Quality-by-Design-Assisted Development of Dexamethasone Nanosuspension-Loaded Thermosensitive in Situ Gel for Ocular Drug Delivery

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ABSTRACT

Background: Dexamethasone exhibits poor aqueous solubility and limited ocular bioavailability, restricting its therapeutic efficacy in ocular inflammatory conditions such as conjunctivitis, uveitis, and post-surgical inflammation. The present study aimed to develop a dexamethasone nanosuspension-loaded thermosensitive in situ gel to overcome these limitations.

Research Design and Methods: Dexamethasone nanosuspensions were prepared by nanoprecipitation using an Eudragit RS 100:RL 100 polymer system with polyvinyl alcohol (PVA) as stabilizer and optimized across five batches (DNS1–DNS5). The optimized nanosuspension (DNS3) was incorporated into a thermosensitive in situ gel composed of Poloxamer 407 and Xanthan Gum, selected following comparative evaluation of pH-triggered, ion-activated, and thermosensitive gelling systems. A 3^2 full factorial design (32 FFD) was applied to optimize Poloxamer 407 (X1: 20–24%) and Xanthan Gum (X2: 0.10–0.30%) concentrations against gelation temperature (Y1), viscosity (Y2), and in vitro drug release (Y3). The optimized formulation (DMSG9) was evaluated for physicochemical properties, in vitro drug release and kinetics, in vitro dialysis membrane permeation, anti-inflammatory activity (protein denaturation inhibition), and six-month stability.

Results: DNS3 exhibited particle size 218.1 ± 1.25 nm, PDI 0.121 ± 0.02, zeta potential 24.2 ± 1.12 mV, and entrapment efficiency 87.13 ± 1.12%. DMSG9 (Poloxamer 407 24%, Xanthan Gum 0.30%) showed gelation temperature 29.5 ± 0.17°C, viscosity 4790 ± 5 cP, pH 7.2 ± 0.01, and drug content 99.42 ± 0.01%. Sustained cumulative drug release of 72.65% was achieved over 8 h, following Fickian diffusion (Korsmeyer–Peppas $R^2 = 0.990$, $n = 0.131$). In vitro dialysis membrane permeation confirmed superior drug penetration with the combined nanosuspension-loaded in situ gel. The optimized formulation demonstrated highest anti-inflammatory activity (78.65 ± 1.31% inhibition of protein denaturation). Six-month stability studies at both ambient (25 ± 2°C/60 ± 5% RH) and accelerated conditions (40 ± 2°C/75 ± 5% RH) confirmed acceptable physicochemical stability.

Conclusions: The dexamethasone nanosuspension-loaded thermosensitive in situ gel is a promising ophthalmic drug delivery system offering improved ocular residence time, sustained drug release, and enhanced anti-inflammatory efficacy for management of ocular inflammatory disorders.

Keywords: Dexamethasone; nanosuspension; thermosensitive in situ gel; Poloxamer 407; ocular drug delivery; Quality by Design; 3^2 factorial design

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INTRODUCTION

Ocular inflammatory conditions including conjunctivitis, keratitis, uveitis, and post-surgical inflammation represent major causes of morbidity worldwide. These conditions are characterised by release of pro-inflammatory mediators that, if inadequately treated, can lead to pain, redness, oedema, and vision impairment. Corticosteroids remain the mainstay of therapy because of their potent anti-inflammatory and immunosuppressive effects. Dexamethasone, a fluorinated glucocorticoid with high receptor affinity and potent anti-inflammatory activity, is one of the most widely used agents in ophthalmic practice.1,2

Despite proven efficacy, topical ocular delivery of dexamethasone faces formidable physiological barriers. Tear turnover, blinking reflex, nasolacrimal drainage, and corneal epithelial tight junctions collectively reduce drug contact time and absorption. Less than 5% of a topically instilled ophthalmic dose typically reaches intraocular tissues, necessitating high dosing frequency and contributing to local and systemic adverse effects.3,4 Moreover, dexamethasone's poor aqueous solubility precludes effective formulation as a simple aqueous solution, resulting in incomplete drug dissolution, reduced corneal penetration, and subtherapeutic efficacy.5

Nanosuspensions — colloidal dispersions of pure drug particles stabilised by surfactants or polymers, typically below 1000 nm — have emerged as a powerful strategy for poorly soluble ophthalmic drugs. By reducing particle size to the nanoscale, the Noyes–Whitney and Ostwald–Freundlich equations predict significant increases in surface area, saturation solubility, and dissolution velocity, translating into improved bioavailability.6,7 Nanosuspensions have also demonstrated superior corneal penetration and therapeutic efficiency compared with conventional formulations.8

Even with improved dissolution, rapid precorneal drainage remains a challenge for nanosuspensions. In situ gelling systems address this limitation by transforming from a low-viscosity sol to a gel in response to physiological stimuli — temperature, pH, or ionic strength — upon instillation, thereby prolonging precorneal residence and reducing drug loss.9,10 Among available stimuli-responsive platforms, thermosensitive systems based on Poloxamer 407 undergo sol-to-gel transition at ocular surface temperature (~34–35°C) and offer the additional advantage of rapid gelation without dependence on tear film composition.11,12

Integrating nanosuspension technology with in situ gelation creates a synergistic delivery system: nanosized drug particles enhance dissolution and corneal penetration, while the gel matrix prolongs ocular residence and sustains drug release.15,16 Several studies have confirmed that nanosuspension-loaded in situ gels outperform conventional eye drops by maintaining therapeutic concentrations over extended periods.17,18

Conventional one-factor-at-a-time optimisation is inefficient for multi-variable systems. Quality by Design (QbD), as mandated by ICH Q8(R2), provides a systematic, statistically robust framework for formulation development.24,25 Factorial design allows simultaneous evaluation of multiple independent variables and their interactions, reducing experimental burden while yielding polynomial predictive models.19-22 The present study therefore applies a 3^2 full factorial design to optimise a dexamethasone nanosuspension-loaded thermosensitive in situ gel, and comprehensively characterises the optimised formulation for physicochemical properties, drug release kinetics, membrane permeability, anti-inflammatory activity, and stability.

MATERIALS AND METHODS

Materials

Dexamethasone was received as a gift sample from Lupin Pvt. Ltd, Pune, India. Poloxamer 407 and Poloxamer 188 were procured from Evonik Lab Pvt. Ltd, Mumbai, India. Eudragit RS 100 and RL 100 were sourced commercially. Carbopol 934P, sodium alginate, Xanthan Gum, hydroxypropyl methylcellulose (HPMC K4M, HPMC K15M), and polyvinyl alcohol (PVA) were purchased from Sigma Aldrich, Mumbai, India. Gellan gum and all other reagents were of analytical grade. Simulated tear fluid was prepared as described by Gilbard et al.

