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**Abstract**

HIV/AIDS has been major source of concern all over the world for past few decades. The current treatment regimes include the use of antiviral drugs belonging to the classes of nucleoside-analog reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). The use of highly active antiretroviral therapy (HAART) using multiple drugs, has raised the life expectancy of HIV-infected patients. However, the HIV infections, is yet to be targeted in anatomical reservoirs, such as the brain, testes, gut, liver, kidney, and secondary lymphoid tissue. Potential nanocarriers have been studied and analysed thoroughly to overcome the hurdles in the delivery of antiretroviral drugs for HIV prevention and therapy. This review provides an insight into the life cycle and infection of HIV and various nanoparticulate delivery vehicles used for anti-retroviral drugs. Biocompatible polymeric nanoparticles, liposomes and hybrid nanosystems have been thoroughly discussed. Such nanostructured materials hold great promise for the future of HIV treatment and can be expected to improve the quality of life of HIV victims.

**INTRODUCTION**

The Human Immunodeficiency Virus (HIV) is the causative agent of HIV infection which acts against the infection-resisting CD4 cells of the immune system. The major areas of localization of these viruses are the central nervous system, macrophages and lymphoid tissues. The severe loss of CD4 cells makes the body incapable of fighting infections. The person infected by HIV is left untreated; the viruses can slowly destroys immune system and lead to Acquired Immuno Deficiency Syndrome (AIDS) which is the most advanced stage of the HIV infection. HIV is commonly transmitted through sexual contact, direct blood contact, and from mother to baby. The discovery of the Human Immunodeficiency Virus (HIV) in 1983 accounted for the set of symptoms currently known as AIDS. HIV/AIDS has turned into a global epidemic claiming the lives of millions of adults annually [1]. Figure 1 shows the major areas of infection of HIV.

According to reports published in 2015 by the UN, India has the third-highest number of people living with HIV in the world with 2.1 million in Indians accounting for about four out of 10 people infected with the deadly virus in the Asia-Pacific region. About 36.7 million people are reported to be living with HIV infections globally, of these 1.8 million are children below the age of 15. Approximately 2.1 million individuals have been newly infected by the deadly virus. The use of medicines for treating HIV infection is called antiretroviral therapy (ART). ART involves a daily- HIV regimen which is a combination of HIV medication. Though ART is incapable of curing HIV infection, it can aid infected people to live longer and healthier lives. A world wide statistics showing the number of people living with HIV infection is shown in Figure 2.

Inspite of continued advancement in the modes of treatment and prevention HIV/AIDS, it still remains as an important and unsolved problem for the human race. This pandemic continues to be a major economic and social burden. The absence of a complete cure or curative agents for stopping HIV infection emphasizes the need for seeking out new approaches for HIV/AIDS treatment and prevention [2]. Global overview of HIV infection is shown in Figure 3.

**The virus**

Infection by the HIV, a lentivirus belonging to the family Retroviridae leads to AIDS in primates. The virus is composed
The glycoproteins gp120 and gp41 aid in recognizing the CD4 receptor and the CCR5 or CXCR4 co-receptors on the host cell membrane, and for virus/ cell fusion, respectively. Transcription errors of these genes result in high polymorphism which leads to mutation, thereby increasing the difficulty in developing and targeting drug against this virus. HIV-1 and HIV-2 are the two different types of this virus known to cause infection and disease. The HIV-1 virus is more common, effective, infectious and is responsible for the majority of HIV infections in the world. HIV-2 is has demonstrated slower progression to immunodeficiency and its transmission is less efficient compared to HIV-1 which explains its lower prevalence when compared to HIV-1 [3] (Figure 4).

**Life cycle of HIV**

HIV mainly infects the cells of the immune system. The virus exhibits a great affinity for the cells expressing CD4 receptors. The life cycle of HIV consists of the following steps:

**Binding and fusion:** HIV begins its lifecycle by binding to CD4 receptor and co receptors found on the CD4+ T- lymphocyte. The virus then fuses with the host cell and releases its genetic material (ssRNA) into the host cell.

**Reverse Transcription:** The reverse transcriptase enzyme converts the RNA to DNA within the host cell.

**Integration:** The DNA thus produced enters the host nucleus and gets integrated into the host genome. This integrated viral DNA is called a provirus and it remains inactive for several years.

**Replication:** The provirus then utilises the host machinery to transcribe mRNA which then codes for the HIV proteins. The RNA is transcribed into long chain proteins.

**Assembly:** The viral proteins and the viral RNA move towards the cell surface. They are then assembled into immature non-infective viral particles.

**Budding:** The non infective viruses then push itself out of the host cell. The HIV then release the protease enzyme which then cleaves the long chain proteins into shorter ones, these proteins then associate together to form the infectious virus. Copies of HIV genetic material are present among the strands of messenger RNA. These form new HIV particles, which are then released from the T-helper cell. These are then ready to infect other cells and begin the process all over again [4].

Figure 1: A schematic representation shows the major areas of infection of HIV.

Figure 2: Statistics showing the number of people living with HIV infection in different countries (A: Eastern and Southern Africa, B: Western and Central Africa, C: Asia and Pacific, D: Europe and N. America, E: Latin America and Caribbean, F: East Europe and central Asia).

Figure 3: Global overview of HIV/AIDS infection.

Figure 4: Detailed structure of HIV.
HIV transmission

The most common means of HIV transmission is by vaginal or anal sexual intercourse [5]. Vaginal sexual intercourse has been attributed to viral penetration of the vaginal and cervical mucosa. Upon sexual intercourse, HIV gets transmitted as free virions or associated with macrophages, which are the primary carrier of HIV in semen and vaginal discharges. Rectal viral transmission is also very common. The simple columnar epithelial lining makes the rectum and terminal colon easy for transmission of HIV infection [6]. Other significant means of HIV spread is by transfusion of contaminated blood products, sharing of contaminated needles among intravenous drug users, and transmission from mother-to-child during pregnancy, labor or breastfeeding [7].

HIV pathogenesis

The virus invades the new host and undergoes local amplification at the mucosal site. The infected cells then migrate to the regional lymph nodes where the virus undergoes some mild amplification in the native T cells. From the regional lymph nodes the infection quickly spreads via the T cells to the lymphoid organs especially the gut-associated lymphoid tissues (GALT), spleen, and bone marrow. This leads to a burst in the viral load (acute infection) [8]. The gastrointestinal tract is severely affected by the HIV during the early acute stages of infection leading to a severe loss of CD4+ and CD8+ T cells [9,10]. The acute infection lays the foundation for the establishment of chronic and persistent infection by HIV, which despite vigorous immune response in the early stages is never completely eliminated from the body [11].

