ORIGINAL ARTICLE

Formulation Designing and Optimization of Loteprednol-loaded spanlastic Nanocarriers for Treatment of Ocular Inflammation

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Abstract

Objective: The objective of this scientific work was focused on formulation, optimization and evaluation of loteprednol-loaded spanlastic nanocarriers for effective treatment of ocular inflammation with enhanced transcorneal drug permeation, prolonged corneal residence time, and sustained drug release profile. **Materials and Methods:** The spanlastic nanocarriers of loteprednol was prepared by ethanol injection method using Span 60 and Tween 80 as vesicular components. A 2^3 factorial design was employed in the optimization studies selecting independent variables such as Span 60, Tween 80, and drug content, whereas, the response variables such as particle size, polydispersity index, zeta potential, entrapment efficiency, and cumulative % drug release. The optimized formulation was evaluated for various *in vitro* and *ex vivo* parameters. **Results and Discussion:** The optimized formulation of spanlastic nanocarriers of loteprednol was found to have 207.4 nm particle size, 0.41 polydispersity index, −0.62 mV zeta potential, and 58.23% entrapment efficiency. The cumulative % drug release was 98.45% during 8-h period exhibiting sustained release and Korsmeyer-Peppas model release kinetics. The *ex vivo* drug permeation study across goat eye cornea exhibited apparent permeability (P_{app}) of 11.95 cm/min^{-1} and steady-state flux (J_{ss})of 19.45 mg/cm^2 min^{-1}which was >5 times higher than marketed product. The developed formulation maintained its physical characteristics and possessed all desired product quality attributes in the ideal range. **Conclusion:** The developed loteprednol loaded spanlastic nanocarriers demonstrated all the quality parameters very close to the software predicted values and also exhibited enhanced transcorneal drug permeation which would lead to higher ocular drug bioavailability and need of less frequent drug administration in effective management of ocular inflammation.

Key words: Factorial design, loteprednol, nano-carriers, ocular inflammation, optimization, spanlastic, transcorneal permeation

INTRODUCTION

Conventional ocular formulations encounter various challenges in delivering the drugs to the different segments of eye because of its peculiar anatomy and various ocular barriers which makes it a challenging task for drug delivery scientists to develop the effective topical ocular drug delivery systems. Innovative methods for the non-invasive administration of effective drugs are becoming more popular to ensure patient compliance for eye illnesses.[1] The most commonly used ocular dosage form is topical eye drops which accounts for almost 90% of the currently prescribed ocular medicines.[2] Topical eye drop formulations are non-invasive, rapidly acting, and patient-compliant. A rapid first order absorption into the corneal and conjunctival tissues occur after eye drop solutions are applied in the cul-de-sac.[3] It still faces anatomical and physiological barriers such as the applied drops are quickly washed off due to lacrimal drainage due to which it gets short absorption time of 2–3 min. This

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leads to a very low bioavailability of 3–5%.[4] The human cornea is highly impermeable which leads to precorneal enzymatic drug breakdown and inefficient drug absorption and thus requires need of frequent drug administration in order to achieve the desired therapeutic effect resulting into low patient compliance.[5]

The nanovesicular based drug delivery systems such as liposomes, niosomes, micelles, and nano emulsions have been extensively investigated for enhancing the penetration and bioavailability of drugs across the ocular (corneal) surface.[6] Spanlastic vesicles are the modified/advanced version of niosomes, that is, Span (non-ionic surfactant) based vesicles which can penetrate across the biological membranes due to their elastic/deformable properties.[7,8] The schematic representation of structure of spanlastic vesicle is shown in Figure 1. Drug-loaded spanlastic was developed by Kakkar and Kaur in 2011. They are novel non-ionic surfactant-based nanocarriers for drug delivery to anterior and posterior segment of eye.[9] These spanlastic nanocarriers are consists of Span 60 as a bilayer forming agent and Tween80 as an edge activator which destabilizes the membrane and makes it extremely deformable.[10] They can encapsulate both hydrophilic and hydrophobic drugs. Hydrophilic drugs are encapsulated in the aqueous medium and hydrophobic drugs are encapsulated in the bilayers.[11,12]

The first-generation of elastic vesicles including phospholipids and edge activators are called transferosomes which was developed in 1992 by Cevc and Blume.[13] The edge activators which increase the membrane flexibility of nanocarriers are surfactants such as Tween 60 and Tween 80 or bile salts such as sodium cholate and sodium deoxycholate. The elastic vesicles can deform their original shape up and squeeze down to 10 times of their diameter and pass-through tight junctions of tissues and deliver drug to the target site.[14] The second-generation of elastic vesicles made up of non-ionic surfactant was first described by Van den berg in 1999.[15]

There are various inflammatory eye diseases that affect the ocular surface, for example, conjunctiva, cornea, sclera, uvea, retina, optic nerve and several other regions of the eye. Post-operative ocular inflammation can cause complications

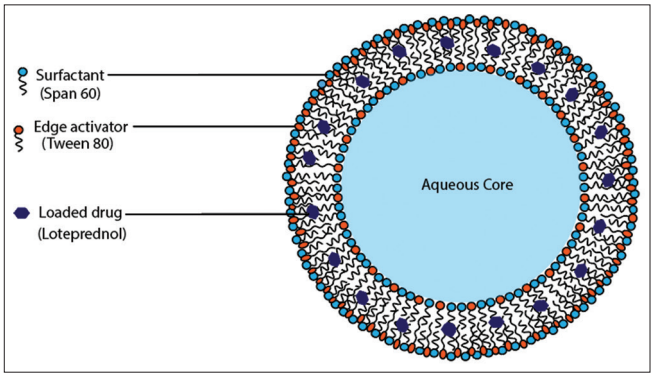

Figure 1: Schematic representation of spanlastic nanocarrier

such as macular cystoid edema and is frequently treated with steroidal medication. It has recently been discovered that some conditions thought to be non-inflammatory (like age-related macular degeneration and macular edema) are also dependent on inflammatory mediators.[16] Diseases which include inflammation of eye such as keratitis sicca, fibrosis, retinal degeneration, and septicemia infections can cause permanent or temporary vision loss if it is not treated properly. Corticosteroids are mainly used for the treatment of the diseases of eye which includes an inflammatory component.[17]

Loteprednol is a mild corticosteroid widely prescribed for the treatment of ocular inflammation. Unlike the other topically administered corticosteroids, the loteprednol have minimal intraocular pressure elevating effect as compared to prednisolone acetate or dexamethasone with long-term use. Loteprednol inhibits the inflammation by blocking the arachidonic acid by producing phospholipase A_2 leading to suppression of the prostaglandins, leukotrienes, and other inflammatory mediators.[18]

The present research paper describes the formulation optimization of loteprednol loaded spanlastic nanocarriers using 2^3 factorial design by selecting some critical formulation components as independent variables and the critical product quality attributes as response variables. The optimized formulation was further characterized and evaluated for desired *in vitro* and *ex vivo* performance parameters.