Preparation of Dexamethasone Nanosuspensions

Nanosuspensions (DNS1–DNS5) were prepared by the nanoprecipitation technique. Dexamethasone (50 mg) and Eudragit RS 100:RL 100 copolymers at varying ratios (1:1 to 1:5) were co-dissolved in 10 mL of acetone–methanol (3:1, v/v) with the aid of a probe sonicator. This organic phase was injected dropwise (syringe, 26-gauge; rate 0.5 mL/min) into 100 mL of aqueous PVA solution (0.5% or 1.0% w/v, pH 6.2) under magnetic stirring at 900 rpm for 2 h. Residual organic solvents were removed by air-drying; the resulting suspension was probe-sonicated to reduce particle size further. The optimised formulation (DNS3) was lyophilised using mannitol (5% w/v) as

cryoprotectant. Batch compositions are presented in Table 1.

Characterization of Nanosuspensions

Each batch was evaluated for particle size, polydispersity index (PDI), and zeta potential by dynamic light scattering (Zetasizer Nano ZS, Malvern Panalytical). Entrapment efficiency (EE%) was determined spectrophotometrically (λmax of dexamethasone) after separation of free drug by centrifugation. In vitro drug release was assessed over 8 h using the dialysis bag diffusion method in phosphate buffer saline (PBS, pH 7.4, 37 ± 0.5°C). All measurements were performed in triplicate and reported as mean ± SD.

Preliminary Optimization of In Situ Gelling Approaches

Prior to final formulation development, six preliminary batches representing pH-triggered (DNSG-P1, P2), ion-activated (DNSG-I1, I2), and thermosensitive (DNSG-T1, T2) systems were prepared and screened based on pH, gelation time, viscosity, and drug content. Gelation temperature was determined by the test-tube inversion method; gelation time by mixing with simulated tear fluid at 34 ± 1°C. The thermosensitive system combining Poloxamer 407 (18%) and Xanthan Gum (0.3%) (DNSG-T2) demonstrated the fastest gelation, highest viscosity, and optimal physiological pH, and was selected as the platform for further optimisation (Table 3).

Optimization Using 3^2 Full Factorial Design

A 3^2 full factorial design (Design Expert software v11.0, Stat-Ease Inc.) was applied with nine experimental runs. Independent variables were X1: Poloxamer 407 concentration (20, 22, 24%) and X2: Xanthan Gum concentration (0.10, 0.20, 0.30%). Dependent responses were Y1 (gelation temperature, °C), Y2 (viscosity, cP), and Y3 (in vitro drug release at 8 h, %). Polynomial quadratic models of the form: $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_1X_2 + \beta_4X_1^2 + \beta_5X_2^2$ were generated by multiple linear regression analysis (MLRA). Statistical significance was assessed by ANOVA ($p < 0.05$), and three-dimensional response surface plots were generated to visualise factor interactions.

Preparation of Nanosuspension-Loaded In Situ Gelling System

The lyophilised DNS3 nanosuspension equivalent to the target dexamethasone dose was reconstituted and incorporated into Poloxamer 407 and Xanthan Gum solutions prepared in cold PBS (4°C) with stirring until homogeneous. The thermosensitive gel was formed upon warming to physiological temperature. Nine batches (DMSG1–DMSG9) were prepared according to the factorial design layout (Table 5).

Characterization of In Situ Gelling Formulations

Each batch was characterised for: (a) pH (calibrated digital pH meter, triplicate at 25 ± 2°C); (b) drug content (UV-visible spectrophotometry after extraction in methanol, filtered through 0.45 µm membrane, triplicate); (c) gelation temperature (test-tube inversion in thermostatically controlled water bath); (d) gelation time (mixing with simulated tear fluid at 34 ± 1°C, stopwatch); (e) viscosity (Brookfield rotating viscometer, 100 rpm, 25°C and 34°C, triplicate); (f) gelling capacity (visual grading: + to ++++ upon contact with simulated tear fluid); (g) mucoadhesive strength (modified balance method using freshly excised goat corneal tissue, reported as dyne/cm²); (h) surface tension (Du Noüy ring method, digital tensiometer, mN/m).

In Vitro Drug Release and Release Kinetics

Drug release was studied using the dialysis bag diffusion method (USP Type II apparatus, 50 rpm, PBS pH 7.4, 37 ± 0.5°C, 500 mL). Samples were collected at 1, 2, 3, 4, 5, 6, 7, and 8 h; replaced with equal volumes of fresh medium to maintain sink conditions; and analysed spectrophotometrically. Cumulative drug release (%) was calculated from a pre-validated calibration curve. Release data were fitted to zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas models. The best-fit model was identified by highest correlation coefficient (R^2); the release exponent (n) from the Korsmeyer–Peppas model was used to characterise the release mechanism ($n \leq 0.45$: Fickian diffusion; $0.45 < n \leq 0.89$: non-Fickian/anomalous).

In Vitro Dialysis Membrane Permeation

Permeation profiles of dexamethasone nanosuspension, dexamethasone in situ gel, and dexamethasone nanosuspension-loaded in situ gel were compared using a Franz diffusion cell with a pre-hydrated dialysis membrane (MW cut-off 12–14 kDa). Receptor compartment contained PBS (pH 7.4, 37 ± 0.5°C, 200 mL). Samples (1 mL) were withdrawn at defined intervals and replaced with fresh PBS. Cumulative percent drug permeated was calculated against a standard curve.

Anti-inflammatory Activity (Protein Denaturation Inhibition Assay)

In vitro anti-inflammatory activity was assessed by the heat-induced albumin denaturation method. Reaction mixtures (2.0 mL test formulation + 0.2 mL fresh egg albumin + 2.8 mL PBS, pH 6.4) were incubated at 37 ± 2°C for 15 min, then heated at 70°C for 5 min to induce protein denaturation. After cooling, absorbance was measured at 242 nm (UV-visible spectrophotometer). Percentage inhibition of protein denaturation was calculated as: % Inhibition = [(A_control − A_test) / A_control] × 100. Four formulations were compared: marketed dexamethasone eye drops, nanosuspension (DNS3), in situ gel (DMSG9 without nanosuspension), and the optimised nanosuspension-loaded in situ gel (DMSG9).

