DESIGN, DEVELOPMENT AND EVALUATION OF NANOFORMULATION FOR BRAIN TARGETING AND BIOAVAILABILITY ENHANCEMENT OF ANTI-VIRAL DRUG

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Ms. Shweta Gupta

Enrollment No: 119997290052

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Supervisor:

Dr. Rajesh Keserla,

Principal & Professor,
Oxbridge College of Pharmacy,
Banglore, India.

Co-supervisor:

Dr. Abdelwahab Omri,

Professor,
Laurentian University,
Ontario, Canada.

Doctoral Progress Committee (DPC) Members:

Dr. Ambikanandan Misra

Professor, Faculty of Pharmacy,
M.S.University of Baroda,
Vadodara, Gujarat, India.

Dr. Narendra P. Chotai

Associate Professor,
A.R.College of Pharmacy, V.V. Nagar,
Anand, Gujarat , India.

Index

1. ABSTRACT

HIV infections causing AIDS is one of the most life-threatening infections and is a leading cause of death. The non-nucleoside reverse transcriptase inhibitors, used for the treatment of HIV infections are reported to have low bioavailability pertaining to high first pass metabolism, high protein binding and enzymatic metabolism. They also show low permeability across blood brain barrier. The CNS is reported to be the most important HIV reservoir site. In the present study, solid lipid nanoparticles (SLN) of efavirenz were prepared with the objective to provide increased permeability and protection of drug due to biocompatible lipidic content and nano-scale size, and thus to develop formulation having potential for enhanced bioavailability and brain targeting. Solid lipid nanoparticles were prepared by high pressure homogenization technique using a systematic approach of design of experiments and evaluated for particle size, polydispersity index, zeta potential and entrapment efficiency. Particles of average size 108.5 nm having PDI of 0.172 with 64.9% entrapment efficiency were produced. Zeta potential was found to be -21.2 mV and the formulation was found stable upto 12 months. The formulated solid lipid nanoparticles of efavirenz were also evaluated for surface morphology using transmission electron microscope and were found to be spherical in shape and non-irritant/harmless with histopathological studies. The optimized SLN were incorporated in *in-situ* gelling system. The efavirenz SLN based gel was evaluated for various parameters like gelation temperature, time, pH, viscosity, transmittance, mucoadhesive strength, spreadability, *in-vitro* and *ex-vivo* release studies. The release of dug was found to best fit in the Zero order release kinetics (R^2 = 0.9936), followed by Higuchi model (R^2 = 0.9899) indicating concentration independent diffusion controlled release. The in-vivo pharmacokinetic studies revealed increased concentration of the drug in brain, when administered through intranasal route indicating its potential for an attempt towards complete eradication of HIV and cure of HIV-infected patients.

2. INTRODUCTION

Poorly soluble drugs are highly prevalent in the pharmaceutical field leading to low bioavailability. These drugs pose a major challenge to the formulation scientists in order to increase the bioavailability and develop a targeted drug delivery system [1]. Drug delivery to brain is even more challenging due to the presence of blood brain barrier (BBB) and brain cerebrospinal fluid

barrier (BCSFB) [2,3].

Viral infections are extremely widespread and are of various types like common cold, influenza, rabies, measles, chickenpox, small pox, herpes, AIDS, mumps, measles, rubella, viral hepatitis, viral meningitis, viral pneumonia, etc. [4,5]. The most life - threatening infection is of HIV causing AIDS. According to the World Health Organization approximately 35 million people worldwide are living with HIV/AIDS including 3.2 million children of less than 15 years age and an estimated 2.1 million individuals worldwide are newly infected with HIV every year [6]. AIDS is the sixth leading cause of death among people aged 25 - 44 in the United States [7].

2.1 Brief description on the state of the art of the research topic

Solid lipid nanoparticles (SLN) are colloidal dispersions or particulates in the size range of 100–500 nm composed of biocompatible lipid matrix that are physiologically well tolerated when administered *in-vivo* [8]. SLN are gaining increased attention during recent years because of various advantages over other colloidal drug delivery systems.