MATERIALS AND METHODS

Materials

The loteprednol drug sample was a kind gift from Piramal Healthcare Ltd. (Pithampur, India). Span 60 and Tween 80 was purchased from Sigma-Aldrich® (India). Analytical-grade chemicals and solvents were used in all experimental work described in this paper.

Preparation of spanlastic nanocarriers

The loteprednol loaded spanlastic nanocarriers were prepared by ethanol injection method as described by Kakkar and Kaur[8] and have also been diagrammatically shown in Figure 2. Span 60 and loteprednol were accurately weighed and dissolved in 10 mL of absolute ethanol and injected into pre-heated (60°C) aqueous phase containing Tween 80 with continuous stirring for 60 min at 500 rpm on magnetic stirrer (Remi®MLH 5). The resultant nanovesicles were sonicated for 5 min in bath sonicator (Citizen®, India) for the size reduction and prevention of aggregation of nanovesicles.

Formulation optimization of loteprednol loaded spanlastic nanocarriers

Optimization of drug loaded spanlastic nanocarrier formulation was done by Design Expert® software (Stat-Ease, USA) using 2^3 factorial design with three factors and two levels. The three independent variables A, B, and C wereconsidered to be the amount of Span 60, Tween 80, and loteprednol, respectively, with their high and low level as shown in Table 1. The response variables selected were particle size (R1), polydispersity index (R2), zeta potential (R3), entrapment efficiency (R4), and cumulative% drug release at 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, and 8h (R5-R11), respectively.[19,20]

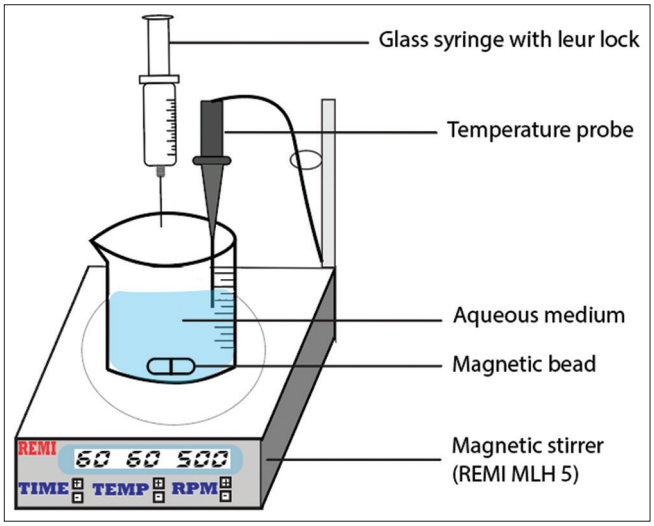

Figure 2: Schematic representation of ethanol injection method

Table 1: Selected independent and response variables

Independent variables	Unit	Low level	High level
A: Span 60	mg	300	500
B: Tween 80	mg	75	125
C: Loteprednol	mg	60	80
Response variables			**Criteria**
R1: Particle Size (nm)			Minimize
R2: PDI			Minimize
R3: Zeta potential (mV)			Maximize
R4: Entrapment Efficiency (%)			Maximize
R5: Cumulative % drug release at 0.5 h			Maximize
R6: Cumulative % drug release at 1 h			Maximize
R7: Cumulative % drug release at 2 h			Maximize
R8: Cumulative % drug release at 3 h			Maximize
R9: Cumulative % drug release at 4 h			Maximize
R10: Cumulative % drug release at 6 h			Maximize
R11: Cumulative % drug release at 8 h			Maximize

PDI: Polydispersity index

Statistical fitting of response variable data

The software suggested 13 optimization trial batches (F1-F13) were prepared and were also evaluated for each response variable. The observed response results [Table 2] were entered in Design-Expert® software and were processed for statistical fitting into a suitable model. ANOVA was used to suggest the best-fit model following statistical validation. The software proposed that the linear model equation was being followed in case of all response variables.[21] On the statistical analysis of experimental data, the software generated the regression equations which indicates how the independent variables affected the experimental response variables.[22] The positive sign before the factor coefficients indicated the favorable (increasing) impact and negative sign indicated the opposing (decreasing) impact on the response variables. The representative equation constructed from a 2^n factorial design is as below.

$$Y = B_0+B_1X_1+B_2X_2+B_3X_3+B_{12}X_1X_2$$
$$+B_{13}X_1X_3+B_{23}X_2X_3+B_{123}X_1X_2X_3$$

Where Y is the measured response, X_i is the level of the i^{th} factor, B_1, B_{12}, B_{123}. represent coefficients computed from the responses of the formulations in the design. B_0represents the intercept.[23]

Analysis of independent and response variables

The relationship between independent and response variables was studied by generating 3D response surface graphs for each response variable and impact of independent variables on each response variable was also studied. Based on the observations recorded, the software was given target of the desired response variable to be minimum particle size and polydispersity index, whereas, maximum zeta potential, entrapment efficiency %, and cumulative % drug release to predict the optimized composition of loteprednol loaded spanlastic nanocarriers. The software predicted an optimized formulation composition having highest desirability value, considering the set constraint (minimum to maximum value) for each response variable.[24]

Characterization of spanlastic nanocarriers

Particle size, polydispersity index, and zeta potential

The particle size, polydispersity index, and zeta potential measurement of prepared spanlastic nanocarriers was done by NanoPartica SZ-100 particle size analyser (Horiba®, Japan) using the dynamic light scattering (DLS) technique. For measurement of zeta potential, the motion of loteprednol-loaded spanlastic nanocarriers under the influence of electric field was studied. The nanocarriers were diluted appropriately with HPLC grade water before measurements and all the measurements were taken at 90° angle of DLS.[23]

Table 2: The 2^3 factorial design formulation of loteprednol loaded spanlastic nano-carrier and their measured responses

