperatures and cost-effectiveness of the biopolymer [197-204].

2.6.6 Relevant Properties of Drugs for Microencapsulation and Release

Oral administration due to its simplicity and painless characteristics is the widely acceptable route most of administration of dosage forms, moreover the medication could be adjusted or terminated at any stage of the therapy. However it has some limitation of food effect leading to variable bioavailability of the drug [147, 148]. Taking into consideration, new molecules should be evaluated for various physicochemical parameters including solubility, polymorphism, pKa, lipophilicity, permeability, and stability [176-179]. Some specific properties of the drug should be considered as predictors for oral bioavailability including logarithmic octanol–water partition coefficient (should be more than 5), Hydrophobicity/lipophilicity of the molecule, particle size of the drug used for formulation development, solubility and dose of the drug [201, 203-206]. Factors affecting bioavailability should include the pharmacokinetics of the drug, i.e., its absorption, distribution, metabolism, and excretion (ADME) other than physicochemical properties. The absorption of hydrophobic drugs is highly dependent on
dissolution from the oral formulation, retention time in the intestine, drug solubility and permeability [40-46].

The drugs having low dose and good oral bioavailability are the most suitable candidate for developing the controlled release parenteral microspheres. The other requirements for injectable microspheres include a constant plasma concentration, prioritized local delivery and longer duration of therapy as in case of certain psychotic disorders, drug addiction or cardiovascular disorders.[47-55].

2.6.6.1 Solubility of drug in Aqueous and Organic media

The hydrophobic drug candidate exhibits poor solubility in water and may/may not have the solubility in various organic solvents. There exists various categories on basis of the solubility of the drug i.e. slightly soluble (1-10 mg/ml), very slightly soluble (0.1-1 mg/ml), and practically insoluble (<0.1 mg/ml) [47]. Steroidal drugs belong to class of poorly water soluble drugs; however, their aqueous solubility varies over at least two orders of magnitudes. Further, even lower aqueous solubility usually in range of a few ng/ml is also exhibited by some of the hydrophobic drugs. High-throughput screening of the molecule and prediction of solubility plays a major role in drug discovery because undesirable pharmacokinetic properties usually accompany the inadequate solubility [47].

The determination of drug solubility in aqueous media is imperative in the initial phase of every microencapsulation study since the aqueous phase is involved in the emulsion based manufacturing process of microspheres [199-201]. The process involves polyvinyl alcohol (PVA) as the stabilizer. The pH dependent solubility of the drug should be scrutinized carefully in case of ionizable drugs. This could be carried out through micro solubility methods addressing the limited availability of drug. In cases dealing with highly water soluble drugs, the pH dependent solubility could be used as a major tool for enhancement of encapsulation efficiency of the drug into the polymeric matrix. [201-205]. Moreover, the solubility studies should be carried out in presence of certain specific excipients including Tween® 20 or Tween® 80 non-ionic surfactants, as they are frequently used in release media. The selection of organic solvents on basis of drug solubility rationally provides an insight for determination of suitable microencapsulation techniques [206].

The octanol-water partition coefficient $K_p$ is indicator of lipophilic/hydrophilic nature for new molecules which is either calculated or determined experimentally.
behavior of drug in biological systems and its relative distribution in two-phase solvent systems can be predicted by $K_p$. The effect of dissolved organic solvent on drug solubility in the external phase during emulsification process will be prominent due to excess solubility of solvents in water rather octanol (0.03% at 20°C)[148].

2.6.6.2 Drug Stability

The organic phase emulsification is the most commonly used microencapsulation methods wherein the drug gets dissolved in presence of suitable solvent [147, 148]. The stress-tests conducted over the drug in relevant solvents at different temperatures i.e. from room temperature to accelerated storage conditions could provide the information regarding the temperature induced degradation. For the new molecules stability studies are mandatory to address the probability of degradation of drug in various conditions including acidic, basic conditions, oxidation, humidity-related, thermal, and photodegradation [200-204]. The manufacturing through emulsification involving ultrasonication may lead to drug degradation, particularly in case of drug candidates having hydrolyzable bonds such as esters [188, 189]. Under the release conditions, the hydrolyzable bonds in the drug molecule may be adversely affected in an acidic microclimate arising due to the accumulation of PLGA degradation products inside the microparticles. PLGA degradation products may cause acylation of the amine groups in the drug, especially primary amines, well recognized for peptides. Upon storage of lomustine loaded PLA microparticles, the antineoplastic drug amenable to hydrolytic degradation pathways was destroyed due to the interaction with PLA [178, 182, 201].

2.7 Microspheres

Microspheres are defined as solid; approximately spherical particles ranging in size from 1 to 1000 mm [207, 208]. These are usually made of polymeric or other protective materials. Active pharmaceutical agents (APIs) and other substances may be integrated within microspheres either as an encapsulated core (microcapsules) or homogeneously dispersed throughout the microspheres [147, 148]. Microspheres have been a widely exploited approach of parenteral delivery system for drugs and other tissue response modifiers (TRMs). TRMs encompass traditional small molecule drugs, enzymes, proteins, DNA, vaccines, and cells. Parenteral microsphere delivery offers numerous biopharmaceutical and physicochemical advantages [43, 149].
Microsphere preparation may augment the chemical stability of the TRMs, extend its residence time at the site of administration, result in physical targeting to the inaccessible compartments of the body, protect the TRM from biological degradation, result in controlled delivery, protect the in vivo environment from the TRM (e.g., immune response to protein therapeutics), [148] and enhance safety (subdivision of the dose can avoid dose dumping problems that may occur on failure of a single unit implant). Besides, microspheres may act as an immune adjuvant when used for vaccine delivery. Microspheres have also proved its efficacy in reducing the dosing frequency when a controlled release product is administered [20-22].

These biodegradable polymers may release the active compound by either diffusion or erosion of the matrix or both. Polymers for injectable microspheres must meet the requirements of tissue compatibility, biodegradation kinetics, drug compatibility, drug permeability, mechanical properties and ease of processing [149, 155]. This method of delivery is vital for those APIs for which no other reasonable dosing technique is offered, e.g., proteins that are rapidly degraded and cleared when administered parenterally. Moreover, several investigations wherein, the plausibility of microspheres to serve the purpose of localized therapy and controlled release for classes of small molecular weight drugs have been reported. [21, 22].

