RESEARCH PAPER

Preparation and evaluation of loteprednol etabonate polylactic acid nanoparticle-based nanosuspension as an ocular drug delivery system

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Received: 28th Feb, 2026; Revised: 5th March 2026; Accepted: 12th April, 2026; Available Online: 20th April, 2026

ABSTRACT

Introduction: Topical ophthalmic formulations of corticosteroids are commonly used to treat a variety of ocular inflammatory conditions. The objective of this study was to develop and evaluate PLA-based polymeric nanoparticle-based nanosuspension for ocular administration.

Methods: Loteprednol etabonate-loaded PLA nanoparticles were prepared by the solvent evaporation method. Optimized formulation was characterized for differential scanning calorimetry, X-ray diffractometry, scanning electron microscopy and drug release. Nanosuspension formulation using PLA-based nanoparticles was evaluated for its suitability for ocular administration.

Results: Nine formulations were developed for PLA-based nanoparticles and optimized based on particle size and entrapment efficiency. DSC and XRD studies revealed that loteprednol etabonate is molecularly dispersed in PLA nanoparticles. Nanosuspension formulation of PLA-based nanoparticles demonstrated suitability for ocular administration based on redispersibility, sterility, corneal permeation, and ocular irritancy studies.

Conclusions: It was concluded that the PLA nanoparticle-based nanosuspension formulation exhibited good corneal penetration as well as did not show ocular irritation and could be a suitable nanosuspension of loteprednol etabonate an ocular delivery system

Keywords: Loteprednol etabonate; Poly Lactic Acid; Nanosuspension; Ocular

How to cite this article: Patkari K, Dwivedi J, Mahajan H. Preparation and evaluation of loteprednol etabonate polylactic acid nanoparticle-based nanosuspension as an ocular drug delivery system. Int J Drug Deliv Technol. 2026;16(34s):99-109. DOI: 10.25258/ijddt.16.34s.11

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Several ocular pathological conditions are characterized by marked inflammatory changes. If left untreated, ocular inflammation at both the anterior and posterior segment levels can lead to temporary or permanent vision loss. Treatment of these conditions involves the administration of topical ophthalmic formulations containing corticosteroids.

Loteprednol etabonate, a corticosteroid developed for ophthalmic use, was developed by maintaining the balance between the therapeutic corticosteroid activity and minimizing adverse side effects[1-2].

The topical application of therapeutic agents provides direct access to the target tissue; however, the ocular surface provides a set of unique challenges for topical penetration. Mechanisms to eliminate foreign material from the ocular surface include blinking, tear flow, and drainage through the nasolacrimal duct. Moreover, the cornea and conjunctiva are naturally covered with a 3- to 40-lm layer of mucus[3-5]. [The outer layer is comprised of secreted and other mucins (cleared rapidly by mucin turnover and blinking), whose primary role is to trap and eliminate allergens, pathogens, and debris (including therapeutic particles) from the eye[6].

Polymeric nanocarriers that can penetrate rapidly the outer mucous layer are likely to be retained at the ocular surface for an extended period and facilitate drug release directly to the underlying tissue. To avoid the barrier presented by the mucous layers, nanoformulation has been developed using polymers that allow for diffusion through the mucus and facilitate an even distribution of the nanoparticles across the ocular surface[7,8].

The objective of this study was to develop the loteprednol etabonate-loaded polylactic acid (PLA) nanoparticles suspension formulation, to improve drug penetration and retention in the ocular cavity.

Formulation and Evaluation of PLA Nanoparticles

Design of experiment

The factorial design (3^2) approach generated by Design Expert software (Trial Version 7.1.6, Stat-Ease Inc., MN) was used to assess the experimental fitness that requires minimum experiments to optimize this nanoparticle formulation. In this analysis, different batches of loteprednol

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etabonate-loaded nanoparticles were designed to study the effect of different variables on the structures of the formulation. We selected two parameters, such as polymer concentration and speed of homogenizer. Methanol was chosen as a solvent as loteprednol etabonate is completely soluble in it. Characterization parameters such as particle size and % EE were considered dependent variables. For each independent variable, the lower and upper levels were represented by a (-1) and (+1) sign respectively, whereas middle value (0), alpha values (–α), and (+α) were suggested by design software as summarized in **Table 1**. From the above nine batches optimized batch was selected by taking into consideration various parameters like the model with a value closer to 1 for correlation coefficient r^2, predicted r^2 and adjusted R which are quality indicators. These values were obtained from simulations indicating the linear, quadratic, and two-factor (2F) interaction model through design expert tools. These models were tested using variance analysis (ANOVA). Less than 0.05 was intended for this p-value to be statistically relevant. The batch with a low particle size and an increased EE percentage was chosen as an optimized batch [9].