Batch code	Independent variable			Response variable										
	A: Span 60 (mg)	B: Tween 80 (mg)	C: Drug (mg)	R1: Particle Size (nm)	R2: PDI	R3: Zeta potential (mV)	R4: Entrapment efficiency (%)	R5: % CDR at 0.5 h (%)	R6: % CDR at 1 h (%)	R7: % CDR at 2 h (%)	R8: % CDR at 3 h (%)	R9: % CDR at 4 h (%)	R10: % CDR at6 h (%)	R11: % CDR at 8 h (%)
F1	400	100	70	334.25	0.92	−0.4	75.52	32.79	50.58	52.63	56.46	58.18	63.24	64.49
F2	500	125	60	335.46	0.85	−0.7	71.81	30.63	41.86	52.41	54.84	56.33	61.31	62.28
F3	500	125	80	353.54	0.94	−0.5	70.84	28.46	58.59	73.95	77.02	79.44	83.96	84.85
F4	300	125	60	348.81	0.42	−0.3	73.88	26.11	43.73	48.85	52.22	55.91	58.38	60.96
F5	500	75	80	270.76	0.63	−0.2	62.41	33.51	55.65	64.31	65.76	66.42	72.44	77.25
F6	500	75	60	136.52	0.32	−0.6	58.21	38.21	65.88	72.61	73.73	84.29	85.64	86.51
F7	300	125	80	182.63	0.33	−0.5	56.29	40.55	68.28	74.11	76.36	89.23	90.96	92.89
F8	300	75	80	162.64	0.12	−0.2	50.57	36.94	61.51	78.17	80.74	83.32	86.89	87.81
F9	300	75	60	153.58	0.45	−0.3	52.45	33.51	53.67	64.26	67.58	68.2	70.74	72.61
F10	400	100	70	232.35	0.44	−0.3	65.65	33.19	55.75	64.12	65.64	66.42	72.46	78.36
F11	400	100	70	238.67	0.45	−0.5	66.62	34.59	55.64	64.32	65.49	66.41	72.44	77.56
F12	400	100	70	235.45	0.57	−0.4	63.24	30.56	56.65	65.31	65.76	65.41	72.8	77.27
F13	400	100	70	252.57	0.54	−0.6	62.86	32.99	54.23	63.73	65.49	65.02	71.84	76.55

*CDR=Cumulative % drug release

Entrapment efficiency

The drug-loaded spanlastic vesicles were separated from free drug using a size-exclusion gel chromatography method. A Sephadex-G25 column was prepared by swelling Sephadex-G25 beads in phosphate buffer saline and washing with milli-Q water. The column was then packed completely and the vesicles were poured into the column and centrifuged at 500 rpm for 30 min. The separated vesicles were collected and lysed using absolute ethanol and analyzed on UV-spectrophotometer at 245 nm for estimation of drug content.[25] The entrapment efficiency was calculated by the formula given below:

$$EE\% = \frac{(Total\ amount\ of\ drug\ added - Amount\ of\ free\ drug)}{Total\ amount\ of\ drug\ added} \times 100$$

In vitro drug release

The *in vitro* drug release study of drug loaded spanlastic vesicles was performed by dialysis membrane bag method[5] as schematically shown in Figure 3. Dialysis membrane of 12000–14000 Da molecular weight cut-off (Himedia®) was treated and formed into the bag by closing at one end. Developed spanlastic nanocarrier formulation (1 mL) was filled into dialysis bag and closed in another end also. This bag was dipped into 200 mL of freshly prepared PBS (7.4 pH) release media and stirred at 500 rpm and 37°C using hot plate magnetic stirrer. A 5 ml sample of release media was withdrawn at 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, and 8 h time interval.[11,26]

Preparation of optimized formulation

The software predicted optimized formulation of loteprednol loaded spanlastic nanocarriers was prepared according to composition suggested by the software and evaluated for each response variable. To validate the optimization study, the software predicted response values were compared to experimentally observed values of optimized final formulation batch. The drug release data (experimentally observed) were compared to predicted values and were plotted in a linear regression graph to examine their correlation.[5]

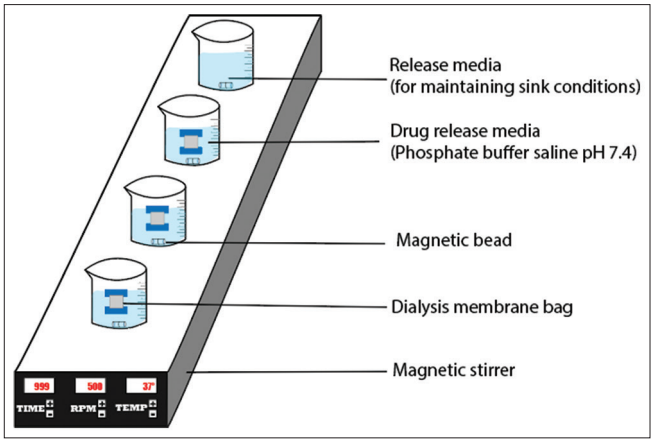

Figure 3: Schematic representation of *in vitro* drug release study

Evaluation of optimized formulation

Microscopy

The shape, size uniformity, and morphological properties of loteprednol-loaded spanlastic nanocarriers was studied on the oil immersion polarizing microscope (Leica®, Switzerland). The physical dispersion attributes and aggregation of the nanovesicles was also optically observed and evaluated.

pH and osmolality

The pH affects the ophthalmic formulations performance, stability, convenience, and safety. Normal tears have the pH range of 6.5–7.6,[27] therefore, ocular formulations having pH in this range does not cause irritation. The human eyes can tolerate the eye drops with osmolality between of 250 and 350 mOsmol/kg.[11] The pH of developed formulation was analyzed by digital pH meter (CyberScan®).The freezing point osmometer (Advanced Instruments®, USA) was used to determine the osmolality of developed formulation.

Drug content

The drug-loaded nanovesicles were mixed with absolute ethanol and vigorously stirred on vortex mixture for lysis of the vesicles and the resultant solution was estimated for drug content. The absorbance was measured at 245 nm in UV spectrophotometer (Shimadzu® 1700, Japan), and the drug content was calculated.[28]

Deformability index

The elasticity of prepared loteprednol-loaded spanlastic nanocarriers was determined using extrusion method. The spanlastic nanocarriers were extruded through polycarbonate membrane of 200 nm pore size (Whatman®) The deformability index (elasticity) was calculated by the difference between size of nanocarriers before and after extrusion using this formula: -

$$\boldsymbol{D} = \frac{J}{T\left[\frac{R_v}{R_p}\right]^2}$$

Where, D is the deformability index (μL/min), J is amount of extrude (ml), T is time of extrusion (min), R_v is the pore size of the prepared spanlastic nanocarriers after extrusion (nm) and R_p is the pore size of the barrier (nm).[11]

Differential scanning calorimetry

Thermal analysis of the compounds can be accurately performed by differential scanning calorimetry, which is

a fast and reliable method for determination of thermal properties and interaction of various compounds such as drugs and excipients.[29] The DSC analysis of loteprednol, Span 60, its physical mixture, and freeze-dried formulation was performed on PerkinElmer® 6000 DSC analyser.The samples (3–3.5 mg) were scanned between 50 to 300°C at the heating rate of 20°C/min and obtained thermo grams were evaluated for any significant differences or the development of any new peaks.

Drug release kinetics study

To predict the rate and extent of drug release from a developed formulation, the drug release kinetics is generally investigated. The *in vitro* drug release data of the optimized loteprednol-loaded spanlastic nanocarrier formulation were plotted in various drug release kinetics models, that is, zero order, first order, Higuchi, and Korsmeyer-Peppas. The R^2 values of each equation were calculated and the best-fitting drug release kinetic model was selected based on the comparison of correlation (R^2) values of different models.

