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Research Article

Development of Solid Dispersion-Based Dapagliflozin In-Situ Gel for Ocular Delivery in Dry Eye Disease

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Abstract
Rapid precorneal elimination of ocular drugs results in low bioavailability and poor therapeutic response. This limitation can be overcome through novel ophthalmic drug delivery systems. The present study aims to develop and evaluate pH-triggered in-situ gels of Dapagliflozin for prolonged corneal residence and sustained drug release in the treatment of Dry Eye Disease (DED). Formulations were prepared using Carbopol 940 as a pH-sensitive gelling agent and HPMC K4M as a viscosity enhancer. Solid dispersion of Dapagliflozin with PEG 6000 was used to enhance the drug's aqueous solubility. The prepared formulations were evaluated for parameters such as physical appearance, clarity, pH, viscosity, gelling capacity, drug content, sterility, and in-vitro drug release. The optimized formulation demonstrated desirable clarity, pH near physiological range, acceptable viscosity with shear-thinning behavior, and sustained drug release for up to 10 hours. It followed Zero-order kinetics, indicating a controlled release profile. The developed Dapagliflozin in-situ gel is a promising alternative to conventional eye drops, offering improved bioavailability, reduced dosing frequency, and enhanced patient compliance.

Keywords: Dapagliflozin, Carbopol 940, HPMC K4M, In-situ gel,Ophthalmic delivery, Solid dispersion, Sustained release

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Introduction
Ocular drug delivery continues to be one of the most difficult problems for pharmaceutical scientists. The eye's special structure prevents the medication molecules from reaching the necessary site of action.[1] Conventional techniques including solutions, suspensions and ointment have not been employed extensively due numerous limitations such increased precorneal elimination, lower drug concentration and blurred vision.[2]. Because eye drops must be installed often, low absorption leads in a shorter duration of effect. The new delivery method based on the idea of in situ gel formation offers an alternate strategy that greatly extends the drug's precorneal residence duration and bioavailability. [3]. In situ gel forming system can be described as liquids dosage form that can be delivered in a drop form and they undergo a phase transition in the ocular cul-desac to form a visco-elastic gel. In situ activated gelling systems on contact to physiological conditions will change to a gel phase [4] Gelation occurs via the cross-linking of polymer chains that can be achieved by covalent bond formation (chemical cross-linking) or non-covalent bond formation (physical cross-linking). In situ gel-forming systems on installation in the conjunctival cul-de-sac form visco-elastic gels due to conformational changes of polymers in response to the physiological environment. In the present study, ophthalmic in-situ gels of Dapagliflozin

were developed based on a pH-triggered gelation system. Dapagliflozin, a SGLT2 inhibitor, is primarily used in the management of type 2 diabetes mellitus by promoting urinary glucose excretion. Recent studies have indicated its potential in treating dry eye disease (DED) due to its anti-inflammatory and osmoprotective effects on ocular surface tissues. However, its poor aqueous solubility limits its ocular bioavailability. To overcome this, solid dispersion techniques using hydrophilic carriers such as PEG 6000 were employed to enhance its solubility. The in-situ gel system, incorporating polymers like Carbopol 940 and HPMC K4M, was formulated to convert into a gel upon contact with the tear fluid pH (~7.4), thereby prolonging ocular residence time and enabling sustained drug release. The optimized formulation aims to improve therapeutic efficacy and patient compliance in the management of dry eye disease.

Dry eye disease (DED) is a complex ocular surface syndrome marked by neuropathic discomfort, hyperosmolarity, tear film instability, and ocular surface inflammation. Chronic inflammation, specifically through the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, is implicated in the pathophysiology of DED, according to an increasing amount of evidence. [(5)]Activated by stress signals (e.g., oxidative stress, hyperosmolarity, or microbial toxins), the NLRP3 inflammasome is a cytosolic protein complex that cleaves pro-caspase-1 into active caspase-1, which in turn promotes the maturation and release of pro-inflammatory cytokines such as IL-1β and IL-18. The inflammatory cascade on the ocular surface is intensified by these cytokines, which exacerbates DED symptoms and damages epithelium and tear film integrity.

According to new research, dapagliflozin may be able to prevent the NLRP3 inflammasome from activating, which would lessen tissue damage and the release of inflammatory cytokines. [6] In ocular tissues, Dapagliflozin is hypothesized to exhibit anti-inflammatory actions by: Reducing oxidative stress and cellular hyperosmolarity, which are important activators of NLRP3, lowering levels of IL-1β and IL-18, two important mediators of ocular surface inflammation, and directly inhibiting NLRP3 inflammasome activation, potentially via SGLT2-dependent pathways.[(7-8)] However, dapagliflozin's bioavailability in traditional ocular formulations is limited due to its poor aqueous solubility. In order to improve drug solubility, the current work uses solid dispersion technology with Polyethylene Glycol 6000 as a hydrophilic carrier. When exposed to the tear fluid, the pH-sensitive in-situ gel matrix containing the tailored solid dispersion allows for site-specific medication release.