Stability Studies

Per ICH Q1A(R2) guidelines, stability of the optimised DMSG9 formulation was evaluated for six months under ambient conditions (25 ± 2°C/60 ± 5% RH) and accelerated conditions (40 ± 2°C/75 ± 5% RH) in amber glass vials. Parameters assessed at 0, 1, 2, 3, and 6 months: appearance (visual), pH, drug content, gelation time, and viscosity.

RESULTS AND DISCUSSION

Preliminary Optimization of In Situ Gelling Approaches

Table 3 presents the evaluation data for the six preliminary in situ gel systems. All formulations fell within the acceptable ophthalmic pH range (6.2–7.0 ± 0.03), indicating minimal potential for ocular irritation. Among the gelling approaches, pH-triggered systems (DNSG-P1, P2) demonstrated relatively slower gelation (28–35 s) and lower viscosities (2255–2478 cP), suggesting limited precorneal retention. Ion-activated formulations (DNSG-I1, I2) improved both parameters; the gellan gum-based DNSG-I2 was notably superior to sodium alginate-based DNSG-I1.

The thermosensitive systems exhibited the most favourable properties. DNSG-T2 (Poloxamer 407 18% + Xanthan Gum 0.3%) outperformed DNSG-T1 in every parameter: fastest gelation time (8 ± 1 s), highest viscosity (4228 ± 18 cP), physiologically optimal pH (7.0 ± 0.03), and highest drug content (99.4 ± 0.3%). The synergistic rheological effect of Xanthan Gum on Poloxamer 407 networks — attributed to polymer chain entanglement enhancing gel strength — has been reported previously and supports the selection of DNSG-T2 as the optimisation platform.

3² Factorial Design: Polynomial Equations and ANOVA

The 3² factorial design generated the following quadratic polynomial equations relating the coded independent variables to each response:

Y1 (Gelation Temperature) = +33.79 − 0.88A − 2.90B − 0.30AB + 0.017A² − 0.23B²

Y2 (Viscosity) = +2884.44 + 393.33A + 1003.33B + 105.00AB + 143.33A² + 343.33B²

Y3 (In Vitro Drug Release) = +88.32 − 3.37A − 9.26B − 1.06AB − 0.27A² − 2.96B²

where A = Poloxamer 407 concentration (%) and B = Xanthan Gum concentration (%). Negative coefficients for A and B in Y1 indicate that increasing either polymer concentration reduces gelation temperature, driving the transition closer to and below ambient temperature — desirable for thermosensitive ocular gels. The strong negative coefficient for B in Y3 confirms Xanthan Gum as the primary determinant of controlled drug release; higher Xanthan Gum creates a denser gel matrix that retards dexamethasone diffusion. ANOVA results (Tables 6–8) confirmed statistical significance of all three models ($p < 0.01$). R^2 values for Y1 (0.9980), Y2 (0.9914), and Y3 (0.9790) indicate excellent model fit. Adequate precision values exceeded 4.0 for all responses, confirming adequate signal-to-noise ratio. The 3D response surface plots illustrating factor interactions are presented in Figure- 1.

Table 6. ANOVA for quadratic model of Gelation Temperature (Y1)

Source	SS	df	MS	F-value	p-value	Significance
Model	55.61	5	11.12	300.30	0.0003	Significant
A – Poloxamer 407	4.68	1	4.68	126.41	0.0015	—
B – Xanthan Gum	50.46	1	50.46	1362.42	<0.0001	—
AB	0.36	1	0.36	9.72	0.0526	—
A²	5.56E−4	1	5.56E−4	0.015	0.9103	—
B²	0.11	1	0.11	2.94	0.1849	—
Residual	0.11	3	0.037	—	—	—
Cor. Total	55.72	8	—	—	—	—

Abbreviations: SS: sum of squares; df: degrees of freedom; MS: mean square. Model F-value = 300.30.

Table 7. Model fit statistics for Gelation Temperature (Y1

Std. Dev.	**0.19**	**R^2**	**0.9980**
Mean	33.64	Adjusted R^2	0.9947
C.V. (%)	0.57	Predicted R^2	0.9781
PRESS	1.22	Adeq. Precision	48.154

Abbreviations: Std. Dev.: standard deviation; C.V.: coefficient of variation; Adeq. Precision: adequate precision.

Table 8. ANOVA for quadratic model of Viscosity (Y2).

Source	**SS**	**df**	**MS**	**F-value**	**p-value**	**Significance**
Model	7.289E+06	5	1.458E+06	69.41	0.0027	Significant
A – Poloxamer 407	9.283E+05	1	9.283E+05	44.20	0.0069	—
B – Xanthan Gum	6.040E+06	1	6.040E+06	287.57	0.0004	—
AB	44100.00	1	44100.00	2.10	0.2432	—
A^2	41088.89	1	41088.89	1.96	0.2564	—
B^2	2.358E+05	1	2.358E+05	11.22	0.0440	—
Residual	63011.11	3	21003.70	—	—	—
Cor. Total	7.352E+06	8	—	—	—	—

Abbreviations: Factor A = Poloxamer 407 (%); Factor B = Xanthan Gum (%). Model F-value = 69.41.

Table 9. Model fit statistics for Viscosity (Y2).

Std. Dev.	**144.93**	**R^2**	**0.9914**
Mean	3208.89	Adjusted R^2	0.9771
C.V. (%)	4.52	Predicted R^2	0.9180
PRESS	6.026E+05	Adeq. Precision	23.606

Table 10. ANOVA for quadratic model of In Vitro Drug Release (Y3).

Source	**SS**	**df**	**MS**	**F-value**	**p-value**	**Significance**
Model	605.15	5	121.03	27.94	0.0102	Significant
A – Poloxamer 407	68.21	1	68.21	15.74	0.0286	—
B – Xanthan Gum	514.86	1	514.86	118.85	0.0017	—
AB	4.45	1	4.45	1.03	0.3854	—
A^2	0.15	1	0.15	0.034	0.8653	—
B^2	17.48	1	17.48	4.04	0.1381	—
Residual	13.00	3	4.33	—	—	—
Cor. Total	618.14	8	—	—	—	—

Abbreviations: Factor A = Poloxamer 407 (%); Factor B = Xanthan Gum (%). Model F-value = 27.94.