Table 1: Independent variables: Factors and levels for full factorial design

Factor	Level		
	-1	0	+1
X1:Polymer (PLA Concentration) (mg)	50	100	150
X2: Speed of Homogenizer (RPM)	800	1000	1200

Preparation of Loteprendol-loaded PLA nanoparticles

Loteprednol etabonate-loaded nanoparticles were formulated using a solvent evaporation approach. Nanoparticles were prepared using different ratios of the drug: polymer concentration. and speed of rotation as depicted in table 2. The solvent evaporation technique has been widely used to prepare drugs loaded with biodegradable nanoparticles. In short, organic solutions were prepared with different quantities of biodegradable polymer (PLA) and 50 mg of the drug with 10 mL of methanol. In 20 mL of aqueous solutions containing different PVA concentrations (1 percent), the organic phase was added dropwise and magnetically stirred. The volume of the nanoparticle dispersions was concentrated to 10 mL under reduced pressure using a vacuum rotavapor (KNF LABS RC 600, Switzerland) after 30 min of agitation at varying speeds. The aggregates were separated via a 0.45 µm syringe filter by filtration. The non-encapsulated drug was separated at 20,000 rpm at 4 °C for 1 h by ultracentrifugation (Beckman Coulter). The supernatant was discarded and, to eliminate excess surfactant, the isolated nanoparticles were washed twice with distilled water. The washed particles were resuspended and freeze-dried for 48 h in 5 mL of water solution containing 5 percent (w/v) mannitol as a cryoprotectant. The lyophilized nanoparticles for investigation[10].

Table 2: Experimental design for formulations containing

Formulation Code	Code Factor Level		
	Trail no	Factor X1	Factor X2
F1	1	-1	-1
F2	2	0	-1
F3	3	+1	-1
F4	4	-1	0
F5	5	0	0
F6	6	+1	0
F7	7	-1	+1
F8	8	0	+1
F9	9	+1	+1

6.3 Optimization of drug Loaded Nanoparticles

6.3.1 Mean Particle Size and Polydispersity Index Measurements

Malvern Zetasizer (Nano ZS 90, Malvern Ltd., UK) was used to estimate Mean particle size and polydispersity index (PDI) for prepared batches. The standard behind the assessment of particle size in a zeta sizer is based on the scattering of light after passing through the sample cell. The fluctuation in light scattering strength is calculated based on the particles in the sample. The sample was initially dispersed in double distilled water and then held in a polystyrene sample cell (with a 10 mm diameter). The evaluation was done at an angle of 90°with an equilibration time of 2 min at 25 °C.

Percentages of Encapsulation Efficiency (%EE)

The encapsulation efficiency of loteprednol etabonate in the polymeric nanoparticle was calculated by extracting and quantifying the encapsulated loteprednol etabonate. The polymeric nanoparticles were triturated in 25 ml of methanol and subjected to room temperature shaking for 12 hr to fully dissolve the particles. The ensuing solution was diluted with proper dilution and the concentration of the drug in methanol was determined by spectrophotometry by measuring UV absorbance at 242 nm.

EE of the drug = (Amount of encapsulated drug)/(Total amount of the drug)X100 ..Eqn no(1)

Differential Scanning Calorimetry(DSC) studies

Loteprednol etabonate, PLA, and loteprednol etabonate-loaded nanoparticles have been studied for thermal behavior using differential calorimeter scanning (Mettler

Toledo DSC 1 Star System, Zurich, Switzerland). The sample (2 mg) was exactly weighed and placed in a 40 µL standard aluminum pan. This pan was sealed, pierced with a pin on top, and positioned in the sample receptacle of the DSC instrument. Samples were intense at 10 ºC/min rate from 0-300 ºC. The sample cell was purged with nitrogen gas at 40 mL/min flow rate throughout the thermal measurement[11].

X-Ray Diffraction(XRD) Studies

X-ray diffractogram patterns of Loteprednol etabonate, PLA, and loteprednol etabonate-loaded nanoparticles have been recorded (Bruker AXS, D8 Advance, Germany). A current of 30 mA was used to produce Cu-Ka radiation at 40 kV voltages to carry out estimations. The X-ray diffractogram was run at 2 °/min and 2 °/2 cm/2 map velocity scanning rates[12].

Scanning Electron Microscopy (SEM) Examination

Using the scanning electron microscope (JEOL-JEM 2100) at an accelerating voltage of 200 kV, morphological analysis of loteprednol etabonate-loaded PLA nanoparticles was carried out. By using phosphotungstic acid solution (2 percent w/v), a drop of nanoparticle suspension samples was mounted on a copper grid and stained. The samples were air-dried and analyzed afterward[13].

Formulation and evaluation of nanosuspension

About 50 mg of drug-equivalent polymeric nanoparticles were suspended in 10 ml of sterile water for injection. The formulation was filled in a glass vial till further use was maintained at 2-8^{0}C. The whole experiment was carried out in an aseptic manner under laminar airflow. The vial was terminalized by an ethylene oxide sterilizer.

Redispersibility of Nanosuspension

The exact quantity (25 ml) of each nanosuspension was maintained for 30 days in a stopped measuring cylinder that was kept undistributed at room temperature. The suspension was shaken vigorously after 30 days to redistribute the sediment and, if any, the existence of deposits and cake was reported and the ease of redispersibility was evaluated.

Clarity testing of nanosuspension

To determine the clarity of the reconstituted suspension product, an externally clean vial was examined under bright lighting conditions against the black and white background.

$_P$H measurement

The instrument with a combined glass electrode is used for this purpose. The pH was measured by immersing the electrode in contact with nanosuspension and performed in triplicate.

Drug Content

The drug content of loteprednol etabonate nanosuspension was determined by diluting 1mL of the formulation with 10 mL methanol followed by content analysis with a UV-visible spectrophotometer at 242 nm (Shimadzu 1700i).