Ex vivo transcorneal drug permeation

The transcorneal drug permeation study of optimized loteprednol-loaded spanlastic nanocarrier formulation was done using goat cornea on the modified Franz diffusion cells (Permegear®Inc., USA).The freshly excised goat eyeballs were acquired from the nearby slaughter house and stored in phosphate buffer saline solution (pH 7.4) at 4°C. The corneas were removed gently and thoroughly cleaned before mounting on modified Franz diffusion cells (12 mm in diameter, 8 mL in volume, and 28 mm in spherical joint).The jacketed receptor chambers of diffusion cells were filled with simulated tear fluid (pH 7.4) and maintained at 37 ± 0.5°C by water bath (Thermo fisher®, USA). One ml of loteprednol-loaded spanlastic nanocarrier formulation was added on to corneal membrane in the donor chamber and covered with a glass cover slip. On regular intervals, 1 mL sample from the receptor chamber was withdrawn and refilled with equal volume of fresh media. After filtration of withdrawn sample, it was analyzed using UV-Spectrophotometer (Shimadzu® 1700, Japan). The marketed product of loteprednol eye suspension was studied as control sample. The permeation parameters were calculated using below mentioned formula:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A \times 60 \times C_0}$$

$$J_{ss} = P_{app} \times C_0$$

Where, P_{app} is apparent permeability coefficient, J_{ss} is drug permeation flux, $\frac{dQ}{dt}$ is the slope of steady state portion of graph plotted between the drug's amount in receptor chamber (Q) versus time (t). A is the exposed corneal surface area (1.13 cm^2), C_0 is the drug's initial concentration taken in donor chamber, and 60 is the conversion factor from hour to minutes.

RESULTS AND DISCUSSION

Formulation design of loteprednol-loaded spanlastic nanocarriers

The loteprednol-loaded spanlastic nanocarriers were prepared by ethanol injection method using Span 60 as bilayer forming agent and Tween 80 as an edge activator. The edge activator would help in flexibility in the bilayer membrane of nanocarriers and would make it ultra-deformable.[8] Based on the pre-optimization studies, ethanol injection rate was selected as 0.5 mL/s rotation speed of aqueous phase to be 500 rpm. Span 60 is sorbitan monostearate with an HLB value of 4.7 and longer fatty acid chain length which increases the entrapment of lipophilic drugs such as loteprednol. Ethanol was evaporated during stirring for 60 min at 60°C

Optimization study of loteprednol-loaded spanlastic nanocarriers

The 2^3 factorial design was used for optimization study which suggested total 13 optimization trial batches.[24] The mean particle size, polydispersity index, zeta potential, entrapment efficiency and cumulative % drug release are essential specifications and so were selected as response variables to be achieved in desired range.The target constraints were selected as minimum particle size, minimum polydispersity index, maximum zeta potential, maximum entrapment efficiency, maximum cumulative % drug release at 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, and 8 h, respectively.[30] The 3D-response surface plots were generated for each response variable. Each response variable of the spanlastic nanocarrier formulation was evaluated along with the interactions between different independent variables used at various levels and the relationship between each independent and response variable was studied.[5,19]

To derive the relationship between different independent and response variables, the mathematical models for study interaction of factors; that is, A-Span 60, B-Tween 80, and C-drug, were analyzed for test of fit using the Design-Expert® Software.[22]

Effects on particle size

Particle size results of different formulations of spanlastic nanocarriers are given in Table 2. The particle sizes of spanlastic vesicles were found between 136.5 nm and 348.8 nm. The effect of formulation variables on the particle size (R1) is shown as a three-dimensional response surface plotin Figure 4. It confirmed that there was significant increase in particle size with increase in Span 60 and slight

increase in size on increasing Tween 80. The Design Expert® software suggested linear model for results and equation generated is given below.

Particle size = 250.93–92.09A–3.72 B+7.64C–4.44AB–6.16AC+4.19BC–5.07ABC

The positive value before a factor in the regression equation indicates that the response value increases with the factor and vice versa. The value of the correlation coefficient (R^2) was found to be 0.9857.

Effects on polydispersity index

The linear model equation was used by the optimization software to determine the relation between polydispersity index (R2) and various levels of independent variables. In different optimization trial batches (F1-F13), the polydispersity index (R2) values ranged from 0.12 to 0.94. The 3D response surface plots [Figure 5] depict the effect of drug, Span 60, and Tween 80 on the polydispersity index of formulation batches. When the amount of Span 60 and Tween 80 increases in the formulation, the polydispersity index value increased significantly; however, the amount of the drug had no significant effect on the PDI value.

The software suggested that linear regression equation explaining the relation between independent and response variable is given below:

PDI = 0.5438+0.2387A+0.0612B+0.0412AB+0.0963AC–0.031BC–0.116ABC

Effects on entrapment efficiency

Drug entrapment efficiency data of loteprednol-loaded spanlastic nanocarriers are given in Table 2 and were found between 50.57% and 75.52%. The DesignExpert® software suggested the linear model equation for this response variable. The value of correlation coefficient (R^2) was found to be 0.9817, indicating a good fit.

EE% = 63.7+9.32A–1.76B–1.32C–1.11AB–0.369AC+0.089BC–0.079ABC

The effect of formulation variables on the % entrapment efficiency (R2) is presented in Figure 6 as a three-dimensional response surface plot which showed that there was significant increase in entrapment efficiency with increase in the amount of Span 60 and slight increase when amount of Tween 80 was increased.

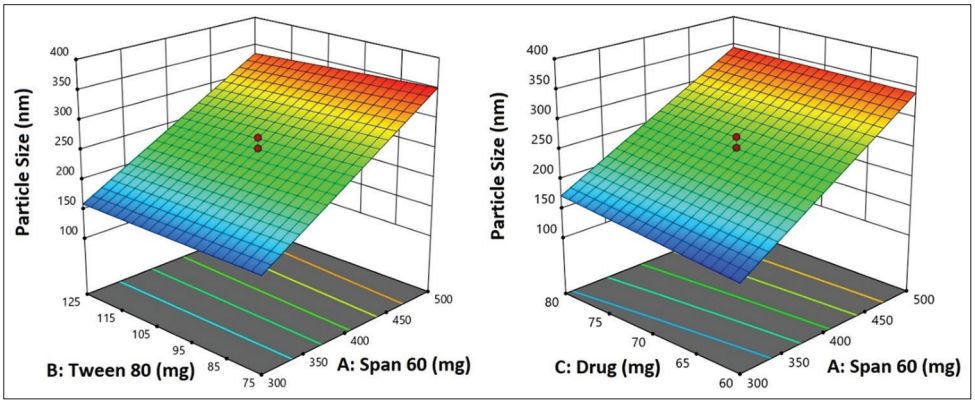

Figure 4: Response surface plots showing effect of Span 60, Tween 80, and drug on particle size of spanlastic nanovesicles

