Journal of Natural Remedies
DOI: 10.18311/jnr/2026/50696

INFORMATICS JOURNALS
Research Article
eISSN: 2320-3358 | Vol 26(1), 2026 | pp. 161-167

Blink Activated Healing: Design of a pH-triggered *In Situ* Catechin Ophthalmic Gel

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Abstract

Background: Ocular drug delivery poses significant challenges due to rapid precorneal drug elimination, poor bioavailability and patient non-compliance arising from frequent instillations. To overcome these limitations, a gel system activated by pH variation was successfully prepared. **Aim:** The current research emphasises the development and assessment of a pH-responsive in situ ophthalmic gel formulated with catechin. **Method:** An ophthalmic *in situ* gel sensitive to pH was formulated using HPMC-K4M and Carbopol 940, which transforms from a liquid to a gel when exposed to tear fluid. **Result:** The developed formulations were analysed to determine their drug content, physiological pH compatibility, refractive index and gelling capacity. *In vitro* drug release studies demonstrated sustained release over 12 hours. Stability studies confirmed that the formulation remains stable at room temperature by retaining its gel integrity and drug potency. Sterility testing showed that the formulation remains sterile over an extended period. The MTT assay on Y-79 retinoblastoma cells revealed dose-dependent cytotoxicity of the catechin-loaded in situ gel, with an IC_{50} value of 18.47 µg/mL, significantly lower than that of pure catechin. **Conclusion:** These findings suggest that the developed formulation offers enhanced therapeutic potential and sustained ocular delivery of catechin for effective eye disease management.

Major Findings: The pH-sensitive *in situ* ophthalmic gel of catechin achieved sustained drug release with good stability and sterility. It significantly enhanced anticancer activity, showing a lower IC_{50} than pure catechin, indicating improved ocular therapeutic efficacy.

Keywords: Catechin, *In Situ* Gel, Ocular Drug Delivery, pH-triggered, Sustained Release, Retinoblastoma (Y-79) Cells

(Article Chronicle: Article Received on: 18.07.2025; Revised on: 18.12.2025; Accepted on: 07.01.2026)

1. Introduction

Delivering drugs to the eye continues to be a major hurdle in pharmaceutical development because of the eye's complex anatomical and physiological defences. Although traditional eye drops are commonly used, they usually exhibit low bioavailability, often below 5%, due to precorneal challenges like tear dilution, quick drainage, and blinking. These issues require repeated dosing, which can reduce patient adherencee[1,2].

In situ gel systems offer a promising solution by undergoing sol-to-gel transition upon exposure to ocular conditions such as temperature, pH or ionic strength. Among these, pH-responsive gels, particularly those formulated with polymers such as Carbopol 940 and HPMC-K4M, are especially effective due to their

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ability to gel in the slightly alkaline environment of tear fluid. These systems help to extend the medication's duration on the surface of the eye, improve its interaction with the cornea and facilitate a controlled, sustained release of the medication[3].

Catechin, a polyphenolic compound derived from green tea, exhibits potent antioxidant, anti-inflammatory and anticancer activities[4]. Its application in ocular therapy is promising, particularly in combating oxidative stress-related disorders and ocular tumours such as retinoblastoma. However, catechin's poor aqueous solubility and instability in neutral or alkaline pH environments limit its therapeutic potential when delivered via traditional eye drops[5].

This research aims to create a pH-responsive in situ ophthalmic gel using Carbopol 940 and HPMC-K4M, to prolong ocular retention, safeguarding catechin from degradation and ensuring prolonged drug release. The prepared formulation was assessed for its physicochemical properties, drug content, *in vitro* release profile, stability, sterility and cytotoxic effects on Y-79 retinoblastoma cells using the MTT assay, to evaluate its effectiveness as a potential ocular system.

2. Materials and Methods

2.1 Materials

The Catechin was purchased from Yucca Enterprises, Mumbai and other excipients were obtained from New Neeta Chemicals, Pune.

2.2 Formulation of Catechin *In Situ* Gel

To formulate the catechin-loaded *in situ* gel, Carbopol 940 and HPMC K4M were weighed in a 1:0.5 (w/w) ratio and dispersed in 80 mL of distilled water. To ensure adequate hydration, the solution was allowed to stand overnight, and the polymers swelled. Separately, 15 mg of catechin, along with a small quantity of ethyl paraben, EDTA, and mannitol, was dissolved in a minimal amount of distilled water under continuous stirring until a clear solution was formed. This drug solution was gradually incorporated into the polymer dispersion with gentle stirring to achieve homogeneity. The final volume was brought up to 100 mL using distilled water. The pH of the formulation was adjusted to 5.0 ± 0.1 by carefully adding 20% triethanolamine dropwise, with constant monitoring using a calibrated pH meter. Finally, the gel was filtered if needed and stored in a clean, sterile container for further evaluation[3].