2.7.1 Small Molecular weight Drugs

Parenteral delivery of small molecular weight drugs viz narcotic antagonists, antibiotics, local anesthetics, anti-malarial, anti-cancer drugs and steroids have been investigated in microsphere systems [148]. Furthermore, the local delivery of antibiotics can assure adequate tissue levels at the local site of infection. For example, localized delivery of gentamicin in microsphere systems has been employed successfully in animal models for acute and chronic bone infections [209, 210]. This approach is also used to prevent bone infections that could arise following surgery. Predominantly, some local sites like isolated tissues, such as the inner ear and cancerous tissue are problematic in terms of attaining adequate tissue levels. Localized delivery of steroids to arthritic joints have been accomplished by controlled release microspheres [40-44]. In sheer contrast to drug solutions or suspensions, these delivery systems allow therapeutic concentrations to be maintained at the local site for an extended period of time [51, 53].
Consequently, medication frequency can be diminished which is imperative for patient compliance as intra-articular injections are predominantly painful. Furthermore, the localized delivery of microsphere formulations can also be used to circumvent the side effects often associated with systemic delivery [149, 155]. For instance, systemic administration of elevated levels of antibiotics may lead to organ toxicity, such as kidney damage. Corticosteroids and anti-cancer drugs are two other examples of drug classes with high systemic toxicity. Likewise, two other examples of drug classes with high systemic toxicity include corticosteroids and anti-cancer drugs [211, 212]. The systemic delivery of anti-cancer drugs may cause toxicity to all rapidly dividing cells. Consequently, targeted microsphere delivery systems have been applied to anti-cancer drug delivery to reduce systemic side effects and ensure adequate drug levels in the tumor.

2.7.2 Protein Therapeutics

Over the past two decades, the therapeutic biotechnological products (proteins, peptides, and DNA) have been explored as candidate compounds for delivery in microsphere systems for parenteral delivery. For instance, luteinizing hormone releasing hormone (LHRH) analogs have been investigated and there is currently one microsphere product in the market containing Leuprolide acetate (Leupron Depot®) [148]. Certain successful microsphere based products are available in the market as well (Lupron Depot®, Sandostatin LAR®, Nutropin Depot®, and Trelstar Depot®). Due to low pH and high concentrations of peptidases in gastrointestinal tract (GIT), this biotechnology therapeutics is susceptible to degradation and hence these products are administered parenterally [213].

Localized delivery of these agents is desirable as they are expensive and may give rise to side effects at other sites. Parenteral delivery may also be problematic since these pharmacological agents need to be protected from the environment (e.g., peptidases present at the local delivery site). Consequently, the in vivo half-lives of this therapeutics are usually very short [40, 43]. An additional problem is that immune responses may occur. Thus, currently, delivery systems, such as microspheres, are under investigation to overcome such problems. Microspheres offer an advantage over other dispersed systems (e.g., liposomes and emulsions) in that the former are more stable, can carry higher drug loadings and can result in extended release profiles [52-56]. Extended release is
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advantageous in reducing dosing frequency, as otherwise daily injections are often required for chronic therapy.

For chronic therapy release rates in the order of months can be achieved when relatively hydrophobic polymers such as poly-lactic acid (PLA) and poly-lactic-co-glycolic acid (PLGA) are used [158]. In a research investigation, it was observed that Vascular endothelial growth factor (VEGF) delivered in PLGA microspheres has resulted in new blood vessel growth at the subcutaneous (s. c.) injection site in rats over the one month time frame of study period [40, 149]. This can be compared to no growth of new blood vessels on injection of unencapsulated VEGF. Like most proteins, VEGF has a short half-life in the body and is rapidly cleared from the injection site. The stated problem was resolved in accordance with the slow release of the VEGF encapsulated within biodegradable polymeric microsphere matrix [214-218].

2.7.3 Preparation of Microspheres

Various microencapsulation techniques with numerous advantages have been evolved till date. The selection as well as utilization of a particular technique is dependent on not only the nature of the polymer but also its intended use. The method of preparation has significant effect on the properties of the developed microparticles; hence the required properties should be kept in mind during the selection of specific method of preparation [216, 217]. For the formulation of particles from the biodegradable polymeric matrix it is essential to select an encapsulation process which fulfils the requirements of the ideal controlled release system.

There are certain requirements of the encapsulated polymer particles that should be fulfilled while selecting an optimized method for encapsulation process: 1) Encapsulated drug stability: The stability of the entrapped drug with its biological activity should be maintained during the process of particle formulation and the shelf life of the intended product. 2) Higher yield and entrapment efficiency [147, 148]. The process should also exhibit maximum encapsulation efficiency. The drug to polymer ratio should be optimized in accordance to entrap maximum amount of drug within minimal amount of polymer for reduction of the mass of microspheres to be administered. 3) Reproducibility and batch uniformity: The process should be robust, simple and scalable to be reproduced with same properties and desired release characteristics of the produced microparticles. 4) Tailored release profile with minimal burst effect: The method of
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Encapsulation should be such that by altering the formulation conditions, tailored release profiles of the encapsulated substances could be produced [147].

The selection of process and polymer should be in such a way that minimum of the encapsulated drug is released during the burst phase; which will ultimately support extending the release of the drug for prolonged frame of time. 5) Free flowing tendency and syringeability [207, 208] The process of encapsulation must produce free flowing particles with minimum tendency of aggregation for producing uniform syringeable suspension of the particles as syringeability through hypodermic needles is a major requirement of the developed microsphere system. 6) Residual content of solvent: Last but not the least residual solvent content used for the manufacturing process should be in limits specified by the recommendations of ICH and USP for getting approved by US FDA [147, 219].

2.7.4 Methods of Encapsulation

Numerous encapsulation processes finalized in accordance with the various properties of material to be entrapped, properties of the used polymer as well as the intended uses and desired site of action of developed microspheres to attain the best therapeutic utility.

2.7.4.1 Solvent Evaporation/Extraction Techniques

Solvent evaporation/extraction methods include single emulsion and double emulsion process [220]. Single emulsion is a good method for the fabrication of hydrophobic drugs as only trace of drug is lost during the fabrication, whereas double emulsion is generally designed for the encapsulation of moderately water soluble drugs, which may easily diffuse into the water in the single emulsion method [147].

(A) Single emulsion Techniques

In general, this process is an oil-in-water process. First, the polymer is dissolved in a water-immiscible, volatile organic solvent (the most commonly used one is dichloromethane [DCM]). Then the drug is added to the polymer solution to form either a solution or dispersion (the dispersed particles of the drug should be less than 20 micron) [219]. This mixture is then dripped into a large volume of aqueous solution of surfactant/stabilizer under stirring at a suitable temperature to yield an o/w emulsion. The
solvent such as DCM can be removed by evaporation and subsequently the polymer-drug particles can be hardened. The solvent is evaporated at a reduced stirring speed and/or reduced atmosphere pressure [220].

Finally, the hardened microparticles are obtained by filtration, washing or centrifugation. The free-flowing injectable microparticles can be obtained by lyophilizing the polymer-drug particles. The major problem with this technique is the poor encapsulation efficiency of moderately water-soluble and water-soluble compounds, which partitioned out from the organic dispersed phase into the aqueous continuous phase [219, 220]. Adjusting pH of the aqueous phase to suppress ionization could minimize diffusion into aqueous phase, which led to loss of water-soluble drugs. Drug partitioning between the organic dispersed phase and the aqueous continuous phase can also be reduced by prior saturation of the continuous phase with the same drug [221].

