RESEARCH
ENVIROSAGED
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AIDS is a world-wide pandemic with massive adverse health and financial implications, particularly in all the developing countries. It is a chronic, progressive syndrome characterized by intense viral replication and profound immunosuppression, resulting in the life threatening opportunistic infections. The root cause of the mentioned disease with chronic infection and ultimate fatal outcome is the human immunodeficiency virus (HIV) which has originated from simian immunodeficiency virus (SIV) [1-4]. This blood-borne and sexually transmitted virus exploits CD4 and a chemokine coreceptor for entering to the host cell which ultimately infects and reproduces in helper T cells and macrophages. Ultimately deficiency generates in host’s immune system as a consequence of virally stimulated attrition of CD4 T cells exhibiting in terms of opportunistic infections and manifestations [5-7].

Antiretroviral treatment consisting viral entry inhibitors, reverse transcriptase and viral protease can manage the viral duplication up to some extent, restore the optimum immune system with delay in AIDS progression but the infection could not be eliminated or cured completely. The virus laden dendritic cells, macrophages and CD4 T cells infect the provincial lymphoid tissues [4-6]. Direct contact of virus loaded cells and liable macrophages or CD4 T cells within the lymph node germinal centers leads to a quick increment in viral duplication in only few days of exposure. Local inflammatory responses induced by the virus facilitate viral duplication with development of an acute phase of viremia, leading to dissemination of infection to further lymphoid tissues and organs [2, 3].

The current AIDS therapy with combinations of antiretroviral drugs has highly reduced the HIV-1 related morbidity and mortality, provided the viral load observed in blood plasma could be maintained at undetectable limits [6-8]. However, the major limitation in complete eradication of infective virus is the generation of resistant mutations within the virus and the localization of HIV in certain inaccessible compartments of the body such as the CNS, the lymphatic system and within the macrophages which cannot be reached with the current treatment regimens [9-11]. These sites cannot be targeted by the majority of drugs in the therapeutic concentrations required and the drugs also cannot be maintained for the desired duration at the site of HIV localization.
The lower drug concentrations and minimum residence time at the specific sites of action for therapeutic efficacy, exhibits significantly for non-elimination of HIV from these reservoirs, as well as the development of multidrug resistance against the ARVs [13]. The severe side effects associated with ARV therapy can therefore be attributed to the subsequent higher doses essential for achieving a therapeutic effect, due to the inadequate drug concentrations at the site of action, and/or the poor bioavailability of several ARV drugs [1, 7, 9]. Current strategies investigates to overcome these limitations including 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 leading to better drug utilization [23]. NDDS also provide other benefits associated with such systems.

It’s a challenge in current research scenario to find new treatment strategies as the currently available regimens for the treatment of AIDS involve long term treatment up to several months with frequent dosing. Antiviral agents like stavudine have a very short half-life leading to rapid elimination from the body [266, 267]. This leads to a poor bio-distribution with inefficient and incomplete cellular uptake of the drug for providing therapeutic benefits. Neuropathy and lactic acidosis are the dose dependent side effect of the drug and reduction in total administered dose may reduce the severity of the drug toxicity [267]. In recent years significant attention has been made on the development of modified release drug delivery systems, as the population of patients with chronic complications has increased. These conditions require taking drug for a prolonged duration and/or multiple medicines simultaneously, which ultimately exhibits to non patient compliance [5-8]. The problem may briskly aggravate in case of drugs with shorter biological half-life. Modifying the drug release provides the improved therapeutic efficacy and bioavailability with reduced dosing frequency. However the basic objective of a modified release drug delivery system is to optimize the delivery of medication to avoid the undesired fluctuations in the in-vivo environment where the drug release takes place [132-134]. In order to fulfill the need of a long-term treatment with an anti HIV agent, it is imperative to investigate and formulate a controlled or long acting drug delivery systems to maximize the overall therapeutic benefits.

The theme of the controlled drug delivery systems (CDDS) is to achieve a steady-state plasma level or tissue level with in the therapeutically effective and non-toxic range for an extended period of time. This ideal delivery system requires targeting the drug to
the peculiar organ or tissue for controlling the rate of the drug release to the targeted site in a specific manner [133]. It is a well known fact that the conventional dosage forms neither maintain the drug blood levels within the therapeutic range for an extended period of time nor do they have any provision to control the release of the drug for a specific period of time. For maintaining drug levels within the therapeutic range for a prolonged duration of time, a drug may be administered repetitively using a calculated dosing interval [132-134]. This causes several significant problems including large fluctuations of drug concentrations in the blood exhibiting saw tooth release patterns, frequent dosing for drugs with short biological half-life and patient non-compliance.

