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Formulation and Evaluation of Novel Lipid-Based Nanocarriers for Targeted Ocular Delivery of Prednisolone Acetate

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ABSTRACT

Objective: In the present research work, the aim was to prepare and evaluate prednisolone acetate solid lipid nanoparticles at eye surface for the treatment of ocular inflammation.

Methods: Solid lipid nanoparticles were prepared by high pressure homogenization followed by probe sonication. The amounts of polymers were selected on the basis of optimum quantity required for sustained release of drugfrom preparation and as reported in literature and performed ranging study.

Results and discussion: Glyceryl monostearate, Tween 80 and transcutol P were used as solid lipid, surfactant and co-surfactant respectively. All formulation was evaluated for particle size, zeta potential, entrapment efficiency % drug content and release study. Nine formulations for each approach were prepared and optimized successfully using 3^2 factorial designs. Optimization was done by DoE software version Version 13.0.10.064.

Conclusion: Solid lipid nanoparticles were successfully developed using high pressure homogenization method. Results of the various parameters indicated that prednisolone acetate can be formulated into nanoparticles using GMS thereby improving its ocular permeability. Stability studies indicated no change in coloration or any other physical parameters.

Keywords: *Nanoparticles, GMS, homogenization and prednisolone.*

1. INTRODUCTION

Ocular drug delivery presents a formidable challenge due to the complex anatomical and physiological barriers of the eye, which limit the effectiveness of traditional dosage forms(1). Among the various ocular diseases, inflammation remains a significant therapeutic challenge, necessitating innovative delivery strategies for optimal treatment outcomes. Prednisolone, a potent corticosteroid, offers profound anti-inflammatory effect. The anti-inflammatory activity of prednisolone is due to its inhibitory action on Phospholipase A2 (PLA2) and cyclooxygenase-2 (COX-2) expression. In addition to this, it also stabilizeslysosomal membranes. There by reduce the production of pro-inflammatory cytokines and proteolytic enzymes which are responsible for inflammatory response. These aforementioned events make it a cornerstone in the management of ocular inflammatory conditions.Traditional delivery systems such as eye drops and ointments encounter limitations such as low bioavailability, short residence time, and poor penetration into ocular tissues, leading to suboptimal therapeutic outcomes and potential systemic side effects. Consequently, there is an escalating interest in the development of novel drug delivery platforms that can enhance the ocular bioavailability and therapeutic efficacy of prednisolone(2).Solid lipid nanoparticles (SLNs) have emerged as a promising carrier system for ocular drug delivery due to their biocompatibility, controlled release properties, and potential to overcome the limitations of conventional formulations(3).Furthermore, the application of Quality by Design (QbD) principles in the development of SLNs offers a systematic approach to optimize formulation parameters and ensure product quality, efficacy, and safety.This study aims to design and evaluate QbD-based prednisolone-loaded novel SLNs tailored for enhanced ocular delivery. By employing a systematic and rational approach guided by QbD principles, we seek to optimize the formulation parameters to achieve desirable physicochemical properties, sustained drug release, improved corneal penetration, and enhanced therapeutic efficacy while minimizing potential adverse effects(4).

2. MATERIALS AND METHODS

Prednisolone acetate, Glyceryl monostearate (GMS), Tween 80, Organic solvent (e.g., ethanol) and diethylene monoethyl ether were procured from Loba Chemie Pvt. Ltd., Mumbai and Himedia laboratories Pvt. Ltd., Mumbai.

3. EXPERIMENTAL

Determination of wavelength and preparation of calibration curve

In this study, 10 mg of drug was appropriately weighed and added in 10 mL OF volumetric flask. Afterwards, 10 mL of suitable solvent was added in the 10 mL volumetric flask which gave the concentration of 1mg/mL. Further from the stock solution 0.1 mL of the sample was taken out and volume was made up to 10 mL which gave the concentration of 10 µg/mL. The obtained concentration solution was scanned for the UV analysis for the determination of wavelength. Similarly, for the calibration curve preparation 2 mL, 4mL, 6mL and 8 mL of the solvent was taken out from 10 µg/mL sample which gave the concentration of 2 µg/mL, 4 µg/mL, 6µg/mL and 8 µg/mL, respectively. These prepared concentrations were scanned for UV analysis and obtained absorbances were used for the determination of regression coefficient i.e., R^2.In addition to this, obtained equation from the CC was further used for the determination of solubility studies(5).

Solubility studies

For the solubility analysis 1mL of respective surfactants (Tween 80, Tween 20, Span 20, PEG 200 and Transcutol P) were taken in an individual glass vial. Afterwards, an excess amount of known quantity of prednisolone was added in them. Then vortexed for a time period of 10 minutes. After vortexing all the samples kept in a glass vial were stoppered and agitated for 72 hour and temperature was set at 37 ± 0.2 °C in a shaking water bath. Afterwards, all the samples were transferred to an eppendroff and centrifuged at 10000 g for 15 minutes. In order, to remove the undissolved drug. Then the obtained supernatant was collected in a test tube and diluted up with a suitable solvent. Similarly, for the solubility analysis of drug in lipids, 1 gram of the respective lipids (Palmitic acid, Stearic acid and Glyceryl monostearate) were taken in a glass vial. Further the vials were kept in a sonicator. Sonication was done for 15 minutes and temperature was set at 80°C. Then the excess amount of drug was added in the molten lipids. Further, centrifugation was done at 10000 g for 15 minutes and obtained supernatant sample was diluted with suitable solvent and accessed for the UV analysis(6).

Ternary Phase Diagram (TPD)

TPD was plotted using Triplot version 4.1.2 software prior to the SLNs' optimisation. The ratios of surfactant to co-surfactant, namely 1:1, 1:2, and 2:1, as shown in Table 1, were varied to create a total of 27 formulations for this investigation. Formulations that were opaque were indicated by the multiplication sign, transparent formulations with foam were indicated by the blank star, and formulations that were clear were designated as SLNs and represented by a filled star. In addition, the drug loading (DL), polydispersity index (PDI), zeta potential (ZP), and particle size (PS) of the clear formulations were further characterised(7).

Table 1 Different composition of TPD

Formulation	SL (mg)	Surfactant (µL)	Co-surfactant (µL)	Water(mL)	Observation
(1:1)					
F1 (1:9)	100	450	450	250	Clear with foam
F2 (2:8)	200	400	400	250	Clear with foam
F3 3:7)	300	350	350	250	Clear with foam
F4 (4:6)	400	300	300	250	Clear with foam
F5 (5:5)	500	250	250	250	Opaque
F6 (6:5)	600	200	200	250	Opaque
F7 (7:3)	700	150	150	250	Opaque
F8 (8:2)	800	100	100	250	Opaque
F9 (9:1)	900	50	50	250	Opaque

(1:2)					
F10 (1:9)	100	300	600	250	Clear with foam
F11 (2:8)	200	270	530	250	Clear with foam
F12 (3:7)	300	230	470	250	Clear
F13 (4:6)	400	200	400	250	Clear
F14 (5:5)	500	170	330	250	Opaque
F15 (6:5)	600	130	270	250	Opaque
F16 (7:3)	700	100	200	250	Opaque
F17 (8:2)	800	70	130	250	Opaque
F18 (9:1)	900	30	70	250	Opaque
(2:1)					
F19 (1:9)	100	600	300	250	Clear with foam
F20 (2:8)	200	530	270	250	Clear with foam
F21 3:7)	300	470	230	250	Clear with foam
F22 (4:6)	400	400	200	250	Clear with foam
F23 (5:5)	500	330	170	250	Opaque
F24 (6:5)	600	270	130	250	Opaque
F25 (7:3)	700	200	100	250	Opaque
F26 (8:2)	800	130	70	250	Opaque
F27 (9:1)	900	70	30	250	Opaque