In-vitro drug release study

The Franz-type diffusion cell was used for studying the in vitro release from optimized nanosuspension. A dialysis membrane (Avg. molecular weight cut-off value of 12,000-14,000, Sigma Aldrich) was used as a semipermeable membrane. A 1 mL portion of the nanosuspension-containing loteprednol etabonate loaded PLA nanoparticles sufficient for establishing sink conditions for the assay was placed into the donor compartment. The receptor compartment contained 12 mL of 0.2M Phosphate buffer solution of pH 7.4 maintained at 32°C under mild agitation using a magnetic stirrer. At specific time intervals, aliquots of 0.5 mL were withdrawn and immediately restored with the same volume of fresh phosphate buffer. The amount of drug released was assessed by measuring the absorbance at 242 nm using a UV spectrophotometer (ShimadzuUV-1700i, Japan).

Drug Release Kinetics.

Zero-order kinetics of drug release

Zero-order release kinetics refers to the process of constant drug release from a drug delivery system. $\mathbf{C= C_0+k_0t}$**…….2**

First-order kinetics equation of drug release

The release of a drug that follows first-order kinetics can be represented by Equation 3

$\log C=\log C_0-K_1t/2.303$ ……3

C_0 is the initial concentration of the drug, C_0 is the percent of the drug remaining at time t. The correlation coefficient of the above equation will give information on whether the drug release follows first-order kinetics or not.

Higuchi equation model

The Higuchi model expression is given by equation 4.

$Ct = k_Ht^{1/2}$……..4

where Ct is the percentage of cumulative drug release at time t, k_H is the Higuchi dissolution constant.

Peppas- Korsmeyer equation of drug release

To find out the mechanism of drug release, drug release data were fitted in the Korsmeyer and Peppas model.

$$Ct/C\infty = Kt^n \quad \ldots\ldots 5$$

Where: Ct / C∞ is a fraction of the drug released at time t, k is the release rate constant, and n is the release exponent. The n value is used to characterize different drug release patterns from the dosage form matrix[14].

Ex-vivo trans corneal permeation studies

The in vitro permeation study of the loteprednol etabonate-loaded PLA nanoparticle-based nanosuspension through the bovine cornea was performed using a Franz diffusion cell at 32 °C. A freshly obtained scleral layer was mounted between the donor and the recipient compartments. The nanosuspension was placed on the epithelial-faced surface of the cornea and the opening of the donor compartment was sealed with a glass coverslip and soaked with simulated lacrimal fluid (SLF, composition: 8.3 g of NaCl, 0.084g of $CaCl_2{\cdot}2H_2O$, 1.4g of KCl, and distilled deionized water to 1000 mL). The receiver compartment

was filled with 12 ml SLF at pH 7.4 and stirred with a magnetic bead at 100 rpm[15]. Three milliliters of the sample were withdrawn from the receiver compartment at predetermined time intervals and analyzed for loteprednol etabonate at 242 nm spectrophotometrically.

The permeation (%) or in vitro ocular availability was calculated as follows:

Permeation % = (Amount of loteprednol etabonate permeated in receptor compartment/ Initial amount of loteprednol etabonate in donor) X 100…….eqn. 6

Ex-Vivo Studies (Ocular Irritation Studies)

A chorioallantoic membrane test as a mucous-membrane irritation test was performed for the Draize eye irritation test. For the research, commercially available fertilized white chicken eggs were used without mycoplasma. The hen's eggs were put in incubator trays with the wide ends up for CAM testing; the trays were placed in the incubator, which rotates automatically, and was held at an optimum temperature of 37.5±0.5 °C. On day 5 of incubation and every day afterward, the eggs were candled, eliminating nonviable embryos. The eggshell was scratched by a dentist's rotary saw around the air cell on day 10 of incubation and then pared off. The vascular CAM was revealed after careful removal of the internal egg membranes. Nanosuspension was applied to the CAM surface at a volume of 0.2ml. A set of four eggs was used; two vehicle-only eggs acted as controls. CAM, blood vessels, including the capillary system, and albumen were tested and rated for irritant effects at 0.5, 2, and 5 minutes after exposure following the application of the test drug. The numerical time-dependent scores for hyperemia, hemorrhage, and coagulation were summed to give a single numerical value demonstrating the irritation potential of the test substance on a scale with a maximum value of 21. The mean value of four tests makes possible consideration by arrangement scheme equivalent to the CAM categories[16].

Table 4: Scoring scheme and assessment for irritation testing with the hen's egg CAM.

Effect	Score (Time in minutes)		
	0.5	**2**	**5**
Hyperemia	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5
Score assessment			
0-0.9	Practically none		
1-4.9	Slight		
5-8.9	Moderate		
9-21	Strong		

Sterility Testing

Sterility testing was performed to ensure the sterility of the finished product. Since nanosuspension was administered by the ocular route, the direct inoculation method was ideal to carry out sterility testing. In this process, the specific quantity of sample under test was drawn aseptically from the containers and transferred to fluid thioglycolate medium (20 mL) and Soybean-Casein digest medium (20 mL), individually. The nanoparticles added in the medium were incubated for not less than 14 days at 30°C–35°C in the case of fluid thioglycolate standard and 20°C–25°C in the case of Soybean Casein digest medium. The growth of microorganisms in the medium was observed[17].

