Research Paper

Development and Evaluation of Mucoadhesive in-situ gel of Celosia argentea for Retinal protection

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ABSTRACT

The need for efficient ocular medication delivery systems with extended residence times and improved treatment efficacy is highlighted by the fact that retinal illnesses linked to oxidative stress and progressive retinal degeneration continue to be a significant global cause of visual impairment. The goal of the current study was to create and assess a mucoadhesive in-situ gel for retinal protection that contained Celosia argentea seed extract. Preliminary phytochemical screening of an ethanolic extract of C. argentea seeds revealed the presence of bioactive components such as flavonoids, phenolics, alkaloids, saponins, and betalains. Gellan gum and hydroxypropyl methylcellulose (HPMC) were used as polymers to integrate the extract into an ion-sensitive in-situ gel. Physicochemical characteristics of the prepared gels, including appearance, pH, viscosity, gel strength, spreadability, drug content, mucoadhesive strength, in-vitro drug release, and stability, were assessed. The formulation's safety for ocular application was established by cytotoxicity testing using the ARPE-19 cell line, which also showed good biocompatibility. Acceptable pH, quick gelation upon contact with simulated tear fluid, improved viscosity following gel formation, adequate mucoadhesive qualities, prolonged drug release, and good stability during storage were all demonstrated by the optimized formulation. In summary, the mucoadhesive in-situ gel of Celosia argentea seed extract shown encouraging properties for extended ocular delivery and could be used as a possible retinal protective formulation for the treatment of retinal illnesses associated with oxidative stress.

Keywords: Celosia argentea, Mucoadhesive In-situ gel, Ocular Drug Delivery, Reactive Oxygen species, ARPE 19 cell line, Antioxidant activity.

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INTRODUCTION

Anatomically separated into anterior and posterior portions, the eye is a highly specialized and sophisticated sensory organ that provides vision. The cornea, conjunctiva, aqueous fluid, iris, ciliary body, and lens make up the anterior segment, whereas the vitreous humour, retina, choroid, and optic nerve make up the posterior section. A key component of visual perception, the retina is a multilayered neurosensory tissue that transforms light impulses into neural signals. However, the corneal barrier, blood-aqueous barrier (BAB), and blood-retinal barrier (BRB), among other unique anatomical and physiological features of the eye, severely restrict medication penetration into ocular tissues and provide significant obstacles to efficient drug administration [1,2]. One of the main causes of blindness and irreversible vision impairment in the world is retinal diseases. Progressive retinal damage and neuronal loss are hallmarks of conditions such diabetic retinopathy, age-related macular degeneration (AMD), retinal vein occlusion, retinitis pigmentosa, and glaucoma-associated retinal degeneration [3, 4]. Retinal degeneration has been linked to oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis of retinal pigment epithelial (RPE) cells [5,6]. Reactive oxygen species (ROS) overproduction can harm retinal cells, impairing the retina's structure and functionality. Thus, a crucial field of ophthalmic research continues to be the creation of efficient retinal protective treatments that can lower oxidative

stress and preserve retinal integrity [7]. The most popular method of treating eye conditions is topical ocular administration due to its convenience of use, non-invasiveness, and patient acceptability. Over 90% of currently available ophthalmic formulations are conventional ocular dose forms, such as eye drops, suspensions, and ointments [1,8]. Nevertheless, these formulations have a number of drawbacks, such as poor corneal permeability, nasolacrimal drainage, tear turnover, and rapid precorneal elimination brought on by blinking [9]. Because of this, intraocular tissues typically receive less than 5% of the medicine that is delivered, requiring frequent dosage and decreasing patient compliance [10]. Additionally, repeated dosing may result in decreased therapeutic efficacy, increased systemic absorption, eye discomfort, and variations in drug concentration [11].

When treating posterior segment diseases like retinal disorders, where drug transport is further limited by the blood-retinal barrier, these difficulties are especially important [12]. A lot of work has gone into creating innovative ophthalmic drug delivery systems in order to get around the drawbacks of traditional ocular formulations. Because they can undergo a sol-to-gel transition when exposed to physiological variables like pH, temperature, or ionic strength, in-situ gelling systems have emerged as potential options among these [13,14]. After being injected into the eye, in-situ gels change from low-viscosity solutions to viscoelastic gels. This change improves ocular bioavailability, decreases drug loss by lacrimation, and lengthens precorneal residence time [15]. Additionally, in-situ gels offer controlled and prolonged drug release, which lowers the frequency of doses and enhances therapeutic results [16]. Ion-sensitive in-situ gels for ocular medication delivery have garnered a lot of interest, especially those based on gellan gum. When cations from tear fluid are present, gellan gum gelates, creating a clear gel matrix that extends medication retention on the surface of the eye [17]. To increase gel strength and stability, hydroxypropyl methylcellulose (HPMC) is commonly used as a mucoadhesive and viscosity-enhancing polymer. Gellan gum and HPMC together have been demonstrated to prolong ocular residency time, maintain medication release, and increase patient comfort [18]. Using mucoadhesive polymers is another way to enhance ocular medication delivery. The capacity of a formulation to stick to the mucin layer that covers the surface of the eye is known as mucoadhesion. Mucoadhesive devices increase drug absorption and bioavailability by extending the duration of contact between the medication and the ocular mucosa [19]. Enhanced mucoadhesion is especially useful for treating chronic ocular illnesses that require long-term therapy because it reduces precorneal medication loss and permits longer therapeutic action [20]. As a result, mucoadhesive in-situ gels have become very successful ocular drug delivery systems that combine the advantages of better patient compliance, controlled release, and extended retention.

