DESIGN AND EVALUATION OF EXTENDED RELEASE FORMULATIONS OF SOME ANTI-VIRAL DRUGS

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INTRODUCTION

The existence of the viruses was identified through experiments with filters in the turn of 20th century. Viruses were believed to be small bacteria till the invention of electron microscope in 1931. After the identification of viruses to be accumulation of proteins, the situation changed and they were said to contain genetic material in the form of DNA or RNA. Once they were recognized as distinct biological entities they were soon shown to be the cause of numerous infections. Many viral diseases in humans have been identified and smallpox, chickenpox, hepatitis, polio are some of the important viral diseases that were of great concern to control these infections initially. After the advent of specific treatments, vaccines / antigens, these diseases are effectively controlled.¹

The human immunodeficiency virus (HIV) infection is fast emerging and afflicting larger population across the world. HIV afflicted with tuberculosis has become a global challenge and remains difficult to control. HIV originated in sub-Saharan Africa during the 20th century and over 70 million individuals have been infected by the virus. By 2011, an estimated 35 million had died from AIDS, making it one of the most destructive epidemics in recorded history.² Every year more new HIV infections are emerging out and these AIDS-related illnesses have become a major cause for the deaths occurring in HIV infected patients. Among the 2.5 million children living with HIV/AIDS in 2008, 90% were Africans. Around 1000 children were infected every day. Half of them would have died before the age of 2 years, and all of them before adolescence without proper treatment.³

Human immunodeficiency virus (HIV) is a lentivirus (slowly replicating retrovirus) that causes acquired immunodeficiency syndrome (AIDS),⁴ a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

There are more than 14 antiretroviral drugs, approved by the Food and Drug Administration (FDA) that are available at present. In 1996, the HAART (highly active anti-retroviral therapy) containing at least three antiretroviral drugs was used
for the extension of the life span of HIV infected patients; however this therapy
attracted patient non-compliance owing to dosing frequency and high dose of the drug
and poor organoleptic properties that resulted in decreased bio-availability. UN-AIDS
2005 guidelines recommend the first and second line ARV therapy for HIV
infections. The second line therapy is more expensive and therefore is beyond the
reach of the people in resource limited settings. WHO recommends the first line
approach to have two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs)
and one non-nucleoside reverse transcriptase inhibitor (NNRTI). HAART therapy
recommended by WHO is having lesser pill burden, dietary restrictions, and the
number of daily doses besides, the current therapy is highly potent and when the
patient adherence is optimal to the therapy it is easy to achieve a very high efficacy.\(^5\)

Current HIV therapy followed across the world includes the fixed dose
combination of stavudine (d4T), lamivudine (3TC) and nevirapine (NVP). This
combination sustains the plasma HIV-1-RNA levels (pVL) for a period of atleast 3
years which results in profound suppression of viral replication and is useful even in
treatment - naive patients also.\(^6\)Current pharmaceutical approach for HIV infection
includes stavudine, lamivudine and nevirapine as conventional tablets or suspensions
either as individual drug or in fixed dose combination or stavudine and lamivudine
together and nevirapine alone as extended release formulations. It has been reported
that a single fixed dose combination provides benefits of treatment such as increased
patient adherence to treatment, delayed development of resistance, lowered total cost
including logistical support and reduce the risk of medication errors. The current
pharmaceutical approach as discussed above may be dis-advantageous considering the
benefits of single fixed dose combination.

The major problem of currently available formulations for HIV therapy such
as FDC of stavudine and lamivudine for extended release and extended release
formulation of nevirapine is poor patient adherence and therefore possibility of
resistance to the therapeutic agents that may ultimately result in clinical therapeutic
failure. Therefore, there is a need to design a formulation that may help overcome the
resistance to the therapeutic agents arising from the current pharmaceutical approach
and thus the clinical therapeutic failure. Matrix and reservoir systems are commonly
followed to develop oral controlled release delivery system that offer advantages like
reduced dosing frequency, increased bioavailability, decreased degradation in the GI
tract, improved taste and stability, and is capable of targeting viral reservoirs of the cellular and anatomical regions. However, the major drawbacks of matrix and reservoir systems are that the drug release is affected by factors like pH, presence of food, and other physiological factors. Also, these systems are not suitable for simultaneous delivery of the drugs that have varied physical characters and to control the drug release pattern becomes a challenging aspect.

ART drugs currently used have varied physical characteristics of which solubility is one. Stavudine and lamivudine are freely soluble whereas nevirapine is poorly soluble and therefore this varied physical characteristic of the ART drugs necessitates a suitable design for simultaneous and controlled delivery of these drugs in order to control HIV infection effectively. Oral osmotic drug delivery systems are ideal for simultaneous and controlled delivery of ART drugs with varied solubility characteristics. Oral osmotic systems are distinguished by their ability to release drugs independently of the medium composition and hydrodynamics. Besides, these systems offer potential clinical benefits such as ability to mitigate the food effect, increase patient compliance and treatment tolerance. Furthermore, these systems are used to deliver both poorly soluble and freely soluble drugs. And therefore, the present study aimed to design oral osmotic tablet of stavudine, lamivudine and nevirapine and evaluate the systems for physico-chemical and release characteristics.
REVIEW OF LITERATURE

Most of the diseases that prevailed in the 20th century seem to be a cause of the emerging viruses that are presenting several challenges to the researchers. The anti-viral therapy is either preventive or curative depending upon the infection. Even though certain viral diseases have been eradicated with the help of certain vaccines/antigens, infections like HIV pose a greater difficulty since the therapy is not curative and there is a lot of difficulty in the choice of the anti-viral drugs for HIV therapy.\(^9\)

Antiviral drugs are a class of antimicrobials that includes anti-bacterials, antifungal and anti-parasitic drugs. The major difficulty in developing vaccines and anti-viral drugs is due to viral variation. The emergence of antivirals is the product of a greatly expanded knowledge of the genetic and molecular function of organisms, allowing biomedical researchers to understand the structure and function of viruses, major advances in the techniques for finding new drugs, and the intense pressure placed on the medical profession to deal with the human immunodeficiency virus (HIV), the cause of the deadly acquired immunodeficiency syndrome (AIDS) pandemic. Clinical use of the currently available antiviral drugs is limited by toxic side effects and the risk of emergence of drug-resistant virus strains. These findings indicate that on the one hand, combination therapy should be studied, particularly for the prevention of HIV drug resistance, and that, on the other hand, attempts to develop new antiviral agents should continue unabated.\(^10\)

Most virologists believe that HIV originated in sub-Saharan Africa during the 20th century and over 70 million individuals have been infected by the virus. By 2011, an estimated 35 million had died from AIDS, making it one of the most destructive epidemics in recorded history. Around the globe, 34 million people are living with HIV. At the end of 2012, about 10 million people had access to antiretroviral therapy, which also has prevention benefits. Close to 26 million are eligible for antiretroviral therapy, under WHO 2013 consolidated ARV guidelines. There is a two fold increase in the infected women which was around 17.7 million in 2006. Every year more new HIV infections are emerging out and these AIDS-related illnesses has become a major cause for the deaths occurring in HIV infected patients. But only one in five HIV patients is receiving the basic preventive measures and one in eight people could be tested properly. In the low and middle income countries, the coverage of antiretroviral therapy started increasing only after the introduction of HAART and till date the utilization meets up to 50% only.\(^11\)
Even though there are more than 14 antiretroviral drugs available at present which are approved by the Food and Drug Administration (FDA), only a very few patients experience a successful viral replication with the ART. The disadvantages of the therapy lie in issues like drug toxicity, poor patient compliance, potency problems and pharmacokinetic interactions of the drug. As there may be a chance for a cross-resistance between the currently available drugs, the ART regimens are limited in their effectiveness. 12 There is a need for suppression of total and sustained viral load (VL) to prevent the resistance mutations emergence and to avoid clinical therapeutic failure for which the detection of virological failure, i.e. HIV-1 VL measurement above the threshold of 400 copies / mL must be identified and managed in an early stage itself.2