Stability Study

A stability study for loteprednol etabonate- loaded PLA nanosuspension was performed as per Q1A (R2) ICH guidelines. The reconstituted sample of optimized loteprednol etabonate-loaded nanosuspension formulation was stored in stability chambers at 25 ºC ±2°C / 60 %RH± 5% RH for 3 months. The samples were collected from a stability chamber every month and characterized for drug content, mean particle size, and redispersibility test.

RESULTS AND DISCUSSION

Formulation and evaluation of loteprednol etabonate-loaded nanoparticles

Design of experiment

3^2 design approaches were used in which two dependent variables were used (X_1: polymer concentration and X_2: Speed of homogenizer). A total of nine batches were generated considering three levels -1, 0, +1, α – value was produced by design software. Depending on the results of selected responses (Y_1: particle size and Y_2: % Entrapment Efficiency), the effect of this dependent variable (X_1 and X_2) was studied on the formation of polymeric nanoparticles.

These response (Y_1 and Y_2) were subjected to analysis and fitted to different models like linear, quadratic, or 2FI (two factor interaction). Depending on the highest coefficient correlation values like predicted r^2, adjusted r^2, and actual r^2 a model was selected as displayed in Table 4. The RSM (Response Surface Method) plot as 3D model graphs, demonstrating the effect of X_1: polymer conc and X_2: speed on responses Y_1: particle size and Y_2: % Entrapment Efficiency are as follows (table 4).

Table 4: Characterization of Acyclovir (PLA) nanoparticles

Sample code	X1 PLA (mg)	X2 Speed of Homogenizer (RPM)	Y1 (PS) nm	Y2 (EE) %
F1	50	800	149.2±0.1	91.2±0.11
F2	100	800	145.6±0.31	88.7±0.1
F3	150	800	154.7±0.14	88.2±0.17
F4	50	1000	135.8±0.2	81.8±0.11
F5	100	1000	130.1±0.8	79.1±0.21
F6	150	1000	131.9±0.11	76.6±0.18
F7	50	1200	115.9±0.9	75.7±0.22
F8	100	1200	119.6±0.13	73.1±0.19
F9	150	1200	104.1±0.1	70.9±0.31

Particle size model analysis

As displayed in Table 4, the particle size for the optimized batch of loteprednol etabonate-loaded PLA nanoparticles was found 149.1 nm suggesting it is suitable for ocular administration. The polynomial equation for particle size in terms of coded variables is given as follows in the equation:

$Y_1=131.8778+1.7555X_1-0.111X_2+17.95Y_1+0.722Y_2-2.388X_1Y_1-4.12X_2Y_1+1.44X_1Y_2-2.38X_2Y_2$…….eqn.(7)

ANOVA showed that the independent variables X1 and X2 had a significant (negative effect) effect on the particle size of both nanoparticle formulations. As the value for X_1 and X_2 increase, particle size significantly decreases as confirmed by Equation 7. in the case of loteprednol etabonate PLA nanoparticles (where *p*-value < 0.05) (Table 5). As per the report, reduced particle size with increased PLA concentration may be due to the improvement of a more compact structure of colloidal steric stabilization nanoparticles (Fig 1). The nanoparticles' lesser particle size limits their Brownian movement, rendering the formulation kinetically stable. Increased drug content compared to PLA may also lead to drug adsorption on the surface of the structure of nanoparticles leading to enlarged particle size, and the effects of particle size as shown in (Table 4).

% Entrapment efficiency model analysis

As displayed in Table 4, the entrapment efficiency of loteprednol etabonate loaded PLA nanoparticles was found in the range from 70.9 to 91.2 From the above outcome we could interpret polymeric nanoparticles to be a suitable carrier for anti-infective drug loading with good % entrapment efficiency. The responses observed for all the batches formulated were fixed to different models using design expert software. The quadratic model possessing the highest adjusted and predicted r^2 (*p*-value > 0.05) was selected as being the best fit (Table- 4) The adjusted r^2value (>90%) demonstrated an elevated level of significance for the selected quadratic model. The polynomial equations in terms of coded variables for % entrapment efficiency are given in the equation as follows:

$Y2= 80.58+2.31X_1-0.288Y_2+8.77Y_1-1.422Y_2-0.477X_1Y_1-0.377X_2Y_1+0.322X_1Y_2+0.222X_2Y_2$

…… eqn. (8).

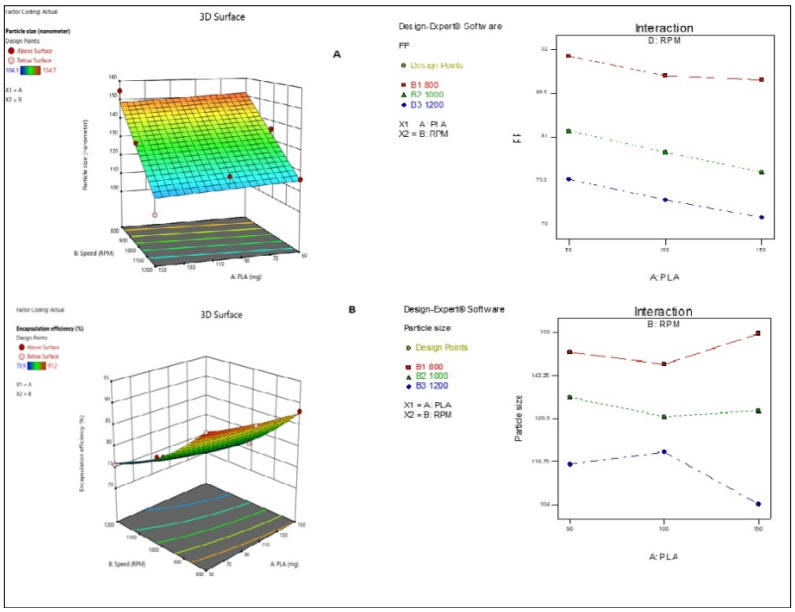

Fig 1: Effect of factors (X_1 and X_2) on responses Particle size (A) and % Entrapment Efficiency (B) of loteprednol etabonate PLA Nanoparticle