The aim of this study include the following:

i. To enhance the aqueous solubility of Dapagliflozin Propanediol Monohydrate (DPM) using the solid dispersion technique.
ii. To develop a pH-sensitive ophthalmic in-situ gel formulation containing DPM-SD for prolonged precorneal retention.
iii. To evaluate the potential of Dapagliflozin in modulating the NLRP3 inflammasome pathway, thereby reducing inflammation and improving therapeutic efficacy in Dry Eye Disease.
iv. Qbd approach used for preparation of in situ gel

Materials and Methods :Dapagliflozin propanediol monohydrate gifted from Centaur Pharmaceuticals (Mumbai) Carbopol 940, PEG 6000 ,HPMC K4M ,HPMC K15 ,Sodium Chloride ,Calcium Carbonate Potassium Chloride, Benzalkonium chloride.

Experimental work of Solid Dispersion:

Preparation of solid Dispersion

1. Weigh Dapagliflozin and PEG 6000 in a 1:5 ratio.
2. Dissolve PEG 6000 in ethanol under heating until completely dissolved.
3. Add Dapagliflozin to the hot solution and stir until a clear solution is obtained.
4. Pour the solution on a flat glass plate and allow solvent evaporation at room temperature or under vacuum.
5. Scrape off the dried film, powder it, and store in a desiccator.

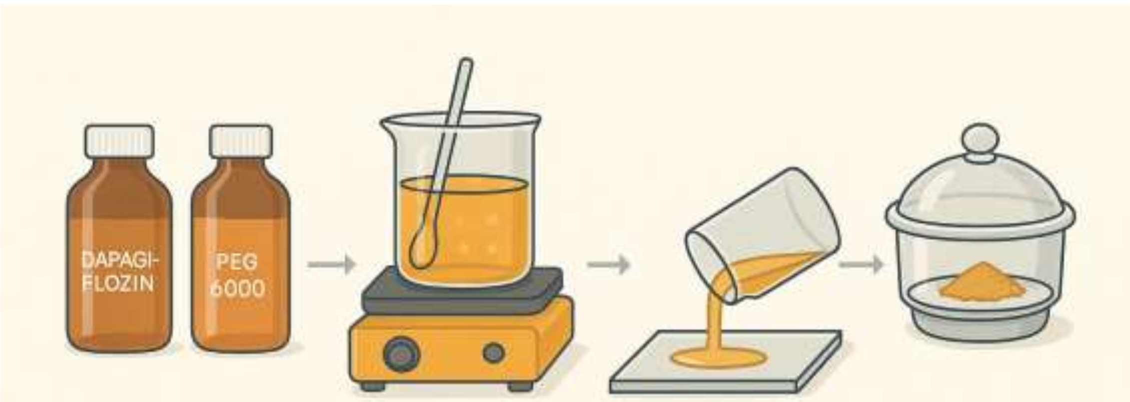


Figure 1: Preparation of Solid Dispersion

Preliminary batches of Solid Dispersion

Preliminary batches of solid dispersion were made to evaluate different carriers and drug: carrier ratio for solid dispersion that is to be incorporated in the ocular in-situ gel formulation.

1. Selection of Carrier

For the development of solid dispersion of Dapagliflozin, suitable carriers were selected based on them been used commonly in solid dispersion technology, percentage yield and dissolution in the in-situ gel formulation. Following carriers were selected for initial screening. These included:

1. PEG 6000
2. HPMC K15
3. HPMC K4

Ingredients	Trail Batches		
	T1	T2	T3
Drug	Dapagliflozin	Dapagliflozin	Dapagliflozin
Carrier	PEG 6000	HPMC K4	HPMC K15
Solvent	Ethanol	Ethanol	Ethanol

Table No.1

Based on the results obtained in initial screening one carrier was further evaluated for different drug: carrier ratios and subjected to further evaluation.

2. Evaluation of Drug Carrier Ratio

From the above-selected carrier, four different drug-to-carrier ratios were formulated and designated as trial batches T4, T5, T6, and T7, respectively. Each batch was subjected to evaluation for to assess the impact of carrier concentration on formulation performance.

Method	Drug : Carrier	Batch	Drug: carrier ratio
Solvent Evaporation	Dapagliflozin PEG 6000	T4	1:1
		T5	1:3
		T6	1:5
		T7	1:10

Table No.2

Evaluation and characterization of Solid Dispersion

FTIR Spectroscopic Analysis

FTIR (Fourier Transform Infrared) spectroscopy was employed to investigate possible interactions between Dapagliflozin, PEG 6000, and ethanol in the prepared solid dispersion. The resulting FTIR spectrum of the solid dispersion is depicted in Figure X. Characteristic absorption bands were assigned and compared with the spectra of individual components to assess compatibility and intermolecular interactions.

Percentage Yield

The percentage yield of solid dispersion formulations was calculated to evaluate the efficiency and reproducibility of the preparation process. It serves as an important parameter to determine the amount of final product obtained relative to the theoretical amount expected, based on the quantities of drug and carrier used. A high percentage yield indicates minimal material loss during processing steps such as solvent evaporation, drying, and handling, thereby confirming the feasibility and scalability of the selected method. Additionally, it provides preliminary insight into the suitability of the carrier and process parameters for further optimization.