To control HIV infection without resume in the viral replication several weeks after withdrawal of the drug, there is a mandatory need for chronic intake of drugs. The main reason for patient incompliance is the dosing frequency and high dose of the drug which pose problem in achieving the pharmacotherapy. Poor organoleptic properties also become a drawback and result in patient incompliance to the regimen and thereby decreasing the bioavailability. Thus, novel drug delivery systems are holding the arena in reducing the dosing frequency and to improve patient compliance. The combination of at least three antiretroviral (ARV) drugs called as the High Activity Antiretroviral Therapy (HAART) used for the extension of the lifespan of HIV-infected patients was introduced in 1996.6

The World Health Organization guidelines for the use of antiretroviral therapy for patients in resource limited countries has been recently updated and it recommends the preferred first-line approach to have two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). It was also reported by world health organization that 95% of the people were adherent to the first line therapy among which 61% were successful with the fixed dose combination of stavudine (d4T), lamivudine (3TC) and nevirapine (NVP). In India, free ART programme was started in April 2004 and further it scaled up very rapidly with 3,50,000 patients which include 21,000 children. Government-sponsored ART program in India uses the generic fixed dose combination of zidovudine (or) stavudine, lamivudine and nevirapine (or) efavirenz. The currently
available ART has proved to be effective in reducing the mortality and morbidity of the infected adults and children.\textsuperscript{13}

Stavudine (d4T) is a synthetic thymidine nucleoside analogue, active against the human immunodeficiency virus type 1 (HIV-1). Stavudine is phosphorylated to active metabolites that compete for incorporation into viral DNA. It is converted intracellularly to the active metabolite stavudine 5'-triphosphate which is then incorporated into the viral DNA by reverse transcriptase resulting in premature ending of the DNA chain elongation. Lamivudine is a synthetic nucleoside analogue with activity against HIV-1 and HBV. It is a nucleoside analogue indicated in combination with other antiretroviral agents for the treatment of human immunodeficiency virus (HIV-1) infection. Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Nevirapine in triple combination therapy has been shown to suppress viral load effectively when used as initial antiretroviral therapy (i.e., in antiretroviral-naive patients).

Though the HIV therapy is a long term process, the main challenges are in the designing of a suitable delivery system that may have the advantages like reduced dosing frequency, increased bioavailability, decreased degradation in the GI tract, improved taste and stability, and is capable of targeting viral reservoirs of the cellular and anatomical regions.\textsuperscript{6} Various dosage form designs are available to control or modulate the drug release to a desired level like matrix, reservoir, or osmotic systems. The major drawbacks of matrix and reservoir designs are that the drug release is affected by factors like pH, presence of food, and other physiological factors.

Several extended release dosage forms were analysed and reported for their usage in HIV therapy. A floating drug delivery system of ritonavir (RN) was developed by Moumita et al (2013) by direct compression technique, using polymers such as different grades of hydroxyl propyl methylcellulose and polyvinyl pyrrolidone in order to prolong the gastric residence time and increase its bioavailability. The formulations took very less time to become buoyant and float for more than 12 hrs, which was sufficient for ER of the drug. Extended-release matrix tablets of zidovudine were prepared by Atul Kuksal et al (2006) using hydrophilic Eudragit RLPO and RSPO alone or their combination with hydrophobic ethyl cellulose and characterized for drug release. The in-vitro drug release study revealed that combining
Eudragit with ethyl cellulose sustained the drug release for 12 hours which suggest that the developed sustained-release tablets of zidovudine could perform therapeutically better than conventional dosage forms, leading to improve efficacy and better patient compliance. Floating matrix tablets of Stavudine (Vidyadhara et al 2012) and Lamivudine (Karunakar et al 2011) developed by direct compression technique, using polymers such as hydroxyl propyl methyl cellulose (HPMC K15M), karaya gum may prolong gastric residence time and increase the bioavailability of the drugs. The floating matrix tablet of Stavudine was a novel approach so as to avoid the disadvantages of anti-retroviral conventional dosage forms. A newer prolong releasing Zidovudine (AZT) bioadhesive vaginal gel was developed by Chatterjee et al (2011), using cold mechanical method to treat HIV infections with increased patient convenience. A generalized protocol, for the further research in this area is surely expected to yield significant outcome with improved drug delivery system. A novel vaginal compactable inserts of zidovudine might enhance the pharmacological effects of (AZT) for once a day administration in the effective management of AIDS in women.\textsuperscript{14}

Nanotechnology-based systems may provide a rationale approach for anti-HIV therapy and prevention. Schäfer et al studied several poly methyl methacrylate based and albumin-based nanoparticles loaded with zidovudine. The rate of uptake of the nanoparticles by the macrophages is based on several factors like physicochemical characteristics, surface morphology, size and their composition. Albumin-based and poly hexyl cyanoacrylate-based nanoparticles (PHCA nanoparticles) loaded with zidovudine and zalcitabine are effective in the prevention of HIV infection of monocytes/macrophages in vitro. The PHCA nanoparticles are effective for increasing phagocytosis and antiviral activity of saquinavir and zalcitabine. Saquinavir nanoparticles with poly (ethylene oxide)-modified poly (epsilon-caprolactone) (PEO–PCL) have 10-fold higher activity than the free drug. Zidovudine-loaded PEGylated-poly (L-lactide) (PEG–PLA) nanoparticles also show similar results as studied by Mainardes et al (2009). Zidovudine is delivered effectively to the interior of cells in its active form, zidovudine triphosphate with the help of poly (ethyleneimine) (PEI) nanogels. Polymeric nanocapsules of PEI and poly (iso-butylcyanoacrylate) are non-cytotoxic systems which may be utilised for increasing the macrophage uptake of zidovudine triphosphate to 10-30 folds. Developments in the delivery of antiretroviral drugs through nanocarrier systems are an interesting approach towards the
improvement of HIV/AIDS treatment. Inspite of all the above facts, certain aspects of these nanosystems are still need to be completely analyzed in order to open road towards human clinical trials. Most of the relevance and applicability of nanotechnology-based systems in the field of microbicides are yet in its infancy and much work is still required.\textsuperscript{15}

Even though the production of a dosage form which meets the needs of all the patients and clinicians is not completely possible, many trials are made in modulating the drug release in a control rate for an extended duration. Several reviews highlight the advantages of the osmotic systems in this regard. Oral osmotic pumps are an alternative to polymeric erodible systems distinguished by their ability to release drug substances independently of the medium composition and hydrodynamics, these systems offer potential clinical benefits, such as being potentially able to mitigate the food effect, increase patient compliance and treatment tolerance. Osmotically controlled drug delivery systems are used to deliver poorly soluble drugs, and water soluble drugs. Review of the osmotic delivery system for the past 30 years shows the development of different types of devices like elementary osmotic pump (EOP), porosity osmotic pump, the push–pull osmotic pump (PPOP), perforated tablet and sandwiched osmotic tablet system (SOTS).\textsuperscript{16}

Various formulation aspects in the development of oral controlled release osmotic drug delivery systems were discussed in earlier studies. The release of drug(s) from osmotic systems is governed by various formulation factors such as solubility and osmotic pressure of the core component(s), size of the delivery orifice, and nature of the rate-controlling membrane. The two-compartment Osmotic delivery systems are very well suited for drugs of different solubility ranges. The drug release rate is dependent upon the permeability of the coating membrane which could be increased by the addition of plasticizers and water-soluble additives in the formulation.\textsuperscript{16}

A number of factors like solubility of the drug, the osmotic pressure generated, size of the delivery orifice and type of the coating membrane and its nature may affect the drug release from an osmotic drug delivery system. The osmotic system which is activated by uptake of water from gastrointestinal fluid initiating very constant drug release over a period of approximately 20 hours after a lag-time of about 2 hours is advantageous for clinical therapy and superior to other twice daily formulations as it could produce plateau-like plasma profiles. \textsuperscript{16}
The influence of core and coating of the formulations on the release profiles from three core tablet formulations having different coating thickness and different ratios of plasticizer was characterized by earlier researchers. The lag time increased with an increase in the thickness of the coating membrane, but decreased with the addition of the hydrophilic plasticizer. Monolithic osmotic pump tablet systems may provide more stable plasma Drug concentration and good in-vitro / in-vivo correlation for a longer period when compared with that of the matrix tablets.  