Therefore, lipid-soluble drugs like steroids are extensively encapsulated by the o/w emulsification process. An oil-in-oil (o/o) emulsification method can also be used to increase the encapsulation of the water soluble drugs [222]. Herein, a water-miscible organic solvent like acetonitrile is employed to solubilise the drug in which PLGA or PLA are also soluble. This solution is then dispersed into an oil phase such as light mineral oil in presence of an oil soluble surfactant like Span to yield the o/o emulsion. Microspheres are finally obtained by evaporation or extraction of the organic solvent from the dispersed oil droplets and the oil is washed off by solvents like n-hexane [147].

The formulation of the microspheres is affected by a number of factors. The key variants that influence the microencapsulation process and the final microsphere product are: (a) the nature and solubility of the drug being encapsulated, (b) the polymer concentration, composition, and molecular weight, (c) the drug/polymer ratio, (d) the organic solvent used (e) the concentration and nature of the emulsifier used, (f) the temperature and stirring/agitation speed of the emulsification process and (g) the viscosities and volume ratio of the dispersed and continuous phases [147, 222].

(B) Double emulsion Processes

The double emulsion process is essentially a water-in-oil-in-water (w/o/w) method and is suitable for encapsulating water-soluble drugs such as peptides, proteins, and vaccines. First the drug is dissolved in water and then the solution is added into the polymer solution with vigorous stirring to form the primary emulsion (w/o). This
emulsion is dripped and gently stirred into a large volume of surfactant water solution to form a secondary (w/o/w) emulsion [223]. Then the emulsion is subjected to solvent removal by evaporation process. The hardened particles are washed and collected by filtration, sieving, or centrifugation. Under suitable drying conditions, the final product is obtained. Some peptide, protein and conventional molecules have been encapsulated using this method [224, 225].

2.7.4.2 Coacervation (Phase separation)

Invariably, this process involves the addition of a third component to the polymer solution in an organic solution thereby reducing the solubility of the encapsulating polymer. At a specific point, the process yields two liquid phases (phase separation): the polymer containing coacervate phase and the supernatant phase depleted in polymer [223]. The drug which is dispersed or dissolved in the polymer solution is coated by the coacervate. Thus, the coacervation process includes the following three steps: (i) phase separation of the coating polymer solution, (ii) adsorption of the coacervate around the drug particles, and (iii) solidification of the microspheres. The polymer is first dissolved in an organic solution. The water-soluble drugs like peptides and proteins are dissolved in water and dispersed in the polymer solution (w/o emulsion) [225].

Hydrophobic drugs like steroids are either solubilized or dispersed in the polymer solution. An organic nonsolvent is then added to the polymer-drug-solvent system with stirring which gradually extracts the polymer solvent [158]. As a result the polymer is subjected to phase separation and it forms very soft coacervate droplets (size controlled by stirring) which entrap the drug. This system is then transferred to a large quantity of another organic nonsolvent to harden the micro droplets and form the final microspheres which are collected by washing, sieving, filtration, or centrifugation, and are finally dried (Fig. 8).
Figure 8: Schematic representation of microencapsulation through coacervation:
(A) Dispersed liquid or solid drug particles, (B) Induction of phase separation, (C) Deposition of microdroplets at the surface, and (D) Fusion into a membrane [223].

2.7.4.3 Spray-drying

The injectable biodegradable PLA and PLGA microparticles have been successfully prepared by double-emulsion and phase-separation methods. However, numerous limitations involved with coacervation method include the formation of particles which are agglomerated, difficulty in mass production, requirement of large quantities of organic solvent, and difficulty in removal of residual solvents from the final microsphere product [226]. While the double-emulsion method requires several steps, rigid control of the temperature and viscosity of the inner w/o emulsion, and is difficult to encapsulate higher concentration of hydrophilic drugs. Contrary to these methods, the spray-dried method is very rapid, convenient, easy to scale-up, involves mild conditions, and is less dependent on the solubility parameter of the drug and the polymer.

Spray drying technique has been extensively exploited in diverse areas such as pharmaceutical, chemical, biochemical and food industries. This process comprises of...
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Converting liquid into powder by spraying a solution or a liquid dispersion through an atomization nozzle in a drying chamber, where it comes into contact with hot air. The solvent evaporates very quickly, leaving solid particles to be collected. Due to the rapid evaporation of the solvent and the reduced pressure involved, a vapor cloud is formed surrounding the product that can protect the product from thermal influences [227].

The temperature of the droplets can thus be kept below the drying air temperature, and for this reason, spray drying can be applicable to both heat resistant and heat sensitive materials. The main components for a spray dryer include air heater, nozzle, spray chamber, fan, cyclone and collecting vessel. The atomization of liquid can be carried out via three different mechanisms: rotary atomization where fluid is introduced into the drying chamber through a rotating wheel; pressure atomization, where feed is brought into the nozzle under pressure; pneumatic atomization, where compressed air and fluid travel separately to the nozzle and are mixed to create a spray of droplets. The difference in the spray feed and the drying air results in co-current, counter-current and combined current apparatus. Counter-current is only suitable for thermally stable products. [226, 227].

In recent years, in particular since the late 1970s, spray-drying technique has been tested to develop microparticles for the controlled release applications. Hydrophilic drugs especially for proteins, hydrophobic drugs as well as bacteria are encapsulated via spray drying technique. Similar to the other fabrication methods, DCM is the most commonly used solvent, other solvent such as hexafluoro-2-propanol, benzene, DCM/chloroform mixture, methanol/DCM were also evaluated [148]. Polymer concentration, inlet and outlet temperatures, spraying rate of the feed, heating medium flow rate are the basic operating parameters to be investigated. However, drug solubility, solvent selection, polymer types and drug loading will also affect the properties of the microspheres produced.

Although spray drying technique offers a lot of advantages over conventional fabrication methods, it has several drawbacks that need attention. One is the formation of fibers due to insufficient driving force available to break up the polymer solution into droplets. Another frequently encountered problem but difficult to overcome is the significant loss of the product due to adhesion of the microparticles to the inside wall of the spray dryer apparatus and vacuum removal. Spray dryer can also produce agglomeration of the microparticles. Efforts have been made to reduce the agglomeration...
and increase the yield by using mannitol as an antiadherent; however, the extent of improvement on the yield was still limited [227]. An improvement on spray drying process was carried out involving the use of the centrifugal disk atomization technique to produce particles of very narrow size distribution, owing mostly to the presentation of chaotic, violent and random interactions during droplet formation.

2.7.4.4 In situ forming Microparticles

In order to overcome the shortcomings related with conventional microspheres like cost-intensive preparation, drying of the system and potentially difficult resuspension, the concept of in situ forming depots was investigated for microspheres. In comparison to in situ implants, microspheres exhibited reduced myotoxicity, easier injectability and reproducible surface area [165]. Starting with relatively large particles prepared by dropping aliquots of drug + PLGA into aqueous medium, the formulations became injectable when preformed o/o emulsions stored until administration or two compartment systems (syringes attached to each other with a syringe connector) with its content being dispersed at the bedside to form o/w or o/o emulsions were investigated [142, 147].