2.2 Advantages of SLN [9-11]

- Biocompatibility of physiological lipids
- Potential for increased permeability due to small size as well as lipid and surfactant contents and hence, enhanced bioavailability
- Increased protection and stability to the encapsulated drug in biological fluids as compared to other colloidal systems, such as liposomes or micelles
- Possibility for passive or ligand-mediated targeting, CNS delivery
- High drug loading capacity
- Suitable for different routes of administration (oral, parenteral, dermal, nasal, ocular, etc.)
- Possibility of controlled drug release
- Less variability in release mechanisms and their kinetics
- Ease of large scale production and sterilization

In the present investigation, an attempt was made to design and formulate solid lipid nanoparticles of the anti-viral drug, efavirenz to increase their bioavailability and overcome the challenges

associated with the drug. The nanoparticles of efavirenz were also proposed to target the drug to brain and increase its bioavailability in brain when administered through intranasal route.

3. Definition of the problem

From the literature review on the subject related to HIV/AIDS and their available therapies, the challenges associated with the current therapies were identified, and from the review emerged the research problem to be addressed.

Current therapies for HIV infections with antiretroviral drugs is effective in reducing plasma viral levels, but are ineffective in eradicating the virus from other sites like CNS due to their inability to reach and accumulate in these cellular and anatomical reservoirs where virus potentially harbours. The CNS is the most important HIV reservoir site [12]. Due to the restricted entry of anti-HIV drugs, the brain is thought to form a viral sanctuary site. This not only results in virological resistance, but also is often associated with the development of complications such as progressive deterioration in mental function, symptoms of motor abnormalities, mild neurocognitive disorder (MDR), HIV associated dementia (HAD), HIV encephalitis (HIVE) and even death in many cases [3,12,13].

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of choice and is recommended as a first line antiretroviral drug used in the high activity antiretroviral therapy (HAART) for the infections of human immunodeficiency virus [14]. Efavirenz (EFV) is a highly lipophilic drug of BCS class II having water solubility of 9.2 µg/ml (pH 8.7) at 25°C and 4.6 as the log P value [15]. Because of low water solubility of the drug, extensive first pass metabolism, metabolism by enzymes, high protein binding, efflux mechanisms, low bioavailability (40-45%) of the drug has been reported [16-20].

Hence, the aim of the present investigation was to design and formulate solid lipid nanoparticles of anti-viral drug, efavirenz to deliver it to the brain in order to increase their bioavailability at the reservoir site of HIV, perform the evaluation studies and compare the formulations.

4. Objective and scope of work

The overall objectives of the research are summarized as:

- To select the right material, process and optimization design for preparation of SLN.
- To provide lipid protection to the drug.
- To avoid extensive first pass metabolism of the drug.
- To select proper route of administration.
- To target the drug to the brain.
- To reduce the efflux of drug.
- To estimate the drug concentration in brain and plasma post-administration of formulation and comparison with existing formulation.

Scope of the research work:

By developing the effective delivery system for the existing drug which can effectively diffuse in cellular and tissue compartments where the virus harbors (reservoir site of HIV) is a potential way in an attempt for completely cure the HIV-infected patients. After clinical trials and fulfillment of other regulatory requirements, the developed formulation may prove to be a boon to the society at large for the complete treatment of the highly-dreaded disease of AIDS.

5. Original contribution by the thesis

The entire work in this synopsis, as well as thesis is original. Extensive literature review was done to identify the challenges associated with the complete cure of AIDS and approaches which can resolve them. Although multitude researchers have worked on development of solid lipid nanoparticles (SLN) for various drugs, the idea of the development of SLN of anti-HIV drug by systematic approach of design of experiment for optimization of various parameters and to target the drug to brain through intranasal route (to avoid various drawbacks associated with oral route) for an attempt towards complete eradication of HIV from its reservoir site was probably yet not investigated by any other researcher.

6. Methodology of research, Results / Comparisons

6.1 Design of Experiment

Literature survey was done to identify and determine the quality target product profile (QTPP),

critical quality attributes (CQA), manufacturing procedures for SLN, various process and formulation attributes having effect on product CQA. Investigations were done by factorial design to minimize particle size and maximize the encapsulation efficiency of the drug in SLN. In the present investigation, different preliminary experiments were performed for selection of suitable excipients/materials which may directly and/or indirectly influence critical quality attributes. The compatibility of the drug-excipients was checked before the optimization of various process and formulation variables. With the selected excipients, various critical process variables identified through literature were optimized sequentially as per the steps involved in the formulation. Finally, the formulation variables were optimized using 3^2 factorial design and design expert software for analyzing the data statistically and graphically using response surface plots [21-23].