Various solubility enhancement techniques such as converting the drug into ionic substance by the addition of an acid or alkali, using solubilizers like (SBE) 7m-β-CD, use of polymers like Polyethylene oxide (PEO) as both suspending as well as osmotic agent are utilized for the delivery of poorly water soluble drugs. Poorly water-soluble drugs like nifedipine are released predominantly in a soluble form by using wicking agents like colloidal silicon dioxide, PVP, sodium lauryl sulphate, etc. A controlled porosity osmotic pump having low water solubility drug, Flurbiprofen was prepared using sodium lauryl sulphate (SLS) as solubility enhancer and evaluated. Release rate studies revealed that less than 10% of drug was released from the system without SLS; while about 75% release was observed from systems containing SLS.

Swellable polymers are used for osmotic delivery of poorly aqueous soluble drugs. In hydrophilic matrix formulations, one of the most commonly used polymers is polyethylene oxide (PEO). It can be used in modulating drug release profile due to its solubility in water, availability in a range of molecular weight/viscosity grades, FDA acceptance, and unique swelling/erosion characteristics. In controlled release formulations, PEO has proved to be an effective alternative to HPMC. Due to its low melting point and very poor tabletability characteristics, PEO consolidates via viscoelastic behavior and fusion.

As the drug release is based on osmosis, it could be controlled by modifying the core, the coating membrane or the orifice diameter and is independent of the pH or hydrodynamics of the system. The release rate of a drug from an osmotic system is directly proportional to the osmotic pressure, a colligative property of the core formulation which is a solution in which a nonvolatile solute is dissolved in a volatile solvent. The osmotic pressure of a solution is dependent on the number of discrete entities of solute present in the solution. The osmotic pressure gradient between the
core compartment and the environmental medium has to be optimized properly for achieving a controlled drug release of the drug. The amount of osmogen has a direct effect on the rate of drug release.\textsuperscript{19}

Delivery orifice is the main aspect behind osmotic delivery systems which may be one or more present in the coating membrane. To achieve a controlled zero-order drug release pattern it is necessary to optimize the size of delivery orifice. The zero-order drug release gets altered due to very small size of delivery orifice as there will be difficulty in relieving the hydrostatic pressure developed within the core through the small orifice. In case of larger orifice size, solute diffusion may occur and that alters the release of drug. Different methods like Mechanical drilling and Laser drilling are used for creating delivery orifices in the osmotic systems.

An important aspect in the formulation of oral osmotic systems is the choice of a rate-controlling membrane. The membranes are semipermeable in nature and thus the drug release is independent of the pH and agitational intensity of the GI tract to a large extent. Materials like CA, HPMC and hydroxyl butyl methylcellulose are used to impart the stability to the wall and to enhance the permeability of the wall. Osmotic systems containing Cellulose acetate (CA) are used for a rate-controlled drug delivery.\textsuperscript{17}

As the osmotic drug delivery systems have the capacity to improve the therapeutic activity of certain drugs, it is been considered as the most attractive technology among the several novel drug delivery systems available in the market. Previous reviews indicate that 75% of the osmotic devices are used to deliver either freely soluble or practically insoluble drugs among which the unitary-core technologies are used to deliver freely soluble drugs, self-emulsifying technologies for practically insoluble drugs and the multilayer core are used to deliver both soluble and insoluble drugs.\textsuperscript{20}

From the above reviews, it is evident that no attempt has been done for the extended and simultaneous release of the fixed dose combination of stavudine, lamivudine and nevirapine and thus, it was planned to design osmotic extended release formulations of these drugs in fixed dose combination used for HIV infection. Initially, elementary osmotic pump tablets of these drugs were developed, followed by sandwich osmotic pump tablets with variable concentration of polymer and
osmogent along with variable orifice diameter and the formulations were evaluated for physico-chemical and release characteristics.
AIM AND OBJECTIVE

The work was planned to formulate and evaluate two different types of osmotic delivery systems – Monolayer osmotic systems i.e. Elementary osmotic pumps and Tri layer osmotic systems i.e. sandwich osmotic tablet systems for the simultaneous and controlled delivery of Stavudine, Lamivudine and Nevirapine from fixed dose combinations. The suitable drug delivery system may be identified by optimization of variables used in the formulation which may be correlated for in-vivo drug release profiles.

The objectives of the study were as follows:

1. Development of analytical method for the estimation of the drugs
2. Improvement of solubility of Nevirapine using micellar solubilisation technique.
3. Preparation of elementary osmotic pump tablets with different variables such as
   - Polymer Concentration
   - Osmogent concentration
   - Orifice diameter
4. Evaluation of elementary osmotic tablets by
   - Fourier Transform – IR spectrophotometry
   - Differential scanning calorimetry
   - Scanning electron microscopy
   - Physico-chemical evaluations : Pre-compression and Post-compression
   - In-vitro drug release study
   - In-vitro kinetic study
   - Statistical analysis
   - Stability studies for optimized formulation
5. Preparation of sandwiched osmotic pump tablets with different variables such as
   - Polymer Concentration
6. Evaluation of sandwiched osmotic tablets by
   - Fourier Transform – IR spectrophotometry
   - Differential scanning calorimetry
   - Scanning electron microscopy
   - Physico-chemical evaluations: Pre-compression and Post-compression
   - In-vitro drug release study
   - In-vitro kinetic study
   - Statistical analysis
   - Stability studies for optimized formulation
   - In-vivo drug release prediction
METHODS

Materials Used

Stavudine, Lamivudine and Nevirapine were obtained from Hetero Life Sciences Ltd., Hyderabad, India. Polyethylene oxide (6,00,000 gm/mol) was purchased from Sigma Aldrich, Bangalore, India. Potassium chloride and starch was supplied from S.D. Fine chemicals, Mumbai, India. Microcrystalline cellulose, magnesium stearate, Cellulose acetate phthalate, Sodium lauryl sulphate and Poly ethylene glycol-400 were purchased from Loba chemie, Mumbai, India. All other solvents and chemicals used were of the analytical grade.

Development of Analytical method

Phosphate buffer pH – 6.8 was selected as the medium because it is most suitable and preferred for the estimation of the drugs in osmotic systems. And moreover, this medium is recommended for dissolution studies on oral osmotic systems.

Standard curve of Stavudine, Lamivudine and Nevirapine

The drugs (10mg) were accurately weighed and dissolved in 100 ml of medium, i.e. Phosphate buffer pH – 6.8 to give a stock solution of concentration 100 µg/ml. From this stock aliquots of solutions were transferred into nine 10 ml volumetric flasks and the final volume was adjusted with the appropriate medium to give concentrations of 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 and 80µg/ml. The absorbance was measured at 266 nm, 271nm and 314nm for stavudine, lamivudine and nevirapine respectively against a blank medium in U.V. Spectrophotometer (Perkin Elmer Lamda-25) based on the validated analytical method.21

Determination of solubility of Nevirapine in distilled water, pH-1.2 and pH-6.8

Solubility of the drug was determined at 25±1ºC. An excess amount of drug was added to three 25ml stoppered flasks containing different solvent systems viz. distilled water, buffer of pH 1.2 & 6.8. The flasks were shaken for 10hrs at 25±1ºC in a mechanical shaker (Orbitrek). These solutions were allowed to equilibrate for the next 24hrs to ensure saturation and then centrifuged for 15 minutes at 1500rpm. The supernatant of each flask was filtered through whatmann filter paper No.14.
filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank at 314nm. The experiment was carried out in triplicate.\(^1\)