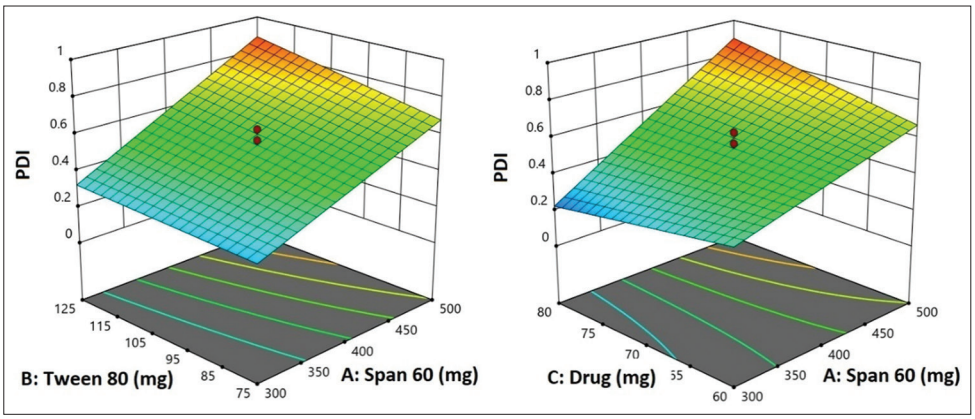

Figure 5: Response surface plots showing effect of Span 60, Tween 80, and drug on polydispersity index of spanlastic nanovesicles

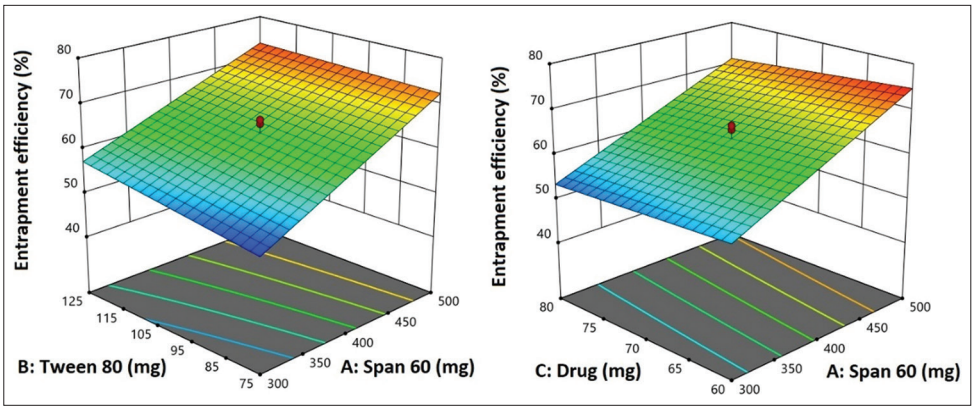

Figure 6: Response surface plots showing effect of Span 60, Tween 80, and drug on entrapment efficiency of spanlastic nanovesicles

Effects on in vitro drug release

The cumulative% drug release data of optimization trial batches are recorded in Table 2 and graphically presented in Figure 10. The cumulative % drug release at 0.5 h varied from 25.155% to 39.862% whereas, at 8h it varied from 90.65% to 98.24% for different level combinations. The effect of formulation variables on % drug release at 1 h, 4 h, and 8 h are shown as a three-dimensional response surface plots in Figures 7-9, respectively. The response plots showed that % drug release decreases with increase in Span 60 content due to increase in alkyl chain of surfactant. Amount of Tween 80 also affects the release of drug from spanlastic nanocarriers. Drug release increases with increase in amount of Tween 80 in the formulation. The linear regression equations given by software describing relationship between formulation factors and cumulative % drug release at various time intervals are as follows:

%CDR at 0.5 h	=	28.56–1.78A+0.47B–0.29C–0.92AB+0.83AC–0.33BC–0.29ABC
%CDR at 1h	=	37.47–0.92A–1.50B–0.751C–0.76AB+2.84AC–0.43BC+0.061ABC
%CDR at 2h	=	44.10–1.20A–1.12B–0.60C–1.10AB+4.36AC–0.59BC–0.58ABC
%CDR at 3h	=	53.71–2.17A–1.22B–0.26C–1.45AB+4.15AC–0.98BC–1.12ABC
%CDR at 4h	=	63.45–2.49A–2.29B–0.0965C–1.21AB+3.62AC–1.56BC–0.83ABC
%CDR at 6h	=	72.91–2.39A–2.47B–0.1172C–1.99AB+3.49AC–1.42BC–0.58ABC
%CDR at 8h	=	83.54–1.59A–2.68B+0.5393C–2.26AB+2.84AC–0.16BC–0.82ABC

Prediction of optimized formulation

Based on the response data observed and target constraints set in the experimental design, the software predicted an optimized formulation [as shown in Table 3] of loteprednol-loaded spanlastic nanovesicles using Span 60 content of 310 mg, Tween 80 content of 120 mg, and drug content of 75 mg with a high desirability value of 0.745. Figure 11 shows the 2D contour plot and 3D response plot with maximum desirability value of the optimized formulation.[31] Correlation between software predicted and practically observed data is shown in Figure 12.

Based on the desired criteria, the suggested formulation having the highest desirability of 0.745 was selected for further studies. Software predicted composition and response variables for optimized loteprednol-loaded spanlastic formulation given in Table 3.

Characterization of spanlastic nanovesicles

Particle size distribution

The particle size of the optimized formulation was found to be in nanorange (207.4 nm) and polydispersity index was 0.41 as shown in Figure 13. It is widely accepted that ophthalmic preparations with particle size smaller than 500 nm do not cause irritation and these nanosized drug carriers are more effective in bio-adhesion to cornea.[32] The PDI value of optimized formulation was found within acceptable range and confirmed that formulation is homogeneous and monodisperse.[33] The spanlastic nanocarrier formulation of loteprednol was found highly suitable for topical ocular administration.

Microscopic study of spanlastic nanovesicles

Optical examination of spanlastic nanocarriers under polarizing microscope at ×100 magnification (oil immersion) confirmed the uniform size, spherical shape, and lamellar structure of nanocarriers as shown in Figure 14. The microscopy showed no particular aggregation and non-uniformity. The spherical appearance of spanlastic nanocarriers confirmed their vesicular properties.