The utilization of plant-derived bioactive chemicals for eye therapy has received more attention lately because of their neuroprotective, anti-inflammatory, and antioxidant qualities. Cockscomb, or *Celosia argentea*, is a medicinal herb in the Amaranthaceae family that has been utilized extensively in traditional medical systems [21]. Flavonoids, phenolic chemicals, alkaloids, saponins, betalains, and polysaccharides are among the physiologically active phytoconstituents found in many plant tissues, especially seeds [22]. These components have been shown in numerous studies to have strong antioxidant and free-radical scavenging properties that can shield tissues from oxidative damage [23, 24]. Additionally, *C. argentea* extracts have been shown in experiments to exhibit anti-inflammatory, hepatoprotective, immunomodulatory, and neuroprotective properties [25]. Antioxidant-rich phytoconstituents of *C. argentea* may have substantial retinal protective potential because oxidative stress is a major contributor in retinal degeneration.

The use of *Celosia argentea* seed extract in mucoadhesive in-situ gel formulations for retinal protection has not received much attention, despite the growing interest in herbal remedies and sophisticated ocular drug delivery methods. The majority of research that is now accessible either concentrates on the pharmacological properties of the plant or separately on the formulation elements of ocular in-situ gels [22,23,24]. Additionally, ocular delivery systems that use natural antioxidants to provide extended residence duration, sustained drug release, higher bioavailability, and increased retinal protective efficacy are still needed. In order to create and assess a mucoadhesive ion-sensitive in-situ gel with *Celosia argentea* seed extract for retinal protection, the current investigation was conducted.

OBJECTIVES

The objective of the present study was to develop and evaluate a mucoadhesive in-situ gel containing *Celosia argentea* seed extract for prolonged ocular retention and retinal protection.

MATERIALS AND METHODS

Collection and Authentication of Plant Material [26]

The whole plant of *Celosia argentea* was collected from Kolhapur, Maharashtra, during late November. Preliminary identification was carried out based on morphological characteristics. Botanical authentication was performed by Dr. Girish Potdar, Department of Botany, Yashwantrao Chavan College of Science, Karad. Mature seeds were separated from the authenticated plant and used for further studies. The seeds were cleaned, shade-dried, and stored in airtight containers until use.

Plant Material Preparation and Extraction [27]

Authenticated *Celosia argentea* seeds were cleaned and shade-dried at room temperature (25–30°C) for three days. The dried seeds were coarsely powdered using a mechanical grinder and stored in amber-coloured containers. About 50 g of seed powder was subjected to Soxhlet extraction. Defatting was carried out using petroleum ether followed by extraction with 200 mL ethanol. The extract was concentrated under reduced pressure and stored under refrigeration until further use.

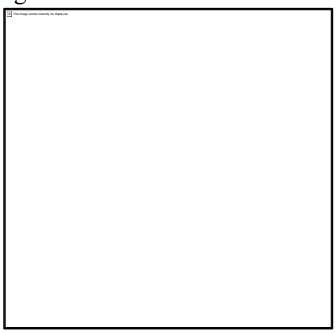


Figure 1: Soxhlet extraction of *Celosia argentea* seeds powder using Ethanol as solvent

Preliminary Phytochemical Evaluation [28]

The ethanolic seed extract was screened qualitatively for major phytoconstituents using standard phytochemical tests. Dragendorff's test was performed for alkaloids, alkaline reagent test for flavonoids, ferric chloride test for phenolics, foam test for saponins, and sodium hydroxide test for betalains. Colour changes and precipitate formation were recorded as indicators of constituent presence. The screening confirmed the occurrence of bioactive compounds responsible for therapeutic activity.

Spectral Analysis of Extract [29]

The ethanolic extract was characterized using UV–Visible and FTIR spectroscopy. For UV analysis, the extract was appropriately diluted with ethanol and scanned between 200–800 nm using ethanol as blank. Absorption maxima (λ max) were recorded for phytoconstituent characterization. FTIR analysis was performed over a spectral range of 4000–400 cm^{-1}. Characteristic peaks were analysed to identify the functional groups present in the extract.

Experimental Design for Optimization [30]

A 3^2 full factorial design was employed to optimize the mucoadhesive in-situ gel formulation. Gellan gum concentration (X_1) and HPMC concentration (X_2) were selected as independent variables. Viscosity (Y_1), pH (Y_2), and gelation time (Y_3) were chosen as response variables. Nine experimental batches (F1–F9) were prepared at three levels of each factor. Design-Expert® software version 13.0.1.0 was used for statistical optimization.

Formulation and Optimization [31]

Nine batches of mucoadhesive in-situ gel containing *Celosia argentea* extract were prepared according to the factorial design. Each formulation was evaluated for pH, viscosity, and gelation time. The obtained data were subjected to statistical analysis to determine the influence of polymer concentrations. Based on optimization criteria, batch F7 showed the most desirable formulation characteristics. Therefore, F7 was selected as the optimized formulation for further evaluation.

Formulation of Optimized Batch [32]

Gellan gum (0.5%) and HPMC (0.55%) were dissolved separately in distilled water with continuous stirring. The polymer solutions were mixed and supplemented with sodium chloride (0.18 g), calcium chloride (0.015 g), and benzalkonium chloride (0.003 g). Three millilitres of concentrated *Celosia argentea* extract were incorporated into the polymeric solution. The final volume was adjusted to 30 mL using distilled water. The prepared formulation was stored in sterile amber-coloured containers under refrigerated conditions until evaluation.

Evaluation parameters of Mucoadhesive in-situ gel

1. Appearance and Clarity [33]

A small quantity of the optimized mucoadhesive in-situ gel was transferred into a clean, transparent glass tube and visually inspected under daylight conditions against both black and white backgrounds. The formulation was evaluated for colour, clarity, transparency, homogeneity, grittiness, and presence of any particulate matter. The formulation was also examined for phase separation or precipitation. Observations were recorded based on visual appearance and uniformity.

2. pH Measurement [34]

The pH of the optimized formulation was determined using a calibrated digital pH meter. Calibration was performed using standard buffer solutions of pH 4.0, 7.0, and 9.2 prior to analysis. Approximately 10 mL of formulation was taken, and the electrode was immersed directly into the sample. Measurements were performed in triplicate at room temperature, and the mean pH value was reported.