**Enhancement of solubility of Nevirapine by micellar solubilisation method**

Different concentrations (0.1, 0.2, 0.4, 0.6 % w/v) of the surfactant, sodium lauryl sulphate were prepared. An excess of nevirapine was added to 10ml each of the surfactant solution taken in 25ml of stoppered flasks and were shaken for 10 hrs at 25±1°C in a mechanical shaker (Orbitrek). These solutions were allowed to equilibrate for the next 24hrs to ensure saturation and then centrifuged for 15 minutes at 1500rpm. Equilibrium samples were withdrawn and properly diluted and filtered through whatmann filter paper No.14, and finally analyzed for concentration of nevirapine under UV-spectrometer at 314nm.\(^1\)

**FTIR – Spectrophotometry**

IR absorption spectrum of the drugs was recorded by potassium bromide dispersion technique in which dry sample of drug and potassium bromide was mixed uniformly and the mixed powder blend was placed in sample holder and an IR spectrum was recorded using FTIR spectrophotometer (Shimadzu-4100 Type A). The same procedure was repeated for drug along with the polymer.\(^2\)

**DSC Studies**

The DSC thermograms of pure drug and coated tablets were recorded. The samples were separately sealed in aluminum cells and set in Perkin Elmer (Pyris 1) DSC (Waltham, MA). The thermal analysis was performed in a nitrogen atmosphere at a heating rate of 10°C/min over a temperature range of 50°C to 300°C.\(^2\)

**Development of Elementary osmotic pump tablets (EOPT; monolayer osmotic tablets)**

Elementary osmotic pump tablets of three types were developed using different variables like concentration of PEO (polymer), concentration of KCl (osmogen) and variable orifice diameter.

- EOPT containing stavudine, lamivudine and nevirapine.
- EOPT containing stavudine and lamivudine.
- EOPT containing nevirapine alone.
Drugs were mixed with polyethylene oxide, KCL, microcrystalline cellulose (MCC) and the powder blend were passed through sieve no: 120 before granulation. This mixture was moistened with 10% starch paste to proper wetness and granulated by passing through sieve no: 14 and were dried at 40°C for 2 hrs. Mixture was again passed through sieve 18. Finally talc, and magnesium stearate was added to the mixture and compacted using 16/32 inch deep concave punches using rotary tablet compression machine with 8 stations (Cadmach, India) fitted with 16/32 in. (12.7mm) punches.

Tablets were coated by using a pan coater using coating solution of cellulose acetate phthalate (CAP) in acetone containing known level of poly ethylene glycol (PEG) as plasticizer. After coating, the tablets were dried over night at 60°C to remove residual solvent. Orifice with various diameter sizes ranging from 0.4mm – 0.8mm were drilled on one side of the surface of the coated tablet manually by a micro drill.  

Evaluation of EOPT

Physico-chemical evaluations

The powder blend of the formulations were evaluated for various parameters like angle of repose, bulk density, tapped density, compressibility index, Hausner’s ratio before compression and the prepared elementary osmotic tablets were evaluated for their physico - chemical parameters like Hardness, Thickness, Friability and % drug content by standard methods.

In-vitro drug release

The in vitro release of the elementary osmotic pump tablets was carried out using 900 ml of pH 6.8 phosphate buffer as the medium in USP II dissolution apparatus at 37°C ±0.5°C and 50 rpm. Five-milliliter samples were taken at 0, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 hrs and filtered through 0.45mm cellulose nitrate filter. Fresh dissolution medium (5 ml) was added after each sampling. Each study was done in triplicate and the mean values utilized for evaluation.

In-vitro release models and Kinetics

In order to describe the kinetics of drug release from controlled release preparations various mathematical equations have been proposed. Drug release data obtained was applied to different drug release models in order to establish the drug
release mechanism and kinetics. Best goodness of fit test (R^2) was taken as criteria for selecting the most appropriate model.\textsuperscript{24}
Optimization of formulation variables

Effect of polymer concentration

The effect of the concentration of Poly ethylene oxide (PEO mol. wt. 6,00,000 g/mol.) on the drug release was studied with the tablets having varied concentration of the polymer (10mg, 20mg and 30mg) and evaluated.

Effect of osmogen concentration

Different osmogen concentrations (10mg, 20mg and 30mg) were used in the formulations to identify their effect on the drug release pattern.

Effect of orifice diameter

To study the effect of orifice on the drug release, the release of the tablets with different orifice size (0.4mm, 0.6mm, 0.8mm) were investigated and compared.

Effect of Coating thickness

The tablet cores were prepared and coated with coating solution having different levels of PEG-400 (10, 20 and 30%) as that of CAP (w/w) and then the properties and drug release characteristics of the coated tablets were compared.23

Effect of pH of release medium

In order to study the effect of pH and to assure a reliable performance of the developed formulations independent of pH, release studies of the optimized formulations were conducted in media of different pH (SGF, pH 1.2 and SIF, pH 6.8) and pH change method (release media was simulated gastric fluid (pH 1.2) for first 2 h, followed by SIF (pH 6.8) for the remaining period). The samples (5 ml) were withdrawn at pre-determined intervals and analyzed after filtration through 0.45mm cellulose nitrate filter. The percentage cumulative drug release of optimized formulations at various pHs was plotted and compared.23

Effect of Agitation speed

In order to study the effect of agitational intensity of the release media, release studies of the optimized formulations were carried out in dissolution apparatus at various rotational speeds. Dissolution apparatus used was USP II at 50, 100 and 150 rpm. Samples were withdrawn at pre-determined intervals and analyzed after filtration through 0.45mm cellulose nitrate membrane filters. The percentage cumulative drug release of optimized formulations at different agitational intensity was plotted and compared.23
Multivariable linear regression analysis

In order to study the influence of the variables upon drug release of EOPTs, the drug release data were analyzed using multivariable linear regression analysis and the linearity of the correlation co-efficient was noted.\(^23\)

Stability studies on EOPT

The optimized formulation was subjected to stability study at 40°C ±2°C and 75 % RH for a period of 90 days in the stability chamber (HTC-3003, Inlab Equipments, India) as per the criterion mentioned in the ICH guidelines and evaluated for physico-chemical properties and drug content.

Development of sandwich osmotic tablets (SOPT; Tri-layer)

The sandwich osmotic tablets were developed in two different approaches using different variables like concentration of PEO (polymer), concentration of KCl (osmogen) and variable orifice diameter as follows:

**Approach – 1** : Sandwich osmotic tablet with stavudine, lamivudine and nevirapine in both drug layers separated by a push layer. (SOPT-A)

**Approach – 2** : Sandwich osmotic tablet with both stavudine and lamivudine at one side and nevirapine at the other side with a middle push layer. (SOPT-B)

The SOPTs were prepared in the same procedure as that of the EOPTs. The orifice of variable diameters (0.4mm, 0.6mm and 0.8mm) were drilled on both the surfaces of the tablet using a micro drill.\(^23\)

Evaluation of sandwich osmotic tablets (SOPT; Tri-layer)

Physico-chemical evaluations

The powder blend of the SOPT formulations were evaluated for various parameters like angle of repose, bulk density, tapped density, compressibility index, Hausner’s ratio before compression and the prepared elementary osmotic tablets were evaluated for their physico - chemical parameters like Hardness, Thickness, Friability and % drug content by standard methods.
Scanning electron microscopy studies

The surface morphology of the coated tablets before and after dissolution was studied using scanning electron microscope (SEM). The samples were placed on a spherical brass stub (12mm diameter) with a double backed adhesive tape. Samples of the coating membrane was dried at 50°C for 2 hrs after dissolution and mounted. The samples were sputter coated for 2min. with gold using fine coat ion sputter (JFC-1600, Joel, Japan) with pressure of 8kg Pascal and examined under SEM (JSM – 6360, Joel, Japan).

In-vitro drug release

The in vitro release of the sandwich osmotic pump tablets was carried out using 900 ml of pH 6.8 phosphate buffer as the medium in USP II dissolution apparatus at 37°C ±0.5°C and 50 rpm. Five-milliliter samples were taken at 0, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h and filtered through 0.45mm cellulose nitrate filter. Fresh dissolution medium (5 ml) was added after each sampling. Each study was done in triplicate and the mean values utilized for evaluation.