Table 5: Regression analysis for measured responses – loteprednol etabonate loaded PLA nanoparticles.

Source	Sum of Square	Degree of Freedom	Mean Square	F value	P Value	Model Significant/Non-significant Relative to Noise
Model	2205.397	5	275.674	2986.21	≤0.0001	Significant
X1 (PS) Linear	2034.34	2	1015.17	34.80	0.0005	Significant
X2 (EE) Quadratic	428.88	5	85.78	289.86	0.0003	Significant
Residual	0.57	6	0.08	-	-	
Core Total	2205.39	8	-	-	-	

Based on the optimization of Y_1 and Y_2, batch F1, was selected as the optimized formulation.

Particle Size Analysis

The results for the mean particle size of loteprednol etabonate loaded nanoparticles formulation was 149.8 nm with 0.29 PDI, (Fig 2).

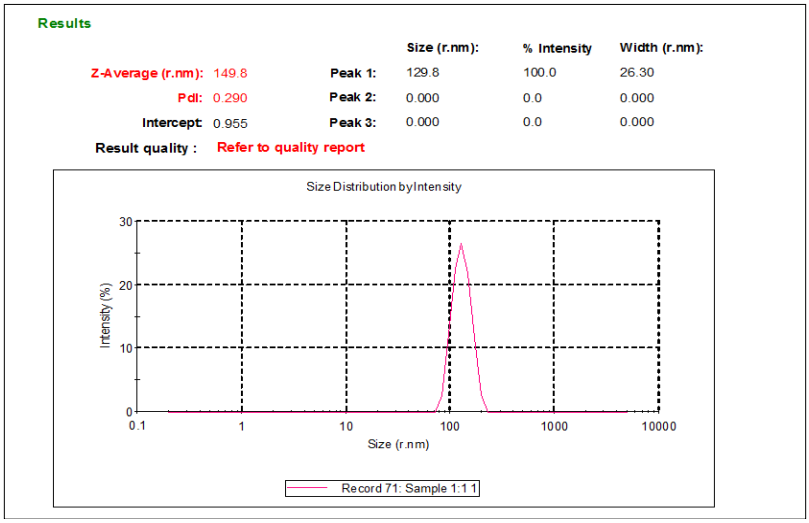

Fig 2: Particle size and PDI of loteprednol etabonate-loaded PLA nanoparticles

Percent of Entrapment Efficiency (% EE)

The percentage of entrapment efficiency of 91.2±0.11 for the F1 batch indicates good drug trapping of loteprednol etabonate inside the core of polymeric nanoparticles. The drug is trapped in the nanoparticles either because of physical trapping due to hydrogen bond and/or Vander Val forces formation or due to drug conjugation with polymer moiety or molecular dispersion of the drug in the polymer. For all the batches, encapsulation efficiency was directly related to the amount of polymer concentration used and speed.

EVALUATION OF OPTIMIZED NANOPARTICLES

Differential Scanning Calorimetry(DSC)

The DSC thermogram of loteprednol etabonate (Fig 3A)indicates an endothermic peak reflecting its melting points at 240.13 °C, DSC thermogram of PLA and loteprednol etabonate loaded PLA nanoparticles displays an endothermic peak at 143°C as shown in Fig 3Band 3C. Absence of peak for loteprednol etabonate in PLA nanoparticles attributed to molecular dispersion of loteprednol etabonate in the polymer matrix. The disappearance of the endothermic peak at 240° C is related to loteprednol etabonate confirmation of the rearrangement of the polymers into nanostructures able to maintain a high amount of the drug in solution.