After injection, the partitioning of the biocompatible solvent into the tissue causes the hardening of the emulsion droplets in vivo. These hardened microspheres depict significant burst release. Current research investigates the hydrophilic substances using o/o emulsions in which the external oil phase act as a diffusion barrier minimizing the burst release depending over the volume and viscosity [147]. The USP and European Pharmacopoeia have granted permission to use the vegetable oils as non-aqueous vehicles. Medium chain triglycerides (MCT) could be used in emulsions for parenteral preparation and acts an alternative non-aqueous Oil phase for in situ formulations. [183, 186, 188].

Since the standard refining procedure, thermal denaturation, might not always eliminate allergenicity, “peanut products should be treated as allergenic unless they have an analytically monitored non-allergenic specification”. Also, allergies are well-known for alternative oils such as sesame, almond, and other oils. Also, alternative oils such as sesame, almond, and other oils exhibit well-known allergies [147].
Melting techniques deals with the basics of avoiding the organic solvents and preparing dispersion or melting the drug in the polymer melt. The process involves the drug/matrix polymer melt to be cooled down and then ground or jet-milled to form non-spherical particles [228]. This method shows resemblance with extrusion spheronization process. The melt should be extruded before complete solidification, or else will lead to formation of unprocessable lumps of the congealed melt especially for large batch sizes. For obtaining the spherical microparticles with a smaller size distribution, the ground melt can be emulsified in a hot solution containing emulsifier or a hot gel [229]. Some of the significant drawbacks of the process include thermal treatment of the drug and the multitude of steps to obtain smooth microparticles. Finally, it should be stated that melt-based encapsulation methods commonly process highly nonporous polymeric matrices, which can lead to undesirably slow release profiles especially for hydrophobic drugs [228, 229].

Methods using Supercritical fluids (SCF)

The conversion into supercritical fluids (SCF) occurs when substances placed above their critical point. The specific phenomenon of SCF is exhibiting the flow properties of a gas (low viscosity) and the dissolving power of a liquid. It has high penetration tendency throughout the materials as it do not depict any surface tension. The solvent power of the same is concerned to its density, which has the tendency of major variations in the vicinity of the critical points and can be governed through the changes in temperature and/or pressure [230-233]. The SCF used as solvent to dissolve the drug and polymer followed by particle formation through rapid expansion from supercritical solution (RESS). In another technique the drug and the polymer melted in SCF afterwards precipitate into particles from the gas-saturated solutions/suspensions (PGSS) after spraying the melt and releasing the gas. In a current research the supercritical fluid extraction of emulsion (SFEE), i.e., a classical o/w emulsion, has been described to reduce the time of solvent removal and polymer precipitation. The major limitation of the technique is requirement of specialized equipments; due to which these techniques are not widely used on the bench scale [152]. Developed microparticles may exhibit high porosity leading to faster drug release due to fast extraction of the organic solvent and the
partitioning of the SCF into the polymer beads, which may afterwards expands during the decompression.

2.7.4.7 Current Trends in Microencapsulation

With the aim to achieve controlled release with improved stability of protein molecules, double-walled microspheres consisting a core (protein-loaded nanoparticles) with polymeric outer shell using (solid-in-oil phase-in-hydrophilic oil-in-water (S/O/Oh/W) emulsification method employing poly(D,L-lactide-co-glycolide), poly(D,L-lactide) and dextran. Protein release from the novel formulation displayed zero-order profile for about 40 days [234]. A novel S/O/O/W emulsion method was used to achieve a sustained release of granulocyte colony-stimulating factor (G-CSF) microsphere from poly(lactic-co-glycolic acid) microspheres. In comparison to S/O/O/W, the control control group fabricated by W/O/W method showed lower bioactivity as reported by proliferation studies on NFS-60 cell line. Moreover, an improved neutrophil ratio (for prolonged period) was observed using microspheres obtained from S/O/O/W method than the control microspheres.

Gelatin microspheres produced via emulsification process and crosslinked with genipin were exploited for local and efficient growth factor release. A sustained release of bone morphogenetic protein 2 was reported form gelatin microspheres with higher loading efficiency (depending on degree of cross-linking) [235]. A sustained release of octreotide acetate, a potent synthetic somatostatin analogue was achieved from biocompatible and biodegradable microspheres composed of poly-lactic-co-glycolic acid (PLGA) following a single intramuscular depot injection. A two-phase bimodal absorption profile was achieved for the two octreotide acetate-containing PLGA microsphere batches with an initial rapid absorption followed by extended absorption [236].

A solid-in-oil-water (s/o/w) technique was used to fabricate Poly(3-hydroxybutyrate) microspheres for controlled delivery of gentamicin. Depending upon the stirring rates, the average size of the microspheres varied from 2 μm to 1.54 μm. At low stirring rates (300 rpm) slightly larger sizes were observed in comparison to microspheres produced at higher stirring rates. The other properties of microspheres (like zeta-potential, surface hydrophobicity, protein adsorption and encapsulation efficiency) were highly affected by presence of residual PVA on the surface of the microspheres. The
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In vitro release of was bimodal with initial burst release followed by a sustained release [237].

A sustained release of levodopa and benserazide was reported from poly(lactic-co-glycolic acid) microspheres injected into dyskinetic rats. The formulation showed better response in lowering abnormal involuntary movements than levodopa + benserazide treated dyskinetic rats suggesting microspheres significant role in improvement of dyskinesia by reducing the expression of pGluR1S831 and pGluR1S845 [238]. A novel formulation consisting drug loaded liposome (chitosan coated) within pH responsive Eudragit S100 microspheres were fabricated for colonic delivery. The formulation prevented drug release within simulated stomach and small intestine conditions with site specific release (large intestine conditions). The stability of α-Chymotrypsin was improved on glycosylation of drug loaded PLGA microspheres. This approach improved encapsulation efficiency as well as reduced burst release effect in comparison to the non-modified (unglycosylated) microspheres [239].

PLGA microspheres loaded with interleukin-1 receptor antagonist showed improvement in interleukin-1 beta mediated degradative changes in the nucleus pulposus which could be highly effective strategy for treating early stage, cytokine-mediated disc degeneration [240]. Microspheres containing BFB0261, a new potent osteogenic compound was prepared using PLA, PLGA, and PLA-PEG. Microspheres constructed from PLA and PLGA polymers were in the range of approximately 10-50 μm while PLA-PEG microspheres showed higher size range (30-45 μm). Upon intramuscular administration, PLGA microspheres showed a fast and complete release within 4 weeks whereas microspheres from PLA and PLA-PEG exhibited sustained profile (> 6 weeks) [241]

An average size of 76 μm was obtained with estradiol loaded PLGA microspheres prepared by a solvent evaporation method. In vitro studies showed a sustained release of estradiol from microspheres for about one month while the in vivo studies (subcutaneous injection into rats) suggested a prolonged release of drug for 50 days with enhanced bone mineral density when compared with control [242]. Ipriflavone PLGA loaded microspheres were prepared for the local treatment of oral bone loss. Formulation showed increase in spongy and total bone mass with good tolerability (temporary/mild inflammation on the injection site) [243].
2.8 Unit/Multiparticulate Systems

2.8.1 Matrix Tablets

Pharmaceutical delivery technology aims at improvement in the therapeutic efficacy of the drug molecules along with a decrease in the associated harmful side effects [244]. Oral drug delivery has been known for decades as the most widely utilized route of administration for systemic delivery of drug entities, owing to the its cost effectiveness, better patient compliance and ease of administration. Depending on the specific requirements associated with a drug molecule, a wide variety of oral pharmaceutical formulations are available in the form of conventional immediate release or novel controlled release, prolonged release, timed release and delayed release delivery systems [245].

