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QUALITY BY DESIGN APPROACH IN THE DEVELOPMENT AND OPTIMIZATION OF ETRAVIRINE HYDROCHLORIDE -LOADED SOLID LIPID NANOPARTICLES

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Abstract:

The purpose of this study was to development and optimization of Etravirine hydrochloride -loaded solid lipid nanoparticles by quality-by-design. Etravirine hydrochloride loaded solid lipid nanoparticles, was prepared by melt emulsification-probe sonication method. Results: Fourier Transform Infra-Red (FTIR) Spectroscopy reveals there is interaction between drug and formulation excipients. % Entrapment efficiency was found to be 96.42. In-vitro drug release studies using Franz diffusion cells revealed a sustained release profile, with approximately 71.40% of EH released over 12 hours, highlighting the formulation's potential for prolonged therapeutic efficacy and patient convenience. The optimized formulation exhibited a particle size of approximately 46.21 nm with a narrow PDI, indicating uniformity and stability of SLNs. Morphological characterization using scanning electron microscopy and transmission electron microscopy confirmed the spherical morphology and homogeneous distribution of EH

Key words: Solid lipid nanoparticles, melt emulsification-probe sonication method, scanning electron microscopy & transmission electron microscopy.

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INTRODUCTION:

Nanotechnology involves the manipulation and control of matter on an atomic, molecular, and supramolecular scale, typically below 100 nanometers. In context of drug delivery, nanotechnology refers to use of nanoscale materials, to deliver therapeutic mediators to precise sites within body. These nanoparticles can enhance medication delivery and therapeutic results by interacting with biological systems on a cellular and molecular level, made possible by their microscopic size.[1-3].

Solid lipid nanoparticles prepared from biodegradable lipids offers numerous advantages like controlled release, high drug loading, low toxicity and ease of large scale production. The ingredients utilized commonly are solid lipids, surfactants and purified water. The lipids include fatty acids, triglycerides, waxes and steroids[4-8].

Nanostructured lipid carriers (NLC), the modified form of solid lipid nanoparticles in which drug in the form of liquid form is encapsulated in lipid matrix are also very popular for brain targeted delivery of drug[9-10].

The potential for a paradigm shift in ocular treatments occurs with the combination of colloidal and in-situ delivery systems which overcome physiological and anatomical limitations of ocular delivery. In terms of improving solubility, stability, targeting, prolonged release, and adaptability, colloidal drug delivery methods are a promising new direction for the pharmaceutical industry. This current review provides an overview of combining in situ gel with niosomes for ocular delivery of many therapeutic agents. An in-depth review has been made focusing on various formulation, characterization, safety, and development prospects of in situ gels loaded with niosomes for ocular administration[11-14].

MATERIALS AND METHODS:

Materials

Etravirine Hydrochloride was received from Hetero Lab Ltd. (Unit-II) Formulation division, Baddi, Dist. Solan, Himachal Pradesh, India, Other chemicals used were of analytical grade.

Methods

A melt emulsification-probe sonication process was used to manufacture SLNs loaded with EH. At first, we made two separate batches: one with precisely measured Dynasan- 118 and span 80, and the other with the necessary amount of EH added after it had melted at 80◦C to produce an oily, transparent phase.Ultra turrax (T25 Basic, IkaWerke, Stanfer, Germany) was used to disseminate pemulen in the DDW at a constant temperature of 80◦C for one minute in a later stage. A queous polymer dispersion is the end product of this process. A probe sonication system (IKASONIC U 200 S, Germany) was used to extract 3 mL of polymer dispersion and inject it into oily phase. Optimized sonication settings were 70 W of power and 3 minutes of sonication. initial emulsion and the remaining polymer dispersion were mixed using an ultra-turrax set to 6,500 rpm for three minutes. Lipid recrystallization enabled nanoparticle formation upon cooling of heated dispersion to room temperature[17].

Factorial design

The HPLC method's mobile phase, wavelength, and flow rate were optimised and chosen using a three-level factorial design after the QTPP and CQAs were described. The effect of the mobile phase's composition, wavelength, and flow rate on the tailing factor, theoretical plates, and peak area were investigated using a three-level factorial statistical screening methodology. Design Expert® (Version 11.0, Stat-Ease Inc.) was used to build a three-factor design. Flow rate, wavelength, and mobile phase concentration were all varied in the design. Ideal response was determined by investigating quadratic response surfaces using a second-order polynomial[18].