6.2 Analytical Methods

6.2.1 UV- spectrophotometric method

UV spectrophotometric methods have been reported for the estimation of efavirenz in formulations [24,25]. Calibration curves were plotted in methanol:waterl::1:1 as solvent for determination of entrapment efficiency using UV-1800 spectrophotometer (Shimadzu) at 247 nm, Linearity was observed in the concentration range of 3-18 µg/ml with regression coefficient value of 0.999. Linearity was also observed with the calibration curves plotted using 40% methanolic phosphate buffer pH 6.4 as solvent. The regression equation determined for the buffer system was used for drug-release studies.

6.2.2 HPLC Method Development & Validation

A simple, sensitive and rapid high performance liquid chromatographic method was developed and validated for the determination of efavirenz in plasma. The method was developed with HPLC using Waters X-Terra Shield, RP18 50 x 4.6 mm, 3.5 µm column and a mobile phase consisting of phosphate buffer pH 3.5 and Acetonitrile. The elution was monitored with the UV-Visible detector at 260 nm with a flow rate of 1.5 ml/min. Tenofovir disoproxil fumarate was used as internal standard. The method was validated for linearity, precision, accuracy, specificity, robustness and data obtained were statistically analyzed. Calibration curve was found to be linear over the concentration range of 1-300 µg/ml. The retention times of EFV and TDF (internal standard) were 5.941 min and 4.356 min respectively. The regression coefficient value was found to be 0.999. The

limit of detection and the limit of quantification determined were 0.03 and 0.1 µg/ml respectively. The developed HPLC method was used for determination of efavirenz in brain and plasma.

6.3 Selection of lipid

Improved permeability of the drug is reported due to lipid content in SLN [16]. It is presumed that higher the solvent capacity of the lipid, higher will be the drug loading potential [26]. Hence, the selection of the lipid was primarily based on the solubility of the drug in lipid. The solubility of the drug was determined in six different lipids - Glycerymonostearate, Glyceryl behenate (Compritol 888 ATO), Glyceryl tripalmitate (Tripalmitin), Glyceryl palmitostearate, Glyceryl distearate and Cetyl palmitate. Amount of drug dissolved in known amount of each lipid at a temperature 5^0C above the melting point of the respective lipid was determined using digital shaker water bath (NOVA Inst Pvt. Ltd., Ahmedabad, India) [27].

Glyceryl tripalmitate (tripalmitin) showed maximum drug solubilizing capacity of 120 $\pm$ 10 mg drug / gram of lipid while glyceryl palmitostearate and cetyl palmitate showed next highest solubilizing capacity of 70 $\pm$ 10 mg drug / gram of lipid. Thus, the solubility of drug in glyceryl tripalmitate was found to be significantly higher than other lipids ($p < 0.05$). In addition, tripalmitin are commonly ingested in food, fully digested and absorbed, and therefore do not present any safety issues [28]. Tripalmitin also has GRAS status as per 21 CFR § 186.1555 and hence was selected for further investigations.

6.4 Selection of Surfactant

SLN are the colloidal system of nanoparticles composed of lipid as matrix and is stabilized in aqueous media by surfactants [29]. Solubilization of endothelial cell membrane lipids and membrane fluidization due to surfactant effect leads to improved permeability [17]. For the selection of surfactant, nanoparticles were prepared with six different surfactants using selected lipid (tripalmitin) and were evaluated for particle size, PDI and entrapment efficiency.

Poloxamer 188 (Pluronic F68) was found to give minimum particle size and PDI with maximum entrapment efficiency. The difference in particle size was found to be statistically significant in comparison to others ($p < 0.05$). Poloxamers are reported to sterically stabilize the nanoparticles and reduce the adsorption plasma proteins or opsonins on the surface of nanoparticles by providing

hydrophilic property to the surface of nanoparticles, thus can prevent the clearance of drug containing SLN from circulation [29]. In general, bulky and non-ionic surfactants are reported to be less toxic than single-chain and ionic surfactants [28]. Hence, Poloxamer 188, also having GRAS status [30] was selected for further investigations for the formulation.

6.5 Drug-Excipients Compatibility Study

IR spectra of pure drug, and the physical mixtures of drug and selected excipients stored at 25 ± 2 ^{O}C, 60% ± 5% relative humidity for a period of 7 days were recorded using FT-IR spectrophotometer (Bruker Alpha-one, Bruker Optik, Germany) in the range of 4000–500 cm^{-1} and compared for any significant change [31, 32]. It was observed that all major peaks of the drug were obtained in the IR spectra and no significant change was observed in the IR spectra of drug-excipients mixture indicating the compatibility of the drug with the selected excipients [14].