3. Viscosity [35]

3.1 Viscosity Before Gelation

The viscosity of the formulation before gelation was measured using a Brookfield Viscometer equipped with Spindle No. 62 at 50 rpm and 25 ± 1°C. Approximately 20 mL of formulation was placed in the sample chamber. Three readings were recorded, and the average viscosity was expressed in centipoise (cps).

3.2 Viscosity After Gelation

The formulation was allowed to gel at 37°C before viscosity determination. Viscosity was measured using a Brookfield Viscometer fitted with Spindle No. 64 at 10 rpm. The measurements were performed in triplicate, and the mean viscosity was expressed in centipoise (cps).

4. Spreadability [36]

Spreadability was determined using the parallel plate method. A known quantity of gel was placed

between two glass plates, and different weights were applied to the upper plate. The diameter of spreading and time required for separation of the plates were recorded. Spreadability was calculated using the equation:

$$S = M \times L / T$$

where S = Spreadability, M = Applied weight, L = Distance moved by glass plate, T = Time required for plate separation.

5. *In Vitro* Drug Release Study [37]

The *In vitro* drug release study was carried out using a Franz diffusion cell apparatus. The formulation was placed in the donor compartment, while phosphate buffer was maintained in the receptor compartment at 37 ± 1°C under continuous stirring. Samples were withdrawn at predetermined intervals and replaced with fresh buffer. Drug release was quantified spectrophotometrically at 257 nm.

6. Antioxidant Activity (DPPH Assay) [38]

Method: DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) Assay

Procedure

- DPPH free radicals were used to determine the antioxidant activity of test substances.
- The microwell plates were filled with 100 µL of various concentrations (20, 40, 60, 80, and 100 µg/mL).
- The samples were covered with 100 µL of 0.1% methanolic DPPH and left in the dark for 30 minutes [10].
- After that, the samples were examined for discoloration, ranging from purple to yellow, and the absorbance at 510 nm was measured using a colorimeter.
- The following formula was used to determine radical scavenging activity:
  DPPH radical scavenging activity (%) = (Absorbance of control- Absorbance of test sample) / Absorbance of control x 100

7. Cytotoxicity Study (MTT Assay) [39]

Method: MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay

Cell line used: ARPE-19 retinal pigment epithelial cells

Model used: 96-well microplate cell culture model

- ARPE-19 cells were cultured for 24 hours at 37°C and 5% CO2 at a concentration of 1 × 10^4 cells/ml in culture medium.
- A concentration of 100µl of cells was planted.
- With 100 µl of culture media and 20, 40, 60, 80, and 100 µg/ml of samples into micro plates (96 wells, tissue culture grade).
- The cell line and DMSO (0.2% in PBS) were incubated in control wells.
- Every sample underwent triplicate incubation.
- To ascertain the percentage of living cells following culture and the control cell survival, controls were kept. Cell cultures were kept in a CO2 incubator at 37°C with 5% CO2 for a whole day.
- Following incubation, 20µl of MTT reagent (5 mg/ml PBS) was added after the medium was entirely withdrawn.
- The cells were cultured for four hours at 37ºC in a CO2 incubator following the addition of MTT.
- Examined the formazan crystal growth in the wells under a microscope. Only live cells converted the yellowish MTT to a dark formazan.
- Following the full removal of the medium. 200µlo of DMSO was added and held for ten minutes.
- Wrapped with aluminium foil, incubate at 37 °C.
- Three samples were examined by measuring each sample's absorbance at a wavelength of 550 nm using a microplate reader [11].

8. Stability Study [40]

The physical stability study was conducted according to ICH Q1A (R2) guidelines for 30 days under controlled storage conditions. The optimized formulation was periodically evaluated for appearance, clarity, pH, spreadability, homogeneity, phase separation, and gelling capacity. Any changes in physical characteristics were carefully monitored and recorded throughout the study period. The formulation was considered stable if no significant changes were observed.

RESULTS AND DISCUSSION

Phytochemical screening

Several physiologically active secondary metabolites were found in the ethanolic seed extract of *Celosia argentea,* according to preliminary phytochemical screening.

Sr. No.	Test	Observation	Conclusion
1.	Alkaloids (Dragendorff's Test)	Formation of orange or reddish-brown precipitate.	Alkaloids may present
2.	Flavonoids (Alkaline Reagent Test)	Intense yellow colour appears and disappears after addition of acid.	Flavonoids may Present
3.	Phenolic Compounds (Ferric Chloride Test)	Formation of blue, green, violet, or black coloration.	Phenolic compounds may present
4.	Saponins (Foam Test)	Formation of stable persistent foam	Saponins may present

5.	Betalains (Visual Colour Test)	Yellow-orange colour → Betaxanthins	betalain pigments may present

Table 1: Phytochemical screening of extract

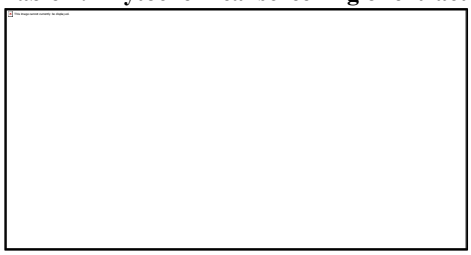


Figure 2: Preliminary phytochemical evaluation of extract

These phytoconstituents show that the ethanolic extract has substantial pharmacological potential, especially in terms of antioxidant and therapeutic properties, which makes it appropriate for additional formulation and research on retinal protection.