Characterization of EH-SLNs:

Particle size analysis

Using the Malvern Zetasizer Nano ZS (Malvern Instruments, UK), we conducted dynamic light scattering (DLS) investigations to determine the size and width of size variation for each batch. Temperature was adjusted to 25 °C, and the nanoparticles were mixed with DDW[19].

Zeta potential (ZP) measurement

A zeta potential assessment tool developed by Malvern Instruments in the UK, the Malvern Zeta sizer Nano ZS, was used to find the surface charge of EH-SLNs. Analysis of all the batches were completed after proper dilution with Double distilled water (DDW)[20]

% Entrapment efficiency (%EE)

For optimized batch (A7), In order to evaluate unentrapped medicine in an aqueous media, the percentage EE of EH-loaded SLNs was determined indirectly using a high- speed cooling centrifuge. The amount of drug entrapped in SLN was determined by subtracting the amount of drug present in the supernatant from the total amount of drug used to create SLNs. In short, a known volume of EH-loaded SLN dispersion was placed in a centrifuge tube, which was centrifuged for 20 minutes at 4° C and 16,000 rpm. A UV- visible spectrophotometer set to 258 nm (λmax) was used to measure the amount of free drug in the supernatant after it had been diluted appropriately. The %EE is calculated using the formula below.

% entrapment efficiency = total drug content – Free drug/ total drug content x 100

Fourier transforms infrared spectroscopy (FT-IR) analysis

Using an FT-IR spectrophotometer-JASCO V5300 (Tokyo, Japan), the FT-IR spectra of drug- lipid melt, lyophilized SLN, Pemulen, Dynasan-118, and the pure drug (EH) were scanned between 400 and 4000 cm−1.For the solid state characterization the adsorbent like kaolin was used.

DSC analysis

DSC-1 STARe (Metler Toledo, USA) System was used to examine thermal behaviour of EH, pemulen, drug-lipid melt, and lyophilized SLN. Within a nitrogen environment, the samples were heated in 40 L aluminium pans at a rate of 10◦C/min, ranging from 20 to 700◦C.

TEM analysis

TEM called a Philips CM 200 was used to create the optimised batch (A7 dispersion) TEM photomicrograph. Carbon-coated Cu grid was carbon-dyed using phosphotungstic acid (2 wt%) and uranyl acetate (1 wt%) after being immersed in the A 7 dispersion. Following staining and curing for approximately 30 minutes, TEM imaging was performed on the sample[19].

In-vitro Drug Release Studies

Diffusion study was carried out by Franz diffusion cells. The cell's receptor area, which held 50 ml of buffer. Dialysis membrane was placed between donor and receptor compartments so that diffusion experiments could be carried out. Before clamping the membrane together, optimised EH-loaded SLN formulations are applied to its surface. Receptor compartment, which had phosphate buffer saline with a pH of 7.4, was kept at 37 °C and continuously agitated with a magnetic bead at 50 rpm. Identical volumes of buffer were added to the 5 ml samples at predetermined intervals. A UV with a maximum wavelength of 258 nm was used to evaluate the samples after they had been diluted to the necessary strength.

RESULTS & DISCUSSION:

Table 1: Results Placket Burman factorial design

Batch Code	Formulation Variables (X)			Responses (*Y*)		
	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	Amt. of lipid (mg)	B:Power (Watt)	C:Sonication time (Min)	Z-Avg (nm) ± SD	PDI ± SD	ZP (mV) ± SD
A1	400	25	03	227.2 ± 3.2	0.198±0.06	-20.2 ± 4.5
A2	400	70	10	229.7 ± 1.5	0.214 ±0.06	-24.1 ± 3.2
A3	50	70	03	250.8 ± 2.0	0.204± 0.04	-18.2 ± 4.1
A4	400	25	10	218.4 ± 1.5	0.194±0.12	-21.8 ± 3.2
A5	56	25	10	247.2 ± 1.5	0.213±0.02	-19.4 ±4.5
A6	50	70	10	274 ± 2.5	0.215±0.05	-24.8 ± 7.1
A7	400	70	03	125.8 ± 2.0	0.192±0.04	-26.6 ± 3.2
A8	50	25	03	248.4 ± 1.5	0.211±0.08	-19.7 ± 4.1

The Plackett-Burman factorial design study evaluated the impact of formulation variables (Factor 1: Amount of lipid, Factor 2: Power of sonication, Factor 3: Sonication time) on the responses of Z-Average (nm), Polydispersity Index (PDI), and Zeta Potential (ZP) forthe formulation process. From the results as mentioned in table 6.16, it is evident that varying these factors influences the nanoparticle characteristics significantly. For instance, increasing the amount of lipid (Factor 1) generally tended to increase the Z- Average size, as observed with batches A1, A2, and A3 showing larger Z-Average values compared to others. Sonication power (Factor 2) and sonication time (Factor 3) also exhibited notable effects: higher sonication power and longer sonication time generally resulted in smaller Z-Average sizes and more consistent PDIs, as seen in batches A2, A6, and A7. The Zeta Potential (ZP) values varied, with factors such as lipid amount and sonication power influencing the surface charge of the nanoparticles.