3. Characterisation

3.1 Melting Point

A small quantity of finely powdered catechin is put into a capillary tube that is sealed at one end in order to use a digital melting point analyser to find out the melting point of catechin. After that, the tube is placed into the analysis device. As the temperature rises, the sample is observed through the display until it starts and finishes melting. The temperature range is recorded as the melting point, and the apparatus is allowed to cool before reuse.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

Using the potassium bromide (KBr) pellet technique, FTIR spectra of catechin, carbopol, HPMC and their physical mixture were recorded. A hydraulic press was utilised to compress the substances into discs, and spectra were collected over the 400–4000 cm^{-1} wavelength range. Drug-excipient compatibility was studied using FTIR analysis.

3.3 Linearity Curve of Catechin by UV Method

A calibration curve for catechin was constructed to assess its linearity in phosphate buffer having a pH of 7.4, covering a concentration range from 10 to 50 µg/ml and measuring absorbance at 279 nm.

3.4 Drug Content

A millilitre of the gel formulation was put into a ten-millilitre volumetric flask and dissolved in ethanol. Ethanol was subsequently added to get the 10 mL volume. A millilitre of this stock solution was taken, and using Simulated Tear Fluid (STF), it was diluted to ten millilitres. With the use of a UV-visible spectrophotometer, the absorbance of the finished solution was measured at 279 nm, and the calibration curve equation was used to determine the drug concentration[4].

3.5 pH

Maintaining an appropriate pH in ophthalmic formulations is essential to ensure drug stability and minimise ocular irritation. The acceptable pH range for such formulations typically falls between 5.0 and 7.4. The pH of the formulated gel was determined using a digital pH meter[7].

3.6 Gelling Capacity

A drop of the formulation was put to a vial holding two millilitres of freshly made Simulated Tear Fluid in order to assess the gelling ability. The time it took for the gel to develop was noted[8].

3.7 Refractive Index

To check the refractive index of the *in situ* gel, a minimal quantity of the sample was applied onto the surface of the lower prism of a calibrated Abbe's refractometer and the refractive index value was read directly from the instrument's scale[9].

3.8 *In Vitro* Diffusion Study

The drug release profile was evaluated *in vitro* using a Franz diffusion cell equipped with a cellulose acetate membrane (25 mm diameter). Approximately 2 grams of the catechin in situ gel were placed in the donor chamber, while the receptor chamber was filled with simulated tear fluid (STF) maintained at pH 7.4 ± 0.2. Samples were taken up to 12 hrs at prearranged intervals. To maintain sink conditions, the receptor compartment was filled with an equivalent volume of new STF after each sampling. The amount of medication released was determined using the calibration curve equation after the absorbance of the collected samples was measured at 279 nm using a UV-Visible spectrophotometer[10].

3.9 Sterility Test

Sterility testing of the formulation was carried out using the Closed-Vial Method to reduce the chances of contamination. The one sterilised test tubes were filled with 10–20 mL of Soybean Casein Digest Medium (SCDM) for detecting aerobic bacteria and fungi, and another sterilised test tubes were filled with Fluid Thioglycollate Medium (FTM) for identifying anaerobic bacteria. Under aseptic conditions, 2 mL of the gel sample was transferred into each pre-sterilised test tube using a sterile syringe and the tubes were then sealed. The FTM tubes were incubated at 30–35°C, while the SCDM tubes were kept at 20–25°C for a period of 14 days. Daily observations of the test tube were made to check for any turbidity or visible microbial growth. The presence of a clear medium indicated sterility, whereas any cloudiness or turbidity suggested contamination[11].

3.10 Stability Studies

A 1-month stability study of catechin gel was conducted in accordance with ICH guidelines, which provide a framework for evaluating the stability of pharmaceutical products under various environmental conditions. Samples were stored at 25 ± 2 °C (room temperature) and 4 ± 1 °C (refrigerated) in airtight containers wrapped in aluminium foil to protect from light. Evaluations were performed at 0, 7, 14, 21 and 28 days to monitor appearance, pH, gelling capacity and drug content. This study helped identify any temperature-dependent changes in the formulation's stability[12].