HIV/host cell interaction: CD4 expressing human cells are the primary targets for the HIV. CD4 is a cell surface protein expressed by macrophages, T cells and dendritic cells (DCs) [12]. The life cycle of HIV-1 is complex and affected by several viral and host factors. Interaction of gp120 (envelope glycoprotein) of HIV with the cell-surface receptor on CD4+ cells is required for the attachment of HIV envelope with the target cell membrane. Once the interaction is established, gp120 undergoes a conformational change that aids its binding to either of the two chemokine coreceptor molecules designated as CCR5 or CXCR4 [13]. Viruses (R5) that prefer CCR5-expressing cell tropism, are responsible for most HIV new infections [14]. After fusion with the cell, the viral core consisting of RNA, reverse transcriptase, and integrase are released into the cell cytoplasm. The core then undergoes disassembly and the RNA is used as template for producing DNA by the viral reverse transcriptase. The DNA then moves into host nucleus and gets integrated into it by the action of the enzyme integrase. At this point the infection becomes irreversible, as the cell is now capable of producing virions [13,15] (Figure 6).

The complexity of HIV/AIDS

HIV infection is associated with very high viral load in the host if left untreated; it leads to the depletion of CD4+ cells, leaving behind a defective immune system. The virus is capable of maintaining reservoirs and protecting itself from the effect of drugs. The viral reservoir then releases progeny into the circulation as long as the patient lives. This makes it one of the most challenging and life threatening diseases in the world. The anatomical and cellular reservoirs within the tissues provide a safe haven for the virus. Anatomical reservoirs are tissues that are inaccessible to antiviral drugs; such regions include the central nervous system, retina and testes. The cells which possess the efflux proteins such as P-glycoprotein, the virus may remain latent and hence escape the action of antiviral drugs. The extracellular virions present on the surface of dendritic cells remain infectious in spite of being bound by many antibodies. They are not susceptible to retroviral drugs as they have not infected any cell. The virions can remain in this form for several months. Dendritic cells within lymphoid tissue trap a large number of extracellular virions on their surface thereby shielding the virus from antiretroviral drugs. The monocytes/macrophages that are found in brain, pulmonary alveoli, spleen and lymph nodes have relatively long life span and the low cytopathic effects of the HIV makes them a persistent reservoir of HIV in spite of the presence of highly active antiretroviral therapy [16]. Such stable and persistent reservoirs make it difficult to eradicate HIV from the body even in the presence of anti retroviral drugs. These drugs in free form have poor local bioavailability and low residence time in these reservoirs when administered systemically [17]. Thus it is of utmost importance to develop new drugs and/or drug delivery systems.
Detection of HIV

The HIV infection is commonly detected by the antibody screening test (immunoassay). The body starts producing these antibodies 2-12 weeks after getting infected by HIV. The current diagnostic strategies make use of blood, oral fluids or urine to detect the antibodies to the virus. Various serological tests such as ELISA (Enzyme Linked Immune Sorbent Assay), Rapid HIV test, and HIV antibody confirmation test 118 are used for diagnosing HIV/AIDS. Several tests have been developed which can detect the antibody or the antigen. All detection tests should be confirmed with western blot or HIV viral load test.

The U.S Center for Disease Control and Prevention (CDC) defines the signs or symptoms of AIDS. People are diagnosed with AIDS when they show certain symptoms such as:

- CD4+ T cell count less than 200 per cubic mm of blood compared with about 1,000 CD4+ T cells (healthy people)
- CD4+ T cells count less than 14% of all lymphocytes.

Recommendations of CDC include testing of CD4+ T cell count for every three to six months in all HIV-infected persons, though the need may vary from patient to patient [8,18].

HIV/AIDS Current therapeutic strategies

The HIV infection is a worldwide health challenge. A cure for HIV/AIDS has been elusive to research for almost 30 years. Early treatments focused on antiretroviral drugs that were effective only to a certain degree. The first drug, zidovudine, was approved by the US FDA in 1987 and till date about25 have been approved and available in fixed dose combinations and generic formulations in resource-limiting settings (to date, only zidovudine didanosine is available as true generics in the USA) [19-21]. The antiretroviral drugs (ARV) are divided into six classes according to their effect on the HIV life-cycle: fusion/entry inhibitors, integrase inhibitors, protease inhibitors, nonnucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside analog reverse transcriptase inhibitors (NRTIs), and multidrug combination products. Tables 1-3 show the list of different classes of drugs approved by US FDA [22].

The emergence of antiretroviral therapy has greatly contributed to the increased life expectancy and quality of life of patients. In the 1990s, a good break through was observed in the knowledge about the disease, advancement in therapeutic resources, increase in life expectancy and epidemiologic profile. The mid-1990s saw the advancement of pharmacology studies and the arrival of protease inhibitor antiretrovirals that gave rise to an anti-HIV agents known as Highly Active Antiretroviral Therapy (HAART), [23,24], where a combination of three or more different classes of drugs are administered simultaneously. The use of the HAART regimen was reported to have been successful in boosting the life expectancy and quality of life of the patients. Despite the success of HAART, latently infected cells can escape the viral immune response and persist for long periods of time [25]. In addition, the HAART could exhibit side effects such as fatigue, nausea, sickness, diarrhoea and lipodystrophy. These symptoms contributed to patients not adhering to the treatment regimen which led to increased blood viral load, a decline in CD4+ T cells, decreased tolerance to anti-HIV drugs, increased opportunistic infections, economic loss and ultimately failure of the treatment [23]. The antiretroviral drugs are exposed to extensive metabolism and the harsh environment of the gastrointestinal tract which result in inadequate oral absorption as well as low bioavailability. The half-life for most anti-HIV drugs is short, which calls for frequent drug administration, which might be difficult for the patient to comply with. Moreover, certain antiviral drugs exhibit poor solubility, low absorption and limited bioavailability. Another limitation of the current HAART regimen is its inefficiency to eradicate HIV from various anatomical reservoirs (e.g., central nervous system (CNS) and gastrointestinal tract) and intracellular sites (e.g.: macrophages, hepatocytes, dendritic cells and langerhans cells) [26-28]. High concentrations of the drugs are essential for eliminating HIV from these reservoirs in order to achieve the desired therapeutic effect. Such large doses may contribute to severe side effects associated with anti-HIV therapy [26,28,29], currently new strategies are being worked out to improve and overcome the limitations of existing therapeutic regimen through the design and development of novel drug delivery systems [26,30].The absence of complete cure for this malady calls for continued efforts in the quest for innovative approaches for treatment.