6.6 Selection of formulation Technique

Various techniques for SLN formulation are reported [9, 33-35]. The selection of the technique was made based on the evaluation of particle size, PDI and entrapment efficiency of the nanoparticles obtained with the trial batches prepared using the most commonly used and reported to be reliable and powerful two techniques.

6.6.1 High Pressure Homogenization

There are two general homogenization techniques (hot homogenization and cold homogenization) which can be used for the production of SLN [9]. In the present study, hot homogenization technique was investigated. The drug was incorporated into the melted lipid. The drug loaded lipidic phase was dispersed in a hot aqueous surfactant solution under continuous stirring to form a coarse o/w emulsion. It was then homogenized at the temperature above the melting point of the lipid using high pressure homogenizer (Panda Plus / GEA Niro Soavi, Parma, Italy) to form o/w nanoemulsion which was cooled to room temperature for solidification and formation of solid lipid nanoparticles [36,37].

6.6.2 Solvent Evaporation method

The lipophilic drug was dissolved in a water-immiscible organic solvent and was emulsified in an

aqueous phase containing the surfactant under continuous stirring on a magnetic stirrer. The organic solvent was evaporated and nanoparticulate dispersion was formed by precipitation of the lipid in the aqueous medium [9].

6.6.3. Comparison of formulation techniques

It was observed that lower particle size with higher entrapment efficiency was obtained by high pressure homogenization. The difference in particle size and entrapment efficiency obtained with two techniques was found statistically significant ($p < 0.01$). The results were found to be in accordance with the findings reported in the literature [9]. There were no chances of residual organic solvent since the high pressure homogenization technique avoids the use of organic solvent. The use of organic solvents presents a major toxicological disadvantage with solvent evaporation technique [16,38]. Another advantage of the high pressure homogenization technique is its scale up feasibility as it easily allows a laboratory, pilot or large scale production [27]. Hence, further investigations were carried out with high pressure homogenization technique.

6.7 Optimization of Process Variables

On the basis of literature survey and a few trial batches, various critical process variables which may have significant effect on the critical quality attributes were identified for each step involved in the formulation and were subjected to optimization. Preliminary optimization of stirring time and RPM of high speed homogenizer (Heidolph silent crusher M) were carried out by conducting the experiments at three different RPM (5,000 to 10,000) for three time durations (10 to 20 minutes) at room temperature. From the results obtained (results not shown) in terms of particle size and PDI, it was observed that as the homogenization time and/or homogenization speed increased, the particle size decreased. It was also observed that with the increase in homogenization time PDI also increased which may be due to formation of foam and more aggregation in the formulation. Better results were obtained by stirring at 10,000 RPM for 15 minutes. Hence further investigations were done with homogenization/ stirring speed of 10000 RPM for 15 min.

To study the effect of temperature on the performance attributes, particle size and PDI were determined for the investigations carried out at three different temperatures (60-80^{0}C). Promising results were obtained at 70^{0}C. It may be because the lipid would have remained at melted condition

at this temperature as well as the temperature is much below the melting point of the drug, hence drug degradation due to temperature remains negligible. In general, higher temperatures result in lower particle sizes which may be due to the decreased viscosity of the inner phase [19].

Critical process variables involved during the high pressure homogenization were pressure and number of cycles. These were optimized using 3^2 full factorial designs with Design Expert 9.0.3.1 software (Stat-Ease, Inc., USA). Thirteen runs were carried out with pressure (500 to 900 bars) and number of cycles (3 to 7) as independent variables at three levels. Particle size and PDI were considered as dependent variables.

ANOVA was applied to determine the significance and the magnitude of the effects of the variables and their interactions. Quadratic model were found to be significant for particle size as well as PDI. Pressure (A), Number of cycles (B) and A^2 were found to be significant model terms ($p < 0.05$), while the model terms AB and B^2 were found to be non-significant for particle size. The individual effect of number of cycles (B) was found to be significant (P value <0.05) and of pressure (A) was marginally significant (P value = 0.0536).

The regression model were also used to generate the contour plots, 3D surface plots and the overlay plot for particle size for analyzing interactions of the independent factors. It was observed from 3D surface plots that as the pressure and/or number of cycles were increased, the particle size and PDI were found to reduce. The results were found in contrast to the investigations for production of SLN with cetyl palmitate as the solid lipid where particle size and PDI was observed to be increased with increase in pressure [18].