In-vitro release models and Kinetics

In order to describe the kinetics of drug release from controlled release preparations various mathematical equations have been proposed. Drug release data obtained was applied to different drug release models in order to establish the drug release mechanism and kinetics. Best goodness of fit test ($R^2$) was taken as criteria for selecting the most appropriate model.

Optimization of formulation variables

The optimized formulation was then subjected to various comparative tests under different conditions to study the following effects of variables:

Effect of polymer concentration

The effect of the concentration of Poly ethylene oxide (PEO mol. wt. 6,00,000 gm / mol.) on the drug release was studied with the tablets having varied concentration of the polymer (10mg, 20mg and 30mg) and evaluated.
Effect of osmogen concentration

Different osmogen concentrations (10mg, 20mg and 30mg) were used in the formulations to identify their effect on the drug release pattern.

Effect of orifice diameter

To study the effect of orifice on the drug release, the release of the tablets with different orifice size (0.4mm, 0.6mm, 0.8mm) were investigated and compared.

Effect of Coating thickness

The tablet cores were prepared and coated with coating solution having different levels of PEG-400 (10, 20 and 30%) as that of CAP (w/w) and then the properties and drug release characteristics of the coated tablets were compared.\textsuperscript{23}

Estimation of Swelling Index

For estimating the swelling index, the tablets were suspended in 5ml of SIF (pH-6.8) and the swelling index was calculated as per the method prescribed by previous studies.\textsuperscript{25}

Estimation of Burst Strength

Burst strength of the exhausted shells, after dissolution was determined to assure that the tablets would maintain their integrity in the GIT. Burst strength was determined as the force required to rupture the shells after dissolution studies. The texture analyzer (Instron 4501, India) with a 5kg load cell and 25 mm aluminium cylindrical probe was utilized for this purpose. Test speed of 0.8 mm / sec with a distance of 2 mm was selected.\textsuperscript{24}

Effect of pH of release medium

In order to study the effect of pH and to assure a reliable performance of the developed formulations independent of pH, release studies of the optimized formulations were conducted in media of different pH (SGF, pH 1.2 and SIF, pH 6.8) and pH change method (release media was simulated gastric fluid (pH 1.2) for first 2 h, followed by SIF (pH 6.8) for the remaining period). The samples (5 ml) were withdrawn at pre-determined intervals and analyzed after filtration through 0.45mm cellulose nitrate filter. The percentage cumulative drug release of optimized formulations at various pHs was plotted and compared.\textsuperscript{23}
Effect of Agitation speed

In order to study the effect of agitational intensity of the release media, release studies of the optimized formulations were carried out in dissolution apparatus at various rotational speeds. Dissolution apparatus used was USP II at 50, 100 and 150 rpm. Samples were withdrawn at pre-determined intervals and analyzed after filtration through 0.45mm cellulose nitrate membrane filters. The percentage cumulative drug release of optimized formulations at different agitational intensity was plotted and compared.23

Effect of Osmotic pressure

In order to confirm the mechanism of drug release, release studies of the optimized formulations were conducted in media of different osmotic pressure. To increase the osmotic pressure of the release media, sodium chloride (osmotically effective solute) was added in SIF and osmotic pressure was measured (Fiske micro-osmometer, 210).26 The pH was adjusted to 6.8±0.05. Release studies were carried out in 900 ml of media using USP II dissolution test apparatus (100 rpm). Release profiles of optimized formulations were compared using model independent pair-wise approach, which included the calculation of ‘difference factor’ $f_1$ and ‘similarity factor’ $f_2$.27

Multivariable linear regression analysis

In order to study the effect of the variables upon drug release of SOPTs, the drug release data were analyzed using Multivariable linear regression analysis. The significance of the correlation co-efficient was noted.23

Stability studies on SOPT

The optimized formulation was subjected to stability study at 40°C ± 2°C and 75 % RH for a period of 90 days in the stability chamber (HTC-3003, Inlab equipments, India) as per the criterion mentioned in the ICH guidelines and evaluated for physico-chemical properties and drug content.

In-vivo prediction study

Known pharmacokinetic properties of drugs and various drug release parameters which were calculated from in vitro release data, were used to predict blood levels of drugs using the method of superposition. The predicted steady-state
plasma levels of the optimized formulation were compared with the desired levels by calculating the percent predicted error (% PD).

RESULTS

Development of analytical method

The absorption maxima of the drugs stavudine, lamivudine and nevirapine were found to be -266nm, 271nm and 314 nm in phosphate buffer pH – 6.8. The absorbance of the solutions was linear with the concentration of the drug in the range of 10 to 30 µg/ml. The analytical method was validated for accuracy, precisions, and specificity according to ICH guidelines. The analytical method described above is specific for the three drugs as there is no drug- drug or drug - placebo interference. The method is also precise because the % relative standard deviation of method precision was found to be less than 2% and that of the system precision was less than 1%.

Enhancement of Nevirapine solubility

Solubility of the drugs is important to imibe water and to generate hydrodynamic pressure inside the osmotic system. There are several methods used to enhance the solubility of poorly soluble drugs like nevirapine. One such approach to overcome the solubility problem is micellar solubilisation technique. Various concentrations of sodium lauryl sulphate were used to examine the solubility of nevirapine and maximum solubility of the drug was found with 0.4 % w/v of Sodium lauryl sulphate.

Drug – Polymer Compatibility studies

The FT-IR spectral analysis is used to examine the interaction if any between the drug and the polymer. The FT-IR absorption spectra of the drugs stavudine, lamivudine and nevirapine showed respective specific peaks at 3522.13, 3329.25 for – OH group; 3425.69, 3213.51 and 3294 for – NH group; 1689.70, 1653.05 and 1647 for C=O group; 1462.09, 1496.81 and 1491 for C=C group; 488,576,642; 461,592,852 and 461, 574, 621 for the aromatic groups which were also seen in the spectra of the mixture of the three drugs along with the polymer at 3419.90 for – NH group; 1685.84 for C=O group; 1496.81 for C=C group; 3329.25 for – OH group; 488.01,576.74,850.64,642.32 for the aromatic groups. The characteristic peaks of the
drugs or polymer were also present in the mixture of drugs and polymer indicating no interaction between the drugs and polymer.
The DSC thermograms of the individual drugs and the mixture of drug and polymer showed no specific change in the endotherms which makes it clear that there is no specific interaction between the drugs and polymer. The endothermic peaks of the drugs corresponding to their melting points were well retained.

The DSC thermograms of pure stavudine, lamivudine and nevirapine were observed with sharp endothermic peaks at 172.21°C, 180°C and 244.80°C respectively which were well retained in the thermograms of the physical mixture of the drugs and the polymer.

**Pre – compression Studies**

The granules of all the EOP and SOPT formulations were found to possess good flow property which was indicated by angle of repose, bulk density, tapped density, percentage compressibility index, and Hausner’s ratio in the acceptable range.

**Post – compression Studies**

The post-compression parameters such as Hardness, thickness, diameter and the swelling index of the EOP and SOPT formulations were studied. Friability of all formulations was less than 1% and weight variation within the acceptable limits as per I.P. The drug content of the elementary and sandwich osmotic tablets was found to be between remarkable values which ensured the uniform distribution of drug in the formulation.

The percentage weight increase due to coating was found to be between 4.87 and 5.67%. All the values were found to be within the USP limits (± 10%). The orifice diameter was measured using optical microscope. The tablets having orifice diameter of specified value ± 0.01% (Average ± SEM) were selected for further studies.

**Scanning electron microscopy**

The surface morphology of the coating membrane before and after dissolution studies was analyzed using scanning electron microscope. It was found that there was no significant difference in the membrane structure and orifice diameter before and after dissolution studies and there were no pores in the membrane. The surface morphology of the membrane appeared similar before and after the dissolution studies. It also confirms that there was no blockade of delivery orifice during drug
delivery. The surface studies also indicate that there is no rupture of the coating during dissolution and the membrane was intact throughout the studies.