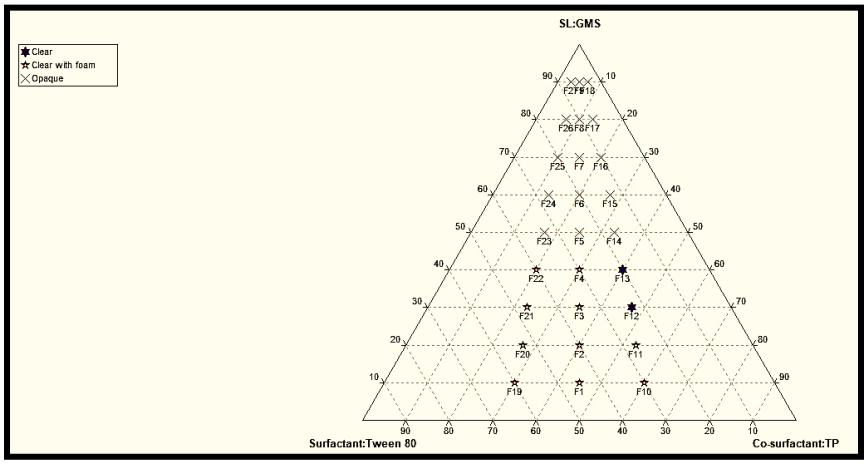

Fig. 1 Representing TPD for SLNs

Experimental Design and Statistical Analysis

SLNs were fabricated by high pressure homogenization followed by probe sonication technique and optimized by using design expert software. In this study, two-factor and two-level factorial design was used to identify the optimal SLNs preparation formula. Independent variables were surfactant concentration (B), solid-to-liquid lipid ratio (A), and A/B ratio. The table outlined both the lowest possible value and the highest possible value for each factor. There was a total of nine batches of formulation created, with one serving as the focal point. After batch analysis, we performed additional analysis by inserting response values. Each trial included three separate responses. There were explanations for the particle size, polydispersity index, and zeta potential.

Particle Size, PDI and Zeta Potential

To quantify the typical size of SLNs, we employed the Zetasizer Nano ZS. Dynamic light scattering is the basis for the zeta-operation. sizer's (DLS). Disposable particle size cuvettes were filled with 1-1.5 mL of diluted SLN formulations. Particle size was determined by scanning this cuvette in an instrument. Distilled water was used to dilute the sample ten times before 1 mL was added to the zeta potential cuvette. The cuvette was placed in the instruments after dilution. The instrument comprises a Helium Neon Laser angled at 90° and cooled to 25 °C(8).

Entrapment Efficiency

The entrapment efficiency of prednisolone acetateSLNs was evaluated by measuring the drug concentration in the supernatant. Before placing 1 mL of the diluted formulation into the ultracentrifuge tubes, the formulation was diluted twice. The tubes were placed in the ultracentrifuge, and the machine was spun at 80,000 revolutions per minute for sixty minutes. For this experiment, researchers utilised an ultracentrifuge (Optima Max XP; Beckman Coulter, USA). We then split the formulation process in two. While the clear solution settled to the bottom of the formulated mixture, the SLNs cake formed on the surface. The clear liquid that had pooled at the tube's base was sucked out and redirected to a new holding tank. As a control, distilled water was used to dilute this supernatant before UV-Spectroscopy at 470 nm was performed(9).

TEM Analysis

Tecnai T20 transmission electron microscopy (FEI CompanyTM, USA) examined prednisolone-loaded SLN morphology. It was time to get the samples ready for examination. A single sample was diluted fifty-fold in double-distilled water. This was then put onto 400-mesh carbon-coated copper grids. After that, a negative stain was made with a phosphotungstic acid solution. High-magnification pictures of the material were taken after the analysis(9).

DSC Analysis

Differential scanning calorimetry revealed prednisolone's thermal properties. Using a DSC (DSC STAR E system, Mettler Toledo, Switzerland) and an intercooler 2P cooling adaptor, pure prednisolone was thermogrammed. The DSC pan was properly sealed after being filled with around 10mg of prednisolone-loaded SLNs. The pan and the reference pan were then put into the DSC instrument and heated between 30°C to 300°C. The rate at which the pan was heated was kept constant at 100°C per minute. An endotherm was recorded while nitrogen gas was vented at 20 mL/min to keep the room inert(10).

***In-vitro* Release Study of prednisolone-SLN**

A dialysis membrane was used to conduct an in-vitro release test on the prednisolone acetate-loaded SLN dispersion. The dialysis membranes were lubricated with synthetic tear fluid (STF). The release investigation was conducted in a 100 mL beaker with STF serving as the release medium. One millilitre of the formulation was added to the dialysis membrane, and it was attached with threads on both ends. It was then stirred in an STF beaker at 100 rpm. I took the aliquots all at once. The same volume of STF was added after the release medium was aliquoted. Using STF as a blank, UV spectroscopy was run on the sample at 470 nm(9).

***Ex-Vivo* Permeation Study of prednisolone-SLN**

The eyeballs of goats were acquired from a nearby butcher shop not long after their removal via surgical procedure. At a temperature of 40 degrees Celsius, the eyes were stored in a standard saline solution (0.9% NaCl solution). In order to remove the cornea, forceps and scissors were used. The cornea and 5-6 mm of the sclera around it were surgically removed. To prevent further tissue damage, this removed cornea was preserved in tear-like fluid by being held in a beaker with air purged out using an air pump [19]. The Franz diffusion device was utilised for the permeability research. Both a donor compartment, where the formulation is kept, and an acceptor compartment, where the releasing medium is kept, make up a Franz diffusion cell. A water jacket surrounds the donor compartment, keeping the study's release medium at the ideal temperature. The 7.8 mL acceptor volume is just right for ocular permeability research. For the release medium, 7.8 mL of artificial tears are placed in the acceptor chamber. Acceptor compartment extensions allow for aliquoting. Clamps were placed between the donor and acceptor chambers, and the goat cornea was then mounted in between. Donor compartment then received 0.5 mL of prednisolone-loaded SLN dispersion. The water jacket kept the release medium at 34^0C while being agitated at 25 rpm constantly. At predetermined time intervals up to 8 hours, 1 mL aliquots were removed from the system, and an equivalent

volume of new simulated tear fluid was supplied to keep the sink at a constant level. Next, UV spectroscopy was used to examine the aliquots at a wavelength of 470 nm, with STF serving as a blank. Following examination, the drug's % release and permeability were determined. Formulas were developed to determine drug permeation characteristics including steady state flux (Jss) and permeability coefficient (Paap). This steady drug flux through the semipermeable barrier is known as the steady state flux(11).

Stability Study

The long-term viability of the formulation was evaluated by testing the stability of prednisolone-loaded SLNs in accordance with ICH recommendations Q1A. The stability of a formulation may provide us with information about how the drug will react with its excipients, how stable it will be over time, and whether or not the drug will escape the formulation. The potential for the suspension to break down while in storage is another factor that can be evaluated in a stability study. It also tests how well a formulation holds up under a variety of storage and environmental conditions. To sum up, 5 mL of prednisolone-loaded SLNs were carefully placed into a glass vial and tightly sealed. The vials were then kept in storage for three months at room temperature (25±2°C/60% 5% RH), accelerated condition (40±2°C/75% 5% RH), and refrigerated temperature (53°C). Particle size, zeta potential, and entrapment efficiency were monitored every month [20] to detect any changes in the formulation(11).

***In vitro* cytotoxicity tests**

The effects of prednisolone loaded SLNs were evaluatedon three types of cell lines such as human corneal epithelial cells (HCEC cells), mouse fibroblast cells (L-929 cells), and RAW 264.7 macrophages, using a method called the3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide(MTT) assay.In this study, culturing of the HCEC cells were done in a mixture ofDulbecco's Modified Eagle's Medium(DMEM)/F12 with certain additives, L-929 cells in RPMI 1640, and RAW 264.7 macrophages in DMEM. Then, the plattingof the cellswas done in a 96-well plate and allowed them to grow overnight in a special environment. After that, different concentrations of prednisolone loaded SLNswere added to each well and incubated for 24 hours. Afterwards, MTT was added to each well and further incubated for another 4 hours. Finally,absorbance of the solution in each well were measured at a specific wavelength using a microplate reader. In addition to this, untreated cells were used as a comparison to measure the effect of prednisolone loaded SLNs on the cell viability. It was calculated by comparing the absorbance of the treated cells to the absorbance of the untreated cells. The untreated cells were used as the control. The experiment was repeated to ensure the accuracy and reproducibility in the results(11).The cell viability was calculated using the following equation:

$$Cell\ viability\ (\%) = \frac{Absorbance\ of\ the\ tested\ sample}{Absorbance\ of\ the\ controlled\ sample} \times 100$$

All experiments were carried out in triplicate (mean ± SD, n = 6). 10

***In vitro* anti-inflammatory efficacy**

In order to evaluate the in vitro anti-inflammatory characteristics of prednisolone loaded SLNs, we measured pro-inflammatory cytokines in the culture medium of RAW264.7 macrophages activated by lipopolysaccharide (LPS) and exposed to different dosages of the medication. Initially, 1×10^5 cells/well of RAW 264.7 macrophages were planted in 24-well plates, and they were incubated for the entire night at 37°C in an incubator with 5% CO2. After this incubation period, cells were challenged with 1 µg/mL lipopolysaccharide (LPS) for a further 24 hours after being pre-treated for 2 hours with either 1 µM Dexp or prednisolone loaded SLNs. After that, supernatants were taken from each well, and the Griess assay and ELISA assay kits were used to measure the amounts of nitrite (NO), tumour necrosis factor-α (TNF-α), and interleukin-6 (IL-6), respectively. For comparison, control cells that had not received any treatments were included(12).