Administration routes: The choice of route of administration depends on the properties of the drug like solubility, bioavailability, accessibility to patient etc. A delivery route is driven by patient acceptability, access to the site of infection, and/or effectiveness in dealing with the specific disease. One of the promising routes for delivering therapeutic compounds is Nasal delivery. Inhaled medications have been available for many years for the treatment of various lung diseases. They are widely accepted as being the optimal route of administration of first-line therapy for asthma and chronic obstructive pulmonary diseases. In recent years, the lung has been studied as a possible route of drug administration for the treatment of systemic diseases, such as diabetes mellitus. The advantage of this type administration route is the availability of large surface area for delivery, high permeability of the nasal epithelium, allowing a higher molecular mass cut-off for permeation (i.e. approximately 1000 Da), the rapid drug absorption rate sometimes almost identical to that of intravenous injections, absence of first-pass metabolism and potential for central nervous system delivery [31-33]. In addition, nasal vaccination has received a lot of attention since the nasal cavity is rich in nasal associated lymphoid tissue (NALT) through which viral infections can be acquired. Intranasal immunisation is straightforward (i.e. administration via drops or sprays) and in general lower doses are required to elicit comparable antibody titres than by oral or other mucosal route of immunisation. Furthermore, intranasal vaccination has proven to be a safe, easy and cost effective means of controlling viral and bacterial diseases. Finally, regarding both nasal vaccination and nasal delivery of therapeutics, it has been shown that nanoscale drug carriers exhibiting mucoadhesive and permeation enhancing properties have a great potential for improving the delivery through the nasal route [31,34].

Major setbacks for anti retroviral therapy for HIV/AIDS: The current treatment regimens do not completely eradicate the virus as the virus resides in ‘cellular reservoirs such as the memory CD4+ T cells and cells of macrophage–monocyte...
Table 1: US FDA approved nucleotide reverse transcriptase inhibitors (NRTIs).

<table>
<thead>
<tr>
<th>Anti viral drug</th>
<th>Oral adult dose/ Frequency</th>
<th>Half-life(Hours)</th>
<th>Bioavailability (%)</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abacavir (ABC)</td>
<td>300 mg/twice daily 600 mg/once daily</td>
<td>1-1.5</td>
<td>83</td>
<td>77</td>
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<tr>
<td>Didanosine (ddl)</td>
<td>200 mg/twice daily 400 mg/once daily</td>
<td>1.3-1.5</td>
<td>21-43</td>
<td>27.3</td>
</tr>
<tr>
<td>Emtricitabine (FTC)</td>
<td>200 mg/once daily</td>
<td>10</td>
<td>93</td>
<td>112</td>
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<tr>
<td>Lamivudine (3TC)</td>
<td>150 mg/twice daily 300 mg/once daily</td>
<td>3-7</td>
<td>82-87</td>
<td>70</td>
</tr>
<tr>
<td>Stavudine (d4T)</td>
<td>30 – 40 mg/twice daily</td>
<td>0.9- 1.6</td>
<td>80-86</td>
<td>83</td>
</tr>
<tr>
<td>Tenofovir disoproxil fumarate (TDF)</td>
<td>300 mg/once daily</td>
<td>4-8</td>
<td>25-30</td>
<td>13.4</td>
</tr>
<tr>
<td>Zalcitabine (ddC)</td>
<td>0.75 mg/every 8 hours</td>
<td>1-4</td>
<td>80-88</td>
<td>76.4</td>
</tr>
<tr>
<td>Zidovudine (AZT)</td>
<td>200 mg/ thrice daily</td>
<td>0.5-3</td>
<td>64</td>
<td>20.1</td>
</tr>
</tbody>
</table>

Table 2: US FDA approved non nucleoside reverse transcriptase inhibitors (NNRTIs).

<table>
<thead>
<tr>
<th>Anti viral drug</th>
<th>Oral adult dose/ Frequency</th>
<th>Half-life(Hours)</th>
<th>Bioavailability (%)</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delavirdine (DLV)</td>
<td>400 mg/ thrice daily</td>
<td>2-11</td>
<td>60-100</td>
<td>0.2942</td>
</tr>
<tr>
<td>Efavirenz (EFV)</td>
<td>600 mg/once daily</td>
<td>52-76</td>
<td>40-45</td>
<td>3-9µg/mL</td>
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<tr>
<td>Etravirine (TMC125)</td>
<td>200 mg/ twice daily</td>
<td>41</td>
<td>unknown</td>
<td>10µg/mL</td>
</tr>
<tr>
<td>Nevirapine (NVP)</td>
<td>200 mg/once daily</td>
<td>45</td>
<td>90</td>
<td>0.007</td>
</tr>
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</table>

Table 3: US FDA approved protease Inhibitors (PIs).

<table>
<thead>
<tr>
<th>Anti viral drug</th>
<th>Oral adult dose/ Frequency</th>
<th>Half-life(Hours)</th>
<th>Bioavailability (%)</th>
<th>Solubility (mg/mL)</th>
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<tbody>
<tr>
<td>Amprenavir (APV)</td>
<td>1200 mg/twice daily</td>
<td>7-10</td>
<td>25-19*</td>
<td>4.91e.02g/L</td>
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<tr>
<td>Atazanavir (ATV)</td>
<td>400 mg/ once daily</td>
<td>7</td>
<td>60-68</td>
<td>4.5</td>
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<tr>
<td>Darunavir</td>
<td>600 mg/ twice daily 800 mg/once daily</td>
<td>15</td>
<td>37</td>
<td>1.8</td>
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<tr>
<td>Fosamprenavir (FOS-APV)</td>
<td>1400 mg/twice daily</td>
<td>7.7</td>
<td>Not established</td>
<td>0.84</td>
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<tr>
<td>Indinavir (IDV)</td>
<td>800 mg/ every 8 hours</td>
<td>1.4- 2.2</td>
<td>30</td>
<td>2.9</td>
</tr>
<tr>
<td>Lopinavir and Ritonavir (LPV/ RPV)</td>
<td>400 mg/100 mg/twice daily/800 mg/100 mg/once daily</td>
<td>4.4/6.1</td>
<td>No data available</td>
<td>No data available</td>
</tr>
<tr>
<td>Nelfinavir (NPV)</td>
<td>1250 mg/ twice daily 750 mg/ thrice daily</td>
<td>3.5-5</td>
<td>80*</td>
<td>6</td>
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<tr>
<td>Ritonavir (RPV)</td>
<td>600 mg/ twice daily</td>
<td>3.5</td>
<td>64</td>
<td>3.9</td>
</tr>
<tr>
<td>Saquinavir (SQV)</td>
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<td>13</td>
<td>4-10</td>
<td>3.8</td>
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<tr>
<td>Tipranavir (TPV)</td>
<td>500 mg/ twice daily</td>
<td>5.5-6</td>
<td>30*</td>
<td>6.9</td>
</tr>
</tbody>
</table>

(*Reported in animal studies)

Lineage [35]. It has been found that in addition to acting as latent reservoirs, macrophages have also been found to aid in the generation of elusive mutant viral genotypes by serving as the host for viral genetic recombination [36]. The secondary lymphoid tissue, testes, liver, kidney, lungs, gut and the CNS act as anatomical reservoirs for the HIV [37-39]. The eradication of the virus from such reservoirs is essential to achieve long term relief for the HIV/AIDS patients. Therefore, there is a great and urgent need to explore new approaches for developing nontoxic, low-dose treatment regimen that provide sustained release and effective eradication of the virus from the reservoirs, thereby eliminating the need for lifelong treatment.