Entrapment efficiency

The entrapment efficiency range of optimization trial batches from minimum to maximum was found to be between 50.57% and 75.52%, respectively. The optimized formulation exhibited

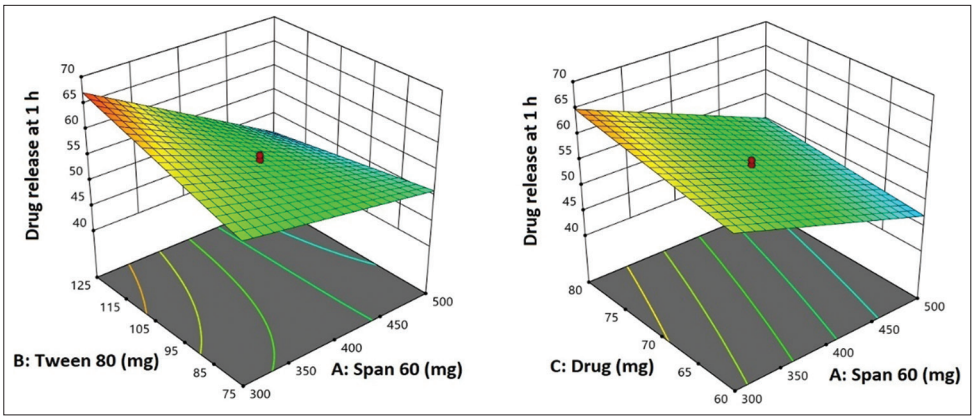

Figure 7: Response surface plots showing effect of Span 60, Tween 80, and drug on cumulative % drug release at 1 h

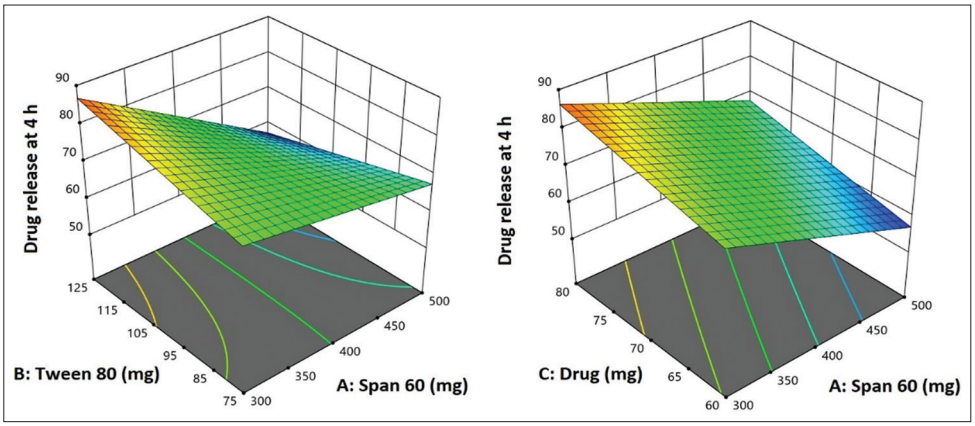

Figure 8: Response surface plots showing effect of Span 60, Tween 80, and drug on cumulative % drug release at 4 h

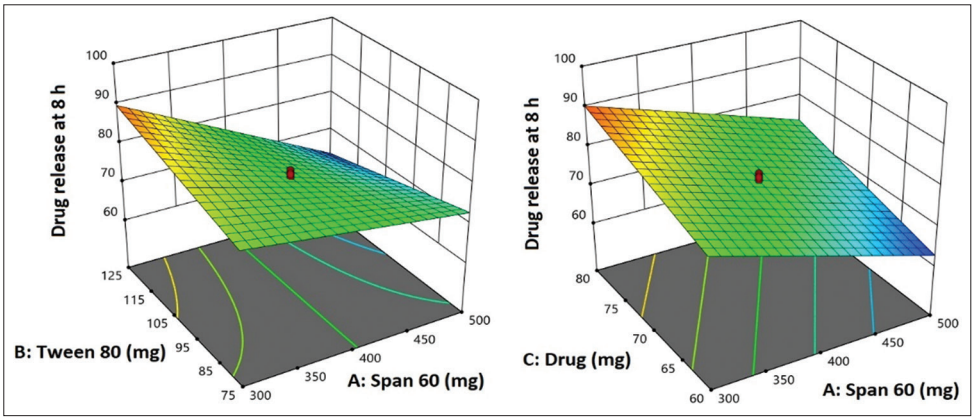

Figure 9: Response surface plots showing effect of Span 60, Tween 80, and drug on cumulative % drug release at 8 h

% entrapment efficiency of 58.23% in comparison to predicted value of 55.72% which was very close to each other.

In vitro drug release

The cumulative % drug release of optimization batches varied for 30.56–40.55% at 0.5 h and 60.96–92.89% at 8 h [Table 2] confirming the sustained release profile of spanlastic nanodrug-carriers. The optimized formulation showed 98.45% release at 8 h in comparison to the predicted value of 92.39% at 8 h. Both values have close agreement between each other. The comparison of drug release profile of marketed product and developed formulation is shown in Figure 15. It can be concluded that the developed spanlastic nanocarrier formulation showed burst release till1 h and then demonstrated sustained release characteristics.[23]

Evaluation of optimized formulation

pH and osmolarity

The stability, tolerability, and safety of ophthalmic formulations are all affected by pH of formulation since normal tears have a pH of around 7.4, ocular products with a pH of 6–8

should not irritate human eyes.[33,34] The pH of the developed spanlastic nanocarriers was found to be 7.42. Human eyes can tolerate osmolality in the range of 250–350 mOsmol/kg. The osmolality of prepared spanlastic nanocarrier formulation was found to be 314 ± 2 mOsmol/kg which comes under the tolerable range for human eye.

Drug content

The drug concentration of loteprednol-loaded spanlastic nanocarrier formulation was determined by UV spectrophotometry.[8] The drug content of the developed formulation was found to be 2.23 mg/mL which was quite suitable for therapeutic application as the marketed products are available with 0.2% w/v (2 mg/mL) strength.

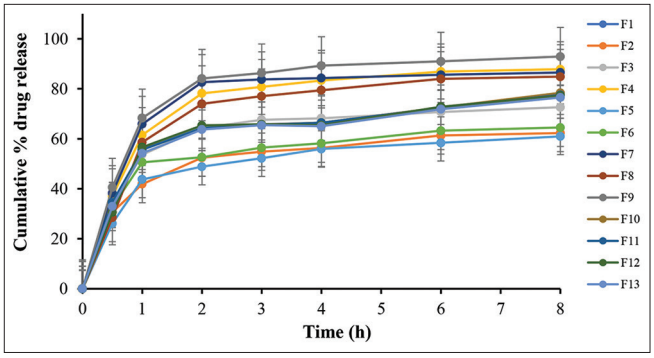

Figure 10: *In vitro* cumulative % drug release profile of optimization batches (Data plotted as mean ± S.D., *n* = 3)

Deformability index

The optimized spanlastic nanocarrier formulation was found to be ultra-deformable with deformability index of 18.64 µL/min. Formulation containing optimization quantity of Tween 80 showed maximum deformability index. Ultra-deformable nature of these elastic nanocarriers helps them to squeeze and cross through tiny pores of biological membranes. The developed spanlastic nanocarrier formulation showed only 3.33% change in average particle size after extruding it through 200 nm polycarbonate filter using a lipid extruder.[35,36]