In conclusion, the Plackett-Burman design highlighted key factors influencing the nanoparticle characteristics crucial for optimizing the formulation process. Further optimization and fine-tuning of these factors could lead to production of nanoparticles with desired size distribution (PDI) and surface charge (ZP), essential for their stability and therapeutic efficacy in pharmaceutical applications. The study underscores the importance of systematic experimental designs in understanding and controlling formulation variables to achieve desired nanoparticle properties effectively.

The different batches of EH-SLN had PDI in the range of -0.192 ± 0.04 and 0.214 ±0.06 as summarized in Table 1. Presents the effect of formulation variables on the PDI of EH loaded SLN, and found to have statistical significance (P value 0.0663, r2 = 0.9729).

The percent drug entrapment of the SLN containing EH in the optimized batch A7 higher drug entrapment efficiency as given in table 6.26.

Table 2: Results of Entrapment efficiency studies

Formulations	Entrapment efficiency (%)
A7	96.42

Scanning Electron Microscope:

The nanoparticles that were produced had an average size of about 46.21 nm and were mostly spherical.

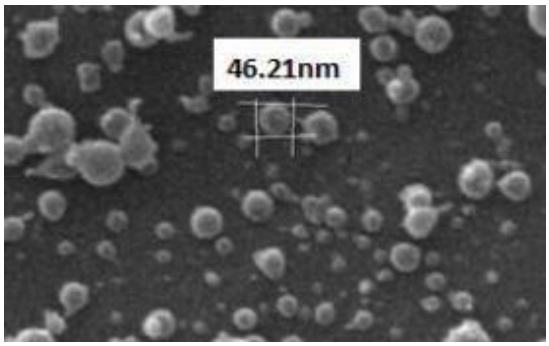


Fig 1: SEM images of optimized batch (A7)

The SEM images showcase nanoparticles from the optimized batch (A7), revealing their predominantly spherical morphology. This uniformity in shape is a positive indicator of the manufacturing process's consistency and precision in controlling particle formation.

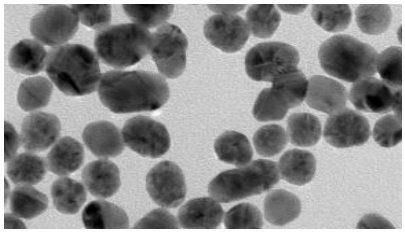
The average size of approximately 46.21 nm, as determined by nanoparticle analysis, underscores the effectiveness of the formulation and processing techniques used. Nanoparticles of this size range are particularly advantageous in pharmaceutical applications due to their enhanced surface area-to-volume ratio, which can improve drug loading efficiency and bioavailability.

The spherical shape observed in the SEM images is beneficial for ensuring uniform dispersion and stability of nanoparticles in various media. Such characteristics are crucial for applications in drug delivery systems, where predictable particle behavior and biocompatibility are essential.

In conclusion, compelling evidence of the successful optimization of nanoparticle characteristics, highlighting their spherical morphology and small size. These attributes collectively support the potential of these nanoparticles for targeted drug delivery and other advanced biomedical applications, showcasing a promising development in nanotechnology research.

Transmission electron microscopy (TEM) analysis

Particles have an average size of 45 nanometers and a size distribution between 30 and 60 nanometers. All of the particles are round. In order to rule out the possibility of other types of metal oxide, electron diffraction was used on the identical sample.



.Fig 2: TEM images of optimized batch (A7)

The TEM analysis provides a detailed view of nanoparticles from the optimized batch (A7), revealing their spherical morphology and size distribution. particle size ranges from 30 to 60 nanometers, with an average size of 45 nanometers. This narrow size distribution is indicative of precise control over the manufacturing process, ensuring uniformity in particle dimensions.
The spherical form observed in the TEM images is advantageous in nanotechnology and pharmaceutical applications. Spherical nanoparticles generally exhibit enhanced stability and dispersibility, which are critical for their effective use in drug delivery systems. This shape also promotes efficient cellular uptake and controlled release of encapsulated drugs, potentially improving therapeutic outcomes.

