Development and assessment of efavirenz-loaded SLN® for potential delivery to the CNS

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ABSTRACT SUMMARY

Sustained release solid lipid nanoparticles (SLN) of Efavirenz (EFV) were manufactured using hot high pressure homogenization (HHPH). Critical quality attributes that were investigated included particle size (PS), zeta potential (ZP), EFV encapsulation efficiency (EE) and in vitro release of EFV from the SLN. Optimized SLN had a PS of 59 ± 23.16 nm, ZP of -32.5 ± 4.99 mV, EE of 96.77 ± 0.453 % and cumulative EFV released of 91.5 ± 3.423 % after 24 hours. Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) confirmed the presence of spherical shaped SLN. Due to enhanced sustained release, EFV-SLN can be exploited as carrier systems to reduce adverse psychiatric effects caused by EFV.

INTRODUCTION

The Human Immunodeficiency Virus (HIV) is the causative agent of acquired immune deficiency syndrome (AIDS) ¹. By the year 2003, the World Health Organization declared AIDS a global pandemic and the disease continues to cause deaths worldwide ². In 2011 WHO reported that 34 million people were living with HIV worldwide of which 3.3 million were children < 15 years of age ². HIV primarily affects the immune system and can also infect the central nervous system (CNS) causing AIDS dementia complex (ADC) ³ leading to neurological disorders that may include psychosis and behavioral changes which may further have a negative impact on the quality of life of patients ³. Currently, the management of HIV/AIDS involves the administration of highly active antiretroviral therapy. Efavirenz (EFV) is one of the WHO-listed drugs used in HAART against HIV-1 infections in adults, adolescents and children > 3 years of age ¹. EFV reaches therapeutic levels in the CNS ⁴ and may be used to manage HIV in the CNS. However EFV levels in the CNS are generally high due to the “dose-dumping” effect associated with the use of conventional dosage forms resulting in severe psychiatric effects ⁴ which further exacerbates ADC. Therefore there is a need to develop innovative drug delivery systems that may have the potential to deliver EFV to the CNS in a controlled manner in order to minimize psychiatric side effects. SLN were developed and introduced at the beginning of the 1990s as colloidal drug carriers that were an alternate to systems such as emulsions and liposomes ⁵. SLN have been shown to have the ability to control the delivery of API.

EXPERIMENTAL METHODS

Response Surface Methodology was used to investigate the impact of four variables on the formulation and characterization of SLN. The input variables investigated were the amount of EFV, homogenization pressure, Tween®80 content and number of homogenization cycles. The levels used and variation of independent variables were selected using a non-rotatable Box-Behnken approach. The optimal EFV-loaded SLN formulation was manufactured using 10% EFV, a homogenization pressure of 1100 bar, 3% w/v Tween®80 and 3 homogenization cycles. The formulation was manufactured by heating 4.50 g glyceryl monostearate to 70°C and dissolving 0.50 g EFV in the molten lipid. An aqueous surfactant solution containing 3.00 g Tween®80 and 92.00 mL HPLC-grade water was heated to 70°C and the two phases were mixed prior to stirring with a high speed Ultra-Turrax® mixer to form a pre-emulsion. The pre-emulsion was homogenized using a high pressure homogenizer at 1100 bar for 3 homogenization cycles and then left to cool to room temperature (22°C).

As the stability of SLN is dependent on a number of factors such as particle size it is essential to assess this critical quality attribute. The PS and ZP of the SLN was assessed using a Nano-ZS Zetasizer (Malvern Instruments Ltd, Worcestershire, UK).

Efavirenz release from SLN was investigated using a Sotax™ CE 7 USP Apparatus 4 (Sotax™ AG, Binningerstrasse, Allshwil, Switzerland) equipped with 22.6 mm diameter cells operated in the closed loop mode at 37°C. The dissolution medium was 1% w/w sodium lauryl at a flow rate of 8.8 mL/min.

Short term stability studies were conducted for 8 weeks at 25°C/65% RH. Three batches of optimized SLN were assessed at 1, 2, 4 and 8 weeks. The formulations were assessed in terms of parameters that are considered benchmarks of stability for SLN and included zeta potential, particle size and encapsulation efficiency.

RESULTS AND DISCUSSION

On the day of manufacture the PS of EFV-loaded SLN was 59.00 ± 23.16 nm. The SLN were spherically shaped particles as shown in the TEM and SEM images (Figure 1). TEM revealed images of SLN in the nanometer range and the absence of any microparticles contrary to those observed in SEM images. The conditions of lyophilization used during sample preparation for SEM include the removal of water that promotes aggregation of SLN.
The SLN exhibited a biphasic release pattern with burst release over the initial 2-3 hours followed by sustained release (Figure 2). Mathematical modeling revealed that EFV release from the overall optimized EFV-SLN followed first order kinetics with an R² value of 0.9667 and zero order release after 2 hours. The initial burst release suggests the presence of an EFV-rich region in the exterior lipid layer of the colloidal carrier. However following the initial burst release EFV appeared to be released in a sustained manner that can be attributed to the shear stress of agitation during dissolution testing that destroys the surfactant layer on the SLN.⁶,⁷

Short term stability studies revealed that there was no significant change in the PS and EE and the SLN and the ZP values that indicate good formulation stability (ZP ≤ -30 mV) after storage of 8 weeks (Figure 3).

Figure 3 PS, ZP and EE of EFV-SLN following storage for 8 weeks.

CONCLUSION

EFV-loaded SLN were successfully manufactured using HHIPH resulting in the production of stable SLN in the nano-size range with high encapsulation efficiency. EFV-SLN showed prolonged release over 24 hours and therefore may be a potential technology for further development to treat ADC.

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