6.7.1 Experimental validation of design space

(For process variables during high pressure homogenization)

Experimental validation of DoE trials was undertaken by preparation and characterization of nanoparticles at the check point batch suggested by software. The observed values (Particle size 259.7 nm and PDI 0.220) were in close agreement with the predicted values (Particle size 267.274 nm and PDI 0.263276) and established the reliability of the optimization procedure. For further reduction in particle size, the effect of sonication was also investigated and sonication time and

amplitude were optimized. The experiments were carried out at three amplitudes for three time durations (data not shown). No significant effect was observed with change in amplitude. Particle size was found to reduce with increase in sonication time but PDI was increased with increased time. Homogenization followed by ultrasonication is reported to be suitable method to produce SLN of 60–380 nm size ranges [39].

6.8 Optimization of Formulation Variables

3^2 factorial design was employed for optimization of formulation variables and Design Expert 9.0.3.1 software was used for statistical analysis by ANOVA, generating model equations and constructing contour plots and 3D surface plots for each response. Amount of drug with respect to lipid and concentration of surfactant were investigated as independent variables at three levels and the critical quality attributes selected were particle size, PDI and entrapment efficiency as responses. A total of 11 experiments were designed by the software with 2 centre points. Experiments were run in random order to increase the predictability of the model. ANOVA was also applied to determine the significance and the magnitude of the effects of the formulation variables and their interactions. ANOVA for Response 1 (Particle size), Response 2 (PDI) and Response 3 (% Entrapment) indicated that linear model best fits for particle size and PDI while quadratic model showed the best fit for entrapment efficiency. P-values less than 0.05 indicated model terms to be significant. It was observed that particle size reduced with increase in drug-to-lipid ratio and increase of concentration of surfactant. The results were found in agreement with the reported observations [40]. PDI was also observed to reduce with increase in surfactant concentration. This may be because high concentrations of the surfactant would have reduced the surface tension and facilitated the particle partition during homogenization. The increase of the surface area during HPH occurs very rapidly due to reduction in size. The process of a primary coverage of the new surfaces competes with the agglomeration of uncovered lipid surfaces [9].

6.8.1 Experimental validation of design space

(For formulation variables)

Experimental validation of DoE trials for formulation variables was undertaken by formulation and characterization of nanoparticles at the check point batch suggested by the software. The observed values (Particle size 108.3 nm, PDI 0.172 and EE 64.9%) were comparable with the predicted

values (Particle size 109.088 nm, PDI 0.186561 and EE 65.3482) establishing the reliability of the optimization procedure.

6.9 Formulation and Optimization of Gel

Different gelling and mucoadhesive agents were screened based on their gelling properties with SLN dispersion. Poloxamer 188 and poloxamer 407 were selected as thermosensitive polymers based on literature [41,42]. Ratio of poloxamer 188 and poloxamer 407 were optimized (1:2) based on their gelling behaviour at different temperatures. Carbopol 934 P was selected and its concentration was optimized (0.4%) in the presence of selected thermosensitive polymers.

6.10 Evaluation of optimized formulation

6.10.1 Particle size, Polydispersity index, Zeta potential and Entrapment efficiency:

The average particle size, PDI and zeta potential of the solid lipid nanoparticles was determined using Zetasizer Nanoseries Nano-ZS, Malvern Instruments, Malvern, UK. Entrapment efficiency was determined by determining the amount of free drug spectrophotometrically at 247 nm in the supernatant after centrifugation of the known amount of nanoparticulate dispersion at 10000 RPM using REMI centrifuge (BL – 135 R) for 15 minutes. The entrapment efficiency was calculated using the equation [43]

Entrapment efficiency

$$= \frac{\text{Weight of drug added in the formulation - Weight of free drug}}{\text{Weight of drug added in the formulation}} \times 100$$

The average particle size, PDI, zeta potential and entrapment efficiency of the solid lipid nanoparticles was found to be 108.3 nm, 0.172, -21.2 mV and 64.9% respectively. Average particle size of less than 110 nm indicated the suitability of the formulation for administration through various routes with the potential of increased permeability and thus enhanced bioavailability of the poorly soluble drug efavirenz. Low PDI value (< 0.2) indicated the narrow distribution of size (monodispersity) and stability of the formulation was indicated by the zeta potential value (-21.2 mV). In the case of a combined electrostatic and steric stabilization achieved by large molecule weight stabilizers, a minimum zeta potential of ± 20 mV is desirable [31]. Nanoparticles in the

range of 180 – 190 nm were achieved with cetyl palmitate as lipid and are reported to be suitable for targeting to brain [11,44]. In general, Z-average size of 20-500 nm are reported depending on the drug, lipid, surfactants and the formulation technique used [40,45,46].