3.11 MTT Assay

From the National Centre for Cell Science (NCCS), located in Pune, India, the Y-79 retinoblastoma cell line was acquired. In 96-well plates, cells were seeded at a density of 1×10^4 cells per well. They were then incubated for 24 hours at 37°C in a humidified atmosphere with 5% CO_2. The cells were treated with varying doses of catechin-loaded in situ gel (1, 5, 10, 25, 50, and 100 µg/mL) and pure catechin following the incubation time. 20 µL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well after an additional 24-hour incubation. The plate was then incubated at 37°C for an additional 4 hours. After this growth media was carefully removed, and 150 µL of dimethyl sulfoxide (DMSO) was added to each well in order to dissolve the formazan crystals. A microplate reader was used to detect absorbance at 570 nm. GraphPad Prism software was used to compute the percentage of cell viability and examine IC_{50} results[13].

4. Results and Discussion

4.1 Melting Point

The melting point of the catechin was found to be 204°C, which was in agreement with the reported value, i.e., 214[15].

4.2 FTIR Analysis

FTIR spectra of the main components of the formulation—pure catechin, HPMC K4M, Carbopol 940 and their physical mixture were recorded. Catechin shows characteristic O–H stretching vibrations at 3394.18 cm^{-1} and 3222.11 cm^{-1}, a C≡C stretch at 2338.79 cm^{-1}, a sharp C=O stretch at 1696.37 cm^{-1}, aromatic C=C stretching at 1616.07 cm^{-1}, CH_3 bending peaks at 1524.30 cm^{-1} and 1478.41 cm^{-1} and a series of C–O stretching peaks at 1363.70 cm^{-1}, 1282.39 cm^{-1}, 1145.73 cm^{-1}, 1088.38 cm^{-1}, and 1031.02 cm^{-1}, along with an out-of-plane vibration at 801.58 cm^{-1}, confirming the presence of multiple hydroxyl, carbonyl and aromatic groups. HPMC K4M displays peaks at 2361.73 cm^{-1} (C≡C or C≡N), 1535.77 cm^{-1} (N–H bend or aromatic C=C), and alkane CH_2/CH_3 bending at 1478.41 cm^{-1} and 1386.64 cm^{-1}. It also shows strong ether linkages, with C–O–C and C–O stretches at 1260.45 cm^{-1}, 1145.73 cm^{-1}, and 1042.49 cm^{-1}, as well as additional C–H vibrations and metal-oxygen bands below 500 cm^{-1}, indicating the presence of a polysaccharide backbone and substituents. Carbopol 940 exhibits C–H stretching vibrations at 3027.09 cm^{-1} and 2946.79 cm^{-1}, a prominent C=O peak at 1696.37 cm^{-1}, C=C stretching at 1558.71 cm^{-1}, CH_2 and CH_3 bending at 1455.47 cm^{-1} and 1409.58 cm^{-1} and C–O stretching at 1168.68 cm^{-1}, with out-of-plane deformations seen at 962.19 cm^{-1} and 801.58 cm^{-1}, characteristic of polyacrylic structures.

The FTIR spectrum of the physical mixture clearly exhibited all the major peaks of catechin, HPMC K4M and Carbopol 940 without the appearance of any new peaks. The slight shifting of peak positions may be attributed to physical mixing and possible hydrogen bonding interactions, but no chemical modification or new bond formation was observed. This indicates that catechin is compatible with the chosen polymers, with no evidence of chemical interaction between the drug and the excipients, confirming their appropriateness for formulation development.

4.3 Linearity Curve of Catechin by UV Method

The maximum absorbance wavelength of catechin was found tobe 279 nm. The linearity curve was developed in PBS solution at pH 7.4 at a concentration range of 10 to 50 µg/mL, and it shows a linear relationship with $R^2 = 0.9909$.

4.4 Drug Content

The drug content of formulations was found to be 97.8 ± 1.53 %, showing that the medication was uniformly distributed throughout the polymeric matrix.

As a result, the observed drug content data confirm that the formulation process was appropriate and show that the created in-situ gel system may be employed for ocular drug delivery with reliability.

4.5 pH

The formulation's pH was found to be 5.6 ± 0.13, which is slightly acidic but within an acceptable range for ophthalmic preparations. The formulation remained in its original form at room temperature and at the measured pH[7].

4.6 Gelling Capacity

Upon contact with simulated tear fluid, the sol-to-gel transition occurred within 30 seconds, forming a stable gel which remained intact for 3 hours.

4.7 Refractive Index

The refractive index of the gel was reported as 1.368. The refractive index of distilled water is 1.333. This shows that the gel is good to be used for the eye[9].