In-vitro release studies

The in-vitro dissolution study of the elementary osmotic tablets (EOPT) and sandwiched osmotic pump tablets (SOPT) was carried out using 900 ml of pH 6.8 phosphate buffer as the medium to identify the rate of drug release at different time intervals. The in-vitro release studies showed that the release profiles of different formulations varied according to the orifice diameter, concentration of polymer and the concentration of osmogen used. All the formulations had a lag time of at least 1 hour. This may be attributed to the fact that the drug release is mainly based upon the osmotic pressure generated internally which is the result of water imbibing of the core that may be varied depending upon the concentration of the polymer, osmogen and the orifice diameter.

Effect of polymer concentration

The drug release profile of various formulations indicates that the amount of PEO had a significant effect on the release rate. With increasing PEO, the release rate clearly slowed down. EOPT Formulations with all the three drugs showed that the percentage release and rate for stavudine and lamivudine was very low in case of 30mg polyethylene oxide and it was higher in case of both 10 and 20 mg. However, the EOPT showed higher release rate in case of 10mg polyethylene oxide and release rate lowered when the concentration was increased to 20 mg. The results also showed that nevirapine release rate decreased markedly as the polyethylene oxide concentration increased.

SOPT formulations (SOPT-A and SOPT-B) showed the effect of polyethylene oxide amount on stavudine, lamivudine and nevirapine release rate. The release rate of the drugs decreased as the concentration of the polymer increased. The concentration of the polymer influenced the drug release of the systems inversely. The desired release rate of the drugs was achieved at median polymer concentration and optimum orifice diameter (20mg polymer and 0.6 mm orifice diameter).

Effect of osmogen concentration

Three different osmogen concentrations (10, 20 and 30mg) were investigated and a direct correlation was observed between the concentration of KCL and the drug
release rate. With an increasing amount of KCL, the release rate was accelerated, because the increasing osmotic pressure made more drug release from the core. EOPT formulations developed by all the three approaches showed that percentage drug release of the drugs increased significantly when KCL concentration was increased from 10 to 30 mg. Similarly, in the SOPT formulations also the concentration of the osmogen was varied and the in-vitro release profiles of these formulations showed that when the concentration of osmotic agent was increased, the release of drug from the formulation also increased. Thus, the concentration of KCl played a direct role upon the drug release.

**Effect of orifice diameter**

The size of delivery orifice must be optimized in order to control the drug release from osmotic systems. To determine the optimal diameter of the delivery orifice in the membranes, orifice was made in the range of 0.4mm, 0.6mm and 0.8mm diameter size. Cumulative percentage drug release profiles of the drugs from these systems were compared. It was found that the size of the delivery orifice significantly increased the rate of release of the drugs. Significant difference existed in the release profiles for orifice diameters of 0.4mm and 0.8mm. The formulations with orifice diameter 0.6mm which was within the optimal range showed a slower and extended release when compared to other formulations. Mean dissolution time (MDT) at various polymer and osmogen concentration and orifice diameters of different formulations was found to be statistically significant (p < 0.0001).

**Effect of coating thickness**

The same core tablets were coated with different coating weights (8%, 12% and 16%). The influence of different coating weights was analyzed and the result showed that the release rate slowed following the increase of the coating membrane thickness. When the coating thickness increased, the weight of the membrane increased which in turn reduced the drug release. MDT between the different formulations at various coating thickness was found to be statistically significant (p < 0.05).

**In-vitro release models and Kinetics**
The in-vitro kinetic analysis of the EOPT and SOPT formulations showed that all the formulations followed the zero-order kinetics ($r^2 = 0.967 – 0.998$) along with the non-fickian transport mechanism.

**Estimation of Burst Strength**

Burst strength of the exhausted shells, after dissolution was determined to assure that the tablets would maintain their integrity in the GIT. The burst strength of the exhausted shells was found to be directly proportional to the weight gain of the coating membrane. When there is increase in the coating weight, the burst strength also increased. No bursting was observed during dissolution. Satisfactory burst strength of more than 320gm was got in the formulations with highest weight gain.

**Effect of pH of release medium**

The EOPT and SOPT formulations were subjected to in-vitro release studies in buffers with different pH, SGF (pH 1.2), SIF (pH 6.8) change method were chosen to carry out the release test. There was no significant difference in the release profile, demonstrating that the developed formulation show pH-independent release. The release profile of the drugs was found to be similar in all the media demonstrating that the developed formulations show pH independent release. The $f_1$ and $f_2$ values were found to be within the acceptable range ($< 15$ and $> 50$ respectively) and they were statistically significant.

**Effect of agitation intensity**

Drug release from osmotic pumps, need to be independent of agitational intensity of the release media. In order to verify effect of agitation intensity on the drug release from EOPTs and SOPTs, the dissolution studies were conducted at three different rpm (50, 100 and 150). The release of the drugs was found to be independent of the agitational intensity. It was observed that the agitation intensity of 50, 100, 150 rpm of dissolution medium had no significant effect ($P >0.05$) on the release rate of the drugs from the osmotic formulations.

**Effect of Osmotic Pressure**

The effect of osmotic pressure on the optimized formulation was studied in the media of different osmotic pressures. Drug release from the formulations decreased as the osmotic pressure of the media increased to 7.81 atm, 13.84 atm, and 21.28 atm. The release was inversely related to the osmotic pressure of the release media.

**Multivariable linear regression analysis**
The multivariable linear regression analysis was utilized to analyze the effect of the different independent variables such as concentration of PEO, concentration of KCl and orifice diameter on the drug release rate of the EOFTs and SOFTs. It was found that the different variables had a significant influence upon the independent variable (drug release). The concentration of PEO had a negative influence whereas the concentration of KCl and orifice diameter had a positive influence upon the drug release.

**Stability studies**

The EOFT and SOFT formulations were subjected to stability study at 40°C ± 2°C and 75% RH for a period of 90 days as per the criterion mentioned in the ICH guidelines and it was seen that the formulations showed no remarkable changes in the physico-chemical parameters and drug content.

**In-vivo prediction study**

The desired steady-state plasma levels of the drugs were predicted from a theoretically designed zero order delivery system. It was clearly evident that the predicted steady-state plasma levels are very close to the desired levels. The absolute % PD was found to be less than 15%, ensuring that the formulation will produce plasma levels close to the desired ones.
DISCUSSION

The findings of the present study show that sandwiched osmotic pump tablet can deliver stavudine, lamivudine and nevirapine simultaneously which ensures greater patient adherence to therapy as well as patients’ compliance that ultimately help control HIV infection effectively. The unique advantage of SOPT is the use of fixed dose combination of stavudine, lamivudine and nevirapine as once a day controlled release formulation. The study has limitations that SOPT was developed with limited variables such as PEO, KCl, and orifice diameter and as such the influence of other variables like polymer molecular weight, type of osmogents, coating materials etc. was not ascertained.

Currently HIV is managed with extended release formulation containing fixed dose combination of stavudine and lamivudine and extended release formulation of nevirapine both individually with a view to effectively control HIV infection. Considering patient adherence with such dosage forms raises the question whether the current approach really improves the patient compliance and adherence to therapy. The dis-advantages of such an approach were dealt with in detail under introduction. Therefore, it necessitates simultaneous delivery with extended release of stavudine, lamivudine and nevirapine in order to reach plasma concentration at therapeutic level of all the three drugs simultaneously that can overcome the problems if any with the current approach for the HIV therapy.

