Orally Delivered Polymeric nanoparticles of Lopinavir: Development and Statistical optimization, *in vitro* and *ex vivo* studies

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ABSTRACT

Nanoparticles (NPs) have been extensively studied for oral drug delivery. NPs are taken up intact by M cells of peyer's patches of gut associated lymphoid tissue (GALT) followed by its systemic circulation thereby preventing first pass metabolism. This mechanism provides a chance to target antiretroviral drugs to lymphatic tissue. Lopinavir has poor oral bioavailability due to extensive first pass effect and limited intestinal uptake due to p-glycoprotein efflux. Main goal of study includes formulation and characterization of Lopinavir NPs for bioavailability enhancement and lymphatic targeting. PLGA NPs were prepared using Nanoprecipitation method. Formulation was optimized by 3^3 full factorial design using Particle size (PS) and entrapment efficiency (EE) as response parameters. Entrapment efficiency, Particle size and Zeta potential for optimized batch were found to be $90.36 \pm 0.426\%$, $169.9 \pm$ 0.231nm, -13.7 mV respectively. HPLC analysis was done using C 18 column (250 X 4.0 mm, 5 µ) at 210 nm. In vitro drug release studies showed the sustained release from NPs for 144h. Ex vivo studies through rat stomach and intestine confirmed that negligible amount of drug was released from NPs in the stomach. Lopinavir loaded NPs can be a suitable alternative to conventional tablet formulation.

Keywords: Nanoparticles, Lopinavir, GALT, Oral, PLGA

1 INTRODUCTION

Nanoparticles can be defined as solid colloidal particles, produced by mechanical or chemical means, which are typically in the nanometric size range (1 to 1000 nm) [1]. Nanoparticles (NPs) have been extensively studied for oral drug delivery. After oral administration of nanoparticles, they could adhere to cells (bioadhesion) or undergo oral absorption as a whole or may be directly eliminated in the faeces. Oral absorption of nanoparticles results in their passage across the gastrointestinal barriers and delivery of the payload into the blood, lymph and other tissues. NPs are taken up intact by M cells of peyer's patches of gut associated lymphoid tissue (GALT) followed by its systemic circulation, therefore preventing first pass metabolism. This mechanism provides a chance to target antiretroviral drugs to lymphatic tissue, which is the major site of storage and replication of HIV.

Lopinavir is a protease inhibitor used for treatment of HIV. The drug has poor oral bioavailability due to extensive first pass effect [2].

Biodegradable polymers have been used in pharmaceutical and medical applications for many years. Poly (D,L-lactideco-glycolide), is a polymer of choice for developing an array of micro and nanoparticulate drug delivery systems as it has excellent biocompatibility, and predictable biodegradability.

Main goal of the present study includes the development, optimization and characterization of Lopinavir NPs for bioavailability enhancement and lymphatic targeting for improved antiretroviral therapy. Therefore, to design a suitable nanoparticulate formulation which would be an effective alternative to the conventional tablet formulation.

2 EXPERIMENTAL METHODS

2.1 Materials

Lopinavir was obtained as gift sample from Aurobindo Pharma Ltd, Hyderabad, India. PLGA was obtained as gift sample from Purac Biomaterials, Holland. Pluronic F-68 was purchased from Hi-media, Mumbai, India. All other chemicals used were of analytical grade.

2.2 Preformulation studies

FTIR studies were performed using FTIR spectrophotometer (Bruker instruments, Germany) to confirm compatibility between Lopinavir and PLGA. FTIR spectra for pure Lopinavir, PLGA, Physical mixture of Lopinavir and PLGA were recorded.