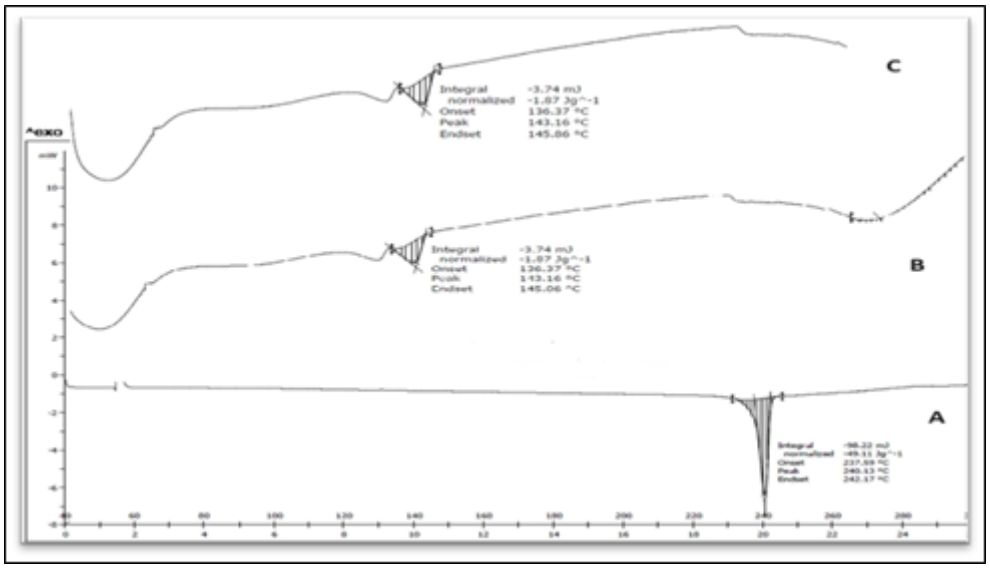

Fig 3: Differential Scanning Calorimetry thermograms of Loteprednol etabonate(A), PLA (B), and Loteprednol etabonate loaded PLA nanoparticles.

X-ray diffractometry (XRD)

Loteprednol etabonate exhibited characteristic peaks between 2θ of 5 and 30º and exhibited sharp diffraction peaks at 2θ values of 8.731°, 10.89°, 16.12°, 32.1° (Fig 4A) whereas, The loteprednol etabonate-loaded PLA nanoparticles sample showed diffraction peaks at 6.162°, 9.888°, 14.842°, 20.675°,25.634°and28.276(Fig 4B), and the absence of diffraction peaks of the drug suggested an effective encapsulation of the drug in the center of nanoparticles(Fig 4C).

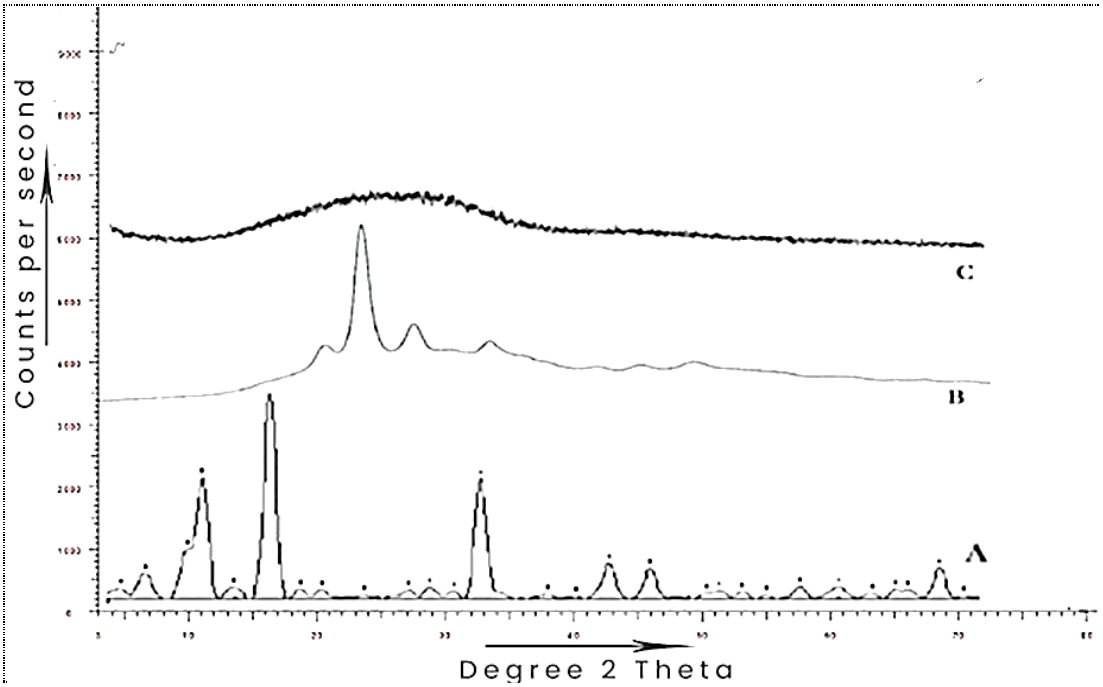

Fig 4: X-ray diffractogram of A) loteprednol etabonate B)PLA C) loteprednol etabonate loaded PLA nanoparticles

Scanning Electron Microscopy (SEM)

Fig 5 shows SEM photographs of loteprednol etabonate-loaded nanoparticles. The morphology review showed that there was a uniform scale distribution for nanoparticles loaded with. The smooth surface of lyophilized nanoparticles can be seen clearly in SEM pictures.

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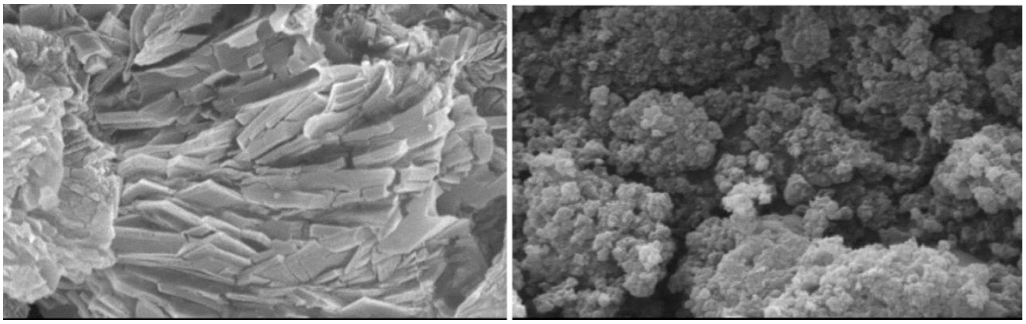

Fig 5: SEM image for loteprednol etabonate loaded PLA nanoparticles.