Differential scanning calorimetry

Evaluation of physical form and drug-excipients interactions can be done by thermal study using differential scanning calorimetry. The DSC thermogram of loteprednol

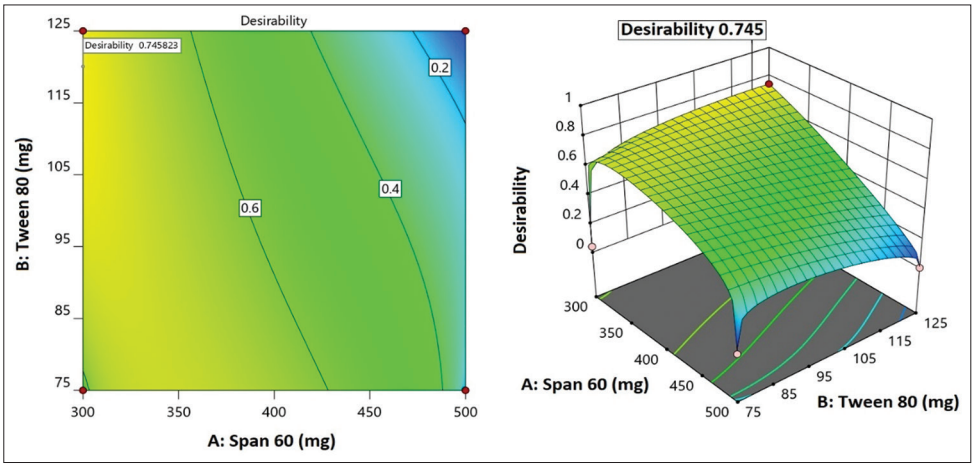

Figure 11: 2-D contour plot and 3-D response surface plot showing maximum desirability value of optimized formulation

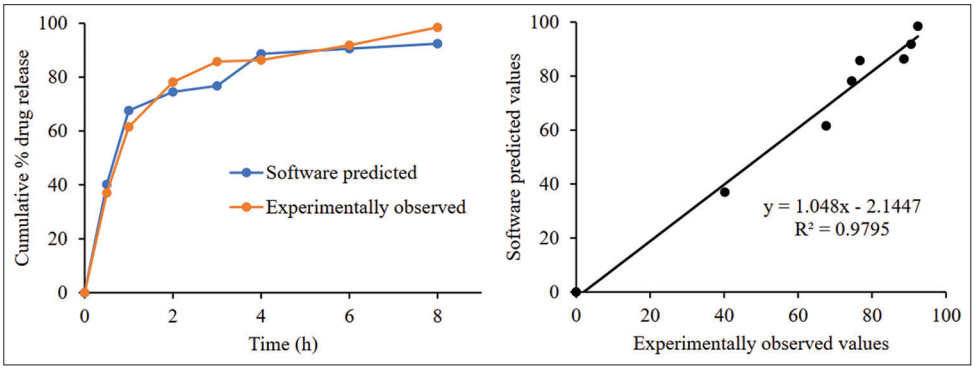

Figure 12: Correlation between software predicted and practically observed data of *in vitro* drug release profile of optimized formulation

Table 3: Predicted composition of optimized formulation

S. No.	Independent variables	Composition	
1	Span 60	310 mg	
2	Tween 80	120 mg	
3	Loteprednol	75 mg	
S. No.	**Response variables**	**Software predicted**	**Experimentally observed**
1	Particle size (nm)	180.6	207.4
2	PDI	0.30	0.41
3	Zeta potential (mV)	–0.47	–0.62
4	Entrapment efficiency (%)	55.72	58.23
5	Cumulative % drug release at 0.5 h	40.19	36.94
6	Cumulative % drug release at 1 h	67.61	61.51
7	Cumulative % drug release at 2 h	74.50	78.17
8	Cumulative % drug release at 3 h	76.73	85.74
9	Cumulative % drug release at 4 h	88.64	86.32
10	Cumulative % drug release at 6 h	90.56	91.81
11	Cumulative % drug release at 8 h	92.39	98.45

PDI: Polydispersity index

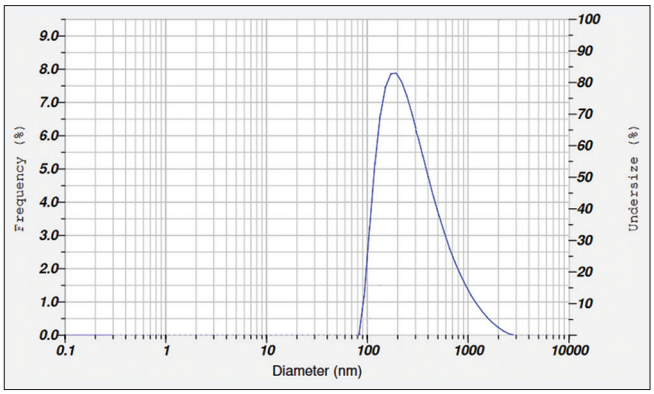

Figure 13: Particle size distribution of loteprednol-loaded spanlastic nanocarriers

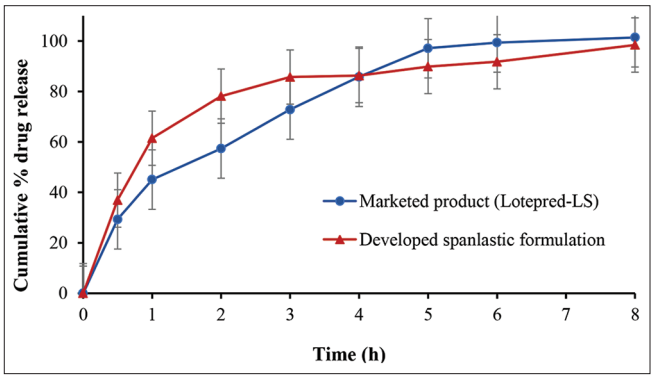

Figure 15: Cumulative % drug release of developed spanlastic formulation versus marketed product (Data presented as mean ± S.D., *n* = 3)