**Nanotechnology in medicine**

Nanotechnology is the engineering of materials, and devices, at an incredibly small scale-between 1 and 100nm. Nanotechnology has exposed incredible applications in the healthcare sector. Nanomedicine is the use of nanostructured materials for preventive, therapeutic and diagnostic purposes [40]. It involves a large number of applications from targeted delivery to regenerative medicine including providing interfaces of nanomaterials with living human material and significantly improving conventional practices [41]. Major goals of nanomedicine in drug delivery are improving drug bioavailability and efficacy, achieving control of pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity and as well as overcoming obstacles due to low drug solubility, degradation, fast clearance rates, decreased biological activity and inability to cross biological barriers such as air-blood barrier and blood-brain barrier. Nanomedicine may also help...
Table 4: A summary of different nanomaterials used for anti-viral drug delivery.

<table>
<thead>
<tr>
<th>Nanomaterial</th>
<th>Antiretroviral drug</th>
<th>The method of testing</th>
<th>Cells/tissue/organs</th>
<th>Ref:</th>
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<td>Synthetic polymers</td>
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<td></td>
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<td>D4T</td>
<td>in vitro</td>
<td>Macrophages</td>
<td>[21]</td>
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<td>PBCA, MMA-SPM</td>
<td>D4T</td>
<td>in vitro</td>
<td>BMVECs</td>
<td>[97]</td>
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<tr>
<td>PBCA, MMA-SPM</td>
<td>AZT, 3TC</td>
<td>in vitro</td>
<td>BBMECs</td>
<td>[98]</td>
</tr>
<tr>
<td>PBCA, MMA-SPM</td>
<td>D4T, DLV</td>
<td>in vitro</td>
<td>HBMECs</td>
<td>[99]</td>
</tr>
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<td>AZT</td>
<td>ex vivo</td>
<td>Wistar rat skin</td>
<td>[40]</td>
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<td>AZT, 3TC</td>
<td>in vivo</td>
<td>Mice</td>
<td>[100]</td>
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<td>AZT</td>
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<td>AZT</td>
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<td>release study</td>
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<td>Macrophages</td>
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<td>HepLL</td>
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<td>Mice and dogs</td>
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<td>[110]</td>
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<td>in vivo</td>
<td>Mice</td>
<td>[111]</td>
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<td>AZT</td>
<td>in vitro</td>
<td>release study</td>
<td>[112,113]</td>
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<td>DPV</td>
<td>ex vivo</td>
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<td>macrophages</td>
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<td>EFV</td>
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<td>Monoc/macroph</td>
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<td>in vitro</td>
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<td>in vivo</td>
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<td>in vivo</td>
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<td>[128,129]</td>
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<td>[130]</td>
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<td>in vitro</td>
<td>macrophages</td>
<td>[131]</td>
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<tr>
<td>Mannosylated liposomes</td>
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<td>macrophages</td>
<td>[132]</td>
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in achieving non-invasive modes of delivering drugs to various parts of the body. Drug characteristics differ substantially with respect to chemical composition, bioavailability, molecular size, hydrophilicity, optimum concentration range (above or below which the drug may be toxic or non-beneficial) etc. Therefore, the design of new drugs can be challenging. The challenges of most conventional drugs include poor bioavailability, in vivo instability, solubility, intestinal absorption, sustained and targeted delivery to site of action, therapeutic effectiveness, side effects and plasma fluctuations of drugs. However, nanotechnology in drug delivery has been designed to address such challenges through the development and fabrication of nanostructures at submicron and nanoscale. Polymeric materials are mainly used and they have multiple advantages [22]. Nanostructured materials have the ability to protect drugs encapsulated within them from harsh environment in the gastrointestinal tract and target the delivery of the drugs to various areas of the body. These materials are also known to facilitate sustained release of drugs, proteins or genes. They are capable of delivering drugs that are hydrophobic; they can bypass the liver, thereby preventing the first pass metabolism of the incorporated drug. They can also increase oral bioavailability of drugs due to their specialized uptake mechanisms such as absorptive endocytosis and are able to remain in the blood circulation for a longer time. The sustained release feature enables drug delivery in a sustained and continuous manner leading to less plasma fluctuations thereby minimizing side-effects caused by drugs. Due to their nano size they are capable of penetrating into tissues and may be taken up by cells thus achieving efficient delivery of drugs to sites of action. Nanostructures were found to be effectively taken up 15-250 times more than the microparticles in the 1-10 μm range. Polymeric nanoparticles have also been known to effectively penetrate the blood brain barrier for management of various CNS related diseases. Research and development of new drugs are capital- and time-intensive. Therefore new drug delivery methods enable pharmaceutical companies to reformulate existing drugs thereby extend the life of products, enhancing their performance, improve their acceptability by increasing effectiveness, as well as increase safety and patient adherence, and ultimately reduce health care costs. Nanotechnology is strategic in developing drug delivery systems which can expand drug markets. Nanotechnology can be applied to reformulate existing drugs. Nanotechnology may also enhance the performance of drugs that are unable to pass clinical trial phases. It provides drug carriers, which may aid in efficient treatment and management of chronic diseases such as cancer, HIV/AIDS and diabetes mellitus [42].

**Nanotechnology approaches in HIV/AIDS management:** The existing nanoparticle-based delivery systems have greatly helped to enhance conventional treatment of HIV/AIDS and also in exploiting the progress in therapeutic strategies such as gene therapy, immunotherapy and vaccine development. The nanotechnology-based platforms for systemic delivery of anti retroviral drugs offer myriads of improvements over the conventional methods. Nanotechnology based therapeutics for HIV have advantages like sustained release, enhanced half-life, improved drug concentrations at target sites, fewer side effects and targeting concealed HIV in anatomically restricted sites. Nanotechnology-based systems improve treatment by maintaining the circulation of drugs at therapeutic concentrations for longer extent. Nanomaterials have also been shown to have therapeutic effects of their own and their great surface to volume ratio improves and alters the distribution of hydrophobic and hydrophilic drugs in tissues. Various nano materials have been found to inhibit viral replication in vitro and it is suggested that these effects are based on structural interference with viral assembly [43-45].