Eye irritation study

Zhang, Yu, et al. (2018) described the use of a modified Draize test to assess eye discomfort resulting from prednisolone loaded SLNs. The guidelines provided in the Institute of Laboratory Animal Resources' Guide for the Care and Use of Laboratory Animals were followed in all animal research. The experiments were approved by Wenzhou Medical University's Institutional Animal Care and Use Committee, with protocol number WMU2019-99. For the investigation, four Japanese White Rabbits, weighing 2.5 kg each, were used. Intraocular pressure (IOP) was assessed before starting medication. Each rabbit was then given 50 microliters of prednisolone loaded SLNs (5 mg/mL) in the left eye and 50 microliters of normal saline (NS) in the right eye as a control. Over the course of the trial, an experienced ophthalmologist recorded changes in intraocular pressure (IOP) and kept an eye out for anomalous symptoms including corneal haze/oedema and conjunctival hyperaemia(13).

Statistical analysis

The results of every experiment were expressed as mean ± SD, and Origin 7.5 (Origin Lab, USA) was used for statistical analysis. The in vitro cytotoxicity and anti-inflammatory efficacy results were subjected to one-way analysis of variance

(ANOVA) with Tukey's multiple comparisons test using GraphPad Prism 7.00 (GraphPad Software, USA). To assess statistical significance, a probability threshold of $P < 0.05$ was chosen(14).

4. RESULT AND DISCUSSION

Determination of wavelength and Calibration curve

The results of the UV study showed that the wavelength of the prednisolone acetate was found to be 243 nm (Fig. 1a). Similar results were found in the study performed by Puet.al., (2017), where the wavelength of prednisolone acetate was obtained at 243 nmDOI: 10.1021/acs.jced.6b00997. Furthermore, the results of CC revealed that the equation obtained from the CC was found to be $y = 0.024x + 0.0307$ and R^2 was found to be 0.9989. This indicated that the all the data fitted in the regression line was found to be linear (Fig 2 A and 2B).

Solubility Studies

The results of the solubility study showed that prednisolone exhibited maximum solubility in GMS assolid lipid, Tween 80 as surfactant and transcutol P ascosurfactant. These excipients were further used for the fabrication of SLNs. Besides these, the solubility of prednisolone in different, surfactants and cosurfactants are given in Fig 2C.GMS is used as a enhancing the solubility of encapsulated drugs by acting as a stabilizing agent, improving encapsulation efficiency, controlling release kinetics, and modifying surface properties. Tween 80 plays a critical role in the formulation of SLNs by enhancing the solubility of hydrophobic drugs, stabilizing the nanoparticle suspension, improving drug encapsulation efficiency, and potentially enhancing the bioavailability of the encapsulated drug. Its surfactant properties are key to achieving stable and effective SLN formulations. Transcutol P plays a crucial role in SLN formulations by improving drug solubility, loading capacity, release kinetics, and penetration into biological membranes, thereby enhancing the therapeutic efficacy of SLN-based drug delivery systems.

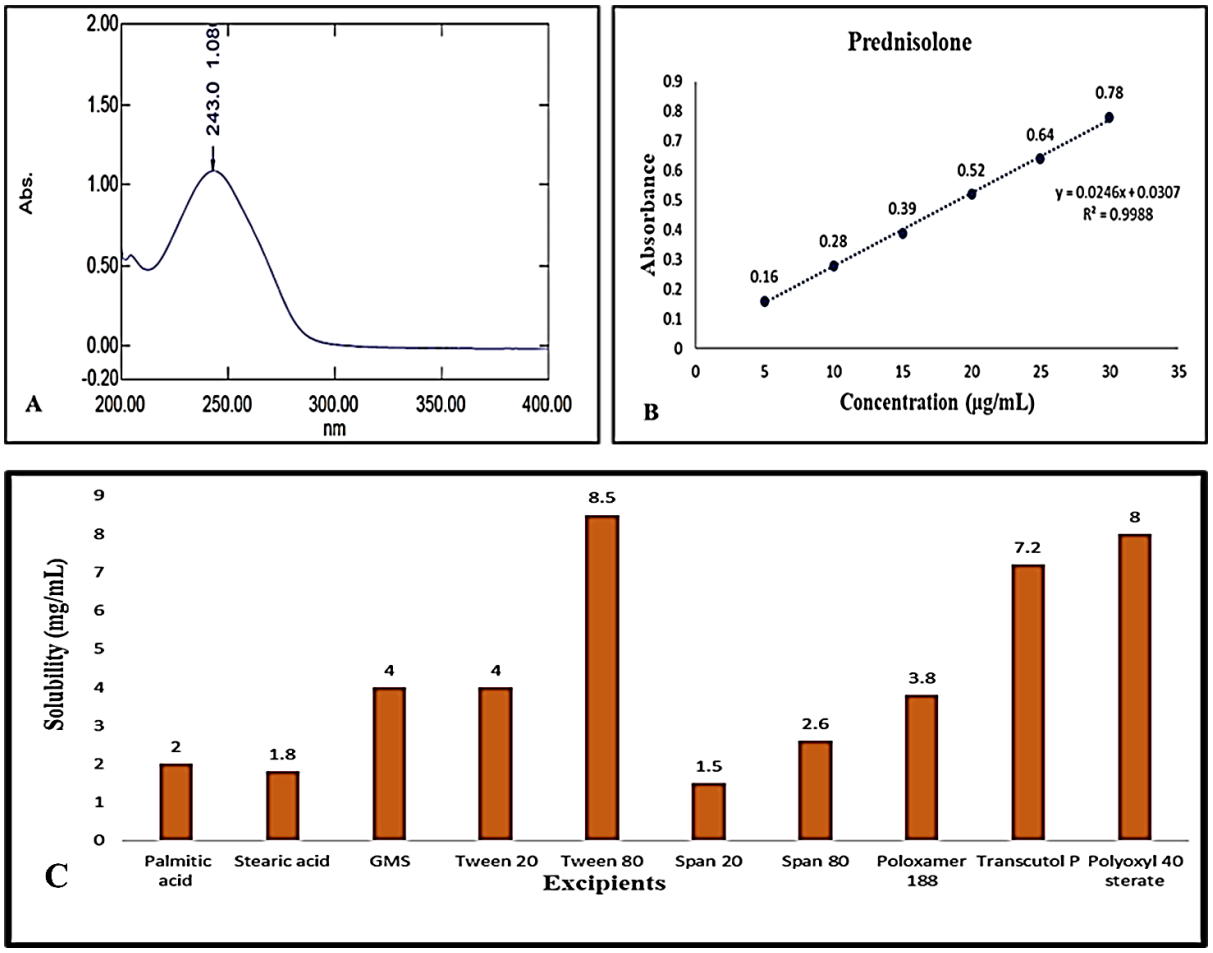

Figure2: Representing [A] Determination of prednisolone wavelength; [B] Linearity of prednisolone; [C] Solubility of prednisolone in different solid lipids, surfactants and Co-surfactants

TPD

In this study, 27 formulations were formulated by varying the ratios of oil and S_{mix}i.e., 1:1, 1:2 and 2:1. The results of the initial screening of TPD revealed that formulations F12 and F13 were clear and were considered as nano emulsions. The PS, ZP, PDI of F12 was found to be 110 nm, -22.9 ± 2.23 mV and 0.243. Similarly, PS, ZP, PDI of F13 was found to be 130 nm, -23.7 ± 2.23 mV and 0.273. These results indicated that formulations are nano in size, having ZP within the limits of ±15 mV to ±30 mV indicated good stability of the formulation. Similarly, the value of PDI was below 0.4 indicated good homogeneity of the formulations. In addition to this, from the TPD the highest and lowest value of oil, surfactant and cosurfactant was obtained which are discussed below.