Controlled release delivery systems have received considerable attention from the pharmaceutical industry over the last few decades. These systems aim at providing continuous delivery of drugs at predictable and reproducible kinetics for a predetermined period throughout the course of gastrointestinal transit. They offer various advantages over the immediate release delivery systems like improved patient compliance, better therapeutic efficacy, reduced side effects and lesser fluctuations in the drug plasma levels [246]. A large number of approaches have been utilized to achieve controlled release of drugs based on the specific product requirements as follows: Gastroretentive systems including altered density formulations, bioadhesive systems, hydrophilic matrices, lipophilic matrices, buccoadhesive systems and osmotic pressure controlled systems. The basic mechanisms by which the drug is released from these systems are diffusion, dissolution, erosion, swelling, osmotic pressure or combination of these [247].

Controlled release systems can be broadly classified into reservoir and matrix systems. In these systems, the release rate of drug is governed by various factors like physico-chemical properties and proportions of the carrier and the drug molecule and the type of carrier system employed [248]. In reservoir devices, a core of drug is surrounded by a polymeric membrane and these are generally prepared by microencapsulation of drug particles or film coating of tablets. In matrix based systems, the dissolved or dispersed drug is distributed uniformly in an inert polymeric matrix. The matrix systems offer significant advantages like ease of manufacturing, cost effectiveness and more reproducibility compared to coated systems [249].
The matrix systems could be classified into hydrophilic matrix systems and hydrophobic matrix systems. A hydrophilic matrix is a homogeneous dispersion of drug molecules within a skeleton in which one or several of the excipients incorporated are a hydrophilic polymer, such as cellulose derivatives, sodium alginate, xanthan gum, polyethylene oxide, or carbopol among others, that swells upon contact with water [247, 250]. Most commercial hydrophilic matrices are obtained by compression, such that in most cases one can speak in terms of a matrix tablet. Thus, the basic operations involved in the preparation of the matrices are the same as those used to prepare conventional tablets, such as mixing and compressing the components [244, 246].

Granulation prior to mixing and the coating of matrix tablets are complementary operations widely used to manufacture matrix tablets. As well as the drug and the release-limiting polymer, other excipients are usually added as diluents, lubricants and anti-adherents. The main problem in the formulation of these systems lies in achieving a suitable rate of drug release to obtain blood concentrations within the therapeutic range over the time desired. Accordingly, this biopharmaceutical and pharmacokinetic aim must be attained with the available technological resources. This is why a deep knowledge of the factors affecting the release rates of drugs is crucial for the correct technological development of sustained release systems [245, 247].

Lipophilic matrices are widely being employed for obtaining sustained release of various drug molecules with diverse properties. Lipophilic matrices owe highly preferred because of their lower cost, natural origin and biodegradable nature. They are also characterized by very low porosity, resulting in better retardation of highly soluble drugs [251]. Many studies have reported the use of lipids like glyceryl monostearate, camauba wax, bees wax etc., for sustained release matrices for highly soluble drugs and enhancement of bioavailability of poorly soluble drugs, especially with solid dispersions, taste masking of bitter tasting drugs, floating of dosage forms and reduction in the gastric-irritation associated with some drugs. Various techniques have been employed for developing these systems like spray-chilling, hot-melt coating, melt granulation and molding.

2.8.1.1 Fabrication of Sustained Release Products

Secondary to the establishment of drug concentration profile and selection of suitable drug molecule for controlled drug delivery, the potential mechanisms available to
achieve the desired profile need to be established. To maintain the drug level at a constant desired value, frequent dosings of drug could be employed to generate a series of peaks and valleys in the blood level profile, whose mean value lies on the plateau of the ideal case [249, 251]. The success of this approach depends on the frequency of the multidoses, as more frequent the dose, the smaller the peaks and valleys and the closer the extreme drug levels will adhere to the plateau value. This is the approach taken with the spansules where four dosage units were employed in each spansule. One dose unit provides the drug in a non sustained form to establish the initial level of drug and the other three doses were intended to release drug at definite time intervals [248-250].

An alternate approach is to employ a continuous release of drug. With this method, a non-sustained portion of dosage form is needed to rapidly establish the therapeutic level of drug in the blood and then, by some suitable mechanism, drug is continuously provided in a zero- or first-order fashion. The various systems used for controlled delivery are reservoir systems, matrix systems, osmotic systems, mechanical systems, swelling systems and controlled release by stimulation [248]. The drug release from these systems is based on dissolution, diffusion, erosion, swelling and osmotic pressure, although one system may involve two or more mechanisms to achieve the optimal control. Reservoir and matrix systems are the most extensively used and studied controlled release systems. In reservoir devices, a polymeric membrane surrounds a core of drug and these are generally prepared by microencapsulation of drug particles or film coating of tablets. On the other hand, in matrix-based systems, the dissolved or dispersed drug is distributed uniformly in an inert polymeric matrix [246-249].

In a recent research percolation theory has been applied to estimate the Hypromellose (HPMC) percolation thresholds and the influence of the polymer viscosity and the initial porosity on these thresholds in carbamazepine multicomponent matrix formulations. Different batches containing two viscosity grades of HPMC as hydrophilic matrix forming polymer, MCC and lactose as fillers, and a lubricant mixture have been manufactured varying the compression pressure in order to obtain matrices with three levels of initial porosity. The results suggested the existence of an excipient percolation threshold between 13 and 15% v/v of HPMC for the different batches prepared [252]. It has been found that the percolation threshold for the polymer is independent on the investigated formulation factors of polymer viscosity and initial porosity of the matrices.
Another experimental design methodology was developed by integrating the response surface methodology and the time series modelling. The major purposes were to identify significant factors in determining swelling and release rate from matrix tablets and their relative factor levels for optimizing the experimental responses. Properties of tablet swelling and drug release were assessed with various factors using a hydrophilic model drug (terazosin) and compared with target values. Based on the results of matrix swelling and drug release, the optimal solutions, target values, and validation experiment results over time were similar and showed consistent patterns with very small biases [251, 253]. The experimental design methodology could be a promising experimental design method to characterize significant factors in the sustained release matrix tablet.

2.8.1.2 Mechanisms of release from hydrophilic matrices

Owing to the rapid “gelification” of the polymers forming them, hydrophilic matrices in contact with water become hydrated instead of disintegrating. This hydration, due to the increase in size of the polymer molecules as a consequence of the entry of solvent (a relaxation of the polymer chains: a decrease in the vitreous transition temperature (Tg) at 37 °C), leads to the formation of a zone in which the polymer passes from the crystalline state to a “rubbery” state known as a gel layer [250-252]. Several transport phenomena take place through this gel layer: the entry of the aqueous medium and the exit of the drug to the outside of the system, and phenomena of matrix erosion. The thickness of the gel layer increases as more and more water enters the system. At the same time, the surface-most polymer chains, which become hydrated earlier than the others, gradually relax until they lose consistency, after which matrix erosion begins.