Characterization of nanosuspension

Redispersibility of Nanosuspension

The product was easily redispersed on shaking after storage at room temperature there is no sign of cake formation or sedimentation after 30 days.

Clarity: In the suspension clarity test, the solution exhibited high clarity, indicating no particulate matter, meeting the stringent quality standards for ocular administration. The formulation after reconstitution was completely clear so there is not any type of visible particulate matter present.

pH

The ideal pH for maximum comfort when an ophthalmic preparation is instilled in the eye should be in the order of 7.2±0.3. The pH value of the prepared PLA nanoparticles suspension was 7.52±0.36 hence no ocular irritation was expected.

Drug content

Drug content for loteprednol etabonate was found to be 98.9± 1.33% which lies in the standard range of 98.5 to 100.5% according to standards.

***In vitro* drug release and kinetics**

The initial slower release of loteprednol etabonate was attributed to polymer swelling after wetting followed by drug release in a sustained manner due to polymer relaxation. loteprednol etabonate loaded PLA nanoparticle, correlation coefficient values (R^2) calculated for these models were: Zero-order (0.951), first order (0.917), Higuchi (0.974), and Korsmeyer–Peppas(0.780) (Fig 6). Also, it was interpreted that the drug release followed Higuchi as the R^2 value for the Higuchi model was found to be near to one compared to other models. Higuchi's model describes negligible drug release from a matrix system due to swelling and dissolution. So, from observation, it can be concluded that loteprednol etabonate-PLA nanoparticles-based nanosuspension follows sustained drug release. The release exponent (n) and K values for the Korsmeyer-Peppas equation were found to be 0.28 and 1.46 respectively (F**ig** 6). depicts the fickian diffusion process. For hetero-dispersed samples, it was found that the particle size distribution affects the value of n varies with the breadth of the distribution and the general shape of that distribution[18].

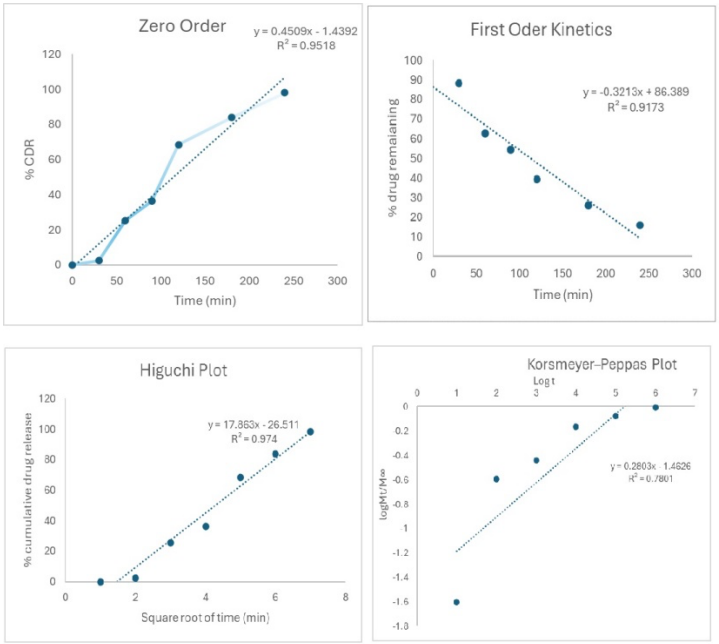

Fig 6: In vitro drug release and release kinetics from nanosuspension formulation

Ex vivo transcorneal permeation studies

The ex vivo transcorneal permeation of loteprednol etabonate from PLA nanoparticle-based nanosuspension formulation and pure drug suspension was conducted on the excised goat cornea for 4h. The nanoformulation showed higher permeation across the cornea after 4h (68.22%) as compared to the pure drug suspension (32.81%) [19-22]

Ocular Irritancy

The Hen's Egg chorioallantoic membrane test was performed to check the eye irritation potential of the optimized nanosuspension formulation of loteprednol etabonate PLA. The experimental results of controls (negative (A) and positive (B)) and test formulation (C) are presented in Figure 7. In the case of 0.9% saline considered a negative control, and test formulation no signs of hyperemia, hemorrhage, or coagulation on the chorioallantoic membrane were observed. However, 1% sodium dodecyl sulphate was applied as positive control; significant damage to the chorioallantoic membrane was observed after five minutes which is slightly irritant, while application of nanosuspension formulation showed no effect on the chorioallantoic membrane after five minutes concerning hyperemia, hemorrhage or coagulation. The irritation score for controls and test formulation is presented in Table 6. The irritation score of the HET-CAM test showed that the developed nanosuspension formulation is essentially non-irritating and possesses good ocular tolerability[23].