Table 11. Model fit statistics for In Vitro Drug Release (Y3)

Std. Dev.	**2.08**	**R^2**	**0.9790**
Mean	86.17	Adjusted R^2	0.9439

C.V. (%)	2.42	Predicted R^2	0.7676
PRESS	143.67	Adeq. Precision	14.870

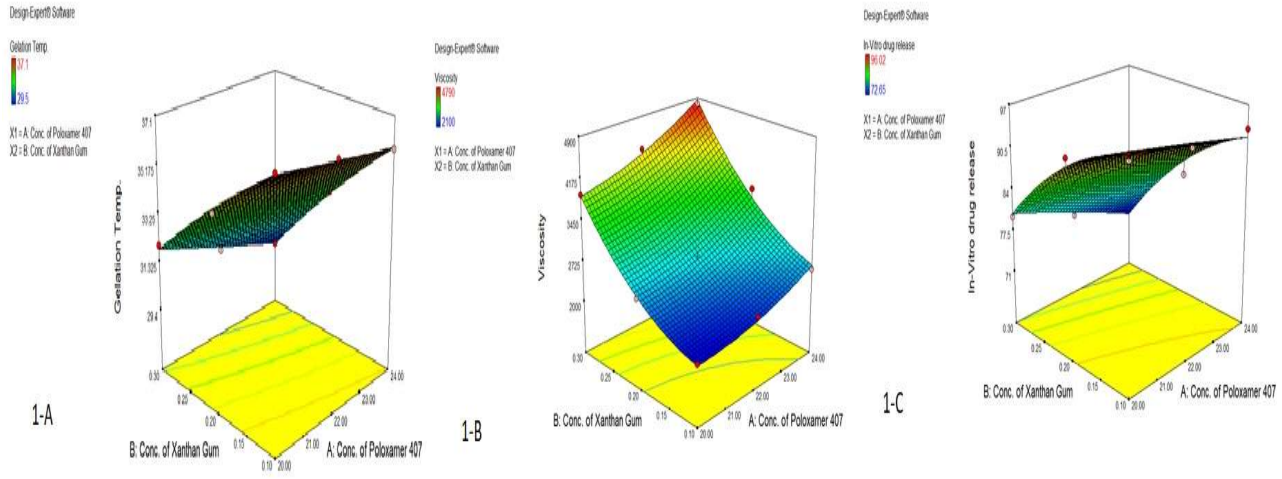


Figure 1-A: 3D response surface plot for Gelation Temperature (Y1) as a function of Poloxamer 407 (X1) and Xanthan Gum (X2) concentrations. Figure 1-B: 3D response surface plot for Viscosity (Y2) as a function of Poloxamer 407 (X1) and Xanthan Gum (X2) concentrations. Figure 1-C: 3D response surface plot for In Vitro Drug Release (Y3) as a function of Poloxamer 407 (X1) and Xanthan Gum (X2) concentrations.

3.3. Selection of Optimized Formulation

Based on the QbD-driven analysis and desirability function, DMSG9 (Poloxamer 407 24% + Xanthan Gum 0.30%) was identified as the optimised formulation. It exhibited the lowest gelation temperature (29.5°C — immediately responsive at ocular surface temperature), highest viscosity (4790 cP — excellent gel strength for prolonged retention), and acceptable sustained drug release (75.80% over 8 h). These parameters collectively satisfy the target product profile for an ocular in situ gel delivering prolonged anti-inflammatory therapy.

Physicochemical Characterization of Nanosuspensions (DNS1–DNS5)

Table 2 summarises the characterization data for DNS1–DNS5. All batches produced nanosized particles (187.9–218.1 nm). DNS3 (Eudragit RS 100:RL 100 = 1:3, PVA 0.5%) was identified as optimal: particle size 218.1 ± 1.25 nm, PDI 0.121 ± 0.02 (indicating a narrow, monodisperse distribution), zeta potential 24.2 ± 1.12 mV (adequate electrostatic stability), and EE 87.13 ± 1.12%. DNS4 and DNS5, despite the highest PVA concentration (1.0%), showed decreased zeta potential (16.4 and 20.4 mV, respectively) and increased PDI (0.456 for DNS4), attributed to PVA-mediated steric shielding reducing surface charge. Cumulative drug release from DNS3 was 97.03 ± 0.33% over 8 h, the highest among all batches. The Ostwald–Freundlich effect at nanoscale particle sizes6 is consistent with the rapid and complete dissolution observed for DNS3.

Table 1. Composition of Dexamethasone nanosuspension batches (DNS1–DNS5) prepared by nanoprecipitation

Ingredient	DNS1	DNS2	DNS3	DNS4	DNS5
Dexamethasone (mg)	50	50	50	50	50

Eudragit RS100:RL100 ratio	1:1	1:2	1:3	1:4	1:5
PVA (%w/v)	0.5	0.5	0.5	1.0	1.0
Distilled water (mL)	100	100	100	100	100

Table 2. Characterization results of Dexamethasone nanosuspension batches (n = 3, mean ± SD). *Optimized batch selected for further studies

Batch	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Cumulative Release (%)
DNS1	210.4 ± 2.11	0.234 ± 0.06	28.1 ± 1.47	83.90 ± 1.11	92.45 ± 1.58
DNS2	214.8 ± 3.24	0.217 ± 0.07	26.3 ± 1.01	85.10 ± 0.88	95.96 ± 0.99
DNS3*	**218.1 ± 1.25**	**0.121 ± 0.02**	**24.2 ± 1.12**	**87.13 ± 1.12**	**97.03 ± 0.33**
DNS4	187.9 ± 2.12	0.456 ± 0.05	16.4 ± 1.14	75.90 ± 1.47	83.12 ± 0.44
DNS5	191.4 ± 2.04	0.127 ± 0.06	20.4 ± 1.09	78.70 ± 0.97	87.14 ± 0.49

Physicochemical Characterization of In Situ Gelling Formulations (DMSG1–DMSG9)

Table 9 summarises the physicochemical profile of all nine formulations. pH values (6.0–7.2) were within the tear fluid compatible range, minimising the risk of irritation. DMSG9 achieved pH 7.2 ± 0.01, most closely matching physiological tear fluid. Drug content increased progressively from 88.78 ± 1.56% (DMSG1) to 99.42 ± 0.01% (DMSG9), reflecting the improvement in drug entrapment with optimised polymer concentrations and confirming uniform drug distribution with minimal processing loss.

Gelation time decreased from 102 ± 2 s (DMSG1) to 45 ± 2 s (DMSG9), with a parallel increase in gelling capacity from + to ++++. This enhanced gelation performance in DMSG9 is attributable to the combined effect of higher Poloxamer 407 (24%) — which promotes more rapid micellar packing and polymer network formation at ocular temperature — and Xanthan Gum (0.30%), which reinforces gel structure through chain entanglement. Rapid gelation is pharmacokinetically advantageous as it minimises drug loss through precorneal drainage before the gel matrix is established.