4.8 *In Vitro* Diffusion Study

A Franz diffusion cell with STF pH 7.4 as the dissolution solvent was used to assess the *in vitro* drug release of catechin from both the pure drug solution and the pH-sensitive *in situ* ocular gel. Up to 12 hours, the cumulative percentage medication release was assessed at predetermined intervals.

The *in vitro* drug release profile clearly demonstrates a sustained release pattern from the *in situ* ophthalmic gel compared to the immediate release from the pure catechin solution, as shown in Figure 1. The pure drug solution released over 99 ± 1.963% of catechin within 6 hours, indicating rapid availability but a shorter duration of action. In contrast, the pH-sensitive in-situ gel showed a gradual and extended release with only 45.5 ± 0.968 % of the drug released at 4 hours and reaching ~95% release by 12 hours. It might be because the polymer changes from a sol to a gel when it comes into contact with tear fluid, creating a viscous barrier that regulates the diffusion of drugs. By delaying drug diffusion, the gel prolongs the drug's

residence duration on the ocular surface and offers sustained drug release.

4.9 Sterility Testing

The *in situ* gel was tested using the Closed-Vial Method. No turbidity or microbial growth was observed in FTM or SCDM over 14 days. Confirming that the formulation was sterile.

4.10 Stability Study

Over the course of 28 days, a stability study was carried out to assess the *in situ* ophthalmic gel formulation's physicochemical stability at room temperature and 4 °C. Samples were examined for physical characteristics, pH, drug concentration and gelling capacity at 0, 7, 14, 21, and 28 days.

The formulation retained its clarity and colourless to slightly opalescent appearance throughout the study period at both storage conditions. No visible signs of precipitation, phase separation or microbial contamination were observed. This indicates that the formulation is physically stable under both conditions for at least 28 days. The use of suitable preservatives and a well-optimised formulation base prevented microbial growth and phase instability. At both storage temperatures, the formulation's pH was determined to be within the permissible ophthalmic range. Minor fluctuations in pH were observed, but these were not significant.

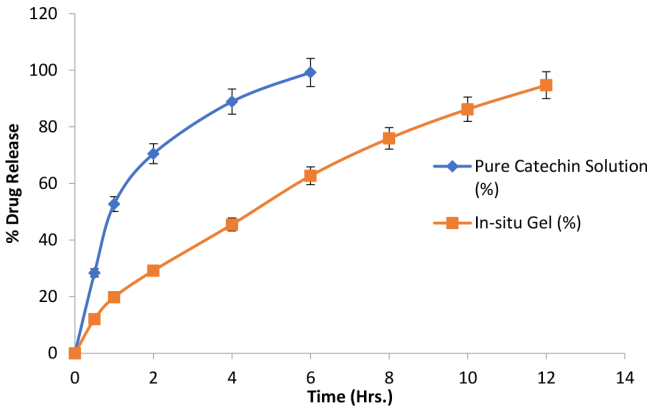


Figure 1. % drug release of catechin.

The slight decrease in pH at 4 °C/ RT may be attributed to minor interactions between excipients and changes in ionisation with temperature (Table 1). However, the pH remained within an acceptable range no adjustment was necessary. The drug content during the stability study was calculated and is presented in the table below. In both storage conditions, the drug content remains in an acceptable range, confirming the chemical stability of the drug over the study period.

The medication seems to be protected from degradation by the formulation. Because molecular activity is lower at lower temperatures, the slower breakdown rate at 4 °C is to be expected (Table 1). However, even at room temperature, the drug remained stable, indicating good formulation compatibility and protection. The formulation maintained its gelling capacity when exposed to STF (pH ~7.4) throughout the study period.

Gelling was immediate and sustained at all-time points under both storage conditions (Table 1). The presence of Carbopol and HPMC K4M maintained their responsiveness. No breakdown or loss of gelling ability was observed, suggesting structural integrity of the polymers was preserved.

On the basis of physical appearance, pH, drug concentration, and gelling capacity, the in situ ophthalmic gel formulation was determined to be stable over 28 days at room temperature and 4 °C. Importantly, the formulation remained stable at room temperature, which is advantageous for practical storage and distribution. The study confirms that the formulation maintains its therapeutic and

Table 1. Stability studies of *in situ* ophthalmic gel of catechin at room temperature and 4 °C