UV–Visible Spectroscopy

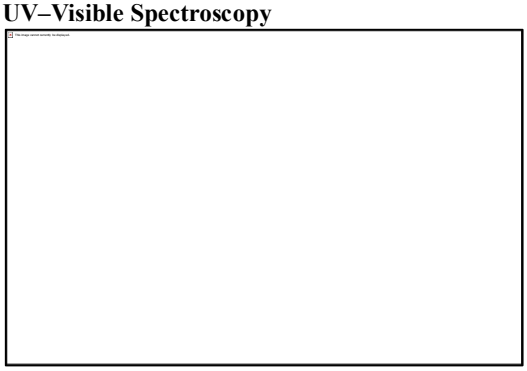


Figure 3: UV Visible Spectral Analysis

The presence of flavonoids and phenolic compounds was shown by distinctive absorption peaks in the UV-visible spectrum analysis of *Celosia argentea* extract. These phytoconstituents are widely recognized for their capacity to scavenge free radicals and exhibit antioxidant activity. The existence of bioactive substances that might support the extract's ability to protect the retina is confirmed by the observed absorption pattern.

FTIR spectral Analysis

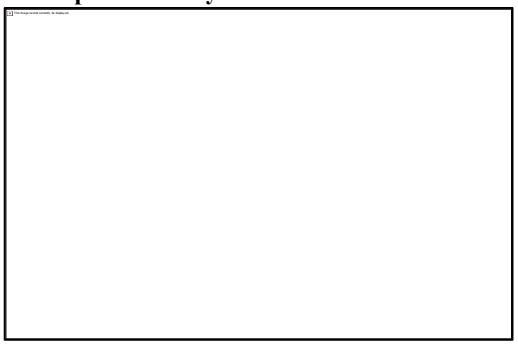


Figure 4: Fourier Transform Infrared Spectral Analysis of Ethanolic Extract of

Literature Value (cm-1)	Observed Value (cm-1)	Vibration Type	Functional Group
3200-3550	3358.36	O-H Stretching	Hydroxyl Group
2850-3000	2973,2923,2854	C-H Stretching	Aliphatic alkanes
1650-1750	1709.87	C=O Stretching	Carbonyl group
1620-1690	1649.28	C=C Stretching	Alkenes or Amides
1400-1500	1455.26	C-H Stretching	Aliphatic methylene Group
1000-1300	1086.61, 1044.64	C-O Stretching	Alcohol, Ether
650-910	879.04, 721.04	C-H Out-of-plane Bending	Aromatic compound

Table 2: Identification of Functional Groups in Ethanolic Extract of *Celosia argentea* by FTIR Analysis

Several functional groups linked to bioactive phytoconstituents were found in the ethanolic extract of *Celosia argentea*, according to FTIR spectral analysis. Hydroxyl groups were suggested by a broad peak at 3358.36 cm^{-1}, whereas aliphatic C–H stretching was indicated by peaks at 2973–2854 cm^{-1}. Peaks at 1649.28 cm^{-1} and 1086.61–1044.64 cm^{-1} indicated alkenes, amides, alcohols, and ethers, while the peak at 1709.87 cm^{-1} indicated the presence of carbonyl compounds. These results confirm that the extract contains flavonoids, phenolics, and other therapeutically significant components.

Experimental Design of Mucoadhesive *In situ* gel formulation

Celosia argentea's mucoadhesive in-situ gel formulation was optimized using a 3^2 complete factorial design and Design-Expert® software version 13.0.1.0. Viscosity (Y_1), pH (Y_2), and gelation time (Y_3) were chosen as response variables, and gellan gum (X_1) and HPMC (X_2) were chosen as independent variables. To investigate the impact of polymer concentrations on the formulation's physicochemical properties, nine formulations were created and assessed.

	Independent variables	Dependant Variables

Run	Gellan Gum (%)	HPMC (%)	Gelation Time (Min)	pH	Viscosity Before Gelation (cps)	Viscosity After Gelation (cps)
F1	0.1	0.55	5.5	6.8	120	420
F2	0.1	0.3	5.2	6.7	95	350
F3	0.3	0.8	5.8	6.9	210	720
F4	0.3	0.3	6.5	6.8	150	500
F5	0.1	0.8	6.2	6.9	140	480
F6	0.5	0.8	5.2	7	260	900
F7	**0.5**	**0.55**	**4.6**	**6.9**	**230**	**800**
F8	0.5	0.3	5.9	6.8	180	650
F9	0.3	0.55	5.8	6.9	190	680

Table 3: Optimization of Ion-activated mucoadhesive In-situ gel

Effect of gellan gum (X1) and HPMC (X2) on gelation time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	26.64	5	5.33	49.65	0.0044	significant
A-Gellan Gum	18.03	1	18.03	167.98	0.0010	
B-HPMC	7.71	1	7.71	71.81	0.0034	
AB	0.2025	1	0.2025	1.89	0.2632	
A^2	0.4356	1	0.4356	4.06	0.1374	
B^2	0.2689	1	0.2689	2.51	0.2116	
Residual	0.3219	3	0.1073			
Cor Total	26.96	8				

Table 4: Gellan gum and HPMC response to the gelation time

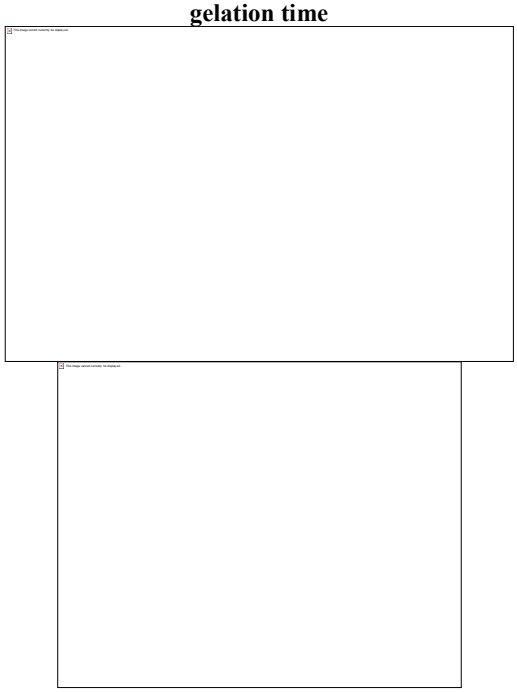


Figure 5: Response surface plots (2D contour and 3D surface) for gelation time as a function of gellan gum and HPMC concentrations.