In the present study, a stepwise approach was followed for designing sandwich osmotic tablets (SOPT) that primarily formed the objective of the present study. With varied solubility characteristics of stavudine, lamivudine and nevirapine it is essential to study the release behavior of these drugs from the elementary osmotic pumps (EOPT). Solubility of a drug plays a crucial role in providing the osmotic effect that helps release the drug. Among the three drugs under investigation in the present study, stavudine and lamivudine are freely soluble and therefore the osmotic pressure development is not difficult. Whereas, nevirapine is sparingly soluble and therefore before designing osmotic system, measures to improve its solubility need to be considered. Based on this, solubility of nevirapine was improved to four folds using appropriate concentration (0.4% w/w) of sodium lauryl sulphate.
Initially, EOPT of the drugs under investigation were developed by different approaches with a view to mimic the proposed SOPT of two compartments. One approach included stavudine, lamivudine and nevirapine; the other approach with stavudine and lamivudine and the third approach containing only nevirapine. In the study, we selected randomly three independent variables such as potassium chloride, poly ethylene oxide and orifice diameter to investigate their influence on the release behavior of the osmotic system. We selected three different concentrations of poly ethylene oxide (PEO; mol wt: 6,00,000 gm/mol) 10mg, 20mg, 30mg; three different concentrations of potassium chloride (10mg, 20mg, 30mg) and different orifice diameter (0.4mm, 0.6mm, 0.8mm).

The pre-formulation study was carried out to examine the interaction between the drugs and the polymer by fourier transform infrared (FT-IR) spectrophotometry and differential scanning calorimetry (DSC) analyses. We observed no interaction between the drug and polymer characterized by absence of any additional peaks in FT-IR spectra or absence or shifting of endothermic peaks in DSC thermograms. The pre-compression parameters such as angle of repose, bulk density, tapped density, percentage compressibility index, Hausner’s ratio and post-compression parameters like hardness, thickness, diameter of the formulations, friability, weight variation, swelling index and drug content were all within the acceptable limits for the dosage forms developed.

PEO is commonly used polymer in designing osmotic systems with controlled release characteristics. It is among various hydrophilic polymers that in the presence of water control the release of the active medicament either by swelling / erosion by forming a hydrogel. It has molecular weight ranging from 1,00,000 to 9,00,000 gm/mol and finds its use as drug entraining polymer for water insoluble drugs or release retardant for highly water soluble drugs. We used PEO with molecular weight 6,00,000 gm/mol for development of EO in all the three approaches as most studies found PEO with this molecular weight suitable for development of EOPs.

The effects of potassium chloride, PEO and orifice diameter on the release rate of the drugs were compared and analyzed by multi-variable linear regression studies. We observed that as the PEO concentration increased, the rate of drug release decreased due to increase in the swelling and viscosity of the core. Besides, there was an increase in the pressure generated by the PEO that hinders the entry of water and a reduction in the drug release rate. Water soluble stavudine and lamivudine can imbibe
more water from the environment and therefore exerts increased hydrodynamic pressure which was increased the drug release rate.

The multi-variable linear regression shows that the correlation co-efficient ($r^2$) was about 0.9 for the respective drugs in all the approaches for EOPs. It was observed, of all the three variables such as PEO concentration (X1), KCl concentration (X2) and orifice diameter (X3); X3 showed the highest absolute value followed by X2 and X1. Orifice diameter seems to be the most influencing factor which has positive correlation with the release rate of the drug. KCl concentration appears to be the secondary factor as the absolute values were found to be approximately four folds lower than that of the orifice diameter; however, KCl positively correlated with the release rate of the drug. PEO concentration showed negative effect on the release rate of the drugs. As the PEO concentration increased, the release rate decreased.\(^2\)

The rate of drug release from osmotic system is affected by the coating membrane thickness. We optimized the coating thickness using varying proportions of poly ethylene glycol (PEG-400) and we observed that 70: 30 ratio of cellulose acetate phthalate and poly ethylene glycol (CAP : PEG) suitable for extended release formulation with 8% tablet weight gain for all the three approaches as said above.

The effect of pH as well as agitation speed on the release behavior of EOPTs was also examined and it was observed the release rate of the osmotic systems was found to be independent of the pH or agitation intensity of the environment. Therefore, the EOPTs developed meet the requirements of osmotic systems. Osmotic systems generally have lag time of 1-2 hours during which the coating membrane imbibes water slowly from the environment to develop osmotic pressure for the release of the drugs. The results of the study showed that the EOPTs utilized 1 hour lag time to build up the osmotic pressure and therefore the EOPTs developed, pass the requirements of the osmotic systems.\(^9\)

EOPTs should ensure their stability over storage without affecting their swelling index, drug content and release characteristics. To examine this, we carried out stability study on the developed EOPs by storing them at 40°C ± 2°C / 75% RH for 90 days as per ICH guidelines. Samples were withdrawn at regular intervals of time and tested for changes in their characteristics. We observed no significant
changes in swelling, drug content and release rate of the drug and therefore, the
developed formulations were found to be stable.
In our preliminary investigation of EOPTs it is understood that the release rate of the water soluble drugs (stavudine and lamivudine) and nevirapine (with improved solubility using sodium lauryl sulphate) was influenced by the PEO concentration, KCl concentration and diameter of the orifice. We observed optimum extended release of all the three drugs from the EOPTs with PEO20mg, KCl 10mg and orifice diameter 0.6mm. The above studies suggest that extended release of stavudine, lamivudine and nevirapine could be possible from the EOPTs. The release data of all EOPTs were fitted in kinetic models and it was found all formulations showed zero-order release ($r^2 = 0.967 – 0.997$) with non-fickian mechanism.

The $f_1$ (difference factor) and $f_2$ (similarity factor) values of the release behavior of the EOPTs were determined in different pH conditions as well as agitation speeds and compared. The $f_1$ and $f_2$ values of all the three EOPs showed values < 15 and >50 respectively and therefore the EOPs are suitable for extended release of the respective drug / drugs incorporated.

Though all the EOPTs showed slow release pattern of the drugs, they did not meet the requirements of once a day extended release formulations according to FDA guidelines. Possibly in the study we limited the variables to PEO, KCl and orifice diameter to design EOPs for simultaneous and extended release of the three anti-HIV drugs and as such the EOPs could not meet the FDA guidelines. The findings obtained from EOPs of stavudine, lamivudine and nevirapine gave an indication to proceed further with the development of SOPT for simultaneous and extended release of the three anti HIV drugs.

SOPT is another approach for simultaneous delivery of water soluble as well as water insoluble drugs. Previous studies reveal the importance of SOPT systems for controlled and simultaneous release of two or more drugs. SOPT systems of nifedipine and metoprolol, theophylline and salbutamol sulphate; rifampicin and isoniacid; metformin hydrochloride and glipizide with simultaneous and controlled release characteristics have been documented. However, to our knowledge no report is available on simultaneous and extended release of three drugs with variable solubility characteristics.
SOPT is a two compartment system containing the drugs separated by a middle push layer that helps simultaneous and controlled release of drugs. In the present study, we followed two different approaches to design SOPT. The first approach included stavudine, lamivudine and nevirapine in both drug layers separated by the middle push layer. The SOPT contains middle push layer and therefore assists in building up osmotic pressure that attributes for slow and simultaneous delivery of the drugs. The second approach included stavudine and lamivudine combination at one side layer and nevirapine alone at the other side layer with a middle push layer. The above approaches were based on examining the influence of the poorly soluble nevirapine on the hydrodynamic / osmotic pressure development in the system, which formed the objective of the study.

Prior to tabletting, the granules were evaluated for pre-compression characteristics and it was found that the granules passed the standards. SOP tablets were developed on both the approaches by standard wet granulation method and evaluated for physico-chemical and drug release characteristics. The physico-chemical properties were within acceptable limits. The morphology of the SOPTs was examined and compared and we observed no significant difference in the membrane structure and orifice diameter before and after dissolution studies and there were no pores in the membrane. The surface morphology of the membrane appeared similar before and after the dissolution studies. It also confirms that there was no blockade of delivery orifice during drug delivery. The surface studies also indicated that there was no rupture of the coating during dissolution and the membrane was intact throughout the studies.

The influence of independent variables like concentration of PEO, concentration of KCl and orifice diameter on the release behavior of SOPTs was examined. The independent variables selected for SOPTs are akin to that followed for EOPTs with a view to find out whether these variables could fit in the SOPTs with extended release characteristics prescribed in FDA guidelines.