It is calculated to determine the efficiency of the formulation process. It is expressed as the ratio of the actual weight of the final product obtained to the total theoretical weight of all the ingredients used, multiplied by 100. The formula used is:

Percentage Yield = (Practical Yield) / (Theoretical Yield) X 100

Table: Percentage Yield of Solid Dispersion Batches

Batch Code	Drug:Carrier Ratio	Theoretical Yield (g)	Practical Yield (g)	Percentage Yield (%)
T4	1:1	2.0	1.81	90.50%
T5	1:3	4.0	3.69	92.25%
T6	1:5	6.0	5.67	94.50%
T7	1:10	11.0	10.34	94.00%

Table no: 3

Dissolution in In-Situ gel

The solubility of the prepared solid dispersion within the in-situ gel base was evaluated to ensure uniform distribution and proper incorporation of the drug. This step was essential to confirm that the solid dispersion dissolves adequately in the gel matrix without phase separation or precipitation. Proper dissolution within the gel system is a prerequisite for consistent drug release, stability, and overall formulation performance. The observation helped in identifying suitable carriers and formulation conditions that allow clear and homogeneous dispersion of the drug in the in-situ gel.

Results:

FTIR Spectroscopic Analysis

3.3.1. Peak Assignment and Functional Group Analysis

The prominent peaks observed in the FTIR spectrum are summarized below, with interpretations based on literature assignments and spectral matching:

Conclusion of FTIR Analysis

FTIR results confirm that the **solid dispersion of dapagliflozin with PEG 6000** results in the formation of a **physically stable system**, with **evidence of hydrogen bonding** and **no chemical degradation**. These findings validate the use of PEG 6000 as a suitable hydrophilic carrier to enhance the solubility of dapagliflozin through physical interaction without altering its chemical structure.

Percentage Yield of Solid Dispersions

The percentage yield of solid dispersion formulations was calculated to evaluate the efficiency of the preparation process. It reflects how much of the expected product was successfully recovered after solvent evaporation, drying, and scraping.

Percentage Yield = (Practical Yield) / (Theoretical Yield) X 100

- The **highest yield (94.5%)** was observed in **Batch T6 (1:5 ratio)**, indicating efficient processing and minimal material loss.
- Batches T6 and T7 had comparably high yields, but T6 was chosen for further gel development due to optimal clarity, solubility, and drug loading

Experimental work of In-situ Gel:

Materials and methods: HPMC K4, Carbopol 940, solid dispersion of drug .

Definition of Quality Target Product Profile and Critical Quality Attributes: The QTPP is a prospective overview of the qualities of a pharmaceutical product that, when taking into account its safety and effectiveness, should ideally be attained to guarantee the intended quality. QTPP serves as the foundation for the product's design development.(19) "In this study, the Quality Target Product Profile (QTPP) was defined to produce a safe, effective, and patient-compliant ophthalmic in-situ gel formulation of Dapagliflozin intended for the treatment of Dry Eye Disease (DED), with desired attributes . A CQA is a physical, chemical, biological, or microbiological property or characteristic of an output material, including a finished drug product, which should meet the predefined requirements to ensure the desired product quality. CQAs are generally associated with the drug substance, excipients, intermediates (in-process materials), and drug products. The QTPP and CQA elements for the resveratrol nanosuspension, with targets and justifications, are defined in Table

Factor	**Target**	**Justification**
Route of administration	Ocular	Topical application to the targeted tissue
Delivery System	Solid Dispersion based in-situ gel	Increase the contact time of drug delivery system with ocular tissues
Drug Content	≥90%	To obtain treatment dose of the API
pH	7.0 – 7.4	For maximum comfort and patient compliance
Clarity	Clear	Increase the patient compliance
Gelling Capacity	Gelation immediately and remained for several or long hours	Affect the contact time of drug delivery system with ocular tissues

Table No 5: QTTP of the Product

- Critical Quality Attributes (CQA)

CQA for In-Situ Gel			
Drug product quality attributes	**Target**	**Is this a CQA?**	**Justification**
Appearance	Transparent/Translucent no particles	Yes	Ensures patient acceptability and safety. Any turbidity or particulate matter may indicate contamination or instability, which could cause ocular irritation or harm.
Tonicity	Isotonic	Yes	To prevent irritation, lacrimation, or damage to ocular tissues. Hypertonic or hypotonic formulations can cause discomfort and reflex tearing, reducing drug contact time.
Sterility	Sterile	Yes	Eyes are highly sensitive; non-sterile products can cause infections, inflammation, or serious ocular complications.
pH	Approximately pH 7.0–7.4 (close to tear fluid)	Yes	Matching physiological pH minimizes irritation and maintains patient comfort, ensuring better compliance.

Table No.6: CQA for product

Initial risk analysis and assessment

To identify all the probable high-risk factors for further study, risk assessment was conducted. The main challenge is to find out which factors and variables (inputs) will show effect on the process of formulations. QbD (ICH Q9) provides tools for methodical assessment of factors/ possible inputs for the identification of those specific causes which have significant effect on the process of formulation. Risks were analysed using the PHA. PHA consists of a table in which each item is indicated by a colour: green, yellow or red (low-, medium- or high-risk, respectively) is shown in tablet no.

Low	Broadly acceptable risk no further investigation is needed.
Medium	Risk is acceptable. Further investigation may be needed to reduce the risk.
High	Risk is unacceptable. Further investigation is needed to reduce the risk.