The contour and 3D surface plots showed that both gellan gum and HPMC significantly influenced gelation time. Increasing gellan gum concentration (0.1–0.5%) decreased gelation time due to rapid ion-triggered gel formation, whereas increasing HPMC concentration slightly increased gelation time because of higher formulation viscosity. Overall, gellan gum exhibited a more pronounced effect on gelation behaviour than HPMC.

Effect of gellan gum (X_1) and HPMC (X_2) on pH

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0578	5	0.0116	7.80	0.0607	not significant
A-Gellan Gum	0.0150	1	0.0150	10.13	0.0500	
B-HPMC	0.0417	1	0.0417	28.13	0.0131	
AB	0.0000	1	0.0000	0.0000	1.0000	

A^2	0.0006	1	0.0006	0.3750	0.5836	
B^2	0.0006	1	0.0006	0.3750	0.5836	
Residual	0.0044	3	0.0015			
Cor Total	0.0622	8				

Table 5: Gellan gum and HPMC response to the pH

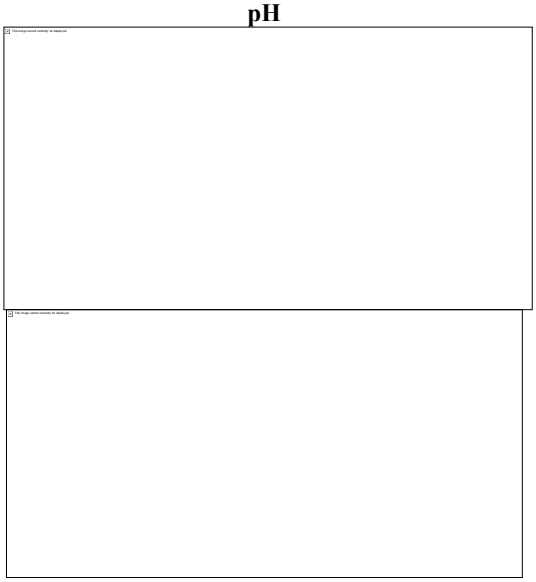


Figure 6: Response surface plots (2D contour and 3D surface) pH as a function of gellan gum and HPMC concentrations.

The ANOVA results indicated that the quadratic model for pH was statistically non-significant (p = 0.0607). Among the formulation variables, HPMC concentration had the most significant effect on pH (p = 0.0131), while gellan gum showed a marginal influence (p = 0.0500). The contour and 3D surface plots demonstrated a gradual increase in pH with increasing concentrations of both polymers, with HPMC exerting the greatest effect.

Effect of gellan gum (X_1) and HPMC (X_2) on viscosity before gelation

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	22972.92	5	4594.58	508.94	0.0001	significant
A-Gellan Gum	16537.50	1	16537.50	1831.85	< 0.0001	
B-HPMC	5704.17	1	5704.17	631.85	0.0001	
AB	306.25	1	306.25	33.92	0.0101	
A^2	312.50	1	312.50	34.62	0.0098	
B^2	112.50	1	112.50	12.46	0.0386	
Residual	27.08	3	9.03			
Cor Total	23000.00	8				

Table 6: Gellan gum and HPMC response to the viscosity before gelation

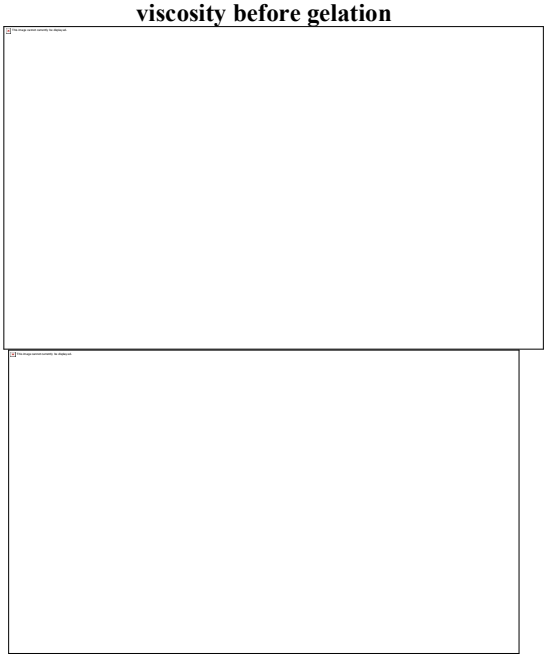


Figure 7: Response surface plots (2D contour and 3D surface) for viscosity before gelation as a function of gellan gum and HPMC concentrations.

The quadratic model for viscosity prior to gelation was very significant (p = 0.0001), according to the ANOVA results, suggesting that formulation variables have a considerable impact on viscosity. Viscosity was greatly increased by both gellan gum and HPMC, with gellan gum having the biggest impact. The viscosity-enhancing qualities of both polymers were confirmed by the contour and 3D surface plots, which showed a progressive increase in viscosity with increasing concentrations.

Effect of gellan gum (X_1) and HPMC (X_2) on viscosity after gelation

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	2.697E+05	5	53942.22	91.03	0.0018	significant
A-Gellan Gum	2.017E+05	1	2.017E+05	340.31	0.0003	

B-HPMC	60000.00	1	60000.00	101.25	0.0021	
AB	3600.00	1	3600.00	6.08	0.0905	
A^2	2222.22	1	2222.22	3.75	0.1482	
B^2	2222.22	1	2222.22	3.75	0.1482	
Residual	1777.78	3	592.59			
Cor Total	2.715E+05	8				