Mucoadhesive strength increased markedly from 21 ± 0.4 g (DMSG1) to 50 ± 0.6 g (DMSG9). Xanthan Gum's anionic character facilitates hydrogen bonding and entanglement with the negatively charged ocular mucin network, directly extending drug residence time. Surface tension decreased from 57.5 ± 0.7 (DMSG1) to 32.4 ± 0.3 dyne/cm² (DMSG9), enhancing corneal wetting and drug absorption.

Table 3. Preliminary optimization of in situ gelling approaches for Dexamethasone nanosuspension gel. *Optimized formulation selected for 3² factorial design.

Code	Method	Polymer System	pH	Gel. Time (s)	Viscosity (cP)	Drug Content (%)	Remarks
DNSG-P1	pH-Triggered	Carbopol 934P (0.3%) + HPMC K4M (0.5%)	6.2±0.03	35±2	2255±18	96.5±0.5	Moderate
DNSG-P2	pH-Triggered	Carbopol 940 (0.4%) + HPMC K15M (0.4%)	6.4±0.02	28±1	2478±22	97.2±0.4	Good
DNSG-I1	Ion-Activated	Sodium Alginate (1.0%) + HPMC (0.5%)	6.7±0.04	22±1	3257±25	97.8±0.3	Better

DNSG-I2	Ion-Activated	Gellan Gum (0.5%) + HPMC (0.4%)	6.8±0.03	15±1	3356±20	98.4±0.4	Excellent
DNSG-T1	Thermosensitive	Poloxamer 407 (18%) + Poloxamer 188 (5%)	6.9±0.02	12±1	3589±24	99.1±0.2	Rapid
DNSG-T2*	**Thermosensitive**	**Poloxamer 407 (18%) + Xanthan Gum (0.3%)**	**7.0±0.03**	**8±1**	**4228±18**	**99.4±0.3**	**Optimized**

Table 4. Factor levels for the 3² full factorial design.

Level	X1: Poloxamer 407 (%)	X2: Xanthan Gum (%)
−1 (Low)	20	0.10
0 (Mid)	22	0.20
+1 (High)	24	0.30

Abbreviations: X1: Poloxamer 407 concentration (%);
X2: Xanthan Gum concentration (%).

Table 5. 3² Factorial design layout with actual factor levels and measured responses (DMSG1–DMSG9). *Optimized batch

Batch	X1 (%)	X2 (%)	Y1: Gel. Temp. (°C)	Y2: Viscosity (cP)	Y3: Drug Release at 8 h (%)
DMSG1	20	0.10	37.1	2100	96.01
DMSG2	22	0.10	36.5	2280	92.00
DMSG3	24	0.10	35.8	2580	90.12
DMSG4	20	0.20	34.5	2600	87.89
DMSG5	22	0.20	33.9	2760	86.52
DMSG6	24	0.20	33.0	3580	83.00
DMSG7	20	0.30	32.0	3890	80.09
DMSG8	22	0.30	30.5	4300	78.52
DMSG9*	**24**	**0.30**	**29.5**	**4790**	**75.80**

Table 12. Physicochemical characterization of Dexamethasone nanosuspension-loaded in situ gelling formulations (DMSG1–DMSG9, n = 3, mean ± SD). *Optimized batch

Batch	pH	Drug Content (%)	Gel. Time (s)	Gelling Capacity	Mucoadhesive Strength (g)	Surface Tension (dyne/cm²)
DMSG1	6.0±0.02	88.78±1.56	102±2	+	21±0.4	57.5±0.7
DMSG2	6.1±0.05	93.80±2.48	94±3	++	25±0.2	54.2±0.2
DMSG3	6.2±0.08	95.12±0.12	90±1	++	28±0.3	50.0±0.1
DMSG4	6.3±0.04	96.36±0.40	87±3	++	32±0.9	48.8±0.4
DMSG5	6.4±0.03	97.84±0.22	84±1	++	35±0.7	44.6±0.8
DMSG6	6.5±0.01	97.97±0.14	71±4	++	38±0.4	41.3±0.7
DMSG7	6.6±0.02	98.05±0.10	60±2	+++	42±0.5	38.9±0.5
DMSG8	6.9±0.04	98.23±0.55	51±3	+++	47±0.1	36.4±0.2
DMSG9*	**7.2±0.01**	**99.42±0.01**	**45±2**	**++++**	**50±0.6**	**32.4±0.3**

Abbreviations: Gelling capacity grading: + (poor) to ++++ (excellent). Gelation time measured on mixing with simulated tear fluid at 34 ± 1°C.

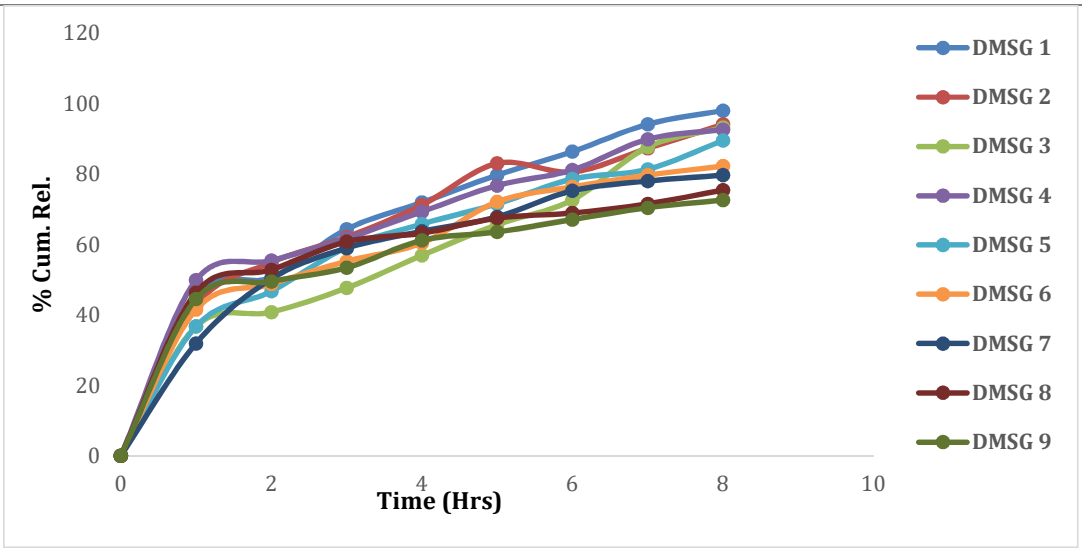
In Vitro Drug Release and Release Kinetics

Table 10 presents the cumulative drug release profiles for DMSG1–DMSG9 over 8 h. All formulations exhibited a biphasic release pattern: an initial burst phase during the first hour, followed by a sustained, controlled release phase. The initial burst likely reflects rapid diffusion of drug particles at the gel surface before the polymeric network is fully hydrated, while subsequent release is governed by diffusion through the swollen gel matrix and gradual matrix relaxation.