DoE

The optimization process of prednisolone loaded NPs utilized a box central composite design; incorporating 3 factors at 3 levels (refer to Table 2.). The chosen model demonstrated noteworthy statistical significance, with all the responses exhibiting a significant P-value below 0.05

Table 2:

Run	A: GMS (mg)	B: Surfactant (uL)	C: Co-surfactant (uL)	Size (nm)	Zeta (mV)	EE (%)
1	350	215	435	115.3	-15.2	86.23
2	300	200	400	123.23	-12.56	77.64
3	434.09	215	435	117.36	-14.2	84.77
4	400	200	400	120.36	-16.35	81.55
5	350	215	435	113.25	-11.79	80.47
6	400	230	400	107.52	-10.02	84.23
7	300	200	470	101	-12.35	89.52
8	400	230	470	132	-11.56	74.03
9	350	215	435	105	-12.36	86.32
10	300	230	470	136.69	-19.35	81.25
11	350	215	493.863	119.54	-13.65	79.22
12	350	240.227	435	139.67	-20.06	76.06
13	400	200	470	103.76	-14.5	88.35
14	350	215	376.137	114.36	-15.6	80.75
15	265.91	215	435	116.32	-14.26	85.36
16	350	215	435	115.32	-13.65	88.06
17	350	215	435	113.57	-12.94	87.44
18	350	189.773	435	100.29	-13.96	88.25
19	300	230	400	109	-15.36	89.36
20	350	215	435	113.74	-11.99	85.36

Effect of independent variables on particle size (Y1)

Understanding the impact of independent variables on particle size (Y1) is crucial for optimizing formulations and achieving desired outcomes. By examining the relationship between various factors and particle size, valuable insights can be gained, enabling us to fine-tune their processes and enhance the performance of their formulations. Here we investigate the effect of

independent variables on particle size, employing advanced analysis techniques to uncover the intricate interplay between the factors and the resulting particle size. By unravelling these relationships, we can unlock key insights that pave the way for improved formulations and enhanced product development. The particles size of prednisolone-loaded NPs was found to be in range from 100.29 nm to 139.67 nm for various factors and level combinations. The effect of independent variables on particle size can be represented by the following regression Equation no. 3.

Polynomial equation = 115.864 + -0.33177 * A + 7.54852 * B + 1.6147 * C + -0.7575 * AB + 0.3025 * AC + 11.375 * BC……...Eq. 3

The regression coefficient value (R^2 of equation 3 was found to be 0.8376, which indicates a good fit between the experimental values and predicted values. The contour plot and 3-D surface plot of particles size are given in Fig 3a. It was observed that particle size of prednisolone-loaded NPs was increased with the increasing concentration of surfactant. The particles size was indirectly proportional with stirring speed.

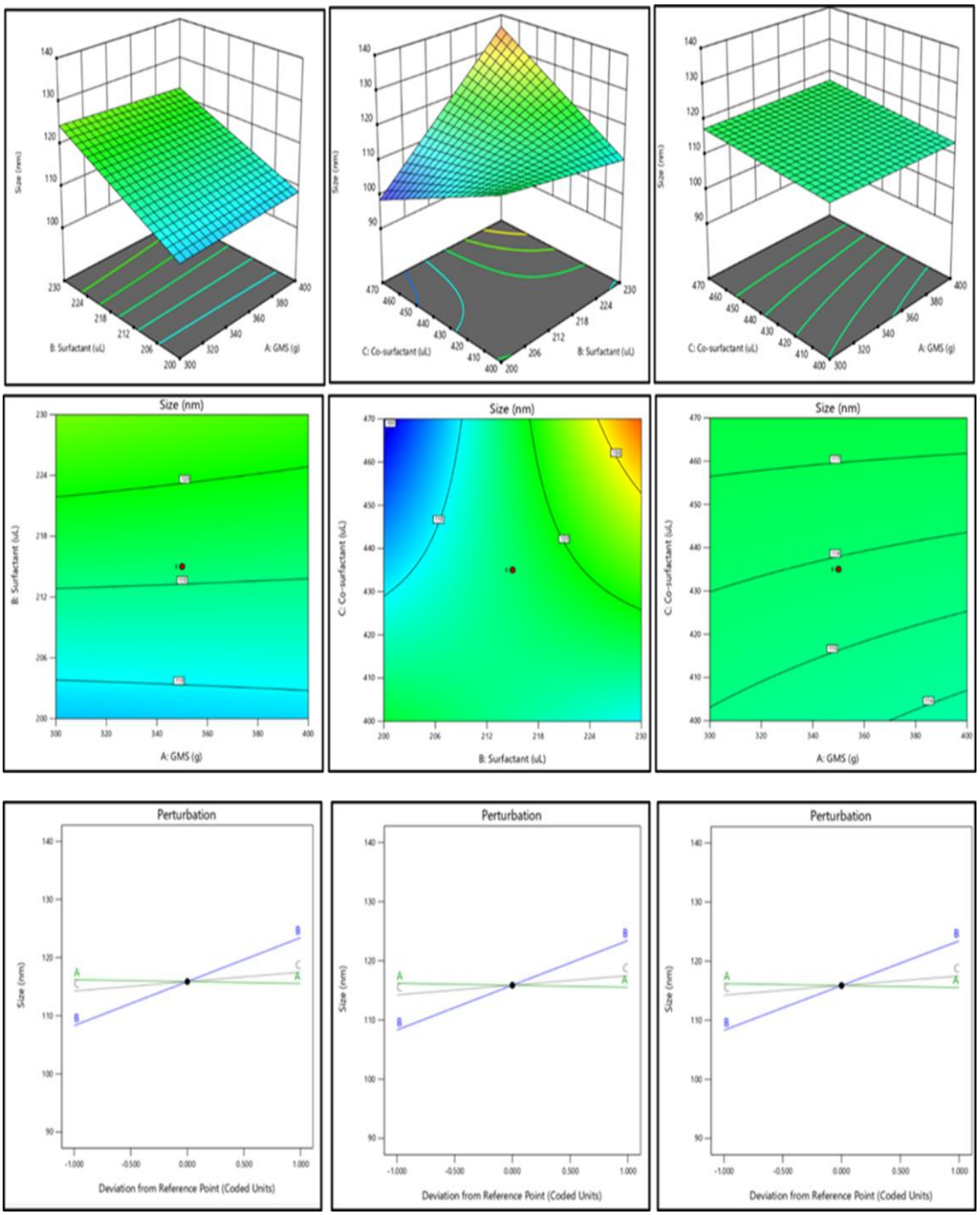

Figure3a: surface, counter plot and Perturation graphs for effect of independent values on particle size.

Effect of independent variables on zeta potential (Y2)

Understanding the impact of independent variables on zeta potential (Y2) is crucial for optimizing formulations and achieving desired outcomes. By examining the relationship between various factors and zeta potential, valuable insights can be gained, enabling us to fine-tune their processes and enhance the performance of their formulations. Here we investigate the effect of independent variables on zeta potential, employing advanced analysis techniques to uncover the intricate interplay between the factors and the resulting zeta potential. By unravelling these relationships, we can unlock key insights that pave the way for improved formulations and enhanced product development. The zeta potential of prednisolone-loaded NPs was found to be in range from -20.06 to -10.02 for various factors and level combinations. The effect of independent variables on zeta potential can be represented by the following regression Equation no. 4.

Polynomial equation= -14.0855 + 0.533864 * A + -0.790002 * B + -0.0139493 * C + 2.38375 * AB + 0.51125 * AC + -0.94875 * BC……Eq. 4

The regression coefficient value (R^2 of equation 4 was found to be 0.5765, which indicates a good fit between the experimental values and predicted values. The contour plot and 3-D surface plot of PDI are given in Fig 3b. It was observed that zeta potential of prednisolone-loaded NPs was increased with the increasing concentration of surfactant and GMS. The zeta potential was indirectly proportional with stirring speed.