Controlled drug delivery systems (CDDS) could eradicate the mentioned complications. CDDS could be precisely defined as the rationalization of drug administration in accordance to the need of condition so that an optimum amount of the drug is used to cure or control the condition within minimum possible time. CDDS provides an extended drug action at a controlled rate by maintaining a constant and effective drug level in the body to provide therapeutic efficacy [139, 140]. It exhibits its potential utility through bearing numerous advantages over conventional dosage forms, i.e. minimized side effects and drug accumulation, reduced blood level fluctuations and total drug amount and last but not the least improved patient compliance with enhanced therapeutic efficacy.

Through the last two decades, not only significant advances in CDDS have been attained but also commercial success and benefits have been availed by several products. Out of various routes of administration parenteral route provides direct entry of the drug molecule into the systemic circulation delivering complete medication to the patient [153, 159]. The potential drawback of short duration of action obtained by IV/IM/SC injection of drug may be accounted for by developing prolonged release depot formulation [40].

Despite the competition from alternative modalities, demand for prolonged release parenteral drug delivery systems has grown exponentially in recent years with a rise of 9.5% annually [43]. Generally parenteral depot systems could minimize the undesired effects by maintaining a constant “infusion like” blood plasma level time profiles. Some other specific potential benefits of CDDS include dose reduction, avoidance of peaks and valleys as well as the enhancement of patient compliance by minimizing the frequency of application [150]. A prompt and quick drug response through parenteral drug administration especially intravenous infusion, exhibits easy and complete absorption of...
drug in systemic circulation. But this requires direct medical supervision and critical observation of the patient. Parenteral drug administration through intramuscular route has a fairly rapid onset of action followed by a rapid decline in the blood-drug level leading to relatively short duration of therapeutic response [151-153].

Considering these shortcomings associated with various modes of parenteral drug administration, injectable depot formulation puts the potential to provide the solution. Parenteral depot formulations can overcome these problems by controlling the drug release over a predetermined time span [40]. These systems can be designed by various approaches as increasing the viscosity of the vehicles, use of water immiscible vehicles, aqueous or oily suspensions, emulsions, salt formation, complexes of drugs, liposomes, encapsulation or co-administration of a vasoconstrictor.

Out of these various means of developing prolonged release parenteral preparations, the application of biodegradable polymeric microspheres is the most suitable one. Drug laden parenteral microspheres are an emerging class of formulation that imparts prolonged residence of therapeutics in the body with significant therapeutic adherence and patient compliance [154]. These polymers have been approved for human use as surgical sutures, implantable devices, and drug delivery systems by the US Food and Drug Administration (USFDA) [155-157].

Biodegradable polymeric microspheres encapsulating ARVs will not only deliver a extended dose in vivo for prolonged duration of time, usually in order of days to weeks to months primarily owing to low degradation rates of the biodegradable polymers but 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 [40, 44, 45]. ARV loaded polymeric drug carriers for targeting to the macrophages 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 [76, 93].

The application of parenteral microspheres formulated using biodegradable polymers to deliver small molecules and macromolecules like proteins, using multiple routes of administration have been studied and successfully used for the treatment of a variety of disease states [150-153]. Some of the advantages of microspheres as drug delivery devices include enhanced stability of protein therapeutics, continuous and controlled drug release, reduced dosage, decrease in systemic side effects, reduced
possibility of dose dumping, reduced frequency of administration; therefore increased patient compliance [154, 155].

Passive targeting of drugs to the MPS by biodegradable polymeric microspheres exhibiting narrow range of particle size distribution might be one of the very appropriate drug carriers for achieving therapeutic drug concentration for extended period of time with minimized chances of drug resistance and cellular toxicity [171]. 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 [179]. It will 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.

Stavudine is available as immediate release tablets which could be given in a dose of 60-80 mg/day [236]. Current research provides the applicability of long acting parenteral microspheres of stavudine in case of failure of highly active antiretroviral therapy (HAART) treatment including the combination therapy regimen. A randomized trial for “Salvage therapy” of stavudine (d4T) has shown that in heavily pre-treated patients, the antiviral efficacy may be associated with the recycling of antiretroviral agents that have been utilized previously [9, 10]. In consonance with the above statement, a recently completed phase II trial revealed that stavudine treatment resulted in delayed progression of clinical disease in patients who had previously been treated with other antiretrovirals. Salvage therapy with stavudine can diminish viral loads to undetectable levels whereas stavudine monotherapy can stabilize CD4 counts and weight loss in heavily pre-treated individuals [11].

Present research investigates the formulation of stavudine loaded microspheres from different grades of biodegradable polymers as a depot and targeted systems for parenteral delivery which will not only provide the patient compliance by reducing the dosing frequency and providing the maximum therapeutic benefits via scavenging of HIV hosted in macrophages but also avoid the plasma level fluctuations of the drug [147, 148].

Prolonged release of stavudine facilitates reduction in symptoms of HIV infection and delay AIDS progression by reducing viral load to undetectable levels. Presenting the drug in the form of parenteral depot formulation and targeted systems [147] will provide an edge 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
Research Envisaged

with damaged hepatic or gastric systems due to complications associated with HIV infections; which are the major indications associated with the disease [266].