Table No.7: Level of risk assessment

CMA / CQA	Concentration of Gelling agent	Concentration of Viscosity enhancer	Concentration of Buffering agent
Isotonicity	Medium	Low	High
Gelling capacity	High	High	Low
Sterility	Medium	Medium	Low

Table No.8: Effect of CMA ON CQA

9.5.1 Optimization using Design of Experiment (DoE)

The formulation of Dapagliflozin ocular in-situ gel was optimized using a 2-factor, 2-level full factorial design, considering two independent variables:

- Factor A: Concentration of Gelling Agent (Carbopol)
- Factor B: Concentration of Viscosity Enhancer (HPMC K4M)

The impact of these variables was studied on the following dependent responses:

1. Drug Content (%)
2. Viscosity (cP)
3. pH

Drug-excipients compatibility studies: Fourier Transform-Infrared spectroscopic studies: Interaction between drug and polymer should be observed to check the compatibility between various ingredients of the formulation by Fourier Transform Infra Red (FTIR) spectroscopy analysis of their physical mixture by employing Potassium bromide pellet method (2 mg sample in 200 mg KBr). The spectrum of each sample was recorded over the range of 400-4000 cm1 and the resolution was 1 cm-1.Compatibility studies were performed for the pure drug Dapagliflozin Propandeiol monohydrate and of Dapagliflozin Propandeiol monohydrate along with physical mixture (PEG 6000).

Formulation Development: Total four formulations have been prepared by employing pH-triggered in-situ gelation method using different concentrations of gelling agents i.e., Carbopol 940 as pHsensitive polymer and HPMC K4 M. The composition of each and every formulation was shown in the formulation Table 1. All the four formulations contain Carbopol 940 as pH-sensitive polymer, HPMC K4M as viscosity enhancer, sodium chloride as an Isotonicity ad justifier, and Benzalkonium chloride as preservative. The method of preparation of ophthalmic in situ gels formulations are as follows:

<u>pH triggered in situ gelation method:</u>

i. Accurately weighed HPMC K4M was dispersed in preheated distilled water using a magnetic stirrer.
ii. Carbopol 940 was gradually sprinkled into the dispersion and allowed to hydrate overnight.
iii. The hydrated solution was stirred continuously using a magnetic stirrer until a homogeneous and clear solution was obtained.
iv. The solid dispersion of the drug was prepared separately by dissolving it in distilled water. This drug solution was then added slowly to the polymeric mixture with constant stirring to ensure uniform mixing.
v. Buffering agents and osmolality adjusters were added to the formulation, followed by benzalkonium chloride as a preservative.
vi. The pH of the final solution was adjusted using 0.1 N NaOH or 0.1 N HCl to match the required physiological range.
vii. The final formulation was filled into sterile amber-colored bottles and subjected to terminal sterilization.

Ingredients	Final Batches			
	F1	F2	F3	F4
SD of Dapagliflozin	1%	1%	1%	1%
Carbopol (mg)	200	200	500	500
HPMC K4M (mg)	500	200	500	200
Benzalkonium chloride	2 drops	2 drops	2 drops	2 drops
Buffering Agent	q.s	q.s	q.s	q.s
Distilled water (ml)	100	100	100	100

Table no :9

Evaluation of Ophthalmic In-Situ Gels

Physical Appearance and Clarity

The prepared ophthalmic in-situ gel formulations of Dapagliflozin were visually examined for their general appearance, color, and the presence of any suspended particulate matter. The clarity of each formulation was assessed against both black and white backgrounds using visual inspection under adequate lighting conditions to ensure optical transparency and absence of turbidity or precipitate.[9]

2. Determination of pH

The pH of the formulated Dapagliflozin ophthalmic in-situ gels was measured immediately after preparation using a calibrated digital pH meter. The pH range was targeted between 6.0 and 7.4, which closely matches the physiological pH of the tear fluid (~7.4), to minimize ocular irritation and enhance patient comfort and compatibility.[10]

3. Gelling Capacity

The gelling ability of the in-situ gel was evaluated by placing a drop of the formulation into a vial containing 2 mL of freshly prepared simulated tear fluid (STF) maintained at 37 ± 0.5 °C. The gel formation was observed visually, and the time required for gelation was recorded. An ideal formulation should form a clear, transparent, and stable gel almost immediately upon contact with tear fluid.[11]

Simulated Tear Fluid (STF) Composition (per 100 mL):

Sodium chloride – 0.670 g

Sodium bicarbonate – 0.200 g

Calcium chloride – 0.008 g

Purified water – q.s. to 100 mL

4. Rheological Studies

The rheological behavior of the Dapagliflozin ophthalmic in-situ gel formulations was evaluated to assess their suitability for ocular administration. Viscosity, an essential parameter influencing the ocular residence time and drug release profile, was measured using a Brookfield Viscometer (model-specific), both before and after gelation. Measurements were taken at room temperature.[12]

5. Drug Content Determination

To determine the drug content, 1 mL of each formulation was accurately measured and diluted to 100 mL with distilled water. From this, 1 mL was withdrawn and further diluted to 10 mL. The absorbance of the resulting solution was measured at 222nm using a UV-visible spectrophotometer. The actual drug content was calculated using a pre-established calibration curve of Dapagliflozin.[13]

6. Sterility Testing

Sterility testing was conducted on the optimized formulation to ensure compliance with ocular safety standards. The test was performed under aseptic conditions using Fluid Thioglycollate Medium (FTM) for aerobic and anaerobic bacteria and Soybean-Casein Digest Medium (SCDM) for fungi, following pharmacopeia guidelines.