**Nanocarrier-based drug delivery systems**

The drug delivery systems which make use of nanocarriers usually comprise biocompatible and/or biodegradable materials that have components like synthetic proteins, lipids, polymers, inorganic materials, and/or a combination of these materials [46]. It would be desirable for the nanocarrier formulation to be non-toxic, biodegradable, biocompatible, stable, and exhibit improved pharmacokinetics and controlled release [47]. Nanoparticles are particulate dispersions of solid particles with a size in the range of 10-100 nm. Nanoparticles are comparable in size to virus or an antibody. Due to this they are capable of entering into smallest capillaries and thereby avoiding rapid clearance by phagocytes from the blood stream, which prolongs their life in circulation. They have been shown to be successful in penetrating a wide range of organs including the CNS. The nanoparticles have been used for delivery of conventional drugs [48], recombinant proteins, vaccines and nucleotides [49, 50]. They have been extensively used in cancer therapeutics, antimicrobial applications and for delivering vaccines, genes and proteins with excellent targeting efficiency. In nanosized drug delivery systems, drugs may be absorbed onto the particle surface or encapsulated in the core of the particles. The use of nanoparticle (NP) mediated drug delivery is advantageous due to high surface area to volume ratio which provides platforms for further modification and tunable size. The size of carrier controls the penetration of materials through the endothelium and further perfusion of the materials through tissues. Size of the delivery system is more than 200nm; it will be eliminated from circulation by the macrophages. The size ranging from 10 to 200 nm is of particular importance in drug delivery system due to their improved bioavailability. The unique properties of the nanoparticles make them very promising and a variety of nanostructures have been widely used, most of which are spherical, such as polymeric micelles, liposomes, calcium phosphate, gold, iron oxide, hydrogel nanoparticles and dendrimers. Hydrophobic drugs present a barrier for administration and to overcome this issue, Dimethylsulphoxide (DMSO), cyclodextrins etc. are used for solubilising or altering the chemical structure of such drugs. The excess use of cyclodextrin may limit the route of administration. Tablets or capsules that are very large in size are highly impractical. The use of nanoparticles is the best alternative to overcome all the limitations of conventional therapy. Nanoparticles enable the incorporation of both hydrophobic and/or hydrophilic drugs in their matrix [42] (Figure 7).

The use of nanoparticles in drug delivery enhances the drug activity. An efficient drug delivery system comprises a pharmaceutically active ingredient and an engineered NP of biological (lipids, polymers etc) or non biological (metals) origin [52]. The nanoparticle mediated drug delivery system...
Dendrimers are highly-branched polymers that are 2.5-10 nm in size [58]. These hyper branched polymers have a unique and controlled structure with topologic features that are ideal for biomedical applications. Various drugs can be incorporated into dendrimers either by covalent conjugation or electrostatic adsorption due to the presence of multivalent surfaces. The dendrimers can be loaded with drugs, by using the cavities present in their cores through hydrophobic interaction, hydrogen bonding or chemical linkage. Their surface can be fabricated with precise spacing of surface molecules and thereby conjugating the targeting moieties. The unique surfaces of dendrimers may be designed with functional groups in such a way that they resist trans-cellular, epithelial or vascular permeation [60].

**Polymeric nanoparticles:** Polymeric nanoparticles are solid, colloidal particles made from macromolecular substances. Their sizes vary from 10 to 1000 nm. Polymeric nanoparticles (NPs) for controlled drug delivery have shown significant therapeutic potential, since drug can be dissolved, entrapped, adsorbed, attached or encapsulated inside the nanoparticles. Nanospheres or nanocapsules are commonly used in drug delivery. Polymeric nanoparticles can protect the drug from degradation (physical

could be an engineered nanoparticle or drug itself formulated in the nano scale and functioning as its own carrier. From an industrial point of view, nanoparticles for pharmaceutical applications are formulated within the size range of 100-250nm or in the suspended form (nano emulsions). With an effective drug delivery system, the drug is released in the diseased target site in the body in a controlled manner, which depends on the competent delivery structure of the carrier molecules. This is possible by biodegradable nanoparticles. So an ideal nanoparticle drug delivery system is one which does not interact with the encapsulated material and does not undergo any chemical change. They possess fast biodegradability, drug delivery and bio-compatibility [53]. It is very challenging to target a drug into the central nervous system by conventional mechanism. It is also due to the blood brain barrier (BBB). Although a life-supporting protective mechanism of the brain, its existence strictly restricts the delivery of most drugs to the brain because they do not cross the BBB in sufficient amounts. Therefore, therapeutic efficiency is diminished because systemic administration of the drug does not lead to an effective concentration in the brain. Pharmaceuticals are notable to cross the blood brain barrier as it is specifically tight at the interface with the brain astrocytes. However the barrier properties may be compromised intentionally or unintentionally by drug treatment allowing passage of nanoparticles [54]. The delivery of drugs by nano carrier has been meticulously studied and reviewed. For extended flow, the size of the carrier should be small enough (<150 nm) in order to flee from being captured and subsequently removed by the resident macrophages in the reticuloendothelial system, such as the liver and spleen [55]. In this regard materials with diameters between 10 and 150nm are chiefly useful due to their better bioavailability and their ability to take advantage of the enhanced permeation and retention (EPR) effect [56].

A variety of nanostructured materials have been used for application in drug delivery, some of the commonly used structures include dendrimer, polymeric nanoparticles, nanocrystal, inorganic nanoparticles, metal based nanoparticles, liposomes etc. A range of functionalities can be incorporated in these nanoparticles which facilitates drug encapsulation within the nanoparticle complex. The surface chemistry of nanoparticles also allows the conjugation of cell-specific ligands for targeted delivery. Coatings to the surface of nanoparticles have been found to increase circulation time for enhanced bioavailability. The incorporation of specific materials on the surface or in the core of nanoparticle enables the storage of a therapeutic agent until the target site is reached which allows the controlled delivery of therapeutics to the target cells [42]. Site specific targeting of nanoparticles can be achieved by avoiding clearance by the reticuloendothelial system (RES), utilizing improved retention and permeation effect. Two types of approaches are applied for using nanoparticle as carrier for drug –

- **Surface bound:** The drug molecules are adsorbed on the surface of nanoparticles
- **Core bound:** In this method, the drug particles are encapsulated inside the nanoparticle and carried to the target.

Drugs can either be loaded onto preformed nanoparticle solution or by adding them to the reaction mixture during the fabrication process. Nature of the interaction between the drug molecules and nanoparticles may be chemical or physical adsorption in which the drug adsorbed on to the surface, without any binding or interaction at all. The type of interaction depends on the chemical structure of the drug and the carrier, the conditions of drug loading etc [57] (Figure 8).

**Dendrimers:** Dendrimers are highly-branched polymers with unique and controlled structure with topologic features that are 2.5-10 nm in size [58]. These hyper branched polymers have chains extending from a center, resulting in a nearly-perfect three dimensional geometric pattern. They are characterized by their polyfunctional core, interior layers, and multivalent surface. The presence of polyfunctional core allows the attachment of several biological moieties. The small size of their multiple terminal groups, narrow molecular weight distribution and ease of incorporation of therapeutics and targeting moieties make them attractive for drug delivery. They can be synthesized from synthetic or natural sources like amino acids, sugars and nucleotides. A number of dendrimer families have been already reported [59], and among them, polyamidoamine (PAMAM) and poly (propyleneimine) (PPI) families have been most widely used for biomedical applications. Various drugs can be incorporated into dendrimers either by covalent conjugation or electrostatic adsorption due to the presence of multivalent surfaces. The dendrimers can be loaded with drugs, by using the cavities present in their cores through hydrophobic interaction, hydrogen bonding or chemical linkage. Their surface can be fabricated with precise spacing of surface molecules and thereby conjugating the targeting moieties. The unique surfaces of dendrimers may be designed with functional groups in such a way that they resist trans-cellular, epithelial or vascular permeation [60].