Table 7: Gellan gum and HPMC response to the viscosity after gelation

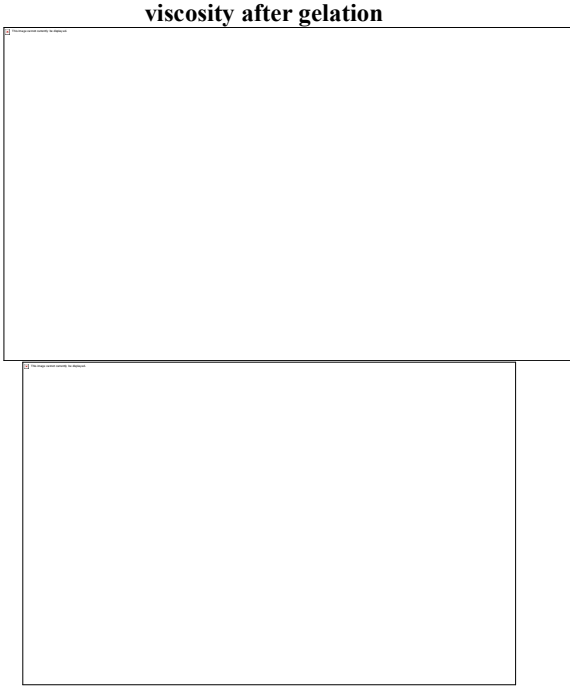


Figure 8: Response surface plots (2D contour and 3D surface) for viscosity after gelation as a function of gellan gum and HPMC concentrations.

The model's appropriateness was confirmed by the ANOVA findings, which showed that the quadratic model for viscosity after gelation was statistically significant ($p = 0.0018$). Post-gelation viscosity was significantly increased by both gellan gum ($p = 0.0003$) and HPMC ($p = 0.0021$), with gellan gum having the greatest impact. There were negligible combination and curvature effects, as indicated by the non-significance of the interaction and quadratic terms. With rising amounts of both polymers, especially gellan gum, the contour and 3D surface plots showed a progressive increase in viscosity.

Evaluation of optimized batch

Sr.	Parameter Evaluated	Observation of F7 formulation
1.	Colour	Transparent to light white
2.	Appearance	Smooth and homogeneous
3.	Clarity	Clear
4.	Grittiness	Absent
5.	Phase Separation	Not observed

Table 8: Evaluation of Appearance and Clarity

There was no discernible turbidity or particle debris in the clear to slightly white optimized mucoadhesive in-situ gel. The uniform distribution of ingredients was shown by the formulation's smoothness, homogeneity, and lack of grittiness. Phase separation was not seen, indicating that the formulation's components were compatible and had acceptable physical stability. These results show that it is appropriate for ocular medication delivery applications. **pH measurement**

Sr. No	Formulation	pH
1.	F7	6.9

Table 9: pH determination for F7 batch

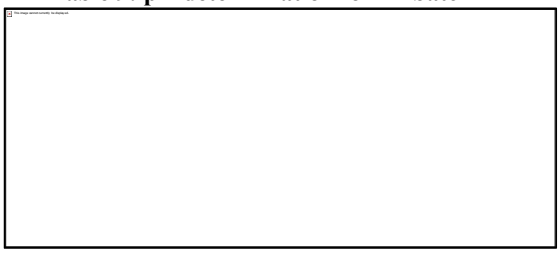


Figure 9: pH measurement

The pH of the improved formulation was 6.9, which is appropriate for ocular delivery and near the physiological pH of tear fluid. This almost neutral pH reduces the possibility of application-related irritation and discomfort. As a result, the formulation is regarded as safe, stable, and suitable for the delivery of drugs into the eyes.

Viscosity

Sr. No.	Formulation	Viscosity (cps) (Before)	Viscosity(cps) (After)
1.	F7	230	800

Table 10: Determination of viscosity before gelation and viscosity after gelation for F7 batch

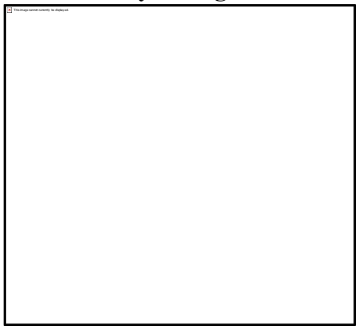


Figure 10: Determination of viscosity after gelation

The improved formulation's viscosity dramatically rose from 230 cps prior to gelation to 800 cps following gelation, indicating a successful sol-to-gel transformation. The creation of a robust gel matrix is indicated by the greater post-gelation viscosity, whereas the low initial viscosity makes uniform spreading and straightforward instillation possible. This increase decreases fast outflow and improves ocular residence time. As a result, the formulation has appropriate rheological characteristics for long-term ocular medication administration.

Spreadability

Sr. No.	Sample	Weight applied (g)	Diameter (cm)	Spreadability (g.cm/sec)
1.	F7	20	0.4	0.13
2.	F7	30	1.2	0.60
3.	F7	40	1.8	1.20

Table 11: Spreadability of F7 formulation

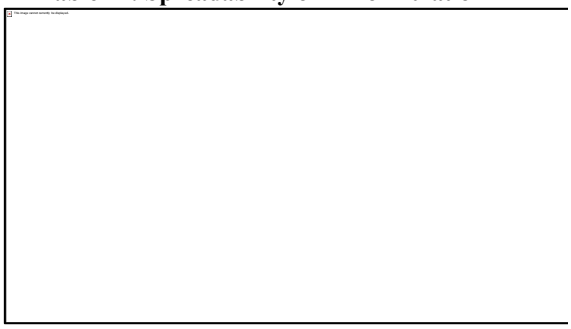


Figure 11: Spreadability of optimized formulation

The spreadability study of the optimized F7 formulation demonstrated a positive correlation between applied weight and spreadability. As the weight increased from 20 g to 40 g, spreadability values increased from 0.13 to 1.20 g·cm/sec, while the spreading diameter increased from 0.4 cm to 1.8 cm. These findings indicate good rheological behaviour and easy spreading of the formulation under applied force. Therefore, the formulation is expected to provide uniform application and improved patient acceptability.