The media were observed periodically for turbidity or microbial growth, indicating contamination.

7. In-vitro Drug Release Studies

The in-vitro release profile of Dapagliflozin from the ophthalmic in-situ gel formulations was evaluated using a bi-chambered Franz diffusion cell apparatus, which consists of a donor compartment and a receptor compartment.

A measured quantity of the formulated gel was placed in the donor compartment, while the receptor compartment was filled with freshly prepared Simulated Tear Fluid (STF) maintained at 37 ± 0.5 °C, mimicking ocular physiological conditions. A cellophane membrane (previously soaked in distilled water for 24 hours) was placed between the two compartments to act as a diffusion barrier.

At predetermined time intervals (e.g., 0.5, 1, 2, 3, 4, 6, and 8 hours), 2 mL samples were withdrawn from the receptor compartment and replaced with an equal volume of fresh STF to maintain sink conditions. The withdrawn samples were suitably diluted, if necessary, and analyzed at 255 nm using a UV-visible spectrophotometer, with STF serving as the blank.[14-16]

8. Stability studies : Stability studies were performed for the optimized formulation F1after subjecting it to sterilization. Sterile gel forming ophthalmic solutions were filled in glass vials and closed with a rubber stopper. The formulations were maintained at room temperature (24±1°C) for 60 days. The samples were withdrawn periodically and estimated for drug release, visual appearance.[17]

9. Isotonicity ensures that the formulation has the same osmotic pressure as biological fluids (e.g., tears, blood, or nasal secretions) to prevent irritation, discomfort, or tissue damage upon administration. The tonicity of ophthalmic in-situ gel was checked by mixing the formulation with few drops of blood and observed under microscope at 45X magnification and observe the effect of formulation on red blood cells like, swelling bursting and cremation. Finally compare the shape of formulation mixed blood cell with RBC alone. [18]

RESULTS AND DISCUSSION

1. Drug–Polymer Compatibility Studies (FTIR)

The FTIR spectra of the physical mixture of Dapagliflozin with polymers such as PEG 6000, Carbopol 940, and HPMC K4M were compared with that of pure drug. The major characteristic peaks corresponding to functional groups of Dapagliflozin were retained with minimal or no shifts in the mixture, indicating the absence of chemical interaction between drug and polymers. Some additional peaks appeared, likely due to the presence of excipients or minor impurities, but no significant peak disappearance or shifting was observed. This confirms that the interaction is physical (entrapment) rather than chemical.

2. Evaluation of In-Situ Ophthalmic Gels

All prepared in-situ gel formulations of Dapagliflozin were evaluated for key pharmaceutical parameters including physical appearance, clarity, pH, gelling capacity, drug content, viscosity (rheology), sterility, and in-vitro drug diffusion.

2.1 Physical Appearance and Clarity

All formulations (F1–F4) were visually inspected against both black and white backgrounds and were found to be clear, transparent, and free from particulate matter. These characteristics indicate good solubilization of excipients and stable dispersion of the drug. No turbidity or precipitation was observed upon storage.

Sr. No	Formulation code	Clarity of Solution	Clarity of Gel
1	1	Transparent	Clear

2	F2	Transparent	Clear
3	F3	Transparent	Clear
4	F4	Transparent	Clear

Table no: 10

2.2 Evaluation of Gelling Capacity

The gelling capacity was assessed by adding one drop of the formulation into a vial containing simulated tear fluid (STF) at pH 7.4. All formulations demonstrated instantaneous gelation and retained their gel integrity for an extended period. Formulations containing Carbopol 940 showed superior gelling strength compared to those with lower polymer concentrations. These results are depicted in Figure 1 and summarized in Table 2.

Sr. No	Formulation code	Gelation Capacity
1	F1	+++
2	F2	++
3	F3	++
4	F4	+

Table no: 11

"+" gelation immediate but dissolves rapidly (1-2 h), "++" gelation immediate and remain for few h (3-4 h), "+++" stiff gel is formed immediately and remain for extended period (more than 6-8 h).

2.3 Rheological Studies

Rheological behavior was assessed using a Brookfield Viscometer. All formulations showed pseudoplastic (shear-thinning) behavior — viscosity decreased with increasing shear rate. At room temperature (25°C), the formulations remained low-viscosity liquids, while at physiological temperature (37 ± 0.5°C) and tear pH (7.4), they underwent rapid gelation, resulting in a significant increase in viscosity. This behavior ensures prolonged ocular residence time, essential for sustained drug release. Viscosity values before and after gelation are presented in Table 4.

Sr. No	Formulation code	Before Gelation Viscosity(cP at 25°C)	After Gelation Viscosity(cP at 37°C)
1	F1	530	1860
2	F2	356	1440
3	F3	111	949.2
4	F4	88.2	1200

Table no: 12

2.4 PH:

A calibrated digital pH meter was used to measure the pH of the formulations. The pH of optimized formulation was found to be 6.8 before gelation, and after undergoing sol-to-gel transition, it increased to 7.4.This pH value correlates closely to the pH range (7.2 to 7.4) of the human eye, indicating that the formulation is appropriate for the ocular surface.