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**Figure 7** The key properties that determine the role of drug carriers [51].
stability during storage and in biological fluids), enhance its transport and distribution (through modification of surface with inserted ligands such as antibodies, surfactants, polymers etc), allow sustained and controlled release and also improve the plasma half-life of the entrapped drug [61]. Polymeric nanoparticles can be engineered for any application by optimizing properties like particle size and surface charge. Both synthetic and natural polymers can be used for preparing drug delivery vehicles. They include natural polymers, albumin, gelatin, alginate, collagen, chitosan and synthetic polymers like polyesters, their copolymers polyacrylates and polycaprolactones.

Inorganic nanoparticle: Silica or alumina are two important classes of ceramic nanoparticles for biological applications. Other inorganic nanoparticles with varying size, shape, and porosity are metals, metal oxides and metal sulfides. Mesoporous silica nanoparticles in addition possess stable mesoporous structures, large surface areas, tunable pore sizes and volumes, and well-defined surface properties which aid inside-specific delivery of drug. Quantum dots, polystyrene, magnetic, ceramic and metallic nanoparticles are other important inorganic nanoparticles that have a central core composed of inorganic materials with fluorescent, magnetic, electronic and optical properties [62,63].

Metal based nanoparticle: Gold nanoparticles (Au NPs) are a suitable platform for development of an efficient delivery vehicle because of the ease of synthesis, functionalization, and biocompatibility. Magnetic nanoparticles of 10–20 nm size with a Fe2+ and Fe3+ core surrounded by dextran or PEG molecules have been used in biomedical field to label biomolecules in bioassays and as contrast agents for MRI etc. They are also used for active targeting in in vivo or for in vitro diagnostics after surface functionalization [62].

Liposomes: Liposomes are spherical bilayered vesicles composed of natural or synthetic amphiphilic lipid molecules [64,65]. They have superior biocompatibility as they are basically analogues of biological membranes. The amphiphilic nature of Liposomes, their biocompatible and biodegradable composition, their simplicity of surface modification, and their unique ability to encapsulate both hydrophilic and hydrophobic therapeutic agents make them excellent vehicle for therapeutic agents. They prevent drugs from degradation, reduce toxicity and side effects and enable site specific targeting. The aqueous interior of liposome may be used to encapsulate hydrophilic drug and the phospholipid membrane may encapsulate hydrophobic compounds. Liposomes are made from naturally occurring phospholipids, so they are free from unwanted toxic or antigenic reactions. They are coated with biocompatible moieties like Polyethylene glycol (PEG) to prolong their circulation time [65]. This type of polymeric coating can also be functionalised in order to provide site for targeting of the liposome to different regions. Phosphatidylycholine and phosphatidyl-ethanolamine are two important classes of compounds for liposomal preparation. Passive diffusion or active extrusion is the possible mechanism of liposomal drug delivery. Due to the poor bioavailability, liposomes are easily cleared by the reticuloendothelial system (RES) (Figure 9).

Solid Lipid Nanoparticles: Solid lipid nanoparticles (SLNs) are nanocrystalline structures made of fatty acids that are solid or semisolid at room temperature. SLNs are a comparatively stable colloidal carrier system in which melted lipid is dispersed in an aqueous surfactant by high-pressure homogenization or microemulsification [66]. SLNs can be made from a wide variety of high melting-point lipids using number of methods [66]. Over the years, they have emerged as a variable substitute for liposomes as drug carriers. They are a new generation of submicron-sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid. SLNs offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interfaces. They are noted for their potential to improve performance of pharmaceuticals, nutraceuticals and other materials. Their hydrophobic core usually entraps the dissolved or entrapped drug [67]. SLNs exhibit certain potential advantages over other polymeric nanoparticles. They have been shown to be taken up by brain and exhibit the least toxicity due to the biodegradable nature of the constituent materials [68]. Their nano-scale dimensions allow them to cross various anatomical barriers and also to bypass the liver. They have high drug entrapment efficiency and render the drug more stable in their lipid matrix and provide a controlled release of the entrapped drug. Their production can be scaled up with excellent reproducibility. SLNs also offer greater drug stability and better control over drug-release kinetics compared to nano emulsions [69]. They are being promoted for intravenous applications. Surface coating of SLNs with hydrophilic polymers or surfactants, such as poly (ethylene glycol) (PEG) minimizes their uptake in liver cells and results in improved bioavailability. Stearic acid-PEG 2000 has been used for their stearic stabilization, whereas the use of complex lipids (mono-, di-, triglycerides of different chain lengths) results in an increased loading efficiency [70,71] (Figure 10).

SLNs incorporate the advantages and eliminate the drawbacks of several colloidal lipid carriers. The drug loading capacity of conventional SLN is limited by the solubility of the drug in the lipid matrix, the structure of the lipid matrix and the polymeric state of the lipid matrix. If the lipid matrix consists of similar molecules (i.e. tristearin or tripalmitin), a perfect crystal with few imperfections is formed. The incorporated drugs are located between fatty acid chains, between the lipid layers
and also in crystal imperfections; highly ordered crystal lattice cannot accommodate large amounts of drug. Hence the use of more complex lipids can be considered desirable for higher drug loading [66].

**Nanostructured lipid carriers (NLC):** NLC are developed to solve the potential difficulties with SLNs in order to increase the drug loading and prevent drug expulsion. Three models have been proposed for the NLCs in the first model, different lipids (like glycerides) composed of different fatty acids are mixed. The use of spatially different lipids creates larger distances between the fatty acid chains of the glycerides and increases general imperfections in the crystal and thus provides more room for accommodation of drug molecules. High drug loading can be achieved by mixing solid lipids with small amounts of liquid lipids (oils). This model is called imperfect type NLC. Drugs exhibiting greater solubility in oils compared to solid lipids can be dissolved in the oil thereby protecting them from the surrounding solid lipids. These types of NLC are called multiple types NLC, and are analogous to w/o/w emulsions since it is an oil-in-solid lipid-in-water dispersion. Drug expulsion is caused by continuous crystallization or transformation of the solid lipids. This can be prevented by the formation of the amorphous type NLC. Here the particles are solid but crystallization upon cooling is avoided by mixing special lipids like hydroxyl octacosanyl, hydroxyl stearate and isopropyl myristate. The NLCs have mainly been investigated in the topical and dermatological formulations [66], in the delivery of clotrimazole [72], ketoconazole [73], other antifungal imidazoles [72], and ascorbyl palmitate [74] (Figure 11).