6.10.2 Evaluation of SLN based gel

The formulated SLN based gel was evaluated for gelation temperature, gelation time, pH, viscosity, transmittance, mucoadhesive strength (determined by two-pan balance method) and spreadability (0.5 g gel between two glass and 100 g weight on it) and were found to be 34^0C, less than 1 minute (at 34^0C), 6, 19000 cps (at 25^0C), 24500 cps (at 34^0C), 92% (10 times dilution, 650 nm), 18150 dynes/cm^2 and 10 cm/gm gel respectively.

6.10.3 Transmission Electron Microscopic Evaluation

The surface morphology of the optimized SLN was investigated using transmission electron microscope (TEM Philips Tecnai 20, Holland). Briefly, it was carried out by operating at an acceleration voltage of 200 kV. A drop of SLN dispersion was placed on grid. Approximately 2 min after sample deposition (1-2 µl), the grid was tapped with filter paper to remove surface water and air dried. Spherical particles were observed with drug incorporated in the lipid matrix.

6.10.4 Histopathological studies

Histological studies were carried out using isolated goat nasal mucosa. Freshly isolated goat nasal mucosa was sectioned in three pieces. One piece was treated with PBS pH 6.4 (as negative control), the other with a mucociliary toxicity agent - isopropyl alcohol (as positive control) and the third one with the SLN formulation [47]. After 24 hours, all the samples were washed properly with distilled water, fixed, processed for dehydration, embedded into paraffin wax and stained with hematoxylin and eosin. DPX was used as mounting medium and microtoming was performed using microtome (model 0126, Yorco, India). The histopathological examinations for determination of damage/irritation due to the formulation were performed using inverted microscope (NIKION TS -100) [41,48]. No significant damage /harmful effects on the microscopic structure of the nasal mucosa treated with SLN formulation for was observed in comparison to that of sample treated with isopropyl alcohol indicating the safety of the SLN formulation for nasal adminstration. This was in accordance with the results obtained by Seju et al [47]. Mucosa treated

with isopropyl alcohol showed heavy loss of epithelial cells.

6.10.5 Drug Release Profile

In- vitro drug diffusion profile was obtained by dialysis-bag/dialysis-sac method [49]. SLN dispersion and plain drug suspension were filled in activated dialysis membrane bags (Dialysis membrane 110 (LA 395), Himedia, cut off 12000 Da) and suspended in glass beakers containing 50 ml methanolic phosphate buffer pH 6.4 [26,47]. The beakers, covered with paraffin film to prevent any evaporative loss during the experimental run were placed on magnetic stirrers. Aliquots were withdrawn from the receptor compartments at periodic time intervals for 24 hours and replaced with equivalent amounts of fresh diffusion medium. The aliquots were analyzed spectrophotometrically at 247 nm. All the experiments were performed in triplicate. The results obtained are as shown in Figure 1. The release of drug from the SLN formulations was found to be more consistent in comparison to that of plain drug suspension. More than 80% drug was found in 24 hours with SLN dispersion and SLN gel in comparison to diffusion of less than 65% drug with plain drug suspension in 24 hours.