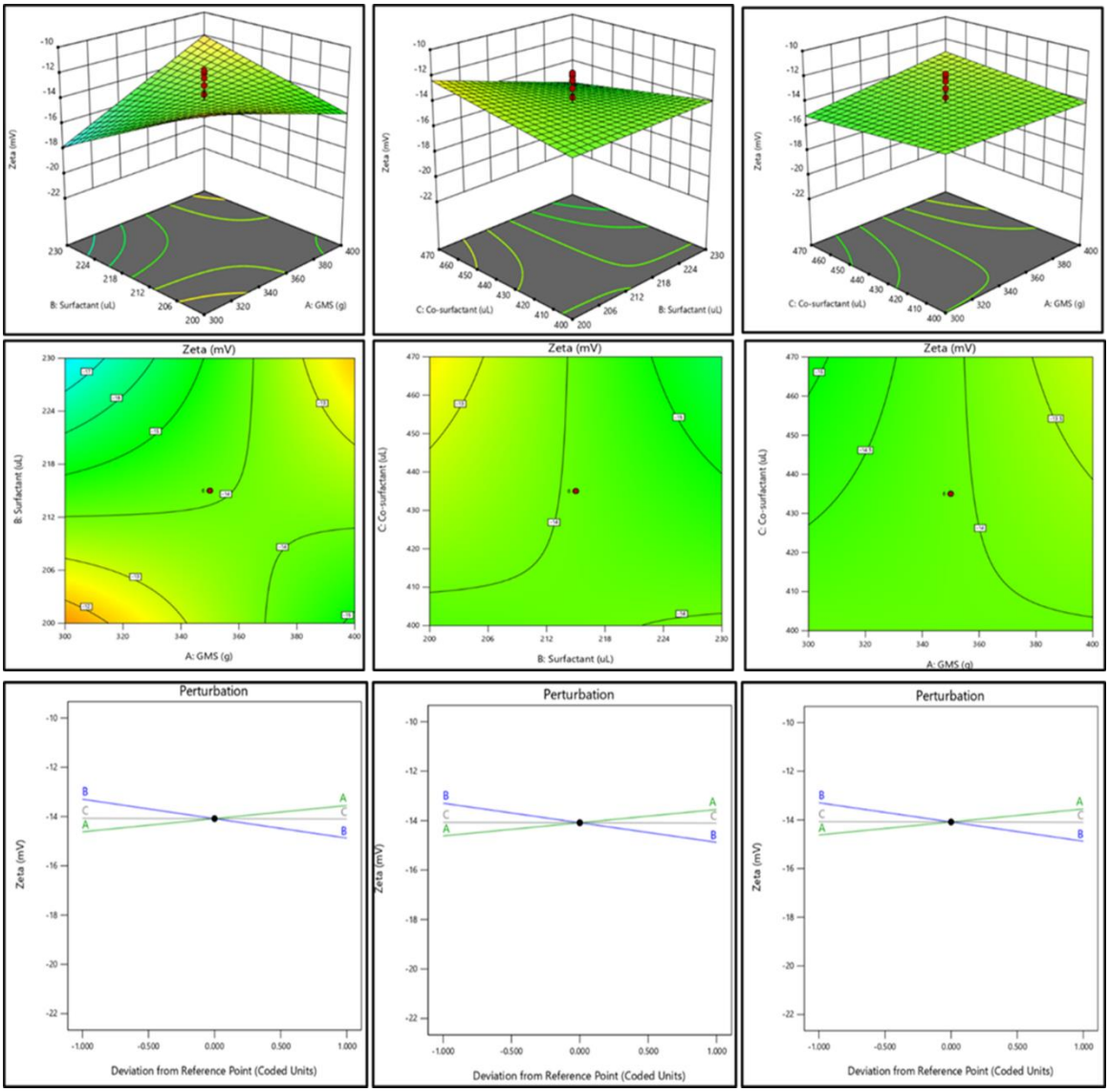

Figure3b: 3-D surface, counter plots and Perturbation graphs for effect of independent values on zeta potential.

Effect of independent variables on Entrapment efficiency (Y3)

The impact of independent variables on entrapment efficiency is investigated in this study. Entrapment efficiency, a critical

factor in the successful encapsulation of active substances within a delivery system, is influenced by various independent variables. Advanced analysis techniques are employed to unravel the underlying mechanisms and explore the relationship between the independent variables and entrapment efficiency. Valuable insights can be gained from this investigation, allowing for the fine-tuning of formulations and the maximization of the encapsulation potential of delivery systems.

The entrapment efficiency of prednisolone-loaded NPs was found to be in range from 74.03% to 89.52% for various factors and level combinations. The entrapment efficiency formulation was found 88.162 %. The effect of independent variables on entrapment efficiency can be represented by the following regression equation.

Polynomial equation= 83.711 + -0.776332 * A + -2.10085 * B + -0.161321 * C + -1.88625 * AB + -0.89625 * AC + -4.62375 * BC……Eq. 5

The regression coefficient value (R^2) of equation 5 was found to be 0.6852, which indicates a good fit between the experimental values and predicted values. The counter plot and 3-D surface plot of entrapment efficiency are given in Fig 3c. It was observed that entrapment efficiency of prednisolone-loaded NPs was increased with the increasing concentration of surfactant and GMS. The PDI was indirectly proportional with stirring speed. The obtained data revealed that an increase in the entrapment efficiency of prednisolone-loaded NPs was observed from 74.03% to 89.52% on increasing the concentration of surfactant and GMS. The potential cause for this could be the higher amounts of lipids available for encapsulation within the NPs. This can lead to improved entrapment efficiency as more drug molecules can be incorporated into the lipid matrix.

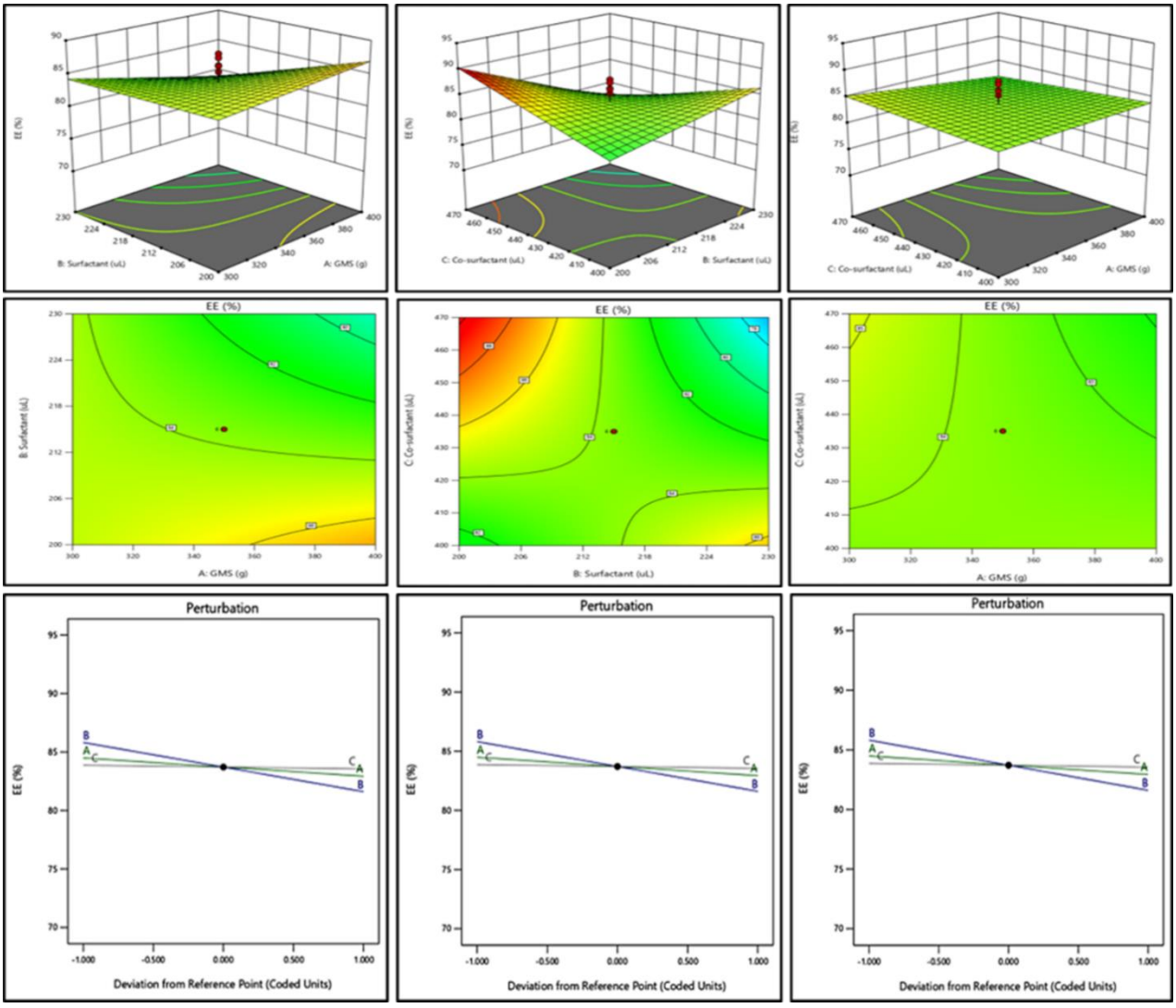

Figure3c: 3-D surface, counter plots and Perturbation graphs for effect of independent values on entrapment efficiency