Drug release was inversely related to polymer concentration. DMSG1 (lowest Poloxamer 407, lowest Xanthan Gum) released 98.02% over 8 h, whereas DMSG9 (highest concentrations) released only 72.65%, confirming the role of a denser gel network in retarding dexamethasone diffusion. The practical implication is that DMSG9 would sustain therapeutic drug concentrations at the ocular surface well beyond the 8 h study period, potentially reducing dosing frequency from every 4–6 h (conventional drops) to twice daily.

Table 13. Cumulative in vitro drug release profile (%) for Dexamethasone nanosuspension-loaded in situ gels (DMSG1–DMSG9) using dialysis bag diffusion method (PBS, pH 7.4, 37 ± 0.5°C, n = 3, mean)

Time (h)	DMSG1	DMSG2	DMSG3	DMSG4	DMSG5	DMSG6	DMSG7	DMSG8	DMSG9
0	0	0	0	0	0	0	0	0	0
1	45.98	42.65	36.72	49.87	36.72	41.54	31.91	46.35	44.50
2	50.61	54.87	40.80	55.43	46.72	48.76	50.24	52.83	49.50
3	64.31	62.09	47.65	61.72	59.13	55.24	58.94	60.80	53.39
4	71.91	71.17	56.91	69.31	65.80	60.43	63.76	63.20	61.17
5	79.69	83.02	65.61	76.72	71.54	72.09	67.83	67.46	63.57
6	86.35	80.61	72.65	81.17	78.57	76.35	75.24	68.94	67.09
7	94.13	87.28	87.65	89.87	81.35	79.69	78.02	71.54	70.43
8	98.02	94.13	93.20	92.65	89.50	82.28	79.69	75.43	**72.65**



[Figure 2: Comparative in vitro dissolution profiles of Dexamethasone nanosuspension-loaded in situ gels (DMSG1–DMSG9) over 8 hrs]

Release kinetics data (Table 11) indicate that the majority of formulations best fitted the Korsmeyer–Peppas model (R^2 = 0.928–0.999). Formulations with n values between 0.844 and 0.882 (DMSG1, 2, 4, 8) followed non-Fickian (anomalous) transport, indicative of concurrent diffusion and polymer swelling contributions. Formulations DMSG3, 5, 6, 7, and 9 exhibited n values below 0.45 (range 0.131–0.442), consistent with Fickian diffusion, suggesting diffusion through the gel matrix as the rate-limiting step. DMSG9 (n = 0.131, R^2 = 0.990) demonstrated predominantly Fickian diffusion, reflecting the highly cross-linked, dense polymer network at optimum concentrations.(Table-14),(Figure -2

Table 14. In vitro drug release kinetics of Dexamethasone nanosuspension-loaded in situ gelling formulations. *Optimized batch. (Correction: DMSG4 Korsmeyer–Peppas R^2 corrected from typographic error 0.099 to 0.999.)

Batch	Zero Order R^2	First Order R^2	Higuchi R^2	Hixson–Crowell R^2	Korsmeyer–Peppas R^2	n-value	Release Mechanism
DMSG1	0.985	0.995	0.987	0.967	0.999	0.844	Non-Fickian
DMSG2	0.953	0.934	0.980	0.965	0.999	0.882	Non-Fickian
DMSG3	0.985	0.872	0.940	0.925	0.953	0.236	Fickian
DMSG4	0.994	0.946	0.980	0.976	0.999	0.849	Non-Fickian
DMSG5	0.972	0.969	0.994	0.988	0.999	0.343	Fickian
DMSG6	0.972	0.983	0.980	0.983	0.994	0.205	Fickian
DMSG7	0.904	0.977	0.965	0.960	0.928	0.442	Fickian
DMSG8	0.946	0.946	0.985	0.971	0.997	0.853	Non-Fickian
DMSG9*	0.976	0.991	0.989	0.984	0.990	0.131	**Fickian**

Abbreviations: n-value interpretation: ≤ 0.45 = Fickian diffusion; 0.45–0.89 = non-Fickian (anomalous transport).

In Vitro Dialysis Membrane Permeation

Table 12 compares the permeation profiles of dexamethasone nanosuspension, dexamethasone in situ gel, and the nanosuspension-loaded in situ gel over 8 h using Franz diffusion cells. The nanosuspension alone achieved the highest cumulative permeation (99.74 ± 0.56% at 8 h), reflecting the rapid membrane availability of nanosized drug particles with large surface area and fast dissolution rate. However, the absence of a sustained release component would result in rapid depletion from the ocular surface.

The plain in situ gel showed the slowest permeation (89.67 ± 0.98% at 8 h), demonstrating the barrier effect of the gel matrix — though the relatively small difference from the nanosuspension at 8 h may reflect the limitations of dialysis membrane permeation as a model. The nanosuspension-loaded in situ gel achieved an intermediate profile (96.86 ± 0.18% at 8 h) with a permeation rate superior to the plain gel, confirming that the nanosuspension component sustains a concentration gradient that drives diffusion through the matrix. The combination thus balances immediate drug availability (from nanosized particles) with controlled delivery (from the gel matrix), which is the intended design objective.(Table-15)

Table 15. In vitro permeation profiles of three Dexamethasone formulations through dialysis membrane using Franz diffusion cell (PBS, pH 7.4, 37 ± 0.5°C, n = 3, mean ± SD).

Time (h)	Nanosuspension (%)	In Situ Gel (%)	NS-Loaded In Situ Gel (%)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	19.52 ± 0.83	9.45 ± 0.41	14.34 ± 0.52
1	29.86 ± 1.11	12.62 ± 0.58	25.65 ± 0.74
2	45.24 ± 1.39	29.36 ± 0.85	39.48 ± 1.07
3	59.18 ± 1.21	45.27 ± 0.92	54.84 ± 1.24
4	80.46 ± 1.07	55.89 ± 1.05	67.92 ± 1.20
5	89.35 ± 0.95	64.78 ± 1.12	76.65 ± 1.41
6	95.72 ± 0.87	74.25 ± 1.18	87.46 ± 1.10
7	97.16 ± 0.70	79.03 ± 1.04	90.58 ± 0.19
8	99.74 ± 0.56	89.67 ± 0.98	96.86 ± 0.18