***In vitro* Drug Release Study**

Time (Hours)	UV Absorbance	% Cumulative Drug Release
0.5	0.128	12.80
1	0.173	17.25
2	0.236	23.60
4	0.274	27.40
6	0.331	33.10
8	0.403	40.25
10	0.441	44.10
12	0.483	48.30
14	0.548	54.75
16	0.592	59.20
18	0.610	61.00
20	0.634	63.40
22	0.699	69.85
24	0.745	74.50

Table 12: *In vitro* Drug Release Study of F7 formulation

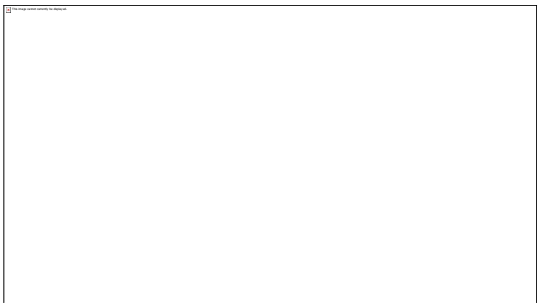


Figure 12: *In vitro* Drug Release at pH 7.4

The improved gel formulation showed a controlled and sustained release pattern over a 24-hour period in the *In vitro* drug release investigation. At 0.5 hours, there was an initial release of 12.80%, which progressively rose to 48.30% at 12 hours and 74.50% after 24 hours. The drug's continued diffusion from the polymeric matrix was confirmed by the consistent rise in absorbance values and drug release. These findings suggest that the formulation may have a longer therapeutic duration and require fewer doses.

Antioxidant Activity

Sr. No.	Sample Code	Conc. (ug/mL)	Absorbance at 510 nm				% Inhibition	IC50 (ug/mL)
			Test 1	Test 2	Test 3	Mean		
1.	Control	-	1.93	1.93	1.93	1.93	-	
2.	Standard (Ascorbic acid)	20	1.34	1.37	1.39	1.37	29.02 %	59.23
		40	1.31	1.29	1.33	1.31	32.12%	
		60	0.95	0.97	0.93	0.95	50.77 %	
		80	0.82	0.85	0.79	0.82	57.51 %	
		100	0.35	0.32	0.32	0.33	82.90 %	

3.	Optimize Batch	20	1.52	1.56	1.51	1.53	20.73%	76.11
		40	1.45	1.47	1.48	1.47	23.83%	
		60	1.08	1.12	1.09	1.10	43.01%	
		80	0.86	0.88	0.92	0.89	53.89 %	
		100	0.65	0.66	0.68	0.66	65.80 %	

Table 13: F7 formulation′s Antioxidant activity by DPPH assay

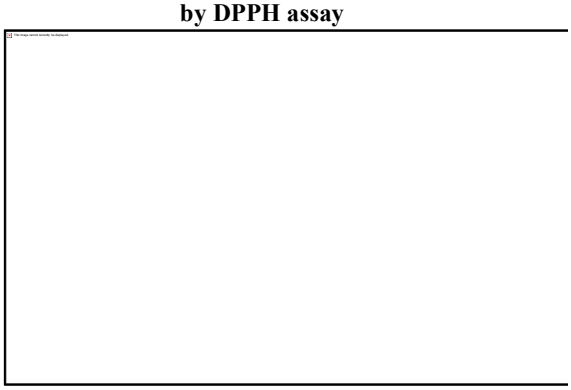


Figure 13: Antioxidant activity by DPPH method

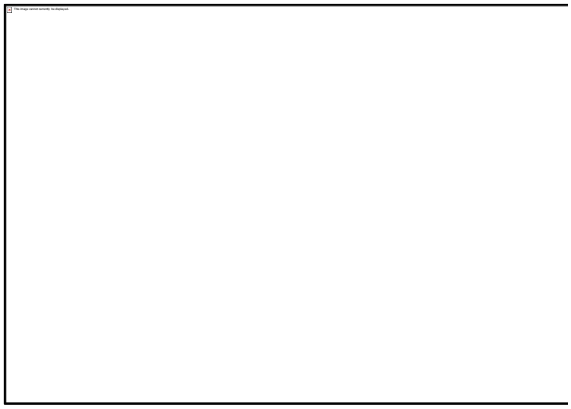


Figure 14: Antioxidant activity Comparison of Standard and Optimized F7 formulation

For both the standard and the optimized batch, the DPPH radical scavenging experiment revealed a concentration-dependent rise in antioxidant activity. The optimized batch demonstrated moderate but efficient antioxidant activity with roughly 66% inhibition at the same concentration, while the standard showed substantially stronger free radical inhibition at all tested concentrations, reaching about 82% inhibition at 100 µg/ml.

Cytotoxicity study

Method used- MTT Assay

Sample Code	**Conc. (ug/ml)**	**Optical Density**			**Mean**	**% Of Inhibition**	**% Of Viability**	**IC 50 (µg/ml)**
Control		1.435						-
Standard Cisplatin	20	0.8115	0.8211	0.8177	0.8177	43.07 %	56.93 %	
	40	0.6955	0.6933	0.6975	0.6955	51.57 %	48.43 %	38.43
	60	0.5888	0.5885	0.5887	0.5886	59.16 %	40.84 %	
	80	0.3778	0.3775	0.3778	0.3777	73.73 %	26.27 %	
	100	0.2366	0.2333	0.2335	0.2334	83.69 %	16.31 %	
Optimize Batch	20	1.4112	1.4115	1.4113	1.4113	1.53%	98.47 %	
	40	1.3889	1.3885	1.3887	1.3887	3.34%	96.66 %	>100
	60	1.3663	1.3662	1.3667	1.3664	4.95%	95.05 %	
	80	1.3400	1.3442	1.3446	1.3442	6.48%	93.52 %	
	100	1.3112	1.3110	1.3115	1.3112	8.57%	91.43 %	