Graphical Optimization of prednisolone loadedSLNs

The CCD indicated that the ideal values for a number of parameters, including surfactant, GMS, and Smix, were 300 microliters and 230 microliters, respectively. As per the design, PS, ZP, and EE of prednisolone can be obtained in the following ranges: 100.29 nm to 139.67nm, -20.06mV to -10.02 mV & 74.03% to 89.52%respectively, for the fabricationof SLNs. The result of the study showed that the PS and ZP of the optimized formulation was found to be 109.23 nm and -21.32mV. Similarly, the EE of prednisolone was found to be 77.83 ± 8.32%. This indicated that the obtained values of optimized formulation in terms of PS, ZP and EE were within the limits of graphical optimization. The good correlation between predicted and obtainedvalue indicated suitability and reproducibility of DoE.

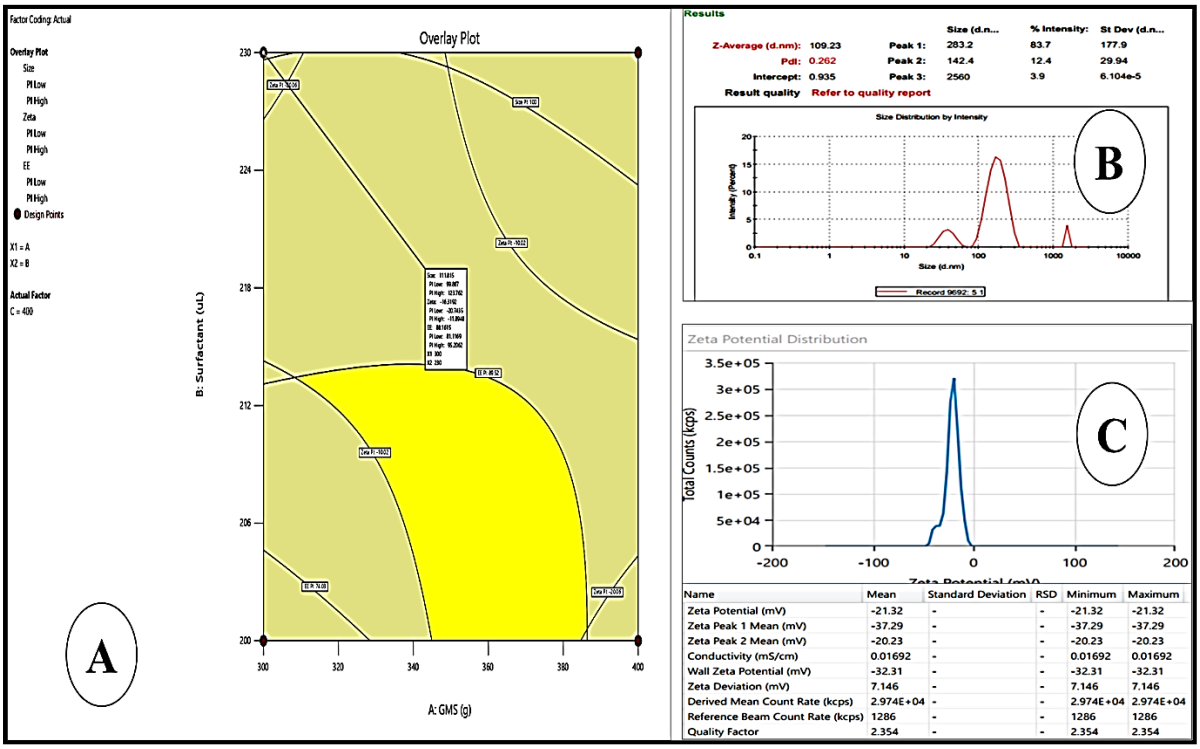

Figure 4: The results of graphical optimization showed that the

FTIR Spectroscopy

The KBr Pellet approach was used to determine the FT-IR spectra of prednisolone acetate (Figure 12). The range of 500–4000 cm–1 displayed distinctive peaks for prednisolone acetate. It was discovered that the peaks at 2361 cm-1 and 2188 cm-1 are caused by the -NH2 group's absorption. A sharp peak of 1747 cm-1 suggests a stretching vibration with a C=O equation. A distinctive peak displaying C-H deformation was seen at 2361 cm-1 and 1651 cm-1. The characteristic peaks at 400–1800 cm-1 displayed the stretching and vibrating of the aromatic ring's carbon (–C=C–). A peak was discovered at 2800 cm-1 for the alkane and 3200 cm-1 for the amine, which matched the reference spectra.

Figure5:FTIR spectroscopy of prednisolone

***In-vitro* anti-inflammatory study**

The measurement of NO level was used to assess the anti-inflammatory potential of SLNs. A rise in NO levels indicates that inflammation is present in the body. It was expected for this investigation that the cells treated with SLNs would have much lower NO levels than both the cells treated with blank SLNs, and cells treated only with lipopolysaccharides (LPS), which was used as a positive control. The results were in line with expectations: cells treated with LPS alone and blank SLNs had a considerably high level of NO; cells pre-treated with prednisolone SLNs and normal control had a significantly low level of NO (p 0.05). The control cells were deemed to have a 100% NO level. The acquired results showed that the drug-containing SLNs could considerably reduce NO production. The percentage of NO in cells treated with LPS was found to be approximately 2.5 times greater than in the control group. In the case of blank SLNs, it was likewise 2.35 times more than that of the typical control. The group treated with SLNs loaded with prednisolone had a 2.36-fold decrease in NO compared to the group treated with LPS alone. Fig. 6 presents the findings.

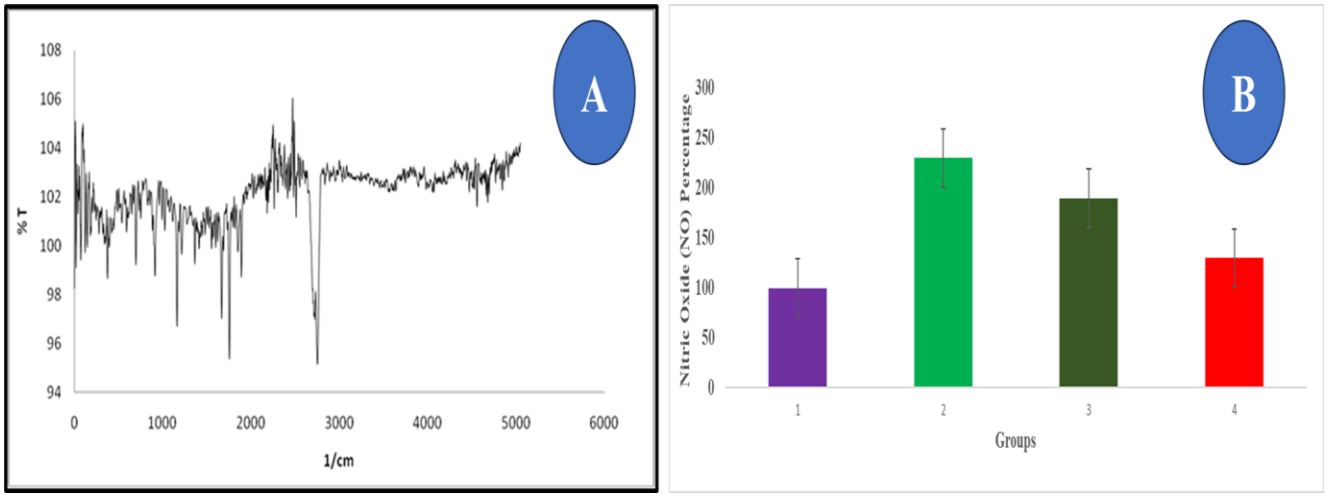

Figure 6: Representing A) FTIR spectroscopy of prednisolone B) Results showing the percentage of NO