2.3 Method of Preparation

PLGA NPs were prepared using Nanoprecipitation method [3]. Solution of PLGA and Lopinavir in Acetone was added drop wise into aqueous phase containing Pluronic F-68 and continuously stirred until the complete evaporation of organic solvent had taken place. The nanoparticulate suspension was then centrifuged at 3,500 rpm for 15 min (3K 30, Sigma centrifuge Osterode, Germany) for separation of free drug. The nanoparticulate suspension was then lyophilized using sucrose as a cryoprotectant. Drug concentration (X_1) , Polymer concentration (X_2) and surfactant concentration (X_3) were optimized by 3³ full factorial design using Particle size (PS) and entrapment efficiency (EE) as response parameters. The coded values and actual values of formulation parameters are shown in Table 1.

2.4 Particle size and zeta potential

The particle size and zeta potential of NPs were measured by malvern zetasizer nanoseries-zs (Malvern, Worcesterchire, UK). Sample was diluted 10 times with distilled water and then it was analyzed for particle size and zeta potential. The electrophoretic mobility (μ m/sec) was converted to zeta potential by in-built software using Helmholtz-Smoluchowski equation.

2.5 Entrapment efficiency

The entrapment efficiency of NPs was determined after separation of free drug from NPs suspension at 3500 RPM for 15 min using Sigma centrifuge (Osterode, Germany). The NPs were dissolved in acetonitrile and then analysed by HPLC (Shimadzu, Japan) using C18 column (250 X 4.0 mm, 5 μ) at 210 nm using Acetonitrile : Buffer (KH₂PO₄) (60:40) as mobile phase.

2.6 Transmission electron microscopy

Transmission electron microscopy (TEM) was performed by TEM (Philips Technai 20, USA) to study the morphology of NPs. PLGA NPs were dispersed in distilled water and one drop was incubated on carbon coated copper grid. This copper grid was fixed into sample holder and placed in vacuum chamber of transmission electron microscope and observed under low vacuum.

2.7 In vitro drug release studies

In vitro release of Lopinavir from PLGA Nanoparticles was evaluated by the dialysis bag diffusion technique. The diffusion medium consists of pH 7.4 phosphate buffer containing Brij 35. The nanoparticulate dispersion equivalent to 1 mg of Lopinavir was placed in the dialysis bag (cut-off 12 000; Himedia, Mumbai, India), which was sealed at both ends. The dialysis bag was immersed in 50 ml of the receptor compartment, which was stirred at 50 rpm and maintained at $37 \pm 2^{\circ}$ C. The receptor compartment was covered to prevent the evaporation of release medium. Samples were withdrawn at regular time intervals (0, 1, 3, 3)6, 12, 24, 30, 48, 60, 72, 96,120 and 144 h), and the same volume was replaced by fresh release medium. The acceptor phase was changed every 3 days to maintain sink condition. The samples were analyzed by HPLC using C18 column (250 X 4.0 mm, 5 µ) at 210 nm using Acetonitrile :

water(60:40) as mobile phase. Similar procedure was followed for Plain drug suspension.

2.8 *Ex vivo* drug release studies

drug release studies were performed on Ex vivo stomach and intestine segments. All animal experiments were approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi, India. Male wistar rats (250-300g) were sacrificed by cervical dislocation. Stomach and a part of intestine were isolated. The isolated organs were washed and cleansed with their respective solutions. 2ml of the nanoparticulate suspension was filled into the stomach which was tied at both the ends. The tissue was placed in an organ bath with continuous aeration at 37°C. The receptor compartment (organ tube) was filled with 30 ml of 0.1N HCl containing Brij 35. At predetermined intervals (15, 30, 60, 90 and 120 min.) of time, aliquots were withdrawn from the receptor compartment. Fresh buffer was used to replenish the receptor compartment. The 10 microlitre of sample was injected and analyzed by HPLC (Shimadzu, Japan) using C18 column (250 X 4.0 mm, 5 µ) at 210 nm using Acetonitrile : Buffer (KH_2PO_4) (60:40) as mobile phase. The percent diffusion of drug was calculated against time and plotted graphically. After 2h, to mimic the in vivo gastric emptying, the solution from the stomach was transferred to the intestine which was then tied at both ends [4]. The receptor compartment was replaced with PBS pH 7.4 containing Brij 35 and the tissue was mounted on the organ tube. At predetermined intervals (30, 60, 120, 180, 240, 300 and 360 min.) of time, aliquots were withdrawn from the receptor compartment. Fresh buffer was used to replenish the receptor compartment. The samples were analyzed by HPLC using C18 column (250 X 4.0 mm, 5 µ) at 210 nm using Acetonitrile:Buffer (KH₂PO₄) (60:40) as mobile phase. The percent diffusion of drug was calculated and plotted graphically. The study was also performed using plain drug suspension following the above mentioned procedure.