**Lipid-Polymer hybrid nanoparticles:** Lipid polymer hybrid nanoparticles are a new class of core–shell-type hybrid systems that typically consist of a polymeric core, coated with single or multiple layers of lipids that constituting the shell. The successful combination of lipids and polymers opened up new systems with great applications in science, medicine, and technology. The LPNs combine the biomimetic properties of lipids and mechanical robustness of the polymeric core to yield a theoretically superior delivery system. They have been extensively used for delivery of both hydrophilic and hydrophobic drugs. They tend to carry hydrophilic drugs inside the polymer and hydrophobic drugs within the lipid bilayer. In this system, the therapeutics are usually entrapped in the polymer core. The lipid layer confers biocompatibility and the PEG outer layer aids in prolonging circulation time and provides steric stabilization. The liposomal layer minimises the leakage of therapeutics and provides sustained release. Various bioactive molecules such as drugs, genes, proteins, etc can be entrapped, adsorbed, or covalently attached in the hybrid system. The LPNs have the ability to carry moderately hydrophilic drugs with high encapsulation efficiency and loading yields. These NPs can be tuned to achieve desirable sustained drug release profile and differential targeting of cells. They have been known to have excellent serum stability. These hybrid NPs can also be used as adjuvants for vaccination [76]. The LPNs can be easily synthesised by self-assembly of the components, hence facilitating cost-effective mass production of these delivery systems [77].
Methods of preparation of lipid-based drug delivery systems

SLNs consist of solid lipid, emulsifier and water/solvent. The lipids used may be triglycerides (tri-stearin), partial glycerides (Imwitor), fatty acids (stearic acid, palmitic acid), and steroids (cholesterol) and waxes (cetyl palmitate). Various emulsifiers and their combinations (Pluronic F 68, F 127) have been utilised to stabilize lipid dispersion. A combination of emulsifiers may reduce particle agglomeration [78]. The lipid matrix of SLNs is made from physiological lipids which decreases the danger of acute and chronic toxicity, thereby acquiring a clear advantage over other means of drug delivery. The choice of the emulsifier depends mainly on the route of administration.

Homogenization: High shear homogenization technique was initially used for synthesizing solid lipid nanodispersions [79]. Microparticles that can hamper its quality may be present in the dispersion. High-speed homogenization method is used to produce SLN by melt emulsification [80], it is a variant of the high shear homogenization method. Olbrich et al., investigated the influence of different processing parameters, including emulsification time, stirring rate and cooling condition on the particle size and zeta potential. It was found that higher stirring rates did not significantly change the particle size, but slightly improved the polydispersity index [81].

Hot homogenization: Hot homogenization process utilizes temperatures above the melting point of the lipid and is similar to the homogenization of an emulsion. High-shear mixing device (like silversion-type homogenizer) is used to obtain a pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature). The quality of the pre-emulsion affects the quality of the final product and it would be desirable to obtain droplets in the size range of a few micrometers. High pressure homogenization of the pre-emulsion is done above the lipid melting point. Usually lower particle sizes are obtained at higher processing temperatures because of lowered viscosity of the lipid phase [82]. This might accelerate the drug and carrier degradation. Better products are obtained after passing several times through the high-pressure homogenizer (HPH) (typically 3-5 passes). High pressure processing always increases the temperature of the sample (approximately 10° at 500 bar) [83]. In most cases, 3-5 homogenization cycles at 500-1500 bar will be sufficient. High kinetic energy of the particles may lead to an increase of the particle size due to particle coalescence.

Cold homogenization: The cold homogenization of solid lipid is considered to be similar to milling of a suspension at elevated pressure. Effective temperature regulation is needed to ensure the solid state of the lipid during homogenization [83]. Cold homogenization has been developed to overcome temperature mediated accelerated degradation of the drug and partitioning and hence loss of drug into the aqueous phase during homogenization. Uncertain polymorphic transitions of the lipid due to complexity of the crystallization step of the nanoemulsion lead to several modifications and/or super cooled melts. The first preparatory step is the same as in the hot homogenization procedure and includes the solubilisation or dispersion of the drug in the lipid melt. The drug containing melt is then cooled rapidly using dry ice or liquid nitrogen to promote homogenous drug distribution in the lipid matrix. The drug containing solid lipid is then pulverized using ball/mortar milling. Particle sizes obtained by this process are typically in the range of 50-100 microns. The SLNs are dispersed in a chilled emulsifier solution and chilled processing aids in particle milling by increasing the fragility of the lipid. The dispersion is subjected to high pressure homogenization at or below room temperature with appropriate temperature control. However, when compared to hot homogenization, cold homogenized samples possess larger particle size and a broader size distribution. Though cold homogenization minimizes the thermal exposure of the drug, it does not eliminate the need for melting of the lipid/drug mixture in the initial step.

Ultrasonication or high speed homogenization: SLN can also be developed by high speed stirring or sonication. The equipment required for this process is commonly available. Broader particle size distribution ranging in the order of micrometers is a potential drawback of this method. This leads to physical instabilities likes particle growth upon storage. Potential metal contamination is also a major problem in ultrasonication. Studies have been performed by various research groups using a combination of high speed stirring and ultrasonication performed at high temperature for making a stable formulation [74].

Solvent emulsification/evaporation: Nanoparticle dispersions can be produced by precipitation in o/w emulsions. The lipophilic material is dissolved in a water-immiscible organic solvent that is then emulsified in an aqueous phase. Upon evaporation of the organic solvent, nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. Lipid nanoparticles of ~ 25 nm have been obtained using this method [84] (Figure 12).

Microemulsion: Gasco and co-workers developed SLN preparation techniques based on the dilution of micro emulsions. They are made by stirring an optically transparent mixture at 65-700 rpm. The mixture is typically composed of a low melting fatty acid (stearic acid), an emulsifier (such as polysorbate 20, polysorbate 60), co-emulsifiers (sodium monooleylphosphatate) and water. The hot microemulsion is dispersed in cold water in the ratio range of 1:25 to 1:50 under stirring. The dilution process is determined by the composition of the microemulsion. According to the literature [85,86], a droplet structure is already contained in the microemulsion and therefore, no energy is required to achieve submicron particle size. Polymer nanoparticles are typically produced with solvents which distribute very rapidly into the aqueous phase (acetone), while larger particle sizes are obtained with more lipophilic solvents [87]. The hydrophilic co-solvents of the microemulsion may also play a similar role in the formation of lipid nanoparticles as acetone in the formation of polymer nanoparticles [74].

Supercritical fluid technique: This is a relatively new technique for SLN production and has the advantage of being solvent-less processing technique [88]. There are several variations in this technology platform for preparation of powder and nanoparticle. SLN can be prepared by the rapid expansion of supercritical carbon dioxide solutions (RESS) method. Carbon dioxide (99.99%) is the good choice as a solvent for this method [89].
Spray drying method: It is an alternative procedure to lyophilisation to produce a drug powder/product from an aqueous SLN dispersion. It is a cheaper method than lyophilisation. This method may cause particle aggregation due to high temperature, shear forces and partial melting of the particle. The use of lipid with melting point >70° is recommended for spray drying [90].

Double emulsion method: For the preparation of hydrophobic drug loaded SLN, a novel method based on solvent emulsification-evaporation has been used [91]. Here the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion (Figure 13).

Synthesis of polymeric nanoparticles

Polymeric nanoparticles have proved to be promising carriers for therapeutic agents. They are attractive vehicles for drug delivery as they can be engineered to prevent degradation of the drug, control release profiles and to achieve targeted delivery thereby lowering the dose-dependent toxicity of the drug. Usually the chosen polymers are biocompatible and biodegradable.