Drug release from matrix systems can be by diffusion, dissolution, erosion controlled or a combination these three mechanisms. It is assumed that the solid drug dissolves from the surface layer of the device first, when this layer becomes exhausted of drug, the next layer begins to be depleted by dissolution and diffusion through the matrix to the external solution. In this fashion, the interface between the region containing dissolved drug and that containing dispersed drug moves into the interior as a front [249-253].
2.8.2 Multiparticulate Systems

Pharmaceutical invention and research are increasingly focusing on delivery systems which enhance desirable therapeutic objectives while minimizing side effects. Recent trends indicate that multiparticulate drug delivery systems are especially suitable for achieving controlled or delayed release oral formulations with low risk of dose dumping, flexibility of blending to attain different release patterns as well as reproducible and short gastric residence time [254]. The release of drug from microparticles depends on a variety of factors including the carrier used to form the multiparticles and the amount of drug contained in them. Consequently, multiparticulate drug delivery systems provide tremendous opportunities for designing new controlled and delayed release oral formulations, thus extending the frontier of future pharmaceutical development [255].

Multi-particulate drug delivery systems are mainly oral dosage forms consisting of a multiplicity of small discrete units, each exhibiting some desired characteristics. Pellets can be defined as small, free flowing, spherical particles manufactured by agglomeration of fine powders or granules of drug substances and excipients using appropriate processing equipment [254, 256]. In these systems, the dosage of the drug substances is divided on a plurality of subunit, typically consisting of thousands of spherical particles with diameter of 0.05-2.00mm. Thus multiparticulate dosage forms are pharmaceutical formulations in which the active substance is present as a number of small independent subunits [255].

The multiparticulates spread uniformly throughout the gastrointestinal tract. High local drug concentrations and the risk of toxicity due to locally restricted tablets can be avoided. Premature drug release from enteric coated dosage forms in the stomach, potentially resulting in the degradation of the drug or irritation of the gastric mucosa, can be reduced with coated pellets because of a more rapid transit time when compared to enteric coated tablets [257]. The better distribution of multiparticulates along the GI-tract could improve the bioavailability, which potentially could result in a reduction in drug dose and side effects. Multiple-unit systems often claim superiority to single-unit modified-release formulations in terms of predictability and reproducibility of behavior in the gastrointestinal tract. Inter and intra-subject variations in bioavailability caused by food effects are reduced. With regard to the final dosage form, the multiparticulates can be filled into hard gelatin capsules or be compressed into tablets [255, 258].
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Of the all multiple unit dosage form, the increased interest of pharmaceutical industry in pellets is evident from the ever growing number of patents and publications in the scientific literature. Pellets are defined as spherical, free-flowing granules with a narrow size distribution, typically varying between 500 and 1500 μm for pharmaceutical applications. Pellets are usually film-coated with one or more layers of polymer film that provide protection of the active ingredient or control the release of the drug [257, 259]. This can be ascribed to myriad of advantages offered by the pellets including smooth surface morphology, spherical shape, a narrow size distribution, good flow properties and uniform packing, low friability, highly reproducible gastrointestinal transit times and reduced potential local irritations with minimum risk of dose dumping.

The pellets can be easily coated due to including smooth surface morphology, spherical shape, narrow size distribution and low friability. In addition, the spherical shape facilitates the mixing of pellets particles with food or juices and hence offers ease of administration. Also, simple mixing of pellets with different release characteristics provide varied drug release profiles; in addition, a more rapid onset of action can be achieved easier with pellets than with tablets [256, 257]. Moreover, several active components, incompatible drugs or drugs with different release profiles can be combined in the same dosage unit in pellets. The dosage forms with different doses can be produced from the same batch by adjusting the fill weight of the pellets. The good flow properties of pellets ensure reproducible die or capsule filling and consequently good content uniformity [260].

Several pelletisation techniques are used for pellet preparation, the most popular being solution/suspension layering, powder layering, direct pelletisation using high shear mixers and conventional or rotary fluid-bed granulators and extrusion-spheronization. Within the various modes of preparation, extrusion/spheronization is the most popular method [254-257]. The extrusion/spheronization pelletisation technique enables the manufacture of pellets with uniform size, shape and density, containing one or more drugs. The process involves a preliminary stage in which dry powders, drug, and excipients are mixed by conventional blenders, followed by addition of a liquid phase and further mixing to ensure homogeneous distribution. The wet powder mass is extruded through cylindrical dies or perforated screens with circular holes, of typically 0.5-2.0 mm diameter, to form cylindrical extrudates [257-261].
Pioneering work in the field employing the hot melt extrusion process as a tool for the manufacture of pellets had revealed the potential for controlled release of polymer embedded drugs and limitations. Microcrystalline cellulose (MCC) is the benchmark to manufacture spherical pellets via extrusion-spheronization. This is because of the fact that wet MCC has proper rheological properties, cohesiveness and plasticity to yield strong and spherical particles [262]. Albeit it is associated with several limitations such as prolonged drug release of poorly soluble drugs, chemical incompatibility with specific drugs, drug adsorption onto MCC fibers. Several investigations have been carried out to explore other materials as potential alternatives aids for extrusion-spheronization to MCC. In an investigation various other materials including powdered cellulose, starch, chitosan, kappa-carrageenan, pectinic acid, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, polyethylene oxide, cross-linked polyvinylpyrrolidone, glycerol monostearate were used. The properties of the different materials and the quality of the resulting pellets in relation to the properties required for an ideal extrusion-spheronization aid for the process was studied [263].

In spite of several limitations associated with microcrystalline cellulose (MCC) such as adsorption of actives, longer dissolution time, and degradation of some sensitive drugs such as ranitidine, still it is the most widely used extrusion spheronization aid. A study corroborates this fact beyond doubt wherein scores of alternative spheronizing aids were characterized and evaluated for their potential as an alternative to MCC based on their intrinsic properties such as solubility, water absorption and retention capacity, rheology, surface properties, binding capacity, drug release, and pellets properties such as sphericity, porosity, and friability with respect to MCC [256-261]. The various aids investigated included cross-linked polyvinylpyrrolidone, carrageenan, chitosan, pectinic acid, modified starches, coprocessed MCC, glycerides, chitosan, sodium alginate, and β-cyclodextrin (CD). The results vividly revealed that none of them succeeded to provide the same flexibility in formulation and processing during extrusion spheronization as observed for MCC (e.g., less water-holding capacity, narrow liquid range providing the correct rheology for extrusion spheronization, addition of binder required to obtain sufficient mechanical strength) [255].