3 RESULTS AND DISCUSSION

Studies based on factorial designs allow all the factors to be varied simultaneously, thus enabling evaluation of the effects of each variable at each level and showing interrelationship among them. The number of experiments required for these studies is dependent on the number of independent variables selected. The data from factorial design and Response surface plots indicated that Particle size and Entrapment efficiency were dependent on X_1 , X_2 and X_3 . Total 27 batches of formulation were prepared by 3^3 Factorial Design. Entrapment Efficiency and Particle size values for all the 27 batches showed a wide variation. Entrapment efficiency and Particle size for optimized batch were found to be 90.36 \pm 0.426%, 169.9 \pm 0.231nm respectively. For orally delivered NPs, particle size has to be kept below 200 nm to avoid first pass effect. The particle uptake is reported to be a size dependent phenomenon, where small sized particles could efficiently be taken up as compared to the bigger sized particles [5]. Zeta potential was found to be -13.7 mV. The high negative zeta potential indicated that the electrostatic repulsion between particles with same charge will prevent the aggregation and increase the stability. The negatively charged particles will be easily uptaken by the Peyer's patches and then translocated to the systemic circulation. FTIR (Fig 1) confirmed no interaction between drug and polymer. TEM studies confirmed spherical shape of NPs with no aggregates.

In vitro drug release studies (Fig. 2) showed the sustained release from NPs for 144 h. *Ex vivo* studies (Fig. 3) confirmed that negligible amount (9.11%) of drug was released from NPs in the stomach while plain drug suspension released nearly 50% of drug in 2h in stomach which would be degraded in acidic environment of stomach.

Table 1: Coded values and Actual values of formulation parameters for Lopinavir loaded NPs for 3^3 full facrorial design , where X_1 is drug concentration, X_2 is Polymer concentration, X_3 is surfactant concentration

	Actual Values		
Coded values	X 1	X ₂	X ₃
-1	5mg	25mg	25 mg
0	10 mg	50 mg	50 mg
+1	15 mg	75 mg	75 mg



Fig 1 FTIR of Lopinavir (A), PLGA (B), Physical mixture (C)



Fig 2 In vitro drug release profile of Lopinavir from plain drug suspension and NPs formulation



Fig 3 Ex vivo Drug release profile of lopinavir from NPs and plain drug suspension, through stomach for initial 2h and through intestine segment for 6h to simulate gastric emptying behaviour.

4 CONCLUSION

The NPs were prepared by nanoprecipitation method and optimized by 3³ Factorial design were found to have entrapment efficiency of above 90 % and particle size below 200 nm. As the size of NPs was below 200 nm, it could be concluded that the NPs would be taken up by M cells of Peyer's patches. Moreover, the NPs had negative zeta potential, therefore they would be more efficiently taken up by M cells. In vitro and ex vivo release studies demonstrated sustained drug release from the NPs. The negligible drug release from the NPs as compared to very high release from plain drug suspension in stomach will prevent degradation in acidic media and avoid first pass effect. The sustained drug release in the intestine from NPs indicates that Lopinavir loaded polymeric NPs can be a suitable alternative to conventional oral tablet of lopinavir by targeting the drug to lymphatic tissue, sustaining the drug release and thereby enhancing the bioavailability.

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