The most commonly used nanoparticles of polymer are nanospheres and nanocapsules. In nanospheres, the drug is loaded in the matrix whereas in nanocapsules, the drug is encapsulated within a thin polymer layer. Various methods of preparation are used for synthesis of polymeric nanoparticles and they include

Nanoprecipitation: It is a single step method with high reproducibility and it results in the formation of uniform-size nanoparticles. In this process the polymer and the drug are dissolved in a water miscible organic solvent. This mixture is then added to an aqueous solution under moderate stirring causing nanoparticles to precipitate instantly due to solvent diffusion into the aqueous matrix. The solvent is then evaporated at reduced pressure and an aqueous suspension of nanoparticles is thus obtained. Nanocapsules are also prepared using the same method. In addition oil is added to the solution of the polymer in order to form an inner oily cavity to accommodate the drug (Figure 14).

Salting out: In this technique two phases that are mixed together to form an oil-in-water emulsion. The oil phase is essentially the polymer solution containing the therapeutic agent in a water miscible solvent. The aqueous phase is a solution or gel containing a colloidal stabilizer and a salting out agent at high concentration which hinders the solvent diffusion. The oil-in-water (o/w) emulsion thus formed is then diluted such that the concentration of the salting-out agent is lowered below a certain threshold value; this enables the organic solvent to rapidly diffuse into the aqueous phase and result in the formation of nanoparticles. Then, the organic solvent is removed by evaporation at reduced pressure. Repeated washing steps are then needed to remove the salting-out agent.

Emulsification diffusion: In the emulsification diffusion method, a partially water miscible solvent and water are mutually saturated. Following which, the polymer and the therapeutic agent are dissolved in the saturated solvent and the stabilizers are dissolved in water, resulting in the formation of a stable emulsion. In the final step, sufficient amount of water is introduced and the solvent diffuses into the aqueous phase resulting in the formation of nanoparticles.

Emulsification evaporation: In this technique the polymer and the therapeutic agent are dissolved in a volatile water immiscible organic solvent. Then this mixture is emulsified with an aqueous phase containing a stabilizer resulting in the formation of o/w emulsion. Ultrasonication or homogenization is carried out to break the emulsion droplets and formation of nanoparticles.

Double emulsion: There are two main types of double emulsions, they are - water-oil-water (w/o/w) and oil-water-oil (o/w/o). In double emulsions the droplets of dispersed phase contain smaller components of another dispersed phase, hence they are commonly referred as “emulsion of emulsions”. They are capable of encapsulating both hydrophobic and hydrophilic molecules either individually or together. Double emulsion solvent evaporation technique is commonly used for preparation of nanoparticulate drug delivery vehicles. In this process, the aqueous phase containing the therapeutic agent is dispersed in an oil phase of the polymer/drug to form the primary emulsion. This primary emulsion is dispersed in outer aqueous phase containing suitable stabilizer to form double emulsion. Evaporation of the organic phase results in the formation of the nanoparticles.
Synthesis and fabrication of lipid - polymer hybrid nanoparticles

Lipid polymer hybrid nanoparticles are usually prepared through two distinct techniques. One technique consists of two-step process wherein the polymer core and lipid shell are separately synthesised and then mixed together to facilitate the formation of hybrid entities. The other technique involves a single step process in which nano precipitation and self-assembly method are employed to prepare hybrid nanoparticles.

Two-step method: The two-step method is the most commonly used method for the development of lipid-polymer hybrid nanoparticles. Preformed polymeric nanoparticles are mixed with preformed lipid vesicles in the conventional two step method. In this technique the lipid vesicles are adsorbed on to the polymeric nanoparticles through electrostatic interactions. They are generally prepared by mixing liposomes and polymeric nanoparticles (PNPs) in which a lipid bilayer or lipid multilayer covers the surface of the polymeric core. The polymer core particles are prepared using any of the several methods available. After preparing the polymeric core nanoparticles, liposomes are prepared by techniques like thin - film hydration and sonication. Mixing of the polymeric nanoparticles and the liposomes followed by vigorous vortexing result in the fusion of liposomes with the polymeric core. The mechanism behind the fusion of the liposomes and the polymeric core particles may be due to an electrostatic interaction between the polymer core and the liposomes. It is important therefore to choose polymer and lipid systems that are compatible with this method [92]. Core-shell nanoparticles find applications in various areas due to their improved physical and chemical properties. Numerous synthesis procedures have been employed for the manufacture of core shell nanoparticles. LPNs generally forms core-shell structure which exhibit high structural integrity, stability during storage, controlled release capability attributed to the polymer core, high biocompatibility and bioavailability owing to the lipid and lipid–PEG layers [93,94]. Large scale production of LPNs can be carried out using soft lithography particle moulding [95], and by continuous nanoprecipitation in a microchannel (Figure 15).

One-step method: One step synthesis involves preparation of lipid-polymer hybrid nanoparticles with a lipid monolayer shell (Figure 16). This technique involves dissolving free polymers and hydrophobic drugs in a water miscible organic solvent and the lipid or lipid-PEG conjugates are introduced into an aqueous solution. The solubilisation of phospholipids in the aqueous solution is facilitated by adding a small amount of water miscible organic solvent to the aqueous solution. The polymer solution is then added drop wise to the lipid-aqueous dispersion. Upon mixing the organic solvent diffuses into the aqueous solution, leaving polymer to precipitate into nanoparticles. The surface of the nanoparticle acts as site for self-assembly of lipids and lipid-PEG. The self-assembly of lipids are promoted through hydrophobic interactions which reduce the free energy of the system. The hydrophobic tails of the lipids project towards the hydrophobic polymer core and the hydrophilic head will face the external aqueous environment. The lipid-PEG conjugate stabilises the self-assembly process by inserting its lipid moiety into the lipid monolayer and PEG moiety outside the lipid monolayer and thereby providing a stealth corona to the nanoparticles. The self-assembly of lipids and lipid-PEG conjugates may be promoted by elevating temperature above the phase transition temperature of the lipids [96]. This is relatively quick and cost effective compared to other existing processes.

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CONCLUSIONS

AIDS and HIV infection has become a life threatening pandemic. The ever-altering nature of the virus and its infection
cycle has created many hurdles for creating an efficient drug delivery system. Several nanocarriers have been studied to enhance the bioavailability, circulation time and therapeutic potential of these drugs. Several types of polymeric, liposomal and hybrid nanoparticles have been synthesised and reported. Such nanosystems have shown great potential in terms of controlled/sustained release of drugs, improved bioavailability, increased circulation time and superior targeting ability. Such nanoformulations can be expected to bring considerable relief to AIDS patients and their caregivers. These systems provide a very bright outlook for the future of drug delivery for HIV/AIDS treatment and these formulations could be modified and extended to deliver drugs for numerous other disease that prevail all over the world.

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