***In vitro* cytotoxicity study**

According to the MTT cell experiments, over 96% of the cells treated with control and blank samples in 12, 24, and 48 hours did not exhibit any signs of cell death. Up to 24 hours, the cells treated with SLNs loaded with prednisolone exhibited above 95% cell viability. At 48 hours later, a small drop in cell viability was noted. However, in the case of both HCEC cells and L-929 cells, more than 88% of the cells were still viable after 48 hours. This demonstrated that the formulations had no harmful effects on the cells. Figures 14a and 14b, respectively, display the cell viability data for HCEC and L-929 cells. Based on the goals of the study, the MTT test is typically used to assess the safety profile of formulations on various cell lines. These goals led to the examination of SLNs' safety and toxicity in HCEC and L-929 cells. The produced formulations were safe against normal cells based on the lack of significant cell killing. This further shown that the created formulation can be successfully used for its intended purpose on the skin of humans and animals.

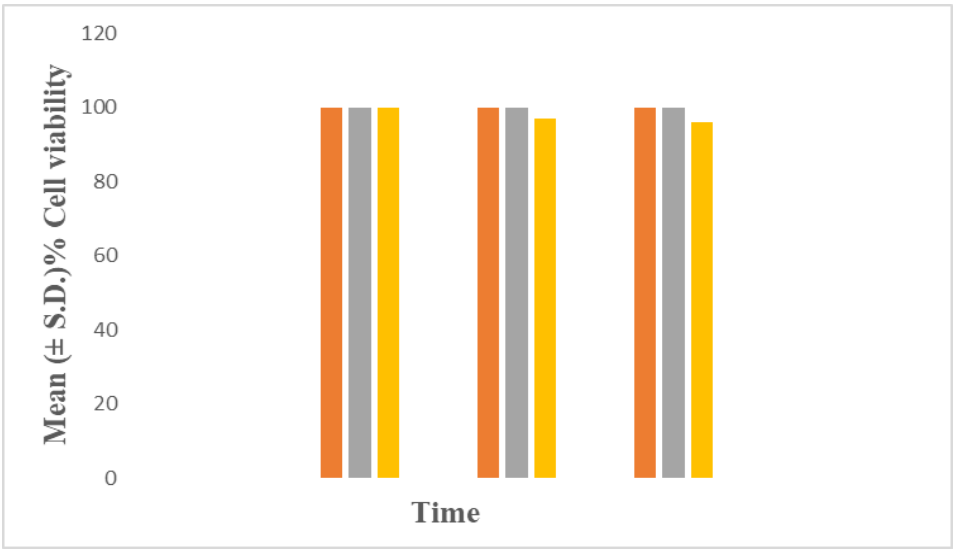

Figure 7a: Results of cell viability on HCEC cell

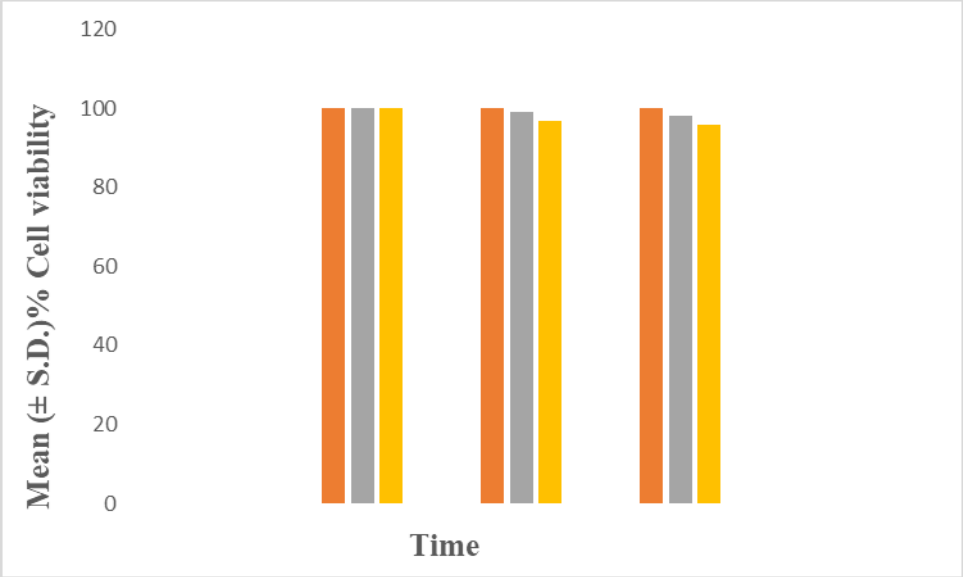

Figure 7b: Result of cell viability on L-929 cell

***In-vitro* release study**

The *in-vitro* release profile of prednisolone from solid lipid nanoparticles (SLNs) was investigated using a dialysis bag method over a period of 12 hours. The results are presented as cumulative percentage release of prednisolone at various time intervals.The release profile exhibited an initial burst release within the first 6 hours, with 45.68% of prednisolone released. This initial burst was followed by a sustained release phase, achieving 94.89%cumulative release at 12 hours. The initial burst release can be attributed to the desorption of prednisolone from the surface of the SLNs, while the subsequent sustained release phase is likely due to the gradual diffusion of the drug from the lipid matrix. This biphasic release pattern suggests that prednisolone-loaded SLNs have potential for providing both an immediate and a prolonged therapeutic effect.(Fig. 8).

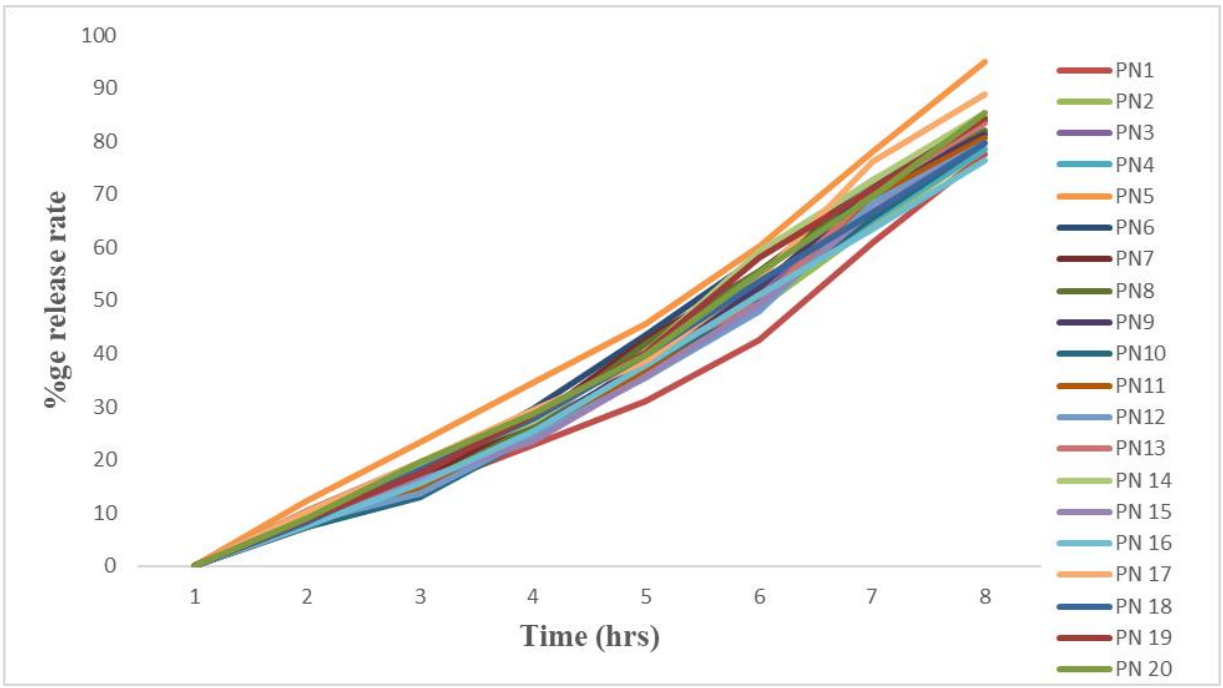

Figure 8: Drug release profile of formulations

Eye irritation study

In this investigation, the skin irritation of the left and right eyes of three rabbits was measured. The results of the Draize tests investigation demonstrated that upon ocular injection of prednisolone in the form of SLNs. After 1 hour, 24 hours, 48 hours,

and 72 hours, the prednisolone treated group showed no signs of redness, opaqueness, or edoema. Furthermore, the rabbits' corneal flow was similarly normal. Thus, was demonstrated that the generated ocular formulation is risk-free and exhibits negligible variation from the normal eye (Fig 9).