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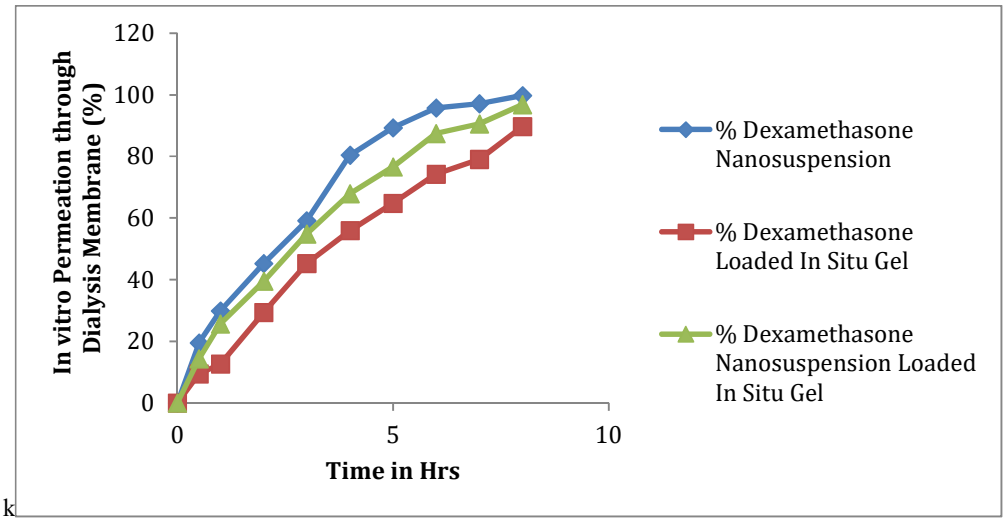


[Figure 3: In vitro permeation profiles of three Dexamethasone formulations through dialysis membrane using Franz diffusion cell]

Anti-inflammatory Activity

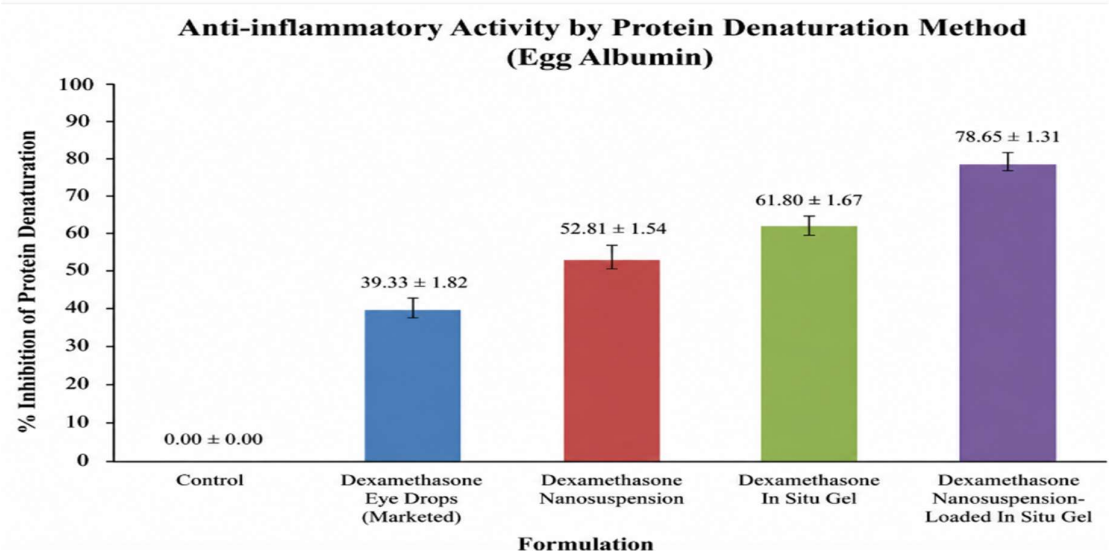
The heat-induced albumin denaturation assay results (Table 13 and Figure 6) demonstrate a clear hierarchy in anti-inflammatory efficacy. The marketed dexamethasone eye drops showed 38.45 ± 1.82% protein denaturation inhibition — the lowest among all formulations, attributable to rapid dilution and drainage from the ocular surface, limiting sustained drug–protein interactions. The nanosuspension (DNS3) significantly improved inhibition (52.81 ± 1.54%) by virtue of increased drug availability from nanosized particles. The plain in situ gel (61.80 ± 1.67%) further enhanced activity through sustained drug retention in the gel matrix.

The nanosuspension-loaded in situ gel (DMSG9) demonstrated the highest inhibition (78.65 ± 1.31%), approximately two-fold greater than the marketed product. This superior activity reflects the synergistic contribution of both technologies: nanosuspension ensures rapid drug solubilisation and availability, while the in situ gel matrix provides sustained release and prolonged drug–protein contact. The relatively low standard deviation (±1.31%) confirms excellent formulation reproducibility.(Table-16),(Figure 3).

Anti-inflammatory activity order: NS-loaded in situ gel (78.65%) > In situ gel (61.80%) > Nanosuspension (52.81%) > Marketed eye drops (38.45%)

Table 16. Anti-inflammatory activity (% inhibition of heat-induced protein denaturation) for Dexamethasone formulations (n = 3, mean ± SD).

Formulation	% Inhibition of Protein Denaturation	Anti-inflammatory Activity
Control	0.00	—
Dexamethasone Eye Drops (Marketed)	38.45 ± 1.82	Least
Dexamethasone Nanosuspension	52.81 ± 1.54	Moderate
Dexamethasone In Situ Gel	61.80 ± 1.67	Good
Dexamethasone NS-Loaded In Situ Gel*	**78.65 ± 1.31**	**Highest**



[Figure 3: Comparative anti-inflammatory activity (% protein denaturation inhibition) of Dexamethasone formulations.

Stability Studies

Tables 14 and 15 report the stability data for DMSG9 under accelerated (40 ± 2°C/75 ± 5% RH) and ambient (25 ± 2°C/60 ± 5% RH) conditions over six months. The formulation maintained visual clarity and homogeneity throughout the study period at both storage conditions, with no precipitation, phase separation, or colour change — indicating excellent physical stability.

Under accelerated conditions, pH decreased slightly from 7.2 ± 0.04 to 6.45 ± 0.05, likely owing to minor polymer chain degradation at elevated temperature. This change remained within the ocular acceptable pH range (6.0–8.0). Drug content declined modestly from 99.78 ± 0.32% to 96.45 ± 0.44% at 6 months, indicating limited dexamethasone degradation within the polymeric matrix. Gelation time decreased from 55 ± 1 s to 50 ± 1 s, and viscosity from 4795 ± 10 to 4702 ± 8 cP — small, clinically acceptable changes that did not impair gel-forming functionality.