Table 14 : Cytotoxicity study of F7 formulation by MTT Assay

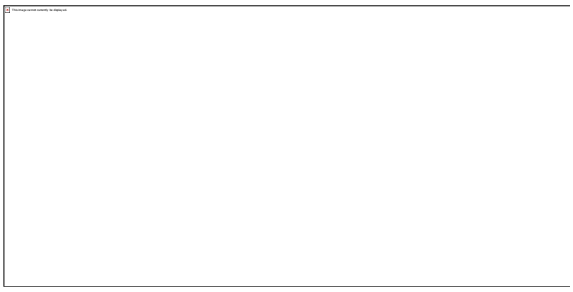
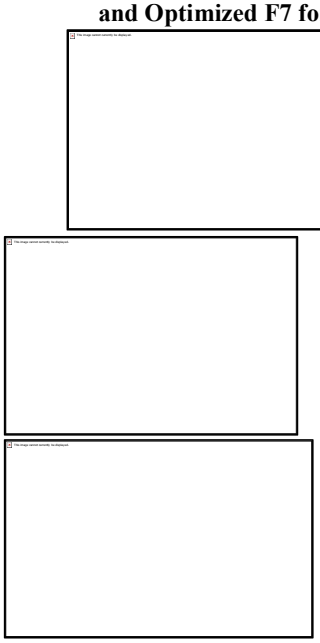


Figure 15: Cytotoxicity Comparison of Standard and Optimized F7 formulation



(a) Control (b) Standard (c) F7 formulation

Figure 16: Microscopic image (a), (b), (c) of ARPE-19 cells for F7 formulation by MTT Assay

Concentration-dependent activity was demonstrated by the MTT experiment, and the tailored mucoadhesive In-situ gel outperformed the standard in terms of retinal cell compatibility and cytotoxicity**.**

Stability Study

Sr. No.	Parameters	Initial (0 day)	7 days	14 days	21 days	30 days
1.	Appearance	Clear	Clear	Clear	Slight pale Yellow	Slight pale yellow
2.	Clarity	Clear	Clear	Clear	Clear	Clear
3.	pH	6.9	6.9	6.9	6.9	6.9
4.	Spreadability	Good	Good	Good	Good	Good
5.	Homogeneity	Uniform	Uniform	Uniform	Uniform	Uniform
6.	Phase separation	Absent	Absent	Absent	Absent	Absent
7.	Gelling Capacity	+++	+++	+++	+++	+++

Table 15: Physical stability study of F7 formulation following ICH Q1A (R2) guidelines

Following ICH Q1A (R2) criteria, a 30-day physical stability investigation of the optimized F7 formulation was carried out. Throughout the course of the investigation, the formulation stayed uniform and did not exhibit phase separation. There was no discernible change in look or clarity, only a minor shift in colour to pale yellow. Good physicochemical stability was indicated by the pH being steady within the permitted range. Additionally, there was no discernible decline in performance while maintaining the gelling capacity. These results show that the developed mucoadhesive in-situ gel formulation has good short-term stability.

Discussion

For retinal protection, the current study effectively created and refined a mucoadhesive ion-activated in-situ gel comprising ethanolic *Celosia argentea* seed extract. Alkaloids, flavonoids, phenolic compounds, saponins, and betalains all of which are recognized for their antioxidant and medicinal properties—were found, according to preliminary phytochemical screening. By detecting distinctive absorption peaks and functional groups connected to phenolics and flavonoids, the UV–visible and FTIR spectrum studies provided additional evidence for the existence of these beneficial phytoconstituents. These results imply that the extract has strong antioxidant activity, which could help protect the retina from damage brought on by oxidative stress. Optimization with a 3^2 complete factorial design showed that polymer concentrations had a major impact on the formulation's physicochemical properties. Because of its ion-sensitive gel-forming capacity, gellan gum had a significant impact on viscosity and gelation time, while HPMC mostly improved viscosity and mucoadhesion. Effective sol-to-gel transition and extended precorneal retention were indicated by the improved F7 formulation's favourable gelation behaviour, acceptable pH (6.9), and appropriate viscosity before (230 cps) and after gelation (800 cps). These results are in line with other studies on ocular in-situ gels based on gellan gum, which found that higher polymer concentrations improved gel strength and residence time. Excellent physical properties, such as homogeneity, clarity, lack of grittiness, and absence of phase separation, were displayed by the formulation, indicating good component compatibility. A sustained release profile with

74.50% cumulative release over a 24-hour period was found in the *In vitro* drug release research, suggesting regulated diffusion of phytoconstituents from the polymeric matrix. Patient compliance may increase and dose frequency may decrease as a result of this extended release pattern. The improved formulation showed 65.80% inhibition at 100 µg/mL in the antioxidant investigation, which showed concentration-dependent free radical scavenging activity. Additionally, even at the highest tested dose, the MTT experiment utilizing ARPE-19 retinal cells demonstrated good cell survival (>91%), indicating great biocompatibility and minimal cytotoxicity. The formulation's short-term stability was established by stability tests conducted in accordance with ICH Q1A (R2) recommendations, which showed no appreciable changes in pH, clarity, spreadability, or gelling capacity. All things considered, the created mucoadhesive in-situ gel is a potential ocular medication delivery method for long-term retinal protection and merits more *In vivo* testing.

CONCLUSION

The current study effectively created and refined a mucoadhesive ion-activated in-situ gel with ethanolic *Celosia argentea* seed extract for possible retinal protection and long-term ocular medication administration. The existence of bioactive phytoconstituents with antioxidant qualities, such as flavonoids, phenolic compounds, alkaloids, saponins, and betalains, was confirmed by preliminary phytochemical screening, UV-visible spectroscopy, and FTIR analysis. Formulation factors were successfully adjusted using a 3^2 complete factorial design; the F7 batch demonstrated appropriate viscosity, fast gelation, and a satisfactory pH. In the MTT assay, the improved formulation showed high spreadability, regulated drug release over a 24-hour period, considerable antioxidant activity, great compatibility with retinal cells, and adequate physical stability during storage. These results suggest that the proposed mucoadhesive in-situ gel is a viable ocular delivery technology that can enhance retinal protection through its antioxidant capacity, improve precorneal retention, and provide prolonged drug release.

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