Sr. No	Formulation code	pH of formulation
1	F1	6.8
2	F2	5.8
3	F3	6.2
4	F4	6.5

Table no: 13

2.5 Drug Content Determination

Drug content uniformity was evaluated to ensure proper drug loading and distribution within the ophthalmic in-situ gel formulations. An accurately measured volume (1 mL) of each formulation was diluted with distilled water in a stepwise manner (1:100 and then 1:10 dilution) to obtain a suitable concentration for spectrophotometric analysis.

The absorbance of each diluted sample was measured at 255 nm using a UV-visible spectrophotometer, and the concentration was calculated using the calibration curve equation:

Drug Content (%) = (Label Claim Actual Drug Content)×100=(10.008.93)×100=89.3%

2.6 Sterility Testing

All the prepared Dapagliflozin ophthalmic in-situ gel formulations (F1–F4) were subjected to sterility testing in accordance with USP guidelines to ensure the formulations were free from microbial contamination.

The test was conducted using two media:

Fluid Thioglycolate Medium (FTM) for detection of anaerobic and aerobic bacteria

Soybean Casein Digest Medium (SCDM) for detection of fungi and aerobic bacteria

A volume of 5 mL from each sterile formulation was aseptically transferred into 100 mL of each medium and incubated at: 30–35°C for FTM 20–25°C for SCDM for a period of 14 days.

The formulations were visually inspected for any signs of turbidity, color change, or microbial growth throughout the incubation period.

Results:

All formulations showed no signs of bacterial or fungal growth in either of the media, indicating that they were sterile and free from microbial contamination.

2.7 In-Vitro Drug Release Studies

The in-vitro drug release behavior of the Dapagliflozin-loaded in-situ gels was studied using a Franz diffusion cell equipped with a donor and receptor compartment. A previously soaked egg membrane was mounted between the compartments to simulate the corneal barrier.

The donor compartment contained the gel formulation.

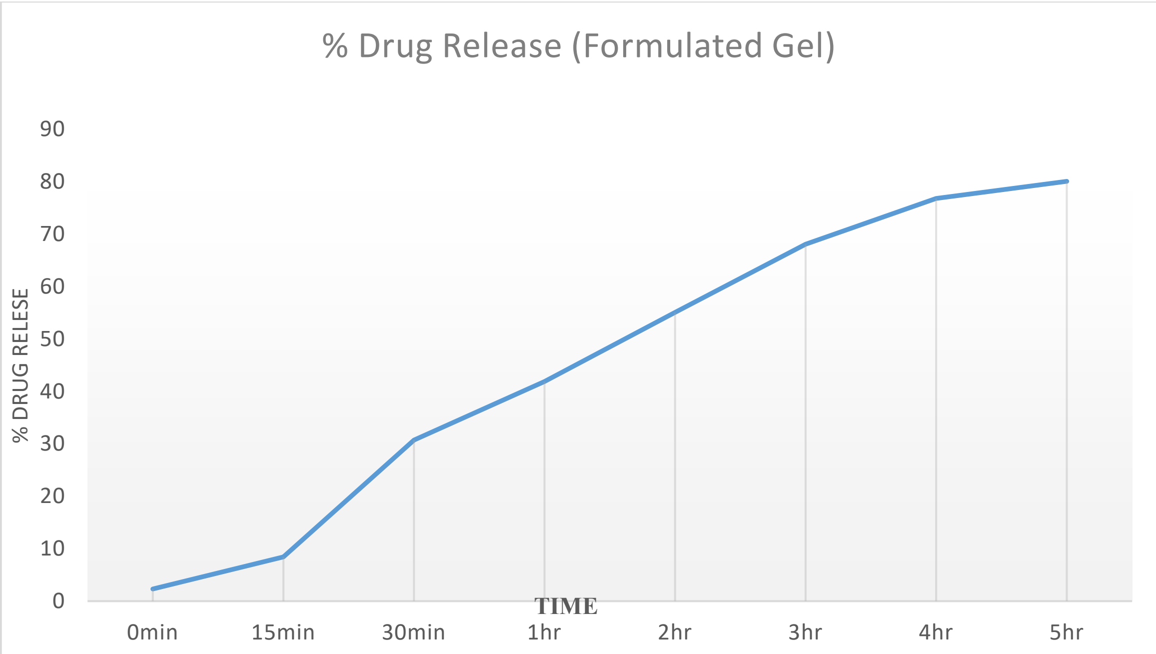
The receptor compartment was filled with simulated tear fluid (STF) of pH 7.4, maintained at 37 ± 0.5°C, and stirred continuously with a magnetic stirrer.

At predetermined time intervals up to 5 hours, 2 mL of the receptor medium was withdrawn and replaced with fresh STF to maintain sink conditions. The withdrawn samples were diluted appropriately and analyzed by UV-visible spectrophotometry at 222 nm to determine the amount of drug released.