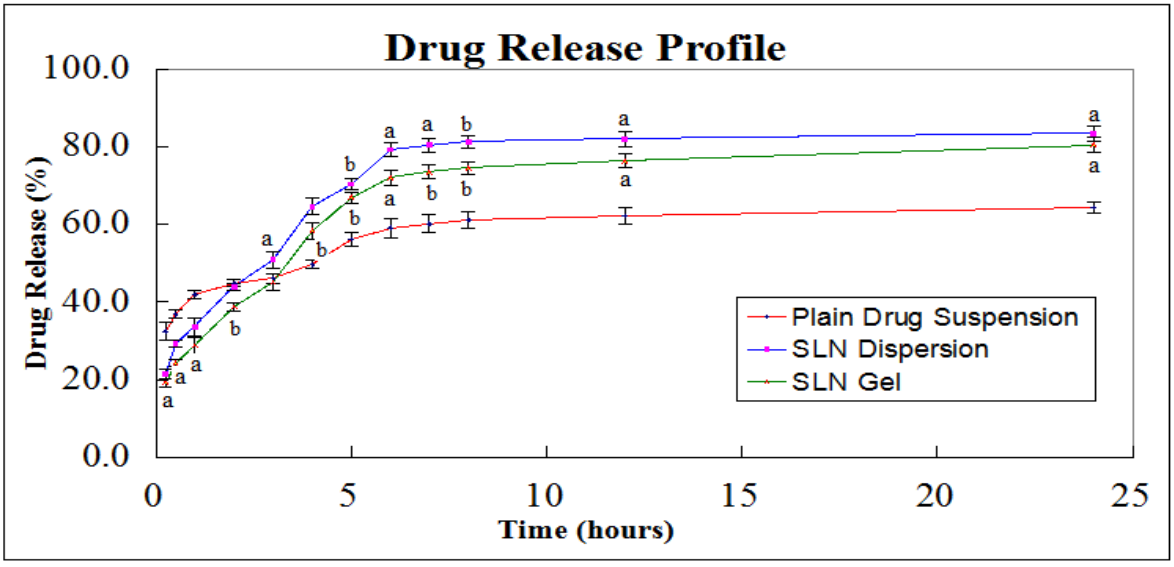

Figure 1: Drug Release Profile (Different letters indicate statistically significant difference relative to plain drug suspension; a indicate $p < 0.01$ and b indicate $p < 0.05$)

6.10.6 *In-vivo* studies

In-vivo studies were performed on adult Wistar albino rats. A protocol for animal studies was approved by Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of

Control and Supervision of Experiments on Animals (CPCSEA), [Protocol no. PIPH 04/15 CPCSEA921/PO/Ere/S/05/CPCSEA, approved on 18th June, 2015]. Animals were housed in polypropylene rat cages. Rice husk was used as the bedding material. Laboratory rat pellet feed and pure drinking water was supplied ad libitum. The rats were divided into two groups. Group I (test group) consisting of 6 animals were administered with the developed SLN formulation (0.25ml equivalent to 0.06 mg efavirenz) intranasally. The second group (standard) consisting of 6 animals were given the marketed formulation – EFAVIR 200 - efavirenz capsules IP orally (powder equivalent to 25 mg efavirenz from capsule dispersed in 1 ml water) [50]. The plasma samples from each animal were collected and the animals were sacrificed by an overdose of pentobarbital sodium at 24 hour. The brains were isolated, weighed, homogenized in PBS pH 6.4 at 5000 rpm using silent crusher homogenizer (Heidolph, Germany), centrifuged and the supernatant were collected for determination of drug concentration [29]. The amount of drug in plasma and the brain homogenate were determined by the method developed and validated for estimation of efavirenz in plasma using HPLC. Brain:Plasma ratio, bioavailable fraction and relative bioavailability were calculated using the formulae:

Brain: plasma = Conc. of drug in brain/Conc. of drug in plasma

Bioavailable fraction

= Bioavailable dose/ Administered dose

Relative bioavailability

=Systemic availability of drug/systemic availability of an oral standard of same drug

The ratio of drug concentration in brain to plasma of 15.61% was achieved with the developed formulation in comparison to 0.104% observed with the oral standard indicating the 150 times more brain targeting efficiency of the formulation through intranasal route. The bioavailable fraction of the drug was calculated to be 0.2454 with the developed formulation while it was found to be 0.0035 with the standard. Relative bioavailability was determined to be 70.11 with the developed formulation indicating 70 times better absorption potential of the efavirenz loaded SLN dispersion in comparison to the orally administered drug powder. The results were found to be in accordance with the similar investigations with different drugs for brain targeting [51].

6.10.7 Stability Studies

The stability of the formulation were assessed under different storage conditions as per ICH

guidelines, namely, 5 ± 3 ^{0}C and 25 ± 2 ^{0}C/60 ± 5% RH [43,52,53]. The samples were evaluated at 0, 0.5, 1, 2, 3, 6 and 12 months for physical appearance, average particle size, PDI and zeta potential. All the studies were conducted in triplicate. The developed formulations (efavirenz loaded SLN dispersion and the gel formulation) were found to be stable for 12 months. No change in the physical appearance of the formulation was observed during the stability studies. No significant change in the Z-average size, PDI and zeta potential were observed during the stability studies when analysed using student's t-test. Thus it can be concluded that efavirenz SLN were stable at long term stability conditions (5±3°C) as well as accelerated conditions (25 ± 2°C/60 ± 5% RH).