In-vitro Drug Release Studies:

Table 3: Results of the in-vitro drug release study

Time (hours)	Cumulative Drug Release (%)
0	0
2	18.50
4	35.80
6	49.20
8	60.10
10	68.30
12	71.40

These results demonstrate the cumulative percent drug release from the EH-loaded SLN gel formulation over a 12-hour period in the Franz diffusion cells. The study shows a sustained release profile, with approximately 71.40% of the drug released into the receptor compartment by the end of 12 hours. This sustained release pattern suggests that the formulation could potentially offer prolonged therapeutic efficacy, maintaining drug concentrations within therapeutic windows over an extended period.

The in-vitro drug release study conducted on the EH-loaded SLN gel formulation, , reveals a controlled and sustained release profile over a 12-hour period using Franz diffusion cells. The results show a gradual increase in cumulative drug release with time, demonstrating the formulation's ability to release EH in a sustained manner. At 2 hours, approximately 18.50% of the drug is released, which increases to 35.80% by 4 hours and reaches 49.20% at 6 hours. This trend continues with 60.10% released by 8 hours, 68.30% by 10 hours, and a final cumulative release of 71.40% at 12 hours.
Illustrating the progressive release of EH from the SLN gel formulation. Such a sustained release profile is advantageous in pharmaceutical applications as it potentially extends the therapeutic effect of the drug, reduces dosing frequency, and enhances patient compliance by maintaining drug concentrations within effective therapeutic ranges over an extended period.

The formulation's sustained release characteristics are likely attributed to the encapsulation of EH within solid lipid nanoparticles. This encapsulation protects the drug, allowing for controlled release and minimizing rapid clearance or degradation in physiological environments. The observed release kinetics suggest that the SLN gel formulation could be suitable for applications requiring prolonged drug delivery, such as in the treatment of chronic conditions where consistent therapeutic drug levels are crucial.

FTIR-Fourier Transform Infra-Red Spectroscopy

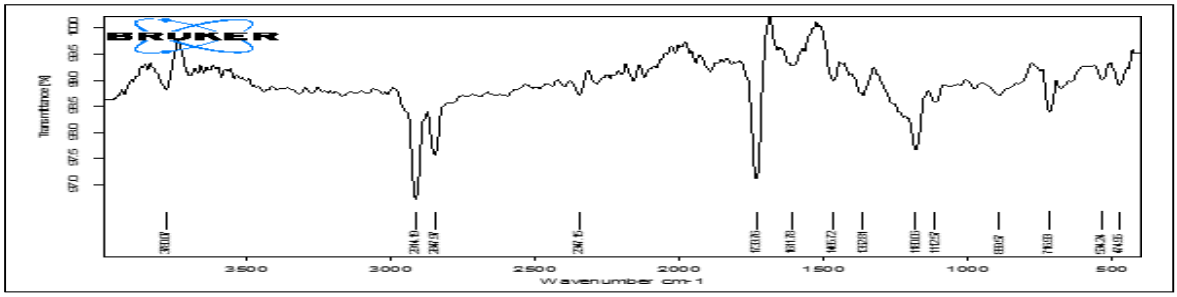


Fig 6.15: FTIR Spectra of EH-loaded SLN

The FTIRspectra confirm the presence and stability of key functional groups essential for maintaining the chemical integrity and molecular structure of EH API and D118 in their formulations, supporting their pharmaceutical application and compatibility in drug delivery systems.

CONCLUSION:

The optimized formulation exhibited a particle size of approximately 46.21 nm with a narrow PDI, indicating uniformity and stability of SLNs. Morphological characterization using SEM and TEM confirmed the spherical morphology and homogeneous distribution of EH within the lipid matrix, essential for sustained drug release and enhanced bioavailability. The comprehensive study on EH-loaded SLN gel formulation represents a significant advancement in transdermal drug delivery systems, offering enhanced bioavailability, sustained release capabilities, and favorable safety profiles. The integration of rigorous pre-formulation studies, optimized analytical methods, and systematic formulation development has provided valuable insights into enhancing EH's pharmaceutical properties and therapeutic efficacy. Future studies should focus on further evaluating pharmacokinetic profiles, clinical efficacy, and commercialization potential, aiming to translate these findings into practical applications for dermatological treatments involving Etravirine Hydrochloride.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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