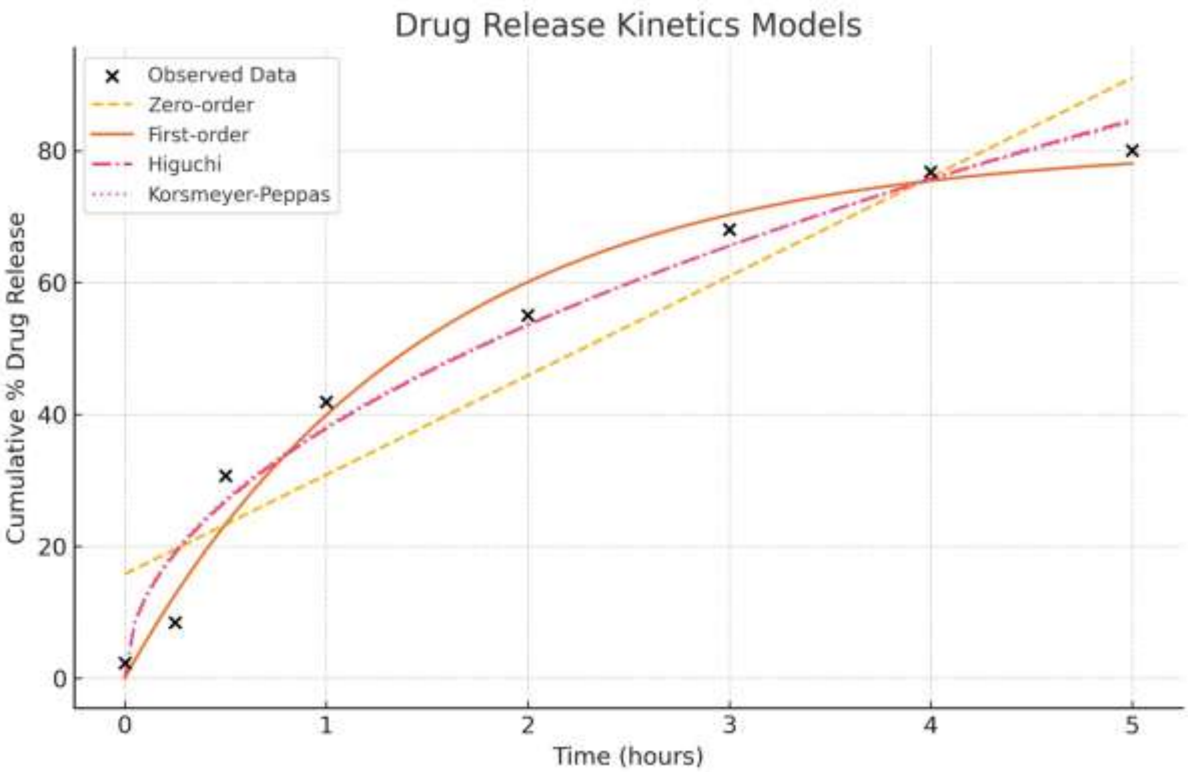
Among all formulations, F1 containing Carbopol 940 and a higher concentration of HPMC K4M exhibited sustained and controlled drug release over 5 hours. The combination of Carbopol 940 as a gelling agent and HPMC K4M as a release retarder was effective in prolonging the release and improving ocular residence time.

Time	% Drug Release (Formulated Gel)
0min	2.32
15min	8.43
30min	30.72
1hr	41.87
2hr	55.05
3hr	68.08
4hr	76.8
5hr	80.05

Table no: 14



Graph no :1



Graph no: 2

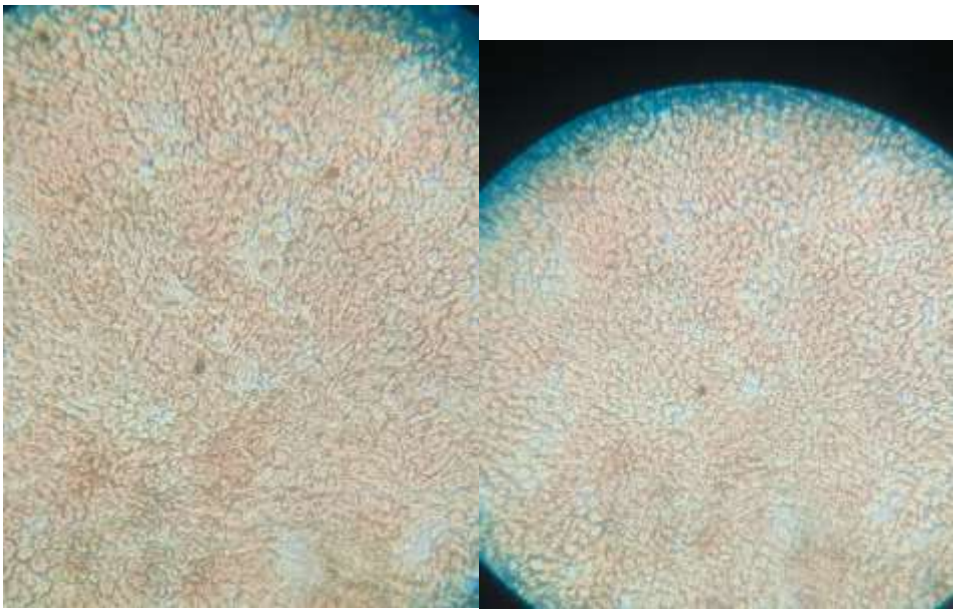
2.8 Stability studies: Stability studies were carried out for 2 months for the characterization of optimized insitu gel.