7. Achievements with respect to objectives

Different lipids, surfactants, process, gelling agent, etc. were screened and statistically analyzed for proper selection. By selection of intranasal route of delivery, the first pass metabolism and enzymatic degradation of the drug in GIT was avoided. By the use of poloxamer reported to reduce efflux mechanism [42], the drug was successfully targeted and released in the brain and thus it could be concluded that the developed formulation has the potential to reach to the latent viral reservoir site for an attempt for complete cure of HIV infections. The analytical method was successfully developed and validated for the estimation of efavirenz in brain and plasma.

8. Conclusion

With the present investigations, it may be concluded that solid lipid nanoparticles of a poorly soluble drug efavirenz were successfully formulated and optimized using the systematic approach of design of experiments (DoE) by high pressure homogenization technique. Thermosensitive gel was prepared with the optimized SLN dispersion. The intranasal administration of the formulation showed 150 times more brain targeting efficiency and 70 times better absorption potential of the efavirenz loaded SLN dispersion in comparison to the orally administered marketed formulation (capsule). Thus, it may be concluded that the developed formulation has better potential to target brain where the HIV viruses are reported to harbor for an attempt towards the complete eradication of HIV with low dose of efavirenz. Hence, the developed formulation, after necessary investigations of clinical trials, has the promising potential for an attempt to completely eradicate HIV reservoir and cure AIDS.

9. Publications/ Patents

9.1 Papers published

- Gupta, S. et al., **2011**. "Self-nanoemulsifying drug delivery system for Adefovir Dipivoxil: Design, characterization, in-vitro and ex-vivo evaluation". Colloids and Surfaces A: Physicochemical and Engineering Aspects, 392(1)5,145-155. [JCR Impact Factor: 2.760, No. of citations: 60].
- Gupta, S. and Rajesh KS. **2013**. "Ophthalmic drug delivery systems with emphasis on in-situ hydrogels". Pharmagene- A genesis journal, 1(2), 79-86.
- Gupta, S. et al., **2013**. "Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self emulsifying systems". ISRN Pharmaceutics, Article ID 848043, Volume 2013, Hindawi Publishing Corporation. [No. of citations: 17]
- Gupta, S. et al., **2016.** "Formulation, optimization and evaluation of colon targeted delivery system for Isradipine". World Journal of Pharmacy and Pharmaceutical Sciences, 5 (5), 632-649. [SJIF Impact Factor 6.041]

9.2 Papers presented

- Gupta, S. et al., 2009. "Controlled release bilayered buccoadhesive tablets of Losartan Potassium: Formulation & Characterization". Presented in IPC 2009, Ahmedabad, Gujarat, India.
- Gupta, S. et al. 2013. "Formulation of Mentholated Hydrogel and its optimization using box-behnken design for treatment of burns and wounds". Presented in AICTE sponsored seminar on "Quality by Design for Better Method Development and Validation" organized by Parul Institute of Pharmacy & Research and Parul Institute of Pharmacy on 18^{th}-19^{th} October, 2013.
- Gupta, S. et al. 2014. "Topical gel containing solid lipid nanoparticles (SLN) with improved efficacy and patient compliance." Presented in VIC-VCCI-Expo 2014.

9.3 Papers in communication

- Gupta, S. et al. 2016. Systematic approach for the Formulation and Optimization of Solid Lipid Nanoparticles of efavirenz by high pressure homogenization using Design of

Experiment (DOE) for brain targeting and enhanced bioavailability.

- Gupta, S. et al. 2016. Development and validation of analytical method for the estimation of efavirenz in plasma.

9.4 Patent filed

Title: Topical Gel containing solid lipid nanoparticles

Patent Application No.: 3658/MUM/2014

Application date: 19/11/2014

Publication date : 20/05/2016

Journal No. - 21/2016

Link :http://ipindiaonline.gov.in/patentsearch/PublishedSearch/publishApplicationNumber.aspx?application_number=to+3fHvLM2HBmJoF1uzwGA==

10. Achievements

- Meritorious academic background.
- Received certificate of merit for reasons of **outstanding academic performance** and for being among the top 0.1% of successful candidate of AISSCE (All India Senior Secondary Certificate Examination) in India. (secured 97 %)
- Qualified in GATE { GATE 2006 & 2007, score : 92 percentile }
- Qualified in NIPER entrance test.
- PhD work selected and guided by International Professor, Dr. Abdelwahab Omri from Laurentian University, Canada.
- Publications in journals of repute with more than 75 citations.
- Reviewer for Elsevier Journal [Impact factor : 3.902].
- Patent filed and published.

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