Besides various other excipients, lipids are useful pelletisation aids. Solid lipid extrusion is a suitable technique to produce oral dosage forms with improved taste properties. A study involved the development of lipid based oral dosage forms (pellets)
containing NXP 1210 as model drug (BCS Class II) with taste masking properties but a fast and complete dissolution. In the study, solid lipid extrusion at room temperature was applied for the formulation development of the BCS Class II drug NXP 1210 [258-261]. The formulations for taste-masked pellets containing poorly soluble drugs were prepared using powdered hard fat (Witocan® 42/44 mikrofein), glycerol distearate (Precirol® ato 5) and glycerol trimeyste (Dynasan®114) as lipid binders and polyvinyl alcohol (PVA)-polyethylene glycol (PEG)-graft copolymer (Kollicoat® IR) and crospovidone (Polyplasdone® XI-10) as solubilizers. Herein, an immediate release of the pellets could be combined with a delayed release at the beginning of the dissolution profile ensuring a taste-masking effect for the bitter tasting drug. Rate of dissolution and duration of the lag-time could be controlled by adapting type and amount of solubilizer [257-259].

Invariably, the pellets obtained from extrusion/spheronization generally do not disintegrate, especially those prepared from MCC. However, recently an investigation revealed that the disintegrating or exploding MCC pellets could be prepared by incorporating PEG 400, polysorbate 80 and CCS, and promising for increasing dissolution of poorly water-soluble drugs [260]. Herein, the MCC pellets formulations containing indomethacin as model drug with low aqueous solubility did not disintegrate and showed slow drug dissolution while the formulations with PEG 400 and/or polysorbate 80 disintegrated within 90 sec. and their drug dissolution was increased. The incorporation of CCS allowed the pellets to explode and disintegrate into two smaller pieces within 5 sec. after contact to an aqueous medium. The increased amount of CCS insignificantly decreased disintegration time or increased drug dissolution [255-259].

2.8.2.1 Formulation Aspects in Multiparticulate Dosage Forms

A broad range of drug-delivery opportunities exist using oral multiparticulate technology. Pellets, beads, and microspheres with coated and/or matrix architecture can be formulated as modified-release (e.g., extended, delayed, pulsed), immediate-release, bioavailability-enhanced, or taste-masked drug product intermediates. These particles can be dosed within capsules, tablets, and sachets [261]. Various methods employed for the production of multiparticulates require different processing conditions and produce multiparticulates of distinct qualities. Drug particles may be entrapped within the multiparticulates or layered around them. Subsequently, these multiparticulates may be modified in many ways to achieve the desired drug release profile [257, 259].
Compression is a pelletisation process in which mixtures or blends of active ingredients and excipients are compacted under pressure to generate pellets of defined shape and size. The pellets are small enough to be filled into capsules. The formulation and processing variables that govern the production of pellets during compression are similar to those that are routinely employed in tablet manufacturing. In fact, pellets produced by compression are nothing but small tablets that are approximately spheroidal in shape [258-262].

Spray congealing is a process in which a drug is allowed to melt, disperse or dissolve in hot melts of gums, waxes, fatty acids etc., and is sprayed into an air chamber where the temperature is below the melting points of the formulation components, to provide, under appropriate processing conditions, spherical congealed pellets. Depending on the physicochemical properties of the ingredients and other formulation variables, pellets with immediate or controlled release behavior can be produced [263].

In a spray-drying process, aqueous solution of core materials and hot solution of polymer is atomized into hot air, the water then evaporates and the dry solid is separated in the form of pellets, usually by air suspension. In general, a spray-drying process produces hollow pellets if the liquid evaporates at a rate faster than the diffusion of the dissolved substances back into the droplet interior or if due to capillary action dissolved substances migrate out with the liquid to the droplet surface, leaving behind a void. In fluidized bed technology a dry drug form is suspended in a stream of hot air to form a constantly agitated fluidized bed. An amount of binder or granulating liquid is then introduced in a finely dispersed form to cause a momentary reaction prior to vaporization. This causes the ingredients to react to a limited extent, thereby forming pellets of active components.
2.9 Drug Profile [264-267]

STAVUDINE

![Chemical Structure of Stavudine](Figure 9: Chemical Structure of Stavudine)

2.9.1 Description

**Category:** Antiretroviral, Nucleoside analogue.

**Synonyms:** Azidodeoxythymidine; Azidothymidine; AZT; d4T.

**Chemical Name:** 1-[5-(hydroxymethyl)-2, 5-dihydrofuran-2-yl]-5-methyl-1H-pyrimidine-2, 4-Dione.

**Formula:** C_{10}H_{12}N_{2}O_{4}

**Molecular Mass:** 224.213 g/mol

**Half Life:** 0.8-1.5 hours (in adults)

**Dose:** 30-40 mg, twice daily

**Solubility:** - At 25°C

- In water-87 mg/ml
- In propylene glycol-30 mg/ml
- In methanol-29 mg/ml
- In ethanol-19 mg/ml

2.9.2 Mechanism of Action

Stavudine, a nucleoside analogue of thymidine, is phosphorylated by cellular kinases to the active metabolite stavudine triphosphate. Stavudine triphosphate inhibits the activity of HIV-1 reverse transcriptase (RT) by competing with the natural substrate thymidine triphosphate (Ki = 0.0083 to 0.032 μM) and by causing DNA chain termination following its incorporation into viral DNA [9, 10]. Stavudine triphosphate...
inhibits cellular DNA polymerases β and γ and markedly reduces the synthesis of mitochondrial DNA.

2.9.3 Antiviral Activity

The in vitro antiviral activity of stavudine was measured in peripheral blood mononuclear cells, monocytic cells, and lymphoblastoid cell lines. The concentration of drug necessary to inhibit HIV-1 replication by 50% (IC50) ranged from 0.009 to 4.0 μM against laboratory and clinical isolates of HIV-1. Stavudine had additive and synergistic activity in combination with didanosine and zalcitabine, respectively, in vitro. Stavudine combined with zidovudine had additive or antagonistic activity in vitro depending upon the molar ratios of the agents tested. The relationship between in vitro susceptibility of HIV-1 to stavudine and the inhibition of HIV-1 replication in humans has not been established [146].

2.9.4 Pharmacokinetic Properties

The pharmacokinetics of stavudine has been evaluated in HIV-infected adult and pediatric patients. Peak plasma concentrations (Cmax) and area under the plasma concentration-time curve (AUC) increased in proportion to dose after both single and multiple doses ranging from 0.03 to 4 mg/kg [266]. There was no significant accumulation of stavudine with repeated administration every 6, 8, or 12 hours.

2.9.4.1 Absorption

Stavudine is rapidly absorbed following oral administration, with peak plasma concentrations occurring within 1 hour after dosing. Stavudine enacts the same systemic exposure following administration as capsules or solution.

2.9.4.2 Distribution

Binding of stavudine to serum proteins was negligible over the concentration range of 0.01 to 11.4 μg/mL. Stavudine distributes equally between red blood cells and plasma.

2.9.4.3 Metabolism

The metabolic fate of stavudine has not been elucidated in humans. Stavudine is mainly eliminated through the kidneys where an active secretion pathway is involved.
2.9.4.4 Excretion

Renal elimination accounted for about 40% of the overall clearance regardless of the route of administration. The mean renal clearance was about twice the average endogenous creatinine clearance, indicating active tubular secretion in addition to glomerular filtration.