The research also explains the feasibility of simultaneous therapy including long acting parenterals and targeted administration of the d4T loaded biodegradable polymeric microspheres for potentially effective and prolonged therapeutic regimen. The therapy will not only provide the stable and extended release of drug but also eradicate the hidden HIV hosted by macrophages [60, 269-271]. The mentioned regime will potentially enhance the clinical efficacy with patient compliance; renewing new hopes for complete cure and improved quality of life in patient with AIDS.

The modified release dosage forms for oral administration i.e. tablets and multiparticulate systems of stavudine were also developed. Matrix tablet is the simplest and most cost-effective method of fabricating a modified release dosage form. Development and evaluation of a typical matrix formulation consisting drug with one or more polymers, excipients such as fillers, a glidant and a lubricant was investigated. In comparison to unit dosage forms pelleted systems offer benefits of homogeneous distribution and better absorption throughout the GIT, increased residence time with lesser susceptibility to dose dumping. Sustained release beadlets of the drug has offered benefits like patient compliance due to single daily dosing, lower incidence of dose dumping and decrease in pill burden [27, 28]. The processing of controlled release beadlets was started from development of pellets through extrusion-spheronization technique, followed by functional coating of suitable polymer using suitable process variables for controlling the release of stavudine for prolonged duration of time [29, 33].

PLAN OF WORK

Following methodology was envisaged for the proposed research work;

- Literature Review

- Preformulation Studies
  - Physicochemical characterization of drug
  - Establishment of analytical procedure

- Polymer Selection
  - Selection of suitable biodegradable polymers for the research work.
Polycaprolactone (PCL)

Poly lactic-co-glycolic acid (PLGA)

- PLGA 85/15

Poly lactic-co-glycolic acid (PLGA) (RESOMER®)

- RESOMER® 505 H
- RESOMER® 504 H
- RESOMER® 502 H

Poly lactic acid (PLA)

Poly (L)-Lactic Acid (PLLA)

Formulation of Modified release Systems

- Formulation of Biodegradable Polymeric Microspheres
  Microspheres were prepared by suitable polymers and process parameters using solvent evaporation method. Various process variables including polymer concentration and viscosity and surfactant concentration in external oil phase were optimized with respect to yield, encapsulation efficiency, shape, size and release parameters.

- Formulation of Unit/Multiparticulate Systems
  Tablets and Pellets were prepared using suitable polymers and process parameters. The pellets were prepared with extrusion-spheronization technique and were further coated with non-aqueous functional coating of ethyl cellulose using air suspension technique in GLATT particle coater.

Characterization of the Developed Modified release Systems

- Characterization of the Microspheres
  Biodegradable microspheres were characterized as follows:
  - Determination of Yield.
  - Determination of Encapsulation efficiency.
  - Effect of release media on surface topography of microspheres
  - Scanning Electron Microscopy (SEM): Surface topography of microspheres was studied using SEM.
  - Particle size analysis: Particle size distribution studies were done using Malvern Mastersizer 2000.
Fourier transform infrared spectroscopy (FTIR): Fourier Transform Infrared Spectra were recorded for the drug loaded microspheres, stavudine and the RESOMER® for verification of chemical integrity and purity of the drug.

Differential scanning calorimetry (DSC): DSC analysis was carried out on polymers, drug and microspheres to investigate any chemical interactions between them.

Residual solvent analysis: It was carried out to determine residual solvent contents in the prepared microspheres for safe use of the same.

X-ray diffraction technique (XRD): Powder X-ray diffraction pattern of microspheres, d4T and RESOMER® were recorded to reveal the molecular dispersion of stavudine into polymeric matrix of the microspheres.

Confocal laser scanning microscopy (CLSM): CLSM was performed to demonstrate thorough distribution of stavudine inside the polymeric microsphere shell.

Evaluation of the Developed Microspheres

- **In vitro** drug release studies
  - Drug release kinetics
- Stability studies
- **Ex vivo** evaluation
  - Histopathological studies
  - Cytotoxicity/cell viability studies
  - Hemolysis studies
  - Cellular uptake/engulfment studies

- **In vivo** studies
  - In vivo pharmacokinetic studies: Pharmacokinetic analysis of the selected formulation(s) was done using female Sprague dawley rats. All studies were carried out in accordance with IAEC (Institutional animal ethical committee) norms.
Characterization and Evaluation of Unit/Multiparticulate Systems

- The prepared unit/multiparticulate dosage forms were subjected to the compendial and non-compendial testing; in-vitro release studies; stability studies.
- The prepared pellets were evaluated for various parameters including: size, shape analysis, elongation, rectang, roundness, angle of repose, flow rate, Carr’s index, hausner’s ratio etc, in-vitro release studies; stability studies.