The drug release profile of the SOPTs indicates that the concentration of PEO had a significant effect on the release rate of the drug. The concentration of PEO negatively correlated with the drug release rate i.e. as the concentration of PEO increased, the release rate slowed down. The mechanisms of slow release from the PEO can be ascribed to the following:
(i) PEO increases the swelling and viscosity of the core as well as that of the push layer which resist the release of the drug delaying the solvent contact with the drug resulting in zero-order release of the drug.\textsuperscript{29}

(ii) PEO increases the hydrodynamic pressure within the system that hinders the entry of water with reduction in the drug release rate.\textsuperscript{18}

The concentration of osmogen (KCl) showed positive effect upon the drug release in contrast to that observed with PEO concentration. A significant influence of KCl concentration upon drug release was seen in almost all the formulations. At lower concentrations, the release was hindered due to lesser pressure generated and vice versa as observed in earlier studies.\textsuperscript{16} The results show a direct effect of the osmogen concentration upon drug release which was influenced by the water imbibing capacity of the core. When the osmogen concentration was increased, there was increase in the water imbibitions that enhances the driving force and thus the drug release. Our findings are in agreement with the earlier reports.\textsuperscript{16}

Orifice diameter is yet other critical parameter that greatly influences the release rate of the drugs. The release data of all the formulations observed in the study clearly indicates the direct influence of orifice diameter on drug release. The formulations with lesser orifice diameter showed lesser drug release than the larger ones. The release was rapid through the larger orifice diameter. Drug particles when presented in viscous suspension may occlude the smaller orifice and therefore reduce the drug release as reported earlier.\textsuperscript{18}

The formulations prepared with different coating levels showed varied drug release pattern. When the coating thickness increased, the weight of the membrane increased which in turn reduced the drug release. When the thickness of the coat increases, the resistance of the coat for water diffusion increases and the rate of water imbibition decreases which in turn decreases the hydrostatic pressure and liquefaction of the core; resulting in decreased drug release.\textsuperscript{29} The drug release studies showed a reciprocal relation of release rate to that of the coating weight which was linear and consistent with the previous studies reported.\textsuperscript{22}

The ratio of CAP / PEG plays a significant role in the drug release of the formulations. It was observed that PEG level in the coating had a marked influence on the drug release. With an increase in the proportion of PEG in the coating solution,
the drug release increased gradually. When the PEG level increased, the porous channels of the membranes increased due to the hydrophilic nature of the polymer, which increased the permeability of the membrane and thus the drug release was also increased.\(^{18}\) When the proportion of PEG increased, the permeability of the membrane increased which in turn reduced the mechanical strength of the coating and thus there was an increase in the drug release rate.\(^{29}\)

While the coating membrane thickness influences the osmotic pressure, the importance of PEG incorporated in the coat was clearly understood in the study. PEG is a hydrophilic plasticizer and also responsible for water imbibing capacity of the membrane and therefore, has influence on the osmotic pressure generated in the system. Compared to EOPTs, the release rate of the drugs was found to be slower with increase in the proportion of PEG. The following may be the possible mechanisms.

(i) As the PEG concentration in the coat membrane increased more water was imbibed resulting in increased viscosity and slow release rate of the drug through the orifice.

(ii) Beyond optimum concentration of PEG resulted in increase in thickness of the coat membrane that further slowed down the release rate of the drug due to more water imbibitions and increase in viscosity.

We observed the above said mechanisms operating in the SOPTs. The findings reveal that it is essential to optimize the ratio of CAP: PEG to achieve the desired release rate which we found at 80: 20.

The interaction between PEO, KCl and orifice diameter and its influence on the release rate of the drugs from both the SOPTs were analyzed by multi-variable regression analysis. The influence of the variables PEO concentration (X1), KCl concentration (X2) and orifice diameter (X3) on the release rate of stavudine, lamivudine and nevirapine was examined. The multi correlation co-efficient was about 0.9 with the SOPTs in both the approaches. X3 and X2 positively correlated and X1 negatively correlated with the release rate of the drugs. As observed with EOPs these variables did influence the release rate of the drugs.

As observed with EOPTs, neither the pH of the release media nor the agitation speed influenced the SOPTs developed by different approaches. The finding shows
that the developed SOPTs may function independent of the environmental conditions of the GIT. The release data of all SOPTs were fitted in kinetic models and it was found all formulations showed zero order release ($r^2 = 0.990 – 0.998$) with non-fickian mechanism.

The release profile of SOPTs were compared and it was observed that both the approaches yielded SOPTs with slow release characteristics of stavudine, lamivudine and nevirapine; however, differences in release rate of the individual drugs were observed between SOPTs developed by the first approach (SOPT-A) which included fixed combination of stavudine, lamivudine and nevirapine in both compartment layers. In contrast, the SOPT developed by second approach (SOPT-B) containing stavudine and lamivudine in one layer and nevirapine alone in the other layer gave extended release rate of all the three drugs simultaneously conforming with the requirements of FDA guidelines for once a day extended release SOPTs. Though both SOPTs prepared by different approaches contained identical proportions of PEO and potassium chloride with same orifice diameter, the underlying mechanisms for variable release pattern of stavudine, lamivudine and nevirapine are not clearly understood. It is hypothesized that solubility characteristics of the sparingly soluble nevirapine seems affected by the presence of the freely soluble stavudine and lamivudine in the same layer. The possible reason may be that the hydrodynamic / osmotic pressure developed in SOPT-A is insufficient and therefore the difficulty in simultaneous release of all the three drugs in controlled manner.

Contrary to the above findings, separation of the water soluble drugs lamivudine and stavudine from the sparingly soluble drug nevirapine by placing them in different compartments as followed in SOPT-B appears to be osmotically effective in releasing the drug simultaneously at a slow rate in accordance with the requirements of FDA guidelines for once a day extended release formulations. The release rate of drugs from the SOPT-B is relatively faster as compared to SOPT-A, may be, the osmotic pressure developed on both compartments was higher than that observed with SOPT-A.

Furthermore, we observed that the osmotic pressure differences between the osmotic system and the external environment changed the release rate of the drugs, thus confirming the mechanism of release of drugs is by the osmotic pressure. In-vivo prediction by method of superposition was performed on the optimized formulation
(SOPT-B) and the results indicated the formulation will produce plasma levels close to the desired ones.

These findings tend to suggest the presence of all the three drugs in the same compartment as seen in SOPT-A may be hindrance to development of required osmotic pressure to efflux the drug due to varied solubility characteristics of the drugs. From overall results of our study, it is understood that whenever soluble and poorly soluble drugs are attempted for simultaneous delivery from SOPT system, the soluble drugs be placed in one compartment and the poorly soluble drug in another compartment in order to achieve the desired results.
SUMMARY & CONCLUSION

- Elementary osmotic pump tablets failed to show simultaneous release of stavudine, lamivudine and nevirapine though showed slow release of the individual drugs.

- Release rate of drugs individually or in combination from EOPTs though slower, did not adhere to the requirements for once a day extended release formulations.

- SOPT-A significantly differed with SOPT-B in the release pattern of the drugs.

- SOPT-A failed to show simultaneous release pattern of the drugs at slow rate.

- SOPT-B showed release pattern of the drugs simultaneously and became eligible for once a day extended release osmotic systems of stavudine, lamivudine and nevirapine.

- Hydrodynamic / osmotic pressure of EOPTs and SOPTs were found to be influenced by the concentration of PEO (polymer), KCl (osmogen) and orifice diameter.

- In-vivo prediction study of the optimized SOPT indicated in-vitro performances correlating well with in-vivo predication.

    Thus, it is clear to suggest that EOPT seems not suitable for simultaneous and extended release of anti-HIV drugs with variable solubility characteristics. SOPT with soluble drugs in one compartment and poorly soluble drug in another compartment appears viable to achieve simultaneous and extended release of stavudine, lamivudine and nevirapine that can ensure better patient adherence to therapy for effective management of HIV infection.
REFERENCE