Day	RT			4° C		
	Ph	Drug Content	Gelling Capacity	pH	Drug Content	Gelling Capacity
0	4.5 ± 0.234	99.2 ± 0.674	30 ± 0.183	4.5 ± 0.234	98.7 ± 1.374	30 ± 0.183
7	4 ± 0.235	98.8 ± 0.356	30 ± 0.177	4 ± 0.235	98.0 ± 1.034	30 ± 0.177
14	4.00 ± 0.236	97.5 ± 1.546	30 ± 0.156	4.67 ± 0.238	97.1 ± 0.937	30 ± 0.189
21	4.00 ± 0.232	96.3 ± 0.927	31 ± 0.178	4.56 ± 0.227	95.8 ± 0.849	31± 0.189
28	4.00 ± 0.237	96.1 ± 1.025	31 ± 0.188	4.48 ± 0.240	95.4 ± 0.462	31 ± 0.190

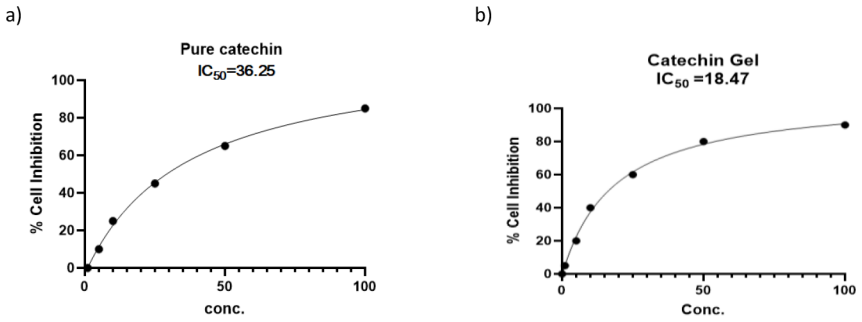


Figure 2. IC_{50} Value of **(a).** pure catechin and **(b).** catechin gel.

physicochemical properties without the need for cold storage.

4.11 MTT Assay

The IC_{50} value of catechin-loaded pH-sensitive ophthalmic *in situ* gel was found to be 18.47 µg/mL against Y-79 cells (Figure 2). In comparison, the IC_{50} of pure catechin was 36.25 µg/mL (Figure 2), indicating improved cytotoxic efficacy of the formulation. The MTT assay revealed a dose-dependent cytotoxicity of the catechin-loaded *in situ* gel formulation on retinoblastoma (Y-79) cells. The lower IC_{50} value of the gel compared to pure catechin suggests enhanced cellular uptake and sustained release from the gel matrix. The gel's pH sensitivity, which promotes extended drug availability at the ocular location, may be the cause of this improvement. The findings indicate that the developed formulation holds potential as a targeted topical ocular therapy for retinoblastoma with minimised systemic exposure.

5. Conclusion

The present study successfully developed and evaluated a pH-responsive ophthalmic *in situ* gel containing catechin for enhanced ocular drug delivery. By employing Carbopol 940 and HPMC-K4M polymers, the formulation demonstrated an efficient sol-to-gel transition upon exposure to tear fluid pH, resulting in prolonged ocular residence time. Physicochemical evaluations confirmed acceptable drug content (97.8%), physiological pH (5.6 ± 0.13), suitable refractive index and effective gelling capacity. *In vitro* diffusion studies revealed sustained drug release over 12 hours, significantly improving therapeutic duration compared to pure catechin solution. The formulation remained sterile throughout 14-day sterility testing, and 28-day stability studies confirmed that the gel retained its appearance, drug content, pH, and gelling behaviour under both room and refrigerated conditions. Cytotoxicity evaluation via MTT assay on Y-79 retinoblastoma cells demonstrated enhanced anticancer efficacy, with the catechin-loaded gel exhibiting a lower IC_{50} (18.47 µg/mL) than pure catechin (36.25 µg/mL), indicating better cellular uptake and prolonged drug activity. Overall, the developed *in situ* gel formulation presents a potential strategy for sustained ocular medication administration, minimising frequent dosing and enhancing patient compliance. It also holds potential as a targeted therapy for ocular conditions like retinoblastoma, offering improved therapeutic effectiveness with reduced systemic side effects.

6. Acknowledgement

The authors sincerely thanks for the assistance of SGMSP's Dnyanvilas College of Pharmacy, Pune, in providing the resources required to complete this study. We are also grateful to our colleagues for their invaluable advice and support during the research.

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Author Contribution

Sonali Devne guided the students, supervised the research work, and prepared the manuscript. Purva Bahekar, Sakshi Andhale, Pooja Jeswani, Sakshi Gopale, Devyani Jangale, and Sharayu Bhagat performed all the experimental work. Vidya Yelam (Kapse), Smita Waghamare, Neelam Bhagdewni, and Pratibha Shelke assisted in data interpretation and manuscript preparation. All authors have read and agreed to the published version of the manuscript.