2.9.5 Dosage and Administration

The interval between oral doses should be 12 hours. Stavudine may be taken with or without food. The recommended doses are based on body weight, for adults <60 kg it is 30 mg bid whereas for adults >60 kg it is scheduled to be 40 mg bid. The recommended dose for pediatric patients is 2 mg/kg/day, not to exceed the recommended adult dose of 40 mg twice daily [266]. The systemic exposure to stavudine is the same following administration as capsules or solution. There is no clinical experience with stavudine in children under the age of 3 months.

2.9.6 Over dosage

Investigations involving the adults treated with 12 to 24 times the recommended daily dosage of stavudine revealed no acute toxicity. Complications of chronic over dosage include peripheral neuropathy and hepatic toxicity. Stavudine can be removed by hemodialysis; the mean ± SD hemodialysis clearance of stavudine is 120 ± 18 mL/min. Whether stavudine is eliminated by peritoneal dialysis has not been studied [266].

2.9.7 Indications and Usage

Stavudine, alone or in combination with other antiretroviral agents, is indicated for the treatment of HIV-1 infection, also indicated for “salvage therapy” for the patients heavily pretreated with other antiretrovirals.

2.9.8 Adverse Reactions

Fatal lactic acidosis has been reported in patients treated with stavudine in combination with other antiretroviral agents. Hence, the therapy with stavudine should be immediately suspended in patients with suspected lactic acidosis. Furthermore, permanent discontinuation of drug (d4T) should be considered for patients with confirmed lactic acidosis. Although, stavudine therapy has rarely been found to be associated with motor weakness, occurring predominantly in the setting of lactic acidosis, nevertheless, the therapy should be discontinued, if motor weakness develops [264]. Stavudine therapy has...
also been associated with peripheral sensory neuropathy, which can produce dose-dependent effects and occurs more frequently in patients being treated with other drugs that have been associated with neuropathy (including didanosine), in patients with advanced HIV infection, or in patients who have previously experienced peripheral neuropathy [266].

Patients should be monitored for the development of neuropathy, which is usually manifested by numbness, tingling, or pain in the feet or hands. Stavudine-related peripheral neuropathy may resolve if therapy is withdrawn promptly, although in some cases, symptoms may worsen temporarily following discontinuation of therapy. Switching the patient with peripheral neuropathy to an alternate treatment regimen should be considered. If switching to an alternate regimen is not suitable and if symptoms resolve satisfactorily after temporary withdrawal, treatment with d4T may be resumed at 50% of the recommended dosage [265]. The efficacy of regimens containing reduced dose of stavudine has not been fully evaluated. If peripheral neuropathy recurs, permanent discontinuation of therapy should be considered. In clinical trials, less than 1% of 466 patients treated with d4T for a median duration of 56 weeks (ranging up to 120 weeks) discontinued therapy because of peripheral neuropathy [266].

The incidence of adverse events may be higher when stavudine is used in combination with other agents with similar toxicities, than when stavudine is used alone. The frequency of occurrence of pancreatitis, peripheral neuropathy, and liver function abnormalities are more in patients treated with the combination of stavudine and didanosine, with or without hydroxyurea. Moreover, the fatal pancreatitis and hepatotoxicity may occur more frequently in patients treated with stavudine in combination with didanosine and hydroxyurea [264, 265]. The pooled database from two clinical trials (AI455-099 and AI455-096) and an ongoing long-term follow-up study for patients completing these two trials (median duration of therapy 56 weeks, ranging up to 120 weeks) revealed that the rates of discontinuation of therapy due to adverse events were 5% for the extended release of d4T regimen and 7% for the immediate-release regimen of the same.
2.9.9 Drug Interactions

2.9.9.1 Drug-drug Interactions

Zidovudine may competitively inhibit the intracellular phosphorylation of stavudine so its combination with d4T is not recommended. Co-administration of stavudine with either doxorubicin or ribavirin should be undertaken with caution, since in vitro data designate that the phosphorylation of stavudine is also inhibited at relevant concentrations by doxorubicin and ribavirin [233]. However, co-administration of drugs during clinical trials revealed no pharmacokinetic interactions between d4T and didanosine, lamivudine (3TC), or nelfinavir. Since, stavudine does not inhibit the major cytochrome P450 isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4; therefore, it is implausible that clinically significant drug interactions will occur with drugs metabolized through these pathways.

2.9.9.2 Drug-food Interactions

Stavudine may be taken without regard to meals. In a study, stavudine absorption was assessed in asymptomatic HIV-infected patients (n=16). Each patient in the fasting state received a 70 mg oral dose of d4T, 1 hour before a standardized meal, and immediately after a standardized meal. The results revealed that systemic exposure to stavudine is not diminished when stavudine is taken with food [266]. The presence of food decreased the rate of absorption but the extent of absorption was not significantly (p = 0.27) affected when when stavudine was taken immediately after a meal. Mean (± SD) $C_{\text{MAX}}$ of stavudine was reduced from 1.44 (± 0.49) µg/mL in the fasting state to 0.75 (± 0.16) µg/mL after a meal, and the median time to achieve $C_{\text{MAX}}$ was prolonged from 0.6 to 1.5 hours. However, mean (± SD) AUC$_{0-\infty}$ values were 2.50 (± 0.71) µg·hr/mL and 2.31 (± 0.55) µg·hr/mL in the fasting state and after meal, respectively, indicating that systemic exposure was similar with or without the presence of food [266].

2.9.10 Drug Resistance

HIV-1 isolates with reduced susceptibility to stavudine have been selected in vitro (strain-specific) and were also obtained from patients treated with stavudine. Phenotypic analysis of HIV-1 isolates from 61 patients receiving prolonged (6-29 months) stavudine monotherapy showed that post-therapy isolates from four patients exhibited IC$_{50}$ values
more than 4-fold (range 7- to 16-fold) higher than the average pretreatment susceptibility of baseline isolates [264, 265]. Of these, HIV-1 isolates from one patient contained the zidovudine-resistance-associated mutations T215Y and K219E, and isolates from another patient contained the multiple-nucleoside-resistance-associated mutation Q151M. Mutations in the RT gene of HIV-1 isolates from the other two patients were not detected. The genetic basis for stavudine susceptibility changes has not been identified.

2.9.11 Toxicology

Several literature reports connote to comprehensive toxicity studies including evaluations for reproductive and genetic toxicities that have been conducted in laboratory animals wherein they have been subjected to multiples exposure of stavudine up to approximately 400 times the human dose. During these pivotal safety studies, performed up to one year duration, no life-threatening toxicity was observed. However, in rats and monkeys, a slight decrease in red blood cell indices, and hepatic alteration were concluded [266]. Further, the toxicity studies on Stavudine neither discerned teratogenicity nor any adverse effect on mating or fertility. Like other naturally occurring nucleosides and other nucleoside analogs, stavudine produced positive responses in one in vivo and two in vitro genetic toxicity assays.