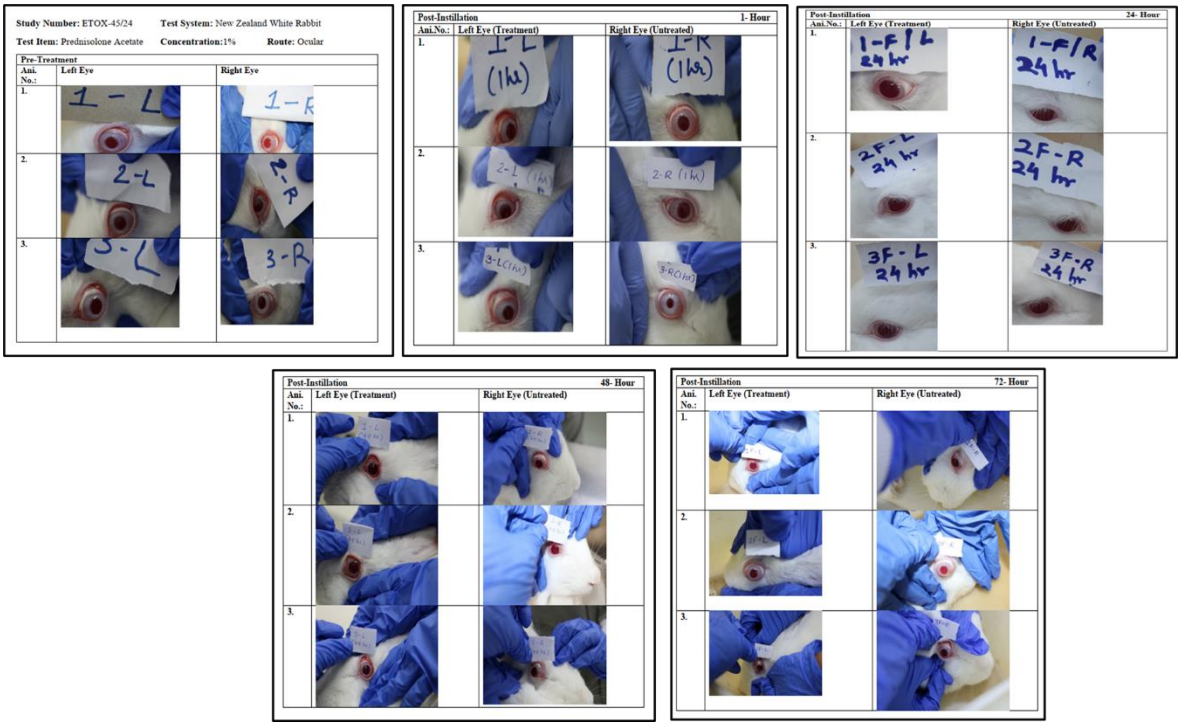

Figure 9 *Invivo* irritation study on rabbit Eye

Ex vivo Corneal Penetration Study

Prednisolone has a higher percent permeability than the optimised formulation, as seen in Table 4. The range of the corneal hydration percentages was 80.32±0.79 to 81.23±0.52%. Given that prednisolone is a tiny molecule (MW=360), it is likely that its permeability is high and unaffected by the formulation effect. Rather than improving penetration, nanosuspensions work to extend the drug's release. Drug absorption into the cornea is facilitated by the attachment of nanoparticles to the corneal surface via hydrogen bonds and electrostatic force. Furthermore, through the opening of the tight connections between corneal epithelial cells, the polycationic Eudragit may enhance corneal permeability. The nanosuspension consequently greatly increased the drug's penetration. The usual range of corneal hydration is between 76 and 80%. Damage to the corneal endothelium or epithelium is indicated by a corneal hydration level that is 3-7% units or higher above the normal value. In every trial, the corneal hydration range indicated no corneal injury. Thus, it can be said that the formulations that have been created have good permeability and do not compromise the integrity of the cornea.

Stability study

It was shown how several optimal SLN parameters were affected by conditions and storage time. The stability investigations over six months of storage in a refrigerator (2 – 6 ºC) and at room temperature (27±1 ºC) were evaluated by comparing the particle size, zeta potential, and entrapment efficiency of SLNs before and after storage under different conditions. Testing was done on the vials holding the stored SLNs to determine the turbidity, particle agglomeration, and particle settling to the bottom. Agglomeration of particles may be the cause of an increase in particle size. There could be less repulsive forces between the particles, which would explain the little decrease in zeta potential. Particle agglomeration and the ensuing little increase in particle size may have also resulted from this. Following storage, the SLNs revealed positive charge. The efficient attachment of the nanosuspension to the ocular surface is aided by its positive charge. Six months of storage did not considerably alter the electrophoretic behaviour. The drug's reabsorption onto the particle surface after dissolution may be the cause of the increased drug entrapment during storage.

Table 3:Stability studies of prednisolone loaded SLNs.

Parameter		**At 27 ± 1°C (RT)**		**At 2-6 °C**	
Time	**Initial**	**3 months**	**6 months**	**3 months**	**6 months**
pH	7.4±1.3	7.4± 1.6	7.6±0.5	7.5±0.8	7.3±1.2
Particle size	109.36±2.6	115.23±3.5	121.56±4.3	105.36±4.5	112.77±2.3
Zeta potential	-12.56±1.3	-19.36±3.6	-15.32±2.5	-20.26±1.6	-16.05±2.7
Entrapment efficiency (%)	85.63 ±2.6	84.55 ±3.2	81.78 ±4.2	86.56 ±2.1	85.66 ±1.2
Particle aggregation	No	No	No	No	No
Settling of particles	No	No	No	No	No
In vitro release					

5. CONCLUSION

Prednisolone, a potent corticosteroid, offers profound anti-inflammatory effect. The anti-inflammatory activity of prednisolone is due to its inhibitory action on Phospholipase A2 (PLA2) and cyclooxygenase-2 (COX-2) expression. In addition to this, it also stabilizes lysosomal membranes. There by reduce the production of pro-inflammatory cytokines and proteolytic enzymes which are responsible for inflammatory response. These aforementioned events make it a cornerstone in the management of ocular inflammatory conditions. Ophthalmic solutions have shortcorneal contact time (law local bioavailability) due to blinking and lachrymal secretion. If residence time is increased, it may improve bioavailability and hencemay decrease dosing frequency and increase patient compliance.

Above mentioned problem was solved by preparing solid lipid nanoparticles of prednisolone acetate, which provides better permeation through ocular membrane for improved anti-inflammatory effect. In this research work, solid lipid nanoparticles were prepared using high pressure homogenization technique followed by probe sonication. Optimized formulation was selected by the use of central composite design (CCD) involving three factors and two levels. A factorial design was used to study the effect of three independent variables (lipid concentration, surfactant concentration and co-surfactant concentration). Their effect was studied on particle size, zeta potential and entrapment efficiency. Further optimized formulation was evaluated for various parameters including particle size, zeta potential, SEM, DSC, entrapment efficiency, %drug release, and *in vivo* studies. *In vitro* data shows that SLNs can emerged as a promising carrier system for ocular drug delivery due to their controlled release properties, and potential to overcome the limitations of conventional formulations and they can be a better alternative to conventional delivery systems like solution, gels etc.

Optimized formulation showed good stability upon storage as per specified guidelines as there were no sign of discoloration and no physical change. All other evaluation parameters were in specified range. Major findings of the present study showed that the Glyceryl monostearate can be used for the permeation enhancer for prednisolone acetate and can be used in the development of a controlled release ocular drug delivery system in the treatment of ocular inflammation.

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Conflict of Interest

There was no conflict of interest stated by authors.

Disclosure Statement

No competing financial interests exist.

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