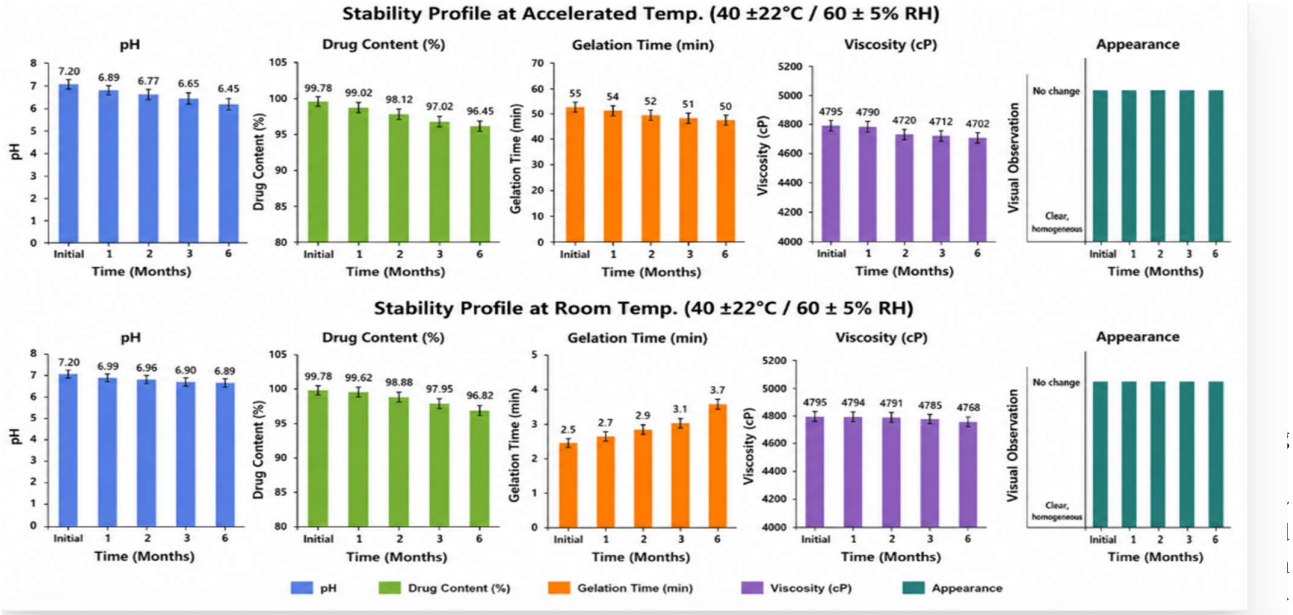
At ambient conditions, the formulation showed notably superior stability: pH changed from 7.2 ± 0.04 to 6.89 ± 0.06; drug content decreased from 99.78 ± 0.32% to 96.82 ± 0.58%; gelation time increased from 2.5 ± 0.1 to 3.7 ± 0.2 min (longer at RT reflects unaltered rapid sol-to-gel responsiveness at ocular temperature); and viscosity remained essentially unchanged (4795 to 4768 cP). These findings confirm that the formulation is chemically and physically stable for at least six months under recommended storage conditions.(Table -17-18),(Figure- 4).

Table 17. Six-month stability profile of optimized DMSG9 at accelerated conditions (40 ± 2°C / 75 ± 5% RH, ICH Q1A(R2)).

Time (Months)	Appearance	pH	Drug Content (%)	Gel. Time (s)	Viscosity (cP)
Initial	Clear, homogeneous	7.2 ± 0.04	99.78 ± 0.32	55 ± 1	4795 ± 10
1	No change	6.89 ± 0.03	99.02 ± 0.35	54 ± 2	4790 ± 8
2	No change	6.77 ± 0.04	98.12 ± 0.42	52 ± 1	4720 ± 9
3	No change	6.65 ± 0.03	97.02 ± 0.39	51 ± 2	4712 ± 7
6	No change	6.45 ± 0.05	96.45 ± 0.44	50 ± 1	4702 ± 8

Table 18. Six-month stability profile of optimized DMSG9 at ambient conditions (25 ± 2°C / 60 ± 5% RH, ICH Q1A(R2)).

Time (Months)	Appearance	pH	Drug Content (%)	Gel. Time (min)	Viscosity (cP)
Initial	Clear, homogeneous	7.2 ± 0.04	99.78 ± 0.32	2.5 ± 0.1	4795 ± 10
1	No change	6.99 ± 0.04	99.62 ± 0.41	2.7 ± 0.1	4794 ± 9
2	No change	6.96 ± 0.05	98.88 ± 0.46	2.9 ± 0.1	4791 ± 8
3	No change	6.90 ± 0.04	97.95 ± 0.52	3.1 ± 0.2	4785 ± 10
6	No change	6.89 ± 0.06	96.82 ± 0.58	3.7 ± 0.2	4768 ± 11



nanosuspension-loaded thermosensitive in situ gel for ocular drug delivery. The nanoprecipitation method yielded a stable, monodisperse nanosuspension (DNS3: 218.1 nm, PDI 0.121, EE 87.13%) that was effectively incorporated into a Poloxamer 407/Xanthan Gum thermosensitive matrix. Application of a 3^2 full factorial design provided a systematic understanding of how polymer concentrations govern gelation temperature, viscosity, and drug release, enabling rational selection of the optimised formulation (DMSG9: Poloxamer 407 24%, Xanthan Gum 0.30%).

DMSG9 demonstrated a gelation temperature (29.5°C) below the ocular surface temperature, ensuring rapid in situ gelation upon instillation; high mucoadhesive inflammatory activity was approximately two-fold superior to marketed dexamethasone eye drops and superior to either nanosuspension or in situ gel alone, confirming the synergistic advantage of the combined approach. Six-month stability data under both ambient and accelerated storage conditions confirmed acceptable physical and chemical stability.

Collectively, the developed formulation addresses three critical shortcomings of conventional ophthalmic dexamethasone preparations: poor aqueous solubility (resolved by nanosuspension technology), limited ocular residence (resolved by thermosensitive gelation and mucoadhesion), and subtherapeutic drug delivery (resolved by sustained release). The formulation shows

strong promise as an improved ophthalmic dosage form for the management of ocular inflammatory disorders. Future work should include ex vivo corneal permeation studies, ocular irritation testing, and in vivo pharmacokinetic evaluation in appropriate animal models.

Declaration of Interest

The authors report no conflicts of interest. Dexamethasone was received as a gift sample from Lupin Pvt. Ltd, Pune, India.

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