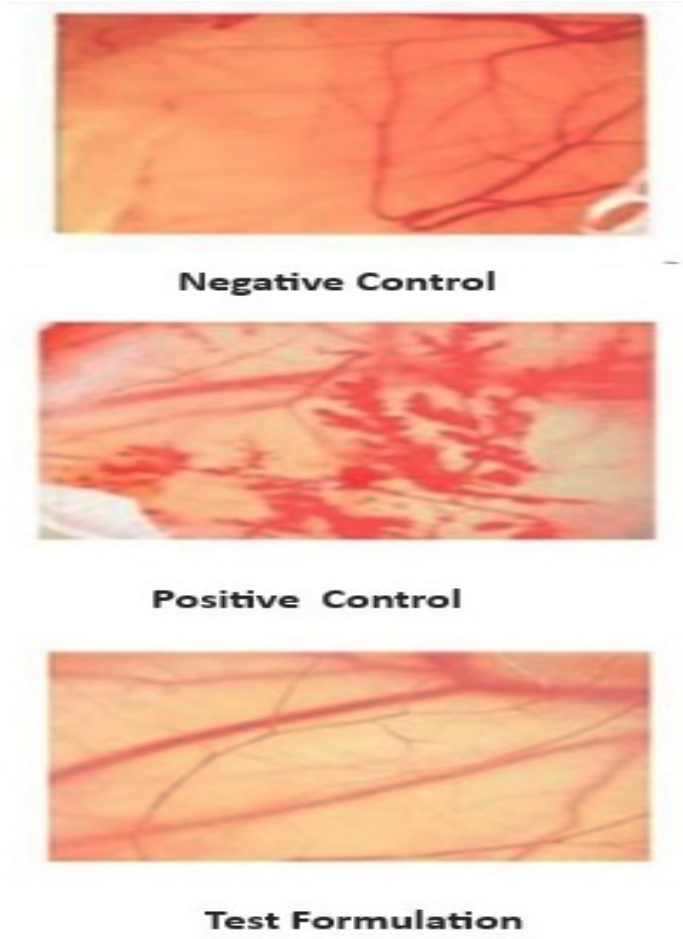

Figure 7: CAM Test of loteprednol etabonate loaded PLA nanosuspension.

Table 6: Irritation scores for loteprednol etabonate loaded PLA Nanosuspension hen's egg chorioallantoic membrane test

Group	Treatment Given	Irritation Score	Observation	Irritation category
Negative control	0.9% NaCl	00	No effect observed	Non – Irritant
Positive control	1% sodium dodecyl sulphate	14	Lysis and Hemorrhage	Severe Irritant
Test	Test Formulation	00	No effect observed	Non - Irritant

Sterility testing

It was found that there was no turbidity and no evidence of microbial growth (Fig 8) when formulations were incubated for 7 days at 30°C to 35°C in case of fluid thioglycolate medium and at 20°C to 25°C in case of Soybean-Casein digest medium demonstrating that formulation passes the test for sterility[24].

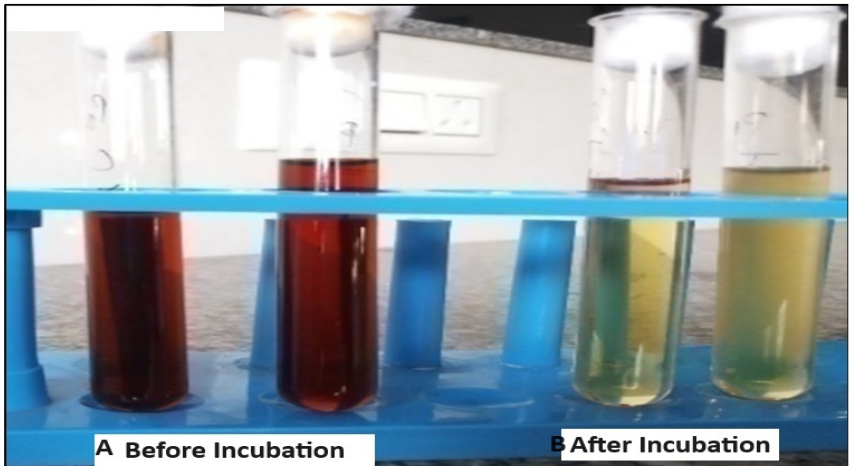

Fig 8: Sterility testing (A before incubation and B after incubation for 14 days)

Short term Stability

The physical appearance of the nanosuspension formulation did not change when samples were stored at 40°C for 3 months. No cake formation or sedimentation was observed. The average particle diameters were 220 nm which was 217 nm before performing stability study. There is no significant change in drug content after stability. It can be inferred from the observed data that the prepared nanosuspension formulation was stable after 3 months of storage at room temperature and 25 ± 2 °C and 60 ± 5% RH.

CONCLUSION

In the present investigation, loteprednol-loaded PLA-based nanoparticles were successfully developed by solvent evaporation techniques. The lyophilized particles exhibited nanoscale size and smooth surface. The nanosuspension of PLA-based nanoparticles exhibited good dispersibility and stability desired for topical ophthalmic application. The nanosuspension formulation demonstrated better penetrability across the excised goat cornea as compared to the pure drug suspension. The nanosuspension lacked an irritation tendency as revealed by the HET-CAM test. The nanosuspension formulation was found to be sterile and stable. However, the clinical benefits of the formulation developed by in vivo studies with humans will decide its appropriateness in clinical practice.

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