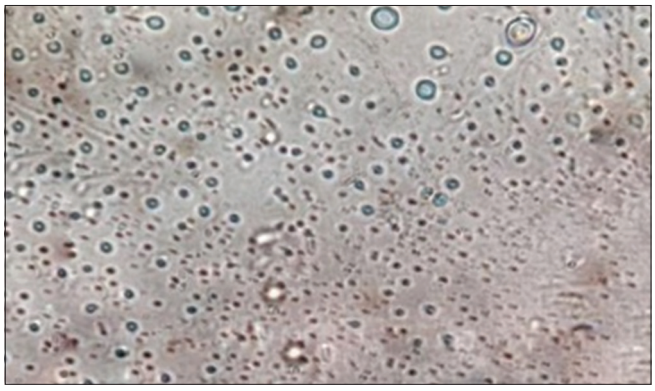

Figure 14: Microscopic view of optimized formulation of spanlastic nanocarriers

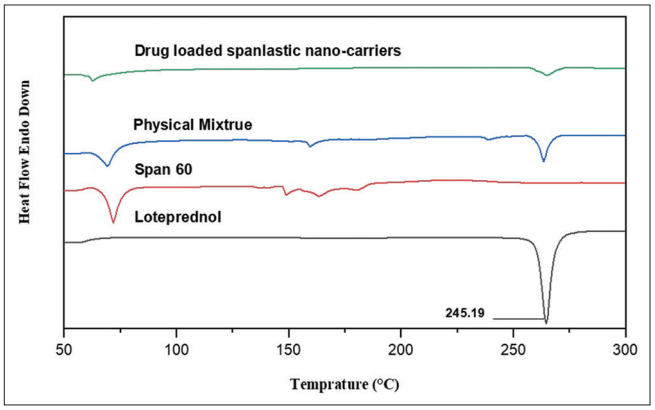

Figure 16: DSC of drug-loaded spanlastic nanocarriers, Span 60, loteprednol, and physical mixture of loteprednol and Span 60

showed a sharp endothermic peak at 245.19°C, Span 60 showed at 75.24°C, and the physical mixture showed a weak peak at 72.16°C [Figure 16].The drug-loaded spanlastic nanocarrier exhibited no significant peaks which suggested that the drug loteprednol is entrapped in spanlastic nanocarriers.[8,29]

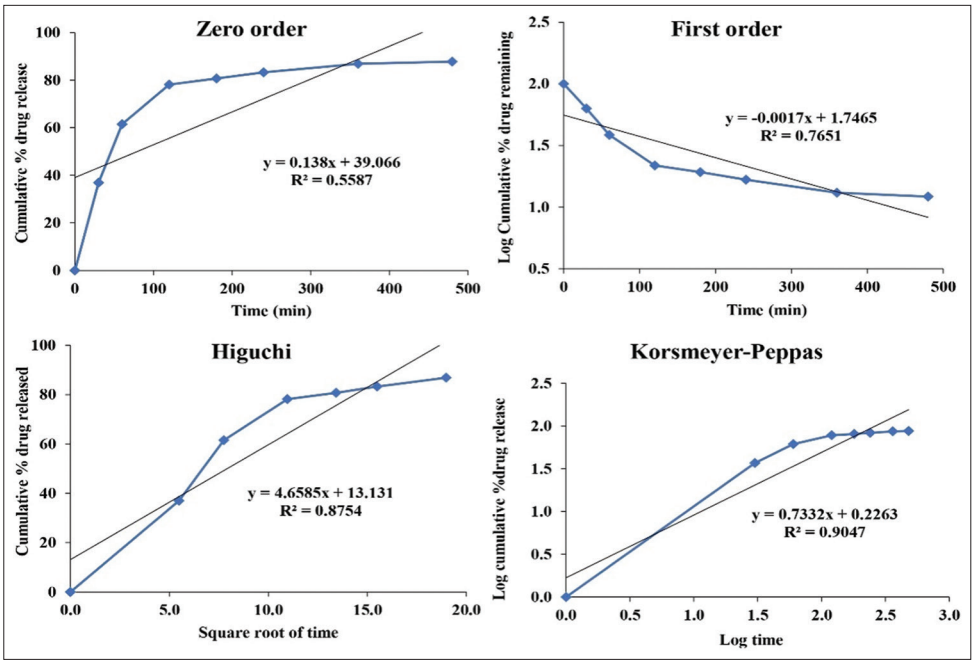

Figure 17: Drug release kinetic model plots of optimized formulation

Table 4: Drug release kinetics model fitting of optimized formulation

S. No.	Kinetic models	Equations	K	R^2
1	Zeroorder	Q_0-Q_t=k_0t	1.38×10^{-1}	0.5587
2	First order	log$Q$=logQ_0-kt/2.303	-1.7×10^{-3}	0.7651
3	Higuchi	Q_0-Q_t=$kt^{1/2}$	4.65×10^{0}	0.8754
4	Korsmeyer-Peppas	log (Q_0-Q_t)=logk-nlogt	7.33×10^{-3}	0.9047

*Q_0: Initial drug amount, Q_t: Remaining drug amount, K_0: Rate constant, t: time

Drug release kinetics study

The *in vitro* drug release data of optimized formulation of loteprednol-loaded spanlastic nanocarriers were fitted into zero-order, first-order, Korsmeyer-Peppas, and Higuchi kinetic models and the model plots are shown in Figure 17. To determine the drug release kinetics, the best fit was confirmed by the highest correlation coefficient (R^2) value. The R^2 value of different kinetic models were found to be 0.9047 (Korsmeyer-Peppas), 0.8754 (Higuchi), 0.7651 (first order), and 0.5587 (zero order). It was concluded that the prepared optimized loteprednol-loaded spanlastic nanocarrier formulation followed Koresmeyer-peppas drug release kinetic model since it had the highest correlation coefficient (R2) value [as shown in Table 4].

Ex vivo transcorneal drug permeation studies

The *ex vivo* transcorneal permeation study was performed for 6-h duration. The developed spanlastic nanocarrier formulation and marketed product demonstrated an apparent permeability coefficient (P_{app}) value of 11.95 cm/min^{-1} and 2.12 cm/min^{-1}, respectively, and steady-state flux (J_{ss}) of 19.45 mg/cm^2 min^{-1} and 3.44 mg/cm^2 min^{-1}, respectively. The developed formulation exhibited marked increase in drug permeation profile (>5 fold higher) in comparison to commercially available conventional eye suspension. Developed loteprednol-loaded spanlastic nanocarrier formulation was far superior to the available suspension of loteprednol.[37]

CONCLUSION

The formulation designing and optimization of loteprednol-loaded spanlastic nanocarriers were well accomplished by 2^3 factorial design. The developed formulation exhibited all the desired response variables such as nanorange particle size, low polydispersity index, and high entrapment efficiency. Nano-size (207.4 nm) of formulation is highly appropriate for strong bio-adhesion and more surface area available for interaction with the cornea in ocular drug delivery. The developed loteprednol-loaded spanlastic nanocarriers exhibited sustained drug release profile and followed Korsmeyer-Peppas release kinetic model. The *ex vivo* transcorneal drug permeation exhibited >5 fold enhanced drug permeation across cornea in comparison to marketed product. Hence, the developed spanlastic nanocarriers of loteprednol have potential to improve ocular bioavailability

of drug. On the basis of all these findings, it can be concluded that the spanlastic nanocarrier based topical ocular drug delivery can be an advantageous approach for effective treatment of ocular inflammation with better patient compliance.

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