Sr. No	Formulation code	Days	Visual Appearance	Drug Content (%)
1	F1	0	Transparent & Clear	80
		30	Transparent & Clear	78
		45	Transparent & Clear	75
		60	Transparent & Clear	74

Table no: 16

Isotonicity

The isotonicity testing of Dapagiflozin in situ ocular gel was performed. It found that there no change in the shape of blood cell (bulging or shrinkage). Which reveals the isotonic nature of the optimized formulation (F1) as showed in figure 1 and 2.



Red Blood Cell alone

Red Blood Cell alone

RBC with optimized formulation (F1)

Figure no 3: Isot

Result of DOE:

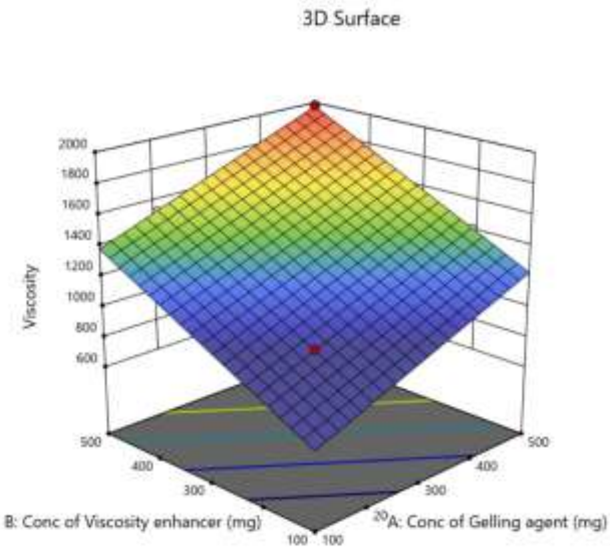


Figure No.:4 3D surface plot for Viscosity

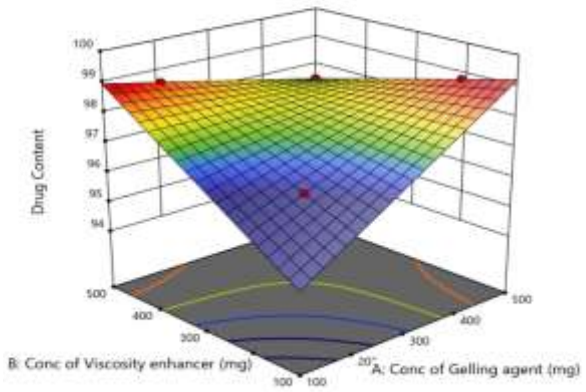


Figure No5: 3D surface plot for Drug content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5.066E+05	2	2.533E+05	1621.16	0.0176	significant
A-Conc of Gelling agent	1.958E+05	1	1.958E+05	1253.16	0.0180	
B-Conc of Viscosity enhancer	3.108E+05	1	3.108E+05	1989.16	0.0143	
Residual	156.25	1	156.25			
Cor Total	5.068E+05	3				

Statistical Analysis Statement:
The statistical significance of the formulation variables was evaluated using ANOVA. The model was found to be highly significant with an F-value of 1621.16 and a corresponding p-value of 0.0176, indicating a strong correlation between the independent variables and the response. Among the variables, the concentration of the gelling agent (Factor A) and the viscosity enhancer (Factor B) had significant effects on the response with F-values of 1253.16 ($p = 0.0180$) and 1989.16 ($p = 0.0143$), respectively. The low residual sum of squares (156.25) further supports the adequacy of the model. These results confirm that both formulation variables play a critical role in determining the performance characteristics of the ophthalmic in-situ gel.

Discussion: From the present investigation, it can be concluded that the pH-sensitive in-situ gel formulation of Dapagliflozin Propanediol Monohydrate (DPM) significantly enhances ocular drug delivery by increasing precorneal residence time and enabling sustained drug release. The formulation utilizes solid dispersion techniques to overcome the poor aqueous solubility of Dapagliflozin, while the in-situ gel matrix composed of Carbopol 940 and HPMC K4M ensures phase transition at physiological pH (~7.4), forming a viscoelastic gel on the ocular surface.Importantly, the potential of Dapagliflozin to inhibit the NLRP3 inflammasome pathway, which plays a critical role in the inflammatory pathogenesis of Dry Eye Disease (DED), adds a mechanistic advantage to this delivery system. The formulation strategy thus addresses both solubility enhancement and site-specific anti-inflammatory action.Additionally, the in-situ gel system offers improved ease of administration, reduced dosing frequency, and better patient compliance, making it a suitable alternative to conventional eye drops. These advantages collectively point toward its promise in managing chronic ocular conditions such as DED.However, to fully validate its therapeutic potential, further in vivo studies are essential to assess its pharmacodynamic performance, ocular tolerability, and long-term safety. Moreover, accelerated and real-time stability studies should be conducted to ensure the formulation's shelf life and physical integrity under storage conditions.In conclusion, the developed Dapagliflozin-loaded ophthalmic in-situ gel represents a novel and effective approach for treating Dry Eye Disease, warranting further investigation for potential clinical translation.

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